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The Syd Wilkins Memorial Fund was set up in 1983-84 by the Australian Branch of the World's Poultry Science Association (WPSA), with the active collaboration of the Poultry Husbandry Research Foundation (PHRF) as it was known at the time, with the help of a major opening donation from Allied Feeds. The purpose of the fund was to honour the many contributions of Syd Wilkins to the Australian poultry industry. The practical use of the Fund was to provide an award, called the WPSA Syd Wilkins Memorial Award, for outstanding work by young poultry scientists working in Australia. The first annual award was made in 1984 but the definition of young has been extended twice from the initial 30 years to the present 35 years.

Syd Wilkins received his tertiary education at Hawkesbury Agricultural College and developed a career as a Poultry Officer in the NSW Department of Agriculture, becoming its Senior Poultry Officer by the late 1950's. In the mid to late 1960's he transferred to Allied Feeds, where he remained as a Senior Executive of the Allied Mills Group until his untimely death in 1982. During all this time he was very active in the affairs of the WPSA Australian Branch, including a period as President. He was also elected as one of the Vice Presidents of WPSA world body in the 1970's, a position he still held on this death. He was actively involved in the conduct of the 1974 and 1978 World's Poultry Congresses in New Orleans and Rio de Janeiro.

Syd Wilkins was also involved with the PHRF virtually from its beginning, and served many years as its Vice-President. He was the recipient of the Australian Poultry Award in 1974. Syd's career was contemporary with the application of the 20th century poultry technology revolution in Australia. In his own low-key and unassuming way he contributed very significantly to its progress.

Previous recipients of the prize are:

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EFFECT OF IB VACCINATION STRAIN DURING REARING ON EGG PRODUCTION AND QUALITY FOLLOWING CHALLENGE WITH IB VIRUS IN THE LAYING CYCLE

M.J. JOLLY[1], J.R. ROBERTS[1] and W. BALL[1]

Summary

In the trial reported here, egg production and egg quality response to challenge at 62 weeks with T-strain IBV were evaluated in 320 vaccinated birds. The birds were vaccinated at 4, 28 and 198 days with one of A3 (A) or VicS (V) vaccine strains to form four vaccination treatment groups AAA, AVA, VVV and VAV. Egg production declined significantly for three weeks post-exposure to T-strain IBV. Moderate negative effects of IBV challenge were found on shell quality measurements, but no deleterious effects were found on internal quality measurements. All birds were still partially immune to some of the egg quality effects of T-strain IBV when challenged late in lay.

I. INTRODUCTION

Since the earliest reports in laying flocks, infectious bronchitis virus (IBV) has been connected with declines in egg production and quality (Sevoian and Levine, 1957). The negative impacts on quality most commonly associated with IBV infection are a thinning of the albumen component, lightening of shell colour, reduced shell thickness, decrease in egg weight and increased incidence of abnormal shell formation (Sevoian and Levine, 1957; McDougall, 1968; Box *et al.*, 1980; Wernery and Daivi, 1984). The importance of IBV to the commercial egg industry is clear, it has the potential to directly impact both egg production and quality.

Currently all pullets destined for commercial egg production in Australia are vaccinated against IBV. Only live attenuated vaccines are presently available for use in this country and the two most widely used vaccines are VicS and A3, both derived from local field strains (Ratanasethakul and Cumming, 1983). The administration protocol for IBV vaccines most commonly involves three vaccinations during the rearing phase, before point of lay. Some producers practice revaccination during the lay cycle. ELISA assessments of circulating IBV antibody titres are used in industry to varying degrees as indicators of immune status and to confirm field infections. Work both within Australia and in other countries has raised questions about the potential negative effects of administering a live attenuated vaccine to a flock in lay (Box, 1988; Sulaiman, *et al.*, 2004). The duration of immunity after vaccination with live vaccines is not clearly understood, but is believed to decline over time from last vaccination (Alexander and Gough, 1978). Work evaluating the impact of vaccine protocols and challenge with IB during lay on a whole flock basis is very limited due to the high costs associated with such trials. Obtaining information about the impacts of vaccination protocol on subsequent egg quality and production, following IB challenge during lay, is fundamental to any future discussion about vaccination best practice. The availability of only live attenuated vaccines and the potential for a decline in immunity with time, results in a conflict between providing the best level of protection in a way that is cost effective, both in terms of revaccination costs, and potential losses in production and product quality. The work presented in this paper investigated whether vaccination during the rearing phase was sufficient to prevent decline in egg production and quality when birds were challenged with IBV late in the lay cycle and whether different rearing period vaccination protocols had a lasting impact on such a response.

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II. MATERIALS AND METHODS

Three hundred and twenty pullets of four different commercial strains of birds were obtained at day old and reared together on litter in isolation sheds at the University of New England. All birds were vaccinated for IBV by coarse spray at day old and at 4 and 14 weeks of age, with one of two commercial live attenuated vaccines, VicS (serotype B, Ignatovic and McWaters, 1991) and A3 (serotype C, Ignatovic and McWaters, 1991). Four vaccination treatment groups were formed by the combination of the vaccine strains given at each age as shown in Table 1. The flock was also vaccinated for Marek's disease at day old and Avian Encephalomyelitis at 12 weeks of age (all vaccines, Fort Dodge Australia).

Table1. Vaccination treatment groups; vaccine strain administered at each vaccination time.

Treatment group	Day old	4 Weeks	14 Weeks
AAA	A3	A3	A3
AVA	A3	Vic S	A3
VVV	Vic S	Vic S	Vic S
VAV	Vic S	A3	Vic S

At 14 weeks of age, the hens were moved to conventional layer cages in two large isolated sheds, at a stocking rate of two birds per cage. At this time the birds were also lightly beak trimmed and divided into groups. Birds from each vaccination treatment were divided into groups of ten with four replicates of each treatment being housed in each shed. The flock was then transferred to a pre-layer diet before being maintained on a commercially formulated layer diet, with regular monitoring of egg production and egg quality.

At 62 weeks of age, the birds in one of the sheds (160 hens) were exposed to T-strain IBV (serotype C, Ignatovic and McWaters 1991) by eye drop of virus suspension $10^{7.4}EID_{50}$/mL) to one in every two birds (IB Challenge group), while the other shed was left unchallenged (Control group). This resulted in each bird within the IB Challenge group receiving an estimated dose of 2×10^5 EID_{50}.

From three weeks before challenge, egg production was recorded daily for each replicate group and averaged over weekly intervals. For statistical analysis of the production data, the challenge period was divided into 3 time intervals, A: three weeks before exposure, B: three weeks immediately after exposure and C: three weeks following interval B.

To compare any inherent differences between the challenge and control groups, egg production was measured and eggs collected in the two groups four weeks prior to challenge. During the challenge trial, eggs were collected for analysis at weekly intervals for 6 weeks after challenge. At each collection, 10 eggs per replicate group (320 eggs per collection) were analysed. Shell quality parameters were assessed including deformation, breaking strength, reflectivity, egg weight, shell weight, percentage shell and shell thickness. Albumen height, Haugh units and yolk colour were assayed as indicators of internal egg quality (Technical Services and Supply, U.K.).

Heparinised blood samples were taken from 5 individually identified birds per treatment group immediately prior to challenge and at 3 weeks after challenge. Plasma samples were analysed using the TropBio® ELISA kit for IBV antibody titres. Statistical analysis was carried out using ANOVA and Fisher's PLSD to establish significant differences between group means. (Stat View 5.0.1, SAS Institute Inc).

III. RESULTS

a) Egg Production

The three weeks prior to challenge, interval A, was analysed separately for differences between vaccination groups unrelated to challenge. The results indicated that the VAV (81.1 ± 2.43), VVV (80.1 ± 1.75) and AVA (75.8 ± 2.35) groups were not different and the AAA (72.3 ± 2.27) and AVA groups were not significantly different ($P < 0.05$). The two groups which received VicS at day old had significantly higher production, immediately prior to challenge, than the AAA group but not the AVA group.

Over the six weeks following challenge with IBV (intervals B and C), comparison of the IB Challenge and Control birds was analysed. The birds infected with IBV had a significantly lower production (66.7 ± 1.34 eggs/hen/100 days) than the Control group (72.15 ± 1.15 eggs/hen/100 days) ($P<0.01$). For the same 6-week post-challenge collection period, the VAV vaccination group (75.6 ± 1.78) had significantly higher production than the VVV (69.5 ± 1.52), AVA (67.2 ± 2.08) and AAA (66.5 ± 1.57) groups, which were not different from one another ($P<0.01$).

Among the three time intervals, for the control and challenge groups combined, A (77.3 ± 1.15 eggs/hen/100 days) was significantly higher than the other two intervals B (70.3 ± 1.31 eggs/hen/100 days) and C (71.6 ± 1.15 eggs/hen/100 days), which were not different ($P < 0.01$).

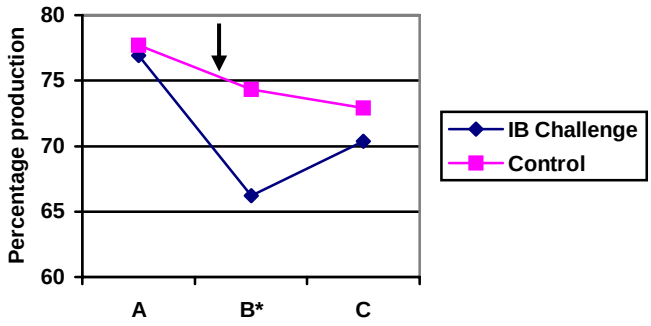

Figure 1. Effect of challenge on egg production at different time intervals relative to challenge ($P <0.05$). Arrow indicates timing of exposure of T-strain group to virus.* indicates statistically significant differences between means at this time interval.

The overall significantly lower production in the IB Challenge group was due almost entirely to the interaction between challenge and time interval ($P <0.05$). For the three weeks prior to challenge (interval A), production was not different between the Control and IB Challenge treatment groups (Figure 1). Over the three weeks following challenge (interval B), production declined slightly in the control group but dropped significantly in the IB Challenge group. During the subsequent three weeks (interval C), production was not significantly different between the Control and IB Challenge groups, although it was lower in both groups than during interval A.

Across the six weeks after challenge there was a significant interaction between the challenge and vaccination group treatments ($P <0.05$). In the VAV and AVA groups there was significantly higher production in the Control than in the IB Challenge groups in this post challenge period (Table 2).

Table 2. Effect of IB challenge on egg production in the four vaccination groups.

	AAA	AVA	VVV	VAV
IB Challenge	65.8 ± 2.72	62.7 [b] ± 2.71	67.3 ± 2.32	71.0 [b] ± 2.82
Control	67.1 ± 1.63	71.7 [a] ± 2.93	71.6 ± 1.92	80.3 [a] ± 1.75
P-value	>0.05	<0.01	>0.05	<0.01

Means ± Standard Error. Different superscripts in columns indicate significantly different means (P <0.05). P-values are for comparison of Control and IB Challenge treatments within each vaccination group.

b) Egg Quality

The baseline collection 4 weeks prior to challenge was to investigate inherent differences between the Control and IB Challenge groups prior to the challenge experiment. There were significant differences between the control and IB challenged groups for both breaking strength and percentage shell, prior and subsequent to challenge, as shown in Figures 2 and 3.

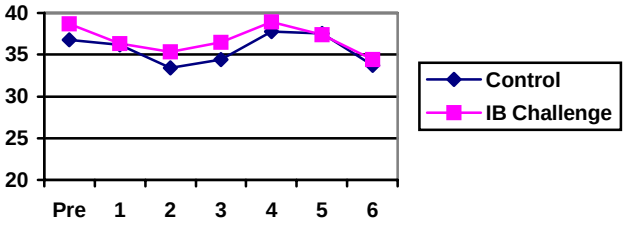

Figure 2. Breaking strength over time in relation to challenge.

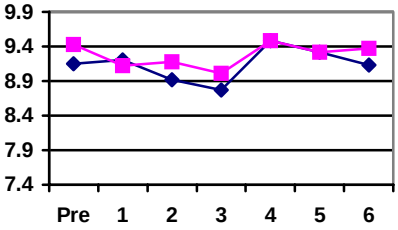

Figure 3. Percentage Shell over time in relation to challenge.

Four weeks before challenge there were significant differences between vaccination groups in breaking strength, albumen height and Haugh units (Table 3). Vaccination group AVA performed best of all groups for all three parameters, although group differences were not large.

The IB Challenge group had significantly lower egg weight, shell weight and yolk colour compared to the Control group for six weekly egg collections following challenge (Table 4). Further, the IB Challenge group had significantly higher shell reflectivity (Table 4), breaking strength and percentage shell (Figures 2 and 3).

Egg weight was significantly lower for the IB Challenged group in all vaccination treatment groups (Table 5). Shell colour was significantly darker (lower reflectivity) for the Control group only within vaccination group VAV (Table 5). Yolk colour was significantly paler for the IB Challenged group within vaccination groups AAA and VVV only (Table 5).

c) IBV Antibody titres

There was a significant interaction between time of blood collection and challenge group (P <0.01) for IBV antibody titres. The challenge treatment groups were not significantly different prior to challenge, while at 3 weeks post-challenge, the titres of IB challenged birds were significantly higher than the Control birds (P <0.01), which had not varied significantly between the two collections (Figure 4).

Table 3. Significance effects of vaccination group at the before challenge egg collection.

	AAA	AVA	VVV	VAV	P-value
Breaking Strength (N)	$37.29^{ab} \pm 0.80$	$39.18^{a} \pm 0.69$	$39.01^{a} \pm 0.67$	$35.48^{b} \pm 0.75$	<0.01
Albumen Height (mm)	$8.41^{a} \pm 0.18$	$8.41^{a} \pm 0.14$	$7.90^{b} \pm 0.15$	$7.94^{b} \pm 0.12$	<0.01
Haugh Units	$89.86^{ab} \pm 0.18$	$90.39^{a} \pm 0.81$	$87.15^{c} \pm 1.00$	$87.84^{bc} \pm 0.76$	<0.05

Means ± Standard Error. Different superscripts in rows indicate significant differences between means (P<0.05).

Table 4. Significant effects of challenge treatment group on egg quality parameters.

	Control	IB challenge	P-value
Egg weight (g)	63.01 ± 0.17	61.68 ± 0.16	<0.01
% Reflectivity	44.82 ± 0.41	45.66 ± 0.44	<0.01
Shell weight (g)	5.75 ± 0.02	5.70 ± 0.02	<0.05
Yolk Colour	11.57 ± 0.03	11.40 ± 0.04	<0.01

Means ± Standard Error.

Table 5. Significant effects of IB Challenge on egg quality parameters within vaccination treatment groups.

		AAA	AVA	VVV	VAV
Egg Weight (g)	Control	$63.84^{a} \pm 0.39$	$61.82^{a} \pm 0.32$	$63.92^{a} \pm 0.30$	$62.47^{a} \pm 0.32$
	IB Challenge	$61.62^{b} \pm 0.31$	$60.94^{b} \pm 0.29$	$62.66^{b} \pm 0.37$	$61.45^{b} \pm 0.32$
% Reflectivity	Control	44.27 ± 0.83	45.22 ± 0.82	46.34 ± 0.74	$43.44^{b} \pm 0.89$
	IB Challenge	44.56 ± 0.94	42.95 ± 0.84	47.43 ± 0.81	$47.56^{a} \pm 0.86$
Yolk Colour	Control	$11.57^{a} \pm 0.06$	11.47 ± 0.07	$11.79^{a} \pm 0.06$	11.45 ± 0.07
	IB Challenge	$11.17^{b} \pm 0.08$	11.58 ± 0.07	$11.37^{b} \pm 0.07$	11.48 ± 0.07

Means ± Standard Error. Means in columns within parameters with different superscripts are significantly different at P<0.05.

The increase in antibody titre of the IB Challenge group was due largely to the response in the vaccination groups that had received VicS at day old. These vaccination groups had lower titres prior to challenge and greater rises in antibody titre after challenge than the two groups that had received A3 at their first IBV vaccination (Figure 5). Only the VVV and VAV vaccination treatment groups showed significant increase in antibody titres after exposure.

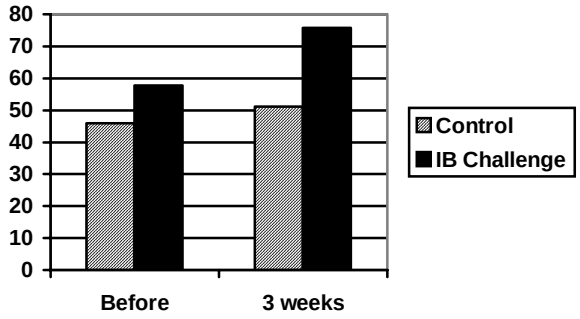

Figure 4. Effect of challenge with T-strain on IBV antibody titre 3 weeks after challenge.

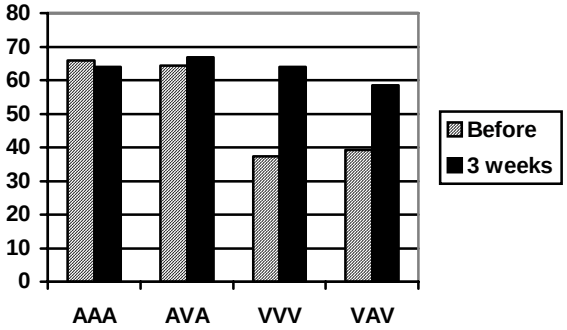

Figure 5. Effect of challenge on IBV antibody titre in the four vaccination treatment groups.

IV. DISCUSSION

This experiment involved birds that had not been revaccinated beyond the rearing phase of the production cycle. The success of the experimental challenge at 62 weeks is shown by the rise in the IB Challenge group antibody titres after exposure. When these birds were exposed to T-strain infectious bronchitis virus, some deleterious effects on egg production and egg quality were recorded.

There was an obvious and significant decline in production over the challenge period, which was unrelated to the effects of the virus. These birds were reaching the end of their first lay period at this time and therefore a decline in the numbers of eggs produced would be expected (Nys, 1986). However, as well as the decline in production with age, there was a significant drop in the IB challenged birds during the three weeks immediately after challenge. The depression in production associated with IBV infection is consistent with the work of others (Sevoian and Levine, 1957; Jones and Jordan, 1972). The significant difference between the IB Challenge and Control groups for the VAV vaccination group across the whole trial period was due to the Control group from this vaccination treatment having a very high level of production for this stage of lay, 80.3%, and as such is perhaps a case of the control group being high rather than, or as well as, the T-strain group having declined in production. All the vaccination treatment groups had numerically lower production in the T-strain infected birds, but between-replicate variation was such, in the VVV and AAA groups, as to prevent statistically significant differences.

The baseline egg collection was carried out four weeks prior to challenge, rather than immediately before. The flock was in the later stages of lay when this trial was carried out, so at this point there are age related changes in egg and eggshell quality. This base-line collection was not included in the statistical analysis of the post-challenge egg collections as

variations related to age, rather than response to challenge, would have influenced the findings. It can be seen from Figures 2 and 3 that the control and IB challenged groups followed very similar patterns over the progressive collections after one group was exposed to virus. Consequently any differences seen after challenge in shell breaking strength and percentage shell are due to inherent differences between the two groups of birds, which were present before challenge occurred.

Based on the one egg collection prior to challenge there appear to be some lasting effects of rearing phase vaccination protocol on egg quality measurements (Table 3). The vaccination treatment groups initiated with the A3 attenuated virus had better albumen quality and shell breaking strength. At this point in the lay cycle, 58 weeks of age, the egg quality would already be on the decline. Hence it may be that the treatment groups, which received A3 at day old, have persisted at higher quality for longer than the VicS at day old groups. These results suggest that use of A3 for vaccination at day old has potential benefits late in lay.

The significant effects of challenge on egg quality measures were on egg weight, shell reflectivity, shell weight and yolk colour. The egg weight of the IB Challenge group following challenge was significantly lower than that of the control group. A decrease in egg weight with IBV infection, along with a decline in production, was one of the first noted signs of the disease in naive birds in the field (Hill and Lorenz, 1956) and it appears to still occur in these vaccinated birds. There was a slight but significant decrease in the mean shell weight of the IB challenged group compared to the control group. This is probably in line with the decline in egg weight, but is too small to account for all of the variation seen in egg weight. The smaller egg weight in the challenged birds is possibly also contributed to by changes in the amount of fluid added to the egg during the formation of the albumen components. This would appear to agree with the work of others, which suggests that some IBV strains target the cells of the upper reproductive tract, and impair albumen formation (Butler *et al*., 1972; Davidson, 1986). However, this has yet to be established for T-strain IBV, traditionally viewed as a nephropathogenic strain (Ignatovic and McWaters, 1991). The shell colour of the IB challenged group was significantly paler than the control group over the six weekly collections following exposure of half the flock to the T-strain virus. A lightening of 2-3% reflectivity is unlikely to be detected by the naked eye or commercial grading equipment and consequently may not be of great practical importance to industry. Despite the very small magnitude of the shell lightening as measured by objective equipment in this case, it coincides with the widely reported subjective assessment of pale shells in association with IBV in fully susceptible birds (McDougall, 1968).

Yolk colour was highly variable between treatment and vaccination groups, with no clear pattern, although it would appear that the yolk colour of the IBV challenged birds became lighter suggesting that these birds were eating less of the pigment-containing feed. The reduction in feed intake with IB infection has been reported by others (Sevoian and Levine, 1957; Heath, 1970) but has not been linked directly to IBV effects, rather a depression in general health. Feed intake measurements were not carried out on the birds in this experiment.

The significant differences between vaccination treatment groups that were noted before challenge in shell breaking strength and albumen quality persisted throughout the post-challenge collections. There were some differences in the response to IB Challenge when compared to the Control group within the same vaccination group. However, there was no clear pattern emerging from the post-challenge results of any of the vaccination protocols, or vaccine strains, being superior in the face of T-strain challenge.

It was expected that a measurable difference would have been detected between the AAA and VVV groups related to the level of homologous protection, since A3 and T-strain

are of the same serotype (Ignatovic and McWaters, 1991). However, S1 glycoprotein sequence analysis has been utilised to divide all Australian strains into two groups with VicS, A3 and T-strain all belonging to Group I (Sapats *et al.*, 1996). Whatever the relationship between the two vaccine strains and the challenge virus used in the study, no vaccination protocol performed consistently better across all the parameters analysed.

The vaccination groups that received VicS at day old had a larger increase in antibody titre and lower titres prior to challenge. The antibody titre reached during the growth phase was not substantially different among vaccination groups (data not shown). This suggests that the circulating antibody titres of these initial VicS groups declined more between the last vaccination at 14 weeks and the time of challenge at 62 weeks of age. These differences in circulating antibody titre prior to challenge did not translate into clear differences in egg quality responses to T-strain infection.

When birds were challenged with T-strain at 62 weeks of age, there was a significant drop in egg production. There were also some modest negative impacts on egg size and shell colour. The internal quality deterioration routinely associated with IBV (Sevoian and Levine, 1957; Box *et al*., 1980; Wernery and Daivi, 1984) was not observed in this trial. Consequently it can be assumed that the birds retained some level of immunity between 14 and 62 weeks of age, an interval of 48 weeks. While being adequate to prevent major direct effects of IBV, this immunity was unable to prevent more generalised effects on production and shell quality. The magnitude of these production and quality effects must be considered in the context of commercial production realities and the cost of ongoing regular revaccination.

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NON-STARCH POLYSACCHARIDES, PROTEIN AND STARCH: FORM FUNCTION AND FEED - HIGHLIGHT ON SORGHUM

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Summary

In terms of structure and chemical composition, sorghum grain is remarkably similar to maize. The digestibility of sorghum starch is lower than that of maize starch and the major factor responsible for this appears to be a subtle difference in the endosperm protein bodies and surrounding protein matrix, which envelop the starch granules. The sorghum prolamin proteins (kafirins) in the protein bodies seem to be more cross-linked than their maize zein counterparts and cross-link more when the grain is subjected to wet-cooking. The cross-linking is primarily due to disulphide bonding. These cross-linked proteins seem to form a barrier to the egress of hydrolytic enzymes and probably also limit starch granule expansion during gelatinisation. Chemical treatments such as addition of sulphites and dry physical treatments such as fine milling, extrusion cooking or popping to disrupt the protein matrix seem to improve starch digestibility and may be of practical value. Selection of floury endosperm type sorghum may also be useful. Mutants with high protein digestibility that are also richer lysine have been identified and it is possible that these traits can be incorporated into sorghum lines with good agronomic properties

I. INTRODUCTION

The chemistry and structure of sorghum grain is unique (Taylor and Dewar, 2001), as is the case with all other cereal grains. Knowledge of and understanding of sorghum's uniqueness is the first step to optimising its exploitation. This paper will examine the unique features of the structure and chemistry of the sorghum grain with respect to nutritional negatives compared to other cereal grains. Possible strategies for alleviating these negatives will be discussed.

II. POLYPHENOLS

The grain of most sorghum cultivars contains higher levels of polyphenols than the grain of other cereal species. The red non-tannin sorghums are highly pigmented with polyphenolic anthocyanins and anthocyanidins. These appear to help protect the grain on the plant from insect and fungal attack. It is possible that they are slightly antinutritional. They bind strongly to the grain starch (Beta, 1999) and protein, but do not significantly affect digestibility (Duodu *et al*., 2002). However, the antioxidant properties of these compounds probably more than outweigh any antinutritional properties.

III. CELL WALLS

The endosperm cell walls of sorghum grain (Verbruggen, 1996) and maize grain (Huisman *et al*. 2000) are rich in water inextractable (insoluble) glucuronoarabinoxylan type pentosans. Unlike for example barley, but like maize, the sorghum endosperm walls are not broken down during germination. Access by endogenous hydrolytic enzymes to the contents of the endosperm cells is via portals in the cell walls (Glennie, 1984). It is therefore probable

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that the cell walls themselves constitute something of a barrier to digestion. The cell walls are also intimately bound to the endosperm matrix protein. The mechanism of attachment is not known, but it is possible that the phenolic acid ferulic acid plays a role (Parker *et al*., 1999) (Figure 1).

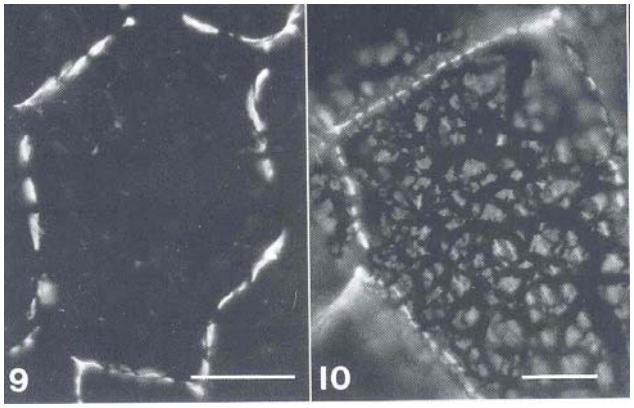

Figure 1. Autofluorescence micrograph of popped sorghum (left) and popcorn (right) fragmented cell walls (from Parker *et al*., 1999)

IV. PROTEINS

a) Protein matrix and protein bodies

As in maize, the sorghum endosperm proteins are of two general types: a matrix and the protein bodies. The matrix envelops the starch granules and the protein bodies (Fig. 2). The matrix protein is probably simply remnants of the endosperm cytoplasmic protein. It is soluble in aqueous alkali and therefore in terms of the Osborne protein classification it can be considered as glutelin-type protein (Taylor *et al*., 1984). However, it is fundamentally different from the wheat glutelins, the glutenins, which are storage proteins. The protein bodies, which are essentially spherical and 1-2 microns across (Fig. 2), are comprised of prolamin proteins (Taylor *et al*., 1984). The prolamin of sorghum is called kafirin. Unlike in wheat and barley, but like in maize, the bodies persist in the mature grain, but are not membrane-bound. Kafirin, like the maize prolamin, zein, comprises three major protein species, designated alpha, beta and gamma (Shull *et al*., 1991). These are small proteins of molecular weight ranging from approx. 16 to 28 k. Alpha, which makes up about 80% of the total kafirin/zein fraction, is concentrated in the core of protein body (the cork of the cricket ball). Beta- and gamma-kafirin/zein are concentration at the surface of the protein body (the leather cover) (Oria *et al*., 1995). Beta- and gamma-kafirin/zein are rich in cysteine and are linked together with themselves (El Nour *et al*. 1998; Duodu *et al*., 2002) and with alpha-kafrin/zein, and probably with the matrix proteins, into oligomers of polypeptides by disulphide bonds. When wet-cooked the degree of linking increases (Duodu *et al*., 2002). We think that large polymers of molecular weight at least 100 k are formed. For reasons not known the degree of cross-linking on wet-cooking with kafirin seems to be greater than with zein. It also appears that some of the cross-links, particularly in kafirin, may not be disulphide bonds, since not all the oligomers can be broken down into monomers with reducing agents that are used for this purpose (Duodu, *et al*., 2002).

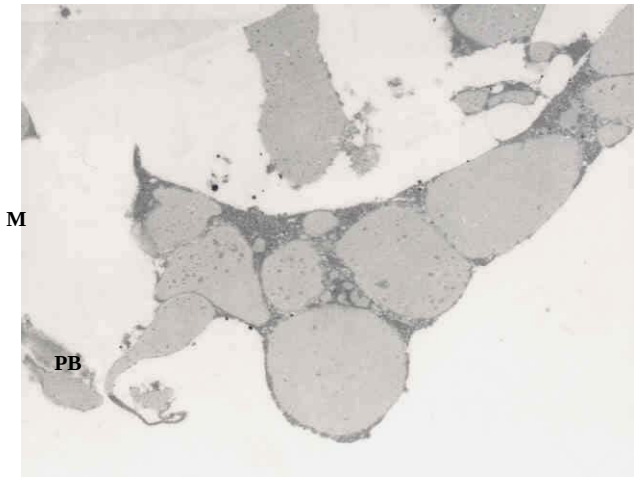

Figure 2. TEM of sorghum protein bodies (PB) and matrix (M)

b) Protein digestibility

The formation of these cross-linked proteins is believed to be the major, but probably not the only, cause of the well-described reduction in the protein digestibility of sorghum on wet-cooking (Table 1) (Duodu *et al*., 2002; 2003). It has been proposed that the cross-linked gamma- and beta-kafirins at the surface of the protein bodies are poorly digestible and that they form a barrier to protease enzymes reaching the readily digestible alpha-kafirin at the core (Oria *et al*., 1995). Strong evidence in support of this theory comes from the research of Prof B R Hamaker and co-workers of Purdue University. They have discovered sorghum mutant lines with high protein digestibility, even when cooked (Oria *et al*., 2000). The protein bodies of these mutants are not generally spherical, but are highly invaginated, in section like the petals of a flower (Fig. 3). Unlike in the protein bodies of normal sorghum, the beta- and gamma-kafirins are concentrated at the base of the invaginations. Hence, they would not constitute a barrier to digestion of the alpha-kafirin. Indirect support for this theory comes from the fact that kafirin polypeptide cross-linking occurs as normal in these mutants (Duodu, 2000).

Table 1. Effect of wet-cooking on the *in vitro* pepsin protein digestibility (g/kg) of whole grain, endosperm and protein body enriched sorghum and maize flours (adapted from Duodu *et al*., 2002).

Grain	Treatment	Whole grain	Endosperm	Protein body enriched
Red med	Raw	591	657	728
sorghum	Wet cooked	305	359	442
White hard	Raw	558	674	743
sorghum	Wet cooked	366	394	635
White hard	Raw	666	674	688
maize	Wet cooked	620	636	674

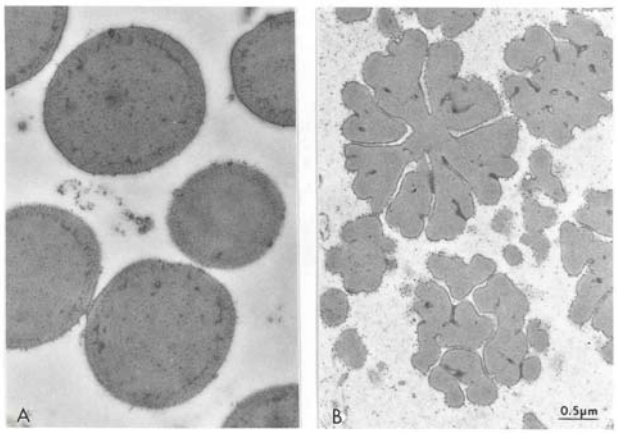

Figure 3. TEM of protein bodies from normal (left) and a highly digestible mutant (right) (From Oria *et al*., 2000)

V. STARCH

Characteristics

With regard to starch, the starch granules of sorghum like those of maize, but unlike those of wheat and barley, are only of one type, large essentially spherical granules of 10-16 microns across (Taylor and Belton, 2002). A minor difference between sorghum and maize is that sorghum contains starch granules in the mesocarp layer of the pericarp (bran) in addition to the normal starch endosperm granules. However, in quantity these are insignificant compared with the number of granules in the endosperm.

It is well known that sorghum starch has amongst the highest gelatinisation temperatures of all starches. The gelatinisation temperature range of 68-78^{o}C is some five degrees higher than that of maize (Taylor and Belton, 2002). It is speculated that the long amylopectin "a" chains which entangle with each other, may be longer in sorghum than in maize. Even if so, this may simply be a consequence of the sorghum kernels being borne on the top of the plant and exposed to direct sun and higher temperature, rather than being in the shade and slightly cooler temperature, as in the case of maize. The high temperature in the sorghum kernels could lead to greater "a" chain synthesis.

a) Starch digestibility

The impact of sorghum's higher starch gelatinisation temperature on starch digestibility depends on the situation. If fully gelatinised, isolated sorghum starch is no doubt just as digestible as maize starch. However, in food and feed systems it is generally flour with much of the cell contents structure intact, and not pure starch, that is consumed. In this situation, sorghum has lower cooked starch digestibility than maize (Fig. 4) (Ezeogu, *et al*., in press). The cause of this appears to be the same as the reduced protein digestibility on wet-cooking. Addition of reducing agents that break protein disulphide bonds increases starch digestibility (Fig. 4) (Zhang and Hamaker, 1998; Ezeogu *et al*., in press). We have also observed that the protein matrix around the starch granules in sorghum appears to remain more intact than that of maize, after the starch has been gelatinized. Both these pieces of evidence indicate that kafirin cross-linking is involved in the lower starch digestibility of sorghum. It can be speculated that the cross-linked proteins in the matrix enveloping the starch granules inhibit the access of amylase enzymes to the granules. Further, the cross-linking of the proteins may inhibit expansion and disruption of the starch granules during gelatinisation, hence, slightly limiting egress of the amylases between the starch molecules.

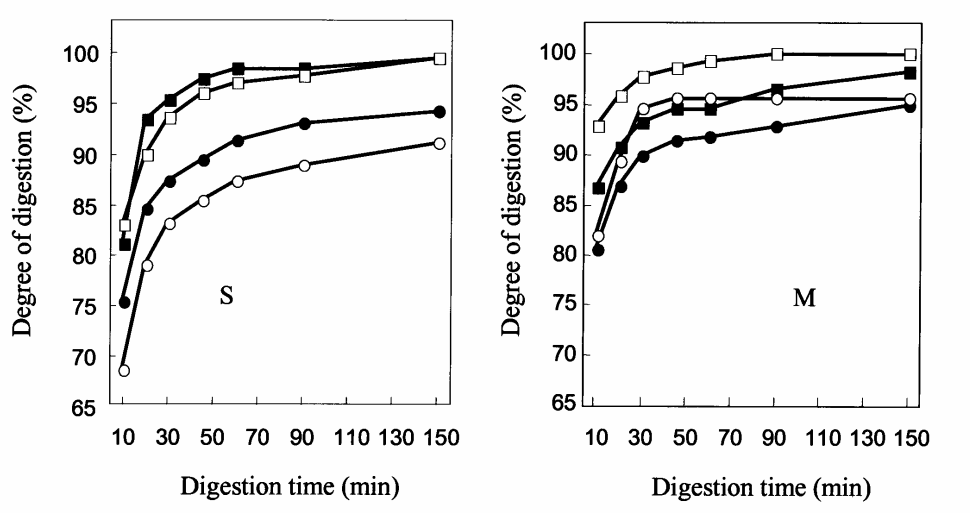

Figure 4. *In vitro* starch digestibility of sorghum and maize floury (squares) and vitreous (circles) endosperm treated without (open symbols) and with a reducing agent (closed symbols) (from Ezeogu *et al.,* in press)

The adverse effects of protein cross-linking on sorghum protein and starch digestibility seem to be predominantly a consequence of protein cross-linking occurring as result of wet-cooking. In poultry feeding, grain is often consumed in the raw (uncooked) state. Notwithstanding this, sorghum has lower starch digestibility and metabolisable energy than maize. The latter cannot be fully accounted for by the slightly lower oil content of sorghum grain, compared to maize. It has long been speculated (Rooney and Pflugfelder, 1986) that the sorghum protein matrix plays a role. There is evidence that there is greater cross-linking of the proteins in the matrix of raw sorghum compared to maize. However, at present the evidence is really only circumstantial. A common method to extract the prolamin proteins from maize or sorghum is to first extract with aqueous alcohol and then to extract with aqueous alcohol plus reducing agent. The theory is that the reducing agent breaks disulphide bonds between the prolamins, extracting the so-called cross-linked prolamins. Analysis of the literature reveals that the relative proportion of prolamin extracted with reducing agent is much higher in sorghum than in maize (Table 2) (Duodu *et al*., 2003), thus indicating that in raw grain the kafirins in sorghum are more highly cross-linked than the zein in maize. Hence, it could well be that these cross-linked kafirins present a barrier to digestion, accounting for the lower starch digestibility and metabolisable energy of sorghum. The problem is to prove this, as the process of analysis necessitates destruction of the native state of the proteins.

VI. STRATEGIES FOR ALLEVIATION OF NUTRITIONAL NEGATIVES

a) Chemical and physical treatments

The idea of using exogenous pentosanases to break down sorghum endosperm cell walls seems at first sight to be attractive. This would remove a potential barrier to digestion of the cell contents. However, it has been found that sorghum cell walls are only hydrolysed to a limited extent by endoxylanase (Verbruggen, 1996). This appears to be because of the high degree of arabinose substitution and the presence of considerable amounts of glucuronic acid in the sorghum arabinoxylans. The use of accessory enzymes to remove the arabinose improves degradation of the arabinoxylan somewhat.

Table 2. Prolamin 1 and prolamin 2 protein fractions (g/kg protein) in raw sorghum and maize (adapted from Duodu *et al*., 2003)

	Sorghum		Maize	
	Kafirin 1	Kafirin 2	Zein 1	Zein 2
	173	245	340	101
	199	351	528	79
	99	153	394	94
	200	440	450	218
	200	330	340	100
Mean	174	304	410	118

Since the protein matrix and bodies both are somewhat poorly digestible themselves and seem to be the major factor reducing starch digestibility, they would seem to be the most important focus area. With regard to digesting them with exogenous protease enzymes, their hydrolysis by proteases is rather slow (Taylor and Boyd, 1986). This is presumably related to their apparently high degree of cross-linking and that they are insoluble in aqueous solution. In fact, the mode of digestion of the matrix and protein bodies by proteases is a process of erosion, primarily at the outer surfaces (Taylor and Evans, 1989).

In *in vitro* studies good success in improving both sorghum protein and starch digestibility has been achieved through the addition of the food grade reducing agent bisulphite/metabisulphite (Zhang and Hamaker, 1998). This is presumed to be due to it breaking protein disulphide bonds. Whether its addition in poultry feed would be effective remains to be seen, since sulphites can have adverse physiological effects, in particular they are allergens.

The simple expedient of soaking the sorghum grain to hydrate it may be of value. Hale (1973) reported that "reconstitution", i.e. storing sorghum grain at 30-35% moisture under oxygen limiting conditions for some 3 weeks, resulted in it having markedly higher protein digestibility, similar to that obtained with steam flaking. We have found that hydration of sorghum grain can be accelerated by soaking in very dilute alkali (Dewar *et al*., 1997). We attribute this to the alkali hydrating the cell wall arabinoxylans.

In ruminant animal feeding, the process of tempering followed by steam flaking is routinely used with sorghum grain feeding as it improves starch and protein digestibility (Rooney, 1992). Steam flaking disrupts the grain subcellular structure, especially the vitreous endosperm, allowing more surface area contact between the digestive enzymes and the protein and starch. The improvement in starch digestibility is also presumably due to the disruption process enabling more expansion of the starch granules during gelatinisation.

According to Hancock (2000) in poultry feeding grain particle size is much more critical in sorghum than in maize. Sorghum needs to be more finely milled than maize. For both soft and hard sorghums, reducing the grain particle size to a maximum of 500 microns resulted in the growth performance of broiler chicks approaching that with maize. This effect is again presumably mainly as a result of disrupting the sorghum grain subcellular structure and also of increasing the grain surface area for enzymic attack. In the poultry industry, the process of pelleting is widely used. The ground grain is conditioned with steam and then passed though a die to create a pellet that reduces dust, improves palatability and feed handling (Rooney, 1992). The steam partially gelatinises the starch so that it acts as binder. It is doubtful whether the protein matrix is disrupted in the process. However, the possible negative impact of what is in effect a wet-cooking process on protein cross-linking does not seem to have been assessed. In this respect, extrusion cooking and popping which are essentially cell disruption dry cooking processes may have advantages. It has been found that

neither extrusion cooking (Hamaker *et al.*, 1994) nor popping (Parker *et al.*, 1999) negatively affect sorghum protein digestibility.

b) Selection and breeding of cultivars

As shown in Figure 3, we have recently found that in wet-cooked sorghum the starch digestibility of the vitreous endosperm is lower than that of the floury endosperm even when the endosperm particles are of similar size. Since in the floury endosperm the protein matrix is much thinner, this suggests that sorghum grain with a high proportion of floury endosperm would have better starch digestibility than grain with a high proportion of vitreous endosperm. There is considerable literature on feeding ruminant animals with maize of different endosperm texture that supports this contention (Philippeau *et al*., 1999; Correa *et al*., 2002).

The improved digestibility of the sorghum mutant lines from Purdue University with the invaginated protein bodies has already been described. These mutants also have additional potential benefits for poultry feed. They are high lysine types (Weaver *et al*., 1998). Some also have high starch digestibility, apparently caused by morphological differences in their starch granules (Benmoussa *et al*., 2004).

VII. CONCLUSIONS

We are making slow but steady progress in understanding why the nutritional value of sorghum, especially with respect to its protein and starch digestibility, is slightly lower than that of other cereal grains. Direct comparison with maize has proved to be useful because of the close similarity in chemical composition and subcellular structure. The evidence points clearly to disulphide bond cross-linking of the beta- and gamma-kafirin proteins being a major factor in reducing protein and starch digestibility. However, what we do not understand is why in view of the great similarity between the prolamin proteins of sorghum and maize, cross-linking should occur to a greater degree in sorghum.

With regard to strategies for alleviating sorghum's nutritional negatives, physical treatments to disrupt the subcellular structure and selection of less vitreous endosperm types appear to be the best options at present. Development of high digestibility, high lysine sorghum lines which have good agronomic characteristics seems to be a good idea but may just be a pipedream. The very beneficial characteristics of sorghum that enable it be cultivated in the semi-arid tropics are probably closely related to its slight nutritional negatives.

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STARCH DIGESTION RATE AFFECTS BROILER PERFORMANCE

H. ENTING[1], J. POS[1], R.E. WEURDING[2] and A. VELDMAN[1]

Summary

In a series of experiments, it was shown that slowly digestible starch (SDS) has a positive effect on broiler chicken performance compared to rapidly digestible starch (RDS). Based on these results, it was hypothesized that a gradual starch digestion may have an amino acid sparing effect and therefore enhance growth efficiency of broiler chickens. Two growth experiments were performed with broiler chickens in order to investigate the effect of SDS on broiler performance and the interaction between starch digestion rate and amino acid level. In the first experiment, differences in starch digestion rate were reached by processing of the starch sources used, while differences in amino acid level were obtained by the addition of casein and glutamine. In the second experiment the birds were fed either a pea/maize based diet (SDS) or a tapioca/maize based diet (RDS). Both types of diet were formulated with five levels of digestible lysine, varying from 8.5 to 11.0 g/kg. The minimal levels of other amino acids varied accordingly. In both experiments the feed conversion ratio (FCR) was lower for broilers on diets containing a high amount of SDS compared to broilers on diets with RDS. Adding extra amino acids decreased FCR for birds on diets with RDS, but not for birds on diets with SDS. In both experiments, this resulted in a significant interaction between starch digestibility rate and digestible amino acid content. It was concluded that SDS seems to have an amino acid sparing effect.

I. INTRODUCTION

Starch is an important energy source for broiler chickens. It supplies more than 50 % of the metabolizable energy in practical broiler diets. Starch digestibility is often considered to be 100 %. However, several authors have reported incomplete starch digestion in broiler chickens for cereal and legume grains (Guillame, 1978; Hesselman and Åman, 1986; Rogel *et al.*, 1987; Yutste *et al.*, 1991; Weurding *et al.*, 2001). Moreover, results from digestibility studies by Yutste *et al.* (1991) and Weurding *et al.* (2001) showed considerable differences in site and rate of starch digestion between feedstuffs. Therefore, it might be important to take starch digestion characteristics into account when optimizing broiler chicken feeds.

In a preliminary experiment, it was shown that performance of broiler chickens was better on a diet with slowly digestible starch (SDS) than on a diet with rapidly digestible starch (RDS; Weurding *et al.*, 2003). Weurding (2002) demonstrated that the improved performance with SDS was independent of starch source and technological treatment of these sources.

In order to explain the positive effect of SDS on bird performance, it was suggested that starch digestion rate might affect metabolic responses of insulin or synchronisation of energy and protein availability (Weurding *et al.*, 2003). SDS could lead to a more continuous supply of glucose which can change insulin response (Björck *et al.*, 2000). Insulin plays a key role in protein deposition during growth (Fox, 1996). Moreover, a more continuous supply of glucose to the posterior part of the small intestine with SDS could prevent the use of amino

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acids as an energy source for the gut wall. In order to test a possible effect of starch digestion rate on amino acid requirements, two experiments were carried out, which are described in this paper.

II. MATERIALS AND METHODS

a) Animals and Housing

Two experiments were performed to investigate the interaction between starch digestion rate and amino acid content in relation to broiler performance.

In experiment 1, 4,080 sexed, new-born Cobb 500 male and female broiler chicks were obtained from Cobroed, Lievelde, The Netherlands, and were divided over 24 floor pens. Each pen contained 85 male and 85 female chicks. The pens were divided in six blocks of four pens. Four dietary treatments were randomly assigned to a pen in each block.

In experiment 2, 6,800 sexed one-day-old male and female broiler chickens of the Cobb 500 strain were used. The chicks were housed in forty floor pens with 85 male and 85 female chicks per pen. The pens were divided in four blocks of ten pens. From d 0-9, chicks were fed a starter diet containing peas, tapioca and maize. From d 9-18, chicks were fed one of ten experimental diets, which were randomly assigned to a pen in each block. Average body weight at the start of the experimental period (d 9) was 230 g.

In both experiments, 23 h light and 1 h dark intervals were used and chicks had unrestricted access to feed and water. In the first experiment, the diets were supplied as mash to maximise the contrasts in starch digestion rate (the processed and unprocessed starch rich feedstuffs were both finely milled) while in the second experiment the diets were provided as pellets. The experimental protocols of both experiments were in agreement with the standards for animal experiments and were approved by the Ethical Committee of Schothorst Feed Research.

b) Diets

In both experiments the diets were formulated according the nutrient standards used in Dutch practice.

Experiment 1: For the grower phase (day 14-30) a diet was formulated with peas and maize as starch sources (PM-0, SDS sources). In order to increase starch digestion rate, expanded and pelleted peas and maize (PM-EP) were used. In order to test the interaction between starch digestion rate and amino acid content, in each diet (PM-0 and PM-EP) protein levels were raised by adding 1% casein and 0.5% synthetic glutamine. These protein additions resulted in an increase of 0.5 g digestible lysine compared to the other diets.

Experiment 2: Two diets containing peas and maize (PM, SDS sources) and two diets containing tapioca and maize (TM, RDS sources) were formulated with digestible lysine contents of 8.50 and 11.00 g/kg respectively. In order to get diets with intermediate digestible lysine contents, diets with the same starch sources were mixed to give digestible lysine contents of 9.13, 9.75 and 10.38 g/kg. During formulation of the diets, the minimum ratio of other digestible amino acids to digestible lysine was equalised in all diets.

c) Statistical Analysis

For experiment 1, data for feed intake, weight gain and FCR were analysed by ANOVA. The effect of processing (PROC, influencing starch digestion rate), the addition of extra amino acids (+AA) and the interaction between these factors on performance were tested using a model including overall mean, block, processing, extra amino acids, interaction terms and a residual error term.

For experiment 2, data for feed intake, weight gain and FCR were analysed by ANOVA. The effect of starch source (SS), digestible lysine content (LYS) and the interaction

between these factors on performance were tested using a model including overall mean, block, starch source, digestible lysine content, interaction terms and a residual error term.

III. RESULTS AND DISCUSSION

a) Experiment 1

Broiler performance from day 14-30 of the first experiment is presented in table 1. The results show that processing of PM diets resulted in a (not significant) increase of the FCR. These results confirm earlier findings of Weurding *et al.* (2003), in which diets with a high content of SDS resulted in a lower FCR compared to diets with a low content of SDS.

Furthermore, the results show a significant ($P = 0.02$) interaction between processing and amino acids for FCR during this period. The addition of extra amino acids did not affect FCR of the birds receiving the PM-0 diet but reduced the FCR of the birds receiving the PM-EP diet from 1.72 to 1.64.

Table 1. Effect of extra amino acids (+ AA) in diets with untreated (0) or expander pelleted (EP) peas and maize (PM) on bird performance, day 14-30.

	PM-0		PM-EP		SEM	P-value		
	0	+ AA	0	+ AA		PROC	AA	PROC x AA
Weight gain, g	1026	1026	1009	1035	11	NS	NS	NS
Feed intake, g	1718	1707	1733	1693	23	NS	NS	NS
FCR, g/g	1.675	1.665	1.718	1.638	0.013	NS	**	*

** = $P < 0.01$; * = $P < 0.05$; NS = not significant ($P > 0.10$); $n = 6$

b) Experiment 2

The results from day 9-18 of the second experiment are presented in table 2. The results show that the weight gain was higher and the FCR was lower for birds on PM diets than for those on TM diets ($P < 0.01$). These results confirm the effect of SDS on broiler performance which was also found in the first experiment. Adding digestible lysine to the diet reduced feed intake linearly ($P < 0.01$) and quadratically ($P < 0.10$) from 662 to 649 g and increased weight gain linearly ($P < 0.01$) from 458 to 480 g. An interaction between starch source and digestible lysine content on FCR was observed ($P < 0.05$). FCR was lower for birds on PM diets than those on TM diets and the difference was most pronounced with lower digestible lysine contents in the diets.

Table 2. Effect of starch source (SS) and digestible lysine content (LYS) on performance of broiler chickens, day 9-18.

	SS	Digestible lysine content (g/kg)					SEM	Effect			
		8.50	9.13	9.75	10.38	11.00		SS	LYS	LYS^2	SS x LYS
Weight gain, g	PM	463	468	476	477	483	3	**	**	0.12	NS
	TM	452	467	469	472	477					
Feed intake, g	PM	655	646	646	639	644	4	**	**	†	NS
	TM	668	669	655	648	655					
FCR	PM	1.414	1.379	1.356	1.339	1.334	0.007	**	**	**	*
	TM	1.478	1.432	1.398	1.375	1.374					

1 PM = pea/maize diet; TM = tapioca/maize diet.

** = $P < 0.01$; * = $P < 0.05$; † = $P < 0.10$; NS = not significant ($P > 0.10$); n = 4.

The positive effect of SDS on broiler performance was confirmed in both experiments. From the results in tables 1 and 2 it is clear that birds given diets containing a high content of SDS grew faster and more efficiently than those on diets with a lower content of SDS. Both experiments also showed a similar interaction between starch digestion rate and digestible amino acid content on FCR of broiler chickens. The second experiment showed that the effect of SDS is more pronounced for birds on diets with low amino acid levels, indicating that amino acid supply and glucose supply are unbalanced in diets with RDS. This could mean that a diet with synchronized starch and protein digestion (e.g. a more gradual starch digestion) results in better performance.

The explanation may be found in hormonal responses to glucose absorption which affect protein deposition. It may be hypothesized that gradual starch digestion results in a lower but longer lasting insulin peak than rapid starch digestion. This hypothesis is supported by data from Björck *et al.* (2000) who reported a correlation between the glycemic index and the insulinemic index. Elevated insulin levels are required for amino acid transport and uptake by body cells (Fox, 1996). On the other hand, asynchrony of starch and protein digestion may increase the oxidation of amino acids to meet the energy demand of gut tissues (Vaugelade *et al.*, 1994; Flemming *et al.*, 1997). When glucose is metabolized in the gut tissues of the posterior part of the small intestine, as may be the case when diets with SDS are fed, amino acids may be spared and can thus be used for muscle growth.

Based on the results of these experiments it can be concluded that SDS results in a lower amino acid requirement compared to RDS. Therefore, SDS seems to have a protein sparing effect. This effect can not fully explain the total improvement in performance when feeding SDS. It is likely that energy utilization is also improved because of the prolonged elevated plasma glucose levels.

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THE ENERGY VALUE OF CEREAL GRAINS, PARTICULARLY WHEAT AND SORGHUM, FOR POULTRY

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Summary

Results from the Premium Grains for Livestock Program were analysed to identify variation in the energy value for laying hens and broiler chickens of cereal grains including wheat, barley, oats, triticale, sorghum and rice. There was wide variation in apparent metabolisable energy (AME) within and between grain species for both layers and broilers. While the range in AME values was similar for most grains in layers and broilers, there were varying responses to specific samples. AME values tended to be higher in layers than broilers for barley, frosted triticale and naked oat samples. More AME was obtained from rice by broilers. There was little relationship between AME content of grains and the amount eaten by layers or broilers. When wheat and sorghum, the most common grains used by the Australian poultry industry, were compared, AME was considerably higher for sorghum in both layers and broilers. The intake of sorghum based diets was also higher for layers, but not for broilers. Layers offered sorghum based diets consumed 13% more AME daily than those offered wheat based diets. However, for broilers, daily intake of AME was similar for sorghum and wheat based diets. Despite a similar daily intake of AME, broilers offered wheat based diets grew 20% faster and used 13% less feed than those offered sorghum based diets. The poor utilisation of energy from sorghum based diets was attributed to a low availability of amino acids, with arginine as possible first limiting amino acid, due to the low content and digestibility of sorghum proteins. In addition, asynchrony in the timing of absorption of amino acids from casein, the main protein source in the experimental diets, and glucose from the delayed digestion of starch granules surrounded by a relatively indigestible protein matrix is thought to have contributed to the lower utilisation of energy from sorghum than from wheat based diets.

I. INTRODUCTION

Cereal grains are the major source of energy for commercial poultry and represent from 60-70% of the diet. However, the capacity of cereal grains to provide energy to birds varies widely between and within grain species (Hughes and Choct, 1999). The amount of energy supplied by a grain depends on both the extent of digestion and the amount eaten. The extent of digestion depends on the adequacy of enzymes within the digestive tract capable of breaking specific chemical bonds in each grain component, accessibility of the enzymes to the chemical components and the time the enzymes and component are associated. Much of the variation between grains in energy digestibility is explained by differences in gross chemical composition (Black, 2000). However, other factors, particularly those that affect the accessibility of enzymes to specific grain components, can affect markedly the digestibility of grain components and availability of energy. The amount of a diet consumed by animals depends primarily on the requirements for nutrients to meet metabolic demands, the volume of the digestive tract and the rate of passage of digesta through the tract (Forbes, 2005). The efficiency with which available energy is utilised for

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chicken growth or egg production depends on its synchronous availability for metabolism in body tissues with amino acids and other essential compounds.

The "Premium Grains for Livestock Program" was established in 1996 by the Grains Research and Development Corporation and other industry funding bodies to examine the capacity of cereal grains to provide energy for different animals including cattle, sheep, pigs, laying hens and broiler chickens. Major aims of the Program were to identify reasons for differences between cereal grains, to develop methods for improving their quality and to enhance marketing opportunities for grains in the Australian livestock industries. Results from the Program are presented to illustrate the variation between grain samples in their capacity to provide energy for both laying hens and broiler chickens. Particular emphasis is placed on the comparison between wheat and sorghum, which are the grains most commonly used within the Australian poultry industry.

II. VARIATION IN THE ENERGY VALUE OF CEREAL GRAINS FOR POULTRY

The cereal grains investigated included wheat, barley, oats, triticale, sorghum and rice and individual samples were collected from a variety of sources with the aim of obtaining the largest possible variation in grain quality. The grains were coarsely milled and comprised 77% of the diets for layers and 80% for broilers. The diets contained 8.5% casein for layers and 15.5% for broilers with added calcium, phosphorus, vitamins and DL-methionine. All diets were cold pelleted. Experiments were conducted with broiler chickens from 22 days of age and with laying pullets. Common grains were included across experiments and statistical procedures used to produce values that were adjusted for differences between experimental periods, cages, birds and experiments. Apparent metabolisable energy (AME, MJ/kg dry matter (DM)) of the diet and of the grain was calculated and total feed intake determined. Growth rate and feed conversion ratio (FCR, g feed/g gain) were determined for broilers.

As illustrated in Figure 1, AME in layers ranged from 12.2-15.6 for wheat, 11.4-14.2 for barley, 12.8-16.1 for oats, 11.8-14.3 for triticale, 14.8-16.3 for sorghum and 13.0-14.8 for rice. Corresponding range in AME values for broilers was 11.9-15.3 for wheat, 10.9-13.6 for barley, 12.1-14.9 for oats, 12.1-14.5 for triticale, 15.3-16.7 for sorghum and 17.6-17.8 for rice. There was little difference in the AME value of the same grains between layers and broilers except layers generally obtained more energy from barley, frosted triticale and the naked oat sample than broilers, suggesting a greater capacity to deal with fibre and with fat than broilers. However, layers obtained less AME than broilers from rice. This difference is difficult to explain because rice is normally highly digestible for most animal species. The results presented in Figure 1 show also that sorghum AME values for both layers and broilers are consistently higher than the AME values for wheat.

There was little relationship between the AME content of cereal grains and the amount of the diet eaten for either layers or broilers (Figures 1 and 2). The range in intake was similar for wheat and sorghum in broilers, but the mean value of 108 g/d was higher for wheat than the mean of 104 g/d for sorghum. With layers there tended to be a higher intake for sorghum than for wheat (120 vs 115 g/d), but the range was larger between sorghum samples. The total intake of AME or energy available to the birds for metabolism was similar between wheat and sorghum for broilers with values of 1.52 and 1.54 MJ/d, respectively. Thus, although sorghum has a consistently higher AME content than wheat, the lower intake of sorghum means that there is little difference in total energy availability between the two grain species for broilers. However, for layers, the mean AME intake was considerably higher for sorghum based diets than for wheat based diets (1.64 vs 1.45 MJ/d), suggesting that layers obtain more energy from sorghum than from wheat based diets.

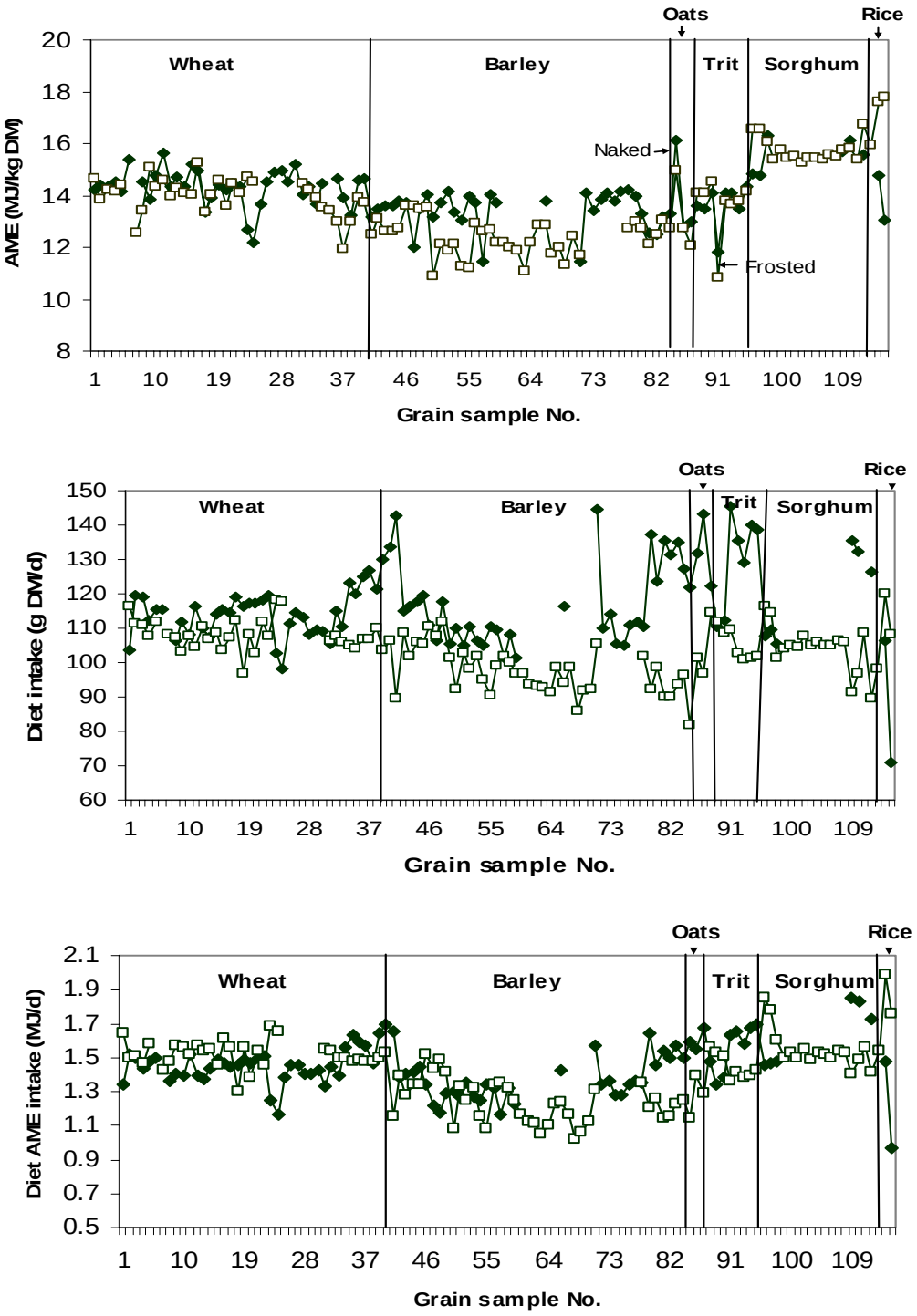

Figure 1. AME, diet intake and AME intake for laying hens (♦) and broiler chickens (□) fed different cereal grains. "Trit" means Triticale.

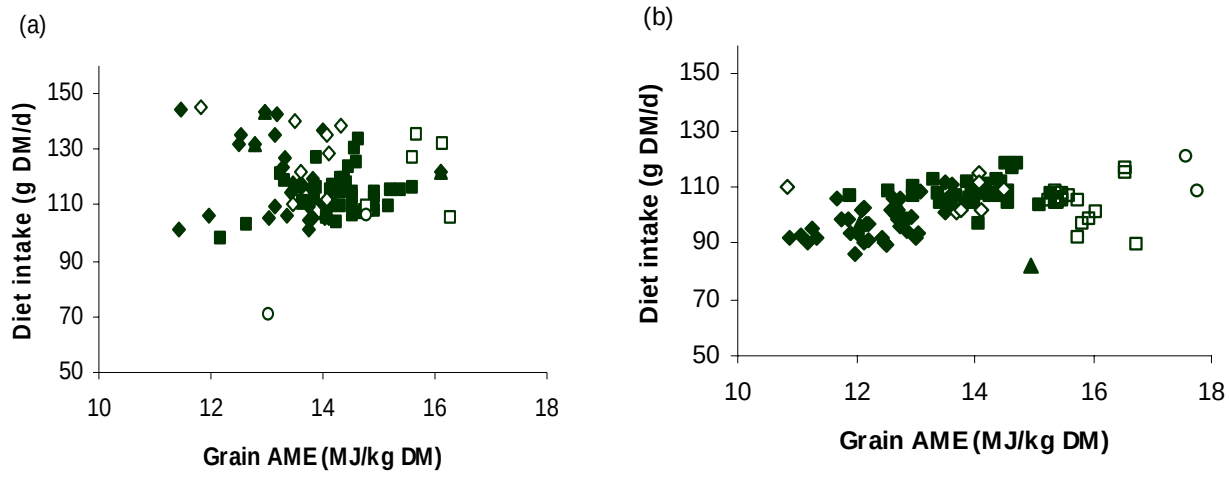

Figure 2. Relationship between diet intake and AME content of the grain for laying hens (a) and broiler chickens (b) given different cereal grains: wheat (■), barley (♦), oats (▲), triticale (◊), sorghum (□) and rice (○).

III. ASSOCIATION BETWEEN AME AND BROILER PERFORMANCE

There is a poor relationship between the AME content of grain and either growth rate of broiler chickens or the efficiency with which feed is converted to body weight gain (FCR) both within and between cereal grain species (Figure 3). However, there are stronger relationships between total daily AME intake and either growth rate or FCR, particularly within grain species (Figure 4). Nevertheless, it is apparent that for the same daily intake of available energy of approximately 1.5 MJ/d (1.46-1.61 MJ/d), growth rate of chickens offered wheat based diets was 20% (61.5 vs 51.0 g/d) higher than for those offered sorghum based diets. Similarly, 17% less feed was consumed for each unit of body weight gain for the chickens offered wheat rather than sorghum based diets (FCR 1.55 vs 1.85).

These observations suggesting that energy available from sorghum is used less efficiently by broiler chickens than the energy from wheat are supported by an experiment conducted in industry by R. MacAlpine (Table 1). Despite diets having similar AME content, replacing sorghum for wheat in diets significantly reduced the efficiency of feed energy use.

Table 1. Effect of wheat and sorghum based diets on efficiency of feed (FCR, feed:gain) and energy use by broiler chicks from 0-35 days of age (R. MacAlpine, unpublished).

Grain base for diet	Diet AME (MJ/kg)	FCR	MJ AME/kg gain
Wheat	12.55 [a]	1.58[a]	19.8 [a]
Wheat: sorghum (50:50)	12.69 [a]	1.59[a]	20.2 [a]
Sorghum	12.82 [a]	1.63 [a]	20.9 [b]

[a,b] Values with different letters differ significantly (P<0.05)

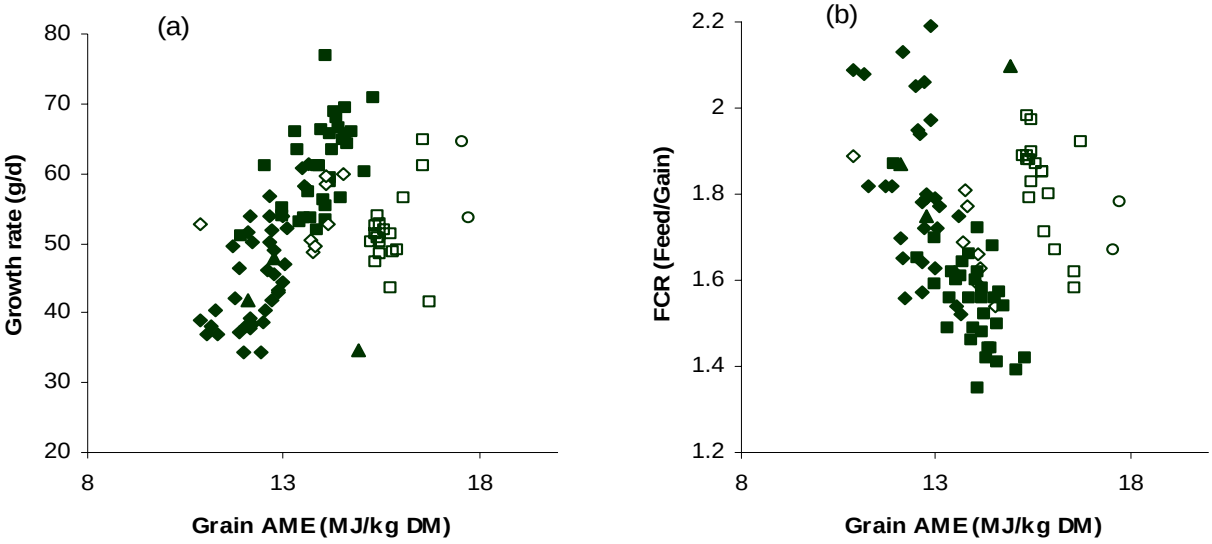

Figure 3. Relationship between grain AME content and growth rate (a) and feed conversion ratio (FCR) of broiler chickens given different cereal grains: wheat (■), barley (♦), oats (▲), triticale (◊), sorghum (□) and rice (○).

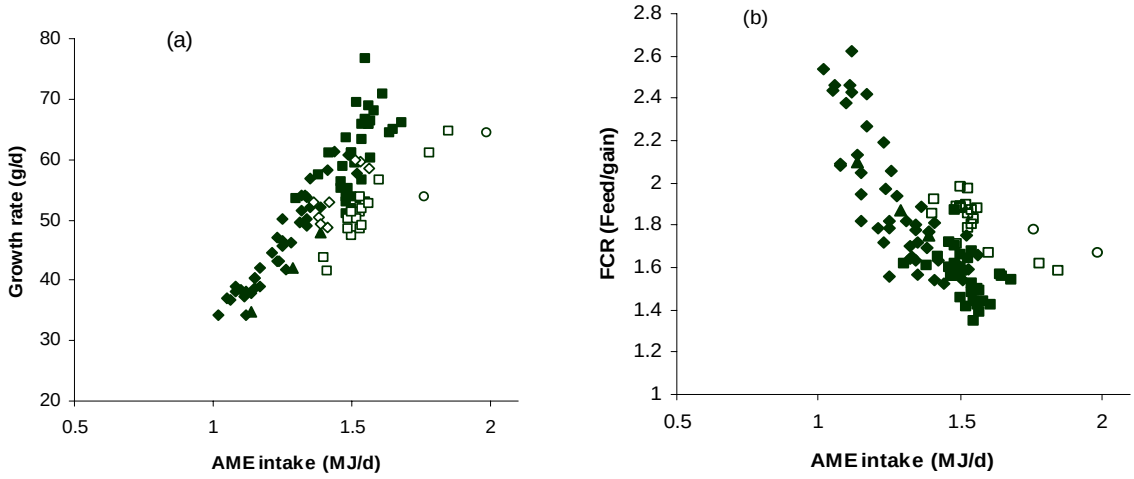

Figure 4. Relationship between total AME intake and growth rate (a) and feed conversion ratio (FCR) of broiler chickens given different cereal grains: wheat (■), barley (♦), oats (▲), triticale (◊), sorghum (□) and rice (○).

IV. POSSIBLE REASONS FOR DIFFERENCES BETWEEN WHEAT AND SORGHUM

There are several possible explanations for the poorer use of available energy from sorghum than from wheat for chicken growth including:

- A deficiency in essential amino acids available for growth due to the lower protein content and digestibility of sorghum proteins containing a high content of disulphide bonds.
- A deficiency of amino acids due to the high tannin and polyphenol content and/or the high phytic acid content of sorghum binding dietary and enzyme proteins and released amino acids thus reducing the digestion of protein and availability of amino acids for growth.

- A deficiency in amino acids due to the inadequate hydrolysis of protein and absorption of peptide chains that are too long and/or of incorrect amino acid structure to be incorporated directly into body proteins.
- A deficiency in some other essential nutrient required for protein synthesis and growth.
- A lack of synchronisation in the timing of the release of amino acids and of energy from starch digestion that results in the catabolism of amino acids rather than their incorporation into body protein.
- A difference between the grain sources in the timing of the release of glucose from starch digestion and its effects on insulin stimulation of protein synthesis.

Results from all grains shown in the Figure 4b with a daily dietary AME intake between 1.46 and 1.61 MJ were analysed to evaluate several of the suggested possible explanations causing the range in efficiency of feed use (FCR) within each grain species and between wheat and sorghum, when available energy was similar.

There was a strong positive relationship between the efficiency of feed use and the crude protein content of the grain in diets as shown by the decline in FCR (Figure 5a). This result suggests that the protein content of the diets may have limited growth rate of the chickens. However, a protein deficiency *per se* would seem unlikely for chickens from 22-29 days of age because the total protein contents of the diets ranged from approximately 23-34% DM. Analysis of the amino acid content of sorghum and wheat proteins shows that sorghum protein contains less arginine, cystine, methionine, lysine and tryptophan than wheat protein. There was a particularly strong relationship between FCR and the daily intake of arginine from grain for both sorghum and wheat up to an intake of approximately 0.9 g/d, suggesting that arginine may have been first limiting amino acid for broiler performance with the sorghum and some wheat based diets used in the experiments (Figure 5b).

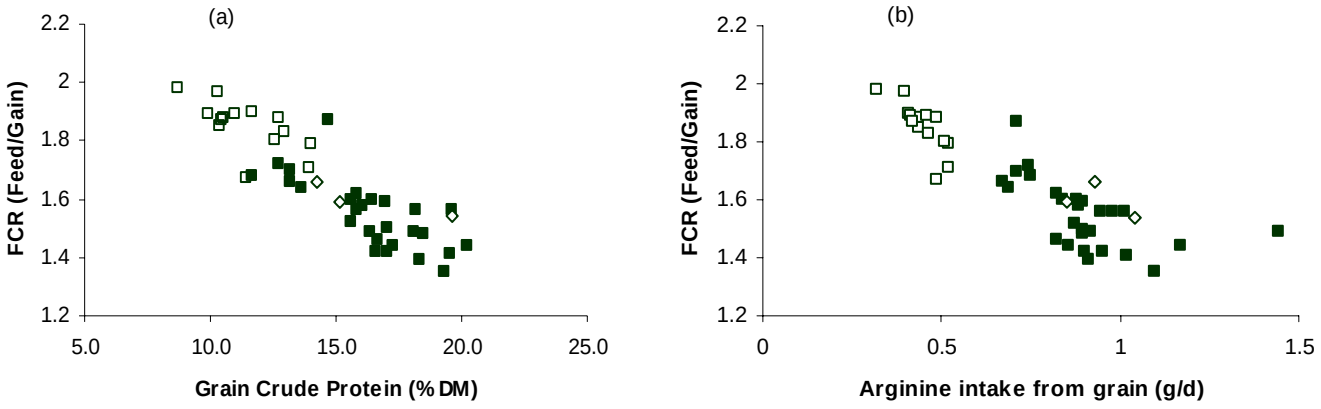

Figure 5. Relationship between feed conversion efficiency (FCR) and grain protein content (a) or arginine intake from grain (b) included in diets for broiler chickens based on wheat (■), triticale (◊) or sorghum (□).

It is known that a small proportion of sorghum proteins, the γ-kafirins, contain high amounts of sulphur rich amino acids that form disulphide bonds and render them relatively resistant to digestion by proteases. The γ-kafirins form on the surface of the protein bodies within the endosperm of sorghum grains and restrict protease enzymes from reaching the more digestible inner proteins of these bodies. Sorghum proteins have been shown to be less digestible than those from other cereal grains because of the presence of these γ-kafirins (Klopfenstein and Hoseney, 1995). However, Silano (1977) reported that the digestibility of

protein ranged from 30-70% in different sorghum cultivars and a recent mutant (P21N) has a digestibility of 85% (Oria *et al*., 2000).

The results from the analyses presented suggest that a protein inadequacy, and particularly arginine as the first limiting amino acid, in the diets with a constant grain and casein content and the lower digestibility of sorghum proteins may have been responsible for the differences in the efficiency of use of available energy from sorghum relative to wheat based diets when the daily intake of AME was similar. If the low content and digestibility of sorghum protein are the main reasons for the poor utilisation of available energy by broiler chickens, there should be differences between cultivars and chicken growth rates should respond to additional dietary amino acids. However, this conclusion is not supported by recent observations from R. MacAlpine (unpublished) who found that the inclusion of 10% additional amino acids in the form of soybean meal and synthetic lysine and methionine to sorghum diets formulated to have adequate protein did not significantly improve FCR in broiler chickens. One possible explanation for the lack of response to additional amino acids may be the presence of anti-nutritional factors.

Tannins are known to bind to digestive enzymes and reduce the digestion and availability of dietary compounds including amino acids in poultry (Nyachoti *et al.*, 1997). Although most commercially available sorghum cultivars in Australia have low tannin contents they contain polyphenols which also have some anti-nutritional properties. Similarly, the phytate content of sorghum is higher than other cereal gains and may bind to amino acids reducing their availability (Selle *et al.*, 2000). The relationships between FCR and total tannin content and FCR and phytic acid content of grains with daily AME intakes from 1.46-1.61 MJ (Figure 6) indicate that neither tannins nor phytic acid are likely to be the reason for the reduced utilisation by chickens of available energy in sorghum relative to wheat based diets.

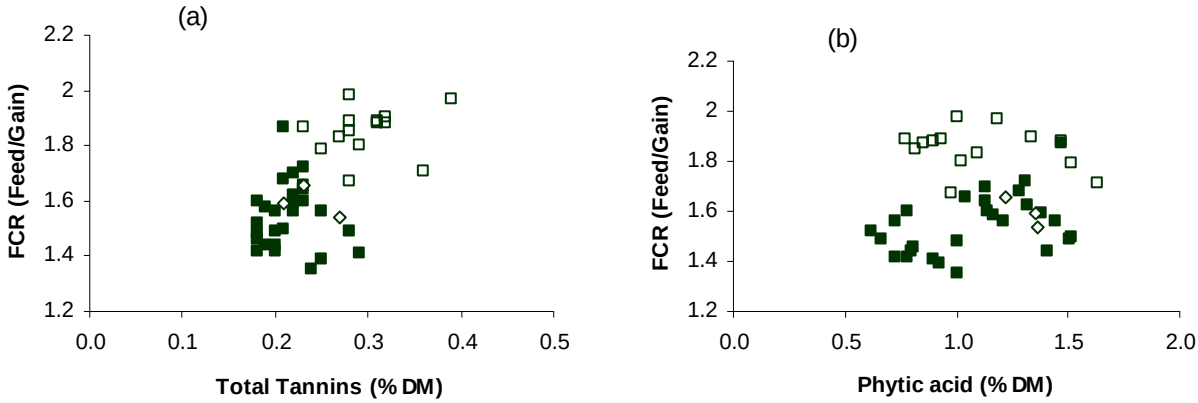

Figure 6. Relationships between feed conversion efficiency (FCR) and the total tannin content (a) or phytic acid content (b) of the grain included in diets for broiler chickens based on wheat (■), triticale (◊) or sorghum (□).

There is evidence that many dietary proteins are not completely hydrolysed to amino acids and are absorbed as small peptides, some of which have an amino acid grouping that cannot be incorporated into body proteins and are excreted from the body. Although such a mechanism occurs, it seems unlikely to be a major reason for the observed differences in the efficiency of use of sorghum and wheat based diets.

Another possible explanation for the poor utilisation of energy available from sorghum is an asynchrony in the absorption of energy providing nutrients and amino acids for protein synthesis. The major source of protein in the experiments was casein which is rapidly digested, whereas the major source of energy was from cereal starch. There is likely to be

considerable differences between sorghum and wheat in the timing of starch digestion. Once the endosperm cell walls of the grains are fractured by the action of the gizzard, starch granules from wheat are readily accessible to amylolytic enzymes. The rate of digestion of wheat starch would then be influenced by factors such as size of the granules, content of resistant starch and viscosity of the digesta. However, the starch granules from sorghum are completely surrounded by a protein matrix which must be disrupted before the starch can be digested. Thus, it is hypothesised that the amino acids from casein in the sorghum based diet are largely absorbed and catabolised before energy was available for protein synthesis from the hydrolysis of starch. This concept of asynchrony may help explain the observations by R MacAlpine that adding amino acids did not improve the efficiency of utilisation of sorghum based diets because the amino acids would have been more rapidly absorbed than glucose from starch. However, the concept does not fit well with the observation that the efficiency of feed use by chickens offered the wheat based diets continued to improve as the protein content of the grain increased, unless the amino acids from casein were so rapidly absorbed relative to the digestion of wheat starch that the more slowly digested wheat protein provided the majority of the amino acids used for growth. The latter idea could explain why chickens continued to improve in performance as protein content of wheat diets increased to over 30% DM.

V. CONCLUSIONS

The analyses presented above suggest that protein availability, with arginine as the possible first limiting amino acid, due to the low content and digestibility of protein restricted the performance of broiler chickens fed sorghum based diets. An asynchrony in the timing of absorption of amino acids relative to glucose from starch digestion also may have contributed to the poor performance of chickens offered sorghum based diets. The asynchrony of amino acid and energy absorption may also have contributed to the observed continuing increase in performance of chickens as the protein content of the wheat grain samples increased to over 30% of the diet. These conclusions need to be supported by experiments in which the and utilisation of amino acids are measured.

The practical implications of these hypotheses for the broiler industry are that the rate of digestion of sorghum proteins needs to be increased and the extent of encapsulation of starch granules with protein matrix reduced either by plant breeding/selection or through processing techniques including the use of effective protease enzymes. In addition, consideration should be given to ensuring that the rate of digestion of dietary protein sources are synchronised with the rate of starch digestion.

The experiments described were conducted with cold pelleted diets and the adverse effects of amino acid deficiency may be exacerbated by the high temperature processing used commonly in industry because of the known reduction in digestibility of sorghum proteins during cooking (Duodu *et al.*, 2002). There appears to be considerable opportunity for research and development funding directed towards improving the content and digestibility of proteins in sorghum and to reduce the extent of encapsulation of starch granules by the protein matrix through plant breeding and selection and through improved processing techniques including the identification of highly effective protease enzymes.

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FIVE SIMPLE "IN-FIELD" METHODS FOR MEASURING SORGHUM END-USE QUALITY"

J.R.N. TAYLOR[1]

Summary

Five simple 'in-field' methods and associated standards have been developed for the determination of the end-use quality of batches of sorghum in terms of total defects, tannin grain, grain colour, grain hardness and germinative energy. A feature of the methods is that they do not require the use of specialised equipment, analytical instruments or laboratory chemicals. They should be of particular use to farmers who grow sorghum or purchase it for animal feed. The methods have been subject to two international ring trials and are currently being evaluated by the International Association for Cereal Science and Technology for provisional draft method status approval.

I. INTRODUCTION

With the aim of promoting sorghum grain trade in southern Africa, five simple “in-field” methods and associated standards were developed for the determination of end-use quality of batches of sorghum grain (Taylor, 2001). In view of the lack of scientific infrastructure in the region, a unique approach was taken. The methods were developed so that they could be understood by, be useful to and could be performed by farmers, grain traders and food processing entrepreneurs. Thus, the methods had to conform to the following strict criteria:

- The methods must be simple to perform, i.e. they should not require a skilled laboratory technician to perform them
- The methods must not require the use of specialised equipment or instruments
- Any chemicals required to perform the analyses must be readily available
- The methods should ideally be rapid
- The methods should be such that they can be performed by those in the sorghum trade, i.e. there must be no necessity to send samples to a specialist organisation to perform the analyses.

Although developed specifically for the situation in southern Africa, they and the approach used has wider applicability, in particular the methods should be useful to commercial farmers who grow sorghum and farmers who purchase the grain for animal feed.

Resulting from a survey of sorghum stakeholders (Taylor, 2001), the five grain end-use quality parameters which were deemed to be the most important were selected. These were total defects, tannin grain, grain colour, grain hardness and germinative energy. This paper will outline why each of these parameters is important, the standard methods for measuring the parameters and the developed simple “in-field” methods.

II. TOTAL DEFECTS

There are many types of defects in batches of grain. Of most concern are those that can potentially affect the safety of the end-user, such as toxic seeds, mouldy grain and non food/feed rubbish such as metal waste. Codex Alimentarius (Codex Alimentarius, 1995) and

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the United States Department of Agriculture (USDA, 1999) standards give maximum permissible levels of sorghum grain defects and some information on methodology. The International Association for Cereal Science and Technology (ICC) has a standard method with full details for the determination of Besatz (also known as dockage, defects or screenings) of wheat (ICC, 1972) which could be applied to any grain. All standard methods for quantifying defects in batches of grain are similar in principle in that they involve sieving and/or manual separation of the various fractions and then weighing to determine the mass of the fractions. Thus, the problem with such methods for the desired application is that they require specialised sieves of a specific mesh size (and often specific hole shape) and a balance weighing at the gram level to at least one decimal place of accuracy.

We have developed a method for determining the quantity of defects without the requirement for a balance or sieve. The method is outlined in Figure 1. It involves manually sorting the defects: extraneous matter, filth, blemished grains, broken kernels and other grains, and spreading them in a single layer of a sheet marked out in one square centimetres, and measuring the area. The principle of the method is that the area of defects was found to be highly significantly correlated with the mass of defects, $R^2 = 0.933$.

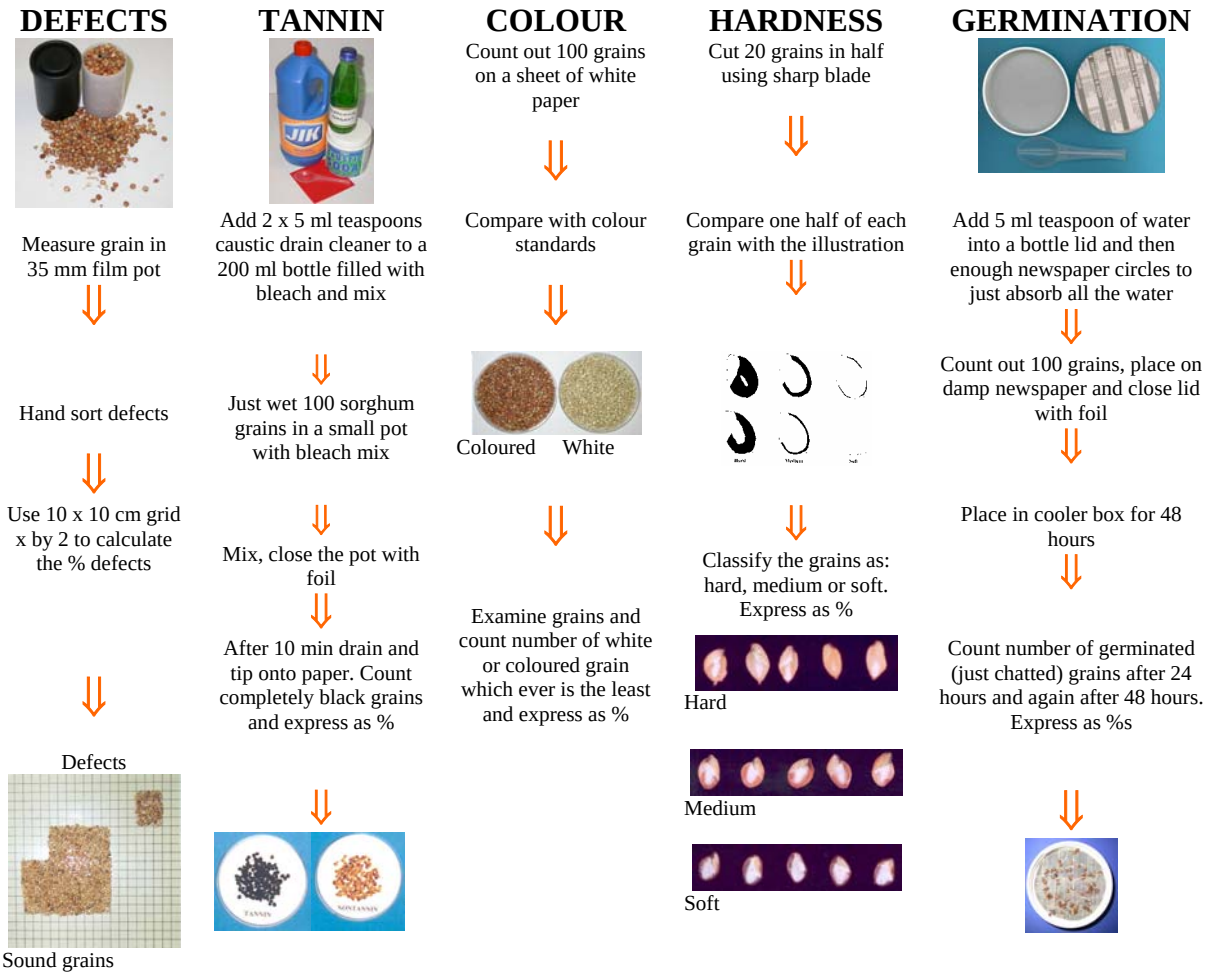

Figure 1. Summary of the five simple "in-field" sorghum end-use quality methods for measurement of total defects, tannin grain, grain colour, grain hardness and germinative energy

III. TANNIN GRAIN

Some sorghum varieties (known as tannin/high-tannin sorghums or bird-resistant/proof sorghums) contain significant levels of condensed tannins (proanthocyanidins or procyanidins). The condensed tannins significantly adversely affect the feed performance of monogastric animals (Hancock, 2000), probably primarily by binding with the sorghum grain proteins, preventing them from being metabolised. The colour of sorghum grain is not indicative of whether it contains tannins, thus tannins need to be measured. The tannin content of sorghum grain can be quantified by methods such as the vanillin-HCl method developed by Burns (1971) or the International Organization for Standardization ferric ammonium citrate method (ISO, 1988). However, of more practical use is to assess the percentage of sorghum grain that is of the tannin type. The US Grain Inspection Service uses the bleach test (Waniska *et al*., 1992) for this purpose. The sorghum grain is heated in a solution of potassium hydroxide in bleach (sodium hypochlorite solution). The reagent dissolves away the pericarp, exposing the black tannin-containing testa layer. Tannin sorghum grains appear completely black.

We have simplified the bleach test to use an easily measured volume of commercial sodium hydroxide (caustic soda, sold in hardware stores as a drain cleaner) instead of a weight of potassium hydroxide and eliminated the requirement to heat the reagent solution. The procedure is outlined in Figure 1. The simplified test gives identical results.

IV. GRAIN COLOUR

The colour of sorghum grain ranges from almost black through to almost white. Sorghum appears coloured mainly due to anthocyanin and anthocyanidin pigments in the pericarp. Sorghum grain colour affects the colour of and preference for foods prepared from the grain. The pigments in red sorghums can also stain the gizzard of poultry.

We have developed a simple visual test whereby sorghum grain is classified either as white or coloured, regardless of signs of surface mould or purplish anthocyanic blotches. The procedure is outlined in Figure 1. The method gives complete agreement with the non-subjective Agtron Color Quality Meter (Agtron Inc, Sparks, USA) and Tristimulus Colorimeter (Hunter Associates, Reston, USA) instruments.

V. GRAIN HARDNESS

The hardness of grain is a measure of its resistance to milling. In sorghum, grain hardness is closely correlated with the percentage of the endosperm that is vitreous. Sorghum endosperm vitreousness affects the digestibility of the starch (Ezeogu *et al*., in press) and probably of the protein. Sorghum grain hardness can be quantified by measuring milling resistance using instruments such as the Tangential Abrasive Dehulling Device (TADD) (Gomez *et al*., 1997). It can more easily be estimated by visually assessing the ratio of vitreous to floury endosperm in grains that have been cut in half longitudinally (Rooney and Miller, 1982).

We have simplified the 5-point scale visual rating system of Rooney and Miller (1982), classifying sorghum grains into soft, medium and hard by comparison with a set of standard illustrations (Figure 1). The simplified method is highly significantly correlated with the original Rooney and Miller (1982) method, $R^2 = 0.815$.

VI. GERMINATIVE ENERGY

That a very high proportion of sorghum grains germinate rapidly and uniformly is of critical importance to farmers and for the end-use of malting. Germinability is a reflection of grain age and handling and storage conditions, and as such as a useful indication of grain quality even for end-uses where the grain is not germinated. There are many germination tests. For sorghum, we routinely use an adaptation of the Brewing Industry Research Foundation (BIRF) barley method (Dewar *et al*., 1993). The quantity of water relative to grain has been optimised for sorghum and the temperature increased to 25^{o}C, as sorghum is a tropical grain.

We have simplified this method for "field" use as outlined in Figure 1. Newspaper circles and bottle lids and foil, instead of filter papers and petri dishes are used and the germination temperature is controlled by the use of a cooler box. Even at germination temperatures of 20^{o}C and 30^{o}C the simplified method is highly significantly correlated, R^2 = 0.899 and R^2 = 0.909 respectively, with our own standard method at 25^{o}C. However, there remains one intractable problem with all germination tests, which is their long duration, up to 72 hours.

VII. CONCLUSION

The developed methods have been subjected to two international ring trials to determine their repeatability and reproducibility. The methods and associated standards have been recommended for use at a meeting of southern African sorghum stakeholders (Chemonics International, 2001). The results of the international ring trials are currently being evaluated by the ICC for provisional draft method status approval. This approach of simple "in-field" methods for determination of sorghum grain end-use quality could be extended and refined for other specific end-uses such as poultry feed. Possible additional useful quality criteria include the percentage of grains with coloured glumes and the percentage weathered (surface moulded) grains.

ACKNOWLEDGEMENT

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VARIATION IN THE NUTRITIVE VALUE OF SORGHUM – POOR QUALITY GRAIN OR COMPROMISED HEALTH OF CHICKENS?

R.J. HUGHES[1] and G. BROOKE[1]

Summary

Sorghum is generally regarded as a relatively consistent source of energy, however, anecdotal evidence from the field in Australia suggests that some commercial flocks respond poorly to sorghum-based diets. A series of at least eight experiments were conducted on each of three sorghum samples to determine their apparent metabolisable energy (AME) values. Near infrared reflectance (NIR) profiles for sub-samples of the sorghum used in each of these experiments were used to predict pig faecal digestible energy (DE) values from a global calibration. AME values for the three sorghum samples were observed to vary widely (15.1±0.58, n=12, range 1.7 MJ/kg; 15.5±0.36, n=9, range 1.1 and 16.2±0.57, n=8, range 1.8 MJ/kg, respectively). In contrast, NIR analysis indicated relatively minor variation in predicted DE values (ranges were 0.2, 0.4 and 0.2 MJ/kg, respectively). It was concluded that variation in AME might be determined in part by the health status of the chickens involved.

I. INTRODUCTION

The Australian chicken meat industry is highly dependent on supply of energy from cereals such as wheat and barley that are known to vary widely in apparent metabolisable energy (AME). In contrast, sorghum is generally regarded as a relatively consistent source of energy (Hughes and Choct 1999). However, this may not always be the case, as anecdotal evidence from the field suggests that some commercial flocks respond poorly to sorghum-based diets. Hughes and Choct (2000) concluded that variation in the nutritive value of sorghum might be associated with the immune status of chickens used in metabolism experiments.

Well in excess of 100 AME experiments have been conducted at the Pig and Poultry Production Institute since it was established in 1996. Many of these experiments have included a control sorghum diet. At the start of each year approximately one tonne of sorghum was purchased from a local grain merchant and put aside for use in AME experiments that took place in the following 12 months. This report discusses variable responses by different batches of chickens fed experimental diets based on three of these sorghum samples in the period 1997 to 1999, inclusive. Each of the sorghums was measured at least eight times with different batches of chickens that were obtained from the same commercial hatchery, and reared in a similar manner in floor pens until placed in metabolism cages at 22 days of age. Contrary to best industry practice, some of these batches of chickens were reared alongside other batches of chickens that were up to three weeks older, which may have compromised the health of the younger chickens.

II. MATERIALS AND METHODS

The AME values of sorghums were determined in repeated conventional energy balance experiments involving measurements of feed intake and excreta output, as described by Mollah *et al.* (1983) with minor modifications, and subsequent measurement of gross energy values of feed and excreta by bomb calorimetry. Day-old feather-sexed broiler chickens were raised in floor pens on a commercial broiler diet to 22 days of age then transferred in single-sex groups of five to metabolism cages in controlled temperature rooms to allow chickens to adapt to the cages. Air temperature was maintained at 26^{o}C at the start of the 7-day experiment and

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lowered daily until it was 23°C at the end of the experiment. Experimental diets contained (per kg) 800 g sorghum, 155 g casein, 20 g dicalcium phosphate, 11 g limestone, 7 g DL-methionine, 5 g mineral and vitamin premix, 3 g salt, and 2 g choline chloride (60%). Dietary treatments were replicated at least four times (two cages of males and two cages of females). Cold-pressed diets were fed for seven days. The first three days enabled the chickens to adapt to the feeds. During the following four days, all excreta were collected and dried at 85°C. Moisture content of excreta voided over a 24 h period was measured. Feed intake was measured during the adaptation and collection phases of the study. Birds were weighed at the start and end of the 7-day period. Dry matter (DM) contents of samples of pelleted and milled feeds were measured. Gross energy values of dried excreta and milled feeds were measured with a Parr isoperibol bomb calorimeter. AME of the grain was calculated by subtracting from the total energy intake the energy contribution of casein, which was assumed to be 20.1 MJ/kg dry matter (Annison *et al*., 1994). Samples were inserted in a quartz window rectangular cell attached to a NIRSystem Model 6500 NIR spectrophotometer. NIR spectra were recorded at 2 nm intervals in the wavelength range 400-2500 nm with Intrasoft International Windows (winISI) NIRS 3 version software. Pig faecal DE values of samples were predicted using a global whole grain calibration (van Barneveld *et al*., 1999).

The usefulness of NIR analysis for predicting the nutritive value of sorghum for broiler chickens was assessed in a single AME experiment involving six different samples of sorghum from a commercial feedmill in Toowoomba, Queensland. Each sorghum diet was fed to eight replicates (four male and four female) each comprising five chickens in a metabolism cage. Pig faecal DE values were obtained as described above.

III. RESULTS AND DISCUSSION

A strong statistical relationship between values for AME determined *in vivo* with chickens in a single experiment and values for pig faecal DE predicted by NIR are evident in Figure 1. This tends to suggest that the nutritive value of sorghum for chickens can be determined solely from NIR properties of sorghum grain.

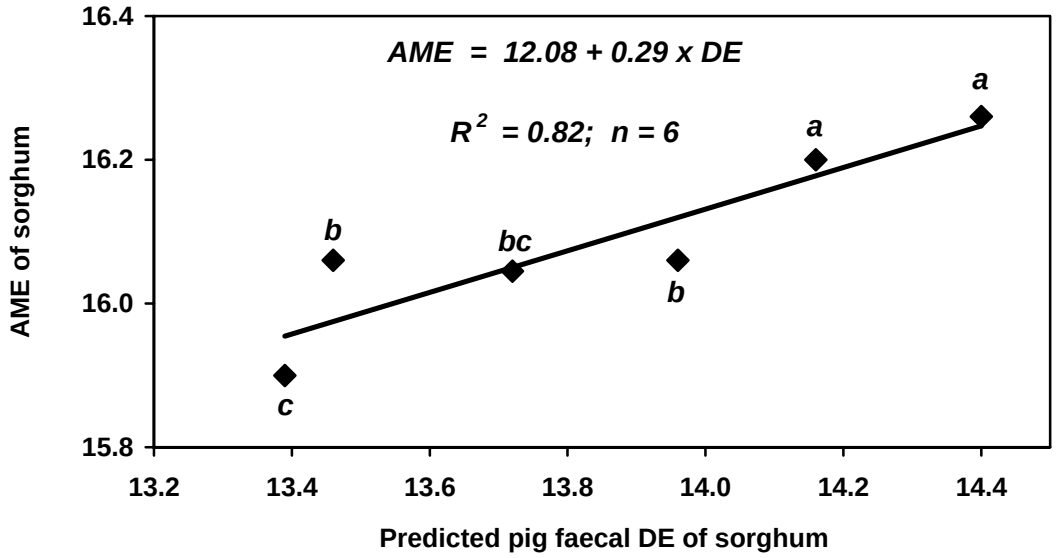

Figure 1. Relation between apparent metabolisable energy (AME MJ/kg dry matter basis) values determined *in vivo* and pig faecal digestible energy (DE MJ/kg as fed basis) values predicted by near infrared reflectance (NIR) analysis. The pooled standard error for AME was 0.04 MJ/kg. Mean values for AME with the same letter are not significantly different (P>0.05).

However, when data for 32 samples of sorghum from several other experiments involving separate batches of chickens were pooled, the statistical relationship between AME and faecal DE disappeared (AME=16.55–0.081xDE; R^2=0.005, n=32). An explanation for this lack of a relationship is that large variation was observed between different batches of chickens, possibly due to differences in ages and health status of donor flocks, and compromised health of chickens when exposed to older birds during rearing.

The results summarised in Figure 2 support the notion that different batches of chickens can show a variable response when fed the same sorghum sample. For sorghum samples A, B and C (Figure 2), the DE values (mean ± standard deviation in MJ/kg as fed basis) predicted by NIR showed very little variation (14.1±0.06, 14.2±0.13 and 14.5±0.07, respectively). In contrast, AME values were 15.1±0.58 (range 1.7 MJ/kg), 15.5±0.36 (range 1.1 MJ/kg) and 16.2±0.57 (range 1.8 MJ/kg), respectively. We did not observe any clinical signs of health problems with different batches of chickens. However, we did notice that batches differed in average weight, and that variation between chickens within a batch was greater if the average weight of the batch was depressed relative to other batches. These observations tend to imply differing degrees of immuno-competence as described by Klasing (1996).

The relatively even gradient from low to high AME within each sorghum sample (Figure 2) implies that bird-related factors influenced the results in a quantitative nature (i.e., with varying degrees of effect) rather than in a categorical way (i.e., all or nothing effects). This is an area that deserves further study, not only to improve experimental precision and accuracy, but also to improve commercial flock performance and uniformity.

IV. CONCLUSIONS

These findings demonstrate that different batches of chickens can respond in a highly variable manner to the same diet. That is, the energy value of the diet might be determined in part by the health status of the chickens involved. Similarly, we expect that variable responses could occur on commercial farms as a result of inadequate cleaning and sanitising of sheds between flocks and/or poor biosecurity practices during grow-out.

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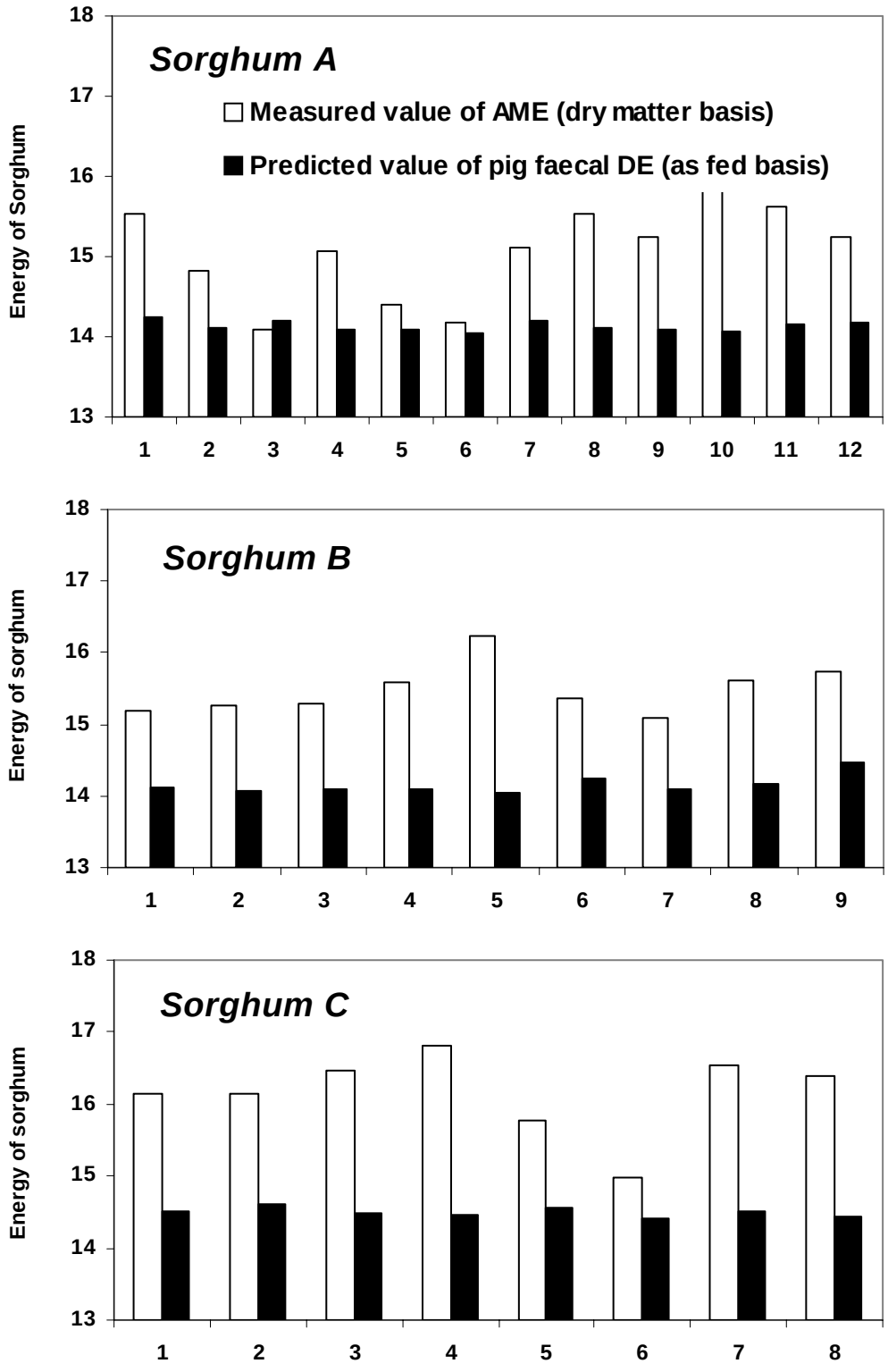

Figure 2. Comparison of apparent metabolisable energy (AME MJ/kg dry matter basis) values determined *in vivo* and pig faecal digestible energy (DE MJ/kg as fed basis) values predicted by near infrared reflectance (NIR) analysis. The numbers on the x-axes indicate the order of the repeated measurements on three sorghum samples.

PHYTATE LIMITS BROILER PERFORMANCE AND NUTRIENT DIGESTIBILITY IN SORGHUM-BASED DIETS

D.J. CADOGAN[1], P.H. SELLE[2], D. CRESWELL[3] and G. PARTRIDGE[4]

Summary

The benefits of adding phytase and/or a multi-enzyme product to nutrient adequate, sorghum-based broiler diets were investigated. Supplemental phytase significantly increased ($P<0.05$) weight gain, AME, the digestibility of total ileal essential amino acids and numerically improved ileal starch digestion ($P=0.066$). The multi- enzyme product significantly increased ($P<0.05$) weight gain and feed intake in the first 21 days, and numerically increased AME ($P=0.058$). The response to all enzymes was greater in the 0 to 21 day period, which may have been due to higher stocking rates and increased pressure on nutrient intake. The present results, supported by previous studies, suggest that the relatively high dietary phytate levels in these sorghum-based diets were limiting the response to the multi-enzyme product alone. Overall, the general response in broiler performance and nutrient digestibility to phytase supplementation, demonstrates that phytate was the major factor restricting nutrient availability and intake in this sorghum-based diet.

I.INTRODUCTION

Increasing dietary phytate adversely affects the apparent metabolisable energy (AME) and ileal digestibility of protein and amino acids (Cabahug *et al.,* 1999; Ravindran *et al.,* 1999). Rutherfurd *et al.* (2002) also showed high levels of phytase (1200 FTU) increased nitrogen digestibility by 6.94% in birds offered diets based on sorghum. The phytate content of sorghum is the highest of all cereal grains (Selle *et al.,* 2003) and these high levels of phytate in sorghum-based diets may limit nutrient availability and broiler growth performance.

The hypothesis tested in this trial is that phytate and other anti-nutrients, such as insoluble arabinoxylan and the kaffarin protein matrix around the starch granule in sorghum-based diets, are limiting bird performance, and that this restriction can be alleviated by adding combinations of enzyme activities, specifically phytase, xylanase, protease and amylase.

II. MATERIALS and METHODS

This study investigated the effects of supplementing nutrient adequate diets, containing sorghum (541-602 g/kg), soybean meal (183-273 g/kg), canola meal (40-60 g/kg), meat and bone meal (60 g/kg) and rice pollard (40-50 g/kg), with phytase (750 FTU/kg feed; produced from E.coli expressed in *Schizosaccharomyces pombe*) and a multi-enzyme product (amylase 400 U/kg feed, protease 4000 U/kg feed and xylanase 300 U/kg feed) in a 2 x 2 factorial arrangement. All diets were steam pelleted and crumbled before applying each enzyme activity in liquid form. Day-old Cobb male broiler chicks (512) were allocated to 32 cages (8 replicates per treatment, 16 birds per cage) and fed a starter diet (11 g/kg digestible lysine, 12.34 kcal/kg ME, ~3.35 g/kg phytate P) for the first 21 days, and then a finisher diet

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(9 g/kg digestible lysine, 12.76 MJ/kg ME, ~3.5 g/kg phytate P) to 42 days of age. Growth rates and feed intakes were recorded at days 0, 21 and 42. Ten birds per pen were selected at random at 21 days of age, euthanased and the contents of the lower ileum were collected and pooled within each pen. Samples of diets and ileal digesta were assayed for amino acids, starch, gross energy and acid insoluble ash (celite 20 g/kg in diet). From 28-31 days of age feed intake and total excreta output were measured quantitatively per pen to determine apparent metabolizable energy (AME). Two-way analysis of variance was used to determine the main effects (phytase and multi- enzyme product) and their interactions by using SPSS (SPSS Inc. Chicago, IL).

III. RESULTS

Overall, birds in this trial (6 birds per cage at 42 days) had an average weight gain of 2,638g; feed consumption of 4,433g and feed conversion ratio of 1.68. A total of 23 birds (4.5%) either died or were culled but these losses were unrelated to treatment ($P = 0.596$). Phytase supplementation significantly increased 21 and 42 day weight gain ($P<0.05$), 21 day feed intake ($P=0.003$) and AME ($P=0.009$). The addition of phytase also increased the digestibility of total essential amino acids ($P=0.044$), including the individual amino acids arginine ($P=0.042$), isoleucine ($P=0.047$), tyrosine ($P=0.035$) and valine ($P=0.013$), although the responses were more pronounced, numerically, when combined with the multi-enzyme product. Supplementation with the multi-enzyme product increased 21 day weight ($P=0.043$) as a result of a higher feed intake ($P=0.030$), however, no other significant ($P<0.05$) improvements in broiler performance or nutrient digestibility were observed. The combined enzyme products significantly ($P<0.05$) increased 21 day gain, 21 day feed intake and AME

IV. DISCUSSION

Enzyme supplementation significantly improved broiler performance, demonstrating that there were anti-nutritional factors in the nutrient adequate sorghum-based diet limiting bird growth. Phytase elicited improvements in both AME ($P<0.05$) and the digestibility of several amino acids ($P<0.10$), whereas the multi-enzyme product showed some improvements ($P<0.10$) in AME alone.

It is curious that enzyme supplementation had significant effects on weight gain and feed intake in the starter phase but these positive effects were not evident in the finisher phase. The removal of 10 out of 16 birds per cage at 21 days post-hatch was a confounding effect; impacting both stocking density and statistical evaluation. The overall mortality rate of 4.5% was quite high, but the majority of losses occurred in the finisher phase, which was an additional disruptive effect.

The lack of response, and sometimes numerically negative effects on 1 to 42 day performance and nutrient digestibility of the multi-enzyme product was possibly due to the increased release and solubilisation of phytate by the xylanase and protease. Non Starch Polysaccharide degrading enzymes have shown to decrease monogastric performance (Cadogan and Selle, 2000) when background levels of phytate are high (eg above 0.30%), and growth is subsequently increased in the presence of phytase. The digestibility of essential amino acids was significantly increased when phytase was added to the diet containing the multi-activity enzyme. The responses to the carbohydrate and protein degrading enzyme was probably limited by the presence of high phytate levels, and furthermore, the enzyme possibly solubilised more phytate and increase its anti-nutritional effects

In summary, the significant improvement in growth, nutrient intake and AME produced by the supplementation of individual and enzyme combination demonstrates there

are components of sorghum that are restricting broiler growth performance. Supplemental phytase produced the greatest improvement in bird performance, energy and protein digestibility, showing that phytic acid was the main restriction on nutrient availability and intake in sorghum based-feeds.

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Table 1. Effects of the multi-activity enzyme and phytase, individually and in combination, on energy and starch digestibility and broiler performance from 1-21, 22-42 and 1-42 days post-hatch

Treatment	1-21 days post-hatch[1]			22-42 days post-hatch[2]			1-42 days post-hatch			AME	Ileal digestibility	
	Gain (g/bird)	Intake (g/bird)	FCR[3] (g:g)	Gain (g/bird)	Intake (g/bird)	FCR[3] (g:g)	Gain (g/bird)	Intake (g/bird)	FCR[3] (g:g)	(MJ/kg)	DE (MJ/kg)	Starch (%)
Control	748	1003	1.34	1868	3386	1.81	2616	4388	1.68	14.19	11.59	91.88
Mutli-enzyme	776	1052	1.35	1792	3296	1.84	2568	4348	1.69	14.38	11.66	92.62
Phytase	791	1062	1.33	1907	3495	1.81	2698	4557	1.66	14.46	11.78	93.12
Multi-enzyme + Phytase	813	1064	1.29	1856	3375	1.82	2669	4440	1.65	14.45	11.79	93.70
SEM	11.527	11.197	0.0155	38.511	81.039	0.0300	40.644	79.780	0.0205	0.0689	0.0775	0.310
Significance (P values)												
Multi-enzyme	0.043	0.030	0.672	0.112	0.206	0.825	0.349	0.329	0.814	0.058	0.777	0.284
Phytase	0.002	0.003	0.165	0.194	0.254	0.962	0.033	0.254	0.239	0.009	0.332	0.066
Multi-enzyme + Phytase	<0.001	0.001	0.113	0.829	0.928	0.902	0.368	0.652	0.219	0.013	0.384	0.046

[1]8 replicates of 16 birds per treatment, [2]8 replicates of 6 birds per treatment, [3]FCR corrected to live weight gain (0.03 per 100g).

Table 2. Ileal amino acid digestibility from 21 day old broilers, offered sorghum based diets supplemented individually with the multi-activity enzyme and phytase, and in combination.

Treatment	Control	Multi-enzyme	Phytase	Enzyme combination	SEM	P values	
	(%)	(%)	(%)	(%)		Multi-enzyme	Phytase
Amino acid							
Arginine	0.822[ab]	0.813[b]	0.827[ab]	0.839[a]	0.0038	0.837	0.042
Histidine	0.751	0.747	0.758	0.765	0.0047	0.844	0.200
Isoleucine	0.758[ab]	0.746[b]	0.765[ab]	0.783[a]	0.0057	0.786	0.047
Leucine	0.783[ab]	0.774[b]	0.792[ab]	0.807[a]	0.0054	0.761	0.055
Lysine	0.823	0.814	0.829	0.835	0.0047	0.882	0.153
Methionine	0.932	0.926	0.936	0.934	0.0051	0.759	0.162
Phenylalanine	0.776[ab]	0.763[b]	0.781[ab]	0.798[a]	0.0054	0.864	0.061
Threonine	0.650	0.640	0.667	0.675	0.0076	0.954	0.104
Tyrosine	0.820[b]	0.814[b]	0.829[ab]	0.841[a]	0.0043	0.690	0.035
Valine	0.774[b]	0.770[b]	0.796[ab]	0.805[a]	0.0058	0.800	0.013
Total essential amino acids	0.779[ab]	0.771[b]	0.789[ab]	0.801[a]	0.0049	0.852	0.044
Aspartic acid	0.695[ab]	0.683[b]	0.699[ab]	0.718[a]	0.0064	0.797	0.131
Serine	0.676[ab]	0.665[b]	0.690[ab]	0.707[a]	0.0072	0.822	0.051
Glutamine	0.779[b]	0.769[b]	0.785[ab]	0.803[a]	0.0052	0.657	0.053
Glycine	0.710	0.707	0.721	0.737	0.0059	0.582	0.078
Alanine	0.751	0.747	0.763	0.779	0.0060	0.633	0.075
Total amino acids	0.759[ab]	0.750[b]	0.768[ab]	0.782[a]	0.0053	0.766	0.054

[a,b] Means with different superscripts in the same row are significantly different ($P<0.05$; LSD).

WET FEEDING OF YOUNG CHICKS

M. FORBES[1], R.D. SLADE[1] and A.Y. YALDA[2]

Summary

Wet feeding of broilers from 11 days has consistently improved weight gain and/or efficiency. Two experiments were conducted to investigate wet feeding at younger ages. Introducing wet food at 3 days improved weight gain and efficiency to 19 days compared to dry feeding throughout, with introduction of wet feed at 7, 10 or 13 days giving intermediate results. In the second experiment, in which chicks were fed either wet or dry from arrival to 10 days and all were then dry-fed to 21 days, the wet-fed birds maintained their higher food intake and weight gain when subsequently dry-fed. Wet feeding of chicks is feasible and can give significant advantage.

I. INTRODUCTION

Wet feeding has been shown to give significant improvements in growth rate and/or efficiency of broiler chickens (Forbes, 2003) but there are some major barriers to the commercial implementation of this method. One of these is a lack of knowledge as to whether newly-hatched chicks can be wet-fed as previous experiments have usually started wet feeding at about 11 days post-hatching. It may be convenient from a management point of view to commence wet feeding as soon as possible, or it might only be possible to wet feed at an early age when the feed intake is low enough for the food to be mixed with water and delivered to the birds by hand.

In preliminary trials we observed that day-old chicks became very dirty with food if it was offered in containers into which the birds could climb; however, when small troughs were used that prevented this, they started to consume wet food immediately it was offered and at 5 days of age the chicks fed wet food were heavier than those fed dry food by 15 g. In the first experiment reported here broilers were fed dry food from arrival and this was replaced by wet food at different ages in different groups, from 3 to 19 days. In the second experiment wet or dry food were offered from arrival to 10 days and subsequent performance on dry food was followed to 21 days.

II. EXPERIMENT 1

Ninety day-old male broiler chicks were divided into 15 groups (6 chicks each) and allocated to 5 treatments each replicated three times. DRY birds were fed dry feed (Dalgety broiler starter) throughout; WET3, WET7, WET10 and WET13 were provided with wet food (2kg water/kg feed, sufficient to give the consistency of sloppy porridge) from 3, 7, 10 and 13 days, respectively, and the experiment finished when the birds were 19 days of age. Wet food was prepared 2 or 3 times a day and given to the birds without soaking.

It will be seen from Table 1 that there was an increase in weight gain during the four days after starting wet feeding and that this advantage was maintained for the rest of the experiment. At 19 days WET3 and WET7 were significantly heavier than DRY. Feed intake (expressed as air-dry feed) was not significantly affected by treatment but tended to be higher with the wet feeds, particularly at the end of the experiment. Feed efficiency was improved in

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almost every case once wet feeding had been introduced, but in no case was this statistically significant, because each mean is based on only three observations. Over the whole period of the experiment the improvement was 13%, comparing DRY with WET3. The other groups were intermediate in efficiency. Carcass weight was significantly increased by feeding wet diets.

Table 1. Main results of Experiment 1.

	Days	DRY	WET3	WET7	WET10	WET13	sed
Body weight (g/bird)	3	77^{a}	78^{a}	77^{a}	78^{a}	77^{a}	2.8
	7	167^{b}	181^{a}	166^{b}	169^{b}	161^{b}	5.8
	11	293^{b}	323^{a}	306^{ab}	287^{b}	294^{b}	9.7.
	15	457^{b}	510^{a}	498^{a}	482^{ab}	451^{b}	12.7
	19	637^{c}	736^{a}	700^{ab}	677^{bc}	675^{bc}	24.2
Food intake (g DM/b)	3-19	848^{a}	884^{a}	889^{a}	862^{a}	884^{a}	16.9
Feed efficiency	3-19	0.66^{a}	0.75^{a}	0.70^{a}	0.70^{a}	0.68^{a}	0.03
Carcass weight (g)	19	422^{b}	508^{a}	492^{a}	489^{a}	483^{a}	20.3

III. EXPERIMENT 2

Ninety-eight day old female broilers were divided at random into two equal treatment groups, DRY and WET, each of 4 replicates. Both treatments were fed a wheat based commercial starter crumb *ad libitum* (crude protein, 190 g/kg; crude fibre, 45 g/kg; total oil, 30 g/kg). WET diets were mixed daily with 2.0 kg tap water per kg air-dry food; refusals were collected the following day, heated to constant weight at 100^{0}C. From 10 d of age both treatment groups were fed starter crumbs in air dry form. Excreta were collected on days 5-7, 12-14 and 19-21 for determination of approximate DM retention. At 10 d and 21 d of age 12 from each treatment group were killed and dissected for body analysis.

Feed intake to 10d were similar for both treatments but LWG and FCE were significantly higher ($P<0.05$) for wet fed birds (Table 2). Following the change to conventional dry diet, the feed intake of the WET treatment birds remained high. Apparent DM retention remained similar for both treatments throughout the trial. At 10 days body and carcass weights of WET treatment birds were significantly greater than for DRY control, an effect that persisted to 21 days. Total empty weight of the intestinal tract was significantly greater in WET treatment birds (+3.19g) mainly due to increases in mean crop, gizzard and duodenum empty weights (+0.22g, +1.28g, +0.70g respectively). By 21 days there were no significant differences in intestinal tract measurements although mean gizzard and duodenum weights still tended to be elevated in wet fed birds (+2.5g and +0.86g respectively). Viscosity of proximal SI digesta samples from wet fed birds on day 10 were significantly lower than those from control as were the pH values of samples from the middle and distal thirds. No significant differences were found in SI digesta viscosity or pH of initially DRY and WET fed birds on day 21.

Table 2. Main results of Experiment 2.

	DRY	WET	STDEV	DRY	WET	STDEV
	0 to 10 days of age			11 to 21 days of age		
Feed intake (g DM)	228	235	16.4	751	782	26.0
Live weight gain (g)	153[a]	172[b]	8.5	446	462	17.0
Feed conversion efficiency	0.67[a]	0.73[b]	0.025	0.59	0.59	0.026
	Day 10			Day 21		
Total gut weight empty (g)	19.7[a]	22.8[b]	2.64	49.8	53.2	6.86
Total gut content (g)	17.7	17.8	3.30	38.0	42.1	11.53
Total gut length (cm)	116.9	119.2	8.4	150.8	146.6	23.7
Viscosity prox. SI (cPs)	4.12[c]	2.75[d]	0.83	3.68	4.00	0.65
21 day body weight (g)	637[a]	672[b]	17.9			
21 day carcass weight (g)	351[a]	399[b]	37.7			

IV. DISCUSSION

The results of the first experiment show that growth and efficiency are improved when the feed is given in the wet form from 3 days of age, to a similar extent to that previously shown with older broilers. In this experiment the feed was mixed with water two or three times per day in view of the high level of feed intake and the small size of the troughs used. The fact that efficiency was improved by such a large margin suggests that it is not necessary to soak feed for a long time, as confirmed with older birds (Yalda and Forbes, 1996).

In the second experiment the improvement in growth due to wet feeding was accompanied by reduced digesta viscosity, previously associated with a reduction in the anti-nutritional effects of non-starch polysaccharides present in cereal based diets (e.g. Philip *et al.*, 1995). One possible mode of action was reported by Yasar and Forbes (1999) who noted that a significant reduction in crypt cell proliferation rate (CCPR) paralleled a similar decline in digesta viscosity to that reported here, representing a saving in terms of bird maintenance and therefore increasing the efficiency of food utilisation, as observed here.

In the absence of any significant differences in FCE, gut measurements or viscosity, the mechanism by which body and carcass weight advantages persist beyond the wet feeding period might involve the relationship between body weight and feed intake. Proportional to body weight, feed intake for WET and DRY treatments respectively was 0.89 and 0.84 at 10 days and 0.86 and 0.84 at 21 days. Thus the increased body weight of wet fed birds at 10 days would appear to promote higher feed intake and faster growth to 21 days.

These results suggest that it might be worthwhile providing commercial chicks with wet food initially, when their food intakes are very low and such provision would not be expensive in terms of machinery or labour. Care must be taken, however, that chicks can easily obtain food without being able to climb into the feeders during the first few days.

Where an investment in automated wet feeding has been made, its use can commence as soon as the birds are able to eat from the troughs.

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SATELLITE CELLS: A REVIEW OF THEIR PHYSIOLOGY, MANIPULATION AND IMPORTANCE TO MUSCLE DEVELOPMENT

S.J. WILKINSON[1] and T.A. SCOTT[1]

Summary

Meat yield of a market age broiler is determined by the number of muscle fibre cells present at hatch. Muscle growth (hypertrophy) is influenced by the activity of satellite cells (located within the muscle fibre) that remain active for up to seven days post-hatch. Satellite cells respond to various stimuli (nutritional, environmental, physiological etc.) and thus potentially may be stimulated to increase meat yield and improve product uniformity**.** In particular, we are interested in increasing the yield of breast meat while being cognisant of associated potential for muscle myopathy.

I. INTRODUCTION

It has been demonstrated that muscle size (meat yield) is a direct function of muscle fibre cell number (as established by embryonic and post hatch (to seven days) satellite cell differentiation to breast muscle cells) and their subsequent growth (hypertrophy). Similarly, it is well known that breast yield from the modern day broiler chicken can vary from 17 to 25% of the birds' body weight; therefore, yield of breast meat (estimated to equate to 60% of the value of the whole bird) can dramatically influence profitability of the industry. Therefore it is important to increase the yield or breast muscle (and therefore value of bird) as well as achieve higher product uniformity. The objectives of this paper are to review the physiology of satellite cells and their function in relation to manipulation during the embryonic and post-hatch stages of development in the broiler chicken.

II. MUSCLE CELL PHYSIOLOGY

The muscle cell, in contrast to most other cells is multi-nucleated. These nuclei are found along the entire length of the muscle fibre with each nucleus exerting control over its associated cytoplasm, termed a DNA unit (Moore and Mozdziak, 2004). The term satellite cell (based on its location) refers to the myogenic precursor cells located within the basal lamina of the muscle fibre adjacent to the sarcolemma (Figure 1). Satellite cells are capable of entering the cell cycle and proliferating and either fusing into existing fibres or fusing with each other to form new fibres (Halevy *et al*., 2003).

There are two mechanisms of muscle growth; hyperplasia (increase in fibre number) and hypertrophy (increase in fibre size). The chick emerges from the egg with a predetermined number of muscle cell fibres that does not alter during the life of the bird. Muscle growth post-hatch is the result of an increase in myofibre size and an increase in DNA content (Halevy *et al*., 2003; Mozdziak *et al*., 2002a); the latter increased DNA content is provided by satellite cells (Hill *et al*., 2003). However, instances of excessive hypertrophy such as that found in fast growing strains of poultry lead to idiopathic myopathy and an increased susceptibility to stress induced myopathy as a result of the muscle growing beyond the available blood supply (Maltby *et al*., 2004; Mitchell and Sandercock, 2004).

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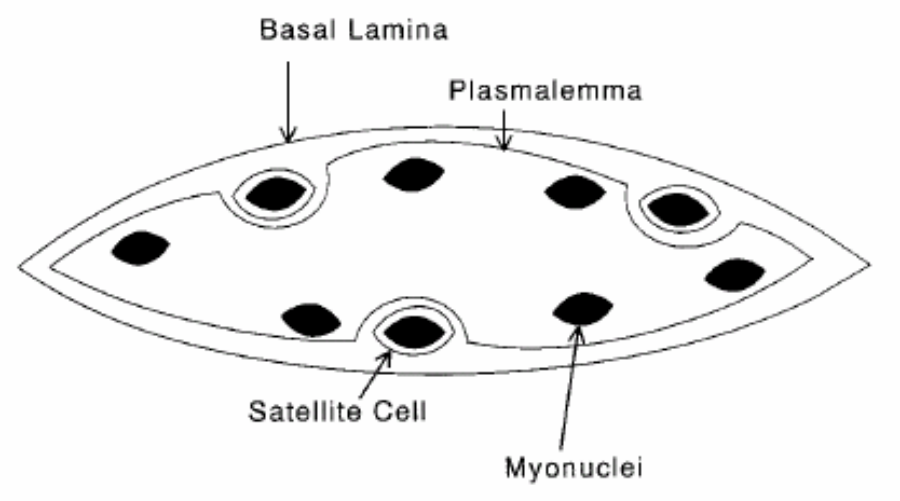

Figure 1. Diagrammatic representation of the location of satellite cells in the muscle cell fibre (McFarland, 1999).

Apoptosis (nucleic turnover) in this instance refers to the removal of the nuclei units from the cell and not the death of the entire cell. In some instances the mechanism of nuclei turnover can compete with muscle growth as a reduction in the number of DNA units available to the muscle cell would result in a decrease in muscle size throughout the life of the animal. Evidence suggests that there is some flexibility in DNA unit size, however once a critical level is reached, the superfluous myonuclei are destroyed (Mozdziak *et al*., 2002b). Apoptosis of myonuclei in post hatch starved chicks has been attributed to irreversible reductions in muscle size (Mozdziak *et al*., 2002b).

At hatch, skeletal muscle of the chick contains a high proportion of proliferating satellite cells that decrease rapidly towards the end of the growth cycle (Halevy *et al*., 2000). This period of proliferation and differentiation has been reported to last only one week post hatch in broilers (Halevy *et al*., 2003) where in mature animals, satellite cells are largely quiescent unless activated by myotrauma (muscle damage). The response to myotrauma results in regulated satellite cell populations migrating to the site of injury and depending on the extent of injury, either fuse to the existing myofibre or produce a new myofibre (Hawke and Garry, 2001). Thus any attempt to increase the number of muscle fibre cells would be best made during the embryonic and early post hatch stages of development.

The process of satellite cell activity and therefore muscle growth is influenced by several growth factors and other stimuli (Figure 2). Secretion of Insulin-like Growth Factor (IGF-I and IGF-II) by skeletal muscle has been determined to be particularly important in the proliferation and differentiation of satellite cells *in vitro* (Hawke and Garry, 2001). This work is supported by studies conducted by Chakravarthy *et al*. (2001) who reported that intramuscular administration of IGF-I resulted in enhanced satellite cell proliferation and increased muscle mass.

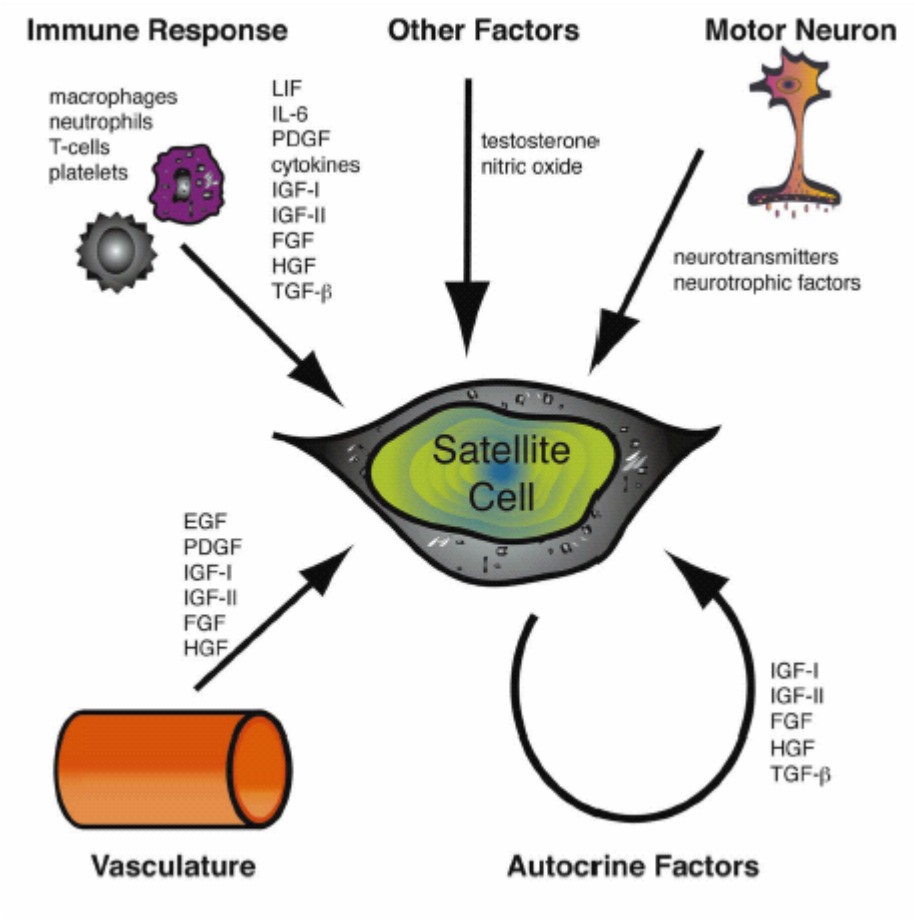

Figure 2. Factors that modulate satellite cell activity (Hawke and Garry, 2001).

III. MANIPULATION OF SATELLITE CELLS

a) Nutrition

The nutrition of the post-hatch chick has also been demonstrated to influence satellite cell activity. Work conducted by Halevy *et al*. (2000, 2003) has shown that starvation of the post-hatch chick for 48 h leads to a decrease in satellite cell proliferation resulting in decreased overall body and breast muscle weight when compared to chicks that were fed within 1 h post-hatch. During this adjustment period, the chick moves from being reliant on the yolk sac (lipid) as a source of nutrients to that of carbohydrate (starch) (Moran, 1995). During this adjustment phase, approximately 2-6% of hatchlings don't survive, and many that do exhibit reduced growth, poor feed conversion, compromised immune status and poor meat yield (through apoptosis (programmed cell death); Uni and Ferket, 2004). If post-hatch starvation occurs, energy is diverted away from the proliferative satellite cells and redirected to mechanisms essential to survival (Moore and Mozdziak, 2004). As the broiler increases its body weight 50-fold from hatch until market age (42 days), the magnitude of this adjustment phase represents a greater proportion of the bird's productive life and therefore importance to manage.

Limitations to satellite cell activity also reside in the underdeveloped gastrointestinal tract of the chick post hatch. Impaired development of the gastrointestinal tract has been associated with post hatch nutritional deficiencies, reduced satellite cell activity and decreased body weight at market age (Noy *et al*., 2001). Practical methods to overcome this

handicap are as yet to be fully elucidated however current research is targeting the administration of specific nutrients directly into the amnion of the of the late term embryo (*in-ovo* feeding) as well as the provision of a post-hatch feed source (early feeding) to stimulate gut development. The provision of a highly digestible early post hatch feed at the hatchery could be more attractive due to the relatively small amounts of feed consumed by the chick.

b) Chick Quality

Further compounding this situation of variable muscle mass at marketing age is the individual effect of the chick – chick quality. Chick quality is also highly variable and plays a significant role in the outcome of the hatching process (hatch %, body weight, mortalities) and inevitably influences the marketing weight/age of the bird (Boerjan, 2004). The vitality and performance of the chick is strongly influenced by breeder nutrition and immunocompetence, as well as the handling and storage of fertile eggs (York, 2004). As genetic potential for increased growth performance and production increases there will be further changes to requirements for embryo and early chick development. Chick quality and vitality is highly dependant on the incubator climate with increased mortalities and delayed development associated with temperatures greater and/or less than optimum (Boerjan, 2004).

c) Environmental

Further opportunities for satellite cell manipulation exist with temperature control both pre- and post-hatch. We hypothesise that these environmental effects may be linked with the nutrient profile of the egg, e.g. the ratio of egg yolk and albumen and fluctuations of specific nutrients in these two egg components. During the final stages of embryonic development, target tissues of the embryo become responsive to signals of heat and cold stress, with development and differentiation being inversely related to temperature. The need for different and highly specific incubation conditions was highlighted by studies that measured oxygen consumption of Ross 308 embryos during incubation; the Ross 308 embryos produce approximately 26% more metabolic heat than traditional meat producing strains (Boerjan, 2004). Exposure to constant high temperatures (> 39.5ºC) inside the hatcher also results in stunted growth and poor chick vitality.

Halevy *et al*. (2001) investigated the effects of early age heat exposure and its affect on satellite cell proliferation and muscle development in chicks. In response to thermal conditioning (37 °C for 24h at 3 days of age) of the chick, increased IGF levels in the breast were observed; this was associated with an increased satellite cell proliferation and differentiation, indicating that IGF-I plays a central role in the immediate stimulation of satellite cell myogenic processes in response to heat exposure. Maximal improvements to satellite cell proliferation and differentiation were achieved when thermal conditioning was applied at day three post hatch and therefore may be applicable to industry as a means to improve breast yield. These results are contrary to the effects of heat exposure to broilers at 6 weeks of age, where exposure to similar temperatures leads to muscle damage (Yahav 1998), indicating that heat exposure at an early age results in an increase in growth factors, important to muscle hypertrophy.

The effects of light on the development and growth of chicks has also been shown to influence body weight and breast muscle weight. Studies by Rozenboim *et al*. (2004) investigated the effects of light on embryonic development, finding that stimulation with monochromatic green light during incubation enhanced development and growth in chicks, enhancing breast and body weight at market age whereas rearing under green light did not produce any noticeable affect. The mechanism for this is yet to be determined however it is

theorised that the use of green light during incubation increases the number of myoblasts therefore leading to increased muscle hypertrophy.

IV. CONCLUSION

The implications of this review indicate that muscle size and therefore meat yield may be enhanced by the stimulation of myogenic precursor satellite cells. Increased muscle fibre cell number would circumvent the increased myopathies of excessive hypertrophy whilst providing increases to meat yield. Methods such as, alternative breeder nutrition, *in-ovo* feeding, enhanced environmental control of incubation and early nutrition may provide practical methods to achieve this, however more research is required to determine the mechanisms of this phenomenon.

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GLYCOMICS - THE NEW FRONTIER IN POULTRY NUTRITION

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Summary

Carbohydrates are at the centre of almost every aspect of biology. In particular, it has been recognised that carbohydrates play a central role in cell-to-cell communication and the defence mechanisms to common pathogens. It is now also known that carbohydrates have a crucial function in the immune response. This growing understanding of the structure and function of carbohydrates opens new opportunities to use carbohydrates as functional feed ingredients in modern poultry diets.

I. INTRODUCTION

The role of indigestible oligo- and polysaccharides as substrates for the microflora in the large intestine of farm animals has been widely discussed in scientific literature. Additionally, it is well known that the microflora plays a key role in the development of the gut associated immune system (GALT) (Fioramonti *et al.*, 2003). It has further been recognised that sugars on the intestinal surface have an important role in the bacterial attachment to the host (Firon *et al.*, 1983, Ofek *et al.*, 1977). However, it has only recently been recognised, that distinct carbohydrates can have very specific biological activities.

Carbohydrates are known to have three main functions. Firstly, they act as a readily available energy source or as components for energy reserves. Secondly, carbohydrates are structural components in the form of cellulose or chitin and, thirdly, carbohydrates are part of protein and lipid structures especially on the cell surface. It is particularly this third function of carbohydrates, which until now, has confused researchers the most. Scientists who studied the structure and role of proteins and lipids were often confronted with the problem of how to remove these carbohydrates in order to truly understand the role of protein or lipids. It is now known however, that the structure of these carbohydrates plays a major role in cell recognition and defines the function of the proteins or lipids they attached to.

Distinct carbohydrate structures can have very specific biological activities. For example, sugars (monosaccharides) combine to form giant molecules such as cellulose; they are already known to regulate hormones, organize embryonic development, direct the movement of cells and proteins throughout the body, and regulate the immune system (Schmidt, 2002). Glycobiology or glycomics is defined as the characterisation of the sugars that make up a cell (Newman, 2004). Glycomics is hugely more complex than genomics (the structure and function of genes in a cell) or proteomics (the structure and function of proteins in a cell). In contrast to the structure of DNA or the structure of protein, the vast number of different monosaccharides and possible connections between these monosaccharides makes it impossible to predict the structure of complex carbohydrates or their function. A simple example is the biological difference between amylose and cellobiose, the building blocks for starch and cellulose respectively. Both carbohydrates are essentially built from 1-4 linked glucose units. However, in the case of amylose, these units are linked in the α configuration and can be easily degraded by the endogenous enzymes of mammalians and avian species. In contrast, cellobiose or cellulose has units that are linked in the β configuration, which makes cellobiose indigestible for mammalian and avian species.

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This example clearly demonstrates the complexity of the structure of carbohydrates. Two glucose units bound at the same site using different binding configurations have dramatically different functions. Continued research in understanding the structure and function of carbohydrates has opened new opportunities to use carbohydrates as functional feed ingredients in modern poultry diets. The gastrointestinal tract of chickens harbours a wide range of carbohydrate-based receptors on its surface, which interact with intestinal cells, the immune system and the microflora in the intestine. The key to successfully using selected carbohydrates to influence intestinal health will depend on the recognition of specific carbohydrate structures and their specific function in the chicken.

II. THE ROLE OF CARBOHYDRATES IN HEALTH AND DISEASE

Carbohydrates are at the centre of cell-to-cell communication. The presence of specific surface carbohydrates enables bacteria and viruses to attach to the cell surface, to colonise and in the case of a pathogen, cause disease. Most bacteria express one or more types of protein polymers, so called fimbriae, which recognise specific carbohydrates (Kelly, 2004). These fimbriae are carbohydrate specific and are classified according to the monosaccharide that most effectively inhibits binding to the intestinal surface (Table 1). However, it has to be pointed out that binding capacity varies widely depending on the specific structure in which the carbohydrate is presented to the bacteria.

Table 1. Carbohydrate adhesins of various bacterial strains.

Bacterial strain	Carbohydrate adhesin
Campylobacter coli	Glucose
Clostridium spp	Glucose
Edwardsiella ictaluri	Galactose, Glucose, Lactose
Edwardsiella ictaluri	Mannose
Enterobacter cloacae	Mannose
Escherichia coli	Mannose, Fucose, Galactose, Glucose
Fusobacterium spp	Galactose, Lactose, Raffinose
Haemophilus influenzae	Galactose, Glucose
Klebsiella pneumoniae	Glucose, Mannose
Salmonella marcescens	Mannose
Serratia ssp.	Mannose, Fucose
Shigella ssp.	Fucose
Streptococcus bovis	Glucose
Streptococcus suis	Galactose

From (Newman, 2004)

Similarly, viruses also attach to cell surface carbohydrates in order to 'enter' the host cell and replicate. The influenza virus for example, binds to sialic acid-galactosyl linkages on the cell surface. However, the binding to specific forms of sialic acid is strain specific. During the resent outbreak of avian influenza in Thailand and many Asian countries a large number of birds died from the disease, whereas the virus affected only relatively few humans. The avian influenza virus binds to the α-2,3 sialic acid-galactosyl linkages and not to the α-

2,6 linkage and the human influenza has the opposite receptor specificity (Matrosovich *et al.*, 1999). The great fear during the AI crises was that pigs would be infected with the avian influenza. In contrast to humans, pigs have receptors for both α-2,3 and α-2,6 sialic acid linkages. Pigs are therefore susceptible to both human and avian influenza and can act as intermediates between the two types of influenza virus.

III. THE USE OF OLIGOSACCHARIDES TO CONTROL HEALTH AND PERFORMANCE

One way to reduce the risk of a bacterial or viral infection is to block the attachment to the intestinal surface either by promoting colonisation with commensal organisms or by blocking bacterial fimbriae. The inclusion of substrates like fructo-oligosaccharides (FOS), transgalacto-oligosaccharides (TOS) or inulin in poultry diets can selectively stimulate the growth of beneficial microorganisms (bifidobacteria, *Lactobacillus* spp.) in the intestine (Bielecka *et al.*, 2002, Ziggers, 2001). These bacteria will colonise the intestine and inhibit the colonisation of pathogens by competitive exclusion.

Xu *et al.*, (2003) has shown that the addition of commercially available fructooligosaccharides (FOS) at 4 g/kg feed resulted in a significant reduction of *E. coli* in the small intestine of 49d old chickens and a significant improvement in weight gain and feed efficiency. However, when FOS was included at 2g/kg or 8g/kg no significant changes in the numbers of *Bifidobacterium*, *Lactobacillus* and *Escherichia coli* were found. Similarly, (Waldroup *et al.*, 1993) reported that the inclusion of 3.75g/kg of FOS had no inhibitory effect on the concentration of *Salmonella spp.* The lack of response at lower dosages and very high dosage could indicate a possible interaction between FOS and bacterial species. It has also been pointed out that *Salmonella ssp*. have the ability to use FOS as a substrate for growth when the concentration of non-complex fructose molecules was increased (Oyarzabal, *et al.*, 1995).

In contrast to FOS, which stimulates the growth of beneficial bacteria, the inclusion of mannan-oligosaccharides (MOS) inhibits pathogen colonisation by blocking the fimbriae that enable these pathogens to attach to the intestinal lining. Mannose is the key carbohydrate in the adhesion process of many pathogens species. Several studies have demonstrated that the addition of MOS to broiler diets can significantly reduce the colonisation of *Salmonella ssp*. and *E. coli* (Fairchild *et al.*, 2001; Spring *et al.*, 2000). Although mannose is the key for bacterial attachment it has been shown that complex α-1-3 and α-1-6 branched MOS are up to 37 times more effective in binding *E. coli* than pure D-mannose (Firon *et al.*, 1983). This type of mannose is found predominantly in the outer layer of the yeast cell wall. This effect clearly demonstrates the importance of glycomics in poultry nutrition. Blocking bacterial attachment sites can also lead to improved immunity by allowing pathogens to be presented to immune cells as attenuated antigens (Ferket, 2004). It is further suggested that MOS has a direct effect on the immune cells in the gastrointestinal tract via its uptake into M-cells located in the Peyer's patches on the intestinal surface. As a result, specific circulatory and secretory immunoglobulin synthesis in the plasma and bile are increased in response to antigen exposure (Cotter, 1997; Savage and Zakrzewska, 1996).

Preventing exposure to potential pathogens or other antigens that trigger an acute immune response is the key for optimal animal growth performance. Cotter *et. al.* (2002), speculated that MOS stimulate the production of mannose binding lectin (MBL), an acute phase protein that aids in phagocytosis. It is suggested that surface mannose present on the mannan- oligosaccharides have a positive effect on the production of MBL by stimulating Toll-like receptors (TLR) on the intestinal surface.

IV. CONCLUSIONS

Research in glycomics has clearly shown that that modulation of the attachment sites in the intestine and the immune response will lead not only to improved overall health but also to improved animal performance. Future research will add to existing knowledge and will open up new applications for functional carbohydrates in poultry diets, which will ultimately improve animal health, and performance.

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UNDERSTANDING BIOENERGETICS OF THE ENTEROCYTE: THE BASIS FOR EFFICIENT GROWTH OF THE CHICKEN

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Summary

Food provides the chicken with the necessary building blocks for body growth and energy for metabolism and work. Macro and micronutrients contained in the diet greatly influence biochemistry and physiology at the level of the cell and of the whole organism, ultimately determining productivity. Energy is derived from carbohydrates, fats and proteins, with 70% of food energy being used to power metabolism and work. The relative proportions of carbohydrates, fats and proteins in feed influence the efficient functioning of cells and tissues. The optimal dietary balance of these compounds is the subject of ongoing debate among nutritionists.

Productivity of the chicken is largely determined by intestine function. Enterocytes, the first layer of cells of the intestinal epithelium, transport and further metabolise nutrients contained in the digested food. To perform their primary function of nutrient transport, the enterocytes continuously adjust their physiology to the ever-changing environment of the intestinal tract. This places them under severe energetic stress. Future research is needed to define the energetic conditions for optimal function of enterocytes. A better understanding of basic biochemical and physiological processes in the intestine will open the way for novel approaches for reducing the costs of production of chicken meat and eggs.

I. INTRODUCTION

Animal production is an integral part of a balanced economy providing food and profit. The need for increased efficiency of production has been the driving force behind research into animal farming in recent times. The poultry industry continues to benefit from new ideas and developments in animal sciences. Classical breeding and progress in molecular genetics and understanding of nutrition contribute to increased productivity (Zhang and Aggrey, 2003).

Studies of cell biochemistry indicate that genetically determined characteristics, such as rate of growth and size, may be significantly influenced by cell physiology, in particular energy yielding reactions (Strohman, 2003). Significant change in poultry productivity can be attributed to diet composition and further, the events associated with its digestion and absorption (O'Sullivan *et al*., 1992 a,b).

This short review draws attention to the need for clearer understanding of the biochemistry and physiology of the intestinal cells, which transport and further metabolise nutrients. Modification of feed, spurred by financial or political concerns, such as reduced use of antibiotics, have direct impact on the function of these cells, which in turn may influence animal productivity and health.

II. FOOD: SOURCE OF BUILDING BLOCKS AND ENERGY

For the heterotrophic organisms, which include chicken, ingested food is the only source of the building blocks for body growth and maintenance (Stryer, 1995). Food also

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provides energy necessary to sustain life processes. From the energy available to the chicken up to 70% is used to power metabolism and animal work (e.g. locomotion and maintaining body temperature). Remaining is converted to body mass as growth or for tissue maintenance and repairs (Kleiber, 1975).

Animals utilise a diverse range of organic compounds for synthesis of the body tissues including organic bases, acids and salts, vitamins, carbohydrates, organic pigments, protein, fatty acids and their derivatives. All of these are either found in food or generated through the digestion and catabolism of food-derived organics. Liberating such organics from the matrix of the food is the primary role of the extracellular enzymes of the gastro-intestinal tract. These compounds are further catabolized by the cells of the intestinal epithelium and then are transported through blood and lymph to other areas of the body (Atkinson, 1977).

Metabolism and work are powered by the potential energy contained within the chemical bonds of carbon and hydrogen atoms of carbohydrates, proteins and fats. The radiant energy of the Sun is locked in the C-H bonds during photosynthesis and liberated during the process of cellular respiration. Hydrogen atoms are stripped off carbon by the attractive force of oxygen and liberated energy is conserved in molecules of adenosine triphosphate (ATP). The carbon skeleton is excreted as carbon dioxide and in the instance of protein, nitrogen is eliminated as urea or uric acid. ATP forms the ultimate link between the energy of C-H bond in a nutrient and the energy required for metabolic and physiological processes of the organism (Nicholls and Ferguson, 2002).

As 70% of the ingested energy is utilised to power metabolism and work, the type and quantity of the compounds delivering energy are of vital importance. Human and animal nutrition science is an ongoing debate about the optimal balance of carbohydrates, protein and fats in the diet (Grundy, 1999). The relative proportions of each of these nutrients are paramount in energy regulation and performance at the level of the cell, tissue and organism, ultimately determining the animal's health and productivity (Robinson *et al*., 2000).

One group of cells in particular, which energy status underlines performance of the whole organism, are the nutrient gathering cells of the intestinal tract – the enterocytes.

III. THE ENTEROCYTE

Enterocytes constitute the first layer of cells of the host in direct contact with the luminal contents of the gut and thus are exposed to a constantly changing physico-chemical environment of passing digesta (Wright and Alison, 1984; Ferraris and Diamond, 1989). These conditions distinguish enterocytes from the other epithelial cells, which are bathed in the homeostatic environment of blood or lymph.

The primary functions of the enterocytes are the transport and further catabolism of the nutrients contained in the ingested food and their transfer to the blood stream. The transport from the intestinal lumen occurs through the phospholipid membrane of the cell's brush border, whereas enterocyte/blood exchange occurs through the basolateral membrane.

The physico-chemical properties of a nutrient determine its mode of transport (Pacha, 2000). Fat soluble and small, non-polar molecules enter by the process of simple diffusion. Cellular proteins located in the brush border facilitate the transport of certain inorganic ions and some organic compounds of low molecular weight. Most nutrients, however, including amino acids, sugars and a number of fatty acids, are transported by membrane located carrier proteins that are driven by energy derived from hydrolysis of ATP (Yan, 2003).

Macro and micronutrients play a multifunctional roles in the anatomy and physiology of the enterocytes. Fats and carbohydrates have other functions apart from supplying enterocytes with energy. Dietary fats play a critical role in the structure and function of phospholipid membranes, determining fluidity and the degree of anchorage of membrane

proteins (Hajri and Abumrad, 2002). Fats, being a component of hormones, also play a role in intra and extracellular signalling and defence responses. Short and long chain fatty acids have been recognised as key players in the regulation and function of numerous enzymes and expression of many genes (Sanderson and Naik, 2000; Jump and Clarke, 1999).

Carbohydrates provide cells with the structural frames for nucleic acids, coenzymes, ATP and carriers of acetyl groups. The characteristics of carbohydrate rings combined with proteins and lipids, determine the physical properties of inner and outer cell membranes. Carbohydrate units play a pivotal role in cell-cell and cell-molecule recognition processes, thus coordinating cell development, repair and defence. Indirectly, through biochemistry of insulin, sugars influence many key metabolic pathways of the enterocyte (Whelan and Manners, 1978).

Proteins play crucial roles in all biological processes of the cell. These compounds provide internal and external mechanical structures, initiate and mediate intra and extracellular signalling, transport and store macro and micronutrients. In addition, proteins regulate cell growth, repair and defence (Stryer, 1995).

Sodium, potassium, hydrogen, chlorine and other inorganic ions are necessary in active cellular transport (Decoursey, 2003). Magnesium, nickel, iron, and other metallic ions form part of coenzymes and prosthetic groups of many catalytic and transporting proteins. In consequence, extra and intracellular concentrations of these molecules influence activities of many biochemical processes of the cell (Newsholme and Start, 1977).

The quantity and quality of nutrients in the gut lumen, which differ with diet and over time after ingestion, constantly alter the physiology of the enterocyte. Change in concentration of one compound or element often create a cascade of physiological reactions, which determine the cell's response towards other nutrients. These interactions are well illustrated by the mechanisms of amino acids transport, changes in digesta fatty acids content and pH. Dietary alteration of a particular amino acid may influence velocity and rate of its transport and structurally related amino acids as well as the transport of other nutrients. Similarly, variable concentrations of carbohydrates, fatty acids, hydrogen or sodium ions dramatically alter cell energetics impacting on transport and storage rate of many key macronutrients and the rate of synthesis of transport molecules (Kilberg *et al*., 1993).

The food composition, particularly carbohydrate content, influences the make up and growth rates of gut microbial communities, consequently leading to constant fluctuation in quality and quantity of nutrients, particularly hydrogen and sodium ions, amino acids, sugars and short chain fatty acids (Savage, 1986).

To maintain its function, the enterocyte is constantly monitoring the biochemistry of its environment and reacting to external changes. These responses exert great thermodynamic demand, making the enterocyte one of the most energetically demanding cells in the animal's body (Thompson, 1992). Therefore, the optimal energy source for the enterocyte is critical for its proper function, which in turn may determine optimal growth and health of the whole organism.

IV. INDUSTRY PERSPECTIVES

The thermodynamic state of the enterocyte determines the level of its primary function of transport and further catabolism of food. Uptake kinetics of most intestinal nutrients, including essential amino acids, is ultimately regulated by the energetic state of the cell. Up to 80% of the energy of the enterocyte is spent on powering its own physiology (Newsholme and Start, 1977).

Much progress has been made in our understanding of the mechanisms and regulation of intestinal transport, but less attention has been paid to the total thermodynamic state of the intestinal epithelium. Future research should attempt to define energy utilisation and the

optimal proportions of the three groups of energy supplying compounds, namely fatty acids, protein and carbohydrates during the diet-influenced thermodynamic states of the enterocyte. The kinetics of the nutrient uptake, its regulation and interaction with other transport processes should be viewed in the overall energetic balance of the cell. Such an approach may allow the elucidation of optimal thermodynamic states of the cell under particular conditions such as the presence or absence of antibiotic growth promoters. This in turn will provide a fuller appreciation of the bioenergetics of food digestion and absorption that will point to strategies to optimise intestinal epithelial function for improved feed utilisation and reduction in faecal losses.

In conclusion, a bioenergetically balanced diet directed at energy savings at the cellular level will also lead to additional energy savings at the level of the whole organism through a reduction in the total amount of food to be processed, an increase in the availability of energy for growth and development, reduced maintenance and repair costs and lowered faecal output. In real terms, taking novel approaches to understanding the digestive physiology and metabolism of poultry can lead to practical ways of reducing the costs of production of chicken meat and eggs. .

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PERFORMANCE, DIGESTIVE TRACT MEASUREMENTS AND GUT MORPHOLOGY IN BROILER CHICKENS FED DIETS CONTAINING MAIZE, WHEAT OR SORGHUM

D.V.THOMAS[1], V. RAVINDRAN[1] and D.G. THOMAS[1]

Summary

The performance of broiler chickens fed diets containing viscous grains (wheat) is generally poorer than those fed diets containing non-viscous grains (maize and sorghum) and the hypothesis that these differences in performance are related, partly, to differences in in relative digestive tract size and intestinal morphology was tested in this study. It was found that the weight gain was greater (P<0.05) in the birds fed the maize diet than those fed the sorghum diet. Weight gain in birds fed the wheat diet was not significantly different (P>0.05) from either the maize or the sorghum diet. There were no significant differences (P>0.05) in feed intake between any of the diets. Although the feed per gain was numerically lower for birds fed the maize diet, the difference was not significant (P>0.05). The type of cereal base had no effects (P>0.05) on the relative weights of the proventriculus, gizzard, liver, pancreas and small intestine. No significant differences (P>0.05) were measured in any of the gut architecture parameters measured.

I. RESULTS AND DISCUSSION

The performance of broiler chickens fed diets containing viscous grains (wheat) is generally poorer than those fed diets containing non-viscous grains (maize and sorghum) and the hypothesis that these differences in performance are related, partly, to differences in in relative digestive tract size and intestinal morphology was tested in this study. Three experimental diets containing wheat, maize and sorghum as the cereal base were formulated. All three diets were formulated to contain similar levels of metabolisable energy and amino acids. The ingredient composition of the diets is shown in the table below.

	Wheat diet	Sorghum diet	Maize diet
Wheat	65.76	-	-
Sorghum	-	61.08	-
Maize	-	-	58.62
Soybean meal	27.34	32.19	35.18
Vegetable oil	2.73	2.09	1.78
Dicalcium phosphate	2.03	1.91	2.17
Limestone	0.34	0.94	0.78
Lysine.HCl	0.34	0.29	0.18
DL methionine	0.24	0.33	0.25
Threonine	0.12	0.08	0.03
Sodium bicarbonate	0.36	0.33	0.18
Salt	0.14	0.16	0.23
Vitamin/mineral premixes	0.60	0.60	0.60

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The wheat-based diet was supplemented with a xylanase (Kemzyme®, Kemin [Asia] Pte Ltd, Singapore). All diets were steam pelleted at 60 ^{0}C. Each diet was fed to six replicate groups (8 birds/replicate) from days 1 to 14 post-hatching. Body weights and feed intake were recorded at weekly intervals, and the feed per gain was calculated on a pen basis. On day 14, the weights of digestive organs and tract were obtained from two birds per replicate pen. Approximately 5 cm lengths of jejunum were removed from two more birds per replicate pen for gut morphological measurements.

The influence of dietary treatments on bird performance, digestive tract measurements and gut morphology are shown in the following table. Weight gain was greater (P<0.05) in the birds fed the maize diet than those fed the sorghum diet. Weight gain in birds fed the wheat diet was not significantly different (P>0.05) from either the maize or the sorghum diet. There were no significant differences (P>0.05) in feed intake between any of the diets. Although the feed per gain was numerically lower for birds fed the maize diet, the difference was not significant (P>0.05). The type of cereal base had no effects (P>0.05) on the relative weights of the proventriculus, gizzard, liver, pancreas and small intestine. No significant differences (P>0.05) were measured in any of the gut architecture parameters measured.

	Wheat	Sorghum	Maize	Pooled SEM
Performance[1]				
Weight gain, g/bird	438[ab]	422[b]	463[a]	10.8
Feed intake, g/bird	568	553	588	13.6
Feed: gain, g/g	1.304	1.324	1.277	0.025
Relative weights, g/kg body weight[2]				
Liver	3.39	3.25	3.23	0.14
Pancreas	0.38	0.34	0.34	0.016
Proventriculus	0.63	0.67	0.62	0.040
Gizzard	1.64	1.87	1.69	0.074
Small intestine	4.73	4.68	4.57	0.155
Jejunal morphology[2]				
Villous tip to crypt base (µm)	810	723	812	130
Villous height (µm)	652	586	660	108
Crypt depth (µm)	149	138	150	34
Epithelial thickness (µm)	33	31	34	5

[a,b] Means in the same row with different superscripts are significantly different (P<0.05).
[1] Mean of six replicates (8 birds/ replicate).
[2] Mean of 12 birds.

In conclusion, under the conditions of the present study, the differences in the performance of broilers fed maize, wheat and sorghum-based diets could not be explained by differences in relative size of the digestive tract or gut morphology.

INFLUENCE OF WHOLE WHEAT FEEDING ON THE PERFORMANCE AND DIGESTIVE TRACT DEVELOPMENT OF BROILER CHICKENS

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Summary

The present study was designed to examine the performance and the changes in the relative growth of digestive organs of broilers over a 5-week feeding period when diets containing ground wheat or whole wheat were offered. The results showed that, throughout the 5-week trial, the birds offered feed containing wheat as whole grain grew slower ($P<0.05$) and consumed less ($P<0.05$) feed than those offered feed containing ground wheat. Feed-to-gain ratio was not influenced ($P>0.05$) by the form of wheat during the first two weeks, but was significantly lowered ($P<0.05$) from three weeks onwards. Over the 5-week period, the feed-to-gain ratio of birds fed the whole wheat diet was five points better ($P<0.05$) than those fed the ground wheat diet. Improved feed efficiency from week 3 onwards coincided with significant increases ($P<0.05$) in the relative weights of gizzard in birds fed the whole wheat diet. Whole wheat inclusion had no effect ($P>0.05$) on the relative weights of crop, proventriculus and pancreas or on the relative weight and length of the small intestine.

I. RESULTS AND DISCUSSION

In most studies, the influence of whole wheat inclusion on the digestive organs has been determined at the termination of the study after 3-5 weeks of whole grain feeding (Forbes and Covasa, 1995; Preston et al., 2000; Svihus and Hetland, 2001). The present study was designed to examine the performance and the changes in the relative growth of digestive organs of broilers over a 5-week feeding period when diets containing ground wheat or whole wheat were offered. Diets containing ground wheat and whole wheat (10 and 20% whole wheat replacing ground wheat during 1-21 and 22-35 days, respectively) were fed to four replicate pens (46 birds/pen) each. Both diets were supplemented with exogenous xylanase (Allzyme PT, Alltech, Inc., Nicholasville, KY). Body weights and feed intake were recorded on a pen basis at weekly intervals. On days 7, 14, 21, 28 and 35, two birds (closest to the mean pen weight) were selected from each replicate pen and fasted overnight and the empty weights of the crop, proventriculus and gizzard, and the weights of pancreas and liver were recorded. The length and weights of different segments of small intestine (duodenum, jejunum, ileum) were also recorded. The digestive tract measurements were collected from 12 day-old chicks to obtain the data points on day 1. Weights of the digestive tract and digestive organs were expressed as a proportion of bird weight. The data on weight gain, feed-to-gain ratio and relative gizzard weight are summarized in the following table.

[1] Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.

Week no.	Dietary treatments		Pooled SEM	Probability, P<
	Ground wheat	Whole wheat		
Cumulative weight gain (g/bird)				
1	101	88	1.8	0.01
2	339	306	5.6	0.01
3	786	738	6.4	0.01
4	1343	1233	21.9	0.05
5	1991	1856	15.9	0.001
Cumulative feed-to-gain ratio (g/g)				
1	1.126	1.159	0.022	0.21
2	1.286	1.309	0.018	0.40
3	1.368	1.331	0.012	0.06
4	1.568	1.512	0.016	0.05
5	1.661	1.590	0.014	0.01
Gizzard weight (g/kg body weight)				
1	45.1	47.0	1.12	0.25
2	33.9	31.6	0.98	0.37
3	15.7	22.3	0.66	0.05
4	11.9	15.9	0.61	0.001
5	10.0	15.2	0.51	0.0001

Throughout the 5-week trial, the birds offered feed containing wheat as whole grain grew slower (P<0.05 to 0.001) and consumed less (P<0.05 to 0.001) feed than those offered feed containing ground wheat. Feed-to-gain ratio was not influenced (P>0.05) by the form of wheat during the first two weeks, but was significantly lowered (P<0.06 to 0.01) from three weeks onwards. Over the 5-week period, the feed-to-gain ratio of birds fed the whole wheat diet was seven points better (P<0.01) than those fed the ground wheat diet. Improved feed efficiency from week 3 onwards coincided with significant increases (P<0.05 to 0.0001) in the relative weights of gizzard in birds fed the whole wheat diet. Whole wheat inclusion had no effect (P>0.05) on the relative weights of crop, proventriculus and pancreas or on the relative weight and length of the small intestine.

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GROWTH RATE OF BROILER CHICKENS GIVEN CONDENSED TANNINS EXTRACTED FROM GRAPE SEED

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Summary

Broiler chickens were fed nutritionally adequate diets supplemented with condensed tannins (CT) extracted from grape seed by a proprietary process. Dose rates of CT were 0, 2, 5, 10 and 30 g/kg. Growth rate, feed efficiency and mortality were not significantly affected by inclusion of CT 10 g/kg or by the dietary addition of antibiotic (Tylosin) in the positive control diet. The reduced feed intake, but no change in feed efficiency, by chickens given CT 30 g/kg suggests that depression of growth rate was mainly due to reduced feed intake rather than to more subtle effects on digestion and absorption of essential nutrients. The antibacterial properties of grape seed CT in concentrations less than 30 g active ingredient/kg feed need to be evaluated.

I. INTRODUCTION

Sustainable production of chicken meat without reliance on antibiotics is an outcome sought by the Australian chicken meat industry (RIRDC, 2004). However, removal of antibiotic growth promoters from the feed is expected to result in reduced feed efficiency, and increased incidence of intestinal disorders due to proliferation of gut pathogens.

Numerous factors can affect susceptibility of chickens to bacterial pathogen colonisation of the gut, including age, stress, general health, feed additives, and genotype (Hafez, 1999). Newly hatched chicks are more susceptible than older ones that have developed resistance through establishment of their own indigenous gut microflora (Stravric *et al*., 1987). Competition for bacterial binding sites in the gut may reduce pathogen colonisation in older chicks (Stravric *et al*., 1987).

Other possible effectors of pathogen colonisation are normal dietary constituents. For example, tannins are plant poly-phenolic compounds that are known to bind protein, forming insoluble protein-tannin complexes, and inhibiting bacterial attachment and growth (Cowan, 1999). However, high dietary concentrations of tannins reduce performance in chickens as well as other livestock (Gualtieri and Rapaccini, 1990; Nyachoti *et al*., 1997). There has been little or no research done to determine whether tannins in the diet influence the commensal bacteria in general, or reduce pathogen colonisation, in particular.

Condensed tannins extracted from grape seed may have potential as an alternative to antibiotic growth promotants in commercial broiler diets. However, a necessary first step is to assess the effect of CT on growth of broiler chickens. The experimental work described in this report examines the effects of dietary concentrations CT from grape seed on feed intake, growth rate and feed efficiency of broiler chickens during a 42-day growth period.

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II. MATERIALS AND METHODS

Cobb 500 male broiler chickens were housed in experimental rearing pens. Each of the four pens was partitioned into six sub-sections to provide a total of 24 experimental plots. A total of 10 chickens was allocated to each plot. Each chicken was eye-drop vaccinated with Eimeriavax 4m to provide protection from coccidiosis without the need to use chemical feed additives, which may have otherwise confounded the results. Chickens were weighed at placement and weekly for six weeks. Feed intake was also measured weekly in each plot.

Six experimental treatments used in this study were:- negative control (no antibiotic used), positive control (Tylosin 15 ppm active ingredient), and CT at 2, 5, 10 and 30 g/kg. The CT product was extracted from grape seed by a proprietary process (Tarac Technologies Pty Ltd). Tylosin was chosen, as it was known by the researchers to be effective in preventing necrotic enteritis, which had occurred previously in these experimental facilities, despite use of conventional antibiotic growth promotants in experimental diets. Antibiotic product and condensed tannin extract were added to the diets by substitution with millrun or wheat in the starter, grower and finisher diets (Table 1), which were formulated to meet the nutrient requirements of this breed of chicken. Starter feed was cold-pressed into pellets measuring approximately 6mm diameter and 4-6 mm in length. Grower and finisher feeds were fed as mash. Each type of diet formulation was fed for two weeks. The six experimental treatments were fed to four replicate groups of 10 chickens each, in a randomised block design.

Table 1. Composition (in g/kg) of starter, grower and finisher basal diets.

Ingredient	Starter	Grower	Finisher
Wheat	69.00	68.50	71.3
Peas	5.00	7.50	13.50
Soybean meal (48%)	10.00	10.00	2.00
Full fat soybean meal	2.00	2.00	2.00
Meat and bone meal (50%)	10.00	7.50	7.50
Blood meal (85%)	2.50	2.50	2.50
Canola oil	0.50	0.50	
Rock phosphate (Palfos)		0.20	
Limestone	0.20	0.65	0.55
Sodium chloride	0.11	0.24	0.12
Sodium bicarbonate	0.05		0.10
Lysine HCl	0.20	0.06	0.03
DL-methionine	0.23	0.20	0.13
Choline chloride (60%)	0.04	0.04	0.09
Premix	0.15	0.15	0.15
Glycanase enzyme product	0.05	0.05	0.05

III. RESULTS

Results for live weight and cumulative feed intake are summarised in Figures 1 and 2. Feed conversion was not affected ($P>0.05$) by dietary treatment at any stage in the 6-week experiment. Mean feed conversion values were 1.38, 1.56, 1.61, 1.66, 1.76 and 1.83 after 1 to 6 weeks, respectively.

There were no significant differences ($P>0.05$) due to diet on feed intake or live weight in the first week of the 6-week experiment. At the end of week 2, there was no significant effect ($P>0.05$) of diet on feed intake, but live weight of chickens given CT 30

g/kg was significantly lower (P<0.05) than all other dietary treatments except CT 2 g/kg. By the end of week 3, the cumulative feed intake of chickens given CT 30 g/kg was significantly lower (P<0.05) than all other treatments that were not different (P>0.05) from each other (Figure 1). Live weight was also significantly lower (P<0.05) due to CT 30 g/kg compared with other treatments. Feed intake in two of the four pens of chickens given CT 30 g/kg seemed more severely affected, hence it was decided to remove these pens from the experiment at this point. The two remaining pens given CT 30 g/kg ate less feed, and gained weight at a slower rate than either the negative or positive controls for the remainder of the experiment. There were no significant differences (P>0.05) between the negative and positive control groups in feed intake or live weight at any stage of the experiment.

Losses due to mortality and culls amounted to 10 out of 240 chickens (4.2%) during the 6-week experiment. Seven chickens died or were culled in the first two weeks. All dietary treatments, with the exception of the positive control containing antibiotic product, lost one or more chickens. Specific losses were; one in the negative control, one in CT 2 g/kg, three in CT 5 g/kg, one in CT 10 g/kg, and four in CT 30 g/kg.

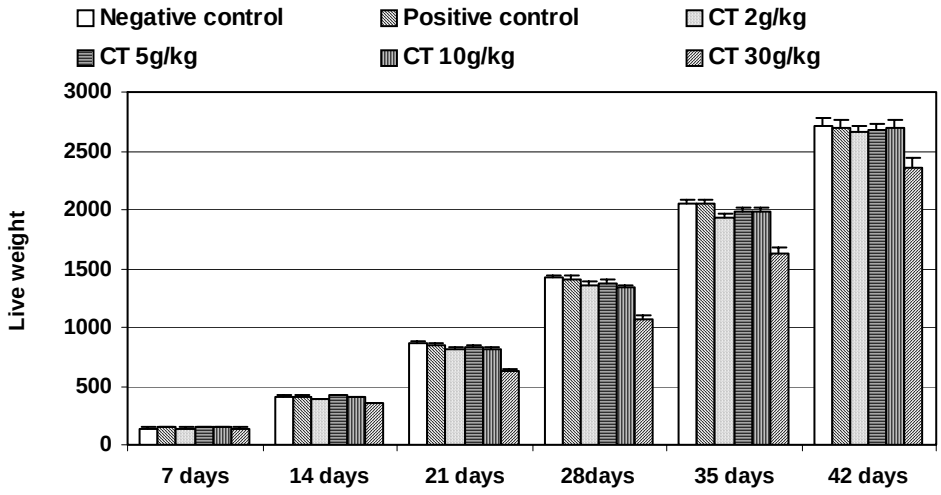

Figure 1. Effects of dietary treatments (CT is abbreviation for condensed tannins) and growth period on live weight (g/bird). Least squares means ± standard errors.

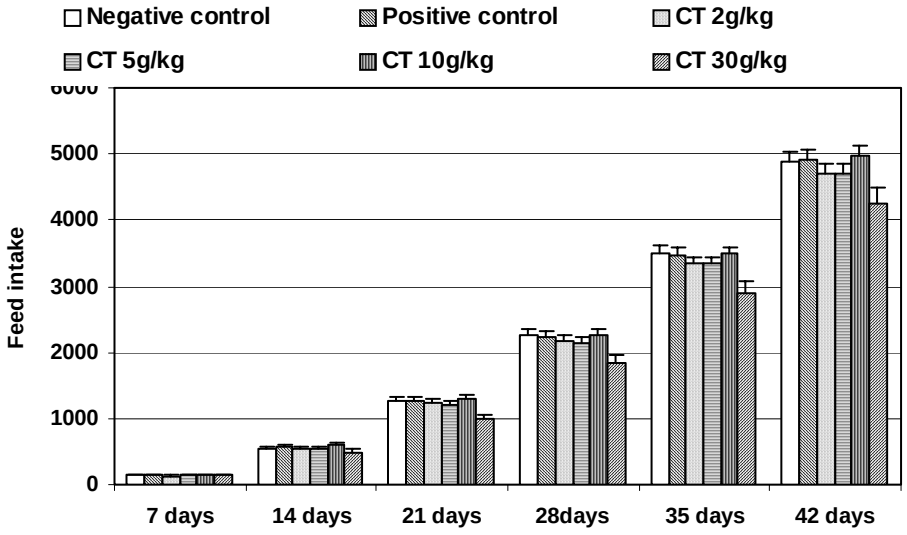

Figure 2. Effects of dietary treatments (CT is abbreviation for condensed tannins) and growth period on cumulative feed intake (g/bird). Least squares means ± standard errors.

IV. DISCUSSION

The highest dietary inclusion rate (30 g/kg) of condensed tannins extracted from grape seed severely depressed feed intake and growth of broiler chickens. The detrimental effects were noticeable within three weeks of feeding this experimental diet. Lower dietary levels of condensed tannins (CT 2, 5 and 10 g/kg) had no observable detrimental effects. It was evident at three weeks that two of the four pens of chickens given CT 30 g/kg were particularly affected. These chickens were removed from the experiment at this point and relocated to a floor pen in another room. Within a few days of eating normal commercial broiler feed, the appearance of these chickens improved noticeably and they seemed to undergo a period of compensatory weight gain. The appearance of the chickens in the other two pens given CT 30 g/kg was comparable with chickens in other treatment groups; hence it was decided to allow these pens to continue in the experiment.

The lack of difference between the negative and positive control groups suggests that the dietary addition of antibiotic product provided no detectable advantage in this particular experiment. This tends to suggest that any anti-microbial effects of condensed tannins were unlikely to be demonstrable under these benign conditions. On the other hand, dietary CT concentrations of 10 g/kg or less were not detrimental to growth and feed efficiency.

Losses due to mortality and culls (4.2%) were typical for this breed from the hatchery concerned, judging on similar patterns of losses in previous and subsequent batches reared at PPPI. The tendency for increased losses in diets as dietary concentration of CT rose suggests that CT may have imposed additional stress on less than healthy chickens. This seems most likely to be due to reduced voluntary feed intake associated with CT. Further work is needed in this area. It would also be desirable to determine whether dietary addition of CT is required after about 2-3 weeks of feeding after placement of day-old chicks.

V. CONCLUSIONS

A dietary inclusion rate of CT 10 g/kg extracted from grape seed did not impair growth performance of commercial broiler chickens. Voluntary feed intake was significantly reduced, and growth rate was retarded, when chickens were given CT 30 g/kg.

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EVALUATION OF A PROBIOTIC PRODUCT ON CECAL COLONIZATION AND ORGAN INVASION OF SALMONELLA ENTERITIDIS IN BROILERS

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Summary

The use of Antibiotics in animal production has been declining worldwide throughout the years. Consumer pressures continue to be the cause of this decline in both EU and Non-EU countries. Recent studies suggest that some natural alternatives offer good efficacy towards the reduction of foodborne pathogenic bacteria. One such alternative is the use of probiotics which speed the development of a protective intestinal microfloral which is ultimately essential for protecting against food poisoning salmonellas and other disease causing organisms. With the rise of food/health crisis the use of competitive exclusion strategy is becoming more accepted among farm producers.
This review describes evidence in which a particular probiotic product decreased colonization of *Salmonella enteritidis* in broilers. The data obtained from this experiment also emphasizes that such a strategy may be a possible alternative not only to reduce avian diseases but also to limit the use of antibiotics in animal husbandry.

I. INTRODUCTION

Antibiotics (Abs) are very powerful and effective tools in preventing pathogenic bacteria from causing infection. For many years it was accepted that animals could be given Abs to optimize growth. However due to political and consumer concerns this act is undergoing extreme scrutiny. For example, recent legislation within the European Union has banned certain antibiotics (EC Council Regulation, 1999). Now many researchers and companies alike are searching for alternatives to antibiotics as methods of disease prevention and growth promoters. Cost effective control of avian disease and food borne pathogens remains a high priority for all sectors of the poultry industry with cleansing and disinfection, vaccination and competitive exclusion (CE) approaches used widely (Ragione *et al*., 2003). Although practicing good management on the farm has shown to reduce pathogens such as *Salmonella* there are other methods to control pathogenic bacteria. Probiotic supplementation has shown great promise in protecting broilers against pathogens by colonization in the gastrointestinal tract (GI) (Nisbet *et al*., 1993).

One of the most common enteric infections worldwide is caused from salmonella species. In the last few years, massive foodborne illness outbreaks have made us aware of the risks involved in our world food supply. Most infections are traced back to the consumption of contaminated foods of animal origin, like beef, poultry and other meat products including eggs.

The intestinal microflora of animals is the first barrier in protecting the host from diseases caused by colonization of pathogens in the gastrointestinal tract (Huang *et al.*, 2003). In modern commercial conditions the chick is hatched in relatively clean conditions thus only having indirect contact with the mother hen. Most of the chicks gut flora starts to develop from the incubator environment and their food. Exploitation of the CE effect is now an accepted part of the overall strategy by which poultry-associated salmonellas are being controlled in some countries (Mead, 2000). From a commercial standpoint it is important that the method used is practical and that it provides that all birds receive a proper treatment.

[1] Biomin IAN
[2] Biomin Erber

A proper method of CE administration is through spray application, which was first developed by Goren *et al*. (1984, 1988), where chicks were treated in the delivery boxes, prior to leaving the hatchery. Later Blankenship and co-workers (1993) combined spraying in the hatchers and providing treatment also in the drinking water upon arrival of the farm. Based on these facts Biomin developed a product combining not only the essential components that are needed to combat with pathogenic bacteria, but also employed this application strategy. Both of which have been studied for many years. Biomin®C-EX and Biomin®IMBO is a probiotic and prebiotic product that can be administered to young chicks in order to competitively inhibit pathogenic salmonella bacteria in the intestinal tract of livestock thus improving food safety. The composition of Biomin®C-EX and IMBO product line is comprised of specific natural agents such as probiotics, prebiotics, and immunstimulating substances.

With the concerns of salmonellosis on the rise an experiment was conducted to confirm the effectiveness of C-EX and IMBO in reducing Salmonella cecal colonization and organ invasion in 49-day-old broilers as well as body weight and weight gain were recorded. Also measured was the concentration of volatile fatty acids (VFA) and lactic acid as well as the pH in the GI-tract were recorded. In this trial, which was conducted at the Universidad Nacional Autónoma de México the results proved to be significant.

Four hundred and fifty day-old chicks were weighed, divided into 6 groups, randomly allocated and reared in cages at isolation units until the age of 49 days. Food and water were given *ad libitum*. Twenty-four hours after oral application of tested products the chicks were challenged with *Salmonella enteritidis*, orally inoculated with 0.25 ml of *S. enteritidis*, which contained $4x10^5$ CFU/ml. The groups and treatments consisted of the following:

Group 1: Control, inoculated with *S. enteritidis*
Group 2: Positive Control (Avi-xy), inoculated with *S. enteritidis*
Group 3: Biomin® C-EX, inoculated with *S. enteritidis*
Group 4: Biomin® IMBO, inoculated with *S. enteritidis*
Group 5: Biomin® C-EX and IMBO, inoculated with *S. enteritidis*
Group 6: Negative Control, not inoculated

Although there were no significant differences ($P>0.05$) on body weights in experimental groups the applications of all three Biomin products, especially the combination of C-EX and IMBO resulted in higher live weights at the end of fattening period compared to controls. pH of cecal contents decreased significantly in groups given Biomin® C-EX, Biomin® IMBO and the combination of both compared with control groups at day 7, 14 and 21. There were no significant differences on the Avi-xy group. The number of chicks with *S. enteritidis* positive organ cultures was significantly reduced compared to control-chicks throughout the whole evaluation period. The combined application of Biomin® C-EX and IMBO resulted in a total reduction of *S. enteritidis* positive organ cultures already at day 21. In general it can be noticed that levels of organ invasion by *S. enteritidis* were much lower compared to Avi-xy when given Biomin® C-EX and IMBO combination. Furthermore the effect of tested products on organ invasion of *S. enteritidis* in broiler chicken; values show number of positive liver and spleen per total of chicken were significantly different from positive control. The data clearly showed that the combination of C-EX and IMBO had reduced the number of *S. enteritidis* samples in liver and spleen at days 7, 21, and day 35. The trial groups that received C-EX and IMBO had a better result when compared to the other 4 experimental groups. All three Biomin products could reduce the concentration of *S. enteritidis* distinctively until day 21. With this data it can be stated that the potential of such a strategy has a great benefit in animal production and could ultimately help safeguard the health of consumers.

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EFFECT OF DIETARY MANNANOLIGOSACCHARIDE LEVEL ON PERFORMANCE AND GROSS MORPHOLOGY OF DIGESTIVE TRACT SEGMENTS OF BROILER CHICKENS

Y. YANG[1], M. CHOCT[1] and P.A. IJI[2]

Summary

The effects of different levels (0.5g/kg, 1g/kg, 2g/kg and a step-down programme) of a mannanoligosaccharide product (MOS) on growth and the gross morphology of gastrointestinal tract of broilers fed a corn-wheat-soy diet were investigated in comparison to a negative control and a positive control (Zn-Bacitracin). The inclusion of MOS in broiler diets improved both weight gain and FCR in the first three weeks but only FCR in the final three weeks compared with controls. MOS did not affect growth performance in a dose-responsive manner. The average weight gain and FCR of birds fed the diet containing 2g/kg MOS group were comparable to the positive control group. MOS addition tended to affect the liver relative weight and the relative length of the small intestine.

I. INTRODUCTION

The mannanoligosaccharide product MOS is a mannose-based complex carbohydrate derived from the outer cell wall of *Saccharomyces cerevisiae*, and has been proposed as an alternative to antibiotic growth promoters. MOS is believed to block type-1 fimbriae thus preventing pathogens from attaching to the intestinal lining (Dawson and Pirulescu, 1999). MOS supplementation can help the gut microflora of younger birds reach gut microbial balance quickly and therefore improve gut health and growth performance. Significant reduction in both *Salmonella* and pathogenic *E. coli* in the caeca (Spring, *et al*., 1995, 2000; Fernandez and Van Gils, 2000) and reduction in *E. coli* populations in the jejunum (Jamroz*, et al*., 2003) were noticed with *Salmonella* or *E. coli* challenged/unchallenged birds. Hooge (2003) reviewed the research results of MOS from 25 pen trials and showed that MOS increased body weight by 1.7%, reduced feed conversion ratio (FCR) by 2.3%, and decreased mortality by 18%, compared with negative controls. Furthermore, MOS lowered mortality by 18% relative to the positive control (antibiotic diets). In the present study, the effects of dietary MOS level on the growth performance and gross morphology of the gastrointestinal tract (GIT) were investigated.

II. MATERIALS AND METHODS

The treatment groups were: 1) a negative control (NC); 2) 0.5g/kg MOS (Bio-Mos®, Alltech, Australia); 3) 1g/kg MOS; 4) 2g/kg MOS; 5) Step-down MOS (2 g/kg MOS 1-7d, 1g/kg MOS 7-21d, and 0.5g/kg MOS 21-42 d, SD MOS); and 6) a positive control (Zinc Bacitracin 50ppm 1-21 d, 30ppm 21-42d, ZB). A total of 384 male broiler chickens (Cobb) were purchased from a local hatchery, weighed and randomly assigned into 8 replicates of 8 chickens per treatment. Pelleted basal diets were formulated with corn, wheat and soybean meal in a 2-phase feeding programme with the starter feed (12.54 MJ/kg metabolisable energy, ME and 22% crude protein, CP) provided from 0-3 weeks of age and the finisher feed

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(12.73 MJ/kg ME and 20% CP) from 3-6 weeks. A commercial xylanase (1g/kg Allzyme^{TM} PT, Alltech, Australia) were supplemented in the basal diet. All the birds were fed *ad libitum* throughout the experiment. Body weight and feed intake (FI) were recorded weekly. Body weight gain (BWG) and FCR were determined weekly and corrected for mortality (Feed intake was corrected for mortality, prior to the calculation of FCR).

At 7, 21 and 42 days of age, one bird per replicate (8 birds per treatment) was killed by cervical dislocation, GIT excised and the gross morphology of the GIT recorded. The proventriculus and gizzard were emptied and weighed. The small intestine was divided into three segments: duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the caecal junction). Each part was emptied by gentle pressure and the weight and length were recorded, as was the body weight of the bird. The weights of pancreas, liver, spleen, and bursa were also measured.

The software package, SPSS, was used for statistical analyses in this study. Data were analysed according to the GLM procedure for ANOVA. The differences between mean values were identified by the least significant difference (LSD). Differences between treatments were deemed to be significant only if the P-value was ≤ 0.05.

III. RESULTS AND DISCUSSION

The values for feed intake, weight gain, and FCR are given in Table 1. The addition of 2g/kg MOS to the diet improved both weight gain and FCR in the first three weeks and only FCR in the final three weeks compared to the negative control. In comparison with the positive control, some MOS treatments showed comparable weight gain and FCR in the first three weeks but not in the final three weeks. This information supports the findings that the addition of MOS to diets can improve the growth performance of broiler chickens (Macdonald, 1995; Kumprecht and Zobač, 1997; Ten Doeschate and Kenyon, 1999; Iji, *et al.*, 2001; Hooge, 2003; Jamroz*, et al*., 2003; Kocher, *et al.*, 2004). Kumprecht and Zobač (1997) reported the optimum dose of MOS for body weight gain was around 1.5 to 3.0g/kg feed. We also found that the dosage of 2g/kg MOS resulted in better growth and feed efficiency which were comparable to the positive control during the whole experimental period. However, we did not observe any dosage-dependent relationship between the level of MOS in the diet and the growth performance of birds.

Table 1. Effects of dietary treatments on performance of broiler chickens

Treatment	0 - 21 d			21 - 42 d		
	FI (g/bird)	BWG (g/bird)	FCR (g/g)	FI (g/bird)	BWG (g/bird)	FCR (g/g)
NC	1160	826^{a}	1.405^{a}	3465	1936^{ab}	1.793
0.5g/kg MOS	1208	872^{ab}	1.386^{ab}	3437	1918^{ab}	1.792
1g/kg MOS	1167	835^{ab}	1.399^{ab}	3439	1923^{ab}	1.791
2g/kg MOS	1203	877^{b}	1.374^{b}	3405	1910^{ab}	1.783
SD MOS	1141	836^{ab}	1.366^{b}	3367	1894^{a}	1.779
ZB	1169	847^{ab}	1.380^{ab}	3482	1995^{b}	1.746
SED	9.9	7.2	0.006	22.7	14.3	0.007

^{a,b}Means within the same column with no common superscript differ significantly (P<0.05).

Supplementation with MOS improved feed efficiency ($P<0.05$), but significance was lost at 42 d ($P>0.05$), which is consistent with the findings of Jamroz *et al.* (2003). The improvement in the growth performance of broiler chicks may be related to the less balanced gut microflora in young than older birds. Ochi *et al*. (1964) found that the adult caecal flora, consisting mainly of strict anaerobes, took up to 30 d to become established by which time bifidobacteria and bacteroides predominate.

Iji *et al.* (2001) reported that different dosages of MOS (1, 3, 5g/kg diet) did not affect the weight of visceral organs (proventriculus/gizzard, pancreas, small intestine) of chicks fed on a sorghum/lupin-based diet between 7 and 28 days of age. In agreement with their findings, we found MOS treatments had no effect on the relative weights of the visceral organs mentioned above as well as spleen and bursa in the present experiment. However, we observed that 0.5g/kg MOS and SD MOS group showed less ($P<0.05$) relative weight of liver compared to the negative control at 7 days of age (Table 2). Also, MOS supplementation affected the relative lengths of the small intestine, especially, when birds were at 6 weeks of age (Table 2) but had no overall effect on the GI tract weight compared with controls. The effects of MOS on GIT development are not well elucidated and further research is needed to confirm the current findings as well as to explore the effects of MOS on gastrointestinal morphology and microflora ecology in young birds.

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Table 2. Effects of diet treatments on the relative weight and relative length of gastrointestinal organs of broiler chickens 7, 21 and 42 days of age

Treatment	Relative weight (%BW)									Relative length(cm/%BW)		
	Proven.[1]	Gizzard	Pan.[2]	Liver	Spleen	Bursa	Duo.[3]	Jejunum	Ileum	Duo.[3]	Jejunum	Ileum
						Day 7						
NC	1.09	4.52	0.53	5.19^{a}	0.10	0.15	2.09	2.76	1.70	11.30	26.23	25.15
0.5g/kg MOS	1.04	4.00	0.45	4.32^{b}	0.10	0.20	2.05	2.54	1.75	11.26	25.43	23.10
1g/kg MOS	0.97	4.23	0.52	4.51^{ab}	0.10	0.19	2.06	2.84	1.63	11.66	26.82	25.37
2g/kg MOS	0.97	4.02	0.48	4.43^{ab}	0.10	0.16	2.03	2.80	1.64	10.72	25.90	24.91
SD MOS	1.09	4.11	0.53	4.24^{b}	0.09	0.19	2.14	2.74	1.81	11.60	27.76	26.75
ZB	1.01	4.17	0.52	4.67^{ab}	0.09	0.16	2.08	2.64	1.66	11.67	25.83	24.00
SED	0.017	0.058	0.013	0.083	0.003	0.006	0.027	0.041	0.033	0.202	0.482	0.552
						Day 21						
NC	0.45	2.03	0.25	3.01	0.09	0.23	0.88	1.50	0.93	2.79^{ab}	6.57	5.71
0.5g/kg MOS	0.46	2.07	0.25	2.94	0.10	0.23	0.90	1.44	0.95	2.98^{ab}	6.70	6.18
1g/kg MOS	0.46	2.16	0.27	3.09	0.09	0.26	0.91	1.44	0.96	2.86^{ab}	6.73	6.27
2g/kg MOS	0.45	2.12	0.26	3.06	0.10	0.26	0.95	1.40	0.94	2.76^{ab}	6.23	5.77
SD MOS	0.43	2.10	0.26	2.88	0.08	0.24	0.88	1.45	0.92	2.66^{a}	6.48	5.95
ZB	0.43	2.05	0.24	2.82	0.09	0.22	0.79	1.33	0.82	3.04^{b}	6.64	6.00
SED	0.008	0.031	0.005	0.062	0.003	0.008	0.015	0.027	0.017	0.049	0.108	0.102
						Day 42						
NC	0.34	1.74	0.19	2.45	0.11	0.16	0.54	0.91	0.66	1.16^{b}	2.60^{ab}	2.62^{bd}
0.5g/kg MOS	0.35	1.66	0.17	2.36	0.11	0.16	0.51	0.89	0.66	1.17^{b}	2.65^{ab}	2.67^{bc}
1g/kg MOS	0.34	1.76	0.17	2.43	0.12	0.16	0.48	0.87	0.67	1.06^{a}	2.62^{ab}	2.35^{a}
2g/kg MOS	0.37	1.77	0.17	2.56	0.12	0.2	0.49	0.96	0.68	1.08^{ab}	2.72^{a}	2.70^{b}
SD MOS	0.32	1.68	0.19	2.31	0.12	0.16	0.46	0.89	0.69	1.08^{ab}	2.51^{b}	2.50^{acd}
ZB	0.31	1.73	0.19	2.32	0.12	0.16	0.53	0.9	0.65	1.17^{b}	2.58^{ab}	2.46^{ad}
SED	0.008	0.031	0.004	0.045	0.005	0.007	0.01	0.016	0.011	0.015	0.03	0.032

[a,b]Means within the same column with no common superscript differ significantly ($P<0.05$).

[1] Proven.: Proventriculus. [2] Pan.: Pancreas. [3]Duo.: Duodenum.

MANAGING THE RISK OF MYCOTOXINS IN POULTRY PRODUCTION

E. BINDER[1] and E.E. ERBER[1]

Summary

Mycotoxins are invisible, odourless and cannot be detected through smell or taste. Due to the complex nature of these naturally occurring fungal contaminants and the elaborate requirements for their analysis a risk management concept has to be installed in order to reduce the risk encountered to a defined and acceptable level. While the pre- or post-harvest prevention of mycotoxin contamination is the preferred strategy for minimising their risk in food and feed, the early identification of mycotoxin-contaminated grains provide the opportunity to direct the moulds most highly contaminated grains into uses by less sensitive species or to take measures to counteract these toxins in specific and proper ways.

I. INTRODUCTION

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by higher animals. *Fusarium, Aspergillus,* and *Penicillium* are the most abundant that produce these toxins and contaminate human foods and animal feeds through fungal growth prior to and during harvest or as a consequence of improper storage following harvest (Bhatnagar *et al*., 2004). Due to modern methods and thanks to a growing interest in this field of research more than 300 different mycotoxins can be differentiated today, although only a limited number is of relevance in animal husbandry.

II. IMPACTS OF MYCTOXINS

The most common mycotoxins known are the aflatoxins due to the fact that they represent some of the most potential carcinogenic substances known so far, and as they are the main hepatocarcinogen in animals, although effects vary with species, age, sex, and general nutrition conditions. Fatty liver or pale bird syndrome and inhomogeneous flocks are the most typical symptoms for such a contamination in feed.

Trichothecenes are a large group of mycotoxins produced by various species of moulds, in particular, those belonging to the genus of *Fusarium*. Approximately 170 trichothecene mycotoxins have been identified up to date, with deoxynivalenol (DON, vomitoxin), nivalenol (NIV), 3- or 15-acetyl-deoxynivalenol (AcDON), and Fusarenon X (FUS-X), as well as T-2 toxin and HT-2 toxin as the most prevalently occurring toxins of this group. An important issue is that some of these closely related compounds occur frequently simultaneously (Fuchs *et al.*, 2004) and are proven to cause synergistic effects (Weidenbörner, 2001). Different types of trichothecenes vary in their toxicity though all of them are acutely toxic. They may cause haematological changes and immune suppression, reduced feed intake and skin irritations as well as diarrhoea and haemorrhages of internal tissues.

Zearalenone is also produced by *Fusarium* species and has strong hyperoestrogenic effects leading to impaired fertility in general.

Ochratoxin A (OTA), which is produced by *Aspergillus* and *Penicillium* species and causes renal toxicity, nephropathy and immune-suppression in several animal species, which entails reduced performance parameters. As it occurs in many commodities and there is a

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certain carry-over B toxins or their metabolites into products of animal origin human intake in some countries can be high.

The most recently described mycotoxins with relevance in human and animal nutrition are fumonisins, which were first reported in South Africa in 1988 and also *Fusarium* mycotoxins. Besides their hepatotoxicity and nephrotoxicity they affect also the immune system.

III. MYCOTOXIN ANALYSIS

Testing for mycotoxins is a complicated process that generally consists of three steps:

(1) Sampling – i.e., selecting a representative sample of a given size from a bulk lot
(2) Sample preparation - comprises the grinding of the sample and taking a representative sub-sample of ground material
(3) The analytical procedure - consists of several processes where the mycotoxin is solvent extracted from the sub-sample, the solvent is purified and the mycotoxin in the solvent is quantified. The mycotoxin value, measured in the analytical step is then used to estimate the lot concentration or is compared to a maximum limit in order to classify the lot as acceptable or unacceptable.

Analytical procedures for the determination of mycotoxins have improved continuously over the past years. Chromatographic methods have been used widely, including thin-layer chromatography (TLC), gas chromatography (GC) as well as high-performance liquid chromatography (HPLC), using a large variety of detector technologies.

The most commonly used system for rapid testing is ELISA (Enzyme Linked Immunosorbent Assay) since it is the fastest and most cost effective system, in case of high sample throughput and quick result requirements. It can also come in qualitative formats like small cups, where an enzymatic color reaction visualizes within five minutes the presence or absence of a toxin at a certain cut-off level, but does not indicate an exact contamination level.

III. MYCOTOXIN PREVENTION

Management practices to maximize plant performance and decrease plant stress can decrease mycotoxin contamination substantially. This includes planting adapted varieties, proper fertilization, weed control, necessary irrigation, and proper crop rotation. But even the best management strategies cannot eliminate mycotoxin contamination in years favorable for disease development. Some fungi, like several *Fusarium* species, are widespread colonizers of crop residues, where the pathogen survives during winter. Thus wheat stubble, corn stalks and rice stubble can be major sources of these moulds, which produce large inocula as temperatures increase in spring. Airborne release of spores may peak during and after rainy periods, distributing the fungal sources over wide distances, and causing epidemics (CAST, 2003).

High moisture content is a significant factor for mycotoxin infestation, with the final “safe” moisture content depending on the crop and the climatic conditions where the commodity is stored, although drying to 15% moisture content or below is widely recognized as being suitable. It should be mentioned that when conditions are generally favorable for fungal contamination it is not uncommon for more than one type of fungus to be involved. During storage, grain is often colonized by a succession of fungi, depending on temperature and moisture levels. Due to these possible interactions of several fungal species, grain may be contaminated with a number of different mycotoxins (Cast, 2003).

IV. COUNTERACTING MYCOTOXINS

It is important to stress the point, that the use of mould inhibitors or preservation by acids can only reduce the amount moulds but does not influence mycotoxins contamination generated prior to treatment . If mycotoxins have been produced earlier they will not be affected in any form by mould inhibitors or acid mixtures, as they are very stable compounds. Thus these toxic compounds remain in the formerly infected commodities even if no more mould can be seen or detected. Since all mycotoxins are quite stable substances no physical or chemical treatment can be applied under practical field conditions, without altering the nutritive value of the grain or causing high cost implications.

The most commonly used strategy of reducing exposure to mycotoxins is the decrease of their bio-availability by inclusion of various mycotoxin binding agents or adsorbents, which leads to a diminishing of mycotoxin uptake and distribution to the blood and target organs. Various substance groups have been tested and used for this purpose, with aluminium silicates, in particular clay and zeolitic minerals, as the most commonly applied groups. An important criterion for evaluation of mycotoxin adsorbers is their effectiveness at high and low pH levels since a product must work throughout the gastro-intestinal tract, thus within a broad pH range. Since the mode of action has to commence in the stomach it must be effective at least at pH level 3. Another important aspect in the evaluation of potential mycotoxin binders is the stability of the sorbent-aflatoxin bond, in order to prevent desorption of the toxin.

The elimination of other mycotoxins than aflatoxins from contaminated feedstuffs by the use of adsorbents has not lead to any satisfactory results so far, as most of the adsorbing agents bind them only weakly *in vitro* and are more or less ineffective *in vivo*. As it is known that in the case of trichothecenes the 12,13-epoxide ring is responsible for their toxic activity and removal of this epoxide group entails a significant loss of toxicity, research focused on the identification of natural processes where this reaction occurs. Biomin® researchers were the first to isolate a pure bacterial strain which is able to bio-transform the epoxide group of trichothecenes into a diene, thus detoxifying all relevant trichothecene toxins by this reaction.

The active isolate is a new species of the genus *Eubacterium,* named BBSH 797. For its application as feed additive, fermentation and stabilisation processes were established and optimized with respect to good and fast growth of the microbe, high biotransformation activity of the resulting product, and economic reasons. For enhancement of stability during storage and within the gastro-intestinal tract, a three-step encapsulation process was implemented. The additive's efficiency in counteracting adverse effects of feed contaminated with trichothecenes was demonstrated in feeding trials with several animal species (Binder *et al*., 2001). Further research by our group led to the isolation of a yeast strain, *Trichosporon mycotoxinivorans* (MTV), which can decompose and thus detoxify ochratoxin and zearalenone. Both, *in vivo* and *in vitro* trials have shown this additives´ high efficacy to counteract these mycotoxins under practical conditions (Schatzmayr *et al.*, 2004).

Based on the knowledge summarized above and information about the overall contamination levels the following mycotoxin detoxification strategy can be recommended:

For any contamination where aflatoxin occurs as the only contaminant a certified binder should be used for reduction of bioavailability of aflatoxins. The adsorber´s certificate should comprise data about its efficacy, i.e., its guaranteed binding capacity at at least two relevant pH levels (e.g. pH 3 and pH 6.5), as well as the absence of any potential hazard, in particular of dioxin. In case of contamination of feedstuffs with other toxins than aflatoxin, the application of proper alternative technologies, like microbial detoxicants, should be considered.

V. MANAGING MYCOTOXIN CONTAMINATION IN THE DAILY OPERATION

Hazard analysis starts with the preparation of a list of steps in the production process, where mycotoxin or mould infestation could occur, and describes preventive measures, like purchasing of raw materials. Many contracts do not mention mycotoxins at all and it could be an improvement to add a clause with maximum acceptable levels of mycotoxin contamination to the contract. The second step in an HACCP system is to determine the critical control points, i.e., which are the materials, products or production steps that have to be monitored for fungal contaminants. One rule of the thumb could be the ratio of tests conducted on raw materials versus tests done on finished products, which could be - for example – nine to one. A third step would be to establish critical limits, which means to determine the maximum tolerable toxin levels. What is the internal risk profile that is acceptable within an operation? Step number four is the establishment of procedures for monitoring the critical control points. This can include procedures for sampling, sample preparation and testing itself, or the outsourcing of parts of or even the full analytical process. Step five covers the establishment of corrective actions, which would comprise the introduction of certain cleaning procedures for silos, bins, hoppers, and elevators into the maintaining plan, as repeated contamination could originate from bins containing materials like wheat bran that have never been cleaned so that contamination might originate from and spread within the same operation and not only from purchased raw materials, or feed supplementation with detoxyfying products. Step six comprises the verification procedures and step seven the documentation and record keeping.

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BROILER LITTER DISPOSAL - A SUMMARY OF ADVANCES

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Summary

The global poultry industry is undergoing many changes, one being the disposal of its broiler litter (BL) due to reduction in land available for cost effective disposal. To date, Australian BL disposal has been achieved by selling the litter as a fertiliser to agricultural sectors. Land availability has decreased due to encroaching urban development, legislative change, decreasing social acceptance, environmental quality issues, and increasing pathogen concerns. Alternative disposal options have developed significantly overseas and have shown potential to solve many of the issues facing BL disposal in Australia. Commercialisation of composting, vermiculture, anaerobic digestion and direct combustion for large scale BL disposal is still in its infancy for Australian conditions but has already been achieved overseas.

I. INTRODUCTION

Broiler production worldwide like other intensive animal systems generates a large amount of biomass including broiler litter (BL). Annually Australia produces approximately 1.6 million tons of BL. Application of BL directly onto land provides a convenient mechanism for disposal (Sharpe *et al.,* 2004) and acts as both a fertiliser and soil addiment (Ribaudo *et al.,* 2003). In excess of 90% of BL is spread on land usually located close to the grower (Vervoort and Keeler, 1999) negatively affecting biosecurity. However, for some poultry producing regions this practice is becoming less cost effective, due to restrictions on land available and costs associated with moving BL to appropriate land (Jackson *et al.,* 2003; Ribaudo *et al.,* 2003). In Australia these restriction will potentially increase due to encroaching urban development, legislative change, decreasing social acceptance, environmental quality issues (Nash and Halliwell, 1999), and increasing pathogen concerns.

Alternative BL disposal options include composting, vermiculture, anaerobic digestion and direct combustion. Research into the feasibility of these options to provide an alternative disposal mechanism for BL, has received increased interest from potential commercial operators. Currently growers in Australia sell BL, which is predominantly disposed of to land and covers the cost of buying new bedding. However, recently some growers in Queensland have had to pay a small fee for BL disposal (McTavish, K. 2004, personnel communication).

II. COMPOSTING

Composting can be defined as the aerobic microbial breakdown of organic matter and can immobilise nitrogen and phosphorus in organic wastes, reducing the risk of soluble P and N entering aquatic systems (Vervoort *et al.,* 1998; Cooperband *et al.,* 2002; Peigne and Girardin, 2004). Composting has been shown to significantly reduce pathogen concentrations in organic wastes due to the heat produced in the decomposition process (Das *et al.,* 2002; Kelleher *et al.,* 2002). Hatchery waste composting systems have reduce *E. coli* by 99.9%, whilst amending hatchery waste with small percentages of BL enabled the composting process to remove all *Salmonella* (Das *et al.,* 2002).

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Trace elements are used by the poultry industry to improve broiler feed conversion ratios, including Cu Zn and As. Composting BL under the right conditions can degrade arsenic in the 3-nitro-4-hydroxyphenylarsonic and 4-aminobenzenearsenic acid forms to more stable arsenate ions $(AsO_4)^{-3}$, potentially limiting contamination of ground and surface waters with arsenicals (Garbarino *et al.*, 2003; Jackson *et al.*, 2003).

Methane (CH_4) and nitrous oxide (N_2O) are considered significant greenhouse gases due to their efficiency in absorbing infrared radiation, with CH_4 and N_2O absorbing 20 to 200 times more infrared radiation respectively then CO_2 (Sommer and Moller, 2000). Composted and surface applied animal manures have been shown to contribute to greenhouse gas emissions, and potentially contribute to global warming and compounds that contribute to acid rain (Hao *et al.*, 2004; Peigne and Girardin, 2004; Sharpe *et al.*, 2004). Nitrogen losses through NH_3 volatilisation during composting, reduces the agronomic value (Vervoort and Keeler, 1999; Kelleher *et al.*, 2002; Tiquia and Tam, 2002). Commercialisation success of large scale composting in Australia will be determined by the agronomic value of the composted product.

III. VERMICULTURE

Vermiculture can be defined as the non-thermophilic biodegradation and stabilisation of organic materials, by interactions between earthworms and micro-organisms (Arancon *et al.*, 2003). Vermiculture in Australia is receiving increasing attention for its ability to breakdown and value-add to organic wastes and produce worm protein (Edwards and Steele, 1997; Bajsa *et al.*, 2003). Casts, vermicasts and vermi-composts are digested organic remains, mucus, and nitrogenous excretory substances, from the worm's intestinal tract (Tripathi and Bhardwaj, 2003). Using a grinding gizzard, worms produce casts that have a much finer texture than either raw or composted wastes, increasing the commercial value due to its soil like texture and lack of unpleasant odour. The casts in comparison to their organic source have been shown to exhibit: more plant available N, P and K; greater organic carbon; lower C/N ratio; and increases in beneficial microbes, enzymes and hormones (Ndegwa and Thompson, 2000; Atiyeh *et al.*, 2002; Arancon *et al.*, 2003; Bajsa *et al.*, 2003; Tripathi and Bhardwaj, 2003).

Vermiculture systems can be established at minimal costs anywhere in Australia (Smith *et al.*, 1999) and can reduce the volume of organic waste by up to 45% (Ndegwa and Thompson, 2000). A vermiculture system using sewage sludge resulted in 4-fold reductions in pathogens that effect humans, and is considered a safer treatment then direct land application of sludge (Eastman *et al.*, 2001; Bajsa *et al.*, 2003). Trials in the USA have shown that the integration of thermophilic composting before vermiculture achieved almost complete pathogen removal (99.9%), without increasing costs in the vermiculture process (Ndegwa and Thompson, 2000). This level of reduction may not be representative of large commercial operations and no current experiments have determined likely reductions. The integration of vermiculture and aquaculture in India has shown that both casts and worm protein can be used as nutrient source for fish and provide benefits to water quality, reducing filtration and water replacement costs (Ghosh, 2004). Issues that will effect the feasibility of large scale vermiculture operations are variations in cast composition (Edwards and Steele, 1997), commercial agronomic value of casts, and the development of industries that use worm protein.

IV. ANAEROBIC DIGESTION

Anaerobic digestion degrades and stabilises organic materials such as BL, producing potentially saleable methane and digestate (Collins *et al.*, 2000). Methane can be captured and used as a renewable energy source, while the digestate can be utilised as a soil improving agent, with good fertilizer attributes (Salminen and Rintala, 2002). BL has been shown to produce more methane than swine or cattle manure and the digestate has higher available nitrogen levels (Kelleher *et al.*, 2002). Steam pressure exerted on BL before digestion has shown improvements in methane capture by reducing the shield effect of lignin by exposing cellulose for increased bacterial consumption (Liu *et al.*, 2002).

While anaerobic digestion has many benefits, there are several limitations to its adoption. Commercialisation of anaerobic digestion requires the BL to be supplied free, or a tipping fee is introduced, or if the digestate can be marketed as an organic fertilizer (Collins *et al.*, 2000). Extraction of gas does not significantly reduce volume of litter, hence the digestate must represent some economic value otherwise disposal will become an issue (PPRP, 2004). BL contains high concentrations of uric acid (Bujoczek *et al.*, 2000) which during initial anaerobic decomposition stages, forms methanogenesis inhibiting ammonia ions. Excess ammonia ions significantly influence the success of using anaerobic digestion for BL disposal (Kelleher *et al.*, 2002; Salminen and Rintala, 2002).

V. DIRECT COMBUSTION

Direct combustion is recognised as an efficient option for generating renewable energy from organic wastes including BL (Abelha *et al.*, 2003). Currently successful large commercial electricity utilities are operating in the UK and are powered solely on BL (Anonymous, 2000). Combustion achieves seven fold reductions in BL volume and results in an odour free and sterile poultry litter ash, with easier marketability than BL (Codling *et al.*, 2002). With advances in gas cleanup and combustion systems being designed and operated specifically for BL combustion, emissions from these systems have been shown to be well below the limits set by air quality standards (Henihan *et al.*, 2002; Henihan *et al.*, 2003).

Issues concerning the use of BL as a fuel include; availability, reliability, cost, transportation and aggregation (Abelha *et al.*, 2003). Development issues include; electricity value, connection costs, future government policies on emissions and the expansion of existing renewable energy sectors (Kelleher *et al.*, 2002). Public concerns have been raised over the emissions from the combustion of fuels like BL, these polluting emissions are considered a major non-economic determinant in the commercialisation DC (Porteous, 2002).

VI. RECOMMENDATIONS AND FUTURE RESEARCH

For these options to be successfully adopted by the poultry industry in Australia, they need to be economically viable, environmentally sustainable and socially acceptable. Currently the most economically viable option is vermiculture as it can be conducted on-site with no capital costs if a contractor is used. A trial with a broiler grower and contract vermiculture specialist is being developed for a Masters project for the Australian Poultry CRC. The trial will utilise all BL and dead birds over a 6 month period and all vermiculture products will be sold by the contractor on behalf of the grower.

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WORLD'S POULTRY SCIENCE ASSOCIATION (WPSA) 'HIGH SCHOOL POULTRY INDUSTRY EDUCATION PROJECT'

P. KENT[1]

Summary

All animal industries require community acceptance to maintain their position in the global market place. The World's Poultry Industry has a high media profile resulting in constant public scrutiny, where acceptance of poultry products is largely dependent upon community perception of how the industry produces these products. However, industry image can be changed through education.

In order to change these perceptions, in the year 2000, members of Queensland WPSA established a sub-committee consisting of WPSA members and representatives from Queensland Education to develop an industry education project sponsored by the poultry industry and run by the Queensland WPSA Sub-branch.

I. INTRODUCTION

One such project which has been achieving success in the State of Queensland, is the 'WPSA High School Poultry Industry Education project' which promotes advancement and knowledge on all aspects of poultry science and the poultry industry and in turn disseminates knowledge to the consumer.

Information which appears regularly about the poultry industry in Australia's national media is, to say the least, misleading. There is a public perception usually reinforced by people with a high standing in the community that:

- Hormones are fed to poultry
- Antibiotics are used indiscriminately
- Alternative layer production provides a more wholesome product
- Free range is an environmentally sounder production system than cages
- Eggs are linked to an increase in coronary heart disease.

All of these issues impact on the industry through loss of consumer support i.e. sale of product.

II. PROJECT OUTLINE

At the beginning of each school year, the Project Co-ordinator determines a calendar of events and invites all high schools having poultry agricultural facilities to take part. Participating schools are provided with a resource pack containing:

- A schedule of project time lines
- The project guidelines and rules
- Industry information and contacts
- Poultry information which provides students and teachers' reference material suitable as a teaching aid.

Participating schools develop a simple research trial which could be, for example, a comparison between the growth of birds' fed diets containing different levels of protein, where the students will measure the response to growth, consumption and products.

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This simple research trial is conducted during the second school term (6-8 weeks). Students are required to undertake a literature review of the Australian poultry industry, which not only helps support their trial outcome but also provides a unique opportunity to learn about the poultry industry, their products and practices.

To date, the project has been implemented annually by approximately 60% of agricultural schools throughout Queensland, and is being expanded nationally.

Throughout the year's activity, the Project Co-ordinator satisfies individual schools requests by providing specific reference material that supports their research and also arranges school visits to the Queensland Poultry Research and Development Centre to see research in progress.

The school project teams complete a final report (100 marks) based on their trial outcomes and a poster (50 marks) that supports their findings; this part of the project is completed in term three of the school year. Students' final reports are sent to the Project Co-ordinator who arranges an independent panel of WPSA and poultry industry members to assess and mark the reports according to a developed trial evaluation sheet which outlines the expected report structure and necessary criteria which need to be satisfied. The project activity culminates in fourth term when representatives from each school present their posters at an awards' presentation day held annually at the Department of Primary Industries and Fisheries', Queensland, Poultry Research and Development Centre. Throughout the day students, teachers and guests are given a tour of the research facilities; selected industry speakers provide short informative talks on their respective roles within the industry. Certificates and prizes, for first $250, second $150 and third place $100 are presented to the students based on marks received for their reports and posters.

The day provides students who are contemplating a career path in the industry, the unique opportunity to meet and talk to livestock professionals, nutritionists, veterinarians, agricultural scientist and poultry academics etc.

All participating schools and industry sponsors are provided with a certificate acknowledging their participation in the project. The day concludes with an informal chicken and egg barbecue sponsored by Queensland WPSA and the Australian poultry industry.

This project provides the Australian Poultry industry with a couple of unique opportunities, firstly due to the animals size and nature making it easy to research, handle and house (eg. stocking rates) compared to larger animals; and secondly provides an easy vehicle where correct practices/ information can move from 'The industry' to consumers.

The Queensland sub-branch of WPSA sees this project as one means of combating the industries' negative image by providing young Australians with the opportunity to obtain correct information. This in turn will be spread word of mouth from the school project team to other classes/schools and carried back to the students home unit. Today's students are tomorrow's parents and consumers. This knowledge reaching several generations will help cement support for poultry industry sustainability both socially and ecologically into the future.

ACKNOWLEDGEMENTS

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- Australian Egg Corporation Limited (AECL)
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- Poultry Research and Development Centre's staff

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Inghams Enterprises Pty Ltd
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Queensland Chicken Growers Council
Ridley Agriproducts Pty Ltd
SPAFAS Australia Pty Ltd
University of Queensland
Alpharma Animal Health
Aventis Animal Nutrition
Brisbane Export Corporation
Darwalla Milling Co
Golden Cockerel Pty Ltd
Intervet Australia Pty Ltd
Janos Hoey
Provimi Australasia
Queensland Egg Farmers' Association
Safe Food Queensland
Sunny Queen Ltd
Australian Chicken Meat Federation

DIET-INDUCED CHANGES IN THE GUT MICROFLORA IN BROILER CHICKENS

B. HUGHES[1]

Summary

Measurement of hydrogen and methane in the breath of chickens is an inexpensive, non-invasive way of assessing the overall metabolic activity of the gut. It provides a simple way of tracking changes in metabolic activity of microflora in experimental animals prior to, during and after administration of different dietary treatments and other therapies likely to alter the profile of gut microflora. Variable changes in concentrations of hydrogen and methane following a switch of diets point to complex changes in the numbers and/or species of bacteria in the gut population, and/or changes in the metabolic activities of those bacteria. Further work is needed to determine the fundamental reasons why bacterial colonisation of the gut is variable and why it can differ substantially between male and female chickens. This is a necessary step towards being able to control the colonisation of the gut in newly hatched chicks and to maintain an ideal microflora for the life of the bird

I. INTRODUCTION

The gut of the newly hatched broiler chicken is free of organisms (Yamauchi *et al.*, 1990). Shortly after hatching, chickens are exposed to ubiquitous microorganisms in the hatchery and on arrival on the farm. These organisms proliferate as they compete for various niches in the microenvironment of the gut (Ewing and Cole, 1994). The profile of the gut microflora is also likely to change with the age of the chicken in response to ingestion of various types and amounts of substrates such as carbohydrate in the diet. As chickens age they will continue to ingest a range of organisms, some of which could be pathogenic (Ewing and Cole, 1994), from a rapidly changing environment as viable organisms are shed by other birds and possibly introduced to the farm from neighbouring properties. McBurney *et al.* (2003) reported that the profile of organisms in the small intestine stabilised at about three weeks of age, with lactobacilli predominating in healthy chickens.

Included among the many and varied types of metabolites of normal gut microflora are hydrogen and methane which can be detected in the breath of chickens (Hughes *et al.*, 2001b). Hughes *et al.* (2001a) used non-invasive breath tests for hydrogen and methane to show that gut microflora competed for energy and other nutrients thus slowing the rate of growth and reducing feed efficiency in healthy chickens. They also showed that antibiotic treatment resulted in an increase in hydrogen concentration in breath of chickens given sorghum but a decrease in chickens given barley compared with the respective control diets. Differences in the amounts of hydrogen produced are indicative of changes in the numbers and/or species of bacteria in the gut population, and/or changes in the metabolic activities of those bacteria. Furthermore, these changes in hydrogen concentration in response to antibiotics were also dependent on the type of grain used in the diet. Presumably, the differential flow of undigested nutrients into the hindgut created different growth media for those hydrogen-producing species of bacteria surviving antibiotic treatment.

This report describes the use of non-invasive breath tests for hydrogen and methane as indicators of changes in the metabolic activity of gut microflora in response to changes to the diet

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of broiler chickens and the age of the chicken at the time of the change. Results from three experiments are presented here.

II. MATERIALS AND METHODS

Batches of day-old, feather-sexed broiler chickens were raised in floor pens on a commercial starter diet prior to transfer to metabolism cages in controlled temperature rooms for three separate experiments. Experiment 1 involved a switch of diet from commercial starter crumbles to an experimental low-AME wheat diet with and without a commercial glycanase enzyme product when chickens were 15 days of age. Breath samples were collected at 1000 h prior to the change from commercial starter diet to experimental wheat diets (day 0), again at 1100, 1200, 1400 and 1600 h, and then six days later. In Experiment 2, commercial starter feed was changed to commercial finisher feed when chickens were 22 days of age. Breath samples were collected prior to the change of diet (day 0), and again six days later. The third experiment involved a change of feed from commercial starter diet to experimental wheat- or barley-based diets, with or without a commercial glycanase enzyme product. In this experiment, the change of diet took place when chickens were 15, 22 or 29 days of age. Three separate groups of chickens from the same rearing batch were used in Experiment 3. Male and female chickens were considered separately in each of the experiments for reasons outlined by Hughes (2003).

Helmets were constructed from capped PVC pipe (40 or 50 mm internal diameter and 95 or 100 mm length, respectively) to suit chickens of different ages and hence size. To collect a breath sample, the helmet was placed over the head and neck of the chicken and held firmly against the shoulders and breast to minimise loss of expired H_2 and CH_4. After 30 s for experiments 1 and 2, and 15 s for experiment 3, a sample of breath was drawn from the helmet into a 10 mL evacuated tube. Hydrogen and methane concentrations were analysed by gas chromatography.

III. RESULTS AND DISCUSSION

a) Experiment 1

Breath hydrogen concentrations differed significantly between males and females at 15 days of age just prior to change in diet (Figure 1), and the change in breath hydrogen concentrations between day 0 and day 6 tended to be greater in females than males but, as Figure 2 shows, these changes differed widely between individual chickens. Addition of enzyme to the diet made no difference to concentration of breath hydrogen. Seven of the 24 chickens used in the study had no detectable methane in breath at the commencement of the study (day 0). However, all 24 chickens produced methane during the following six hours once they began to ingest the wheat-based experimental diet, irrespective of presence or absence of enzyme in the feed. The arithmetic mean value for change in methane from day 0 to day 6 was 12 ppm. These results are consistent with the previous suggestion by Hughes (2003) that post-intestinal events associated with gut microflora were affected by the sex of the chicken.

b) Experiment 2

There was no statistical difference ($P>0.05$) between males and females in breath hydrogen at the start of the experiment (day 0) in contrast with results from Experiment 1. The change in concentration of hydrogen in breath over a 6-day period (Figure 2) was less in females than males (-21 vs -51 ppm), which also contrast with results from Experiment 1.

The observations in regard to expiration of methane (Figure 3) point to remarkably different metabolic activities of gut microflora in birds reared on starter diets with the same

nutrient specifications (but not necessarily the same ingredient composition), and to highly variable diet-dependent changes in activities in individual birds during Experiments 1 and 2. These changes in methane appear to be independent of sex. In the current experiment, all birds had methane in breath on day 0, but only one bird (female) was expiring methane on day 6.

c) Experiment 3

Hydrogen and methane concentrations in breath were highest in the oldest chickens (Figure 4). Addition of feed enzymes to the diet increased the hydrogen concentration in breath from the youngest chickens compared with control-fed chickens, but decreased it in subsequent age groups.

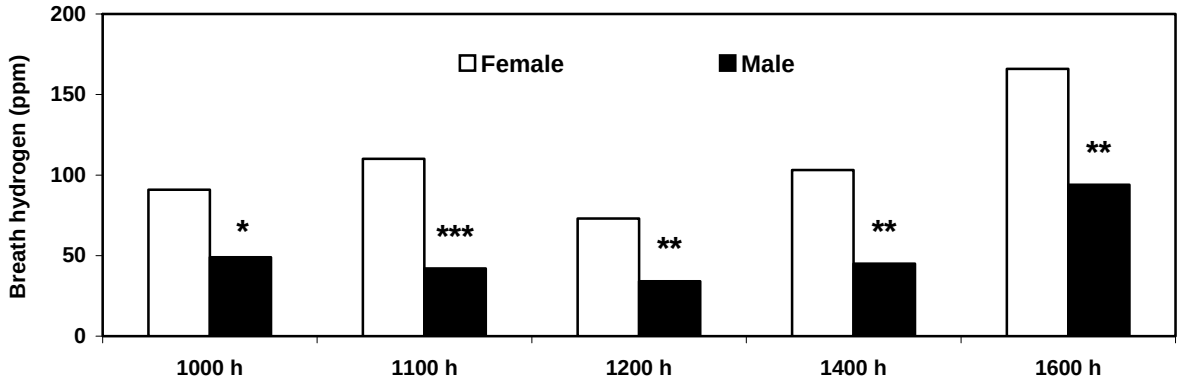

Figure 1. Breath hydrogen concentrations differed between male and female chickens given commercial starter diet (Experiment 1). * P<0.05, ** P<0.01 and *** P<0.001.

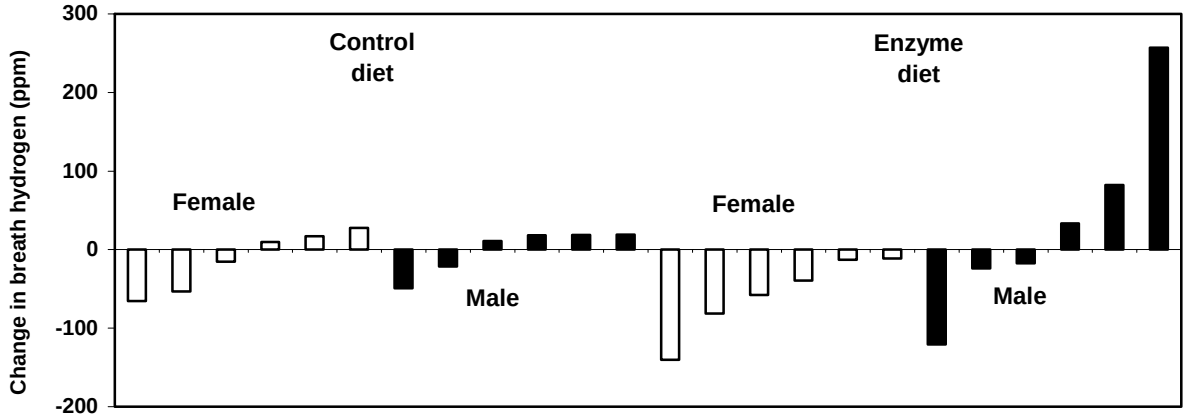

Figure 2. Change in breath hydrogen concentrations from day 0 to day 6 for male and female chickens given a low-AME diet with and without glycanase enzyme (Experiment 1). Each bar represents one chicken.

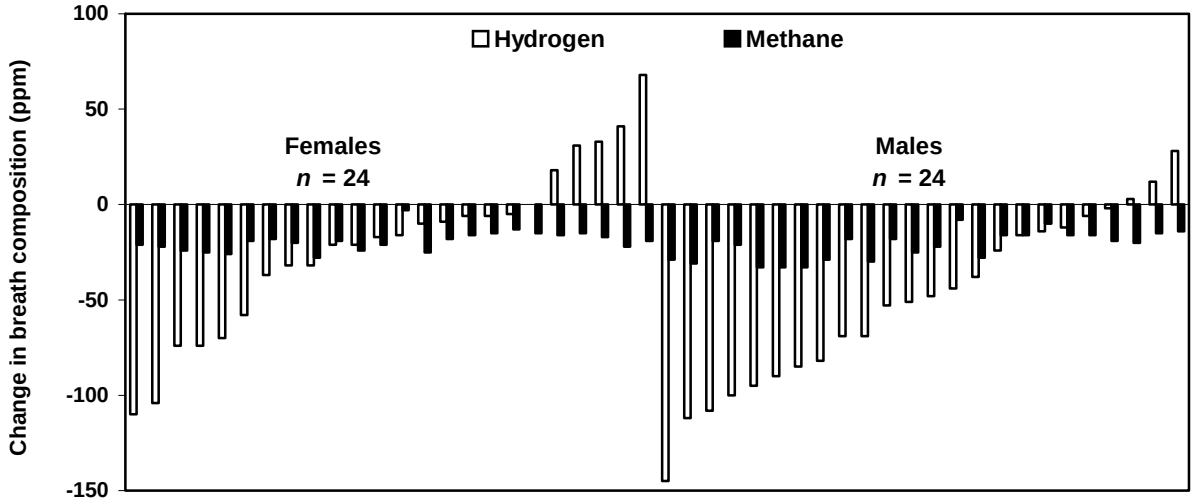

Figure 3. Change in breath hydrogen and methane concentrations from day 0 to day 6 for male and female chickens following a change of diet from commercial starter to commercial finisher (Experiment 2). Each bar represents one chicken.

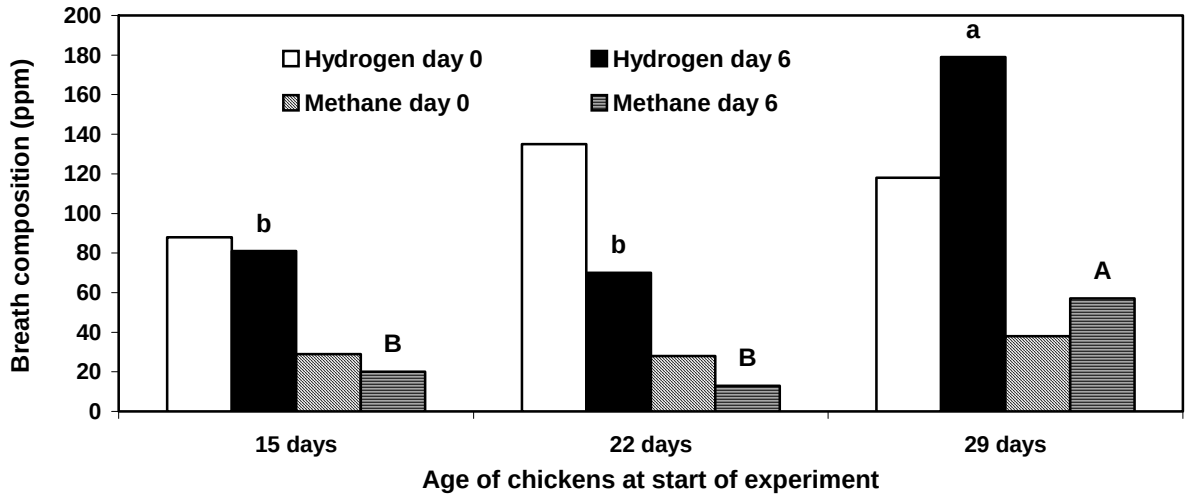

Figure 4. Effect of age of chickens on hydrogen and methane concentrations in breath on days 0 and 6 (Experiment 3). Lower and upper case letters indicate significant differences ($P<0.001$) on day 6 for hydrogen and methane, respectively. Results on day 0 were not significant ($P>0.05$).

IV. CONCLUSIONS

Variable changes in concentrations of hydrogen and methane following a switch of diets point to complex changes occurring in the numbers and/or species of bacteria in the gut population, and/or changes in the metabolic activities of those bacteria. Increases in hydrogen and methane in breath point to increased losses of nutrients from the small intestine to fuel bacterial proliferation in the caeca.

ACKNOWLEDGMENTS

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THE DEVELOPMENT OF MOLECULAR TOOLS FOR MONITORING GUT MICROFLORA OF POULTRY

V.A. TOROK[1], K. OPHEL-KELLER[1] and R.J. HUGHES[2]

Summary

The microorganisms that colonise the gastrointestinal tract during the early post-hatch period form a synergistic relationship with their poultry host, releasing and providing essential nutrients as well as competitively excluding pathogenic species. Microorganisms can also directly interact with the lining of the gastrointestinal tract, which may alter the uptake of essential nutrients and the immunological status of the bird. Recent Australian studies indicate that microbial colonisation of the gut is a variable process and that it can differ substantially between male and female chickens. This report outlines the development and application of terminal restriction fragment length polymorphism (T-RFLP), a DNA-based analytical tool, for examining diet-induced changes in the microbial community of the chicken gut.

I. INTRODUCTION

Gut microbiology and its role in animal health has become increasingly important, particularly now that the prophylactic use of antibiotics in animal feeds to promote growth has been questioned (Grimes, 2000). Gastrointestinal microorganisms have a highly significant impact on uptake and utilisation of energy (Choct *et al*., 1996) and other nutrients (Smits *et al*., 1997; Steenfeldt *et al*., 1995), and on the response of poultry to anti-nutritional factors (such as non-starch polysaccharides), pre- and pro-biotic feed additives and feed enzymes (Bedford and Apajalahti, 2001). Microorganisms can also directly interact with the lining of the gastrointestinal tract (Van Leeuwen *et al*., 2004), which may alter the physiology of the tract and immunological status of the bird (Klasing *et al*., 1999). Current methods for analysis of intestinal flora rely on culturing, which is not only laborious, but more importantly, misses a large part of the uncultivable microflora. Alternatively, DNA techniques have the advantages of being rapid, relatively inexpensive and capable of monitoring gene regions of complex populations.

This report is a brief summary of current work supported by the Australian Poultry Cooperative Research Centre (CRC) to develop DNA-based analytical tools for examining diet-induced changes in the microbial community of the chicken gut.

II. CURRENT MOLECULAR APPROACHES TO THE ANALYSIS OF COMPLEX BIOLOGICAL MICROBIAL COMMUNITIES

Currently the techniques of choice for microbial community analysis in many disciplines are denaturing or temperature gradient gel electrophoresis (DGGE/TGGE) (Muyzer, 1999). These techniques amplify the 16S subunit of bacterial ribosomal DNA by PCR (polymerase chain reaction), and then separate the amplicons on a denaturing gel to visualise fragment size differences in the ribosomal DNA. However, this technique is not conducive to high throughput and there are issues with reproducibility and analysis based on presence/absence/position of bands. To this end, there has been emphasis placed on the need

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to standardize conditions across research groups and the need for good analytical software. Of further concern with DGGE/TGGE and other nucleic acid-based techniques involving PCR is the choice of appropriate primers in order to obtain a true representation of the actual microbial community present.

An alternate technique for bacterial community analysis is terminal restriction fragment length polymorphism (T-RFLP) (Osborn *et al.*, 2000). This technique also amplifies the 16S subunit of the bacterial ribosomal DNA present in biological samples; however, all bacterial sequences amplified are labelled with a fluorescent dye. The amplified and labelled bacterial sequences are cut with sequence specific enzymes. The resulting fragments are separated according to size and detected by fluorescence emission from the incorporated dye by a DNA sequencing machine. Results are converted to graphical profiles where peaks can represent taxonomically related groups and/or strains of bacteria. These can be easily compared between samples to identify changes in bacterial community composition. The T-RFLP technique has great potential for large scale profiling, as 96 samples can be run at a time.

III. DEVELOPMENT OF A HIGH THROUGHPUT APPROACH FOR CHICKEN GUT MICROBIAL ANALYSIS.

The aim of our work is to establish a microbial profiling technique for chicken intestinal microflora based on high-throughput, high resolution fingerprinting of bacterial ribosomal gene regions. This will enable changes in the intestinal microbial community under different environmental conditions to be monitored.

We have elected to use the T-RFLP technique for chicken gut microbial community analysis because of its high-throughput potential. T-RFLP, like all PCR based methods, is influenced by template and therefore DNA extraction procedure. Choice of extraction method can greatly influence the complexity of the resulting profile (Figure), and impact on the knowledge gained.

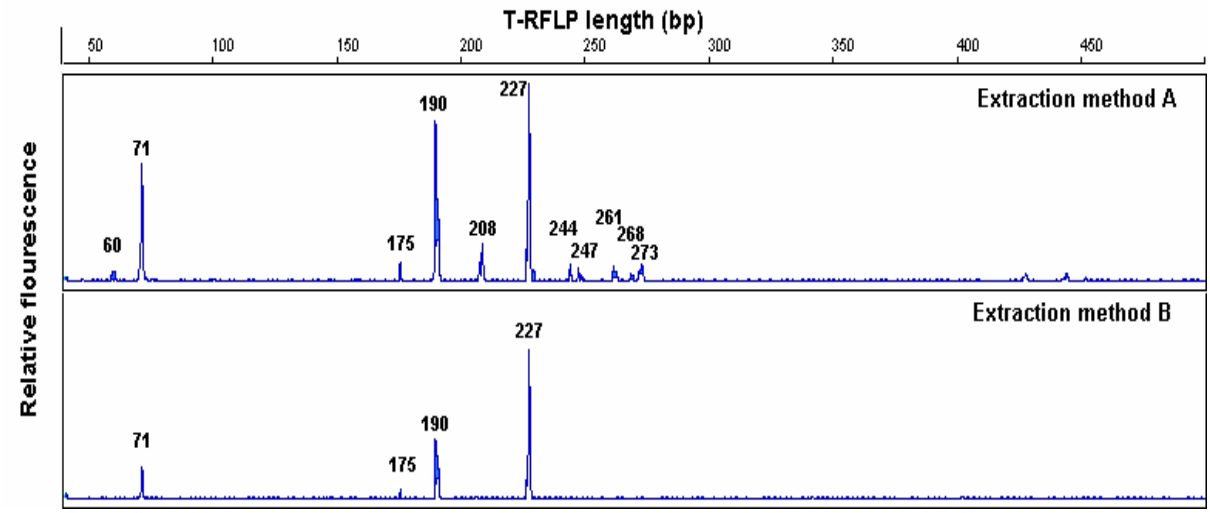

Figure. Comparison of T-RFLP profiles of the 16S subunit of the bacterial ribosomal DNA gene amplified from DNA extracted from the ileum of a single chicken using two different extraction methods.

Peaks in the Figure above represent taxonomically related groups and/or species of bacteria found in the chicken ileum. Numbers above peaks indicate peak position. Peak heights are representative of the proportion of different bacterial groups found in the population. Note that more peaks and, therefore, bacterial groups are detected in the gut sample extracted with method A. Only dominant peaks present in extraction method A are

also represented in extraction method B, indicating that extraction method B is less efficient in recovering all bacterial DNA. This demonstrates the importance of using an appropriate and efficient extraction method. The extraction method used on chicken gut and digesta samples is a modification of a proprietary method developed by SARDI researchers for analysis of soil-borne organisms.

PCR bias is also another important issue; therefore, PCR primer choice and standardized conditions are important when developing a high throughput method. To this end we have: (i) compared various DNA extraction procedures and developed an effective DNA extraction protocol suitable for chicken gut samples, which is also conducive to high throughput; (ii) compared various universal 16S ribosomal DNA PCR primers to ascertain those which perform best; (iii) tested the reproducibility of our method; and (iv) developed quality controls to be included in each T-RFLP run. The selection of restriction enzymes chosen for the T-RFLP analyses were based on those, which showed the greatest theoretical discrimination potential between available bacterial ribosomal 16S sequences in databases. These have given us good preliminary results in our T-RFLP analysis of chicken gut microbial samples. The T-RFLP data will be analysed with multivariate statistical models, such as multidimensional scaling.

IV. INDUSTRY PERSPECTIVES

The use of our developed T-RFLP tool will allow us to define what constitutes an optimum intestinal microflora in an "elite" chicken, and to utilise this technology and benchmark to conduct comparative studies of the effects of dietary manipulations on changes in the "good" and "bad" bacterial population in the gut of chickens. This tool will contribute to an increased knowledge of the chicken gut microbiota, and hence, a better understanding in its role in chicken nutrition, which will form the basis for practical recommendations on novel nutritional strategies for the chicken meat and egg industries in Australia.

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DETECTION OF *LACTOBACILLUS ACIDOPHILUS* SPECIES IN THE GUT OF CHICKENS

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Summary

The bacterial community of the crop, ileum and ceca of broilers was investigated during the first week of life using nucleic acid based microbial ecology techniques and selective bacteriological culture of lactobacilli. The microbial ecology studies revealed that the *Lactobacillus* populations in the ileum and ceca of very young birds were similar to each other and among birds. By day 3, each bird had developed its own unique microbial community. The LAB population in the crop was always more complex than in the ileum and ceca. Species belonging to the *Lactobacillus acidophilus* complex were detected in all organs. Isolates obtained from the selective culture of lactobacilli from the crop and ceca were genetically fingerprinted which revealed that several strains were present in different regions of the gut in the same and different birds. The species and strains present in the distal region of the gut appear to be dictated by an initial colonisation of the crop.

I. INTRODUCTION

The diverse collection of bacteria in the digestive tract, collectively referred to as the gut microflora, plays an important role in the nutrition, health and disease of broiler chickens, other livestock, and humans. The composition of the microflora in the broiler chicken gastrointestinal tract (GIT) is organ-specific, with microbial colonisation occurring in the crop, ileum, and caeca (Sarra *et al*., 1992; Tannock, 1995). Lactobacilli dominate the microflora in the crop and are numerous in the ileum, whereas the microflora in the caeca is dominated by anaerobes. In the 1970s, Fuller hypothesised that the crop microflora provides a bacterial inoculum for the remainder of the gut (Fuller and Brooker, 1974). Different research groups have independently identified the same *Lactobacillus* species (specifically *Lactobacillus crispatus*, *Lactobacillus johnsonii*, and *Lactobacillus reuteri*) in the crop, ileum, and caeca, suggesting that lactobacilli may be a genus that supports Fuller's hypothesis. Understanding how and where lactobacilli colonise the GIT is necessary to rationally derive husbandry methods that utilise feed supplements other than antimicrobial drugs for the efficient and sustainable production of poultry

II. METHODS

A culture-independent technique, based on the species specific sequence of the 16S rRNA gene, was used to analyse the microbial ecology of lactobacilli in the broiler gut (Walter *et al*., 2000, 2001). DNA was extracted from the crop, ileum and caeca of broilers on day 1, day 3, and day 7, and used as template in a PCR reaction that was designed to amplify the 16S rRNA gene of lactobacilli and closely related species. The resulting PCR reactions

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were analysed on a denaturing gradient gel (DGGE) which separates similar sized fragments of different sequence to create a profile of the *Lactobacillus* species present in the gut sample.

Crop and caecal samples were plated onto selective media to propagate lactobacilli from the chicken gut, and 10 colonies were analysed from each section of each bird. The isolates were speciated using molecular techniques (Guan *et al*., 2003). Isolates of the same species were then identified at the strain level using pulsed field gel electrophoresis (PFGE). PFGE is the industry "gold standard" for genetically fingerprinting bacterial strains. Bacterial cells are captured in agarose, the chromosomal DNA is purified *in situ*, cut with restriction enzymes, separated in a PFGE apparatus, and the fragmentation patterns analysed.

III. RESULTS

The aim of our work is to understand the microbial ecology of lactobacilli in the chicken GIT. The objective of the current study was to investigate the development of the *Lactobacillus* microflora in the crop, ileum and caeca of healthy broilers in the first week of life. We had previously determined that the microflora in the crop changed dramatically during this time frame (Guan *et al*., 2003). A sample PCR-DGGE profile of *Lactobacillus* species found in the chicken gut is shown in Figure 1. Analysis of the data revealed that *Lactobacillus* populations in the ileum and caeca of day 0 and day 1 birds were similar to each other and among birds. The crop community, though different from that of the intestinal organs, was similar between birds. By day 3 and again at day 7, each bird had developed its own unique microbial community. The LAB population in the crop was always more complex than in the ileum and caeca. Species belonging to the *Lactobacillus acidophilus* complex (*Lactobacillus acidophilus*, *L. crispatus*, *L. amylovorus*, *L. gallinarum*, and *L. johnsonii*) were detected in all of the organs.

Lactobacilli were also cultured from crop and caecal samples. Isolates of the same species were then identified at the strain level using PFGE (Figure 2).

Comparison of the origin of isolates revealed that several strains of *L. johnsonii*, *L. gallinarum* and *L. crispatus* were present in different regions of the gut in the same and different birds (Table 1). Other strains were only isolated from either the crop or caeca. Remarkably, both sections of the gastrointestinal tract can be inhabited by multiple strains of the same and different species.

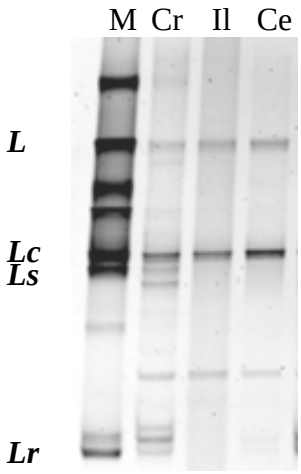

Figure 1

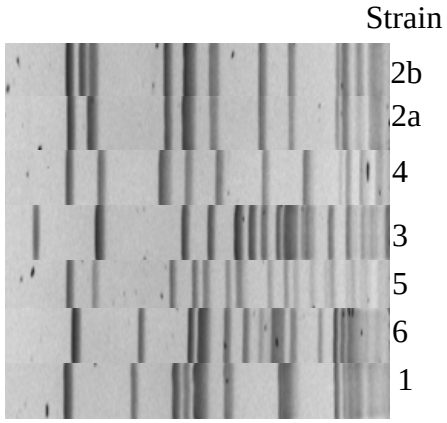

Figure 2

Figure 1: Sample PCR-DGGE profiles of *Lactobacillus* species in the crop (Cr), ileum (Il), and ceca (Ce). Pure cultures used in the marker (M) lane to compare the mobility of the bands in the samples and putatively identify the species present were as follows: *Lj, L. johnsonii; Lc, L. crispatus; Ls, L. salivarius*; and *Lr, L. reuteri.*

Figure 2: Sample PFGE profiles of *Lactobacillus* strains.

Table 1. An example of the data obtained when comparing the origin of the strains.

Bird (age in days)	Strains isolated from the crop	Strains isolated from the ceca
1 (2)	2b	2a, 2b, 5
2 (5)	1, 2a	2a
3 (5)	1	
4 (5)	1	1, 4
5 (8)	1	1, 3
6 (14)	3	3
7 (14)		6
8 (14)	1	1
Strains in multiple birds	1	1, 2a, 3

IV. DISCUSSION

This is the first study to compare the succession of lactobacilli in the crop, ileum, and caeca. The species and strains present in the distal region of the gut appear to be dictated by an initial colonisation of the crop. Our observations support Fuller's hypothesis that the crop community acts as a bacterial inoculum for the remainder of the gut. The microflora in the crop, therefore, is important in the development of the microflora in distal regions of the gastrointestinal tract.

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ISOLATION OF MAREK'S DISEASE VIRUS FROM DUST SAMPLES FROM COMMERCIAL CHICKEN FARMS.

Z. HUSSAIN[1], A.F.M.F. ISLAM[1], S.K. BURGESS[1], P.S. REYNOLDS[1] and S.W. WALKDEN-BROWN[1]

Summary

Marek's disease virus (MDV) replicates in chicken feather follicle epithelium and these cells serve as a source of infection for other chickens. We report the use of chicken dust collected in the field to isolate MDV in specific pathogen-free chickens. Chickens were infected via the respiratory route with MDV-positive chicken dust. Quantitative polymerase chain reaction (q-PCR) assay was used to quantify MDV in dust and spleen samples from the experiment. Two out of five field dust samples successfully induced infections with subsequent MDV recovery. Serological testing of chickens at day 56 post infection indicated that no other major poultry pathogens were transmitted by the infective dust material. Use of poultry dust as primary material for isolating MDV offers potential advantages over fresh tissues and this work has shown that with some refinement it is a feasible approach.

I. INTRODUCTION

Marek's disease is caused by a cell-associated herpesvirus called Marek's disease virus (MDV). Although the initial target cell for MDV infection is the lymphocyte, fully productive infection of feather follicle epithelium cells follows with significant virus shedding in dander from day 10 post-infection (Islam *et al.*, 2004).

The disease is well controlled by vaccination but appears to be evolving towards greater virulence in the face of blanket vaccination (Witter, 1998). Some 80% of Australian broiler chickens and almost all layer and breeder chickens are vaccinated against MD. Although there has been no major outbreak of MD in Australia since 1997 due to adequate vaccinal control, Australian MDV strains may be evolving towards greater virulence. To evaluate this requires isolation and testing of current Australian strains of MDV.

MDV is usually isolated on to cell culture from blood, spleen, or tumour tissues. These require rapid chilled transport from the field to laboratory and immediate processing. They also may contain vaccinal MDV and other poultry pathogens, requiring a series of complex procedures to propagate uncontaminated isolates. In contrast, MDV in dander is less likely to be contaminated by vaccinal virus (Cho and Kenzy, 1975) or other poultry pathogens given the unique mode of transmission of MDV. Furthermore MDV in dander retains infectivity for considerable periods at room temperature (Carrozza *et al.*, 1973) thus removing requirement for chilled transportation and immediate processing at the laboratory.

We report the results of a preliminary experiment to test the isolation MDV from field poultry dust in SPF chickens. Specific hypotheses were: 1) poultry dust containing MDV can be used to infect chickens with MDV; 2) infective dust collected from chicken sheds without clinical MD may produce clinical MD experimentally; 3) the dust infection model will not transmit other infections, and 4) commercial Cobb broilers and SPF white leghorn chickens will not differ in MD susceptibility when challenged with a reference MDV strain.

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II. MATERIALS AND METHODS

The experiment utilized a completely randomized design with 9 MDV treatments as shown in Table 1. MDV challenge was with 5 MDV-positive field dust samples (02Lar, 02Nov, 04Loc, 04Cre, and 04Man), one new cell-culture adapted viral strain from RMIT University, Melbourne (MPF132/5), and a dust sample collected from a previous experiment at UNE using the reference vvMDV strain MPF57 (positive control). One isolator contained unchallenged chickens. MDV was quantified in the dust samples using real-time PCR (Islam et al., 2004). Chicks were infected with 2mg dust each administered intra-tracheally except for the 04Loc treatment for which 2mg of dust was suspended in 100µl of antibiotic solution and administered intra-peritoneally. MPF132/5 (50pfu in 100µl) was also administered intra-peritoneally. Chickens of each treatment group were placed in a positive pressure isolator.

Specific pathogen free (SPF) chickens (SPAFAS Australia) and unvaccinated commercial Cobb broilers were used in the experiment. SPF chicks were transported by air from Melbourne to Armidale on the day of hatch and challenged with MDV on the same day (14-15 chickens per treatment). Broiler chicks were accessed locally on the same day.

At day 14 post-infection (pi), chicken dust was collected from all isolators for quantification of MDV. All dead and euthanased chickens were examined post-mortem for gross MDV lesions. At day 56, five plasma samples were collected per treatment and a pooled sample for each treatment tested for antibody against for a number of chicken pathogens (standard chicken inoculation test serology) at the University of Melbourne. All chickens were then euthanased, weighed and examined post-mortem for gross MD lesions. Spleen samples were also collected to quantify the MDV expressed as MDV viral copy number (VCN) per mg of spleen tissue. Individual spleen samples were submitted to RMIT University for viral isolation on cell culture.

Table 1. Details of the different treatments applied.

Sample name	Origin	Challenge material	Vaccination history	VCN/mg dust	Chickens challenged	Chicken strain	Dose/bird
04Loc	NSW	Dust	HVT	1,648	12	SPF	2mg
04Cre	NSW	Dust	Rispens	31,592	14	SPF	2mg
02Lar	Vic	Dust	Nil	87,179	14	SPF	2mg
02Nov	Vic	Dust	Nil	17,836	14	SPF	2mg
04Man	Vic	Dust	HVT	456	13	SPF	2mg
MPF132/5	NSW	Infected CEF*	Nil	-	14	SPF	50pfu
MPF57	NSW	Dust	Nil	326,562	14	SPF	2mg
MPF57	NSW	Dust	Nil	326,562	15	Cobb	2mg
Control		Nil	NA	NA	15	Cobb	NA

* CEF = chicken embryo fibroblasts

III. RESULTS

Results are detailed in Table 2. At day 14, dust samples from 4 treatments (04Cre, 02Lar, MPF57, and MPF 132/5) were positive for MDV (Table 2). All spleen samples tested from these treatment groups at day 56 were positive for MDV and birds from each of these treatments exhibited gross MDV tumours (range 21-40%). In isolators negative for MDV at day 14 (02Loc, 02Nov, 04Man, Neg control), no MD lesions were observed and all spleen

samples were negative apart from 2 samples with low MDV copy number in the 04Man treatment. In these isolators there was no mortality at all.

Live weight of surviving chickens at day 56 was significantly higher (P<0.01) in groups without MD (732±32g) than in groups showing MD lesions (629±30g) (Figure 1).

Table 2. Summary of results. Blank cells indicate no measurement taken. The control group of 15 chickens had no mortality or MDV by any test.

Treatment	04Loc	04Cre	02Lar	02Nov	04Man	MPF57(SPF)	MPF57 (Cobb)	MPF 132/5
No of birds	12	14	14	14	13	14	15	14
MDV VCN/ mg dust, d14	0	168	25	0	0	1615	-	87
Total mortality	0/12	5/14	3/14	0/14	0/13	5/14	6/15	1/14
Mortality with MD lesions	0	0	1/3	0	0	2/5	4/6	0
Birds with MD lesions, d56	0/12	3/9	3/11	0/14	0/13	3/9	2/9	3/13
Total MD lesions (%,)	0/12 (0)	3/14 (21)	4/14 (29)	0/14 (0)	0/13 (0)	5/14 (36)	6/15 (40)	3/14 (21)
MDV in spleen at day 56 pi (+/total)	0/10	5/5	9/9	0/10	2/10	6/6	-	9/9
Mean MDV in spleen (VCN/mg ± SEM)	0	19635± 11,721	7285± 1402	0	17.9± 33.5	4188± 3755	-	12971± 5216

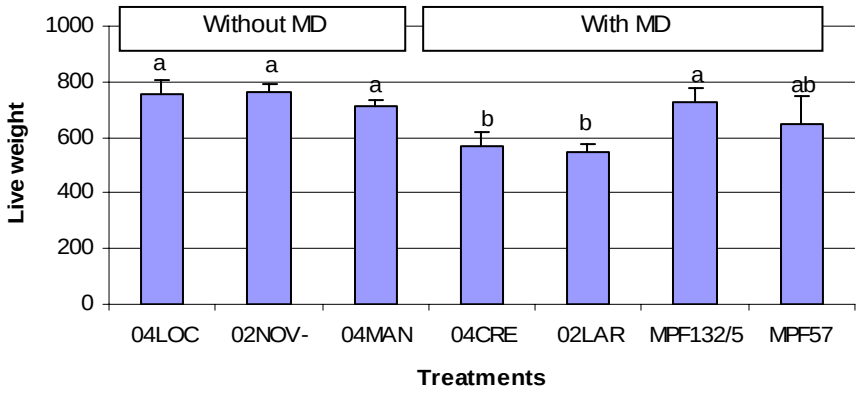

Figure 1. Live weight of surviving SPF chickens at day 56, by treatment group. Bars not sharing a common letter are significantly different (P<0.01).

Serological results are summarised in Table 3. Pooled sera for all treatment groups were negative for major poultry pathogens apart from positive MDV serology in the four groups for which MDV was present in dust at d14, and in which gross MD tumours were observed. With regards to virus growth on culture at RMIT following the experiment, all PCR positive spleens showed evidence of MDV growth in cell culture and no PCR-negative spleens showed evidence of MDV growth in cell culture.

Table 3. Serology results for pooled plasma samples from day 56.

Pathogen	Test used	Results
Avian adenovirus (Gp 3) EDS, NDV	HI	All negative
Marek's disease virus	AGP&IFA	*Positive/Negative
Big liver & spleen, H.enteritis, AIV, Avian adenovirus 1	AGP	All negative
MG, MS, SP	RSA	All negative
AE, IB, AL, ILT, Avian reovirus, CAV, IBD	ELISA	All negative
Reticuloendotheliosis virus	ELISA/IFA	All negative

*Positive - 04Cre, 02Lar, MPF57, and MPF 132/5. Negative - 02Loc, 02Nov, 04Man, Neg control)

IV. DISCUSSION

In this study we have shown that field chicken dust containing MDV can be used to infect chickens experimentally followed by successful isolation of MDV from spleen, thus supporting hypothesis 1. Two of five field dust samples produced positive infections and viral recoveries. These were the samples with the highest original MDV content in dust. A 3rd sample with relatively high viral content (02Nov) has since been used to successfully infect chickens using a higher initial dose rate. Overall these results suggest that dust samples with a viral content of 10,000 VCN/mg dust or greater are most suitable for virus isolation.

Hypothesis 2 is supported as sample 02Lar came from an unvaccinated broiler flock not reporting MD tumours, yet induced tumours in 29% of birds in this experiment. Hypothesis 3, that the dust infection model will not transmit other pathogens is also supported by the experimental data as the serological data indicate that no other significant chicken pathogens were transmitted. This will require further verification over time. Hypothesis 4 is supported, with few differences between commercial broiler and SPF chickens in the level of MD induced. This is interesting given that the commercial broilers contain maternal antibody against MDV serotype 1.

One finding of major interest was that all mortality observed in the experiment was in isolators in which MDV was shown to be present. In the other isolators, no birds died during the whole experiment. This suggests that the early mortality observed in the experiment without the presence of MD tumours is nevertheless MD-associated, Early mortality syndromes have been reported for MD overseas (Witter *et al.,* 1999). These data provide experimental support for their occurrence in Australia.

This experiment was not designed to formally pathotype the various strains, as the initial infective doses and methods of infection varied. However the data suggest that the three new MDV isolates could be potentially as virulent as the vvMD reference strain MPF57, the most virulent Australian isolate to date.

ACKNOWLEDGMENTS

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DYNAMICS OF MAREK'S DISEASE VIRUS AND HERPESVIRUS OF TURKEY SHEDDING IN FEATHER DANDER OF BROILER CHICKENS

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Summary

In an attempt to measure the excretion dynamics of HVT and MDV via feather dander, broiler chickens were vaccinated with HVT or sham-vaccinated at day old and than challenged with MDV at days 2, 4 and 7 post-vaccination. The effect of the interval between vaccination and challenge on the protection of vaccine was determined. MDV and HVT were quantified in the dust samples collected at various days post-placement. There was an effect of the interval between vaccination and challenge on protection, higher the interval, lower the protection. Shedding of HVT was recorded from day 9 to 58 post-vaccination with a peak at around day 20. MDV was recorded in the dust from day 7 to 56 post-challenge with a peak at around 30-35 days post-infection. MDV shedding was significantly reduced by HVT vaccination, higher the protection less the shedding. Identification of HVT and MDV in the same dust sample can be a useful tool for monitoring vaccinal presence and MDV status of a chicken flock by analysing a single dust sample.

I. INTRODUCTION

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by a cell-associated herpesvirus called MD virus (MDV). Chickens are infected with MDV via the respiratory route. Following uptake in the lungs the virus infects lymphocytes where initial viral replication occurs. Virus is then transmitted to the feather follicle epithelium (FFE) where fully productive viral production occurs and large amounts of virus are excreted to the environment in feather dander (Calnek *et al.*, 1979). MDV in host cells such as lymphocytes, is infective but infectivity is lost if the integrity of the host cell is lost. However virus excreted in dander remains infective in the environment for a long time and is the main means of MDV transmission (Gilka and Spencer, 1993). MDV shedding in feather dander is thought to commence two weeks after infection (Carrozza *et al.*, 1973).

A naturally occurring turkey herpesvirus (HVT) is antigenically related to MDV, does not produce disease in turkeys or chickens, but maintains horizontal transmission within turkey populations. HVT used as a vaccine against MD and is not thought to transmit horizontally between chickens unless they are infected at around 8 weeks of age, in which case limited horizontal transmission has been reported (Cho, 1976; Cho and Kenzy, 1975). HVT replication in the FFE has been reported for a short period during weeks 2 and 3 post-infection (Zygraich and Huygelen, 1972). With the advent of fully quantitative methods for measuring MDV in poultry dust samples (Walkden-Brown *et al.,* 2004) it is of interest to determine whether this replication in the FFE is manifest as HVT in dander.

As for other live vaccines, the interval between vaccination and challenge is an important determinant of vaccine efficacy. If so, varying the interval between HVT-vaccination and MDV-challenge may produce variable protection. MDV shedding rate may also vary with the protection status of chickens. In this paper we report the excretion dynamics of MDV and HVT in feather dander in broiler chickens in an experiment in which

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the intervals between HVT-vaccination and MDV-challenge varied, giving rise to chicken populations of different protection status against MD.

II. MATERIALS AND METHODS

One hundred and fifty HVT-vaccinated (8,000pfu s/c at hatch) female Cobb broiler chickens were placed in 6 positive pressure isolators, 25 chickens in each isolator. Fifty more sham-vaccinated chickens were placed into two other isolators. Chickens of two sham- and two HVT-vaccinated isolators were challenged with 100pfu of MDV (strain MPF57) at 2 day post-vaccination (dpv) intra-peritoneally. HVT-vaccinated chickens in two isolators were challenged with MDV at 4 dpv and those in the remaining isolators were challenged at 7 dpv.

Dust samples were collected from each isolator between days 9 and 58 after placement. Chickens dying during the experiment were examined post-mortem for gross MD lesions. At day 56 post-challenge, all survivors were euthanased and examined post-mortem for MD lesions. The protective index of each challenge group was calculated as previously described (Islam *et al.*, 2001).

DNA was extracted from 5mg of dust using DNeasy kits (Qiagen Pty Ltd, VIC Australia) and quantified by spectrophotometry (BioRad SmartSpec, TM3000). MDV and HVT were quantified using a real-time PCR described previously (Islam *et al.*, 2004). MDV quantity was expressed as viral copy number (VCN) per mg of dust while HVT quantity was expressed as calculated concentration of HVT in arbitrary units, in the absence of absolute quantification for this assay.

III. RESULTS

There was an increase in protection with increasing interval between vaccination and MDV challenge. Protective index and percent MD to day 56 for the various vaccination-challenge treatments is presented in Table 1.

Table 1. Incidence of gross MD lesions (%MD) to day 56 post-challenge and protective index (PI) of sham-vaccinated or HVT-vaccinated broiler chickens challenged with 100pfu of MDV intra-peritoneally at various intervals after vaccination.

Isolator	Vaccine	Vaccination-challenge interval (day)	MD positive	MD Negative	Total[1]	% MD	Mean %MD	PI
18	Sham	2	16	2	18	88.89	74.36^{A}	0.00
20	Sham	2	13	8	21	61.90		
5	HVT	2	6	8	14	42.86	38.71^{B}	47.94
7	HVT	2	6	11	17	35.29		
17	HVT	4	6	17	23	26.09	23.26^{BC}	68.72
19	HVT	4	4	16	20	20.00		
6	HVT	7	4	15	19	21.05	16.67^{C}	77.59
8	HVT	7	2	15	17	11.76		

[1]Total chickens present at day 31 post-challenge when the first mortality with MD lesions was observed.

Excretion of HVT in dander started from 9 dpv, the first dust sample collection day. The rate of excretion increased sharply at around 20 dpv and than decreased slowly up to 58

dpv, the last dust sample collection day. No HVT was detected in the dust samples collected from unvaccinated chickens. The calculated concentration of HVT is presented in Figure 1.

Marek's disease virus was first detected in the dust collected at day 7 post-challenge in both sham and HVT-vaccinated chickens. Viral copy number was significantly higher overall in sham-vaccinated than HVT-vaccinated chickens (P=0.005). In sham-vaccinated chickens MDV shedding increased rapidly from day 14 post-challenge, peaked at around days 35-40 and than declined to day 56 post-challenge. In HVT-vaccinated chickens MDV increased slowly from days 14 to 21 and then tended to plateau at a lower level than in sham-vaccinated chickens for the remainder of the experiment (Figure 2A).

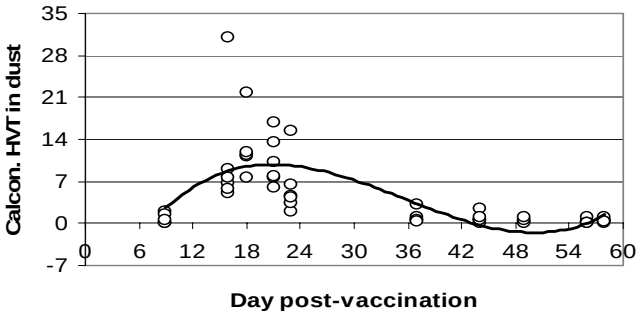

Figure 1. Excretion of herpesvirus of turkey (HVT) by broiler chickens vaccinated at day old with 8,000pfu of HVT vaccine. Each point represents one isolator.

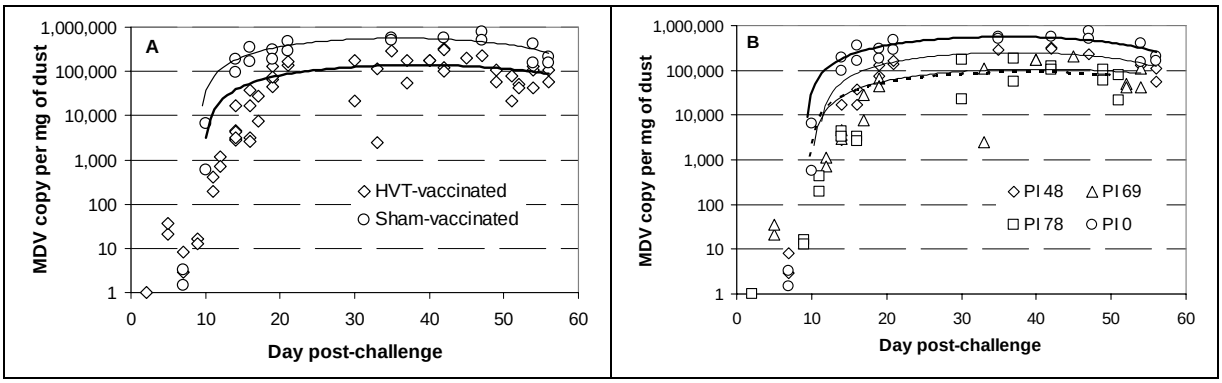

Figure 2. MDV copy number per mg of dust sample at various days post challenge in vaccinated and unvaccinated chickens (left panel) and in chickens with various levels of protection (right panel).

The rate of MDV shedding was also influenced by the timing of challenge post-vaccination and thus the vaccinal protection status of the treatment (Figure 2B). Sham-vaccinated chickens shed MDV at the highest rate. An intermediate level of shedding was observed in the day 2 challenge group (PI 48) and low levels of shedding were observed in the day 4 and day 7 challenge groups (PI 69 and 78, respectively). These two groups shed significantly less MDV than unvaccinated chickens (P=0.01). The PI 48 group also shed less MDV than unvaccinated chickens between days 14 and 56 (P=0.02).

IV. DISCUSSION

This study has demonstrated for the fist time the shedding of HVT in dander by chickens following vaccination with HVT. The study also demonstrated that MDV shedding may start as early as from day 7 following infection with MDV, and that HVT-vaccination significantly reduces MDV shedding into the environment.

The finding of significant HVT shedding in dust supports earlier reports of HVT replication in FFE (Zygraich and Huygelen 1972) and PCR detection of HVT in feather follicle extracts between days 14 and 42 (Handberg *et al.*, 2001). This raises the obvious issue of the efficiency of lateral HVT transmission in broiler chickens. There is little evidence of efficient spread of HVT between young chickens to date, but the issue requires resolution with the improved methods for measuring virus now at our disposal.

Significant shedding of MDV may start much earlier than the 2 weeks previously reported (Carrozza *et al.*, 1973). We observed MDV load of 600-7000 and 200-400 copies per mg dust at day 10 in unvaccinated and HVT-vaccinated chickens respectively; and very limited shedding (VCN 21-36/mg dust) was found as early as day 7. However, infectivity of this early MDV in dust was not confirmed. Using conventional PCR, MDV was identified in feather tip at day 14-16 post-challenge (Davidson and Borenshtain 2003; Handberg *et al.*, 2001). MDV detection in dust at day 7 in our study might be due to higher sensitivity of our method, or dust samples may contain more virus than feather tip in the early stages of infection. Our finding of early shedding is supported by the finding of Cho *et al.* (1996), who detected aggregates of lymphocytes in the perifollicular dermis in MDV-infected chickens as early as day 7.

Vaccination with HVT overall reducing peak MDV shedding by approximately four fold but the extent of reduction was dependent on the PI. A PI of 78% reduced peak viral shedding by more than five fold. It is unclear at this point whether this reduction reflects a uniform reduction across the group of birds, or simply a greater proportion of individual birds in which shedding is dramatically reduced.

Identification of MDV and HVT in the same dust sample is now feasible and may be a new turning point in the routine monitoring of MDV status of chickens using dust samples.

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MEASURMENT OF THE EFFECT OF STORAGE TEMPERATURE AND DURATION ON THE IN VIVO INFECTIVITY OF MAREK'S DISEASE VIRUS IN FEATHER DUST

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Summary

A study was conducted to assess the in vivo infectivity of Marek's disease virus (MDV) in feather dust stored at various temperatures for a period of 90 days after collection. One-day old commercial chickens (10 per temperature group) were inoculated with MDV containing chicken dust by intra-tracheal insufflation. Spleen samples were collected at day 7 post-infection and MDV was quantified. The proportion of chicks infected was significantly greater at lower temperatures (-80, -20, 4^{o}C) than higher temperatures (10, 26 and 37^{o}C) but there was no detectable decline in infectivity during storage at any temperature. There was no correlation between the copy number of MDV in the spleen of infected chicks at day 7 and the infectivity of dust (proportion of chickens infected).

I. INTRODUCTION

Marek's Disease (MD), an important disease in the poultry industry. It is caused by a cell-associated alpha herpesvirus called MD virus (MDV) (Biggs, 2001). The enveloped infectious virus, produced in the feather follicle epithelium, is vital in the natural transmission of MD. It is shed from the host in feather dander (Calnek *et al.*, 1970). Persistence of MDV viability in poultry house dust is of major importance in the transmission of the disease (Carrozza *et al.,* 1973). There are a number of factors that impact on the survival, and therefore the transmission, of MDV. However heat inactivation appears to be the main reason for the loss of MDV infectivity (Carrozza *et al.,* 1972).

The viability of MDV in dust stored at different temperatures has varied considerably between experiments (Table 1) which may be due to the different MDV strains used, method of determining MDV presence and type of chicken used.

Quantitative polymerase chain reaction (PCR) technology has recently been utilised to detect and quantify the viral load in both dust and spleen samples (Walkden-Brown *et al.,* 2004). This study used this method to determine the decay in infectivity of MDV in poultry dust over time, when stored at a wide range of temperatures from -80^{o}C to 37^{o}C. The main hypotheses were tested in this study namely 1) viral DNA in the dust as measured by qPCR will not be affected by storage temperatures and time; 2) infectivity of MDV in dust will be reduced over time, particularly at higher temperatures and 3) Infectivity of MDV in dust will be positively related to MDV viral copy number in infected chicks 7 days post infection.

II. MATERIALS AND METHODS

Infective feather dust was collected from a MDV challenge experiment in which unvaccinated commercial broiler chickens were inoculated with MDV (strain MPF 57). The original experiment produced gross MD lesions in 77% of chickens and the dust was collected between 42 and 49 days post-inoculation (dpi). Dust was weighed into 2 mg aliquots and stored at various temperatures (-80ºC, -20ºC, 4ºC, 10ºC, 26ºC and 37ºC). Ten chickens were inoculated with dust held at each storage temperature using intra-tracheal

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insufflation at days 14, 28, 42, 56 and 90 using the method describe previously (Underwood 2003). Initial infectivity was also measured at day 0 of dust storage using 10 chickens.

Table 1. Literature reports of the *in vivo* infectivity of Marek's disease virus in feather dust samples stored for variable periods at various temperatures

Temp-erature	Storage period	Study method	End point	Results	Reference
-20°C	252 days	0.5mg dust intra-tracheal inoculation	Histology, AGP test	+	Carrozza *et al,* 1973
-70°C - 200°C				+	
				+	
4°C	3 years	One-day old chickens, intra-abdominal inoculum	CKC culture	+	Calnek and Hitchner 1973
Room Temp	28 days	Two-day-old chickens, injected inoculum	Histology	+	Beasley *et al.* 1970
Room Temp	42 days	Two-day-old chickens, injected inoculum	Histology	-	Beasley *et al.* 1970
Room Temp	44 days	Day old chicken infection	Histology	+	Jurajda and Klimes 1970
Room Temp?	205 days	0.5mg dust intra-tracheal inoculation	Histology/ AGP test	-	Carrozza *et al.*, 1973
Room Temp	1-8 months	Two old chicken, intra-tracheal inoculation	Histology/ AGP test	+	Carrozza *et al.* 1972
Room temp	8-13 months	Day old chicken, intra-abdominal inoculum	CKC culture	+	Calnek and Hitchner 1973
37°C	21 days	One-day old chickens, intra-abdominal inoculum	CKC culture	+	Calnek and Hitchner 1973
37°C	28 days	0.5mg dust intra-tracheal inoculation	Histology/ AGP test	-	Carrozza *et al.* 1973

One-day-old commercial layer cockerels were used to test infectivity. The chickens were obtained from a flock vaccinated with serotype-1 MDV and were therefore positive for MDV-1 maternal antibody. Chickens were inoculated on the day of hatch (day 0) and spleen samples were collected from individual birds at 7 dpi for quantification of MDV.

DNA was extracted from 10 µg of spleen and 5 µg of dust using DNeasy® tissue kit and stored at -20 ºC. The extracted DNA was quantified using spectrophotometric analysis (BIO-RAD, SmartSpec TM 3000) and MDV was quantified using real-time PCR described previously (Islam *et al*., 2004) with viral quantity expressed as viral copy number (VCN) of MDV per mg of sample. Spleen samples were accumulated to the end of the experiment and then randomised (stratified for storage temperature and period) before DNA extraction and assay for MDV. Quantitative PCR processing of MDV DNA extracted from dust samples was carried out randomly at the end of the experiment.

Analysis of variance (ANOVA) was used to test the effect of storage period and temperature and their interaction on VCN using Super ANOVA® (Abacus Concepts, Berkely, CA, USA). The absence or presence of MDV in chicks was also analysed by ANOVA following binomial coding with S-plus 2000 (Mathsoft Inc., Cambridge MA, USA).

III. RESULTS

The number and percentage of positive chickens for each of the treatments over time is shown in Table 2. Using the binomial approach, there was a significant effect of storage period (P=0.005) and temperature (P= 0.036) on *in vivo* infectivity of MDV-infected dust, however there was not a significant (P= 0.208) interaction between them. The effect of storage period showed no particular pattern except high variability between days but effect of

temperature was due to higher infectivity at lower (-80 to 4˚C) compared to higher (10 to 37˚C) storage temperatures.

Table 2. Number of MDV-positive chicks at day 7 after inoculation with chicken dust collected from MDV-infected birds and stored for various periods at different temperatures.

		Storage period (days)					
		14	**28**	**42**	**56**	**90**	**Total**
Temperature (°C)	-80	5/10 (50%)	4/10 (40%)	4/10 (40%)	2/10 (20%)	5/10 (50%)	20/50 (40%)
	-20	2/9 (22%)	7/10 (70%)	1/10 (10%)	0/10 (0%)	6/10 (60%)	16/49 (33%)
	4	4/10 (40%)	7/10 (70%)	2/9 (22%)	7/10 (70%)	5/10 (50%)	25/49 (51%)
	10	0/10 (0%)	3/10 (30%)	1/9 (11%)	2/10 (20%)	4/10 (40%)	10/49 (20%)
	26	2/10 (20%)	4/9 (44%)	1/10 (10%)	0/9 (0%)	5/9 (55%)	12/47 (26%)
	37	7/10 (70%)	2/10 (20%)	0/9 (0%)	2/10 (20%)	2/10 (20%)	13/49 (27%)
Total		20/69 (29%)	27/68 (40%)	9/67 (13%)	13/69 (19%)	27/69 (39%)	96/293 (33%)

Storage period and temperature did not have any significant effect (P=0.58 and 0.74 respectively) on MDV copy number in spleen recovered from infected chickens, nor was there significant interaction between these effects (Figure 1A). There was no correlation (r=0.044) between MDV content in spleen at day 7 of infected chickens and the percentage of chickens MDV positive (infectivity) (Figure 1B).

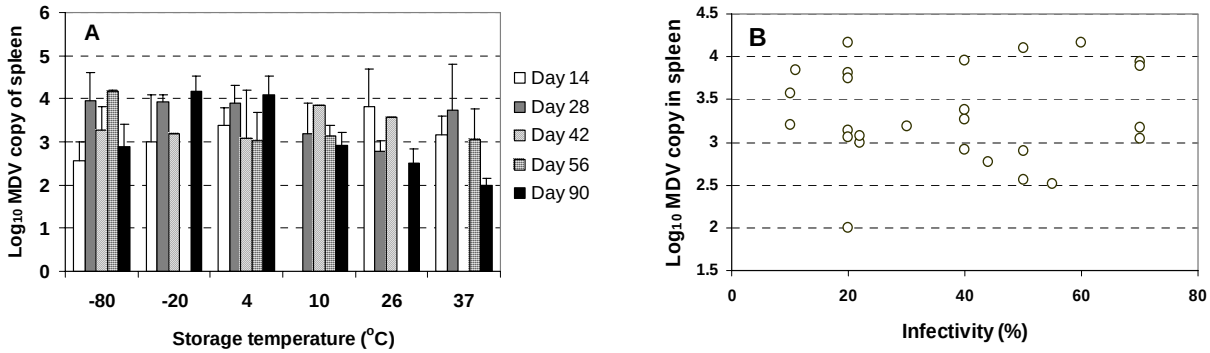

Figure 1: A. Mean MDV copy number per mg of spleen at day 7.
B. Correlation between MDV content and dust infectivity.

There was a significant (p= 0.001) effect on storage time, but not temperature (P= 0.157), on the concentration of MDV in dust. The mean $\log_{10}$ VCN in dust for day 0, 14, 28, 42, 56 and 90 were 4.95, 5.28, 5.58, 5.52, 5.51 and 5.58 respectively.

IV. DISSCUSSION

Our 1st hypothesis, that the quantity of MDV DNA in chicken dust will not be affected by storage temperature or duration, was partially supported as there was no significant effect of temperature on MDV content. However, there was a significant change in virus content over time, with increasing levels over the first 14 days before levels plateaued thereafter. This may reflect a true phenomenon with enzymic or other factors increasing extraction yield of viral DNA during the early post-shed period, or it may reflect differences in extraction efficiency due to laboratory causes, despite the use of standard kits.

The second hypothesis was also only partially supported, as the *in vivo* infectivity of dust was lower after storage at higher temperatures compared to lower temperatures. However, contrary to the hypothesis there was no decline in infectivity with storage time to 90 days. Our infection model showed high variability in the rate at which chickens were infected at various different time periods. This may be due to variation in the challenge method, although the same operators and equipment were used for each inoculation. There may also be variability in the genetic resistance levels between different batches of commercial chickens may also be partly responsible, although this would not explain the large variation between treatments on any given day of storage.

As there was no association between the infectivity of dust for any particular group, and MDV content of spleen of MDV positive chickens at day 7 post-infection, the third hypothesis was rejected. This result is not completely unexpected as 7 days is a long period for viral replication to occur in the host, and it is reasonable to assume that host factors may influence MDV content after 7 days to a greater extent than initial infective dose.

The main finding of this study is that dust stored at all temperatures, including 37˚C, remained infective for 90 days with no apparent decline in infectivity over this period. This finding is inconsistent with Carrozza *et al.* (1973), who reported loss of infectivity after 28 days storage at 37˚C. This can probably be explained by the greater infective dust dose in the present experiment and the more sensitive molecular determinants of infection used.

The prolonged retention of infectivity of MDV at even adverse high temperatures reported in this study reinforces the importance of cleanout and disinfection procedures in controlling the disease. The study is ongoing and infection rates after longer storage periods will continue to be assessed.

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EFFECTS OF HVT VACCINATION-CHALLENGE INTERVAL AND EXTERNAL CONTAMINATION LEVEL ON MAREK'S DISEASE TRANSMISSION AMONG BROILER CHICKENS – A MATHEMATICAL MODEL BASED ASSESSMENT

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Summary

The effects of HVT vaccination-challenge interval and external contamination level on Marek's disease (MD) transmission in one shed of broiler chickens were evaluated on the basis of the simulations of a mathematical model. Results of the simulations demonstrated that increases in vaccination-challenge interval are associated with exponential decreases in MD tumour and death cases. An assessment of the effects of HVT vaccination-challenge interval with external contamination level of 107 viral copy numbers per cubic metre air (VCN/m^3 air) suggested that an increase in vaccination-challenge interval from 0 to 5 days, decreased tumour cases at day 50 from 43.5% to 15.8% and death cases at day 50 from 20.5% to 5.1%. MD tumour and death cases at day 50, however, were only influenced by external contamination level within a narrow range (1-5×106 VCN/m3 air). These model-based simulations have showed that the longer the HVT vaccination-challenge interval, the fewer MD tumour and death cases. External contamination level over 5×106 VCN/m3 air had little effect on disease outcomes.

I. INTRODUCTION

Marek's disease (MD) is a worldwide problem in chicken farming. The economic impact of MD on the poultry industry was thought to be about US$1-2 billion annually (Morrow and Fehler, 2004). Effective vaccination was the main strategy for MD control in broiler flocks (Bublot and Sharma, 2004) and the main vaccine used in Australia is herpesvirus of turkeys (HVT) vaccine. The efficacy of vaccination is considered to be greatly influenced by the HVT vaccination-challenge interval and background contamination level (biosecurity status). Background contamination may be of internal origin (carryover from previous batch) or external origin (from infected birds in other sheds or other farms). MD is transmitted by inhalation of infected feather dust (dander).

This paper describes our efforts in evaluating the effects of HVT vaccination-challenge interval and initial external contamination level on MDV transmission in a shed of boiler chickens based on the mathematical model assessment (Gao *et al.*, 2004). The simulations assume that initial challenge is due to external contamination via airborne dander from other flocks, or residual contamination from the previous batch.

II. METHODS

For each value of vaccination-challenge interval between 0 and 15 days and each value of initial external contamination level from 0 to 10^7 MDV viral copy numbers (VCN)/m^3 air, the numbers of broiler chickens in the different disease stages of MD over time in one shed were

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obtained from the simulations of our mathematical model (Gao *et al.*, 2004) Figure 1). The mathematical software MATLAB (Ceanet, Sydney) was used for all simulations. The effects of varying HVT vaccination-challenge interval and level of airborne contamination were based upon the experimental results of Islam *et al.*, (2004) using HVT-vaccination of commercial Cobb broiler chickens at hatch and challenge at various times post vaccination with serotype-1 MDV strain MPF-57. MD infection probability (P) that an uninfected bird challenged with MDV load (V, VCN/m^3 air) becomes infected is expressed as $P = \theta V$ if $V \leq 1.162\times10^8$, and $P = 1$ if $V > 1.162\times10^8$, where V is VCN/m^3 air; θ is the increase in rate of MD infection probability per unit of increase of VCN/m^3 air (the increase in P per day per VCN/ m^3 air) and is taken to be 8.6×10^{-9} based on the data from isolator experiments at University of New England that 100% of birds will get infected in 14 days of continuous contact with MDV load of 8.3×10^6 VCN/m^3 air.

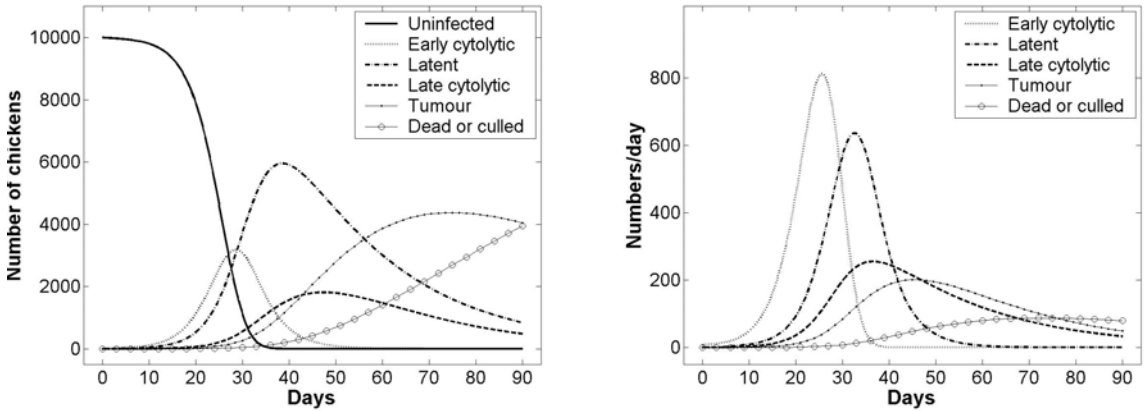

Figure 1. Model simulation of the numbers of chickens in different stages of MD under the assumption that there are 10,000 broiler chickens in one shed with no vaccinal protection and a low level of initial challenge (10^5 VCN/ m^3 air) (**Right plot**). Epidemic curves of MD incidence (new cases per day) at various disease stages (**Left plot**).

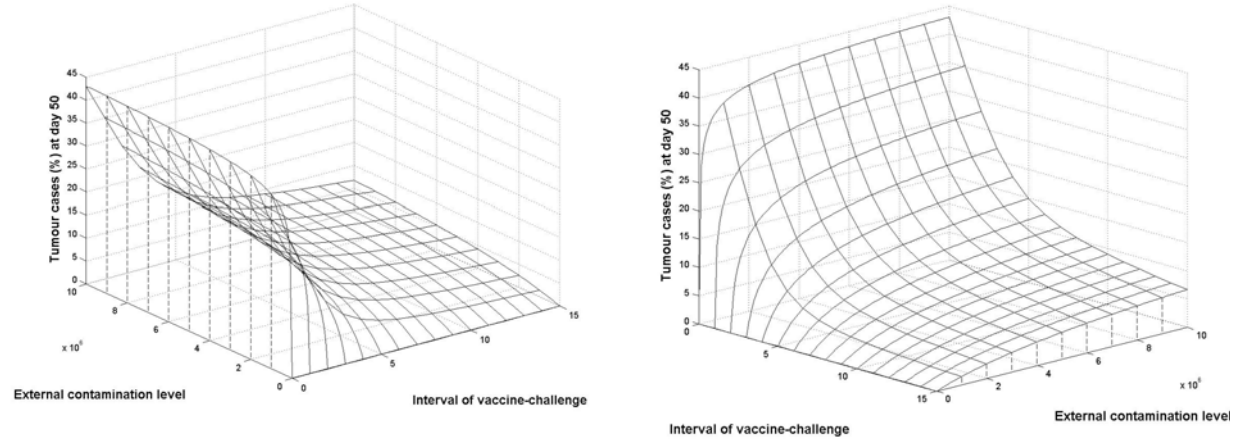

Figure 2. Mathematical model based simulation surface depicting the effects of HVT-vaccination-challenge interval (varying from 0 to 15 days) and external contamination level (varying from 0 to 10^7 VCN/m^3 air) on MDV transmission in terms of tumour cases (%) at day 50. The **left plot** is the front elevation of model simulated surface and the **right plot** is the side elevation.

III. RESULTS

To illustrate the mathematical model-based simulations, the following example was based upon a flock of 10,000 unvaccinated broiler chickens reared from day 0 in a shed which was not contaminated with MDV and in which no chickens were infected at the time of placement. The example assumes all birds were vaccinated with cell-associated HVT (CaHVT) virus at hatch (day 0) and external contamination occurred from days 0 to15 with varying contamination level from 0 to 10^7 VCN/m^3 air. The response surface of tumour cases (%) is shown in Figure 2.

Increases in HVT vaccination-challenge interval were associated with exponential decreases in tumour cases. An increase in vaccination-challenge interval from 0 to 5 days resulted in a decrease of tumour cases from 43.5% to 15.8% at a fixed external contamination level of 10^7 VCN/m^3 air (the left plot in Figure 3). However increases in external contamination level above 5×10^6 VCN/m^3 air caused only very small increases in tumour cases. An increase in external contamination level of 2-fold (100%) from 5×10^6 to 10^7 VCN/m^3 air corresponded to an increase in tumour cases from 42.8% to 43.5% with a vaccination-challenge interval of 0 (the right plot in Figure 3).

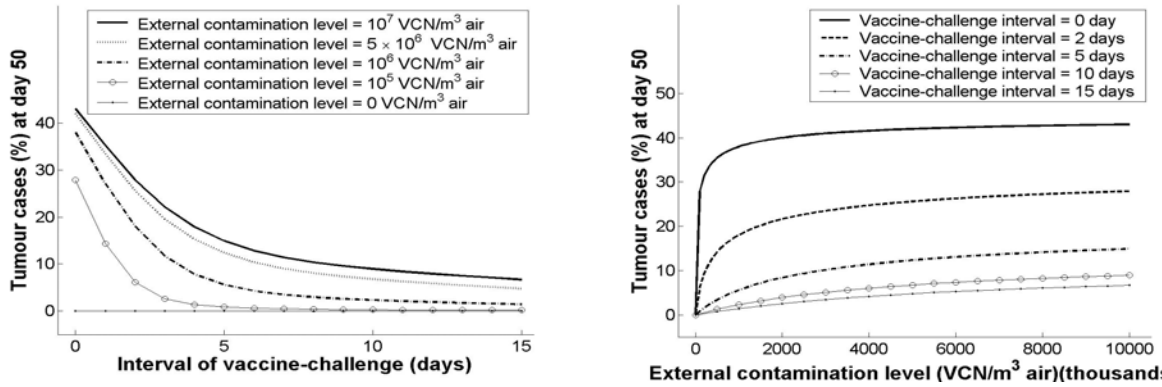

Figure 3. Effects of HVT vaccination-challenge interval on tumour cases at day 50 under five different levels (VCN/m^3 air) of external contamination based on simulation of mathematical model (**Left plot**). Effects of external challenge level on tumour cases at day 50 with five different HVT vaccination-challenge intervals based on simulation of mathematical model (**Right plot**).

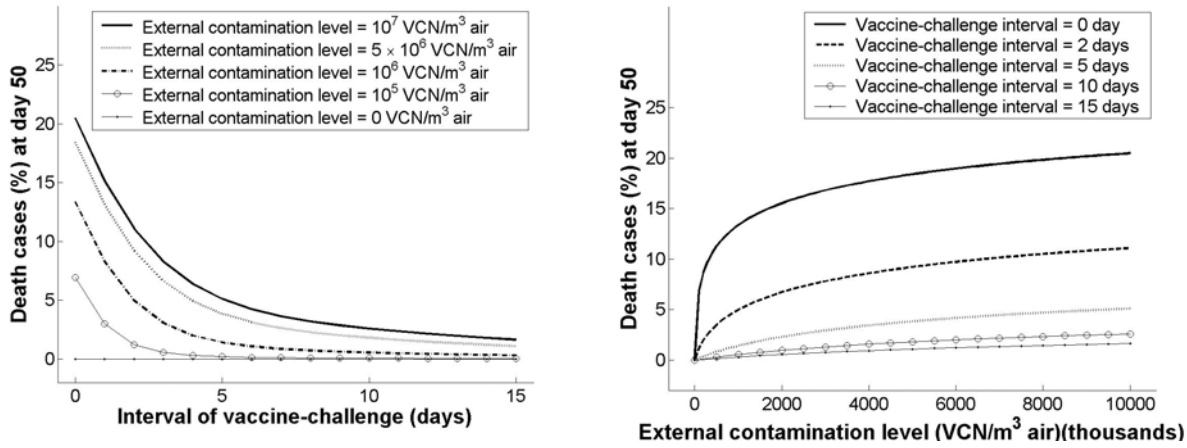

Figure 4. Effects of HVT vaccination-challenge interval on death cases at day 50 under five different levels of external contamination (MDV VCN/m^3 air) (**Left plot**). Effects of external challenge level on death cases at day 50 with five different HVT vaccination-challenge intervals (**Right plot**).

The simulated surface of MD death cases shows a similar pattern to that of tumour cases. Effects of HVT vaccination-challenge interval and external contamination level on death cases are shown in Figure 4. An increase of 5 days in HVT vaccination-challenge interval from 0 to 5 days incurred a 75% reduction in death cases from 20.5% to 5.1% with external contamination level of 10^7 VCN/m^3 air (the left plot in Figure 4). An increase in external contamination level of 2-fold (100%) from 5×10^6 to 10^7 VCN/m^3 air corresponded to an increase in death cases of 2.1% from 18.4to 20.5% with 0 HVT vaccination-challenge interval (the right plot in Figure 4).

IV. DISCUSSION

The results of model-based simulations showed that increases in the HVT vaccination-challenge interval are associated with marked reductions in MD tumour and death cases (Figures 3 & 4). This is consistent with the experimental results (Islam *et al.*, 2002, 2004) that a longer HVT vaccination-challenge interval corresponded with a higher vaccinal protective index. This makes biological sense as the efficacy of live viral vaccines is dependant upon the establishment of the vaccinal virus and development of vaccinal immunity prior to challenge. These results support maximizing vaccination-challenge intervals and thus use of *in ovo* vaccination as a means of reducing early challenge following vaccination.

The model also suggests that initial external contamination level is important in determining the level of MD in a flock with MD increasing with increasing contamination to a threshold of around 5×10^6 MDV VCN/m^3 air (Figures 3 & 4). This is a prediction of the model which requires further validation, and determining the relationship between air contamination with MDV and infection rate in exposed birds is something our research group will be pursuing. In practical terms these simulations support the importance of good shed cleanout between batches and location of broiler farms in low density areas

All simulated results presented here are based on our mathematical model of Marek's disease epidemiology in broiler chickens. The data that the simulations are based on were from isolator experiments and fixed point challenge with high doses of MDV. This is probably the reason why HVT-vaccinated chickens still have significant MD (tumour cases, 15.8%) even with a challenge interval of 5 days (Figure 3). While the model requires continual validation against field and experimental data, it has already been proving extremely useful in identifying critical areas in the epidemiology of Marek's disease where information is lacking.

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NEWCASTLE DISEASE (ND) VACCINATION PROGRAMS FOR AUSTRALIAN BROILER CHICKENS

C. JACKSON[1] and G.J. UNDERWOOD[2]

Summary

The results of a number of laboratory and field trials on the efficacy of an experimental live ND V4 vaccine have been discussed in relation to their impact on the current ND eradication program in Australia. Evidence for reduced efficacy associated with ND maternal antibody (Mab) and when used in combination with infectious bronchitis (IB) vaccines raised concerns about the likely outcomes from the eradication program. Whilst some additional studies could assist in further evaluation of the now preferred day-old spray vaccination method, several alternate vaccination programs could be adopted in the interim.

I. INTRODUCTION

Evidence has been obtained that Australian broiler chickens can be protected against virulent ND virus challenge by vaccination from one week of age with the V4 strain of NDV (Bell, 1990; Arzey and Arzey, 2000). However, those studies were mainly undertaken in broiler chickens derived from parent flocks that had been exposed to endemic lentogenic field strains of NDV, which therefore had low levels of maternally-derived antibody (Mab). Recent use of inactivated vaccines as part of the current ND eradication program in Australia designed to provide long term protection to breeder flocks has resulted in high levels of ND Mab transfer to most broiler flocks. Vaccination of progeny from such flocks, particularly at day-old has resulted in lower than desirable levels of active ND antibody. This paper describes experiments that confirm this observation and makes suggestions as to how best to interpret responses to ND vaccination.

II. INTERFERENCE TO EFFECTIVE VACCINATION

Vaccination of chickens with live ND vaccines in the presence of ND Mab has been contra-indicated by a number of studies undertaken overseas (Allan, 1973; Westbury *et al.*, 1984). These contra-indications were based largely upon the poorer development of active antibody following use of live lentogenic vaccines. Studies on the response to V4 vaccine virus have produced similar results (Kim *et al.*, 1978; Westbury *et al.*, 1984). Generally it was found that maternal antibody HI titres above 2^3 would delay and depress the active antibody response to the live V4 vaccine. However, it was also generally concluded that young chickens vaccinated by the oro-tracheal/ spray routes were more resistant to challenge than non-vaccinated or parenterally vaccinated chickens due to a combination of passive antibody and local/ cell-mediated immunity (CMI). Holmes (1979) presented evidence that stimulation of local immunity can bypass interference by maternal antibody. More recently, Reynolds (2000) showed that whilst CMI may play a role in protection in the face of ND Mab, it also requires neutralising antibody to be present.

Further studies by Reynolds (2000) demonstrated that local secretory antibody at the mucosal surface provided protection when chickens with high levels of maternal antibody

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were challenged with virulent virus. Hence, protection may be obtained in the absence of significant levels of active ND antibody.

Hatcheries are also under pressure to apply infectious bronchitis (IB) vaccines concurrently with the live ND vaccines at day-old. Overseas, concurrent administration of these two vaccines has remained controversial despite the marketing and wide sale of combined IB and ND vaccines. Vaccine manufacturers have generally followed recommendations by Winterfield (1984) to increase the ND vaccine component some 2 to 3 $\log_{10}$ over the titre of the IB vaccine component.

III. REGULATORY VACCINATION REQUIREMENTS

Removal of current ND vaccination exemptions for Western Australia and the implementation of legislature in Tasmania will result in all states and territories obligated to mandatory vaccination programs by the end of 2004 as described in a set of Standard Operating Procedures (SOPs) developed by the Technical Working Group (TWG) of the ND Management Group (NDMG). The SOP for ND vaccination of broiler chickens recommends that chickens be preferably vaccinated with live V4 strain ND vaccine at 7-14 days of age. However, the SOP also allows farmers the option of vaccinating at day-old provided they can show evidence of equivalence to the preferred program. Adequacy of the response to vaccination is defined as the mean haemagglutination inhibition (HI) titre of the flock being at least log 2^3 with at least 66% of the individual samples reaching a HI titre of 2^3 by 35 days of age. The latter program retains control of ND vaccination in hands of the hatchery where it has been claimed that improved uniformity (coarse-aerosol spray vaccination) and lower cost can be achieved than through administration by drinking water undertaken by broiler growers.

IV. STUDIES BY BIOPROPERTIES PTY LTD (BPL)

Over the past two years, as part of the application for registration, BPL has undertaken a series of laboratory and field studies on the safety and efficacy of a new seed of the V4 vaccine virus in chickens. It has used the existing registered live V4 vaccine as a benchmark in many of the trials. Whilst the trials were designed towards satisfying the regulatory requirements, they also touched on many of the variables described above that can impact on the success or failure of the live ND V4 vaccine relative to SOP requirements.

The following studies have been undertaken with BPL V4 vaccine:

1. The ND antibody response of SPF chickens vaccinated at 14 days of age
2. The ND antibody response of SPF chickens vaccinated at 14, 42 and 70 days of age
3. The ND antibody response of SPF chickens vaccinated at day-old.
4. The ND antibody response of ND Mab negative broilers vaccinated at day-old.
5. The ND antibody response of ND Mab positive broilers vaccinated at day-old
6. The ND antibody response of Mab positive commercial broilers vaccinated at either 7, 12 or 17 days of age.
7. The ND antibody response of commercial broilers vaccinated at day-old and 17 days of age compared to vaccination at 17 days of age only
8. The ND antibody response of SPF chickens vaccinated at day-old with combined ND V4 and IB vaccine compared to ND V4 and IB vaccines alone.
9. The ND antibody response of SPF chickens vaccinated three weeks after vaccination with Infectious bursal disease virus (Strain V877)
10. Field trials of ND V4 vaccines in commercial broiler chickens in two states.

V. SUMMARY OF RESULTS FROM THE STUDIES

SPF and Mab negative broiler chickens responded rapidly (within 14 days) and exceeded the SOP titre requirements by a wide margin (>2.0 Log_2 HI units). However, the ND antibody response in Mab positive broilers was delayed and inferior to Mab negative broilers. Antibody levels declined with age to approximate the SOP minimum level where they remained before increasing marginally by 35 days of age. Unvaccinated in-contact chickens exceeded the SOP minimum level by day 28 post-vaccination. Commercial broiler chickens responded more rapidly and exceeded the SOP minimum requirements when Mab titre levels were lower at the time of vaccination. Mab levels above 2^3 were associated with delayed and lower active antibody responses. A single ND V4 vaccination at 17 days of age gave a superior active antibody response to a day-old vaccination followed by vaccination at 17 day of age. The ND antibody of SPF chickens to a combined ND + IB vaccine exceeded the SOP minimum titre by a wide margin. However, the antibody response was lower than that of ND V4 given alone. The ND antibody response in SPF chickens that had been previously vaccinated with an IBD vaccine exceeded the SOP minimum titre by a wide margin. In field trials involving some 36 broiler flocks (2.5 million birds), the experimental BPL vaccine provided active antibody levels that exceeded the minimum SOP titre in 91% of flicks compared to 64% following the administration of the reference ND V4 vaccine. Broiler flocks were vaccinated at 10 days of age when the mean Mab titre was 2^2.

VI. RECOMMENDATIONS FOR BROILER VACCINATION IN AUSTRALIA

The studies undertaken by BPL produced results that were consistent with data previously observed overseas and in Australia on the efficacy of ND V4 vaccines. Interference by ND Mab was clearly evident with ND V4 vaccines particularly when they were administered in the face of high levels of ND Mab. The delayed and lower humoral response observed in these studies due to the presence of high Mab levels suggests the possibility that field viruses more virulent than V4 may have a greater opportunity for replication in these flocks if they are able to replicate in the presence of higher levels of Mab than the V4-strain vaccine. Concurrent administration of IB vaccine with ND V4 vaccine at day-old could further reduce the active antibody response. This could be particularly so when arbitrary proportions of the two vaccines are chosen rather than use of a correctly formulated combined vaccine.

As vaccination at day-old has now become the currently preferred program by most companies, the results described above should raise concerns as to the overall efficacy of this type of program. Whereas, broiler chickens with low levels of ND Mab could respond adequately and possibly seed the broiler shed, a high level of transfer from breeder flocks could well lead to inadequate humoral antibody responses. In the absence of adequate humoral antibody responses from day-old vaccination, dependence on local antibody and CMI for life-long broiler protection is contrary to well established overseas recommendations (MAFF 1974). These overseas programs normally recommend a second vaccination at about 18-21 days of age to stimulate high levels of neutralising antibody.

The control of endemic virulent and precursor viruses in Australia following the 1998-2002 outbreaks is partly dependent on the objective of the ND V4 vaccine out-competing those viruses. Although there is evidence that ND V4 vaccine will reduce the excretion of precursor viruses (Daniels, 2003), the level and frequency of challenge from such viruses is not well understood. The continued application of sub-optimal programs could well encourage the evolution of further precursor or virulent endemic strains.

VII. FUTURE DIRECTIONS

The TWG of the NDMG should consider whether the current recommended SOPs meet the overall objectives of the eradication program. Additional information on the response to vaccination under field conditions is urgently needed. Evidence of protection following day-old vaccination should be obtained through challenge studies or though evaluation of CMI/ local immune responses to ND V4 vaccine.

In the interim, a number of alternate programs could be considered, as follows:

a) Delay primary vaccination until mean flock ND Mab levels fall below 2 [3]
b) Following day-old spray vaccination, revaccinate at about 18-21 days of age
c) Reduce ND Mab levels in broiler progeny by reducing the administration of inactivated vaccines to breeding stock.
d) Optimise the application of ND V4 and IB vaccines when used together.

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AN EVALUATION OF DIETARY OMEGA-3 FATTY ACIDS ON AVIAN IMMUNITY

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Summary

The effect of feeding broiler chickens an enriched source of marine omega-3 (n-3) fatty acids on their immune function was examined. The n-3 supplement was fed between days (d) 0-42 or d22-42. Generally feeding n-3 for either period of time increased total serum immunoglobulin (Ig) titres, but reduced the antigen-specific immune response following vaccination. Further, supplementation induced some changes in blood derived Ig isotypes but had no effect on T-cell subsets.

I. INTRODUCTION

As the polyunsaturated fatty acid (PUFA) profile of chicken meat reflects that of the chickens' diet (Walisundra *et al*., 1989), feeding broilers n-3 enriched diets is one possible mechanism to provide humans with n-3 enriched food sources (Howe *et al.*, 2002). PUFA's are known to affect the immune response in humans (Calder, 2001). Studies in chickens have demonstrated varying effects of PUFA's on the avian immune response (Wang *et al*., 2000; Sijben *et al*., 2002). Therefore, this study was designed to assess the impact of feeding 10% PorcOmega (POM), a patented fish meal supplement enriched with n-3 fatty acids, on immune function in chickens.

Day old male broilers were randomly allocated to dietary treatment groups: Control – received the basal diet, POM 0-42 - receiving the POM supplemented diet from d0-42 and POM 22-42 - which received the POM supplemented diet from d22-42. At 21 and 35 days of age all birds received a subcutaneous vaccination of T. toxoid emulsified in an equal volume of Freund's incomplete adjuvant. Blood samples were collected on d 21, 28, 35 and 42 for analysis of total immunoglobulin (Ig) and T. toxoid-specific IgM and IgG antibody titres using an enzyme-linked immunosorbent assay. On d 29, 37 and 43 peripheral blood derived $CD4^+$ and $CD8^+$ T cell subsets, and IgM^+, IgG^+, IgA^+ and Ia^+ cellular subsets of humoral immunity were assessed via flow cytometry (Muir *et al.*, 2002)

Birds fed the POM diet generally demonstrated an increase in average total serum immunoglobulin (Ig) throughout the study compared to the control birds. At days 21, 28 and 35 birds fed POM 0-42 had significantly higher ($P<0.05$) average total serum Ig compared to the control birds. Birds fed POM 22-42 had average total serum Ig titres between those of the control and POM 0-42 treated birds. Anti-T. toxoid IgM titres of birds fed POM 0-42 were lower than the control birds on days 28 ($P<0.05$) and 35 (not statistically significant). Birds fed POM 22-42 did not demonstrate any significant change in anti-T. toxoid IgM titres compared to the control birds on either days 28 or 35. Similarly anti-T. toxoid IgG antibody titres of both the POM treated groups were lower than the control birds on d 28 (not statistically significant) and 35 ($P<0.05$ for POM d22-42).

The POM treatment had no significant effect on peripheral blood-derived T-cell subsets on either d 29, 37 or 43. On d 29 birds fed POM 0-42 had an increase in the mean

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percentage of IgG^+ (P=0.08) and IgA^+ (P=0.06), and on day 43 birds fed POM 22-42 had a higher percentage of IgA^+ (P<00.5) cells compared to the control group. Interestingly, POM 0-42 had a significant increase (P<0.05) in the percentage of Ia^+ cells on day 37 compared to birds fed the basal diet.

This study has identified the potential for POM to increase the total circulating levels of immunoglobulin in chickens. However, that response was not a systemic response to the vaccine antigen. Therefore, the actual character of the enhanced immune response needs further elucidation. Interestingly, the systemic antibody response to the vaccine antigen in birds fed the POM diets was not enhanced, and, in some instances, it was significantly lower than the control birds.

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ALPHA TOXIN SEQUENCES OF AVIAN ISOLATES OF *CLOSTRIDIUM PERFRINGENS*

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Summary

Clostridium perfringens causes several diseases in humans and animals, and produces an alpha toxin that is thought to be a key virulence determinant of Necrotic Enteritis (NE) in chickens. Previously, the alpha toxin from mammalian-derived strains of *C. perfringens* has shown to be highly conserved in sequence and biochemical properties. Recently, characterisation of the first alpha toxin gene isolated from an avian strain of *C. perfringens* from a diseased swan (SWCP), showed that the deduced toxin had only 80% amino acid sequence identity with other *C. perfringens* alpha toxins. We report here that 25 isolates of *C. perfringens* strains collected from chickens diagnosed with NE from a number of different outbreaks and locations have a highly conserved alpha toxin sequence that is significantly different from the SWCP isolate. We have shown that the predicted alpha toxin from these isolates have at least 98% amino acid sequence identity to alpha toxin from mammalian isolates. Therefore, it is concluded that divergent alpha toxin sequences are not common in avian isolates.

I. INTRODUCTION

Clostridium perfringens is a widely distributed pathogen (Hatheway, 1990) commonly isolated from the environment and the gastro-intestinal tract of birds and mammals (Hein and Timms, 1972; Willis, 1984). *C. perfringens* isolates are classified into five types (A to E) according to the production of four major toxins (alpha, beta, epsilon, and iota toxins) (MacLennan, 1962; McDonel, 1980). The alpha toxin has been implicated in several diseases (Rood, 1998), including Necrotic Enteritis (NE) in chickens (Baba *et al.*, 1992; Long and Truscott, 1976). The alpha toxin structural gene (*plc* or *cpa*) has been isolated and characterised from several strains of *C. perfringens* (Ginter *et al.*, 1996) and the encoded proteins found to be highly conserved in all but one recently identified strain (Justin *et al.*, 2002). This strain (SWCP) was isolated from a diseased swan. Justin *et al.* (2002) found that the SWCP alpha toxin had only 80% amino acid sequence identity to the other *C. perfringens* alpha toxins and questioned if this difference in sequence was typical of all avian isolates. In this study we examined the encoded alpha toxin sequences from a range of isolates of *C. perfringens* derived from chickens to determine if the divergent SWCP alpha toxin sequence is common in avian isolates.

II. METHODS

The *C. perfringens* strains used in this study were isolated from chickens displaying clinical signs of NE (Al-Sheikhly and Truscott, 1977). Genomic DNA was prepared as template for PCR by boiling crude cells in water for 3 mins. PCR conditions and reaction concentrations were as described by Meer and Songer (1997). PCR primers were designed from the sequence of *C. perfringens* strain 13 (Shumizu *et al.*, 2002), and two PCR products, together encompassing the complete *plc* gene, were amplified and sequenced from each of

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the 25 strains to determine the amino acid sequence of the encoded alpha toxin. Each alpha toxin gene was sequenced twice, using independently generated templates, to confirm that the changes were not due to sequencing or PCR errors.

III. RESULTS

In each isolate the full-length sequence was predicted to be 398 amino acids. The toxins were all highly conserved in amino acid sequence (Figure 1), and only five different alpha toxin sequence types (I–V) were identified from the 25 isolates sampled from several different NE outbreaks from different locations. All the alpha toxin sequence types from the chicken isolates closely resembled the toxin from the human isolate strain 13 (Shimizu *et al.*, 2002), with greater than 98% identity, but differed considerably from the swan isolate (SWCP), with only 82-84% identity. The SWCP isolate has between 67-70 amino acid differences from the chicken isolates, and of all the changes in the SWCP sequence, only two amino acid changes are found in the field isolates reported here.

IV.DISCUSSION

Sequence type I has only one amino acid difference from the strain 13 sequence and was the most common alpha toxin found in the sampled group. The threonine to alanine substitution is within the putative signal peptide sequence (Titball *et al.*, 1989) and would not be present in the mature protein and therefore cannot affect the properties of the mature toxin (Ginter *et al.*, 1996). Alanine is also found in this position in the alpha toxin signal sequence from strain NCTC8237 (Leslie *et al.*, 1989). Sequence type II has two amino acid differences compared to the strain 13 sequence, and includes the threonine to alanine change at position 13, and an isoleucine to valine substitution at position 373. Sequence type IV contains two amino acid changes – the threonine to alanine change (position 13), and a leucine to methionine alteration (position 54). The latter amino acid substitution is also seen in the alpha toxin from *C. perfringens* strain 8-6 (Saint-Joanis *et al.*, 1989) and the phospholipase C from *Clostridium novyi* (Tsutsui *et al.*, 1995). The type V alpha toxin sequence contains three amino acid substitutions compared to strain 13 – the common threonine to alanine substitution at position 13, an aspartic acid to alanine change at position 202 (also found in the SWCP sequence), and an alanine to threonine substitution at position 205. The most distinct alpha toxin sequence type seen in this study was type III. It contains six amino acid changes compared to the strain 13 alpha toxin, including the isoleucine to valine substitution at position 373, and a methionine residue that replaces a lysine residue at position 54. Overall, the amino acid differences detected in this study were minimal compared to the sequences differences observed between SWCP and strain 13. The differences that were found in the alpha toxin sequences of the chicken isolates were all the result of single base substitutions, and did not significantly alter the predicted physical properties of the encoded proteins (MW 45.5kDa, pI 5.58, overall negative charge). Plating each of the strains onto egg-yolk agar (Awad *et al.*, 1995) produced a zone of precipitation around the colonies, indicating that each strain was able to produce functional alpha toxin, although the levels of toxin activity varied. Toxin activity of some strains with identical alpha toxin sequence was markedly different, indicating that the variable toxin levels must be due to differences in strain growth or expression rather than differences in specific activity.

None of the predicted amino acid differences occurred in the active site (Guillouard *et al.*, 1996; Nagahama *et al.*, 1997; Nagahama *et al.*, 1995) or in the calcium binding pocket in the C-terminal domain (Justin *et al.*, 2002), regions of the protein thought to play a key roles in membrane-protein interactions. The valine to isoleucine change (position 373) is located in

a region that is predicted to be a flexible surface-exposed loop linking two helices (Ginter *et al.*, 1996). However, this change is a conservative substitution that is unlikely to affect the tertiary structure of the protein. In regions of the protein that are reported to be important for structural integrity, such as between residues 87-95 and 100-118 (Naylor *et al.*, 1998), there are no amino acid changes in the chicken isolates.

Williamson and Titball (1993) showed that the protective antibody response to alpha toxin is directed against the C-terminal domain of the protein (amino acids 247-370 in the mature toxin). Unlike SWCP, none of the chicken isolates have any amino acid changes in this C-terminal domain of the toxin. Therefore, it is predicted that animals vaccinated with the C-terminal domain would elicit an immune response against the alpha toxin from these chicken-derived strains of *C. perfringens*.

In conclusion, the *C. perfringens* strains from chickens suffering Necrotic Enteritis have highly conserved alpha toxin sequences that closely resemble those of the alpha toxin found in mammalian isolates of *C. perfringens,* but are significantly different from that of the SWCP isolate obtained from a diseased swan, the only avian derived strain previously characterised. These results are encouraging for the development of diagnostic tests and vaccines for the control and treatment of *C. perfringens* infections of commercial chickens as they signify that vaccines and tests used for other *C. perfringens* infections may be able to be used in this host species.

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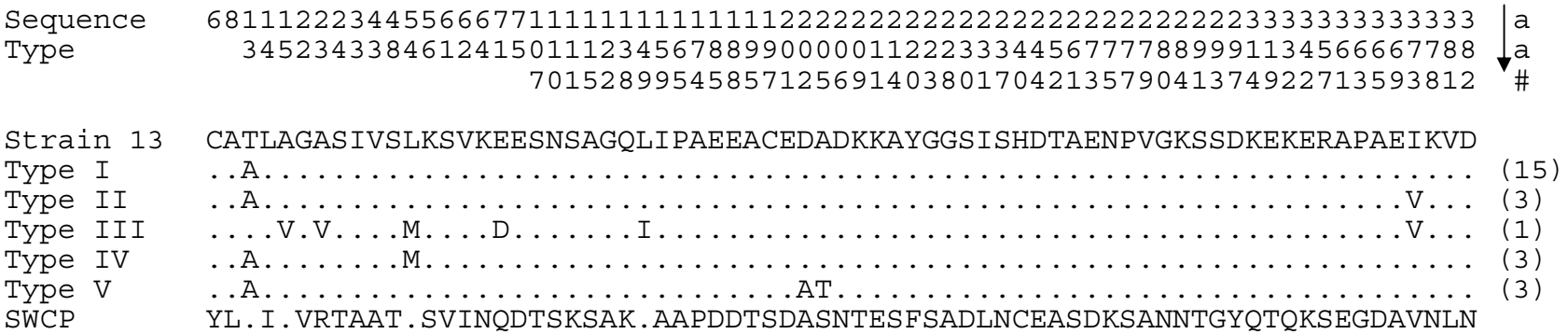

Figure 1. Non-conserved amino acids in a multiple alignment of five alpha toxin sequence types (I-V) from chicken isolates. The sequences of alpha toxins from the swan isolate SWCP (Justin *et al.*, 2002) and strain 13 (Shimizu *et al.*, 2002) are included for comparative purposes. Conserved amino acids at which every amino acid is the same, including within the SWCP alpha toxin, are not shown. Amino acid sequence positions of the non-conserved amino acids are shown above the alignment, and refer to the full length alpha toxin protein (397aa). Numbers are shown running vertically down, as depicted by the arrow. Amino acids are represented by the single letter code. The number of strains with the specific alpha toxin sequence type is shown in parentheses after each type sequence.

GENETIC BACKGROUND OF AN INDIVIDUAL DETERMINES THE SENSITIVITY OF INNATE COMPONENT OF HUMORAL IMMUNITY FOR A NOVEL ENVIRONMENTAL STRESSOR

B. HANGALAPURA

Summary

Future poultry husbandry aims at enhanced animal welfare, with minimal use of preventive medical treatments. These husbandry conditions will resemble more natural or ecological conditions. Under such farming systems, animals will undergo various physical and climatic stresses (cold, heat, wind, etc), infectious diseases and social stress. So the animals under such future conditions must be able to cope with much more dynamic environments than nowadays, preferably without an increase in production costs and risk of diseases. Therefore, there is a need to understand how environmental stressors affect immune system of chickens. In the present study, an attempt has been made to understand the effect cold exposure (10^oC) for a period of 10 weeks on both innate and adaptive components of humoral immune system of chickens selected for high (H) and low (L) antibody responses. H line birds had significantly higher innate and adaptive humoral immunity when compared with L line birds. Cold exposure significantly suppressed innate component of humoral immunity only in H line birds but not in L line birds. However, no significant effect was found on adaptive component of humoral immunity in both lines. Present study suggests that sensitivity of innate component of humoral immunity for environmental stressors is determined by the genetic background of an individual.

I. INTRODUCTION

Future poultry husbandry conditions will resemble more natural or ecological conditions. Under such farming systems, animals will undergo various physical and climatic stresses (cold, heat, wind, etc), infectious diseases and social stress. So the animals under such future conditions must be able to cope with much more dynamic environments than nowadays, preferably without an increase in production costs and risk of diseases. Moreover, genetically determined differences in susceptibility to environmental stressors have been proposed. Therefore, there is a need to understand how environmental stressors affect the immune system of chickens with different genetic background.

II. MATERIAL AND METHODS

a) Experimental design

In the present study, ISA warren brown layer lines divergently selected for high (H line) or low (L line) antibody responses to SRBC for 22 generations were used. At 20 days of age, two groups of 10 hens each of H and L line were randomly assigned to control (CG) or treatment group (TG). CG was kept according to routine procedures for layer hens in brooder cages throughout the experiment except for the feed. TG was housed in one of the two climate chambers in similar cages (Verstegen et al., 1987) which were maintained at 21.7 ± 1.9^oC with relative humidity (RH) of 58±8% (control temperature). After a three day acclimatization period (23d of age), temperature of the climate chambers was decreased to 10 ± 0.4^oC (RH 70±1%). After 6 weeks of cold stress, CG and TG birds were immunized (s.c) with 1 mg Complete Freund's adjuvant (CFA) / bird. On a weekly interval one ml blood samples were collected. Plasma samples from all sampling days were used to measure

natural antibody titers binding lipopolysaccharide (LPS). Plasma samples from d 0, 1, 7, 14 and 21 after immunization were used to measure specific antibody titers to *Mycobacterium butyricum*.

b) Assay for antibody level determination.

Antigen specific antibodies binding to *M. butyricum* or natural antibodies binding LPS were determined in individual plasma by indirect two-step ELISA as described by Hangalapura *et al*., (2004).

III. RESULTS

a) Specific antibody responses to *M. butyricum* (MB).

There was no significant effect of CS on specific antibodies binding MB in both H and L line birds (Table 1). MB binding plasma antibody levels were significantly higher in H line than L line birds (Table 1).

b) Natural antibodies binding LPS.

Plasma natural antibodies binding LPS were significantly suppressed in the H line birds, whereas plasma Nabs binding LPS were significantly enhanced in the L line birds (Table 1). Level of natural antibodies binding LPS was significantly higher in H line than the L line birds (Table 1).

IV. CONCLUSION

Present findings demonstrate that the model environmental stressor does not affect the adaptive humoral immune responses. However, the effect of a novel environmental stressor on the innate component of humoral immunity is determined by the genetic background of an individual.

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Table 1. Effect of Control (CG) v/s Cold Stress (TG) on average *Mycobacterium butyricum* (MB) binding specific and Lipopolysaccharide (LPS) binding natural antibody levels of chicken lines divergently selected on High and Low antibody responses to sheep erythrocytes

		Antigen	
Line	Treatment	MB	LPS
High	CG	7.63[a]	5.15[a]
	TG	7.52[a]	3.96[c]
Low	CG	6.07[b]	3.14[b]
	TG	5.87[b]	4.12[c]
	SEM	0.27	0.29
	Main effects		
	Treatment	NS	NS
	Line	***	***
		H>L	H>L
	Treatment x Line	NS	***

[a-c]Means within a column lacking a common superscript differ significantly ($P < 0.05$).
*** $P < 0.001$

INFLUENCE OF DIVERGENT SELECTION BASED ON RESPONSE TO SHEEP RED BLOOD CELLS ON OTHER IMMUNOLOGICAL TRAITS IN WHITE LEGHORN CHICKEN

S. KUMAR[1] and B.M. SHIVAKUMAR[2]

Summary

Influence of divergent selection based on humoral response to sheep red blood cells (SRBC) at 5th day post immunisation on other immunological traits viz., mercaptoethanol resistant (MER) and sensitive (MES) antibody titres, serum concentrations of IgG and lysozyme (LZM) and cell mediated immune (CMI) response was assessed in White Leghorn chicken by standard methods and analysed by least squares analysis of variance. Divergent lines differed significantly for SRBC response, MES titre, IgG concentration and CMI response ($P<0.01$) and MER titre ($P<0.05$). High SRBC line generally had higher estimates than low SRBC line except CMI response. Findings suggested for incorporation of humoral response to SRBC in the breeding programs to improve general immunocompetence of the birds.

I. INTRODUCTION

The economics of poultry production needs reduction of inputs. Innate resistance to diseases is under genetic control (Gavora, 1993); hence, breeding for non-specific resistance is a viable approach for its genetic improvement. Bi-directional selection for response to sheep red blood cells (SRBC) has been found to alter the immune the components on chicken immune system (Siegal and Gross, 1980; van der Zijpp and Nieuland, 1986; Boa Amponsem *et al.*, 2001). So, assessment of the influence of such selection on other immune traits is mandatory. Hence, the present study was envisaged to study the influence of divergent selection based on antibody titres against SRBC on other immune traits in IWG-White Leghorn chicken. IWG strain has been selected for 25 generations for higher 40-week part period egg production, but never for any immunological parameter. In this study, antibody response to SRBC at 5^{th} days post infection (dpi), measured through hemagglutination (HA) test (van der Zijpp and Leenstra, 1980), was included as divergent criteria for developing high and low SRBC lines and its influence was assessed on other important immunological traits viz., mercaptoethanol (ME) resistant (MER) and sensitive (MES) antibodies, serum concentration of IgG and lysozyme (LZM) and cell mediated immune (CMI) response.

II. METHODS

Three hundred fifty four chicks belonging to high (189) and low (165) SRBC lines were evaluated for various immunological traits viz., mercaptoethanol (ME) resistant (MER) and sensitive antibodies (Ab), serum concentration of IgG and lysozyme and cell mediated immune response. The MER (IgG) Ab were assayed after treating the serum with 2 β-ME (Martin *et al*., 1989). The MES Ab, representing IgM Ab, were calculated as the difference of total Ab titre and MER Ab titre The serum IgG concentration was determined by single radial immunodiffusion (SRID) method on agarose plate (Manicini *et al*., 1965). Serum LZM concentration was determined by Lysoplate assay (Lie *et al.*, 1986). The CMI response was

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measured as "Foot web index", using intra-dermal (i/d) injection of Phytohaemagglutinin-A (Corrier and Deloach, 1990).

III. RESULTS

The least squares analysis of variance by full-sib model revealed significant differences ($P<0.05$) for MER and ($P<0.01$) for HA, MES, IgG and CMI traits in divergent lines. The serum LZM concentration remained unaffected by this divergent selection. The least squares means for HA, MER and MES Ab titres, LZM concentration, CMI response and IgG conc. were 10.42±0.40, 3.63±0.20, 6.79±0.23, 2.75±0.05µg/ml, 0.39±0.02mm, 12.75±0.30mg/ml, respectively in high SRBC response line and the corresponding values in low SRBC response line were 7.35±0.42, 2.89±0.21, 4.50±0.25, 2.79±0.06µg/ml, 0.52±0.03mm and 8.58±0.32mg/ml.

IV. DISCUSSION

Similar to the present observation, Siegal and Gross (1980) and van der Zijpp *et al.* (1983) reported significant differences in the HA titres in the divergent SRBC response lines of White Leghorn even after first generation of selection. Similarly, Martin *et al*. (1989) and Boa Amponsem *et al*. (2001) also observed higher MER and MES antibody titres in the high SRBC line. Serum lysozyme level remained unaffected with the divergent selection for SRBC response, the same was reported by van der Zijpp and Nieuwland (1986). Birds of high SRBC line had higher IgG concentrations than LRBC line. Earlier reports also revealed similar trend (Boa Amponsem *et al*., 2000). The observation of lower CMI response in high SRBC response line was similar to that reported by Kreukniet *et al.* (1994). The findings revealed that divergent selection based on sheep RBC response had significant effect on many immune traits; high line generally had higher values than the low line except for CMI response. It may be suggested that breeding of poultry for improvement of disease resistance along with layer traits could be achieved by appropriate breeding programs.

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CADMIUM DOWNREGULATES T- AND B-LYMPHOCYTE BLASTOGENESIS IN POULTRY

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Summary

Avian Lymphocytes isolated from the spleen of apparently healthy birds were treated with NOEL (No Observable Effect Level) $x10^{-2}$, NOEL$x10^{-3}$, NOEL$x10^{-4}$, NOEL$x10^{-5}$, NOEL$x10^{-6}$ & NOEL$x10^{-7}$ concentrations of cadmium chloride for 30, 60, 90 & 120 min. For the investigation of apoptosis, transmission (TEM) and scanning electron microscopy (SEM), agarose gel electrophoresis, Annexin-V biotin and avidin peroxidase conjugate staining was done. T- and B-Cell blastogenesis was assessed by Lymphocyte stimulation test (LST) using Concanavalin A (Con A) and Lipopolysaccharide (LPS) as mitogen, respectively. Both TEM and SEM revealed shrunken cells, margination of chromatin, karyorehxis, budding and phagocytised apoptotic bodies. Agarose gel electrophoresis revealed DNA fragmentation. Annexin ' V tagged apoptotic lymphoid cells exhibited a brown colour on their surface. There was significant reduction in delta OD of the lymphocytic cultures stimulated by Con A and LPS mitogens. From the present investigation, it can be concluded that cadmium chloride exerts its deleterious effects on avian lymphocytes via apoptosis even at very minute concentrations (NOEL$x10^{-7}$) and short exposure time (30 min.), which may lead to immunosuppression in poultry.

I. INTRODUCTION

Little work has been done regarding *in vitro* exposure of heavy metals in very minute concentration on cultured chicken lymphcocytes for 120 min. Use of cadmium, an environmental pollutant belonging to heavy metals, is in metal coating, paints, enamels, plastics, stabilizers of PVC, nickel-cadmium batteries, solders, low melting alloys, production of semiconductor and photovoltaic devices (Peterson and Alloway, 1979). Immunosuppression, due to cadmium, has been recorded in mice (Koller *et al.,* 1975; Krzystynaik *et al.,* 1987) and calves (Agrawal and Chauhan, 1997; Chauhan and Agrawal, 1998, 1999; Singh *et al.,* 2000). We hypothesized that cadmium induced immunosuppression could be due to apoptosis of T and B lymphocytes In the present study, the influence of NOEL dose of cadmium chloride on apoptosis as well as T-cell and B-cell blastogenesis of cultured avian lymphocytes has been recorded.

II. MATERIALS AND METHODS

Commercial preparation of cadmium chloride was purchased from Loba Chemie, India. *In vitro* cultures of avian lymphocytes from spleen were treated with NOEL$x10^{-2}$, NOEL$x10^{-3}$, NOEL$x10^{-4}$, NOEL$x10^{-5}$, NOEL$x10^{-6}$ & NOEL$x10^{-7}$ doses of cadmium chloride for different intervals i.e. 30, 60, 90 and 120 min. NOEL dose of cadmium chloride was taken as 1.61 ppm (Institoris *et al.,* 1999). Apoptosis in avian lymphocytes was detected by transmission and scanning electronmicroscopy (Malorni *et al.,* 1998; Chauhan, 2003), DNA fragmentation (Hermann *et al.,* 1994) and immunoperoxidase technique (Chauhan, 1998).

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Blastogenesis of T and B lymphocytes was assayed by lymphocyte stimulation test (Chauhan, 1998).

III. RESULT AND DISCUSSION

a) Scanning and transmission electron microscopy

A plethora of findings contributing to the knowledge of the process, e.g. the role of subcellular structures and organelles, have been widely published (Kerr *et al*., 1994; Allen, 1987; Allen, 1997; Bellomo *et al*., 1994; Sun *et al*., 2000). The nuclear outline often becomes convoluted prior to dissolution of nucleus into discrete fragments that scatter throughout (Singhal and Chauhan, 2001). Extensive chromatin clumping, cytoplasmic condensation, membrane blebbing and nuclear fragmentation was observed in cells undergoing apoptosis (Kerr *et al*., 1994). (generally these descriptive paragraphs would be better placed at the start of a section so the uninitiated reader can understand the significance of the reported findings)

The avian lymphocytes treated with NOELx10^{-2}, NOELx10^{-3}, NOELx10^{-4}, NOELx10^{-5}, NOELx10^{-6} & NOELx10^{-7} concentrations of cadmium chloride for 30, 60, 90 & 120 min were found to be shrunken and denser. The chromatin was pyknotic and packed into smooth masses applied against the nuclear membrane. Karyorhexis was observed in many cells. Detection of apoptotic bodies as well as condensed chromatin material revealed late as well as early stage of apoptosis, respectively. The changes were most prominent in cells treated with cadmium at NOELx10^{-2} concentration for 120 min while least in cells treated with cadmium at NOELx10^{-7} concentration for 30 min.

b) Apoptotic cell detection by immunoperoxidase

Exposure of isolated avian lymphocytes to NOELx10^{-2} dose of cadmium for 1 hr resulted in 44% Annexin-V positive apoptotic cells. The cells which were undergoing apoptosis displayed brown colour on their surface as a result of Annexin-V binding and immunoperoxidase staining. Few cells also exhibited vesicle like structures, probably representing apoptotic bodies.

Different changes on the surface of apoptotic cells such as the expression of thrombospondin binding sites, loss of sialic acid residues and exposure of phospholipids like phosphatidylserine (PS) were previously described (Wyllie *et al*., 1984). In the early stages of apoptosis, a change in the composition of the cell membrane occurs, and phosphatidylserine is translocated to the outer leaflet of the cell membrane, where it functions as a marker for recognition by other cells (Fadok *et al*., 1992).

The expression of Annexin-V to cell membrane, as observed during the current study, supported the idea that it is a feature of the early as well as late stage of apoptosis

c) Electrophoresis of apoptotic DNA

DNA isolated from avian lymphocytes treated with NOELx10^{-2}, NOELx10^{-3}, NOELx10^{-4}, NOELx10^{-5} & NOELx10^{-6} concentrations of cadmium chloride for 30, 60, 90 & 120 min and NOELx10-7 concentration for 60, 90 & 120 min revealed fragmentation of DNA. The DNA isolated from untreated or control cells showed intact band.

Fragmentation of chromatin into single or multiple nucleosomes is biochemical hallmark of apoptosis and one of the easiest ways to distinguish programmed cell death and toxic necrosis (Herrmann *et al*., 1994). Thus, in the present investigation, fragmentation of DNA with its ladder pattern indicated apoptotic cell death as a result of heavy metal exposure.

d) Cell proliferation assay

In the present study, there was significant reduction in delta OD of the lymphocytic cultures stimulated by ConA and LPS mitogens. Maximum reduction in blastogenic activity

was seen in cells exposed to NOELx10^{-2} dose of cadmium chloride for 120 min and minimum reduction in NOELx10^{-7} dose treated cells for 30 min in comparison to controls (Table 1 and 2). Present investigation indicates that cadmium chloride causes decrease in T and B cell blastogenesis even at very minute concentrations and short exposure time such as NOELx10^{-2}, NOELx10^{-3}, NOELx10^{-4}, NOELx10^{-5}, NOELx10^{-6} and NOELx10^{-7} concentration for 30, 60, 90 and 120 min.

Table 1. *In vitro* effects of cadmium on T-cell blastogenesis of avian splenic lymphocytes (Mean delta OD±SE)

Exposure time (min)	Control	Concentration of Cadmium (N=NOEL)					
		Nx10^{-2}	Nx10^{-3}	Nx10^{-4}	Nx10^{-5}	Nx10^{-6}	Nx10^{-7}
30	0.426 ±0.003	0.160 ±0.002**	0.173 ±0.007**	0.199 ±0.007**	0.285 ±0.003**	0.264 ±0.003**	0.249 ±0.009
60	0.497 ±0.012	0.107 ±0.004**	0.107 ±0.003**	0.183 ±0.005**	0.163 ±0.004**	0.224 ±0.006**	0.258 ±0.014**
90	0.454 ±0.011	0.105 ±0.007**	0.156 ±0.002**	0.126 ±0.002**	0.166 ±0.016**	0.274 ±0.009**	0.223 ±0.011**
120	0.383 ±0.003	0.182 ±0.004**	0.116 ±0.006**	0.104 ±0.005**	0.218 ±0.003**	0.238 ±0.017**	0.196 ±0.010**

**Significant difference ($P \leq 0.01$); *Significant difference ($P \leq 0.05$)

Table 2. *In vitro* effects of cadmium on B-cell blastogenesis of avian splenic lymphocytes (Mean delta OD±SE)

Exposure time (min)	Control	Concentration of cadmium (N=NOEL)					
		Nx10^{-2}	Nx10^{-3}	Nx10^{-4}	Nx10^{-5}	Nx10^{-6}	Nx10^{-7}
30	0.468 ±0.005	0.150 ±0.006**	0.170 ±0.006**	0.178 ±0.012**	0.163 ±0.009**	0.218 ±0.009**	0.390 ±0.016**
60	0.407 ±0.005	0.147 ±0.009**	0.174 ±0.003**	0.189 ±0.003**	0.191 ±0.010**	0.212 ±0.012**	0.351 ±0.011**
90	0.406 ±0.008	0.141 ±0.008**	0.176 ±0.009**	0.177 ±0.007**	0.184 ±0.011**	0.229 ±0.009**	0.270 ±0.002**
120	0.412 ±0.005	0.142 ±0.009**	0.160 ±0.006**	0.173 ±0.016**	0.185 ±0.006**	0.199 ±0.012**	0.222 ±0.002**

**Significant difference ($P \leq 0.01$); *Significant difference ($P \leq 0.05$)

Significant decrease of *in vitro* lymphoproliferative response to allogenic antigens, LPS and Phytohaemagglutinin-A (PHA) antigens, and inhibition of the primary IgM response to sheep erythrocytes were correlated with a marked decrease in spleen cell viability at 5-8 days after aerosol cadmium exposure. (Krzystyniak *et al*., 1987). Cadmium causes decrease in blastogenic activity of T- and B- lymphocytes along with reduction in bovine CD_4^+ and bovine CD_8^+ cells in peripheral blood of calves (Chauhan and Agrawal, 1999).

In the present study, results indicated that heavy metals, such as cadmium, induce apoptosis in avian lymphocytes leading to suppression of both cell mediated and humoral immune response. Due to enhancement of apoptosis, cells die without performing their

function eventually leading to lower immune competence and increased susceptibility to various infections. The present study also advocates that the cell culture system can be used as a promising replacement for animal experimentation.

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THE IMPACT OF PELLETING AND ENZYME SUPPLEMENTATION ON FEED VALUE OF TWENTY-FIVE CANADIAN WHEAT SAMPLES

T.A. SCOTT

Summary

Variation in market weight and age in broiler chickens is of major economical importance to the industry. The science of feeding broiler chickens to obtain efficient and uniform growth relies on the bird's ability to adjust intake to maintain its requirement for nutrients. However, there is increasing evidence that the physiological control of appetite in broiler chickens has been eroded by increased selection for growth and that growth is being limited by the bird's capacity to ingest, digest and convert dietary nutrients into body tissue. Therefore, limitations in intake associated with wheat-source, as presented here, have a direct effect on growth but are not related to either energy level or availability. It is also apparent that although pelleting and enzyme supplementation increase the bird's total intake, these treatments do not reduce variation in intake and growth of broilers given wheat from different sources. This data also indicates that enzyme supplementation and/or pelleting affect variation between specific sources of wheat. It is therefore imperative that we address these challenges and determine how variation in intake, growth and utilisation of wheat-based diets by broilers can be reduced.

I. INTRODUCTION

Realising the genetic potential of broiler chickens is closely associated with our capacity to provide diets that will supply balanced levels of nutrients to meet the broiler's requirements for maintenance, growth and tissue partitioning. It is assumed that an increase in knowledge of the available nutrients in cereal grains, such as wheat, will facilitate achieving set energy levels to which other nutrients can be proportionally added to the diet to maintain appropriate nutrient (i.e. amino acid) to energy ratios. It is also assumed that there are no major limitations in the broiler's ability to alter its intake of an ingredient, all other things being constant when the metabolisable energy of the ingredient varies, thereby maintaining a constant energy intake. Contrary to this, Scott (2004a,b) and recent unpublished work from the Premium Grains for Livestock Program (PGLP; personal communication with J. Black, 2004) demonstrated no consistent relationship between food intake and AME of the ingredient/diet.

There is ever increasing evidence that the definition of feed value of cereal grains needs to be modified to not only reflect nutrient composition and availability, but also include measurement of nutrient intake and retention of nutrients as meat or eggs (Scott 2004a,b). This is based on concerns that there may be limitations in the broiler's capacity to consume wheat-based diets which are not related to energy levels in the diet, and this limitation in intake has a direct effect on growth rate. This paper will discuss how measures of feed value, as discussed above, are also impacted by processing (pelleting) and enzyme supplementation.

II. MATERIALS AND METHODS

Twenty-five wheat samples from across western Canada were presented for bioassay evaluations of feed value within six months of harvest (2002). Each of the grain samples was

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also subject to a number of physicochemical analyses. For bioassay evaluation, each of the 25 wheat samples was ground, and one portion was steam pelleted (<80 ^{o}C) and then re-ground; treatments were compared in mash diets thereby minimising confounding by physical feed form. The mash and pelleted (re-ground) wheat samples were included (80%) in a basal diet (Scott 2004a); then each of these diets was split and one portion was fed as is, the other was supplemented with 0.5 g xylanase/kg diet (Danisco Animal Nutrition, Marlborough UK). Each of the resultant 100 diets was fed *ad libitum* to a cage of six male broilers (4 to 17 d of age), and this was repeated in a series of four separate bioassays to provide sufficient replication. Each of the diets contained 1.0 % acid insoluble ash marker to facilitate determination of nutrient retention based on excreta samples collected at 16 d and ileal digesta collected at 17d of age; digesta viscosity of two samples of ileal digesta were measured for each cage of birds. Total feed intake, body weight gain and feed conversion ratios for the 4-17 d period were calculated. The average values for physicochemical and performance measures for the 25 wheat and respective 100 dietary treatments are presented in Table 1.

A series of standard lab-bench measurements were conducted on each sample of wheat. The analyses were conducted by Agricore United, NorWest Laboratories and Danisco Animal Nutrition and are listed in Table 1.

III. RESULTS AND DISCUSSION

Table 1. The mean, standard deviation and range (minimum vs maximum) of physico-chemical and overall performance (for 100 diets) measurements of 25 wheat samples.

Laboratory Analysis	Mean	Std Dev	Minimum	Maximum
1000 kernel wt (g)	39.3	4.98	27.4	50.0
Bulk density (g/l)	374	12.4	356	403
RVA – Measure of sprouting	256	92.3	58	413
MJ ME – calculated	13.6	0.23	13.3	14.2
MJ ME DM basis – calculated	15.6	0.15	15.4	16.2
TDN – ADF	77.3	1.24	74.9	79.6
Ash	1.65	0.158	1.43	2.04
Crude Fat	1.80	0.235	1.40	2.20
Crude Fibre	2.17	0.359	1.50	2.70
Crude Protein	16.16	1.752	12.20	19.90
NDF	19.94	1.677	17.10	22.90
For dietary parameters (100 diets)				
Feed Intake (4-17d) g/b/d	41.7	2.70	35.9	48.1
Body wt gain (4-17d) g	440	27.8	382	506
FCR (4-17 d) g feed: g gain	1.49	0.060	1.38	1.64
AME (excreta 16 d) MJ/kg	13.3	0.85	10.3	15.1
Digesta viscosity (cPs)	45.8	34.48	8.6	100.7

The dietary treatments (wheat source, processing and enzyme supplementation) and their interactions were tested for the variables measured on each diet (broiler performance and digesta viscosity). When interactions between wheat source and either processing and enzyme supplementation were significant, the data was presented by determining the correlation coefficients between grains for the respective processing or enzyme supplementation treatment.

It is evident from Table 1 that there is considerable variation between sources of wheat with respect to physico-chemical measurements often employed as a means of predicting feed value. It is also evident that there is considerable variation (range) between the combined dietary treatments (wheat source, form (pellet vs unpelleted) and enzyme supplementation) with respect to feed intake (34%), body weight (33%), feed conversion ratio (19%), AME (47%) and digesta viscosity (1070%) based on the difference between the minimum and maximum values expressed as a percent of the minimum value. Individual or combined physiochemical parameters did not provide good prediction of broiler performance. This supports previous work (Classen *et al.*, 1995) and unpublished work from our laboratory using more than 100 different chemical and physical measures of wheat.

The data in Table 2 presents the statistical model for the performance parameters measured on the dietary treatments and presents the mean values for the main effects of pelleting and enzyme supplementation across the 25 wheat sources. Table 2 also includes a summary of the correlations between the broiler performance parameters of the 100 dietary treatments. This data strongly supports previous observations (Scott 2004a,b) that the variation in growth rate of the birds was directly related (r^2=0.91) to feed intake. Further, there was no relationship between feed intake and AME levels of the various diets.

Table 2. The effect of pelleting and enzyme supplementation on 25 sources of wheat when *ad libitum* fed to broiler chicks from 4 to 17d (italicised values indicate significance of main effects and interactions; mean±std dev are presented for main effect treatments).

	Feed intake g/b/d	Body wt gain g	FCR (4-17d)	AME MJ/kg	Digesta viscosity cPs
Pelleting (P)	*0.01*	*0.01*	*0.02*	*0.01*	*0.06*
Pelleted/ground	45.5±3.22	455±38.2	1.50±0.075	13.4±1.02	48.3±38.1
Unpelleted	39.8±3.17	425±33.1	1.49±0.066	13.1±0.84	42.8±34.6
Enzyme (E)	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*
With E	42.6±3.46	456±33.7	1.45±0.052	13.6±0.85	17.1±14.7
Without E	40.7±3.65	422±35.2	1.53±0.064	13.0±0.92	75.5±24.9
Wheat Source (WS)	*0.25*	*0.23*	*0.01*	*0.01*	*0.06*
Interactions					
P x E	*0.09*	*0.01*	*0.01*	*0.03*	*0.13*
P x WS	*0.24*	*0.18*	*0.01*	*0.01*	*0.17*
E x WS	*0.86*	*0.65*	*0.02*	*0.01*	*0.08*
P x E x WS	*0.99*	*0.91*	*0.53*	*0.01*	*0.94*
Correlations					
Body wt gain	0.91**	1.00			
FCR	-0.19**	-0.55**	1.00		
AME	0.03	0.17**	-0.29**	1.00	
Digesta viscosity	-0.22**	-0.37**	0.44**	-0.15*	1.00

*,** indicate significant (P<0.05, P<0.01) correlation coefficients.

There were no significant interactions between the main effects of wheat source, feed form and/or enzyme supplementation for feed intake measurements. There were however, significant effects of both feed form and enzyme supplementation. The average feed intake was 5.7 g (14%) greater when the wheat samples were pelleted. Feed intake was approximately 5% higher with enzyme supplementation across the 25 sources of wheat. It is interesting that there was no significant effect of wheat source on feed intake over the 100 diets even though there was a 12 g/b/d (34%) difference in feed intake between diets (Table 1). No overall significant interactions between grain sources between enzyme and/or

processing treatments suggests that there is a marked variation in how pelleting and/or enzyme supplementation impact individual wheat sources (to be discussed in detail later) and that although variation between wheat sources for specific dietary treatments is high, this is reduced when averaged across dietary treatments. This supports previous observations (Scott 2004a,b) that variation in feed intake (and subsequently growth) is complex and the use of enzymes and/or processing does not alleviate it.

Data in Table 3 demonstrates significant interactions between feed form and enzyme supplementation for body weight gain, FCR and AME levels of diets. It is appropriate to discuss the two-way interactions between body weight and FCR, however, there was a significant three-way interaction for AME and this will be discussed separately.

Table 3. The interactions (mean ± std dev) between pelleting and enzyme supplementation on measurements of feed value.

	Feed intake g/b/d	Body wt gain g	FCR (4-17d)	AME MJ/kg	Digesta viscosity cPs
Pelleted					
With E	44.6±2.67	476±26.5a	1.45±0.043c	13.8±0.59a	17.9±17.6
Without E	42.3±3.24	432±33.3b	1.55±0.064a	13.0±0.87c	82.9±22.1
Unpelleted					
With E	40.5±2.80	435±26.6b	1.46±0.058c	13.4±1.02b	14.6±11.2
Without E	39.2±3.38	414±35.0c	1.51±0.060b	12.9±0.95c	70.9±26.0

a,b,c Mean values with different letters are significantly ($P<0.05$) different.

The overall increase in body weight with enzyme supplementation was proportionally higher when the diets were pelleted, although pelleting significantly improved body weight gain regardless of whether it was supplemented with enzymes or not. Similarly, the pelleted wheat based diets demonstrated a proportionally larger difference in FCR when diets were supplemented with enzyme, part of which was associated with a higher FCR in pelleted as compared to unpelleted wheat sources. If FCR was corrected for a fixed body weight rather than body weights determined at fixed ages, it would be apparent that pelleting and enzyme supplementation had an additive effect on FCR. However, this does not appear to be related to improvements in AME, *per se*.

There was a significant two-way interaction between wheat processing and wheat source for FCR, and this is depicted in Figure 1. The mean response in FCR with pelleting was negligible (0.8%); however, for individual wheat sources the percentage effect on FCRof pelleting varied from -3.9 to 6.2%. There was a 0.6 % mean reduction in FCR with pelleting of diets supplemented with enzyme and a 2.3% mean increase in FCR with pelleting of non supplemented diets. The correlation between FCR values obtained across the 25 wheat-source diets (with and without enzyme) for unpelleted and pelleted diets was moderate ($r^2=0.46$, $P<0.01$). Although the interactions were not significant for feed intake and body weight, on average there was an increase of 9.5% in feed intake and 7.3% in body weight in the pelleted-grain diets. There was a significant two-way interaction between enzyme supplementation and wheat source for FCR, and this is illustrated in Figure 2. On average, for the 25 pelleted or unpelleted wheat-based diets there was a 5% reduction in FCR with enzyme supplementation; however, this varied from 1 to 10% depending on the source of wheat. Enzyme supplementation decreased mean FCR by 3.7%, and 6.8% in the un-pelleted and pelleted wheat-based diets respectively. The correlation coefficient between FCR values obtained across the 25 diets with and without enzymes (forms combined) was low ($r^2=0.20$), indicating that there is minimal association between measurements of FCR before and after enzyme supplementation. It is often assumed that poor quality wheat would typically

respond more from enzyme supplementation. When we calculated the enzyme response (% change in FCR with enzyme supplementation) it did indicate that wheat samples with high FCR have high negative (as in FCR is lowered) enzyme responses (r^2=-0.68; data not presented).

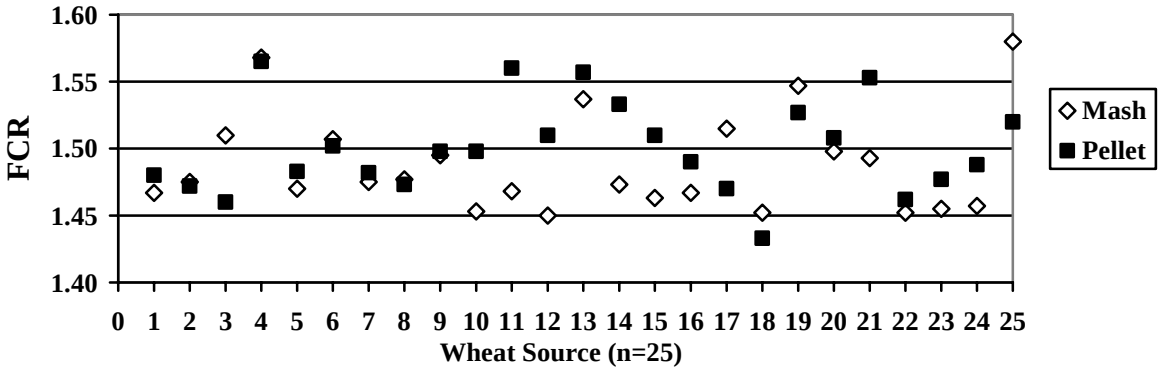

Figure 1. Represents the FCR of 25 wheat sources fed as mash (open box) and with the grain pelleted, ground and fed as a mash (solid box) *ad libitum* to broiler chicks from 4 to 17 d.

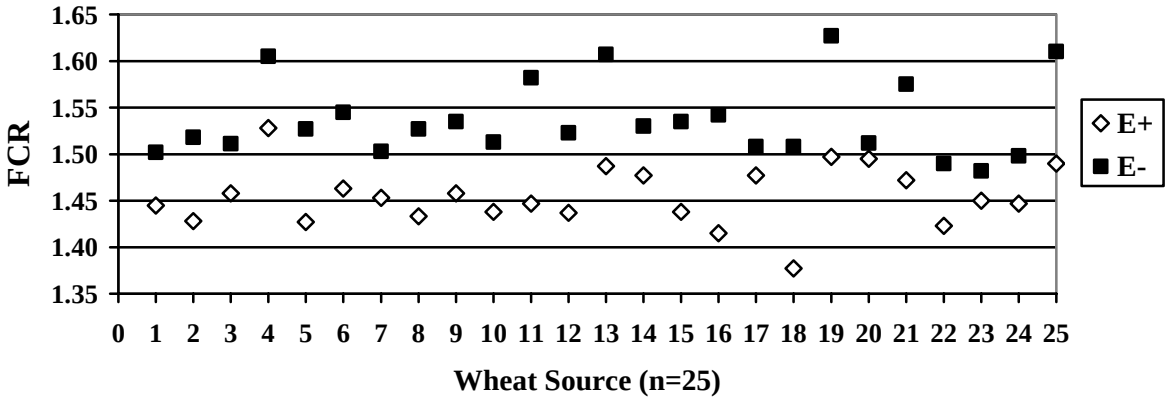

Figure 2. Represents the FCR of 25 wheat sources with (E+; open box) and without (E-; solid box) xylanase supplementation for broiler chicks *ad libitum* fed diets from 4 to 17 d.

There was a significant three-way interaction for AME (MJ/kg diet) and this is presented in Figure 3. It is difficult to define what makes this interaction significant as it involves 100 different dietary treatments. In an attempt to illustrate this, correlation coefficients were calculated for the 25 sources of wheat fed in the four feed form by enzyme combinations. There was no relationship between determined AME for mash diets without enzyme or for pelleted diets with or without enzyme, but there was a reasonable correlation (r^2=0.52) with AME of mash diets with enzyme. This data indicates that it is imperative when determining AME values for a wheat source that the feed form most commonly fed be used in the bioassay.

As discussed previously, there was considerable variation in feed intake between the 25 wheat sources, but this was only significant when the comparisons were made for

individual dietary treatments (e.g. with or without pelleting and enzyme supplementation; Figure 4). This begs questions as to the cause of the variation in feed intake, and the reason(s) that this variation does not relate to dietary energy? Our only explanation for this relates to a limitation in capacity to increase feed intake, that as was discussed previously we feel may be associated with variation in hydration rate of the grain component of the diet. It may not be expected that this would vary significantly with enzyme supplementation, but it is readily apparent that both rate and capacity of hydration are changed dramatically with pelleting. Further work to identify the limitation in feed intake and to maximise this to achieve both optimum growth and feed conversion are required.

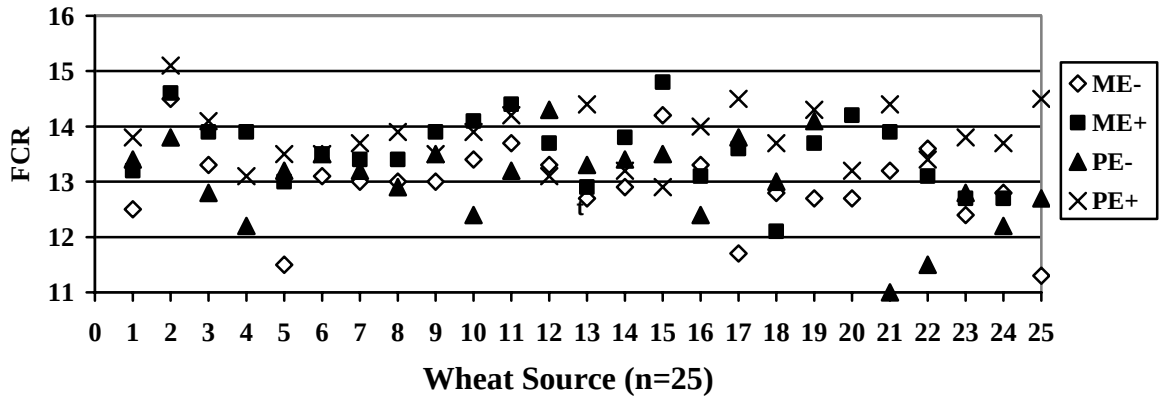

Figure 3. Represents the AME (MJ/kg) for 25 wheat samples fed with (P) or without (M) pelleting and with (E+) or without (E-) xylanase supplementation

ACKNOWLEDGMENTS

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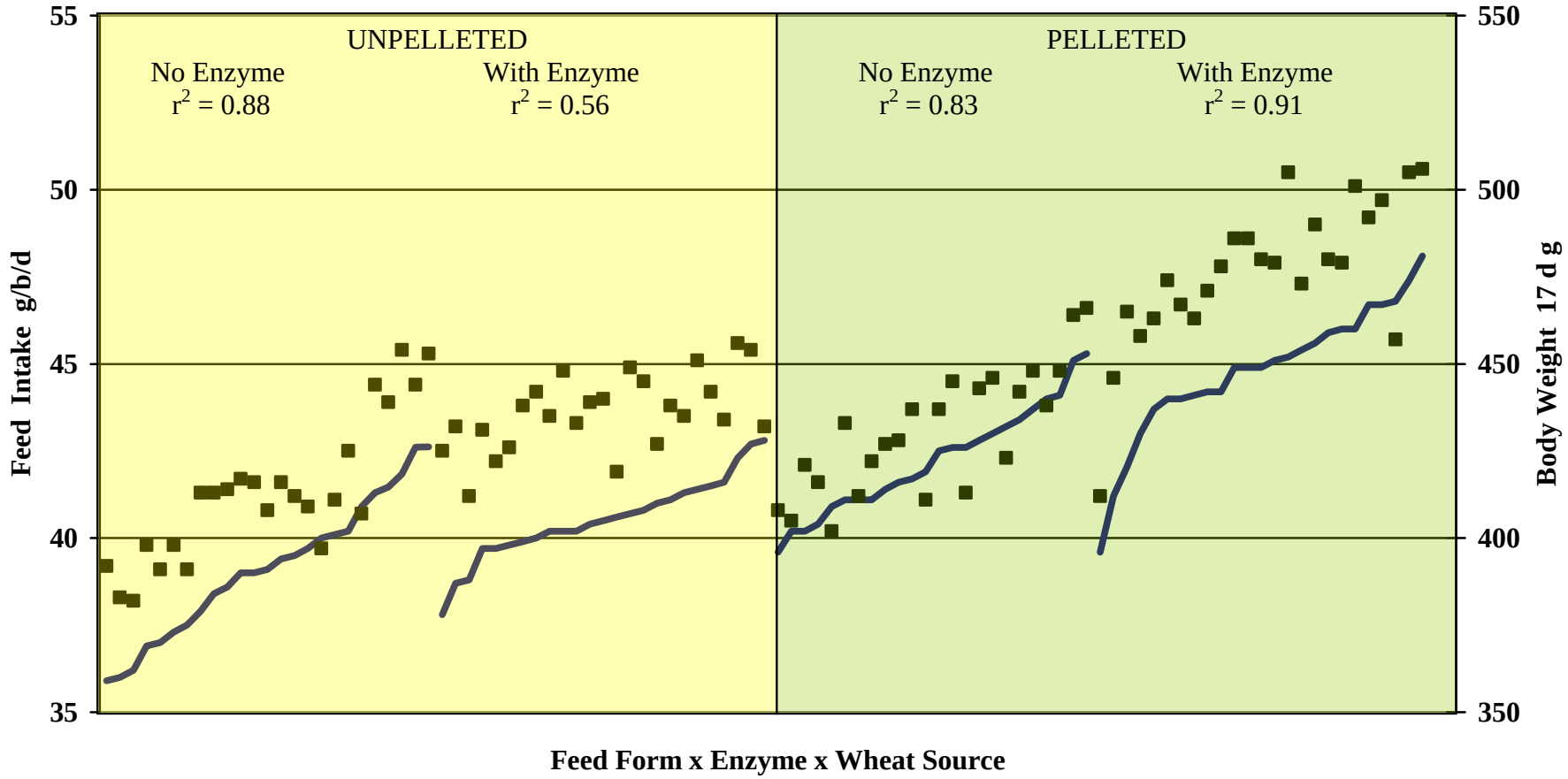

Figure 4. Illustration of relationship between feed intake (solid line) and body weight (points) of individual sources of wheat. Note: wheat sources within feed form and enzyme categories are not presented in consistent order, but by ranking with respect to feed intake).

WHY DID THE CHICKEN CHOOSE THE FOOD?

J. M. FORBES[1]

Summary

Food intake by poultry is influenced by the food energy content, but not in order to maintain a strictly constant intake of ME. Similarly, dietary concentration of protein, fibre, minerals and vitamins can affect intake. When conditions allow, chickens can make nutritionally-wise choices between foods. The mechanisms underlying food intake and choice include pathways from receptors in many parts of the digestive system and those sensitive to metabolism whose signals are proposed to be additive in their effects on the CNS. It is postulated that animals, including birds, modulate their eating behaviour to minimise the total discomfort due to over- or undersupply of nutrients and other food resources; this takes into account many factors simultaneously and an unvalidated model is proposed which illustrates the principle. In practice choice feeding of whole wheat and high protein compound food is popular in Northern Europe.

I. INTRODUCTION

It has frequently been said that food intake is under the control of many factors. These include energy, protein and amino acids, bulk and fibre, as well as micronutrients. In each of these cases both the concentration in the food and the "requirement" of the animal under consideration are important. We can reasonably start from the point that animals eat according to their requirements. However, most foods are likely to contain nutrients in ratios different from those optimal for the animal so that eating to meet the needs for one resource will result in over-or under-eating other resources.

Given a choice of two foods animals can in theory meet their requirements for two nutrients by eating appropriate amounts of the two foods; requirements for *n* nutrients could, again in theory, be met by choosing appropriately between *n* different foods of appropriate composition. There are numerous examples of chickens selecting wisely when given a choice between two foods differing in their content of an essential nutrient. In those cases when wise selection does not occur there is often a rational explanation (Forbes and Kyriazakis, 1995).

Given that birds have evolved in an environment in which there are normally many different sources of food, it could be argued that to have only one food available, as in most modern production systems, is unnatural and a special case. We should be able to learn about the control of intake of a single food, therefore, by considering how poultry control their choice between foods. However, the great preponderance of studies of the control of food intake has been with single foods.

II. FOOD COMPONENTS AFFECTING INTAKE

a) Energy

It has been said that "Animals eat for calories" because changes in the metabolisable energy concentration of the diet are followed by compensatory changes in intake, and changes in animals' energy requirements are followed by appropriate changes in food intake. These changes are only approximate, however, as illustrated by the results of De Groot (1972) with laying hens. There was a consistent increase in ME intake with ME concentration increasing from 10.5 to 13.5 MJ ME/kg; although food intake decreased over this range

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(Figure 1a) [DMI (g/day). = 202-7.3 ME concentration (MJ/kg) (r^2=0.92)] this was insufficient to maintain ME intake at a constant level (Figure 1b). As a result both egg weight and body weight gain increased with increasing ME concentration and it is clear that the intake of ME is not maintained constant in the face of changing dietary ME concentration.

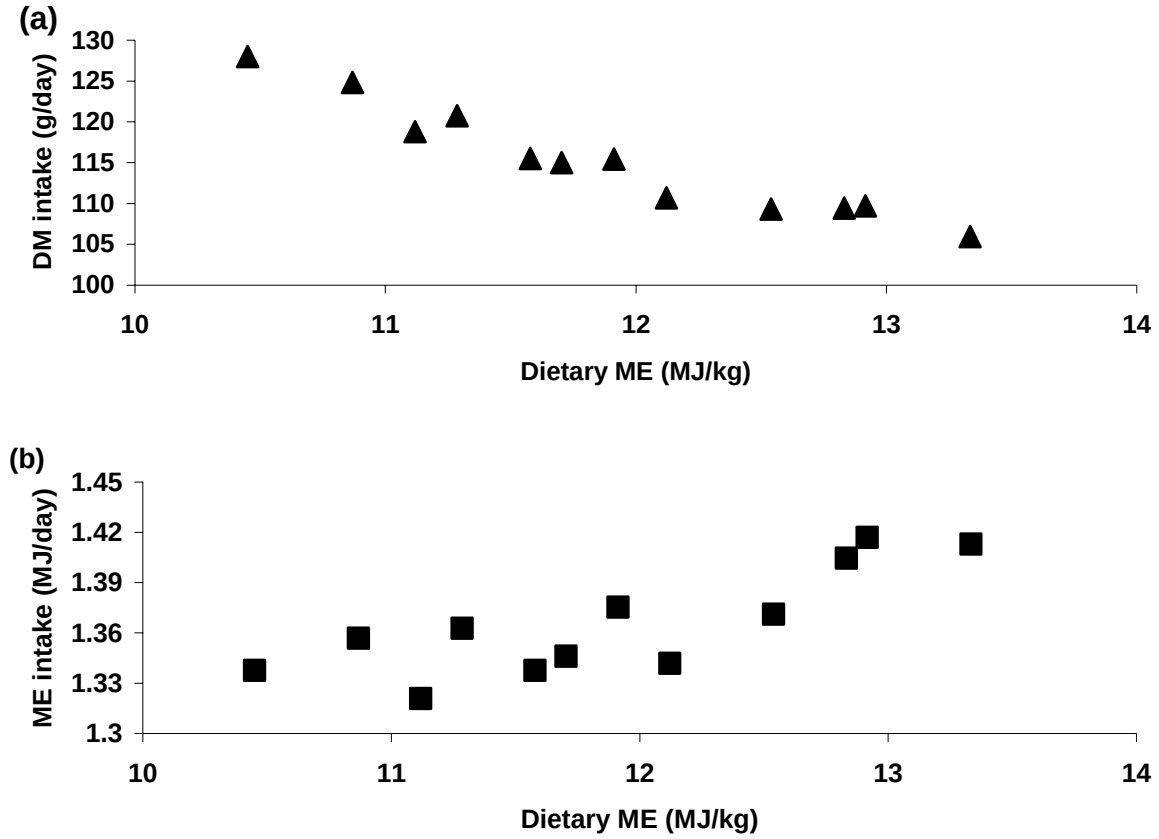

Figure 1. a, DM intake; b, ME intake of laying hens offered foods with ME concentrations ranging from 10.5 to 13.5 MJ/kg (de Groot, 1972).

Given a choice between two foods with different concentrations of ME, it would be expected that birds would choose predominantly that with the higher concentration of ME. However, such experiments have invariably used foods with other differences in composition (e.g. protein content), so this aspect of the appetite for energy is unclear. What is clear is that there is more to the control of food intake than simply energy.

b) Protein and amino acids

There is little effect of the protein content of the food on its intake by broiler or layer chicks unless gross deficiency or excess are achieved. With foods as low as 115g protein / kg daily food intake by broilers was not significantly different from that with more normal foods containing 172 or 225 g / kg (Shariatmadari and Forbes, 1993). However, daily intake was very much lower when the food contained 65 g protein / kg. At the upper end of the scale, intake of a food containing 280 g / kg was not affected, except during a period of hot weather. Feeding an excess of protein results in deamination of the excess amino acids which generates ammonia, which is toxic, and heat, both of which cause reduced intake.

Under normal conditions, therefore, the protein content of the diet does not have a major effect on intake. When a choice of two foods is offered, one providing a lower ratio of protein: energy than is optimal for the birds in question, the other with a higher P:E ratio,

birds have demonstrated a clear ability to select a diet close to optimal (Forbes and Shariatmadari, 1994). There is no doubt, therefore, that birds sense an excess or deficiency of protein intake and correct it if they are given the chance. However, if that choice is not available to them, they can cope with a moderate deficiency (but grow more slowly and deposit more fat) or excess (by deamination) of protein.

c) Amino acids

Growing chicks are particularly susceptible to imbalances of amino acids in the diet. Animals' ability to store amino acids is very limited, so the effects of a deficiency of an essential amino acid on metabolism are rapid and severe, the accumulation of the other amino acids causing toxic effects and reduced food intake in an attempt to avoid these effects.

As with protein in general, choice feeding with foods containing excessive and deficient concentrations of an amino acid allows the bird to exercise its ability to learn what mixture of the foods provides the optimal intake (e.g. Steinruck *et al.*, 1990 for methionine).

d) Fibre

Indigestible and slowly digested components of the diet occupy space and distend the stomachs and intestines (Savory, 1992; Razdan and Pettersson, 1994; Jorgensen *et al.*, 1996). It is possible that distension of the digestive tract wall sets a fixed limit to food intake but it seems unlikely that this would be a fixed limit. This will be examined below where the dietary content of neutral detergent fibre (NDF) will be used as an index of bulk.

e) Minerals and vitamins

As with essential amino acids, so there are effects of deficiencies and excesses of minerals and vitamins. Effects of calcium, phosphorus, selenium, zinc, ascorbic acid (vitamin C) have all been demonstrated in chickens (Forbes, 1995) and there are, no doubt, effects of other minerals and vitamins as well, as seen in mammals.

Given the opportunity, birds select a mixture of deficient and excessive foods to achieve an intake of the nutrient in question that is within the non-toxic range: zinc, vitamin B_6, ascorbic acid. Perversely, selection for an appropriate intake of sodium has not been demonstrated (Hughes, 1979), even though such selection is very accurate in some mammals.

III. MECHANISMS

In order for nutrients to influence food intake or choice they, or their effects, must be sensed. The visual appearance, smell, taste and texture of food are not reliable indices of the nutritional value of food but nutritional properties can only be reliably assessed after food has been eaten. Gastric and intestinal chemoreceptors respond to chemical products of digestion as well as the osmolality of digesta (Shurlock and Forbes, 1981b). It is, however, only after absorption that the yield and mixture of nutrients available can be assessed with reasonable accuracy by the animal.

The liver is the first organ to receive most of the nutrients absorbed – glucose, the major product of digestion with cereal-based foods, is sensed by the liver as are other oxidisable substrates, such as lysine and fatty acids (Forbes, 1988). Evidence that the liver senses glucose comes from experiments such as those of Sherlock and Forbes (1981a) in which glucose infused into the general circulation via the jugular vein had little effect on food intake whereas the same amount infused into the liver via the coccygeomesenteric vein caused a highly significant depression. Lysine also depresses intake via the liver (Shurlock and Forbes, 1984) and its effect can be blocked by section of the vagus nerves carrying information from viscera to CNS (Rusby *et al.*, 1987).

We can build up a picture in which numerous families of receptors are stimulated as digesta pass through the digestive tract and nutrients are absorbed, each transmitting

information to the CNS (Figure 2). There is convergence of this information at several levels (see Forbes, 1996 for discussion with respect to ruminants) resulting in an integrated signal as to the general state of the digestive and metabolic systems, which the animal can take into account when making its feeding decisions.

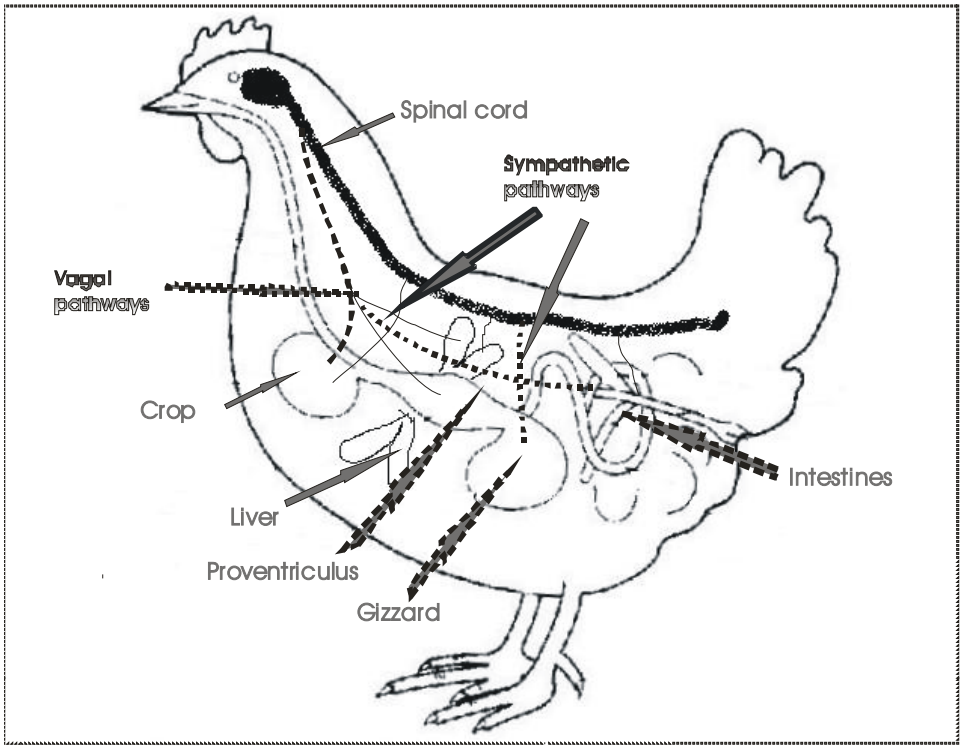

Figure 2. Diagram of chicken showing major visceral organs and their innervation.

IV. INTEGRATION: MINIMAL TOTAL DISCOMFORT

How can we bring these diverse factors involved in the control of intake into a single framework? One way would be to calculate the intake likely to be attained with each of the factors: energy, protein, fibre …. and to take the smallest of these as our prediction of intake (e.g. Poppi *et al.*, 1994 for cattle). However, this is to assume that once one factor limits intake the other factors have no involvement. Assume, for example, that birds are offered foods with ever-increasing levels of fibre. Intake increases to compensate for the reduction in available energy concentration, mediated by such factors as liver sensation of glucose, until the point is reached at which fibre intake suddenly limits intake. Beyond this point intake decreases to maintain a constant fibre intake and the signals from the liver either disappear or are ignored by the CNS. It seems most unlikely that information from one set of receptors would be suddenly ignored when a small increase occurs in the strength of signals from another set of receptors. Yet, how else are the various signals converging on the CNS to be integrated? What common currency is available so that the various factors involved can be integrated in a model?

We have seen how animals behave when offered a choice of foods: they appear to select in a manner designed to minimise the discomfort of an imbalanced diet – the discomfort of too little or too much of a nutrient to support its current physiological needs – the discomfort of gut distension – the discomfort of excessive oxidation in the liver. Given the convergence of signals into the CNS, we propose that the discomforts from various sources are additive (e.g. glucose and lysine). It is reasonable to propose that animals act to

minimise discomfort, in this case the total discomfort – Minimal Total Discomfort (MTD, Forbes, 1999).

a) Example

This example takes a growing broiler with optimal intakes of 1.4 MJ ME and 30 g protein per day, with a capacity of 25 g NDF /day before distension becomes uncomfortable, offered a single food containing 11.5 MJ ME, 22 g protein and 25 g NDF/kg (no attempt is made to simulate a specific real situation, and only three food resources are used for the sake of simplicity). Initially a level of food intake is proposed and the quantities of ME, CP and NDF in this weight of food are calculated. These are subtracted from their optimum intakes 'required' by bird in question and these 'errors' are squared and summated, giving total discomfort (TD). (Note that NDF intakes below the threshold do not generate discomfort.) The calculations are repeated for progressively higher and lower intakes until the intake giving MTD is found. Figure 3 plots relative discomfort for the three resources, and TD, for the bird and food specified above.

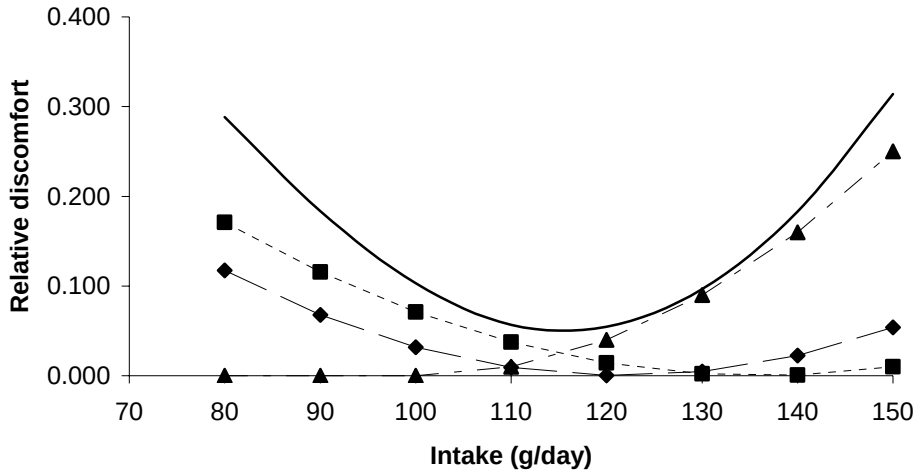

Figure 3. Relative discomfort due to ME (diamonds), CP (squares), NDF (triangles) and total discomfort (continuous line) for broiler chickens at food intakes from 80 to 150 g/day.

In this example discomfort due to ME is minimal at an intake of 120g /day, that due to CP at an intake of 130 g/day while discomfort due to NDF is minimal up to an intake of 100 g / day. Summation gives a total discomfort curve whose minimum is at an intake of 115 g / day at which point discomfort is not zero as the animal is suffering slight distension from NDF and slight and moderate deficiencies of ME and CP, respectively.

With the parameters given above the model predicts that DM intake is depressed with diets of NDF contents above 25g / kg. This is probably not realistic as Leeson *et al*. (1996) found that dilution of a standard finisher feed with up to 50% sand and oats resulted in a 100% increase in feed intake in a fast-growing broiler strain; i.e. complete compensation for dietary dilution. However, it is not the purpose of the present exercise to validate the model, simply to propose it as a working hypothesis.

Simulation of the effects of CP content (Figure 4) predicts little effect on daily intake, but with marked variation in the discomfort associated with protein supply. In reality, as observed above, extremely low protein content results in greatly depressed intake, a feature not replicated by the model in its present form; it will be necessary to incorporate a further factor, *viz*. a reduction in optimal ME intake during protein deprivation, in order to encompass this situation.

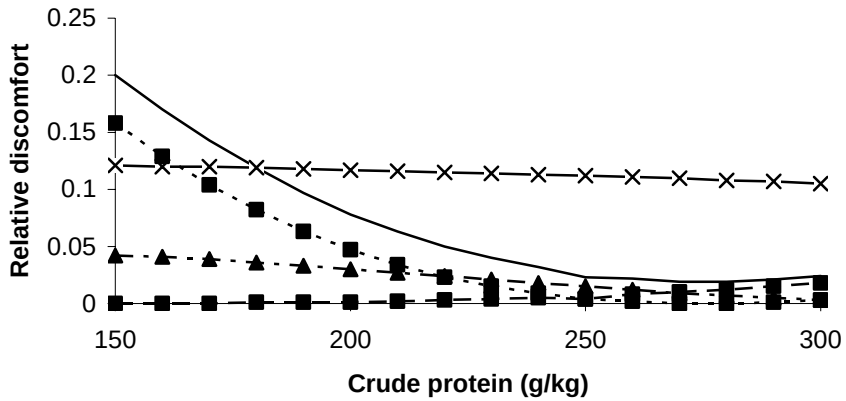

Figure 4. Predicted voluntary food intake (kg / day) and discomforts due to ME, CP, NDF and total (see Figure 3 for definition of symbols, animals and foods) for foods of different CP contents. X, food intake (kg/day).

The optimal CP concentration for birds of this type is around 270g / kg, which is higher than that used commercially, but the specification of the bird in this case includes a high optimal protein intake (30 g / day).

b) Diet selection

The MTD model outlined above is easily adapted to the diet selection situation. Intakes of each food are changed progressively and the outcome in terms of TD examined to see at which combination of intakes of the foods MTD occurs. For example, with a high protein food (HP) containing 320 g CP / kg, offering low protein foods (LP) with CP content ranging from 140 to 300 g / kg gives the predictions show in Figure 5.

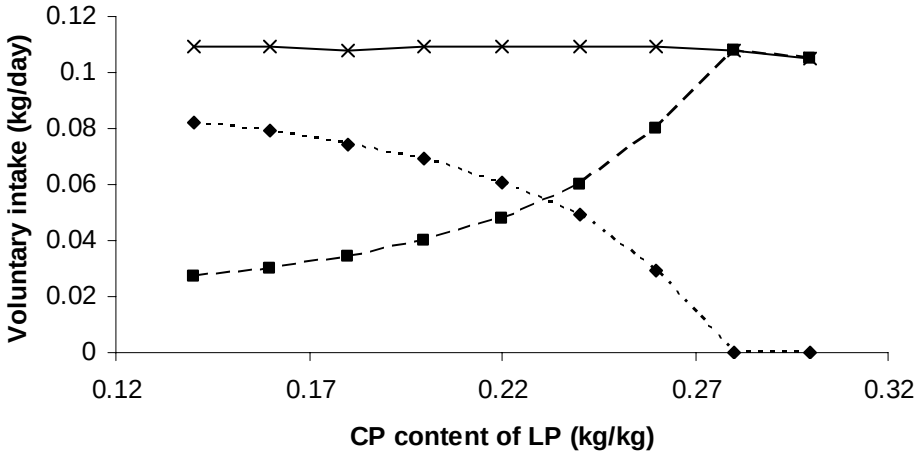

Figure 5. Predictions of food intake and choice by broiler chickens offered a choice between HP with 320 g CP / kg and LP of different CP contents. Solid line, total intake; diamond, HP intake: squares, LP intake.

As the CP content of LP increases so the bird's selection for LP increases, up to a CP content of 280 g / kg. Above this the bird eats only LP, which provides an excess of CP resulting in an increase in discomfort and a slight reduction in total food intake.

c) Limitations of model

The MTD model presented here is intended as a proposal for dealing with the multiple factors that are involved in the control of food intake and selection. No attempt has been made to optimise the model and it is therefore not appropriate to compare it with actual observations. Indeed, there are no measurements of 'discomfort' with which to compare the predictions made above.

Those who question the point of the sort of speculation presented here must either believe that there is no importance in understanding and predicting food intake and choice by poultry, or must have some alternative (and better) proposals.

V. PRACTICAL CONSIDERATIONS

Through practical experience and scientific research, we know approximately how much birds might be expected to eat, and how they are likely to perform, when given conventional foods. The commercial challenges are twofold: how to fine tune the diet according to the raw materials (e.g. variety of cereal) used and the method of preparation; and how to cope with new types of raw material (GM?) and new production methods (organic; transgenic birds?).

In predicting the feeding value of a novel material, whether this be a new conventional variety of wheat or a radically new type of food, it is not sufficient to measure its ME content and to assume that birds will eat the same amount of ME from this food as from other foods. Not only is ME intake not constant between diets with different concentrations of ME, it is also influenced by the content of other nutrients and resources such as protein (and amino acid balance), fibre, minerals and vitamins.

While optimal diets can theoretically be achieved by offering choices between two or more foods, in practice there is considerable variation between individuals and it is not yet certain whether these are due to differences in requirements; do fast-growing males choose an appropriately higher proportion of a high protein food than slow-growing females? An option used widely in Europe is to offer broilers whole wheat grains in choice with a high-protein compound food. It is to be noted that broilers stimulated by injection with corticosterone to gain more fat but less protein chose to eat a significantly higher proportion of whole wheat than controls (Covasa and Forbes, 1995), suggesting that requirements can drive food choice.

Commercially it is usual to start by offering very small amounts of wheat and increasing this as the optimum protein:energy ratio declines with age of bird and their capacity for bulk increases. Note that a specific training period, in which whole wheat and compound feed are offered separately in alternate periods, is not necessary (Covasa and Forbes, 1996).

Why did the chicken chose the food? The academic answer is: In order to take in the quantity and mixture of foods necessary to optimise its chance of reproducing and passing on its genes to the next generation. The practical answer is: That intake and choice are governed by the bird's nutrient requirements, which sounds simple enough. However, a complex of factors is involved and we do not yet have a sufficiently good understanding of these in order to be able to predict intake and choice with the degree of certainty required by the poultry industry.

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GENETIC ASPECTS OF FOOD INTAKE AND FOOD UTILISATION EFFICIENCY FOR GROWTH IN CHICKENS

R.A.E. PYM[1]

Summary

The effect of selection for increased growth, appetite or feed efficiency in chickens on direct and correlated responses in the performance traits and on aspects of nutritional physiology and appetite regulation were studied in progeny from the three lines after twelve generations of selection. Relative to selection for growth rate, selection for improved feed efficiency resulted in: a reduced response in growth rate but no increase in food intake, a greater improvement in feed efficiency whether measured to the same age or weight, a reduction in body fat, an improvement in metabolisability of dietary energy, a higher net availability of ME for gain, a lower fractional rate of protein breakdown, a longer gastro-intestinal tract and a slower rate of ingesta passage, and a greater capacity to maintain nutrient intakes and meet nutrient requirements on low nutrient density diets. The results suggest that birds selected directly for appetite or long term for growth rate alone, may be eating near to gastrointestinal capacity and that the more recent emphasis on improved nutrient utilisation in modern broiler breeding programs, increases the capacity of birds to meet nutrient requirements on diets varying in nutrient composition.

I. INTRODUCTION

Broiler chickens have been selected for increased growth rate for many generations, and whilst the selection pressure on growth in commercial broiler breeding programs has been somewhat relaxed in more recent times to accommodate aspects of performance that have suffered because of the early high emphasis on juvenile growth rate (e.g. body conformation and composition, leg and skeletal abnormalities, ascites, reproductive performance) there is still upward selection pressure on growth rate in most commercial lines as evidenced by the continued improvement in growth performance in commercial broilers.

One of the very logical consequences of selection for growth rate has been a substantial increase in appetite, but also a considerable improvement in feed efficiency as observed in commercial broiler chickens from the 1960s to the 1980s. The dramatic increase in growth rate due to selection for increased liveweight at a given age over this period was accompanied by a marked increase in food intake (g/d) but also a marked reduction in the age at processing (at the same body weights), and the improved feed efficiency observed was largely due to the shorter growing period with its commensurately reduced maintenance requirements..

It was not until the mid 1980s that commercial broiler breeders began to incorporate direct measures of food intake as selection criteria in their broiler breeding programs. Hitherto it was generally assumed that adequate selection response in feed efficiency was achieved through correlated response to selection for growth rate, through the above mechanism. Reports in the 1970s and 1980s (Guill and Washburn, 1974; Pym and Nicholls, 1979; Sorensen, 1984: Chambers, 1987; Leenstra and Pit, 1987) alerted breeders to the fact that there was considerable variation in feed efficiency to a given body weight that was not attributable to variation in growth rate alone. In most commercial broiler breeding programs, selection has been applied to males pre-selected on early body weight and tested for two to

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three weeks in single cages (Emmerson, 1997). Selection has typically been based on an index which theoretically optimises economic selection response with respect to growth and feed efficiency by incorporating liveweight at the beginning and end of the test period, and food intake over the test period (Pym 1990).

In order to understand the genetic relationships between growth rate, food intake and food utilisation efficiency, it is necessary to study the direct and correlated responses to selection for these traits and to look at the underlying physiological factors that contribute to variation in the three traits. In this paper, direct and correlated responses measured in three lines in a selection experiment will be used to illustrate these relationships. Genetic effects upon dietary nutrient density regulation of food intake will also be discussed

II. THE GENETIC AND PHENOTYPIC RELATIONSHIPS BETWEEN GROWTH RATE, FOOD INTAKE AND FCR

In the selection experiment reported by Pym and Nicholls (1979) and updated by Pym (1985), lines of chickens were selected for either: increased 35-63 d weight gain (line W), increased 35-63 d food intake (line F) or decreased 35-63 d FCR (line E) for 12 generations. A randomly mated control (line C) was also maintained. Individual food intake was measured on all birds in single bird cages between 35 and 63 days of age. The mash diet used over the food intake measurement period throughout all generations of the selection experiment contained 13.0 MJ ME and 210 g CP/kg. Each line was generated from a mating between 16 males and 48 females with a total of approximately 480 chickens tested per line across four hatches.

Direct and correlated responses in weight gain, food intake and FCR in the three lines over 12 generations of selection, are shown in Figure 1. Selection for increased gain in line W resulted in a substantial increase in gain, a moderate increase in food consumption and, as a result, a moderate improvement on food efficiency. Selection for increased food consumption in line F, however, resulted in only a moderate improvement in gain, a substantial increase in food consumption and, as a consequence, a marked deterioration in food efficiency. Selection for improved food efficiency in line E resulted in a moderate improvement in gain, essentially no effect upon food intake and, as a result, a marked improvement in food efficiency. The dramatic difference in response between the W and F lines clearly demonstrates that selection for growth rate and food intake are by no means synonymous.

Initial (35d) liveweight was essentially unaffected by selection for improved efficiency in the E line, whereas there was substantial correlated increase in this trait in the W and F lines. That initial weight did not increase in line E is not surprising, given that such selection would favour birds that grow well but which are lighter initially with its associated lower maintenance requirement. Age-constant measurement of food efficiency penalises the faster growing birds as they must carry their heavier average weight, with its higher maintenance cost, over the fixed time period. The commercial broiler breeder is, however, interested principally in optimising growth rate and food efficiency to a given weight and it is important that comparisons are made on this basis and that the focus of commercial selection is to maximise growth and efficiency over the entire growth period to the chosen slaughter weight.

In a study of age- and weight-constant measures of growth, intake and FCR in the 12th generation of the above lines, notwithstanding the fact that it took and extra three days (with its associated maintenance cost) for the E line to achieve the same weight gain as a the W line, the former line still had a lower FCR (2.42±0.02 c.f. 2.60±0.04 respectively). This begged the question as to what factors might be involved in the improved feed efficiency of the E line and a number of possible factors were identified.

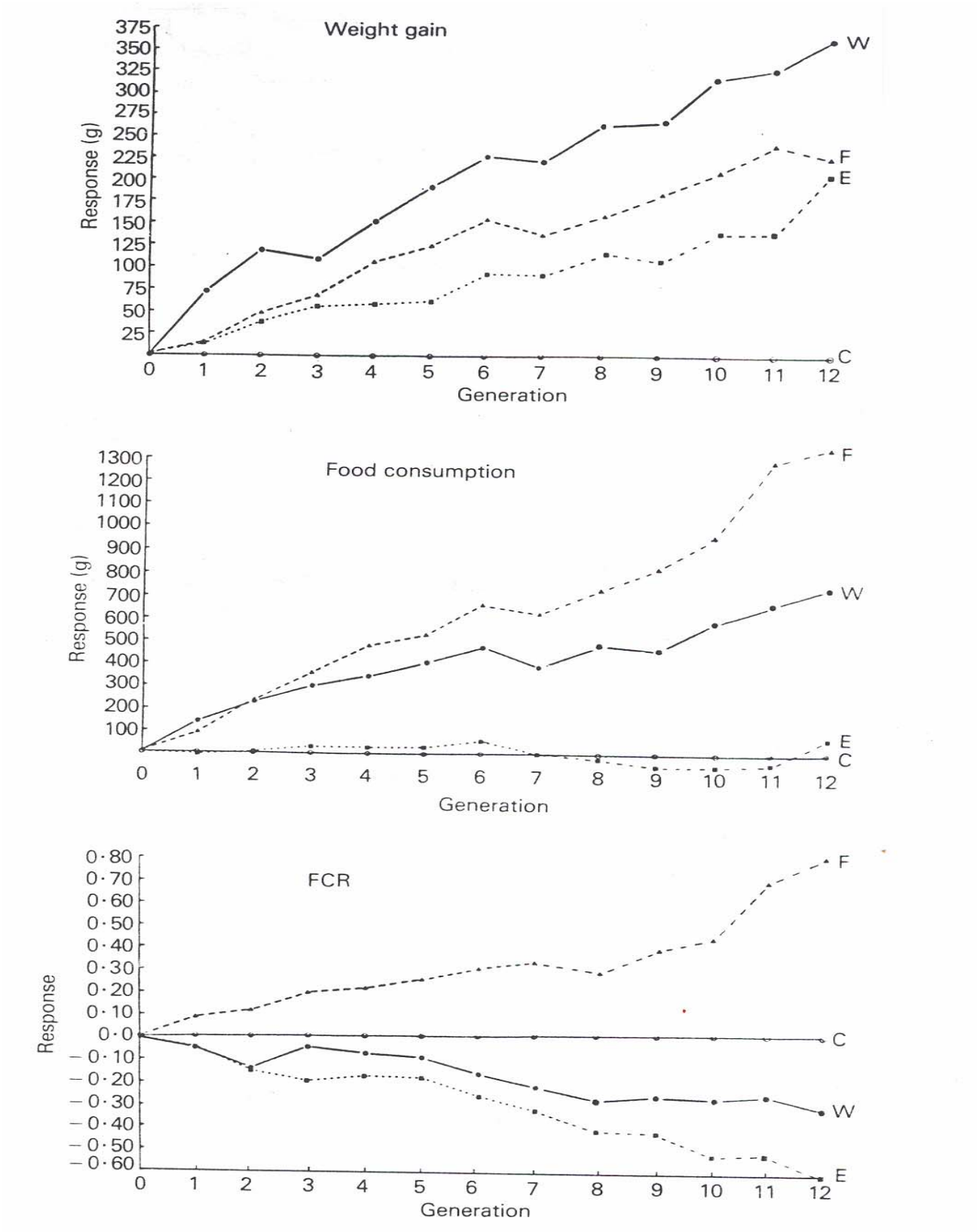

Figure 1. Direct and correlated responses to 12 generations in 35-63 d weight gain, food consumption and FCR in three selection lines of chickens (expressed as deviation from control line, sexes combined). Line W selected for increased 35-63 d weight gain, line F selected for 35-63 d food consumption, line E selected for decreased 35-63 d FCR, randomly selected control line C.

III. PHYSIOLOGICAL FACTORS CONTRIBUTING TO FOOD UTILISATION

Factors considered as possible explanations for the observed better feed efficiency in the E line (Pym 1990) were:

- A reduction in external losses – i.e. food spillage
- A reduction in the energy content of the gain i.e. partitioning of water, fat and protein
- An increase in digestibility or metabolisability of dietary nutrients
- A reduction in daily maintenance requirements per unit liveweight
- An increase in the net availability of Metabolisable Energy for gain
- An increase in the net efficiency of protein utilisation through a reduction in protein breakdown rate.

A number of studies were undertaken with the lines to determine the physiological and other factors that contribute to variation in growth and food efficiency. Taking the above suggested factors in turn:

Feed spillage in the study was essentially eliminated by the use of feed-saving grids on top of the feed in the troughs and as such feeding behaviour in its possible effect upon feed spillage, was not a factor in the study. There is, however, significant genetic variation in gustatory behaviour (e.g. Masic, 1974; Barbato *et al.*, 1980) between broilers and layers or high- and low-growth rate lines, which may well impact upon metabolic processes and energetic efficiency, particularly under group feeding conditions. If these factors are important, it argues for some method of measuring individual food intake in group rearing conditions.

As found in a number of studies during the progress of the selection experiment (Pym and Solvyns, 1979; Pym, 1985), and as shown in Figure 2, correlated response in growth-related change in body fat, was significantly reduced in the E line, substantially increased in the F line but essentially unaffected in the W line. This is in keeping with the above expectations based on the energetic cost of fat deposition. It does, however, demonstrate that selection for increased growth rate does ***not*** result in an increase in fatness at given body weights.

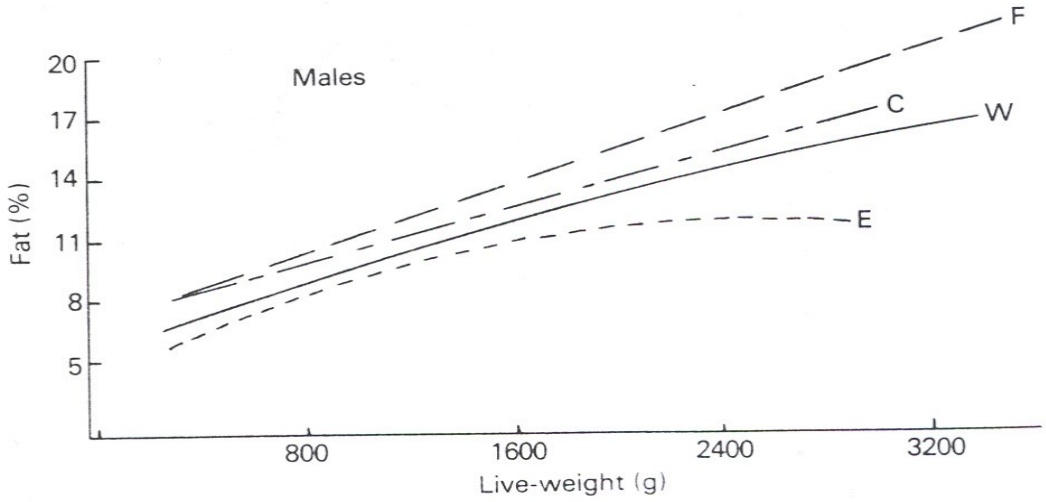

Figure 2. Growth-related changes in fat expressed as a percentage of body weight in male chickens sampled from the four lines after nine generations of selection.

It has hitherto been assumed that there was limited genetic variation in digestibility or metabolisability of dietary nutrients (Fowler, 1962; Blaxter, 1968), although some relatively small differences in metabolisability of dietary energy between strains and breeds of chickens had been reported (Sibbald and Slinger, 1963; Proudman *et al*., 1970). In the present study, metabolisability of dietary energy was measured on a number of occasions throughout the progress of the selection experiment. Line differences in percent metabolisability increased significantly between 9 and 12 generations of selection, as shown in table 1 (Pym, 1985).

Table 1. Line average metabolisability of dietary energy (%) after 9 and 12 generations of selection in the four lines.

	Line			
Generation	W	F	E	C
9	75.4±0.9	73.8±0.8	77.0±0.2	76.0±0.3
12	73.0±0.3	62.7±1.8	76.0±0.3	72.7±0.5

Selection for increased food efficiency thus resulted in a correlated increase in ME whereas selection for increased appetite had the opposite effect. The dramatic decline in ME in the F line between generations 9 and 12 suggests the possibility of a deleterious mutation in this line. Many of the birds in this line by generation 12 were characterised by having very high faecal output. It is suggested that the hitherto perceived limited genetic variation in this trait was a reflection of a lack of any attempt to exploit that variation through selection.

A respiration calorimetry study of the four lines at generation 10 (Pym *et al.*, 1984) showed significant line differences in the components of both energy and nitrogen metabolism, as shown in Table 2.

Table 2. Daily maintenance energy requirements (kJ ME/kg W), net availability of metabolisable energy (NAME) for gain (%) and net N retention efficiency (%) in the four lines at generation 10.

Line	Daily maintenance energy requirements	Net availability of metabolisable energy for gain	Net N retention efficiency
W	671±15 ^{c}	68±5 ^{b}	38±8
F	866±14 ^{a}	76±4 ^{ab}	50±6
E	701±13 ^{c}	85±6 ^{a}	56±10
C	742±11 ^{b}	73±4 ^{ab}	53±6

The high maintenance requirement in the F line are likely due to very poor feathering in this line and the role that feathering plays in insulation to body heat loss. All birds in this line expressed either slow or retarded feathering, whereas birds in lines E and W were all rapid feathering. Line differences in activity were not apparent. It is likely that the significantly higher NAME in the E than in the W line birds contributed to the above observed difference in growth-constant FCR between the lines. Differences in net protein retention efficiency were also in line with the above differences in FCR, although standard errors were large.

Measures of protein turnover were made on birds from the four lines after 12 generations of selection (Tomas *et al.*, 1988 and 1991). Whilst there were no differences between the lines in the fractional rate of protein synthesis, the fractional rate of protein breakdown was significantly lower in the E line than in the three other lines and significantly

higher in the F line than in the W or C lines. As a consequence protein accretion rate was highest in the E line, lowest in the F line and intermediate in the W and C lines.

Overall, in comparison to selection for increased growth rate, selection for improved feed efficiency resulted in an improvement in growth constant FCR through: i) a reduction in body fat, ii) an increase in metabolisability of dietary energy, iii) an increase in the net availability of metabolisable energy for gain and iv) an increase in the net utilisation of protein through a reduced rate of protein breakdown. The combined effect of these is that direct response to selection for improved feed efficiency would appear to reduce the FCR ratio by resisting increase in food intake whilst favouring a moderate increase in growth rate.

IV. GENETIC VARIATION IN APPETITE CONTROL

A number of reports have demonstrated genetic differences between breeds, strains and selected lines of birds in their response to factors regulating food intake. An exhaustive series of studies carried out with the high (H) and low (L) weight lines of Siegel (1962) provided insight into the effects of growth rate selection on food intake regulation and feeding behaviour. Dunnington *et al.* (1987) found that the L line birds were less able to compensate for a 24 h fast than the H line. This was considered due to the smaller gastrointestinal tracts (Cherry *et al.,* 1987), general hypophagia (Burkhart *et al.,* 1983), and modified feeding rhythms (Barbato *et al.,* 1980) of the L line birds. Nir *et al.* (1978) demonstrated that food consumption in meat-type, but not egg-type chickens approaches the capacity of the gastrointestinal tract. Burkhart *et al.* (1983) provided evidence of a depressed sensitivity of the satiety centre, located in the medial hypothalamus, in meat-type birds to explain their inability to increase intake of low nutrient density diets. Lesioning of this region of the hypothalamus resulted in an increase in food consumption and induced obesity in egg-type chickens (Lepkovsky and Yasuda, 1966) whereas such lesioning of Siegel's H and L lines produced the expected hyperphagia and obesity in the L line but not in the H line (Burkhart *et al.*, 1983).

One of the best recognised appetite regulatory mechanisms in mammals and birds is the energostatic control mechanism wherein the animal eats to meet its energy requirements (Fisher and Wilson, 1974; Forbes, 1986). Response in food intake and growth rate to variation in dietary nutrient density was measured in the selected lines of Pym and Nicholls (1979) and in two commercial broiler strains (A and B) (Iskandar, 1988). Birds were given five diets varying in nutrient density using dilution of the high nutrient density diet with finely ground rice hulls to achieve a range of diets with constant energy: other nutrient ratios but varying in ME from 9.5 to 13.9 MJ ME/kg. Growth rate and food intake on the diets were measured over growth intervals of 35-61 d for the experimental lines and 21-47 d for the commercial strains. The two growth periods were chosen because of the marked disparity in growth between the experimental lines and the commercial strains.

As shown in Figure 3, there was a substantial increase in food intake with decrease in dietary ME in all lines except the F line selected for high food intake and commercial strain B, which showed essentially no increase. This suggests that these birds were already eating near to gastrointestinal capacity. As such, these birds showed a much more pronounced decrease in growth rate on the lower nutrient density diets than the other lines.

As a measure of gut capacity, the amount of food eaten in a 4-h feeding period following a 24 h period of starvation (Newcombe and Summers, 1984), was measured in the six lines on the five diets and is presented in Figure 4. Food intake in the W, E and C lines increased with decreasing dietary ME whereas there was a marked decrease in intake in the B strain, with essentially no response in the F line and A strain. Responses in the E line and B strain were quite dramatically in the opposite direction.

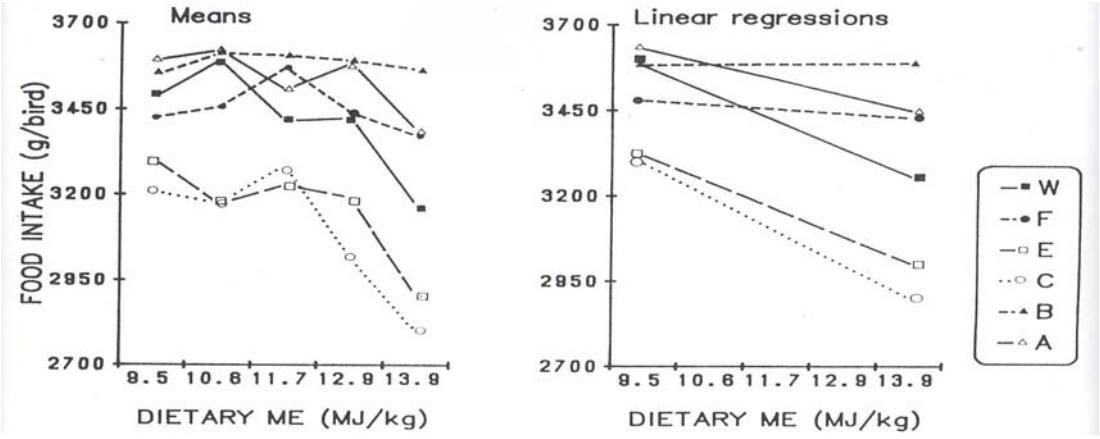

Figure 3. Food intake (means and linear regressions) of chickens (sexes combined) from the four selection lines (W, F, E and C) and two commercial broiler strains (A and B) given five diets varying in nutrient density.

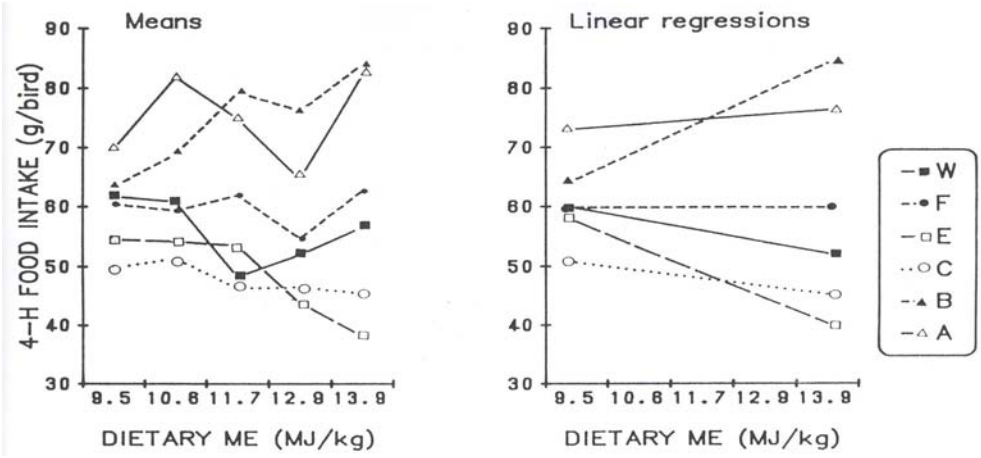

Figure 4. Food intake in a 4 h period following a 24 h fast (as a measure of gastro-intestinal capacity) in chickens from the four selection lines (W, F, E and C) and two commercial strains (A and B) given five diets varying in nutrient density

Food retention time, measured using ferric oxide in a gelatine capsule, and small intestine length in the six lines are shown in Table 3. Food retention time was greatest in line E and Strain A and least in line F whilst small intestine length in the experimental lines was greatest in line E, and in the commercial strains greater in strain A than strain B.

Table 3. Food retention time (minutes) and small intestine length (cm) in birds from the four experimental lines and two commercial strains

Line/strain	Food retention time	Small intestine length
W – Selected for ↑ weight gain	168 ^{ab}	127 ^{a}
F – Selected for ↑ food intake	161 ^{a}	127 ^{a}
E – Selected for ↓ FCR	181 ^{b}	136 ^{b}
C – Unselected control	166 ^{ab}	130 ^{ab}
A – Commercial broiler strain	184 ^{b}	161 ^{d}
B – Commercial broiler strain	173 ^{ab}	145 ^{c}
$LSD_{0.05}$	18	7

In a study of the glucostatic mechanism for appetite control in the lines, using a technique similar to that described by Sherlock and Forbes (1981), Iskandar and Pym (1990) infused varying concentrations of glucose into the hepatic portal system of birds from each line following an 18-hour fast. The birds were then offered one of three diets varying in nutrient density and food intake was measured over the next 1, 3, 6 and 24 h. Whilst there was little effect in the W, F and C lines, in the E line there was a linear reduction in 6 h food intake with increase in dietary nutrient density indicating that this line had a capacity to meet its nutrient requirements across the range of dietary nutrient densities offered, which was not evident in the other lines. There was no difference between the lines in their relative response to increasing glucose infusion levels indicating no apparent line effect upon the glucostatic mechanism of appetite control.

Because of the different growth intervals, it is appropriate to make separate comparisons between the experimental lines and the commercial strains. In understanding the differential response in the latter comparison, it is relevant to note that strain A had been selected for about 6 generations for improved feed efficiency using a selection index incorporating individual growth rate and food intake, whereas strain B had been selected essentially for growth rate alone. The response of the F line and B strain to variation in dietary nutrient density shown in Figures 3 and 4 is in keeping with the findings of Burkhart *et al*. (1983) and Nir *et al.* (1978), namely that these genotypes were essentially incapable of increasing intake under the stimulation of reducing dietary energy. Strain A, on the other hand showed a capacity to increase intake under this stimulation (Figure 3). The disparity between the genotype responses in Figures 3 and 4 are probably related to dietary bulk density and ingestibility limitations. The strong negative regression of intake on dietary ME in the E line is due more to this line eating less of the high density diet than the other lines, than to it eating more of the low density diet than the other lines.

The difference between the W and F lines and strain B in their response, may be attributable to the relatively short period of selection in the experimental lines in comparison to the commercial strain. Whilst there was undoubtedly significant pressure upon appetite in the W line, it was not so intense or sustained as to affect satiety mechanisms. In the F line, however, with direct selection for appetite, such “impairment” appears to have taken place in a relatively short period of selection and that this line, like strain B, is more or less eating to gut capacity. Given the different bulk densities of the five diets, the ability of strain B to consume a similar amount of each diet under ad libitum feeding (Figure 3), suggests that they achieved this by increasing meal frequency rather than by increasing meal size, since increase in the latter appears to be beyond their capabilities, as indicated in Figure 4. Strain A would appear to have significantly greater capacity to increase meal size. The more recent emphasis on direct improvement in food utilisation efficiency in commercial broiler breeding programs is thus likely to have improved the ability of birds to adjust intake in response to variation in dietary nutrient density.

Selection for feed efficiency in the E line and A strain appears to have resulted in correlated increases in food retention time and small intestine length. Such reduced ingesta flow rate would appear to be logically related to a greater opportunity for nutrients to be digested and absorbed. By way of contrast, selection for appetite, either directly (line F) or indirectly through long term selection for growth rate (strain B), would appear to result in a shortening of the GI tract and an increased ingesta flow rate.

V. CONCLUSIONS

Modern broiler selection programs continue to place emphasis on feed efficiency and nutrient utilisation. At least one breeding company is involved in automated data collection for measurement of "hatch to slaughter" food intake of individual birds. The impact of diet and feed form on performance and body composition is also assessed. This approach addresses concerns about the impact of feeding behaviour, activity and social interaction on the efficiency with which birds convert food into body tissue. There is no doubt that profound gains have been made in improving food utilisation efficiency of broilers through past selection of birds under the single bird cage environment, but the technology is now available to move past this and comprehensively address all the factors impacting upon food utilisation efficiency under commercial growing conditions.

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THE MACROBIOPHYSICS OF DIGESTION: IMPLICATIONS FOR THE POULTRY INDUSTRY

R.G. LENTLE[1]

Summary

I discuss work which relates the physical properties of feeds and digesta to digestive efficiency in poultry and outline recent work on the effect of the physical properties of digesta on flow. Digesta which contains significant proportions of solid matter has high non Newtonian viscosity which suppresses turbulent mixing and causes laminar flow to prevail; a situation which runs contrary to the predictions of chemical reactor theory. Digesta that contains significant proportions of flexible fibrous material behaves as a weak gel which allows extrusion of the liquid phase during peristalsis. This augments plug flow by disengagement of adhesion of the digesta plug to mucus and mucosa but also may aid permeation of the plug by digestive secretions. Preliminary work shows that efficient digestion of digesta that contains significant quantities of rigid solid material is related to the ease of passive permeation which is governed by particle size and by the viscosity of the fluid phase

I. THE GROSS EFFECTS OF THE PHYSICAL PROPERTIES OF FEEDS ON DIGESTIBILITY AND DISEASE

A species survival though evolutionary time is related to its ability to gain nutrients efficiently. A number of mechanisms may serve to optimise nutrient gain, for example the search for food items, the choice between food items, and the nutrient composition of food items. In nature these mechanisms are ameliorated by satiety thus giving time for other activities necessary for survival. The exigencies of commercial production, in selecting for nutrient gain, may reduce the efficiency of these ameliorative mechanisms. In the latter scenario, the digestive process is likely to be the ultimate arbiter of nutrient gains. Whilst nutrient gain is influenced by nutritional density, it has been increasingly recognised that the influence of particular food items on the physical properties of digesta may also modulate digestive efficiency (Hetland and Svihaus, 2001).

With regards to nutrient gain in poultry, two particular findings indicate a likely influence of the physical properties of digesta on digestibility; the fact that digestibility is adversely influenced by fibre content of feeds (Dusel *et al*., 1997; Malathi *et al*., 1997; Masonnier *et al*., 2001) and the fact that these adverse effects may be ameliorated by the addition of specific enzymes that degrade such fibres (Malathi and Dvegowda, 2001; Svihus and Guillard, 2002).

Other investigations have more directly correlated gross physical properties with digestibility. Thus digestibility has been related to the viscosity of the feed (Malathi and Devegowda, 2001) and to particle size (Carre *et al*., 1998). Similarly, a number of particular dietary elements have been identified that adversely influence viscosity and digestibility. Thus, whilst the incorporation of linseed into the diet markedly increases the viscosity of ileal digesta, decreases gross fat digestibility, digestibility of single fatty acids and dietary metabolisable energy and depresses weight gain, these adverse effects can be partially overcome by using 'demucilaged' linseed (Alzueta *et al*., 2003). Again, the feeding of linseed

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mucilage to broiler chicks significantly increases the viscosity of their jejunal digesta and has significant adverse effects on crude fat and fatty acid digestibility and AME (Rebole *et al*., 2002).

Other investigations have shown that some agents that influence the physical properties of digesta may selectively impair the digestibility of particular nutrients. Thus, the addition of carboxymethyl cellulose (CMC) to the diet of growing chickens significantly increases the viscosity of the liquid phase of their digesta and the ratio of liquids to solids, and adversely effects the digestibilities of C16:0 and C18:0 fatty acids but has no effect on the digestibilities of C18:1 , C18:2 or C12:0 or C14:0 fatty acids (Smits *et al*., 2001). Finally it is of note that the addition of particular agents which effect the physical properties of digesta may influence pathogenesis. Thus the addition of CMC to a wheat based diet significantly reduces feed conversion efficiency and weight gain in broiler chickens infected on day 21 with Eimeria acervulina (Banfield *et al*., 2002).

Whilst a body of work links the physical properties of food or digesta to digestive efficiency there have until recently been no attempts to gain an understanding of the mechanisms by which this is brought about. The following describes our investigations of some physical parameters that may influence the onflow of digesta and the efficiency of the digestive process.

THE IMPORTANCE OF MIXING WITHIN THE GUT LUMEN

The advent of confocal microscopic and genetic techniques has afforded us a detailed knowledge of the mechanisms by which nutrients are transported from the intestinal lumen into the enterocyte and beyond. However, relatively little is known of the mixing environment within the intestinal lumen.

Glycocalyceal extensions of the enterocyte microvilli may enclose an ‘unstirred water layer’ that necessitates the transit of nutrient and secreted substances by simple diffusion (Johnson and Gee, 1981). Depletion of the rate of nutrient entry into this layer will impair concentration gradients, extend the mean diffusion times and impair absorption efficiency. Efficient intraluminal mixing is therefore important for the maintenance of optimum rates of diffusion across the unstirred water layer and thus for the efficiency of digestion.

Apart from its influence in optimising rates of nutrient absorption, the efficiency of intralumenal mixing influences the establishment and maintenance of commensal facultative and obligate anerobic populations of intestinal microflora (Borriello, 2002). The establishment and maintenance of a balanced ecosystem of mucosal and luminal microbial populations is increasingly recognised as important for the maintenance of appropriate immune response, the prevention of invasion by pathogens and the proper development of the juvenile gut so as to create ‘colonial resistance’(Snel *et al*., 2002). Moreover, soon to be published work by Jeffery Gordon of the Washington School of Medicine suggests that certain enteral microflora can boost nutrient gain from digesta and upregulate the hosts storage mechanisms by suppressing fasting induced adipocyte factor.

The early human neonatal lumen is rich in oxygen and the zone of relative anoxia in the central lumen is only established following successful enteral colonisation by facultative anerobes (Mackie *et al*., 1999). In poultry there is similar early colonisation by facultative with subsequent colonisation by obligate anerobic species (Snel, 2002). Concomitant with enteral colonisation the balance of the immune response shifts from predominantly humoral to cellular immunity (Goddeeris *et al*., 2002).

Changes in the viscosity of digesta may influence the ease of establishment of pathogenic microflora by influencing the ease of mixing of the marginal oxygenated and the central lumenal anoxic zones. Thus dietary supplementation with CMC increases the

viscosity of ileal digesta which may aid the establishment of enterotoxigenic *E coli* and *Brachyspira pilocoli* (Hopwood *et al*., 2002) in young pigs.

THEORY; THE PHYSICS OF INTRALUMINAL MIXING

Mixing of reactants within the gut can occur in two broad ways, macromolecular mixing, where masses of reactants move in relation to one another, for example by convection or turbulence, or micromolecular mixing where individual molecules of reactant admix via diffusion (Levenspiel, 1972). The latter process is slow over large distances (Crank, 1975). Thus, at body temperature, the distance moved by a diffusing molecule of sucrose in 10 hours is 0.43cm (France *et al*., 1993). Given that the diameter the small intestine of the chicken is of the order of one cm, then a molecule of sugar situated at the centre of the lumen would take ten hours to diffuse to the periphery for absorption.

Highly efficient macromolecular mixing can occur during turbulent flow when groups of molecules gain sufficient momentum to admix randomly with adjacent material. The ease with which this can occur is proportional to the velocity of flow and the density of material and inversely proportional to the viscosity of the medium (which latter is equivalent to the frictional force impairing the build up of momentum). The relationship between these parameters can be expressed as a Reynolds number, lower values of this number indicating a lower likelihood of turbulent flow. Watery fluids such as blood flowing in biological tubular systems tend to have very low Reynolds numbers indicating that turbulent flow is unlikely and laminar flow, where fluid layers slide over each other in an orderly manner, is most likely. Given that digesta contains significant quantities of solid material which further increases viscosity, then Reynolds numbers are likely to be very low and turbulent flow unlikely in tubular portions of the gut (Lentle *et al*., 2002). Thus laminar flow conditions have generally been assumed to prevail in regards to the flow of digesta through the GI tract which would effectively preclude mixing across the radial dimension. The validity of this assumption is supported by studies showing that the laminar flow model is able to accurately predict the uptake of substances from watery solutions perfused though isolated gut segments (Levitt *et al*., 1988).

Recently the technique of spatiotemporal video mapping (Hennig *et al*., 1999) has been used to measure the extent and velocity of radial and longitudinal movements during rapid peristaltic contractions induced by lumen distension and by instillation of nutrients (Decanoic acid) (Gwynne *et al*., 2004). The velocity vectors obtained by these workers are generally insufficient to give Reynolds numbers associated with turbulent mixing. However, it is worthy of note that recent modelling of the flow of simple watery fluids though the rat ileum using spatiotemporal data indicate that vorticeal flow may be induced by sudden reversals of flow as the contracting segment propagates along the intestine (Jeffery *et al*., 2003). Thus turbulent mixing may occur when the intestine is occupied by simple watery solutions.

Thus far we have considered macromolecular mixing by movement of masses of digesta in relation to one another. However, macromolecular mixing can also occur by movement of the fluid phase relative to the solid phase i.e. permeation. Such relative movement is dependent on both the physical properties of the digesta and the hydraulic conditions within the lumen. Thus, provided that the digesta will sustain compression during the application of force and not ‘flow away’, compression of the matrix, (either directly by muscular contraction or indirectly via the application of hydrostatic pressure), may cause fluid phase to travel to an area of lower pressure (Weems, 1982). Spatiotemporal analysis of small intestinal contractions shows that certain patterns of small intestinal movement may be conducive to such relative movement (Gwynne *et al*., 2004). Thus zones of relative dilatation

of the gut lumen are often found adjacent to regions in which there are stationary or propagating contractions (Gwynne *et al*., 2004).

DETERMINING THE CONDITIONS FOR OPTIMUM DIGESTIVE EFFICIENCY; THE USE OF CHEMICAL REACTOR MODELS.

In the latter few decades, a number of studies have been made of the manner in which the flow of reactants through the digestive tract approximate that of ideal chemical reactors under the assumption that natural selection will tend to optimise the digestive process (Penry and Jumars, 1987). Thus the rumen has been characterised as a 'continuous stirred flow reactor' where highly efficient mixing must occur at all points within the lumen 'global mixing'. Conversely, the small intestine should approximate a 'plug flow reactor' (PFR) in which there is perfect radial mixing within consecutive radial 'slices' but minimal mixing in the direction of travel i.e. axially. However, under laminar flow conditions, digestion in the small intestine will not approach such optimality as efficient mixing of the digesta within successive radial 'slices' does not take place and there is axial mixing. Thus, those physical characteristics of digesta which promote laminar flow and decrease turbulence in successive segments of small intestine may be expected to decrease digestive efficiency.

PRACTICE; THE PHYSICAL PROPERTIES OF DIGESTA IN RELATION TO MIXING

a) Viscosity

The apparent viscosity of herbivore digesta is high and non Newtonian in both the proximal and distal parts of the small intestine in spite of relatively low dry matter content of the former. The high apparent viscosity will inhibit the establishment of turbulence and promote laminar flow. This will occur even when there are sudden reversals of flow though the contracting segment as described by Jeffery *et al*. (2003), as high apparent viscosity will tend to decrease flow though the narrowed segment and to counter any sudden increase in flow velocity.

The non-Newtonian characteristics of herbivore small intestinal digesta will cause shear near the stationary wall of the intestine to promote a lowering of apparent viscosity. This effect will promote flow in the peripheral zone of the digesta occupying the lumen whilst the more viscid axial core will tend to move as a plug with uniform velocity (Silvester 1985). Similarly, shear thinning may augment flow though contracted segments of small bowel but will tend to damp down any turbulence in the lower shear conditions that prevail within the dilated segments lying proximal and distal to the contracted segment (Sweeney and Patrick, 1977; Walton *et al*., 1981).

b) Rheometry

The extent to which the solid phase may be elastically compressed and the degree to which whole digesta flows laterally on application of stress may be determined rheometrically as the ratio of elastic modulus (G') to the loss of viscous modulus (G'') i.e. G'/G''. This can be determined by placing the digesta in an annular ring on a rheometer and measuring the torque across the annulus when the central plug undergoes rapid alternate clockwise and anticlockwise rotation. This motion causes the sinusoid of strain to be 90° out of phase with strain rate. Now the stress sinusoid of an elastic solid is in phase with deformation whereas the stress sinusoid of a viscous liquid is in phase with the strain rate. Thus by relating deformation to phase we can distinguish elastic modulus (G') from the loss or viscous modulus (G'').

The results of our rheometric analyses show that digesta from the proximal and distal small intestine of an herbivore behaves as a weak gel, the higher elastic modulus indicating an ability to sustain compression without flow. Thus, under appropriate physiological conditions, compression of digesta can occur causing the fluid phase to flow within the solid phase.

c) Permeametry

It remains to show that the hydraulic forces necessary for compression of the digesta plug lie within the physiological range of pressures that exist in the intestinal lumen. The range of hydraulic forces generated during intestinal propulsive and segmentative movements are variously reported between 2.5 cm (Gwynne *et al*., 2004) and 20cm of water (Hightower, 1968). Our work, using static hydraulic pressures applied to fresh herbivore digesta in a vertically oriented permeameter, shows that significant compression of the gel structure and extrusion of the fluid phase from the solid phase of small intestinal digesta can occur at pressures lying within the reported physiological range.

d) Augmentation of plug flow

Given that extrusion of the fluid phase occurs in the area into which propulsive action is forcing digesta it can be hypothesised that a peripheral layer of less viscid fluid interposing between the viscid digesta plug and mucus layer could disengage any tendency of mucus to adhere to digesta (Denny, 1988) and thus expedite flow.

We tested this hypothesis by comparing the viscosity of herbivore small intestinal digesta determined with a capillary viscometer lined with a cylindrical section of fresh distal small intestine to that obtained using conventional rotatory viscometry. The viscosity profile of small intestinal digesta was again high and non-Newtonian, but the apparent viscosities were an order of magnitude below those obtained by rotatory viscometry, indicating that plug flow was being augmented during capillary flow. Such augmentation may be viewed as physiologically advantageous as it prevents trauma to the delicate intestinal mucosa during on-flow of digesta, but similarly could be viewed as disadvantageous in that by expediting flow it may decrease residence time. However the latter effect may be compensated to some extent by the physiological effects of luminal particulate matter which is reported to slow the rate of peristalsis (Larson and Schultz, 2002)

It is notable that 'Hookean' elasticity of the solid phase of digesta may permit both egress and ingress of fluid from the digesta plug during phasic segmental peristalsis. Thus enzymatic components secreted by glandular elements within the intestinal walls (succus entericus) may be admixed with expressed fluid and drawn into the solid matrix of the digesta during lofting of the solid phase on relaxation following compression. Such admixed digestive enzymes would be re-expressed, along with soluble products of enzymatic digestion, in a subsequent contraction. In all, this process will augment the efficiency of digestion of the solid phase. However, this effect is likely to be reduced if intestinal compression exceeds the elastic limit of the matrix as this would lead to irreversible compaction with concomitant reduction of void spaces and permeability. Judging by the progressive increase in dry matter content of digesta from proximal to distal sites in the gut it seems likely that irreversible compression of digesta occurs during distal progression, presumably consequent on digestion reducing the strength of the matrix.

d) Inactivating the 'no-slip' function of mucus

Recent advances have shown that intestinal mucus has diverse functions (Allen *et al*., 1998) which include the formation of a microhabitat for the establishment of organisms that contribute to competitive exclusion of pathogens (Bourlioux, 1997) as well as forming a

barrier to adherence of intestinal pathogens (Moncada *et al*., 2003). Thus optimal protection demands an intact mucus layer (Moncada *et al*., 2003) yet digestion demands close interaction of mucosa with digesta to achieve efficient digestion. The significant elastic properties of herbivore mucus would enable it to adhere to food particles (Denny, 1988) thus promoting mucosal adherence to the digesta plug which may delay its passage and promote digestion. One outcome of our results, showing augmentation of plug flow under hydrostatic pressure, is that the 'no slip' effect of mucus may be disengaged during propulsive actions such a peristalsis and segmentation. As intestinal mucus forms a significant barrier to the diffusion of small uncharged molecules (Khanvilkar, 2001) it is likely that any fluid expressed from the periphery from the digesta plug will accumulate between the mucus layer and the digesta plug. This would provide a shear plane that effectively lubricates the plug during peristalsis regardless of the physical character of the epithelial surface.

OTHER FACTORS MODIFYING MOVEMENT OF THE FLUID PHASE OF DIGESTA

It is appropriate at this point to discuss other physical factors which may interact with the physical dynamics of digesta and influence digestive efficiency.

a) Particle size spectrum

Whilst feeds with finer particle sizes have a relatively greater surface area available for digestion they have smaller void spaces between adjacent particles when in packed arrays (Dullen, 1979). The latter effect results in a reduction of mean diameter of the pores available for permeation. In mixtures of large and small particles there may be relative movement of small particles within the void spaces between larger particles which may lead to progressive obturation of larger spaces during phasic fluid flow.

The effects of variation in particle size spectrum on digestibility are difficult to asses as the type of milling and setting up of the mill (Koch, 1996) along with the physical characteristics of the feed material that is being milled (Wright and Vincent, 1995) lead to differences in the shape, size, and distribution of particles that often preclude meaningful comparisons.

b) Viscosity of the fluid phase

The adverse effect of agents which increase fluid phase viscosity on digestibility is supported by extensive publications regarding the effectiveness of dietary gums in decreasing the efficiency of glucose and cholesterol absorption (Doi *et al*., 1979; Ebihara *et al*., 1981; Davidson *et al*., 1991).

If the apparent viscosity of the fluid phase contained within the digesta plug is increased by the presence of such agents, then the ease of expression of the fluid contained within the matrix during compression will be reduced (Dullen, 1979). This effect is likely in situations where steaming and subsequent pelleting of feeds has caused solublisation of starches and particles are agglomerated. Under such conditions any increase in digestibility from increase in surface area may be offset by local increases in the viscosity of the liquid phase.

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REFLUX OF DIGESTA AND ITS IMPLICATIONS FOR NUTRIENT DIGESTION AND BIRD HEALTH.

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Summary

Reflux is the anti-peristaltic induced, retrograde movement of digesta in the gastrointestinal tract (GIT). Previous research has characterized the occurrence and frequency of reflux, as well as the possible effects it has on the well-being of the chicken. Reflux may be both beneficial or harmful depending on the general health of the bird. In a healthy bird, it may present the opportunity for prolonged exposure of digesta to the enzymatic and mechanical systems of the GIT, thus leading to an increase in digestion and absorption time in the upper intestine. On the other hand, pushing caecal contents up into the intestine may increase the chance of undesirable organisms colonising the intestine, possibly resulting in subclinical infections. Another important implication of digesta reflux in chickens may relate to the use of the marker technique for determination of ileal digestibility of nutrients since the technique assumes constant, one-way flow of digesta throughout the GIT. This paper presents a brief review on the digesta reflux phenomenon and speculates how reflux could affect the general health of the chicken and how it may be manipulated by diet.

I. INTRODUCTION

The growing broiler obtains the necessary nutrients from feed, via a series of enzymatic reactions that hydrolyse the macromolecules of the feedstuffs into a form that can be absorbed into the blood and then transported throughout the body. During this complex process, food is travelling along the GIT and a proportion of it may be lost through excretion before digestion and absorption are complete. Loss of nutrients through excretion depends on a variety of factors including, the nutrient composition of the diet, the characteristics of the microbial population in the GIT, which may be both harmful and beneficial, competing for nutrients. Loss of nutrients through excretion is difficult to prevent but digestion and absorption may be slowed by the reflux, reverse peristalsis, of material from the lower GIT backup into the gizzard.

II. THE OCCURRENCE OF DIGESTA REFLUX IN THE CHICKEN

The phenomenon of reflux of duodenal contents into the gizzard has been widely accepted for over thirty years (Duke *et al*., 1972). In fact, the brownish colour of the gizzard cuticle is believed to be due to the reflux of bile pigments from the duodenum (Sturkie, 2000). Basha and Duke (1999) have carried out extensive studies on gastric and duodenal motilities of turkeys. Their research focused on the small intestinal reflux, which refers to the antiperistaltic contractions moving intestinal contents into the gizzard. Evidence of this was first obtained through the cineradiographic studies of Dziuk and Duke (1972), in which it was reported that the contents of the upper small intestine of adult Wrolstad turkeys were refluxed in the muscular stomach at irregular intervals. Additional evidence for the existence of

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retrograde movement of digesta was provided through the examination of marker distribution throughout the GIT (Oguro and Ikeda, 1974), measurement and characterization of smooth muscle electrical pulses (Roche and Ruckebusch, 1978; Martinez *et al*., 1995a,b; Jimenez *et al*., 1994) and analysis of digestive enzyme distribution along the GIT. The small intestinal reflux is a result of paired high-amplitude duodenal contractions that propagate orally at a high velocity. Gastric activity dies down during this period and then rapidly recovers to normal gastric activity (Duke *et al*., 1975).

Clench and Mathias (1992) and Jimenez *et al.* (1994) characterized a unique gut motility response to fasting in chickens. Fasting appeared to enhance antiperistaltic motility in the GIT, and extended fasting resulted in a characteristic motility pattern labelled a "rhythmic oscillating complex" (ROC). ROCs are highly organized motility patterns that spontaneously occur in fasted birds and often during periods of darkness. ROCs last approximately six minutes and consist of four consecutive patterns of spike bursts progressing in an orad direction. The result of an ROC is the re-establishment of motility patterns in the stomach and duodenum associated with satiety for a short period of time. It has been postulated that ROCs are a mechanism by which birds can recycle food from the caeca or distal intestine, therefore maximizing its nutrient resources, during fasted periods (Clench and Mathias, 1992). There are similarities between small intestinal refluxes (SIRs) in turkeys and ROCs in chickens.

III. IMPLICATIONS OF DIGESTA REFLUX FOR NUTRIENT DIGESTIBILITY

The benefits of digesta reflux have not been firmly established, and the phenomenon has been generally less studied in poultry, perhaps due to their size (Duke, 1982). In turkeys, intestinal reflux appears to enhance nutrient utilization (Basha and Duke, 1999). Reflux has been clearly characterized in fasted animals but as yet little work has been carried out investigating the effects different diets may have on motility patterns. Hetland *et al*. (2003) found that chickens fed a high fibre diet showed an increase in the concentration of bile acids in the gizzard suggesting increases in gastroduodenal reflux. Sklan *et al.* (1978) have reported a rapid rate of reflux from the duodenum to the gizzard. Digestive enzymes and bile salts, usually associated with the duodenum were also found in the gizzard at lower concentrations. Such factors would facilitate the digestion of nutrients in the gizzard, resulting in an increased availability of nutrients to the bird. A major difference suggested was that duodenal-gizzard reflux was more rapid and continuous in the chicken than in turkeys (Sklan *et al.*, 1978). Retrograde movement of duodenal contents to the gizzard re-exposes the feed, now mixed with and partially digested by pancreatic enzymes, to the enzymatic systems of the gizzard and the change in the pH reactivates peptic digestion. The shuttling of digesta between the gizzard and duodenum increases the time the feed is exposed to digestive enzymes and absorption in the upper intestine is favoured by retrograde movement. This theory would explain why high fibre diets appear to induce an increase in reflux, maximizing the nutritive value of the robust feed.

It is likely that similar events would arise when other feed factors that are not digested by animal enzymes are present in the diet. Such factors, including fibre (non-starch polysaccharides) are high in temperate cereals such as wheat, barley, oats and rye. Wheat constitutes a considerable proportion of poultry diets in Australia. Wheat quality, particularly on account of non-starch polysaccharides (NSP) in Australia is very variable between years and regions (Annison, 1993; Choct *et al.*, 1999; Hughes and Choct, 1999). This variation is partly responsible for the wide differences in

individual productivity among poultry flocks. Australia is also the leading producer of lupins and many other legume seeds, which are included as alternatives to soybeans in poultry diets (Petterson and Mackintosh, 1994; Hughes *et al.*, 2000). These seeds are rich in raffinose-series oligosaccharides, factors that cannot be digested by animal enzymes. Apart from these anti-nutritive factors, the effects of regular nutrients such as protein and fats on digesta reflux have not been previously investigated. Poultry diets vary from one growth phase to another, as does the nutrient composition of diets that are use by industry. Such variation is known to affect the productivity of poultry. Previously, knowledge of the mechanisms involved in growth discrepancies has been limited to relationships between different nutrients, nutrient deficiencies and imbalances. No investigations have been made as to the role of changes in pattern of digesta reflux on feed utilization. Infusion of fatty acids, triglycerides and complex carbohydrates into the ileum results in an "ileal brake", delaying gastric emptying and intestinal transit time (Martinez *et al*, 1995a). This mechanism is associated closely to gastric motility and is an adaptive response to ensure complete digestion and absorption of the meal in species with shorter GITs. This mechanism has been more closely studied than reflux but it is possible that they share similarities in that they both appear to be a reaction to complex diets, with the aim of obtaining the maximum nutritive value from the feed.

III. IMPLICATIONS OF DIGESTA REFLUX FOR THE HEALTH OF CHICKENS

The proportions of different microbial species and their location within the GIT of poultry have been previously studied (Apajalahti *et al.*, 2001). Some of these species, *e.g.* Lactobacilli are beneficial, but some strains of species such as *Salmonella, and Escherichia coli* can be pathogenic. Normal peristaltic movements are able to move species from the upper GIT to the lower gut, but antiperistalsis (digesta reflux) has the opposite effect and it is not known to what extent this will pose a health risk. It is envisaged that organisms carried in the refluxed digesta may colonise the upper part of the tract if the conditions are right. For example, in birds fed diets based on non-viscous grains, such as corn and sorghum, there is little fermentation in the small intestine. However, when they are fed diets based on viscous grains, such as wheat, barley and rye, fermentation in the small intestine increases rapidly (Choct *et al.*, 1996). When digesta viscosity increases, digesta passage slows down and oxygen tension in the intestine decreases, creating a relatively stable environment with a low level of oxygen for anaerobic microflora to establish (Smits *et al.*, 1998). The implications of such a change are many, including proliferation of pathogens and changes in nutrient utilisation.

IV. CONCLUSION

It is evident that reflux occurs, but the extent and pattern of reflux in different classes of poultry are not understood. In addition, identification of dietary factors that influence reflux is of practical significance as the information may be used to formulate diets that take advantages of its positive aspects and avoid the negative side.

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EFFECT OF LOW DENSITY FEEDS ON PERFORMANCE OF BROILER BREEDERS AND THEIR OFFSPRING

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Summary

In an experiment with 1,440 Cobb 500 female broiler breeders in the rearing period and with 1,260 Cobb 500 female and 126 Cobb 500 male broiler breeders in the laying period, the effect of low density feeds on performance of broiler breeders, egg composition, embryonic development and offspring performance were studied. The experiment included 3 treatments of 6 replicates each: a control group with practical AME-levels (treatment 1) and 2 groups in which the AME content of both rearing and laying feeds was decreased by 1.26 (treatment 2) or 2.51 (treatment 3) MJ/kg. Results showed no differences in live weight of broiler breeders between treatments at the end of the rearing period. There was a tendency for a delayed development of the reproductive tract at the lowest AME level. During the laying period, treatment 2 gave a significantly higher laying percentage compared to treatment 1 and 3. Egg weight significantly increased with decreased AME. The increase in egg weight was accompanied by a significantly higher albumen/yolk ratio and a significantly better development of the area vitellina externa. Weight of day old chickens was also higher at lower AME levels and mortality was significantly lower with low density feeds at 60 weeks of age. It was hypothesised that increased egg weights, albumen/yolk ratios and embryonic development were due to changes in reproductive development and that changes in offspring performance were related to this.

I. INTRODUCTION

Due to the high growth potential, broiler breeders are fed restrictedly during the rearing and laying period in order to prevent health and reproduction disorders (Katanbaf *et al*., 1986; Hocking *et al*., 1994). The restriction in nutrient intake is particularly strong during the rearing period. In current feeding programmes for broiler breeders, the daily energy intake is restricted to 1.3 times the maintenance requirement in the period from week 12 to 15. This can result in chronic stress and hunger feeling (Gross and Siegel, 1983, 1986; Hocking *et al*., 1993; Savory *et al*., 1996; De Jong *et al*., 2002).

In order to reduce hunger feeling in broiler breeders, qualitative instead of quantitative restriction of feed and nutrient intake has been studied. In studies with pigs, it was found that dilution of feeds by inert materials or low energy feedstuffs might reduce chronic stress and hunger feeling (Brouns *et al*., 1994; Ramonet *et al*., 2000). However, studies in broiler breeders in this field have been limited and results were contradictory (Zuidhof *et al*., 1995; Savory *et al*., 1996; Savory and Lariviere, 2000).

Smulders and Enting (unpublished) reduced nutrient levels in broiler breeder feeds by 11 and 23 % by changing the feed composition. They found a significant reduction in heterophil/lymphocyte HL-ratios at six weeks of age and a significant reduction in mortality of broiler chickens from broiler breeders that were given low density feeds. Based on these observations, a second experiment was carried out to get more insight of the effect of low density feeds on broiler breeder and offspring performance. This paper presents results of this second experiment.

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II. MATERIAL AND METHODS

(a) Animals and housing

In total 1,620 Cobb 500 day old female broiler breeders were bought from a breeding company (Cobb Europe, Putten, The Netherlands). Day old chickens were placed in 18 floor pens of 4.5 x 3 m with 90 chickens per pen. Each pen was fitted with laying nests, that were closed during the rearing period. Each treatment was replicated in 6 pens.

In week 22, the number of female broiler breeders was reduced to 70 per pen and 7 male Cobb 500 broiler breeders were placed in each pen. Light, temperature and feeding schedules were according to guidelines of the breeding company. Feed levels were adjusted when live weight of the birds deviated more than 5 % from the recommended live weights. Birds were vaccinated according to the standard vaccination programmes and beaks were trimmed at day 4.

(b) Dietary treatments

The experiment included three treatments. In treatment 1, standard broiler breeder diets were given with an AME content of 10.88 MJ/kg in the rearing period and 11.72 MJ/kg in the laying period. In treatment 2 and 3, the AME content of the feeds was lowered by 1.26 and 2.51 MJ/kg respectively by inclusion of low density feedstuffs in the feeds (palm kernel meal, wheat bran, lucerne, wheat gluten feed and sunflowerseed meal). In the low density feeds, digestible lysine, calcium, retainable phosphorus and linoleic acid were lowered to the same extend as the AME content. The intake of first limiting nutrients was kept constant between treatments by increasing the feed allowance to the same extend as the AME content was decreased.

(c) Measurements

Live weight of the birds was determined every 3 weeks. Feed intake, laying percentage and egg weight were recorded on a daily basis. In week 24 and 26, 2 birds of every replicate were sacrificed by cervical dislocation and animal, oviduct and ovary weight were recorded, as was oviduct length. Albumen/yolk ratio and embryonic development were determined in 440 eggs of treatment 1 and 3 at week 29 and 41 weeks of age. Embryonic development was measured after 24, 36, 48, 72 and 264 hours of incubation according to stages published by Hamilton (1952).

(d) Statistical analysis

Data were statistically analysed by the analysis of variance method. Treatment means were compared by least significant differences (Snedecor and Cochran, 1967). The generalised model used in the analyses of variances included mean, block, treatment and residual variation. Differences between treatments were considered significant at $P<0.05$. Linearity between the AME content of feeds and performance parameters was tested with generalised model including mean, block, AME, AME^2 and residual variation. Results of the embryonic development were subjected to Student's t-test to find significant differences.

III. RESULTS AND DISCUSSION

During the rearing period, no significant differences in live weight were observed between treatments. At the end of the rearing period, the target weight of 2,550 grams was almost reached. In Table 1, the development of the reproductive organs between week 24 and 26 is given. In week 24, treatment 3 gave the lowest ovary and oviduct weights, which were not significantly different from treatments 1 and 2. The changes in oviduct and ovary

development between week 24 and 26 gave significant differences between treatments. The faster growth of the reproductive tract between week 24 and 26 resembled a delay in the onset of the development of the reproductive tract when feed intake or day length is more restricted during rearing (Hocking, 1996; Bruggeman *et al*., 1999). This would indicate less efficient utilisation of the low density feeds.

Table 1 Effect of diet density on ovary and oviduct weight and length in week 24 and development of ovary and oviduct between week 24 and 26

Treatment - AME, MJ/kg	1 - 10.88	2 - 9.62	3 - 8.37	P lin.	P quadr.
Week 24					
Weight ovary, g	24.6	24.9	15.9	0.407	0.611
Weight oviduct, g	37.6	38.6	33.9	0.704	0.736
Length oviduct, cm	48.5	50.2	48.0	0.954	0.786
Week 24-26					
Growth ovary, g	30.0[a]	34.8[ab]	53.3[b]	0.016	0.354
Growth oviduct, g	27.1	18.2	40.1	0.170	0.499
Increase in length oviduct, cm	10.1	9.1	14.7	0.486	0.545

Low density feeds did not have a negative effect in laying percentage (Table 2). In treatment 2, laying percentage was significantly higher compared to treatment 1. A similar effect was found by Zuidhof *et al*. (1995) when feeds were diluted with 15% oat hulls. During the laying period, no differences in live weight of the birds were observed. Egg weight increased when low density feeds were given. There was a significant linear relationship between the AME content of the feed and egg weight (P=0.002). The higher egg weight might be related to the higher growth rate of the reproductive tract between week 24 and 26. Smulders and Enting (unpublished) did not find an increase in egg weight when low density feeds were given only during the laying period.

Table 2 Effect of diet density on laying percentage and egg weight in the period from week 26-60

Treatment - AME, MJ/kg	1 - 11.72	2 - 10.46	3 - 9.20	P lin.	P quadr.
Feed intake, g/hen/day	156.5[a]	176.2[b]	199.9[c]	<0.001	<0.001
Laying percentage	57.7[a]	60.2[b]	58.0[ab]	0.771	0.021
Egg weight, g	65.4[a]	65.9[ab]	66.5[b]	0.002	0.773

Table 3 presents the effects of low density feed on albumen/yolk ratio and embryonic development.

The higher egg weight that was obtained with the low density feed was associated with a higher albumen/yolk ratio. The low density feed resulted in significant increase in the growth of the area vitellina externa and the embryo in eggs of 29 week old hens. In week 41, these differences were less clear. Day-old chicken weight was significantly higher for the low density diet in week 41. These results support the findings of Latour *et al*. (1996) and Ohta *et al*. (1998) that egg composition can affect embryonic and chick development. At the end of the growing period, the only significant differences in live weight of broiler chickens were observed with young breeders (Table 4). Mortality rate was significantly lower in broiler chickens of 60 week old broiler breeders when low density diets were given.

Table 3 Effect diet density on white/yolk ratio, area vitellina externa and embryo and chick weight of 29 and 41 week old broiler breeders

Treatment - AME, MJ/kg	Incubation period, hr	1 - 11.72	3 - 9.20
Week 29			
Albumen/yolk ratio		1.707[a]	1.775[b]
Area vitellina externa, mm^2	24	58.5[a]	83.3[b]
	48	700.0[a]	910.1[b]
Weight embryo, g	264	1.674[a]	2.039[b]
Weight chicken, g	504	35.9	36.3
Week 41			
Albumen/yolk ratio		1.594[a]	1.612[b]
Area vitellina externa, mm^2	24	84.3[a]	99.9[b]
	48	837.1	858.4
Weight embryo, g	264	3.263	3.241
Weight chicken, g	504	41.8[a]	42.8[b]

Table 4 Effect of diet density on growth rate and mortality of offspring

Treatment - AME, MJ/kg	1 - 11.72	2 - 10.46	3 - 9.20
Week 29			
Live weight, day 38, g	2,125[a]	2,185[b]	2,131[a]
Mortality, day 0-38, %	3.3	3.8	3.2
Week 41			
Live weight, day 38, g	2,336	2,345	2,332
Mortality, day 0-38, %	3.0	2.9	3.5
Week 60			
Live weight, day 38, g	2,257	2,262	2,280
Mortality, day 0-38, %	5.4[b]	3.7[ab]	3.5[a]

Based on the results of this study, it can be concluded that low density feeds can affect the development of the reproductive tract, egg production, egg size and composition, embryonic development and broiler performance. Differences in egg production may be related to digestibility of feeds. Egg size and egg composition seem to have an effect on embryonic development. It is hypothesised that differences in egg size and egg composition are due to differences in reproductive tract development.

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EGG NUTRITION FOR HEALTH PROMOTION: HIGHLIGHTS FROM THE SYMPOSIUM IN BANFF, CANADA

J.ROBERTS

Summary

This report summarises the main papers presented at the Third International Symposium on Egg Nutrition for Health Promotion, held in Banff, Canada in April, 2004. There are many marketing opportunities for eggs in forms other than shell egg and unmodified egg product. Eggs can be enriched with substances beneficial to human health such as selenium, zinc, vitamin E, folate, lutein, choline, phytoestrogens and omega-3 long chain fatty acids. Substances that inhibit the growth of microorganisms can be extracted from egg white and egg shell membranes. Eggs can be used for antibody farming and such antibodies can be used to treat lung infections in people with cystic fibrosis, dental caries, the organism that causes stomach ulcers and the organism responsible for diarrhoea in children in developing countries. Products from eggs are also used in the cosmetics industry. The challenges for the wider industry are to ensure that these 'ovo-nutraceuticals' and 'bio-medical products' can be produced cost-effectively, to increase the range of products available on the market, to increase market penetration and be competitive against rival products.

I. INTRODUCTION

The Third International Symposium on Egg Nutrition for Health Promotion was held in Banff, Canada on April 18-21, 2004, chaired by Dr. Jeong S. Sim from the University of Alberta. The first symposium was held in 1992 and the second in 1998. The papers presented at the third Symposium will be published in a Post-Symposium Book "The Amazing Egg: Nature's Perfect Functional Food for Health". Ordering information is supplied at the end of this paper. The papers presented highlighted the fact that the hen's egg is, indeed, an amazing natural product and that there are many marketing opportunities for the hen's egg, in addition to selling it as shell egg and raw egg product. However, some of the presenters highlighted the economic realities associated with the production and sale of the wide range of egg chemicals and this will be discussed further.

II. PROCESSING TECHNOLOGIES FOR OVO-NUTRACEUTICALS AND BIO-MEDICAL PRODUCTS

The introductory speaker, Dr. G.W. Froning of the University of Nebraska-Lincoln, described the hen's egg as a highly nutritious food containing high quality protein, twice as much unsaturated fat as saturated fat and an excellent source of minerals including iron and phosphorus and all the vitamins except vitamin C. In the U.S., egg consumption has increased between 1994 and 1999-2003 and about 30% of consumption is egg products such as liquid egg white, liquid egg yolk, liquid whole egg, extended shelf-life whole egg, frozen egg white, salted egg yolk, sugared egg yolk, salted whole egg, sugared whole egg, dehydrated egg products, and manufactured egg products. It is possible to influence the composition of the egg in terms of levels of selenium, zinc, vitamin E, folate, lutein and omega-3 fatty acids. Lutein and zeaxanthin which reduce the incidence of macular degeneration of the eye are easily transferred through the diet of the hen into the egg where they have a high bioavailability. Choline, which is important during pregnancy and may assist with dementia such as Alzheimer's Disease, is found in other foods (beef liver, beef

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steak, cauliflower) but has a better bioavailability in the egg. Lysozyme is easily separated from egg albumen and can be modified in various ways. Lysozyme is used to prevent gas formation in cheese, increase the shelf life of meat products, enhance foaming of wines, as a food sweetener, in cold remedies and mouth washes. Shell membranes also contain enzymes which lyse gram positive bacteria (lysozyme), gram negative bacteria (β-N-acetylglucosamidase) and shell membrane extracts have been shown to inhibit *Listeria*, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*. Proteins from the organic matrix of the egg shell have been shown to inhibit *Pseudomonas, Bacillis* and *Staphylococcus* and pancreatin-treated water soluble yolk protein has been shown to inhibit *Streptococcus mutans*. Avidin from eggs binds biotin and can be used as a pesticide. Transgenic maize containing avidin resists post- and pre-harvest pests and has been found safe when fed to mice. Biopolymer edible films and coatings made from egg white proteins are used to encapsulate foods and cosmetics, as carriers of antioxidants and flavours in the food, chemical and pharmaceutical industries. The main challenges in relation to products from eggs are the difficulty of extracting some proteins economically and the problem of allergies.

Dr. H.R. Ball of Michael Foods, U.S.A. discussed the challenges facing the egg industry in the development and marketing of "ovo-nutraceuticals" and "bio-medical products". The composition of the egg is altered by regulating the hens' dietary intake or immune system and extracting egg components. These products are not produced by genetic modification. The proportion of eggs consumed as product is currently 30% but is expected to reach 40% in the U.S. It was pointed out that there is currently a limited range of offerings of ovo-nutraceuticals and biomedical products and that market penetration is relatively small. Challenges facing the expansion of these markets include costs of production, scale of operation, regulatory issues, consumer awareness and receptivity, the competitive environment and the finding of outlets for the residual egg components that remain following the extraction of the target compound. Unless such outlets are available, the enterprise is not cost-effective. Dr. Ball discussed the business model adopted by Michael Foods. For shell eggs such as enriched eggs and egg product such as dried eggs, the proprietary position is that there are patents of limited scope, the know-how to produce them exists and there are relatively low competitive barriers. For extracted egg components, there are higher capital investment requirements, higher barriers to entry and higher risks.

Dr. D.U. Ahn from Korea described advances in the extraction from egg yolk of components such as immunoglobulin, lipids, phospholipids and yolk proteins. Egg yolk is diluted and then subjected to freeze-thaw cycling. Various methods (ultrafiltration, precipitation with ammonium sulphate) are used to concentrate the product. Factors such as temperature, pH and salt all influence the process.

Methods of reducing the cholesterol content of shell eggs were discussed by Dr. R.G. Elkin from The Pennsylvania State University, U.S.A. Between 15% and 30% of individuals exhibit a hyper-response to dietary cholesterol. In order to manipulate cholesterol levels, different parts of the cholesterol biosynthetic pathway may be targeted. Established approaches include genetic selection (this appears to be effective only in increasing cholesterol levels), alteration of the hen's diet, hormones, non-pharmaceutical biochemicals, pharmacological agents. The use of various nutrients, non-nutritive factors or pharmacological agents have, at best, reduced egg cholesterol by 10% whereas oral administration of statins, garlic paste or pharmacological amounts of copper reduced egg cholesterol by 46%, 32% and 34% respectively. Dr. Elkin expressed the opinion that further reductions in egg cholesterol levels will be achieved by manipulation of key genes associated with the uptake and synthesis of lipids.

III. NUTRITIONAL ENHANCEMENT OF SHELL EGGS

Dr. P.F. Surai from the Scottish Agricultural College, Auchincruive, discussed the importance of adding antioxidants such as selenium, vitamin E and lutein to the diets of hens in order to increase the levels of these substances in the egg. These antioxidants have potential benefits in themselves and may also be used to reduce lipid peroxidation in omega-3 enriched eggs, thus reducing the fishy taste. Dr. J.E. Dvorska of the Sumy National Agrarian University, Ukraine, described the advantages of using organic selenium, as opposed to inorganic selenium, in the diets of hens to increase the selenium content of eggs.

The health benefits accruing from xanthophylls in eggs were outlined by Dr. D.J. McNamara of the Egg Nutrition Centre, Washington D.C., U.S.A. The xanthophyll carotenoids, lutein and zeaxanthin, have been used in the poultry industry for many years as cosmetic colouring agents for egg yolks and broilers, often in combination with red carotenoids. In humans, lutein and zeaxanthin accumulate in the lens of the eye and also the macular region of the retina, helping to maintain normal visual function and protecting against macular degeneration and cataracts. There is also some evidence that lutein is protective against cardiovascular disease and some cancers (breast, colon). Eggs can provide lutein in a form that is highly bioavailable and the lutein content of eggs can be modified by manipulation of the diets of the hens.

Dr. S. Leeson of the University of Guelph, Canada, discussed the relatively low transfer efficiency of lutein from the diet to the hen's egg and possible mechanisms for increasing this efficiency. Lutein is found in spinach, broccoli, alfalfa, corn gluten and marigold meal. Lutein absorption is related to fat utilization and digesta viscosity.

Dr. C.A. Adams, from Kemin Europa, discussed the difference between a nutrient and a nutricine. Nutricines include organic acids and antioxidants that are used to maintain feed quality and hygiene, flavours used to increase feed intake, enzymes used to increase digestion and absorption, organic acids and oligosaccharides that modulate the function of the gastrointestinal tract, carotenoids, glucans and herbal extracts that modulate the immune system and antioxidants that reduce oxidative stress and reduce the incidence of some non-infectious diseases. Dr. Adams discussed the carotenoid, lutein, in further detail, pointing out that, while they were previously used as a cosmetic colouring agent in eggs and broilers, they are now considered an important nutricine in poultry and human health. Lutein absorbs free radicals so helps protect against oxidative damage and supports the immune system. Lutein is a naturally occurring carotenoid which usually occurs with zeaxanthin and occurs in all green leaves, whereas barley, rice, sorghum, wheat and oilseed meals are low in lutein. Dr. Adams outlined the benefits of lutein in protecting the chick during hatching and presented data indicating that lutein can improve immune function in dogs and avian species.

Dr. House of the University of Manitoba described how eggs can be enriched with folate and that the form of folate found in eggs is more available than crystalline folic acid. Excess crystalline folic acid can risk masking the symptoms of pernicious anaemia (vitamin B12 deficiency), whereas the folate in eggs does not cause this problem. Dietary folate is known to be important in the human diet for normal cell division, synthesis of red blood cells and metabolism of proteins and amino acids. It is also important in reducing the incidence of neural tube defects (e.g. spina bifida) and miscarriages. There is also some evidence that folate reduces the incidence of cardiovascular disease as it is a cofactor in the metabolism of homocysteine and elevated levels of homocysteine are an independent risk factor for cardiovascular disease. As Alzheimer's Disease and dementia are linked to cerebrovascular disease, folate may have a role in reducing the incidence of these diseases also.

Many nutriceuticals currently targeted at older women contain soy isoflavones (phytoestrogens). Dr. M.A. Ottinger described how the eggs of Japanese quail can be

enriched with the soy isoflavone genistein which has a structure similar to the female hormone, oestradiol.

IV. EGG LIPIDS

Dr. Cherian from Oregon State University described the process of feeding conjugated linoleic acid (CLA) to chickens in order to produce CLA-enriched eggs. CLA is a natural trans fat that is produced in the rumen of ruminant animals so is therefore found mainly in dairy and beef products. The interest in CLA isomers arises from evidence that they possess anticarcinogenic properties and can modulate the immune system. They have also been shown to improve feed efficiency and decrease body fat deposition in pigs and broiler chickens (Watkins et al., 2000). Evidence suggests that more than 3 grams per day needs to be consumed by humans in order to accrue health benefits. Because CLA is found in fat, reduced consumption of fat in dairy and beef products means that less CLA is consumed from these sources. The incorporation of CLA into eggs is an easy and efficient way to increase CLA intake. Addition of CLA to the diets of hens changes yolk shape by making the yolk more round but there is no effect on egg weight, Haugh units or the nutritional value of the eggs. CLA ingestion was found to increase the deposition of lipid droplets in the livers of laying hens but not broilers. When CLA enriched eggs were stored under refrigeration for 40 days, the water content of the yolk increased. Long term storage (3-4 months) resulted in a reddish colour in the yolk.

The presentation of Dr. A.P. Simopoulous from the Centre for Genetics, Washington D.C., U.S.A., was delivered by Dr. Sim who explained the evidence that human diets in earlier times had a ratio of omega-6 to omega-3 essential fatty acids of approximately 1 whereas most present day Western diets have a ratio of from 15 (U.K.) to 16.7 (U.S.A), as compared to Japan where the ratio is 4. This means that the current Western diets contain excessive amounts of omega-6 fatty acids and are deficient in omega-3 fatty acids. This imbalance is thought to contribute to diseases such as cardiovascular disease, cancer, and inflammatory and autoimmune diseases. One way of increasing the consumption of omega-3 fatty acids is via enriched eggs.

Omega-3 fatty acids have been shown to be beneficial in the human diet in terms of affecting plasma lipid levels, visual function and child growth and development. Dr. Jones from McGill University in Canada addressed the benefits of the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish oils and alpha-linolenic acid (ALA) from plant oils in reducing plasma triglyceride levels in humans. High triglyceride levels are a risk factor for cardiovascular disease and stroke. However, these compounds appear to have little effect on low-density lipoprotein (LDL) cholesterol, another risk factor. There is potential for an improved effect on plasma lipids if EPA and DHA are conjugated to plant sterols as a study conducted with guinea pigs showed that both triglycerides and LDL cholesterol were reduced by such a combination.

The importance of dietary omega-3 fatty acids in visual function was addressed by Dr. Suh from the University of Manitoba, Canada. The long chain polyunsaturated fatty acids (LC-PUFA, C20:4n-6 and C22:6n-3) are essential for normal visual development in infants. These fatty acids are found in mothers' milk and it has been recommended that they be added to infant formula. There is also evidence that LC-PUFA have a role to play in reducing the incidence of degenerative diseases of the retina of the eye such as retinitis pigmentosa and macula dystrophy.

More general aspects of the importance of omega-3 polyunsaturated fatty acids (DHA and arachidonic acid, AA) in child development were addressed by Dr. Makrides from the Child Health Institute in Adelaide and Dr. Clandinin from the University of Alberta, Canada.

Dr. Makrides commented on the fact that there is now a tendency to use iron-fortified cereal as the first semi-solid food for infants whereas, traditionally, eggs and brains were fed. Egg allergies affect approximately 3% of infants under three years of age. Dr. Clandinin described the benefits of supplementation of diets with DHA and AA in improving mental and psychomotor development and development of the immune system in children and saw the main role of eggs as being during the weaning period. Dr. Clandinin made the comment that infant formula manufacturers are not willing to reformulate their product to incorporate the omega-3 fatty acids, they just want a small volume of AA and DHA to add to existing formula. Enriched eggs remain a potentially important source of the omega-3 fatty acids in the diets of infants, children and adults.

V. ANTIBODIES FROM EGGS

Hens are able to deposit antibodies into the yolk of the egg for the purpose of protecting the developing chick. These yolk antibodies are collectively referred to as IgY which stands for immunoglobins from yolk. Antibody-farming technology exploits this capability of the hen to produce a range of different types of antibodies in the eggs by immunizing the hen with specific substances and is an easy and inexpensive process. Hens are immunized several times and then require booster doses every 2-3 months. The antibodies, which are stable for several months, act by preventing adhesion of microbes to epithelial cells, inhibiting the growth of microbes and neutralizing the toxins produced by microbes. Dr. Larsson from University Hospital, Uppsala, Sweden discussed the production of yolk antibodies for oral treatment of cystic fibrosis patients against *Pseudomonas aeruginosa* infections in their lungs. The life expectancy of cystic fibrosis patients is 40-50 years and the main killer of these patients is *Pseudomonas aeruginosa.* This organism is impossible to eradicate but treatment with the yolk antibodies prolongs the time between active infections, reduces the need for antibiotics, postpones the onset of chronic infections and helps to preserve lung function. The formulation of the yolk antibodies has now been changed to contain 6 strains. Dr. Larsson discussed the need for a double blind study to determine the effectiveness of the product and the fact that it requires orphan drug status (that is, it has a limited market).

Dr. Sunwoo described the production of a new product, NutraGuard^{TM} for treatment of people with coeliac disease. This disease was first described in 1888 and can be controlled by a gluten-free diet which was first introduced in 1950. The symptoms of the disease are chronic diarrhoea, weight loss, anaemia, bone pain, behavioural changes, gastrointestinal disease and delayed growth. There is a genetic basis to the disease which interacts with environmental factors. NutraGuard^{TM} contains specific IgY along with vitamin E, DHA, folic acid and selenium and can be administered as capsules or added to foods.

Dr. Schade from Humboldt-University in Germany outlined the ongoing work that is attempting to develop IgY anti-prion antibodies for use in assays to detect the presence of prion diseases such as bovine spongiform encephalopathies (BSE-assays). Prions are proteinaceous infectious particles consisting of 151-190 amino acids. It has proved difficult to produce specific antibodies in mammals because of the similarity of the amino acid sequences to those found in other mammalian proteins. However, the use of a different class of animals, the birds, has resulted in some breakthroughs. Dr. Schade explained how a shortage of BSE positive and negative brain material and blood samples was hampering the research.

Antibodies from egg yolk can even be used to treat dental caries, as described by Dr. Smith from the Forsyth Institute, Boston, U.S.A. *Streptococcus mutans* is the main cause of dental caries and infection of humans does not occur until the final primary tooth eruption.

Once a person is infected, they are always infected. Immunisation of hens with *S. mutans* glucan binding protein B results in the appearance of antibodies in egg yolk that have been shown to protect against dental caries in a rat model. The best time for this product to be applied would be at the time of initial *Streptococcus mutans* colonization and after mechanical/chemical cleaning of teeth.

The organism *Helicobacter pylori* causes gastric ulcers in humans. *Helicobacter pylori* specific IgY prepared from the yolk of hens immunized with has been shown to be effective in treating *Helicobacter pylori* infections, as described by Dr. Kim from Dankook University, Korea. Apparently, approximately half the world's population is infected with *H. pylori*, with the incidence being higher in developing countries (80-90%) than in developed countries (10-50%). The antibodies, which are usually given in combination with antibiotics, appear to work by inhibiting the adhesion of the bacteria to the epithelial cells of the stomach. Dr. Sarker from Bangladesh described ongoing work that is investigating the use of egg yolk antibodies for the treatment of human rotavirus which causes diarrhoea in children and is a major problem in developing countries. Dr. Korhonen from Finland described how the same principle of "designer" antibody production can be achieved in bovine milk, following immunization of the cow.

VI. EGG MARKETING STRATEGIES

The final session of the symposium focused on egg marketing strategies which are essential if the potential additional markets for eggs are to be realised. Dr. Watson from the University of Arizona, U.S.A., discussed the egg as a vehicle for delivery of a range of products such as fruit bioflavenoids. Administration of a product JuicePlust® to older people (60-86 years) resulted in improved indicators of immune function. As the active ingredients are fat soluble, it was suggested that they could be incorporated into eggs to produce a functional food supplement with the potential to reduce the incidence of cancer. Dr. De Meester from Belgium described the process of development from Dr. Sim's Designer Egg to the Columbus® Concept. The Columbus® Concept is the return of alpha-linolenic acid (ALA, C18:33), described as "wild fatty acid" to the rations of land-based bred animals so that their fatty acid ratios (ω6: ω3) returned to 1:1, the ratio characteristic of fat deposits in wild animals. As discussed in Section IV, above, such a change would have health benefits for these animals and the humans who consume them.

Dr. Juneja from the Taiyo Kagaku Company of Japan gave an extremely entertaining and informative presentation on the wide range of marketing opportunities for egg nutriceuticals. He outlined the uses for sialic acid which he described as the "sugar" in egg. It is found in all parts of the egg: yolk, white, chalazae, yolk membrane, egg shell, in order of diminishing content. Sialic acid has been shown to inhibit rotavirus. Dr. Juneja referred to the work mentioned in Section V and suggested that IgY against *Streptococcus mutans* could be added to foods such as chocolate and chewing gum. He amused the audience by describing the obsession that young Japanese women have with their hair and, particularly, the prevention of "split ends". Hair cuticle IgY has been shown to repair damaged hair and can be added to shampoo. The use of egg shell calcium has marketing potential as the calcium contained in it is highly bioavailable and it doesn't give a chalky taste. Products utilizing egg shell calcium are already on the market. The egg shell membrane can be used to treat wounds as it promotes collagen synthesis, absorbs moisture, releases proteins and has antimicrobial activity. Yolk protein contains all the essential amino acids required by 2-5 year old children. Lysozyme obtained mainly from egg white is able to lyse gram positive bacteria (but not gram negative ones). Yolk lecithin is a safe and natural source of AA, DHA, cholesterol and choline as an additive for infant formula. Dr. Juneja finished up by

describing how his company has won awards for its products: in London in 1997 for sialic acid; Frankfurt in 1998 for Suntheanine and Sunphenon and in Paris in 1999 for Sun Active Fe and Sun GY.

The importance of egg lecithins was further discussed by Dr. Lange from Degussa Food Ingredients, Germany. Sales of egg lecithin have increased from 1995 to 2002 by 340% worldwide, 475% Far East, 320% Europe and 245% U.S.A. Of the lecithin sold, 60% is used in infant formula, 15% in injection solutions for clinical nutrition, 15% in cosmetics and 10% as functional foods. The composition of egg lecithin (the fatty acid profile) can be optimized by pure vegetable feeding of hens and use of different production technologies. Infants are not able to synthesise AA and DHA from fatty acid precursors so they need to obtain these either from mothers' milk or infant formula. There is competition from other fatty acid sources for inclusion in infant formula. Egg lecithin is used for total parenteral nutrition and injections because of its emulsifying properties. In cosmetics, it emulsifies and stabilizes, acts as a skin nutrient and moisture regulator of skin, protects skin and hair and acts as a carrier for pigments and liposomes. Egg lecithin has a range of applications in food products.

Dr. Basu from the University of Alberta, Canada, discussed the fact that the designer food concept is currently very strong. He described the period 1920-1950 as the vitamin era, 1970-1990 as the mega-vitamin era and 1990 to the present day as the functional food era. He defined functional foods as foods that encompass potentially healthful products including any modified food or food ingredient that imparts benefit beyond the food that they contain. Dr. Basu mentioned the dilemma resulting from the fact that there is no universally accepted provision in the current food regulations for functional foods. The situation is further complicated by the fact that some foods work synergistically with other foods.

VII. CONCLUSIONS

In conclusion, the egg of the hen is a natural product which, by virtue of its role in nature has many properties that can be exploited by humans. It can be used as a means of delivering substances beneficial to human health in a form that is natural and highly bioavailable. The hen's egg can be used for the extraction of its component parts and it can be used for the production ("farming") of antibodies to a wide range of agents. However, the commercial realities of the production of this range of products are an important determinant of the extent of the market for the ovo-nutraceuticals and bio-medical products from eggs.

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INVESTIGATIONS INTO THE EFFECT OF FEEDING LAYING HENS COMPLETE DIETS WITH WHEAT IN WHOLE OR GROUND FORM AND ZEOLITE PRESENTED IN POWDERED OR GRIT FORM, ON PERFORMANCE AND OOCYST OUTPUT AFTER BEING CHALLENGED WITH COCCIDIOSIS

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Summary

A preliminary investigation was conducted to examine the effects on performance of feeding a complete balanced layer diet to laying hens late in lay (70 weeks of age) for 16 weeks where wheat was presented in whole and unground forms and zeolite (insoluble grit) was presented in powdered (<250 μm) and particulate forms (2.0-4.00 mm), a total of 4 treatments. There was no significant effect of grain form on egg production, egg weight, feed intake, egg mass or feed conversion. Zeolite presented in grit form significantly improved egg production, egg mass and feed conversion with whole grain with grit having significantly better egg production, egg mass and feed conversion compared to birds fed the ground grain with zeolite in powdered form. The apparent metabolisable energy (AME) of the diets was determined before and after the same birds were challenged with vaccine strains of coccidia. Before and after the coccidiosis challenge the addition of zeolite in grit form gave a significant improvement in the AME of the diet compared to those diets where zeolite was provided in powdered form. The form of grain had no effect on the AME of the diets either before or after the coccidiosis challenge. Birds fed on whole wheat had a significantly lower (2.5 times) oocyst output than birds fed on ground wheat. The zeolite form had no effect on oocyst output. These experiments demonstrate that balanced diets containing wheat in whole form have economic and health benefits when fed to laying birds. The use of insoluble grit in diets, particularly in diets containing whole wheat have nutritional benefits.

I. INTRODUCTION

The renewed interest in alternative forms of poultry management, such as free range and organic poultry keeping, where the natural social and physical functions of the birds are fundamental, now requires answers on how best to feed, manage and control disease, in particular, coccidiosis, without chemotherapy. The practice of whole grain feeding is encouraged in these alternative management and feeding systems. Feeding whole grain based diets also provides an opportunity to reduce costs through not having to process the grain prior to feeding. Research has shown that whole grain feeding has significant effect on gizzard development (Cumming, 1990) and have unchanged or improved egg production and feed efficiency equal to that of birds fed on all mash diets where the grain has been pre-ground prior to feeding (Blair *et al.*, 1973; Karunajeewa & Tham, 1984; Ouart *et al.*, 1986). In addition, the use of insoluble hard grit may play a major role by improving bird performance and feed efficiency compared with birds fed whole grain without grit (Cumming, 1990). Whole grain feeding in conjunction with grit has been shown to reduce oocysts shedding in crossbred cockerels challenged with coccidiosis (Cumming, 1990).

The purpose of experiment 1 was to investgate the feeding to laying hens late in lay (70 weeks and older) a complete layer diet with the wheat presented in whole and ground form, and with zeolite presented in powder and insoluble grit form (2 to 4 mm) on bird

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performance. The purpose of experiment 2 was to investgate the effect of a coccidiosis challenge on these same birds on oocyste output and the change in apparent metabolisable energy (AME) of the diets before and after the challenge.

II. METHODS

Two experiments were conducted, the first involved a total of 128 laying birds, 70 weeks of age were divided into 4 treatments with 4 replicates (blocks). Within the treatments there were 2 factors, wheat presented in ground or whole form and zeolite presented in grit or powdered form. Each replicate consisted of 8 birds in 4 cages, 2 birds per cage. Birds were fed the same specification wheat-based diet for layers eating 100 g/head/day and consisted of wheat (whole or ground) 600.0, Soybean meal 167.0, Meat meal 80.0, Tallow 20.0, Sunflower oil 12.0, Sodium bicarbonate 1.1, Choline chloride 1.0, DL-methionine 1.7, Yolk pigment 2.0, Vitamin/Mineral premix 2.0, National Zeolite (powder or grit form). All values are expressed in g/kg.

Since the birds had not been fed whole grain previously, birds on the whole grain treatment had the proportion of whole grain increased by 10% (of the diet) each week until 100% of the grain was in whole form, a period of 6 weeks. Diets were fed *ad libitum* for 17 weeks. Water was freely available. Egg production was recorded daily and feed intake and egg weight was recorded weekly.

The second experiment had a total of 48 birds, selected from experiment 1, 12 birds were selected from each treatment and allocated to 48 metabolism cages. The birds were allowed to settle into the cages for 2 weeks and the apparent metabolisable energy (AME) was determined according to the conventional total collection method outlined by Fisher (1988). AME was corrected to zero nitrogen balance using the constant 36.5 kJ/g nitrogen retained.

The birds were challenged with a commercially available coccidiosis vaccine (Eimeriavax 4m) sourced from Bioproperties Pty Ltd, Australia. Eimeria species of coccidia in the vaccine were *acervulina, brunetti, maxima, necatrix and tenella.* Immediately after the challenge clean collection trays were placed under the birds to collect faeces. Faeces were collected for 3 successive days, pooled and a 50 g sample taken for oocyst count determination. Oocyst count was determined according to the method outlined by Work Instruction 33 (Animal Research Institute, Qld)). After 1 week the AME of the diets was again determined. The results were subjected to analysis of variance and oocst count was transformed using the $\log_e(X+1)$ transformation prior to analysis.

III. RESULTS AND DISCUSSION

Table 1 summarises the performance data after 16 weeks into the first experiment. There was no significant effect of the way wheat grain was presented to the birds (ground or whole) on egg production, egg weight, feed intake, egg mass or feed conversion. These results are consistent with those found by other researchers (Blair *et al.*, 1973; Karunajeewa, 1978; Karunajeewa & Tham, 1984; Ouart *et al.*, 1986). This demonstrates there is an opportunity for making a significant economic savings by not having to grind the wheat, the major component of the diet, without any adverse effects on bird performance. Zeolite presented in grit form significantly improved egg production, egg mass and feed conversion but did not influence daily feed intake or egg weight. Hard insoluble grit has not routinely been used in commercial diets for poultry for decades. However, there is evidence from earlier years (Heuser and Norris, 1946; Balloun and Phillips, 1956; Scott and Heuser, 1957) that there is an increase in egg production when insoluble grit was fed in conjunction with

whole grain. Our work would support the work of these early researchers. There is less evidence of a need for insoluble grit when all-mash laying diets are fed. Again, earlier researchers (MacIntyre and Jenkins (1952); Fuller (1958)) observed no increase in egg production or feed efficiency from feeding grit with all-mash layer diets. However, our work would support the feeding of grit even when the grain was ground.

Table 1 Performance and gizzard weight of 70 week old laying hens fed a diet with grain presented in whole or ground form and zeolite presented in powdered or grit form

		Lay %	Egg wt. G	Egg Mass g/b/day	DFI g/b/day	FCR gFeed/gEgg
Grain Form						
	Whole	79.5	64.4	51.1	103.6	2.04
	Ground	76.5	64.6	49.5	103.3	2.10
	Pooled SE	1.23	0.50	0.91	1.01	0.032
Zeolite Form						
	Grit	80.9[a]	65.2	52.7[a]	104.3	1.98[a]
	No Grit	75.1[b]	63.8	47.9[b]	102.5	2.15[b]
	Pooled SE	1.23	0.50	0.91	1.01	0.032
Grain Form x Zeolite Form						
Whole	Grit	82.5	63.7[ab]	52.5	104.4	1.99
	No Grit	76.4	65.0[ab]	49.7	102.8	2.08
Ground	Grit	79.2	66.7[a]	52.8	104.2	1.98
	No Grit	73.8	62.6[b]	46.1	102.3	2.22
	SE	1.74	0.70	1.29	1.43	0.046
Significance						
Grain Form		NS	NS	NS	NS	NS
Grit		**	NS	**	NS	**
Grain Form x Grit		NS	**	NS	NS	NS

[ab]Means within a column for grain form or grit with the same superscript do not differ a P<0.05

Table 2 summarises the AME of the diets before and after a coccidiosis challenge and the back-transformed means of oocyst number output in the faeces in the second experiment. Before and after the coccidiosis challenge the addition of zeolite in grit form gave a significant improvement in the AME of the diet compared to those diets where zeolite was provided in powdered form. The form of grain had no effect on the AME of the diets either before or after the coccidiosis challenge. This is consistent with the findings of McIntosh *et al.* (1962) who found no consistent effect of the grain form on the ME of the grain. Whole grain fed in conjunction with zeolite in grit form significantly improved the AME of the diet both before and after the coccidiosis challenge. Again, this is consistent with the findings of McIntosh *et al.* (1962) who found grit feeding consistently increased the ME of cereal grains. They also found the responses due to grit were greater when whole, rather than ground or pelleted grains were fed. There was a significant interaction for the AME of the between grain form and zeolite form after the coccidiosis challenge. This indicated that the diet which contained whole grain had a significantly inferior AME when zeolite was not presented in grit form, but had a superior AME when zeolite was in grit form. Ground grain with and without zeolite in grit form had the same AME and was an inferior AME to the diet with whole grain and zeolite grit but superior to the diet with whole grain and zeolite in powdered form. This AME interaction did not occur prior to the coccidiosis challenge.

Birds fed on whole wheat had a significantly lower (2.5 times) oocyst output than birds fed on ground wheat. This is in agreement with Cumming (1990) who found that when

grain was fed in whole form to crossbred cockerels challenged with coccidiosis, oocyst shedding was reduced. The zeolite form had no effect on oocyst output.

Table 2 Performance of 70 week old laying hens fed a diet with grain presented in whole or ground form and zeolite presented in powdered or grit form

		AME Before MJ/kg DM	AME After MJ/kg	Oocyst Count Back-Trans Means Number/g faeces
Grain Form				
	Whole	13.82	13.42	54208[b]
	Ground	13.68	13.66	140877[a]
	Pooled SE	0..125	0.127	
Zeolite Form				
	Grit	14.07[a]	13.86[a]	88545
	No Grit	13.44[b]	13.22[b]	86246
	Pooled SE	0.125	0.127	
Grain Form x Zeolite Form				
Whole	Grit	14.22[a]	14.08[a]	55409[a]
	No Grit	13.42[b]	12.76[b]	53032[a]
Ground	Grit	13.92[ab]	13.64[a]	141496[b]
	No Grit	13.45[b]	13.68[a]	140261[b]
	Pooled SE	0.176	0.179	
Significance				
Grain Form		NS	NS	**
Zeolite Form		***	***	NS
Grain Form x Zeolite Form		NS	***	NS

[ab]Means within a column for grain form or grit with the same superscript do not differ a P<0.05

IV CONCLUSIONS

These experiments demonstrate that balanced diets containing wheat in whole form have economic and health benefits when fed to laying birds. The use of insoluble grit in diets, particularly in diets containing whole wheat have nutritional benefits.

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ECONOMICS OF ENERGY REQUIREMENTS FOR LAYER STRAINS

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Summary

When laying hens are allowed unlimited access to feed they tend to eat enough to satisfy their energy requirements. Many strains of layer, however, are far from perfect at judging their energy needs resulting in birds receiving too little or too much energy to support optimum health and performance. Unless the nutrient profile of the diet is carefully matched to the birds' feed intake, shortages or wastage of many nutrients occurs resulting in significant economic loss. ISA Brown and Hyline Brown hens were fed 10 diets representing five metabolisable energy (ME) levels (ranging from 10.3 to 12.2 MJ/kg) and two densities (fixed or floating). The economic analysis of production suggests that, under the pricing structure for ingredients on the market as at June 2003 in South East Queensland, the feed cost per tonne increased as the energy level and nutrient density of the diet increased. However, the feed cost per bird per day tended to be higher for lower energy diets due to the higher daily intake needed to meet their nutritional requirement. Higher returns were received from birds fed medium to higher energy levels due mainly to a reduction in daily feed costs.

I. INTRODUCTION

Economically optimum energy levels in poultry diets depend on a number of uncontrollable factors, including the prices paid by feed mills for cereals and cereal by-products. Recommended metabolisable energy (ME) levels for specific strains of bird may differ substantially from the levels appearing in the solutions of least cost diet (LCD) formulation runs (when ME is allowed to float). Poultry suppliers usually recommend energy dietary levels of 11.5-12.0 MJ/kg for imported brown egg strains, but there is no evidence to support these recommendations under Australian conditions. In practice neither the breeder's recommendation nor the LCD solution is likely to be the economic optimum. The nutritionist must make some judgment concerning (a) the effect of energy level on feed intake (which affects the levels of all other nutrients in the diet specification) and (b) the possible effect of energy level on production, whether mediated through its effect on feed intake or otherwise.

The responses of high-producing imported layers to changes in energy content of diet are not well documented. Historical information relating mainly to white leghorn strains or hybrids suggests that, within a normal range, the ME content of the diet has little effect on any performance factor other than feed intake (Hill, 1956). Because laying hens in Australia are invariably fed *ad libitum*, the relationship between dietary energy concentration and feed intake is of considerable economic importance.

Early models assumed that laying hens consume only enough feed to meet their energy requirement, but it was later shown that the adjustment of feed intake to different dietary energy levels is imperfect, particularly in heavier strains of bird (Morris, 1968). The response is thus strain dependent and may also be non-linear.

It is crucially important to understand that feed intake responds to changes in dietary ME concentration and the effective cost of a diet is not its price per tonne but the cost of the amount consumed by the flock. The predicted feed intake determines the levels of expensive

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nutrients that must be included in the diet to meet requirements for production. If feed intake is higher than expected nutrients will be wasted; if lower, the intake of some essential nutrients may be too low to support maximum production.

The objective was to provide information enabling the formulation of diets containing the most cost effective energy levels in different circumstances and was part of a larger study comparing the effect of different diet densities and metabolisable energy levels on the performance of two imported brown egg layer strains (Singh *et al*., 2004).

II. METHODS

Two experiments were conducted using ISA Brown and Hyline Brown layers, the first aimed at measuring the effects of dietary apparent metabolisable energy (AME) level on feed intake, egg production and egg weight throughout the greater part of the laying period, the second aimed at establishing the relationships between dietary energy level, feed intake, energy intake, egg mass, bodyweight and bodyweight gain.

In a factorial randomised block design experiment, diets containing five metabolisable energy (ME) levels and two densities (fixed and floating) were fed to two bird strains (ISA Brown and Hyline Brown) hens housed in two-bird cages. Each treatment combination was represented by six 8-bird replicates. The nominal ME values of the diets were 11.0, 11.3, 11.6, 11.9, 12.2 MJ/kg, while the actual ME values obtained by metabolism studies, using layers, were 10.32±0.21, 10.76±0.22, 11.20±0.20, 11.60±0.29, and 12.19±0.19MJ/kg for diets where density was allowed to float and 10.78±0.26, 11.14±0.27, 11.50±0.26, 11.86±0.25, and 12.23±0.25/kg for diets where the density was fixed. Amino acids, total protein, calcium and phosphorus were maintained in approximate proportion to the nominal ME levels. The trial ran for 48 weeks (18 –66 weeks of age).

The average price growers received for their eggs in June 2003 in South-East Queensland was $1.35 per dozen. Egg producers are paid on a per dozen basis not per kilo of egg mass, therefore the economic analysis was based on a per dozen basis. Since average egg weight in the experiment on all diets was between 60g and 64g, egg grade was not an issue in the economic assessment. The prices of feed ingredients were obtained from Applied Nutrition Pty. Ltd. and are average prices available as at early June 2003. A feed manufacturing cost of $60 per tonne was added to the raw material costs of each diet. This is a feed industry average manufacturing figure per tonne of feed.

III. RESULTS

Daily intake of amino acids of birds on all diets exceeded the minimum amount required for best performance according to their respective breeder standards. Nutrient (amino acid) density was clearly more than adequate for the feed intake of the birds and resulted in an intake of nutrients surplus to requirements. Therefore, the absence of significant differences in egg production and egg weight between dietary treatments was expected. There was no difference in egg production between strains of birds but egg weight was different between strains. This extra egg weight for the ISA Brown strain has no economic advantage since egg producers are paid on a per dozen basis. Average egg production and egg weight over the production period was more than commercially acceptable for both strains of birds. Daily energy intake increased as the energy level of the diet increased even though daily feed consumption decreased as the energy level of the diet increased. Clearly, the birds were not able to fully compensate feed intake to a constant energy intake as the energy level of the diet increased. This extra energy intake did not influence bird performance.

The economics of feeding ISA Brown and Hyline Brown hens on diets ranging in ME content from 10.5 to 12.5 MJ AME/kg are shown in Table 1. The cost of the diet per kg is relatively flat at energy levels at and below 11.5 MJ AME/kg, based on the prices paid for the ingredients used in the experiment. However price/kg rises as AME content increases from 11.5 MJ/kg upwards. The minimum energy cost is at the higher dietary energy level, mainly because of the lower feed intake of high energy diets. The feeding cost per bird per day is lowest at the highest energy level and so is the cost per dozen eggs. With eggs priced at $1.35/dozen, the margin over feed cost per dozen eggs increased by 3.5 cents as dietary energy increased from 10.5 to 12.5MJ/kg for ISA Brown hens and 1.25cents increase for Hyline Brown hens fed diets ranging from 10.5 to 12.0 MJ/kg. Thus it is more profitable to feed high energy diets to modern brown egg layers.

Table 1. Economics of feeding ISA Brown and Hyline Brown layers diets with different metabolisable energy levels using June 2003 feed and egg prices.

ISA Brown

Diet ME[1] (MJ/kg)	Feed intake (g/day)	Diet cost (cents/kg)	Energy cost (cents/MJ AME)	Feeding cost (cents/day)	Feed cost /dozen eggs (cents)	Margin (cents/dozen eggs)
10.5	122.6	42.0	3.93	5.14	72.64	62.37
11.0	121.7	42.7	3.87	5.20	71.86	63.14
11.5	118.7	43.6	3.81	5.17	71.46	63.55
12.0	115.1	44.4	3.75	5.11	70.78	64.22
12.5	112.6	45.2	3.70	5.09	69.13	65.87

Hyline Brown

Diet ME[1] (MJ/kg)	Feed intake (g/day)	Diet cost (cents/kg)	Energy cost (cents/MJ AME)	Feeding cost (cents/day)	Feed cost /dozen eggs (cents)	Margin (cents/dozen eggs)
10.5	124.2	42.0	3.93	5.20	72.13	62.88
11.0	124.4	42.7	3.87	5.32	72.88	62.12
11.5	122.8	43.6	3.81	5.35	73.29	61.72
12.0	117.1	44.4	3.75	5.20	70.88	64.13
12.5	113.2	45.2	3.70	5.12	71.85	63.15

[1]Major nutrients are included at concentrations proportional to ME level.

IV DISCUSSION

The relative pricing of low and high energy ingredients will influence the relative cost of diets as their energy level and nutrient densities are increased. Therefore, as ingredient price relativities change, the most profitable diets will be different at a particular point in time. In this trial, under the pricing structure for ingredients on the market as at June 2003 in South East Queensland, the feed cost per tonne increased as the energy level and nutrient density of the diet increased. However, feed cost per bird per day tended to be higher for the lower energy diets. This is contributed, to some degree, by the higher cost of unit energy (MJ) of the diets when the energy level in the diets is low, reflected by the relative high cost of energy in lower energy ingredients compared with higher energy ingredients as at June 2003. At this time, higher returns were received from birds fed medium to higher energy levels due mainly to a reduction in daily feed cost of the birds due to lower feed intake.

Fixing the bulk density of feeds had the effect of increasing the cost of feed between $2-$3 per tonne. However in this experiment, this did not adversely affect the economics, and in fact, the fixed group tended to produce more eggs and converted feed more efficiently resulting in a lower feed cost per dozen eggs. However, care should be taken on how this is interpreted since performance differences (egg production, egg weight and feed intake) between the fixed and floating bulk densities were not statistically significant. This economic

difference may well be coincidental and a reflection on the size of the experiment rather than a true effect of bulk density *per se*. Justifying an increase of $2-$3 per tonne by fixing the bulk density needs further investigation in a larger scale experiment designed to pick up smaller differences in performance.

The experimental results of this project give confidence to nutritionists developing minimum cost diets for laying hens. It demonstrates the ability of birds to adjust their feed intake according to the energy level of the diet when given a similar set of environmental conditions. Manipulating nutrient density and energy level of the diet, in line with changes to the relative value of raw materials on the market is a major way of minimising daily feed costs. It cannot be overstressed the importance of knowing the daily feed intake of birds (or daily energy intake required) so that nutrient density and energy level can be adjusted to ensure adequate intake of critical nutrients in order to maintain bird performance and minimise nutrient surpluses. The use of controlling bulk density of the diet and its influence on economic returns needs further study.

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INTERACTION OF CEREAL GRAIN SOURCE AND DIETARY LEVEL LUPINS IN BROILERS

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Summary

An experiment was conducted to investigate the effect of cereal grain source and dietary level of lupins on the performance of broiler chickens from days 1 to 17 post-hatching. The experimental diets consisted of maize, sorghum or wheat as the sole cereal grain and 0, 100 or 200 g/kg lupins. There was no interaction of cereal grain source and dietary lupin level in growth performance of broiler chickens, which is an important observation for feed formulation.

RESULTS AND DISCUSSION

Lupins (*Lupinus angustifolius*) are promoted as a source of dietary protein for broilers. When diets are formulated, it is assumed that the nutrients within each ingredient are additive. However, this assumption may not always be correct because of the possible presence of antinutritive factors in lupins and cereal grains (Maquardt, 1993). The objective of the study was to examine the relationship between cereal grain source and dietary level of lupins on the performance of broiler chickens.

Day-old male, broiler chicks (Cobb) were obtained from a local hatchery and reared in tiered battery brooders. A temperature of 32 ± 1° C was maintained during the first week and gradually decreased to approximately 23° C by the end of the trial. Experimental diets contained maize, sorghum or wheat as the sole cereal source, lupins at level of 0, 100 or 200 g/kg diet and other minor ingredients were formulated to meet nutrient requirements for broilers. Each experimental mash diet was fed to six pens of six birds from days 1 to 17 post-hatching. Individual body weight and pen feed intake were recorded at the beginning and end of the experiment and feed efficiency was calculated. Excreta were collected from days 14 to 17 and dried in a force air oven at 80^{o}C for AME determination. At day 17, digesta was collected for measurement of viscosity.

Cereal grain source did not affect feed conversion, but significantly ($P<0.001$) influenced body weight gain, feed intake, AME and digesta viscosity (see the Table below). There were inverse relationships between the lupin content of the diet and AME. Dietary lupin level increased and digesta viscosity increased, did not appear to affect bird health (mortality 1.6%). There were no interactions between cereal grain source and dietary lupin level.

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Effect	Weight gain (g/b/d)	Feed intake (g/b/d)	Feed/gain (g/g)	AME (MJ/kg DM)	Digesta viscosity (cPs)
Cereal grains					
Maize	31.4[b]	48.7[b]	1.42	14.0[a]	2.44[b]
Sorghum	33.4[a]	51.7[a]	1.38	13.2[c]	2.84[b]
Wheat	27.8[c]	43.4[c]	1.38	13.7[b]	8.49[a]
SEM	0.48	0.82	0.013	0.07	0.55
P value	<0.01	<0.01	0.10	<0.01	<0.01
Lupin level					
0	32.2	49.2	1.39	14.2[a]	2.44[b]
100	31.1	47.9	1.39	13.8[b]	4.72[ab]
200	30.7	48.0	1.39	13.5[b]	5.11[a]
SEM	0.45	1.01	0.011	0.11	0.65
P value	0.22	0.79	0.96	0.01	0.11
Interaction	0.69	0.93	0.65	0.85	0.91

Means within the columns with different superscripts differ P <0.05

Cereal grain source did not affect feed conversion, but significantly (P<0.001) influenced body weight gain, feed intake, AME and digesta viscosity (see the Table below). There were inverse relationships between the lupin content of the diet and AME. Dietary lupin level increased and digesta viscosity increased, did not appear to affect bird health (mortality 1.6%). There were no interactions between cereal grain source and dietary lupin level.

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ENERGY AND AMINO ACID DIGESTIBILITIES OF PEARL MILLET HYBRIDS WHEN FED TO BROILERS

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Pearl millet (PM) has great potential to become a significant alternative to sorghum as a feed grain crop, particularly in the more marginal growing conditions of Western Queensland and North West NSW. It is draught tolerant and very efficient in producing high grain yields. However data on Australian developed PM hybrids is inadequate to characterize their nutritive value. It was, therefore, the objective of this study to compare the chemical composition and nutrient digestibilities of three PM hybrids (PM31, PM3 and PM4)) grown at Biloela Research Station and compare them with a good quality sorghum (S). Results of the chemical composition, AME and IAA coefficients are given in the table.

Chemical composition (g/kg DM)					AME (MJ/kg)				
	PM31	PM3	PM4	Sorghum		PM31	PM3	PM4	Sorghum
Protein	136.9	14.75	14.31	12.44	Uncorrect	14.04 [a]	13.81 [b]	14.06 [a]	13.23 [c]
Ash	20.0	21.0	21.0	12.0	N correct	13.57 [a]	13.27 [a]	13.59 [a]	12.91 [b]
Fat	64.0	64.0	64.0	28.0	DM	15.64 [a]	15.26 [b]	15.43 [a]	14.73 [c]
Ca	0.60	0.40	0.50	0.40					
P	4.3	4.4	4.5	4.6					
CF	19.0	21.0	21.0	22.0					

Amino acids (g/kg DM)					Ileal digestibility coefficient (%)			
					PM31	PM3	PM4	Sorghum
Protein					74	78	78	78
Arginine	5.56	6.77	6.31	3.8	74	84	82	80
Cystine	1.87	2.19	2.22	1.8	72	74	73	69
Glycine	3.76	4.31	3.94	3.1	62	71	67	67
Histidine	2.64	3.03	2.88	2.4	77	83	84	75
Iso Leucine	5.19	5.89	5.78	4.4	78	85	83	81
Leucine	12.63	14.38	14.07	15.1	83	87	87	87
Lysine	2.83	3.18	3.09	2.3	72	76	75	69
Methionine	1.90	3.34	2.47	1.5	85	86	88	85
Phenylalan	6.15	6.89	6.80	5.9	80	87	86	85
Serine	5.98	6.82	6.42	4.8	69	78	76	73
Threonine	4.47	5.18	4.94	3.5	75	74	75	66
Tryptophan	2.60	2.92	2.70	1.2	82	79	77	90
Tyrosine	3.46	3.94	3.88	4.2	72	81	81	83
Valine	6.57	7.46	7.21	5.5	80	83	84	79

[a-c] Means in the same row with different superscripts are significantly different ($P<0.05$)

Pearl millet hybrids contained nearly 2% more protein than sorghum. The fat content of PMs were three times that of S. Ash, Ca, P and CF were similar for all the grains tested. The total amino acid profile of the PM hybrids is much better than S. PM hybrids contain nearly 50% more lysine, methionine, threonine and tryptophan than S. The uncorrected, nitrogen corrected and AME corrected for DM of S were significantly lower ($p<0.001$) than the PM hybrids. AMEn of S was 0.6 MJ lower than PM hybrids and nearly 0.7 MJ lower AME on a diet dry matter basis ($p<0.001$). Nitrogen corrected and diet DM corrected AME of PM31 was significantly higher than PM3 ($P<0.01$) but was similar to PM4. The digestibility of

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cystine, lysine and threonine in PM were higher than that in S whereas the digestibility of the other amino acids in the PM hybrids was very similar to S and within the range recorded by Ravindran *et al*.(1999). Data suggest that PM can easily be substituted for S in broiler diets.

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THE EFFECT OF CASEIN PHOSPHOPEPTIDE AND 25-HYDROXYCHOLECALCIFEROL ON TIBIAL DYSCHONDROPLASIA IN GROWING BROILER CHICKENS

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Summary

The influence of dietary casein phosphopeptides and 25-hydroxycholecalciferol on the incidence of tibial dyschondroplasia in 14-day-old commercial broiler chickens was investigated. A standard broiler diet was used as the control with three experimental treatments using the control diet supplemented with 10 g/kg casein phosphopeptide, 14 g/kg casein phosphopeptide or 69 μg/kg of 25-hydroxycholecalciferol. Those birds fed the diets supplemented with 14 g/kg casein phosphopeptide or 25-hydroxycholecalciferol had a significantly lower ($P<0.05$) incidence of tibial dyschondroplasia than both the control and 10 g/kg casein phosphopeptide treatments. The body weight of birds fed the 10 g/kg casein phosphopeptide diet or the 25-hydroxycholecalciferol diet was significantly higher than birds fed the control diet.

I. INTRODUCTION

Skeletal disorders are a continuing concern for the chicken meat industry worldwide, and are responsible for losses in production as well as poor welfare. Abnormalities of the cartilaginous growth plate of the tibiotarsus have been identified as a significant component of these skeletal disorders in many commercial and experimental strains throughout the world. Research has indicated that the incidence of these abnormalities can be up to 50% in 14-21 day-old commercial broilers (Vaiano *et al.*, 1994).

Two of the most common conditions affecting the growth plate in growing broiler chickens are rickets and tibial dyschondroplasia (TD) which are believed to have different aetiologies. Rickets is caused by a deficiency of calcium, vitamin D or phosphorus and there is characteristic histopathology seen depending upon the deficiency. Tibial dyschondroplasia on the other hand is primarily an abnormal development of physeal cartilage (Riddell, 1997) and, similar to rickets, is characterised by a thickening of the growth plate cartilage. Tibial dyschondroplasia is also associated with calcium deficient diets (Edwards and Veltmann, 1983), high dietary phosphorus and altered vitamin D_3 metabolism (Parkinson *et al.*, 1996), but the aetiology is not believed to be a simple mineral or vitamin deficiency.

The use of in feed vitamin D metabolites such as 25-hydroxycholecalciferol (25-OH-D_3) and 1,25-dihydroxycholecalciferol has been shown to reduce the incidence of TD in broilers (Edwards, 1990, Rennie and Whitehead, 1996). It is not clear whether this response is mediated by the vitamin D metabolite per se and/or by an increase in calcium/phosphorus absorption and partitioning to bone.

Phosphopeptides derived from the enzymatic hydrolysis of casein have also been shown to enhance bone calcification. In vivo studies indicate that the administration of CPP can promote calcification and reduce the thickness of the growth plate in growing broiler chickens (Kusuhara *et al*., 1992). Research indicates that the ability of CPP to stimulate calcium absorption from the intestine may be able to operate independently of vitamin D metabolism (Mykkanen and Wasserman, 1980).

The aim of this experiment was to examine the ability of CPP to influence the incidence of TD in comparison to the effects mediated by 25-OH-D_3. The CPP is designed

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for in-feed inclusion and is likely to stimulate calcium and phosphorus absorption independent of vitamin D function.

II. MATERIALS AND METHODS

Three hundred and twenty mixed sex Cobb commercial broiler chickens (one day-old) were divided into four treatment groups and housed in 32 compartments in a battery brooder (8 compartments per treatment with 10 birds per compartment). Treatments were as follows; Treatment 1 - control group fed a conventional broiler starter diet; Treatment 2 – conventional starter diet plus CPP at 10 g/kg; Treatment 3 - conventional starter diet plus CPP at 14 g/kg; Treatment 4 - conventional starter diet plus 25-OH-D_3 at 69 μg/kg.

All treatments were formulated to provide 10 g/kg calcium, 8 g/kg total phosphorus and 75 μg/kg (3000 IU/kg) of vitamin D_3. The total phosphorus content of approximately 8 g/kg is slightly above conventional levels (6 g/kg) with the intent of increasing the incidence and severity of TD. All diets were fed *ad libitum* and contained 233 g/kg crude protein and had a ME of 13.3 MJ/kg. For the complete diets see Table 1.

Table 1. Composition of treatment diets

Ingredient	Dietary composition (g/kg)			
	Control	10 g/kg CPP	14 g/kg CPP	25-OH-D_3
Wheat (14% CP)	639.8	637.0	634.7	639.1
Soybean (48% CP)	209.1	208.5	207.7	209.2
Tallow	57.0	56.8	56.6	57.0
Fishmeal (65% CP)	66.5	66.3	66.1	66.6
Dicalcium Phosphate	13.9	5.3	3.8	14.0
Limestone	9.2	9.2	9.1	9.2
Vit., Min. Premix*	1.0	1.0	1.0	1.0
Salt	2.4	2.4	2.4	2.4
DL Methionine	0.1	0.1	0.1	0.1
Sodium dihydrogen orthophosphate	2.0	3.5	4.5	1.0
25-OH-D_3	-	-	-	69 μg/kg
CPP		10	14	
Calcium	11	10	10	11
Total Phosphorus	8.1	7.7	7.9	8.0

* Standard broiler vitamin and mineral supplement to meet NRC recommendations

The birds were maintained on these diets until 14 days of age. At 14 days of age average body weight and total feed consumption for the two week period was calculated and feed conversion ratio (kg feed consumed / kg live weight gain) determined.

At 14 days of age all birds were sacrificed and sagittal sections of the left proximal tibiotarsus were examined. The width of the physeal plate from just below the articular cartilage to the beginning of the hypertrophic zone was measured, and those birds with a physeal plate width of less than 3 mm (measured by vernier micrometer) were considered normal. Those birds with a width of greater than 3 mm were considered to have growth plate abnormalities characteristic of TD. The incidence was expressed as a percentage of the treatment group population. Twelve tibias were randomly selected from each treatment and retained for bone ash (expressed as a percentage of defatted dry weight) determination.

Statistical Analysis

A student's *t* test was used for analysing differences between treatments.

III. RESULTS

Table 2. Average body weight, average percentage of birds affected with growth plate lesions greater than 3 mm, average bone ash and feed conversion (kg feed/kg weight gain) in each of the four dietary treatments (standard error in parentheses). Means in the same column without a common superscript are significantly different (P<0.05).

Treatment	Body weight	% birds with growth plate lesions >3 mm	Bone ash (%)	Feed conversion
Control	407.9^{a} (12.1)	33.5^{a} (7.3)	39.4 (0.7)	1.35^{a}
10 g/kg CPP	451.3^{b} (8.7)	25.3^{a} (4.9)	39.2 (1.1)	$1.27^{a,b}$
14 g/kg CPP	$428.3^{a,b}$ (9.4)	7.9^{b} (3.4)	40.3 (0.6)	1.33^{a}
25-OH-D_3	451.9^{b} (9)	7.3^{b} (3.2)	40.6 (0.5)	1.25^{b}

a) Body weight

The body weight of birds' fed the control diet was significantly lower (P<0.05) than those birds fed the diet containing 10 g/kg CPP or the birds fed the 25-OH-D_3 diet (Table 2). The body weight of the birds fed the 14 g/kg CPP diet was greater than the control birds, however this difference was not significant (P>0.05) (Table 2).

b) Incidence of growth plate abnormalities by gross examination

The birds fed the control diet or the 10 g/kg CPP had a significantly higher (P<0.05) incidence of growth plate abnormalities than those birds fed the diets containing 14 g/kg CPP and 25-OH-D_3 (Table 2).

c) Tibial ash percentage

There was no significant difference in tibial ash percentage between any of the 4 treatment groups. However there was a trend that the ash percentage tended to be lower in both the control and 10 g/kg CPP treatment birds when compared to those birds fed the 25-OH-D_3 and 14 g/kg CPP diets (Table 2).

d) Feed conversion

The 25-OH-D_3 treatment had a significantly lower (P<0.05) feed conversion than the 14 g/kg CPP and the control treatments (Table 2).

IV. DISCUSSION

In an experiment by Mitchell *et al.* (1997), 25-OH-D_3 was included in diets at three different rates, 0, 5, and 40 μg/kg, and fed to a broiler strain with a low incidence of TD. The incidence of TD at 16 days for birds fed the 0, 5 and 40 μg/kg diets was 61, 38 and 16% respectively, whilst tibial ash was 36.5, 39.3 and 40% respectively. These results indicate only marginal changes in tibial ash (39.3 to 40%) between the 5 and 40 μg/kg treatments, even though the incidence of TD has decreased from 38% to 16%. Edwards *et al.* (1990) and Edwards (1992), indicate that maximum tibial ash in 15-16 day old broiler chickens is approximately 40% and birds affected with severe cartilaginous growth plate lesions can be

as low as 32%. In the current experiment, the changes in TD and tibial ash are similar to those described by Mitchell *et al.* (1997) for a broiler with low genetic susceptibility.

At the higher dose rate of 14 g/kg CPP, 25% of the dietary calcium is provided by the dicalcium phosphate bound to the peptide compared to 21% in the 10 g/kg CPP. The higher dose of peptide appears to produce a disproportionately large decrease in the incidence of TD. Whether this effect is mediated by the additional 4% of the total calcium which is bound to the peptide, or by some effect of the peptide in the intestine stimulating uptake of non-peptide bound calcium is unknown.

Mitchell *et al.* (1997) showed a 10.5% increase in live weight at 16 days of age for broilers fed a diet supplemented with 40 μg/kg of 25-OH-D_3, compared with controls fed a diet containing cholecalciferol at 27.5 μg/kg (1100 IU/kg) alone. In the current experiment the live weight gain to 14 days in the 25-OH-D_3 treatment, in comparison to the control treatment, was approximately 10% higher, while feed conversion declined from 1.35 to 1.25 (~ 7%). With the CPP included at a rate of 10 g/kg, the growth rates of the birds have been enhanced by 10% in comparison to the control birds, and this has been demonstrated to occur independently of the effect on growth plate abnormalities. These growth responses are worthy of additional analysis to determine what are the mechanisms responsible.

Clearly the dietary CPP at a dose rate of 14 g/kg has a similar capacity to reduce the incidence of growth plate abnormalities as 25-OH-D_3. These findings suggest that an increased efficiency of calcium and phosphorus absorption from the intestine may be as important as the supply of vitamin D metabolites for the effective differentiation of the metaphyseal chondrocytes. The possibility remains that some of the effects of the CPP's may be mediated through effects on vitamin D metabolism.

The current experiment demonstrated that both CPP and 25-OH-D_3 can significantly decrease the incidence of TD in the young broiler. It will be important to assess whether these products can act in an additive or synergistic fashion because the mechanisms by which the two products stimulate calcium uptake and partitioning to bone may be different. Research undertaken by Mykkanen and Wasserman (1980) indicates that CPP can work independently of vitamin D function. The possibility remains therefore of a superior chelation and solubility of dietary calcium and phosphorus in the intestine for absorption created by the CPP, and then an enhanced uptake and partitioning to bone triggered by 25-OH-D_3.

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THE RESPONSE OF CHICKEN NONLYMPHOID LEUKOCYTES TO STRESS

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Summary

This study was designed to show that administration of ACTH causes redistribution and/or reduction of circulating leukocytes resulting in an increased H/L ratio and decreased nonspecific cytotoxic activity of blood. At 33 weeks of age, 24 laying hens were randomly assigned to four different groups: ACTH- and/or saline-injected, handled and/or nonhandled (control) hens. ACTH-treated hens showed an increase in heterophil, basophil, and eosinophil percentage, and a decrease in lymphocyte and monocyte percentages, and result an increased H/L ratio (0.73). Nonspecific cytotoxic activity of the whole blood against *Saccharomyces cerevisiae* was decreased by approximately 60% as compared to other treatment and control hens. This study demonstrated that high corticosterone concentrations may help redistribute leukocytes among compartments, blood, lymphoid and nonlymphoid tissue, thereby bringing the cells required for the nonspecific defence into the circulation, but reducing their activity.

RESULTS AND DISCUSSION

All haemopoietic cells are derived from pluripotent stem cells which give rise to two main lineages, one for lymphoid cells and other for myeloid cells. While the common lymphoid progenitor has the capacity to differentiate either to T cells or B cells, the myeloid cells differentiate into heterophils, basophils, eosinophils, macrophages (i.e. monocytes), Langerhans, Kupffer and dendritic cells, and megakaryocytes (Fox and Solomon, 1980). Peripheral blood contains several cells that are involved in immunity but most, spend very little time in the vascular system. Nonlymphoid (myeloid) leukocytes are a component of nonspecific defense, phagocytosis and nonspecific cytotoxic immunity (McCorkle, 1998). The effectiveness of the nonspecific cellular system of resistance is greatly enhanced by the activity of B and T lymphocytes in the state of specific immunity. There is evidence that avian leukocyte cell response to stress may vary depending on the sensitivity of the haemopoietic/lymphoid tissue to corticosterone (Siatskas and Boyd, 2000), and the importance of the stressor involved to survival (Besedowsky and del Rey, 1996). Stress has been shown to cause lymphopenia and heterophilia in chickens (Gray *et al.*, 1989) and the heterophil to lymphocyte (H/L) ratio appeared to be a reliable indicator of stress in avians (Gross and Siegel, 1983). Little is known, however, of how stress alters the circulating nonlymphoid cell level and activity in chickens.

This study evaluated nonspecific immunity associated with elevated plasma corticosterone in laying hens. At 33 weeks of age, 24 laying hens were randomly assigned to four different groups: ACTH- and/or saline-injected, handled and/or control hens. For an *in vitro* evaluation of nonspecific cytotoxic activity, blood were collected from the wing vein into 5 ml microcollection tubes contain lithium heparin, and the test was performed as described by Kahl *et al.* (1989). Blood smears were prepared directly from peripheral blood and were stained using a modified Wright and May-Grunwald methods (Shini, 2004). Cellular-mediated immunity was evaluated after wattle test measurements (called cutaneous basophil hypersensitivity to phytohaemagglutinin), which was conducted in hens *in vivo* as described by Smits *et al.* (1999). Additionally, plasma corticosterone concentration was measured by RIA (Downing and Bryden, 2002). All blood samples were taken before and

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seven days after the commencement of treatments, between 9:00 and 10:00 a.m., immediately after each hen had been removed from its cage. The entire procedure did not take longer than 45 to 50 seconds (Wingfield *et al.,* 1982).

The data showed that administration of ACTH and handling caused an increase ($P<0.001$) in plasma corticosterone, associated with sequential effects on non-specific immune parameters. ACTH-treated hens and handled hens, demonstrated an increased H/L ratio, which correlated positively ($P<0.001$) with corticosterone concentrations. Furthermore, nonspecific cytotoxic activity of the whole blood against *Saccharomyces cerevisiae*, which is due to the activity of cellular defence mechanisms of granulocytes, thrombocytes and monocytes, but not lymphocytes and erythrocytes, was decreased in ACTH-treated hens by approximately 60% as compared to other treatment and control hens. ACTH also reduced significantly ($P<0.001$) the wattle swelling response. This response in chicken involves macrophages, basophils, heterophils and B lymphocytes, and is orchestrated by cytokines secreted by T lymphocytes. All these cells proliferate after mitogenic stimulation, though were suppressed by corticosterone i.e., ACTH treatment.

This study demonstrated that endogenous levels of corticosterone contribute to the control of nonspecific immune cell level and function. The circulation of non-specific immune cells as well as their traffic through and within structures could be essential to an efficient physiological response to stressors. As is the case with other types of sensory cells, immune cells may serve as receptor-sensorial cells of stress defence receiving signals from inside and outside of the body and informing the CNS about immunological events. Elevated corticosterone concentrations may help redistribute leukocytes among compartments, blood, lymphoid and nonlymphoid tissue, thereby bringing the cells required for the nonspecific defence into the circulation and/or upregulate their function. This event may be a possible negative feedback mechanism of immune function to a nonspecific agent (i.e. elevated levels of corticosterone), and vital to the successful development of a subsequent specific immune response.

Further research is required to explore enhancement of the chicken immune system through upregulation of nonspecific immunity, and potentially providing an adjuvant-like promotion to the vaccines used.

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EFFECTS OF GROUP SIZE AND SPACE ALLOWANCE ON LAYING HEN WELFARE

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Summary

The effects of housing hens at 8/cage at space allowances of 750 and 1500 cm^2/hen and 16/cage at 750 cm^2/hen were examined from 29-36 weeks of age. A range of behaviour observations and physiological and morphological measurements were made. Hens housed in groups of 16 had higher corticosterone concentrations in eggs and a lower immune response compared to groups of 8 hens at 1500 cm^2/hen. Within the range of treatments used, group size had a greater potential impact on welfare than space allowance.

RESULTS AND DISCUSSION

Despite the presence of furnished cages in the market place and the considerable support for such designs of cage, particularly in Europe, there has been little comprehensive assessment of hen welfare as a consequence of such furniture. This preliminary experiment examined the effects of housing hens at eight/cage with a space allowance of 750 and 1500 cm^2/hen and 16/cage with a space allowance of 750 cm^2/hen, as part of a larger experiment on the welfare of hens in furnished cages. The larger experiment involved 20 treatments using 66 Victorsson cages in a controlled environment shed, maintained at 17-23 °C with a 16:8 h light:dark cycle and a light intensity of 20 lux that was decreased to 5 lux at 28 weeks of age, due to a slight increase in cannibalism. The cages were 1.2 x 0.5 m (width x depth) and 0.45 m high at the rear. 1500 cm^2/hen was provided by removing the panel between 2 cages ie. 2.4 m width. The experimental design was a 3 (nest box) x 3 (dust bath) x 2 (perch) factorial + 2 added controls (space and group size) in a rectangular lattice. The experimental unit was the cage and all cages were sampled during an 8-week period commencing at 29 weeks of age. There were 3 and 54 replicates for the double and single cage treatments, respectively. The following samples and measurements were taken: video observations for timing of egg laying, blood for immunology, blood and eggs for corticosterone concentrations, as measures of stress, body weight, claw length and scores for feather damage and cover, foot condition, pecking wounds/injuries and keel bone deformation, as measures of injuries and body condition.

Only data close to or $<P=0.05$ are tabulated. Group size and space allowance had no effects on the majority of the observations and measurements ($P>0.05$). In the 16 hen, double cage treatment corticosterone concentrations in egg albumen were higher ($P=0.021$), adrenal responsiveness to exogenous ACTH was reduced ($P=0.034$) and the ability of cells to produce IL-6 was reduced ($P=0.051$).

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Parameter[1]	Mean values			SE between double cages	SE (max)	P value
	Double 16	Double 8	Single 8			
Egg B *(ng mL^{-1})*[1]	1.75[a]	1.48[a]	1.53	0.128	0.107	0.021
B response to ACTH *(% change)*[2]	2.84[a]	3.23[a]	3.01	0.129	0.105	0.034
IL-6 production test *(cpm)*[2]	3.63[b]	3.79	3.91[b]	0.187	0.153	0.051

[1]B = corticosterone concentrations; [2]$\log_{10}$ values of counts per min; [a]P<0.05; [b]P=0.051.

This experiment showed evidence of both stress and immunosuppression in the large group size treatment, presumably due to social factors, although this was not examined in this preliminary experiment. The data presented and other data on corticosterone concentrations make it equivocal whether the stress response was an acute or a chronic response. Further research on space allowance and group size is clearly warranted.

FROM CAGES TO EXTENSIVE SYSTEMS: IS THIS TREND BETTER FOR THE WELFARE OF LAYING HENS?

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Summary

In recent times, market forces, public attitudes and legislation have all lead to a largely worldwide decrease (see Introduction) in the use of conventional cages for laying hens and a trend towards housing in large group systems. We investigated whether a large group system provided good welfare standards for all birds. By tagging birds with transponders, we found that a minority of hens in a large group were victimised; they were smaller than other birds, stayed under the perches where it was relatively dark, and showed extensive feather damage. The range in use of the area under the perches suggests that there are degrees of victimisation, rather than an all or nothing phenomenon. The design of large group systems should provide resources that allow successful social avoidance.

I. INTRODUCTION

In recent times, the use of conventional cages for laying hens has attracted public attention and, in Europe at least, legislative measures which have questioned their suitability as a type of housing which safeguards animal welfare. The result is that there is in Europe a present trend towards housing of laying hens in large group systems. Driving this trend has been the opinion that welfare is not safeguarded in battery cages. The latter view has largely been supported by scientific evidence- laying hens in conventional cages are prevented from expressing normal dustbathing, foraging, nesting and perching (Baxter, 1994).

Many large group systems for housing laying hens, in contrast, cater for the limitations imposed by cages by providing a large area, litter, nest boxes and perches. This has lead to the assumption, in our opinion unfounded, that laying hen welfare is protected in large group systems.

One of the most obvious causes for concern for the welfare of laying hens in large group systems is the unnaturally large group sizes. It is clear that hens do not try and establish a peck order in large groups (Hughes *et al*., 1997), resulting in low levels of aggression. However, in large groups there is the potential for a few individuals to receive disproportionate amounts of aggression, sometimes these birds are referred to as victimised birds, and these individuals could experience very poor levels of welfare. Research into the problem of victimised birds has been limited, mainly because it is very difficult to obtain detailed information on individual birds within a large group.

Our initial observations of hens in a perchery pen containing 1000 birds suggested that some birds remained under the perches and appeared to be repeatedly pecked by other birds, suggesting that these were victimised birds. In the present experiment we aimed to learn more about these particular birds. We developed a novel technique using a transponder system that automatically recorded the presence of tagged birds at various sites. This allowed continuous long-term measurement of the time that birds of known identity spent at particular areas of a perchery system. Subsequent recapture of the birds fitted with transponders also provided the opportunity to perform behavioural tests and measure physical condition thereby

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providing, as far as we are aware, a hitherto unattained level of information on the association between individual behaviour and location and condition in a large flock.

II. METHODS

One thousand, non-beak-trimmed ISA Brown laying hens reared in large groups on litter were housed at 16 weeks of age in a perchery pen measuring 11.0 x 5.0 m which contained central 3-tier vertical wood perches (measuring 5 x 5 cm and providing 19.4 cm of perch per bird) up to a height of 1.8 m over wooden slats. The slatted area (175 cm wide) was the darkest part of the perchery and was relatively well delineated with two longitudinally placed feed troughs, eight support beams (approximately 40 x 30 cm) and the perch frame. Individual rollaway nest boxes lined with Astroturf (one nest box per 5 birds) were also provided.

A leg-band containing a transponder[2] (approximately 10 g and a diameter of 2 cm) that emitted a unique identification number was placed on the leg of 80 birds at 33 weeks of age. Only birds on the floor were caught for tagging in an attempt to increase the likelihood of tagging victimised birds. Eight antennae measuring 50 x 50 cm were placed on the slats for 3 weeks and on the litter for a further 3 weeks. Antennae were placed about 1 m apart and 10 cm from the divider between the litter and slats so as to provide a reasonable spread in location. The bird identity, antenna location, date and time on the antenna was recorded each time a tagged bird walked on an antenna by a 486 PC running specialist software[3].

At 39 weeks of age birds were caught and each individual, of known identity, undertook a social avoidance test. The test involved placing the tagged bird at one end of a corridor measuring 2 x 0.5 m located in an adjacent room to the perchery. The bird was positioned with the body oriented at and as close to the side as possible. Next to the bird and outside the corridor was a wire cage (1.5 x 1.0 m) containing 5 birds from the same pen. The latency to move away from the group of birds and furthest distance reached in one minute were recorded.

Following the social avoidance test each bird was weighed. Feather scores were then taken for the head, neck, back and tail regions. These regions were chosen, as they were most likely to be pecked by other birds. One experienced experimenter assessed feather score on a scale of 1 (complete feather cover) to 5 (mostly devoid of feathers.

III RESULTS AND DISCUSSION

There was a large variation in the daily duration recorded on the slats (i.e. the area under the perches) with eight birds never recorded on the slats and other birds recorded for up to three hours. On average birds spent 22.9±5.3 min/day on the slats from 11.6±2.5 observations. A median duration on the slats of 2.2 min/day from 2.5 observations indicated an exponential distribution as shown in Figure 1. This distribution suggests that some birds are spending disproportionate amounts of time on the slats, and support our initial observations that some birds use this area considerably.

[2] MID Trovan®, Weymouth, U.K.
[3] Fingerprint®, Bristol, U.K.

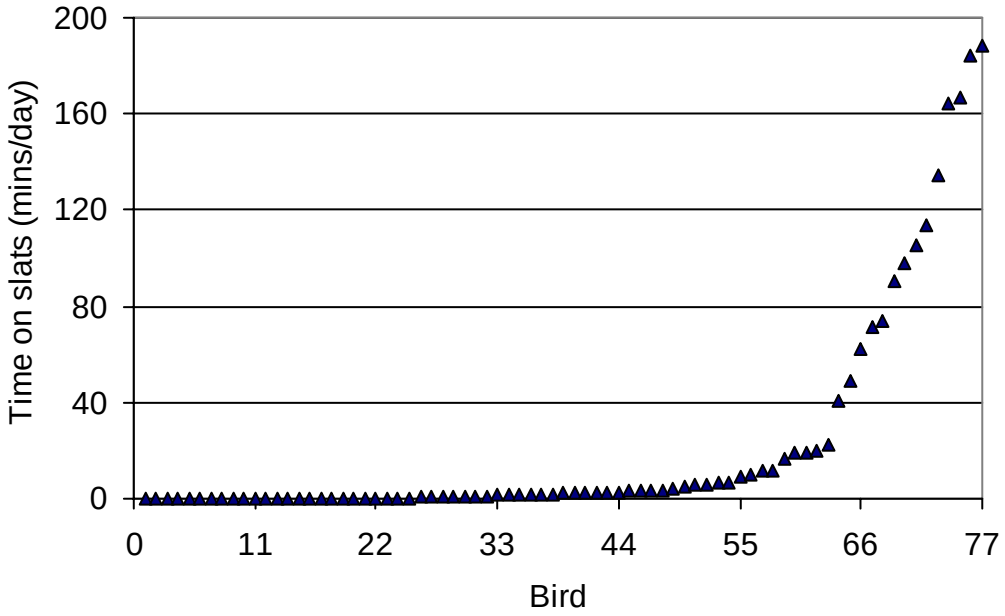

Figure 1. Duration that individual birds spent on the slats

On average birds spent 11.3±1.9 min/day on the litter from 6.2±2.0 observations. The time spent on litter was less variable than time spent on the slats, with maximum durations of 118.7 and 188.4 min/day respectively (Bartlett's variance test, $F=15.2$, $P<0.0001$). A significant positive correlation was found between time on the litter and slats (Spearman rank order correlation coefficient, $r = +0.23$, $N=77$, $P<0.05$), indicating that there was individual variation in use of floor areas as a whole irrespective of whether it was litter or slats.

The social avoidance test was undertaken to investigate if birds that spent considerable amounts of time on the slats were more likely to avoid other birds. On average birds took 30.6±2.6 s to move a maximum of 26.9±1.2 cm away from a group of hens in the avoidance test. Birds that moved sooner reached a further distance away from the group of birds (Pearson correlation coefficient, $r =-0.48$, $N=69$, $P<0.001$). However, the duration on the slats was not related to either the latency to move away from a group of hens (Pearson correlation coefficient, $r =-0.01$, $N=69$, NS) or the maximum distance reached (Pearson correlation coefficient, $r =0.14$, $N=69$, NS). Thus we found no evidence that birds that used the slats did so as a means of avoiding other birds, though it is unclear whether avoidance may be specific to the perchery, and hence not observable in our test.

In general, feather cover for the back and tail regions was very poor, with about half the birds displaying a score of 5 (mostly devoid of feathers). No birds showed complete feather cover for any region. As some body areas recorded few good scores, grouping of scores was undertaken to provide appropriate sample size for analyses. Birds with the worst feather cover for the back, tail and head regions spent significantly more time ($P<0.05$) on the slats than birds with better feather scores (Table 1), consistent with the idea that the former birds may have been receiving more pecks than the latter.

Table 1. Mean duration (± SE) spent on the slats for birds with different levels of feather cover and results of one-way ANOVA

Feather	body region (min/day on slats)			
Score group	Back	Tail	Head	Neck
2	*	*	8.3(±2.9)	10.2(±6.9)
3	*	*	44.8(±16.5)	10.9(±4.6)
4	7.3(±3.1)	2.6(±0.7)	*	8.7(±5.3)
5	24.9(±8.2)	26.7(±7.5)	*	45.2(±20.9)
DF	1,68	1,68	1,68	3,66
ANOVA, F	4.2	8.3	12.9	1.6
Sig. level	0.05	0.01	0.001	NS

Birds that spent little or considerable amounts of time on the slats were lighter than birds that spent an intermediate amount of time on the slats (third order polynomial fitted line, regression analysis $F_{1,68}$= 6.6, P<0.0001). A third order polynomial fitted line was also predicted bird weight following depopulation (regression analysis, $F_{1,36}$ = 2.7, P<0.01). Thus birds that were recorded for little (or no) time on the slats and birds that spent large proportions of time on the slats weighed less than birds that spent intermediate amounts of time on the slats, and this was observed both at 39 weeks and at 72 weeks of age.

IV CONCLUSION

A minority of hens in a large group stayed on the slats under the perches where it was relatively dark for a considerable amount of time. These birds were also smaller than other birds and showed extensive feather loss to the back and head regions suggesting that they may have been pecked repeatedly. Combined, the distribution, weight and feather cover of these birds suggests that they may be recipients of repeated aggressive acts, and show characteristics of victimised birds. The range in use of the area under the perches suggests that there are degrees of victimisation, rather than an all or nothing phenomenon. Since this study was restricted to one housing system (perchery) at one farm, it would be worthwhile to further examine the phenomenon of victimisation in other systems.

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PRE-LAYING BEHAVIOUR OF HENS

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Summary

The interpretation of hen behaviour in the pre-laying period for hen welfare is contentious. This preliminary experiment of 18 pairs of hens aged 45-48 weeks examined the effects of cages with and without a nest box (NB) on behaviour in the "searching" and "sitting" phases of nesting using hens with or without previous experience of a NB. Half of the 18 hens with access to a NB chose to lay in the NB (nest layers). For the other nine hens with a NB and the 18 hens without a NB, oviposition occurred on the wire cage floor (floor layers). Nest layers were less active and performed more sitting behaviour than floor layers in the hour before laying. A behaviour termed "following behaviour" was recorded for over half of the floor layers but none of the nest layers. The interpretation of pre-laying behaviour for hen welfare requires further investigation.

I. INTRODUCTION

From studies of the pre-laying activities of domestic hens, it is generally accepted there are two phases of behaviour involved in oviposition (Sherwin and Nicol, 1992). Beginning ~1 h prior to oviposition, the activity level of hens increases in a phase of behaviour termed "searching" in which hens appear motivated to seek a nest site. In this phase hens increase locomotion and perform behaviours such as inspection of potential nests. Once hens have selected the preferred nest site the "sitting" phase commences, which includes the adoption of a sitting posture interspersed with nest-building activities such as scratching the floor/litter, rotating the body on the nest and collecting litter if available.

Activities performed in the searching phase are goal-directed or appetitive behaviours, occurring when hens are motivated to find a suitable nest for oviposition (the consummatory behaviour). Thus, Appleby and McRae (1986) and Duncan and Kite (1989) showed that hens were motivated to lay their egg in a nest box, and if a nest box was not available hens performed more nest-searching behaviour (Cooper and Appleby, 1995; Freire *et al*., 1996). While an increased occurrence of appetitive behaviour may indicate a stronger motivation to achieve the consummatory phase, it does not necessarily indicate that increased pre-laying locomotion reflects increased frustration and thus a potential welfare problem. For example, using an aversive task approach, Freire *et al*. (1997) suggested that hens were only weakly motivated to reach the nest site during the searching phase, although the motivation to gain access to a nest site increased near the start of the sitting phase. Further, Cooper and Appleby (1997) compared hens that were consistent and inconsistent in their use of nest boxes. While no apparent difference between the two classes of hens was detected in hens' motivation to use a nest box, the inconsistent hens were less responsive to the cues provided by nest boxes than consistent hens. Based on evidence of motivation of hens to lay in a nest box, increased time spent in pre-laying behaviours in the absence of a nest and increased vocalisations indicative of frustration when access to a nest is blocked, it has been concluded that there is convincing evidence of the importance of a suitable nest site (see Keeling, 2004). However, it is also known that even in the presence of a nest not all birds lay in the nest with reports of 0-80% of eggs being laid on the floor (Sherwin and Nicol, 1993) and the question remains of what do hens perceive as a suitable nest site?

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There is reasonable knowledge of the environmental cues that influence pre-laying behaviour by hens, including the presence of a nest box, litter compared to wire flooring, level of light, genotype and social position (Appleby *et al*., 1993; Freire *et al*., 1996). However, the importance of pre-laying searching behaviour and sitting posture to the welfare of laying hens is less-well understood. The objectives of this preliminary experiment in hens that were experienced with laying with or without a NB were to measure activity levels of hens around oviposition, the performance of key behaviours in the "searching" and "sitting" phases of nesting and to examine the use of a NB.

II. METHODS

Commercial Hyline Brown hens aged 45-48 weeks were used in this preliminary experiment, in a controlled climate shed. The hens were part of a larger experiment investigating the effects of different cage "furniture" on hen welfare (ie nest box, dust bath and perch; Victorsson Trivselburen 8-bird Furnished Cages, Sweden, measuring 1.2 m wide, 0.5 m deep and 0.45 m high at the rear of the cage). The NB measured 0.24 m wide, 0.5 m deep and 0.27 m high at the front of the cage and had a solid ceiling, rear and sides, apart from an entrance opening in one side wall. A blue vinyl flap covered the front of the NB while the NB floor was overlain with "astro turf" (0.37 m x 0.22 m x 15 mm thick).

For the present experiment two Victorsson Trivselburen Furnished Cages positioned back-to-back and with all furniture removed were used as the experimental cages. The 36 focal hens (18 pairs) selected for the experiment were from six specific "home" cages (8 hens per cage) that were without dust baths or perches. Three of these "home" cages however contained a nest box (NB treatment) and three were without a NB (No NB treatment). There were nine replicates in time and the NB was randomly allocated to one of the 2 experimental cages for each replicate. At about 1600 h on day 1 of each replicate, a pair of hens was selected on an *ad hoc* basis from a home cage containing a NB and transferred to the experimental cage in which an identical NB had been fitted. Similarly, a pair of hens from a home cage without any furnishings was moved into the other experimental cage. Video recording commenced from the time the hens entered the cages using black and white video cameras with in-built infra-red (IR) lights and time-lapse video recorders. The cameras were positioned to provide views of the two cages from overhead, in front and inside the cages, as well as inside the NB. The two focal hens per cage were marked on the back with a carbon-based dye for individual identification under IR light. The Observer behaviour recording program supplemented with the Support Package for Video Analysis (version 4.0 for Windows; Noldus Information Technology, 1997; Wageningen, The Netherlands) was used to continuously record the activity and location of each focal hen. To allow habituation to the cages there was a minimum of 24 h from placement in the cages to the commencement of data collection from 4 h of video records, commencing 2 h pre-laying on the day that both hens in the pair laid an egg on the same day. The mean (± std dev) time to the first egg laid on the observation day was 47.0 ± 10.7 h (min. 38.4 h, max. 67.5 h). Most hens laid one egg during this "habituation" period, although only the times and locations of oviposition were recorded from the video record. As the experimental cages were of the same design as the home cages, and the pairs of hens originated from the same home cage, it was assumed that this would be a sufficient period of habituation.

A comprehensive list of behaviours was developed to enable the measurement of hen activities, while hen movement was tracked by dividing the video image into nine similar-sized areas excluding the NB or the equivalent area in the No NB cage and the frequency of hens occupying the different locations in the cage. The 4 h observation period was divided into eight, 30-min periods for analysis. Statistical analysis was conducted using a restricted maximum likelihood analysis using the GenStat Committee (2000) statistical package on

individual hen data with random effects for home cage, replicate and pair of birds and fixed effects of laying status of the bird and its cage-mate and presence of a NB. The random effect of pair of birds accounted for any correlation between the two birds in each pair.

III. RESULTS

Of the 18 focal hens in the NB treatment, nine laid in the NB and nine laid outside the NB on the wire floor. These hens were thus termed "nest layers" and "floor layers", respectively. Based on observation of these hens on consecutive days, only one hen was observed to change her location of laying between days, ie laid in the NB and on wire floor on different days. Every combination of "pairing" was observed, with both hens laying in the NB or on the cage floor, one of each laying in the NB or on the cage floor and overlap or separation of both oviposition and nesting behaviour within a pair of hens (ie both hens laid eggs within the same 4-h period or with a clear separation of time periods).

Table 1. Pre-laying behaviours of nest layers and floor layers

Behaviour	Time pre-laying (min)	Nest layers (n=9)*	Floor layers (n=27)*	sed	P value
Stand stationary (min)	120-60[a]	60.1 (45.8)	53.3 (38.6)	3.69	0.122
	60-30[a]	47.7 (16.4)	51.1 (18.1)	3.64	0.395
	30-0[b]	0.74 (5.5)	1.06 (11.5)	0.083	0.00053
Walk (min)	120-60[a]	16.5 (4.8)	17.3 (5.3)	2.17	0.711
	60-30[a]	13.9 (1.7)	21.9 (4.2)	2.14	0.0028
	30-0[a]	5.5 (0.3)	22.7 (4.5)	2.77	1.8×10^{-6}
Areas entered (Freq.)	120-60[c]	9.7 (93)	9.9 (98)	1.45	0.869
	60-30[c]	5.1 (26)	8.5 (73)	0.97	0.0031
	30-0[c]	1.7 (3)	9.5 (90)	0.93	7.4×10^{-9}
Sit stationary (min)	120-60[a]	26.6 (12.0)	23.9 (9.8)	5.17	0.575
	60-30[a]	32.5 (8.7)	22.7 (4.5)	6.41	0.076
	30-0[a]	65.6 (24.9)	35.7 (10.2)	5.35	1.2×10^{-5}

*: Mean values with back-transformed values in parentheses. [a,b,c] = angular, $\log_{10}$ and square root transformations, respectively.

Hen activity level around oviposition was assessed by the time hens spent walking and the frequency of entering areas 1-9 of the cages. Darkness strongly reduced hen activity. As the 2-h pre-laying period included darkness for six hens, the time in the dark was used as a co-variate in the analysis. These six hens alternated between standing and sitting in the dark and none were observed to locomote. After adjusting for darkness, there were no differences in the activity measures in the period 120-60 min pre-laying. However, in the 1 h before laying, nest layers were less active than floor layers; nest layers performed less ($P<0.01$) walking behaviour (excluding following behaviour) and entered areas 1-9 less frequently ($P<0.01$) than floor layers. No other treatment effects were observed. A behaviour termed "following behaviour" was observed. Following behaviour, defined as the focal hen walking or running but following the other hen as she moved about the cage, was only observed in the floor layers (16 of the 27 hens) and occurred during ~5% of the total time in the period 60 min pre-laying to 30 min post-laying and the peak frequency of occurrence was in the 30 min prior to oviposition.

IV. DISCUSSION

This small experiment that involved observations on egg-laying behaviour in 18 pairs of hens has provided more questions than answers. An interesting observation was "following behaviour" which only occurred in hens that laid eggs on the wire floor, irrespective of the presence of a nest box. This behaviour occurred during pre- and post-laying periods and involved the hen (follower) appearing to attempt to remain close to the other hen (followed), including when the followed hen was locomoting. When the followed hen was stationary and standing, the follower would often sit next to her and the follower would put her head under the body of the followed hen. A similar behaviour was described by Kite *et al.* (1980) in which hens appeared to follow and attempt to crawl underneath pen-mates. The reasons for this behaviour are unknown as are the reasons why it occurred in 59% of floor layers and occurred both pre- and post-oviposition. One explanation is that when a nest box was present which was utilised for egg laying, this environment provided appropriate cues for nest site selection. Another explanation is the follower hen may have derived cues for nest site selection from the followed hen.

This experiment has shown, as reported in other studies (Cooper and Appleby, 1997), that the use of the NB for egg laying is highly variable between birds. In this experiment where experienced (with a NB) hens were housed in pairs in a cage with a NB, 50% of hens laid in the nest box and 50% laid on the wire cage floor outside the NB. This is similar to the daily egg production records for the three NB treatment home cages, recorded over 10 months, in which 55.8% of eggs were laid in the NB. These data raise the question of hens' preference for egg laying location. While the literature suggests hens are motivated to seek a "preferred" location for egg laying, the data from this experiment could be interpreted to suggest that either the nest box or the wire cage floor may be preferred locations. Alternatively, by one hen making a choice, this may or may not force the other hen into a less-preferred location. This experiment only used pairs of hens and the possible combinations for preference presumably become more complex in commercial settings of group sizes of 5 to 20 hens, particularly when the number of nest sites (boxes) is limited. Clearly further research is required to answer the following types of questions: What is the biological significance of following behaviour, including any relationship with nest site selection? Is consistency of nest site selection associated with a preference for that site, or are some birds forced to choose a less-preferred site? Are these behaviours the same with larger group sizes? Are there any implications for welfare?

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THE EFFECTS OF A SIMULATED CYCLONE AND DIETARY INTERVENTION ON PRODUCTION OF COMMERCIAL HENS IN SAMOA.

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Summary

The onset of cyclones in the South Pacific region is usually associated with a drop in commercial egg production, with performance taking over two weeks to normalize. It is postulated that during a cyclone, nutrient use in layers changes as a result of increase in catabolism and reduction in anabolism. Consequently, the impact of a simulated cyclone induced stress on production traits of commercial layers in Samoa was investigated. The treatments encompassed a control and simulated stress with and without a vitamin and mineral cocktail intervention. Hens that were subjected to stress with intervention maintained acceptable levels of egg production, and feed efficiency during and after the cyclone stress. The percent hen day egg productions during the stress were 68.3%, 46.9% and 74.6% for the control, stress and stress with intervention treatments respectively. The practical implications for these observations is the ability to sustain egg production during cyclones with dietary intervention.

I. INTRODUCTION

The South Pacific Island countries are located along one of the world's most frequent hurricane and cyclone belt, which spans from about longitudes 127^{o} East to 130 ^{o} West and between latitudes 30 ^{o} South and 20 ^{o} North. Cyclones usually occur in the months of November to April, and during cyclones agricultural activities are exposed to the forces of nature. Poultry are most vulnerable to cyclones and susceptible to flash floods, high humidity, reduced photoperiod and reduced light intensity. Consequently, there is a high incidence of mortality, disease (especially with young stock), growth suppression and reduced egg production, which might cease all together depending on the severity of the cyclone.

Poultry producers rely on egg production as their only or major source of income. Consequently any disruption in production or mortality results in financial losses for farmers. For example after cyclone "Heta" struck Samoa in January 2004 commercial chicken producers observed a drop in egg production of 20-60%, and it took over 2 weeks for egg production to be restored (Personal communication).

Considering the effect cyclones and hurricanes have on livestock it is surprising that no research have been conducted in the region to examine the effects on egg laying chickens. This study was therefore designed to investigate if problems associated with stress as a result of a cyclone could be controlled by vitamin and mineral dietary supplementation by providing additional allowances of ascorbic acid (vitamin C), vitamins A, E, D_3 and thiamine.

II. MATERIALS AND METHODS

The strain of layers used in this study is the Shavers Starcross 579, which is the most predominant egg laying strain in the country. The experimental design was a completely randomized block design, with ninety-six hens divided into the following three treatments: Control (no stress), Stress (+) intervention and Stress (-) intervention. Stress was simulated

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for 7 days by reduced photoperiod - 3 – 4 hours of daylight (use of blind), gale wind (use of electric fan -50 cm), hurricane, thunder and lighting (recorded noise and flashing light). Intervention was based on the oral administration (to individual hens) of the following cocktail: ascorbic acid 250 mg, vitamin E 300 mg, vitamin A 200mg, calcium 105 mg and phosphorus 22.5 mg.

Each treatment consisted of two replicates with 16 hens per replicate. The hens were raised on deep litter floor with wood shavings as litter material. The housing was open sided natural type housing with stocking density of 0.2 m^2 per hen. All hens were approximately 48 weeks old and were fed a commercial layers diet (imported from Australia) with water provided *ad-libitum*. The experimental layout was configured to associate each group with their specific treatment, and the total duration was 4 weeks encompassing a 1 week preliminary period, 1 week pre stress, 1 week stress and 1 week post stress. The following parameters were recorded, egg production, feed intake, egg weight, mortality and egg abnormalities. Data were analyzed using the Gen Stat software programme (Genstat, 2002), and the differences between means determined by L.S.D. at the 5% level of significance.

III. RESULTS

There were no significant differences for the following production traits prior to the commencement of the simulated stress: - feed intake, kg feed per kg egg, egg weight and percent hen house egg production (Table 1). However, the hens that were subjected to simulated cyclone stress without intervention (-) had significantly ($P< 0.05$) reduced feed intake, feed efficiency and percent hen day production compared to the control and the stress with intervention (+) group (Table 2). In contrast there were no significant differences between all groups for egg weight, which ranged from 60-62 grams. Compared to the pre stress levels, the percent drop in hen house egg production was 25.8% and 4.3% for the group without (-) and with (+) intervention respectively. In contrast the control group laid more eggs as observed by 6.85% increase in percent hen day egg production. This however was not significantly different from the group with (+) intervention.

Post simulated cyclone stress effect on the production traits is shown in Table 3. The recovery from stress is more noticeable with feed efficiency or feed per kg egg for the stress without (-) intervention group which is reflected in improvement in percent hen day by approximately 10.4%. During the course of this study no mortality was recorded and the hens were treated humanely, behavioral responses (gregarious, acoustic, feeding and drinking) to stimulated cyclone stress were normal.

IV. DISCUSSION

A major observation from the current study was a 25% drop in egg production when layers were subjected to stimulated cyclone stress. However, hens that were subjected to stress with intervention maintained acceptable egg production levels during and after the stress. In an earlier study Bollengier-Lee *et al.* (1999), noted that increasing the dietary supplementation of nutrients such as vitamins and electrolytes improved post recovery of chickens from heat stress. Similarly, Puthpongsiriporn *et al.* (2001), observed from their study that additional allowances of ascorbic acid (vitamin C) vitamins A, E, D_3 and thiamin can improve bird performance. This is because the vitamins help to control the increase in body temperature and plasma corticosterone concentration which are usually elevated during stress (Lim *et al.*, 2001). In-addition, vitamin E has been shown to protect cell membranes, boost the immune system and improves nutrient transport across cell wall membranes (Kolb and Seehawer, 2002), so additional dietary supplementation may be advantageous during

adverse weather condition or stress. Rao *et al.* (2004), reported that the absorption of vitamin A declines at high temperature and noted beneficial effects from three-fold increase for broiler breeders during heat stress. During cyclones birds will be subjected to stress which can be defined as physical or psychological tensions associated with external stimulants (reference). There are many different types of factors that can initiate stress in a commercial laying flock. Some of these include:- (i) feed and water management, (ii) type of housing and stocking density, (iii) disease control and general management, (v) fright and excitement from the presence of strangers, (vi) fatigue and illness, (vii) temperature, and abrupt weather changes.

For effective strategies to be developed that can sustain egg production during and after a cyclone, it is important to understand the relationship between cyclones and egg production. This is because some studies have shown that a drop in egg production and decline in eggshell quality is a key indicator of stress in layers. Consequently, the most logical physiological explanation for a drop in egg production in the current study might be from mobilization of body reserves for non-productive purposes, including reduced synthesis of certain nutrients (e.g. ascorbic acid). Additionally, the cumulative effects of these factors might be due to a shift in metabolic activities from catabolic to anabolic processes. This might explain why stress intervention was very effective in reducing the impact on production of the simulated cyclone. The supplementary nutrients provided appeared to meet the requirement for both stress and production during the adverse weather conditions.

In summary, this study further confirms the observations of commercial egg producers in Samoa, who had previously reported 20-30% drops in egg production during cyclones (Ajuyah, pers. comm.). The average egg producer in the country has approximately 3000 laying hens with an average hen day production of 80%. If we assume a drop in production of 30% during a cyclone the loss in production would be approximately 60 dozen eggs per day which is equivalent to $312 per day ($1 AUD = $2.0 WST). For an average farmer this is a significant loss in income, especially when there are no insurance policies available for livestock producers in the region to protect against cyclones. This study has shown that a simple strategy can be employed to sustain commercial egg production during and after a cyclone. (Comment on the cost of the intervention. Should the dose be included in the feed or would the farmer need to give oral treatment through drinking water).

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Table 1:Production traits for commercial Brown Egg layers prior to simulated cyclone.

Treatment	Feed intake (g/bird/day)	Egg wt (g)	Hen day (%)	Feed conversion (Kg feed/kg egg)
Control	103.8	60.45	61.45	3.74
Stress (-)	100.0	60.25	72.7	4.50
Stress (+)	103.1	60.55	78.9	5.14

(-) = no intervention; (+) = with intervention (vitamins and mineral).

Table 2: Production traits for commercial Brown Egg layers during simulated cyclone

Treatment	Feed intake (g/bird/day)	Egg wt (g)	Hen day %	Kg feed/kg egg
Control	121[a]	60.09	68.3[a]	4.98[a]
Stress (-)	82[b]	60.55	46.9[b]	2.42[b]
Stress (+)	91[b]	60.70	74.6[a]	3.91[a]

(-) = no intervention; (+) = with intervention (vitamins and mineral).
[a-b]Mean in the same column followed by the same letter are not significantly different (P>0.05).

Table 3: Production traits for commercial Brown Egg layers after simulated cyclone

Treatment	Feed intake/bird/day (g)	Egg wt (g)	Hen day %	Kg feed/kg egg
Control	118.2[a]	60.57	67.7[a]	4.94[a]
Stress (-)	102.6[b]	60.34	57.3[b]	3.64[b]
Stress (+)	105.2[a]	62.02	74.0[a]	4.90[a]

(-) = no intervention; (+) = with intervention (vitamins and mineral).
[a-b]Mean in the same column followed by the same letter are not significantly different (P>0.05).

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HYDROLYSIS OF LUPIN PECTIN BY PECTINASES FOR BROILERS

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Summary

We tested the hypothesis, that a combination of two pectinases, polygalacturonase (PG) and pectin methyl esterase (PME) would break down pectin more completely and reduce the water-holding capacity and viscosity of dehulled lupins more than PG alone. Dehulled lupins were incubated without (no enzyme) and with PG, PME, and PG + PME for 1 hour in acid media. PG + PME significantly reduced the viscosity of the solution, its water-holding capacity, cell-wall materials, pectin, molecular weight, methyl esters and long chains of pectin more than PG or PME, except for viscosity of dehulled lupins treated with PG. PME significantly reduced the methyl esters but, conversely, increased viscosity and water-holding capacity, more than the control. PG reduced these parameters more than the control and PME, but did not reduce the methyl esters and pectic chains. These results suggest that PG alone is not capable of demethylating the pectin chain and PME cannot break down the glycosidic bonds. The combination of PG and PME improved the breakdown of pectin and reduced the water-holding capacity, the long chains of pectin and methyl esters of dehulled lupins by 1 – 3 fold compared with PG alone. By using these two pectinases in broiler diets it should be possible to include a higher proportion of dehulled lupins into the diets without compromising the growth performance or increasing wet droppings.

I. INTRODUCTION

A major stumbling block for the degradation of cell walls by any pectinase is the existence of pectin methyl ester radicals attached to the C_6 atom of galacturonic acid units along the pectin chain. The pectinase, polygalacturonase (PG), targets specifically the glycosidic bonds that join the galacturonic acid units together. But when cell walls are treated with PG only 11% of the bonds are broken because the methyl ester radicals block the binding sites of PG to the glycosidic bonds along the pectin chain (Endo, 1964a,b; English *et al*., 1972; Ali *et al.*, 2001). In addition, the methyl esters through their cross links with neighbouring polymers via divalent ions such as Ca^{++} and Mg^{++} are directly responsible for the properties of water-holding capacity and viscosity (Northcote, 1958; Grant *et al*., 1973; Jarvis, 1984). Increased water-holding capacity is the main cause for an increase in water intake and hence an increase in wet droppings. Higher viscosity is also undesirable as is implicated in poor digestion of nutrients and, consequently, depressed weight gain and food utilization (Erdman *et al*, 1986; Langhout and Schutte, 1996; Langhout *et al.*, 1999, 2000).

However, these radicals can be removed by a specific enzyme, pectin methyl esterase (PME) which strips off these radicals along the pectin chain and hydrolyses them into methanol and hydrogen ions. When PME does this, many of the branches are destroyed, leaving mainly smooth, linear chains of galacturonic acid units which are 4 to 10 times more susceptible to attack from PG (Jansen and McDonnell, 1945; Endo, 1961; Christgau *et al.*, 1996). As a consequence, PME improves the hydrolytic activity of PG for complete degradation of pectin and reduces the viscosity and water-holding capacity of feedstuffs containing pectin. Lupin seems a particularly good target for the action of PME because lupin

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kernel contains 8 – 11% pectin within cell-wall lattices, and the majority of this, between 80 – 90%, is methyl esterified (Konovalov *et al*., 1999). Therefore, we tested this hypothesis by incubating dehulled lupins with PG, PME and PG+PME and measured the breakdown of pectin and the reduction of water-holding capacity and viscosity.

II. MATERIALS AND METHODS

Ten grams of dehulled lupins of narrow leaf (*Lupinus angustifolius*, var. Quilinock) were incubated without (control) and with 1,400 units of PG (ROHAPECT MPE) and PME (ROHAMENT PL) and PG+PME. The incubation mixture was prepared by dissolving ground dehulled lupins in 70 ml acid buffer (42g citric acid + 280ml 1M NaOH + 200ml 1M CaCl2, pH = 3.9) according to the assay method described by the enzyme manufacturer. The analytical procedures and measurements described in a previous *in vitro* experiment (Ali *et al*., 2001) were followed in this experiment. The determinations of methyl ester and methanol were carried out according the method of Wood and Siddiqui (1971). Analyses of the data were carried out by performing ANOVA in a complete randomised design. Four experimental treatments (control, PG, PME and PG+PME) and 12 replicates per treatment were analysed for each measured trait using the statistical package Genstat (5 release 4.1, IACR Rothamsted). If ANOVA of any treatment was statistically significant, orthogonal contrasts were used to compare the differences among their means.

III. RESULTS

The combination of enzymes, PG+PME, reduced ($P<0.05$) cell walls by 27%, pectin nearly 50%, molecular weight of pectin by 56% and the length of pectin chains by 65%. Methyl esters were reduced by a similar amount (64%) and methanol was increased by 116%. This combination reduced viscosity by 7%, water-holding capacity by 15% and filtration rate by 9% (Table 1). When PME was used alone viscosity and water-holding capacity both increased ($P<0.05$) and filtration rate decreased (Table 1). PG used alone induced similar changes compared with the two pectinases but the extent of change was much less with the exception of viscosity. PG had no effect on methanol or methyl esters.

Table 1. Effect of PG, PME and PG+PME on physical-chemical properties of dehulled lupins *in vitro* (mean±SEM).

Parameters	Control	PG	PME	PG+PME	LSD[#]
Cell-wall materials (% DM)	23.4±0.9	20.3±1.2	23.5±1.0	17.1±0.9	2.8
Pectin (% DM)	10.9±0.8	8.6 ±0.3	9.1±1.0	5.8±0.3	1.9
MW of pectin (kilodaltons)	135±5	101±5	128±6	59±3	14
Length of pectin chain[§]	63.0±2.8	56.1±2.6	61.2±2.2	22.3±1.6	6.7
Methanol (µg/ml incubation media)	8.8±0.8	9.6±0.8	14.6±1.5	19.0±1.8	3.7
Methyl esters of pectin chain (%)	20.1±0.9	18.6±1.0	9.7±0.6	7.3±0.5	2.2
Viscosity (m.Pas/sec)	1.69±0.01	1.48±0.01	1.91±0.03	1.57±0.03	0.09
Water-holding capacity (g:g)	3.56±0.17	3.19±0.15	4.10±0.21	3.04±0.13	0.48
Filtration rate (µl/sec)	69.4±2.6	79.0±3.6	47.0±1.6	63.2±2.7	7.9

Least significant differences (LSD) were applied to the single degree of freedom orthogonal contrasts at the 5% level of probability as follows: 1) control vs enzymes, and 2) PG and PME vs PG+PME. § Number of galacturonic acid units per pectin chain.

IV. DISCUSSION

There was unequivocal evidence that the hypothesis was supported because the combination of pectinases was superior to either pectinase alone. PG and PME together were very effective at breaking down pectin (50% reduction) and reducing cell walls (27%). Other parameters measured confirmed these observations. For example, molecular weight of pectin was more than halved and the length of pectin chains was reduced by 3 fold. As anticipated, the methyl esters on the pectin were reduced by a similar magnitude (3-fold) and they were converted to methanol and hydrogen which more than doubled.

Surprisingly, the breakdown of pectins and cell walls did not change filtration rate and the reductions were smaller than anticipated in viscosity and water-holding capacity. There are at least four possible reasons. First, removal of methyl esters from the cell-wall matrix by PME gives more non-methylated galacturonic acid chains and these can be cross-linked by Ca^{++}. In turn this leads to gel formation and an increase in water-holding capacity and viscosity (Penn *et al.*, 1966; Nichols and Deese, 1966; Gupta and Nichols, 1962; Willats *et al.*, 2001). Second, PME itself requires large amounts of water to convert methyl esters (CH_3COO) into methanol (CH_3OH) and H^+. This also increases water-holding capacity of the pectin. Third, PME converts insoluble to soluble pectin, causing the coagulation of soluble pectin to form a gel and hence to retain large amounts of water for formation of the gel network (Kertesz, 1951; Oi and Satomura, 1965; Ben-Arie and Lavee, 1971). Finally, the result could be dose dependent. Just enough PME is required to expose the right number of sites on pectin so that PG can break down the maximum number of glycosidic bonds. Too much PME could be detrimental if it produces too many radicals because the radicals themselves are responsible for water-holding capacity (Endo, 1964ab; Pressey and Avants, 1982). Therefore, had we chosen a lower dose of PME, the reductions in viscosity and water-holding capacity may have been greater because the production of methyl ester radicals may have been less. Before this *in vitro* work is tested in broilers the appropriate combination of PG and PME needs to be determined more precisely.

The combination of PG and PME used did not achieve complete breakdown of pectin which was contrary to expectations. There is a PME that can be extracted from plants and it removes blockwise nearly all of the methyl esters from the pectin chain. The common PME comes from microorganisms and this only removes a portion of the radicals because it acts randomly (Sajjaanantakul and Pitifer, 1991; Kester *et al.*, 2000; van Alebeek *et al.*, 2000). We used the commercial PME from microbial origin which can only hydrolyse about half of the methyl esters. Complete breakdown of pectin requires other pectinases such as exo-PG, pectin lyase and rhamnogalacturonase for cleavage of the terminal, methylated and rhamnogalacturonan glycosidic bonds of the pectin chain, respectively (Rombouts and Pilnik, 1980; Sakai *et al.*, 1993).

The main conclusion from this work is that two pectinases are required to break down pectin chains in dehulled lupins. First, PME is required to remove the methyl ester radicals to expose the glycosidic bonds of pectin. Second, PG is then required to break down the glycosidic bonds to destroy pectin lattice. This synergistic action between PME and PG could make inroads towards greater inclusion of dehulled lupins, up to 20%, into broiler diets.

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EFFECTS OF A MULTI-ENZYME PRODUCT ON METABOLIZABLE ENERGY AND NUTRIENT DIGESTIBILITY OF DIFFERENT BARLEY BASED DIETS

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Summary

In order to understand the interaction between barley cultivars and energy response to enzyme (Rovabio™ Excel) addition, an apparent metabolisable energy (AME) study was performed in broiler chickens. Barley viscosity or digesta viscosity was only correlated with fat digestibility of barley-based diet without enzyme or with fat digestibility improvement by enzyme addition. No barley characteristics measured in the present trial could explain the variability of the AMEn of barley without enzyme supplementation. However, the AMEn improvement by enzyme addition can be explained by two barley characteristics: dry matter content and water soluble: insoluble ß-glucan ratio of barley.

I. INTRODUCTION

Barley with NSP enzyme supplementation can be most extensively used for animal feeding in hot climates because its adaptation to dry climates and drought hardiness or when wheat or corn prices are high. Extensive work has been done to study the effect of enzyme addition to poultry diets on the energy value of barley (Rotter *et al*., 1990, Friesen *et al*., 1992, Villamide e*t al*., 1997). However, excepting Villamide *et al.* (1997), the barley cultivar effect on the NSP enzyme efficiency was not studied, neither were the reasons for the enzyme response variability fully explained.

The objective of this study was to determine the effects of Rovabio™ Excel on metabolisable energy and nutrient digestibility of diets containing different barley cultivars and to establish relationships between some barley characteristics and their response to enzyme addition.

II. MATERIALS AND METHODS

Two hundred and eighty eight day-old (Ross 308) male broiler chicks were brooded in 96 metabolisable cages. During the first 10 days, chicks were housed at three birds per cage and fed with a commercial diet based on corn-soybean meal. At day 10, chickens were weighed and distributed into 12 groups of 24 birds/group allocated at two chickens per cage. Six experimental diets were tested with or without enzyme (Rovabio™ Excel) each containing 50% of either barley selected from six cultivars: Sunrise, Gaelic, Naturel, Sultane, Graphic and Escarlett. Barley cultivars were selected on basis of its viscosity value to obtain diets with low and high viscosity value. The composition of basal diets is shown in Table 1. The feed used did not contain any anticoccidial drug, or antibiotic growth promoter or any other probiotic feed additive. A balance period was performed from 21 to 24 days of age (Bourdillon *et al*., 1990) to determine the apparent fat and nitrogen (N) digestibility and AME of the diet. Barley cultivars and diets were also analysed for dry matter, ash, crude fibre, N, ether extract, water extract viscosity, total and soluble pentosans and total and insoluble β-glucans. Diets were analysed for dry matter, N, crude fibre, ether extract and β-glucanase activity. Fat content of diets and excreta were measured by extraction after acid hydrolysis with petroleum spirit. Nitrogen content of excreta was measured by Kjeldahl's method and uric acid content of excreta according to the method described in Marquardt (1983).

Statistical analysis was performed using StatView 5.0 software program (Abacus Concepts INC. Berkley, California). A 6 x 2 factorial analyses of variance was used to assess the effects of barley cultivars, enzyme addition and their interactions.

Table 1. Composition of experimental diet

Ingredient (g/kg)	Experimental diet
Barley	500
Maize	75
Soybean oil	34
Soya full fat extruded	150
Soybean meal 48	197
DL-Methionine	3.28
Lysine-HCl	1.70
Calcium carbonate	11.7
Dicalcium phosphate	18.6
Salt	3.30
Minerals and vitamins[1]	4.0
Calculated analysis	
Metabolisable energy (MJ/kg)	12.6
Crude protein (%)	21.6
Crude fat (%)	8.2
Lysine (%)	1.24
Methionine (%)	0.63
Met + Cys (%)	0.96
Calcium (%)	1.0
Available phosphorus (%)	0.45

[1] One kg of feed contains: Vitamin A: 12000 IU; Vitamin D_3: 5000 IU; Vitamin E: 30 mg; Vitamin K_3: 3 mg; Vitamin B_1: 2,2 mg; Vitamin B_2: 8 mg; Vitamin B_6: 5 mg; Vitamin B_{12}: 11 μg; Folic acid: 1,5 mg; Biotin: 150 μg; Calcium pantothenate: 25 mg; nicotinic acid: 65 mg; Mn: 60 mg; Zn: 40 mg; I: 0,33 mg; Fe: 80 mg; Cu: 8 mg; Se: 0,15 mg; Etoxiquín: 150 mg.

III. RESULTS AND DISCUSSION

The analytical composition of barley cultivars (Table 2) identified two groups of barley: low viscous (2.13-2.34 cp) and high viscous (3.42-4.92 cp) varieties which also correlate with the soluble β-glucan fraction (r^2=0.89). This confirms previous observations on relationships between water soluble NSP and viscosity value of water extract (Dusel *et al*., 1997).

The AMEn and fat digestibility of barley cultivars were significantly different (Figure 1). The N and dry matter digestibilities of barley cultivars were also significantly different (data not shown). Such variation was also reported by Villamide *et al*. (1997) with a range 2,852 to 2,963 kcal/kg DM, whithout any relationship between AMEn of barley without enzyme and their chemical composition. In the present experiment, the only significant relationship observed were between fat digestibility and extract viscosity of barley (p=0.03, r^2=0.63) and digesta viscosity. Maisonnier *et al.* (2001) pointed out that fat digestibility is the parameter most affected by the increase of digesta viscosity. However, in the present experiment, barley viscosity only explains 63% of the variability in fat digestibility. As for

wheat (Maisonnier *et al*., 2004), barley viscosity does not appear to be the only factor explaining ME variability in barley-fed broiler chickens.

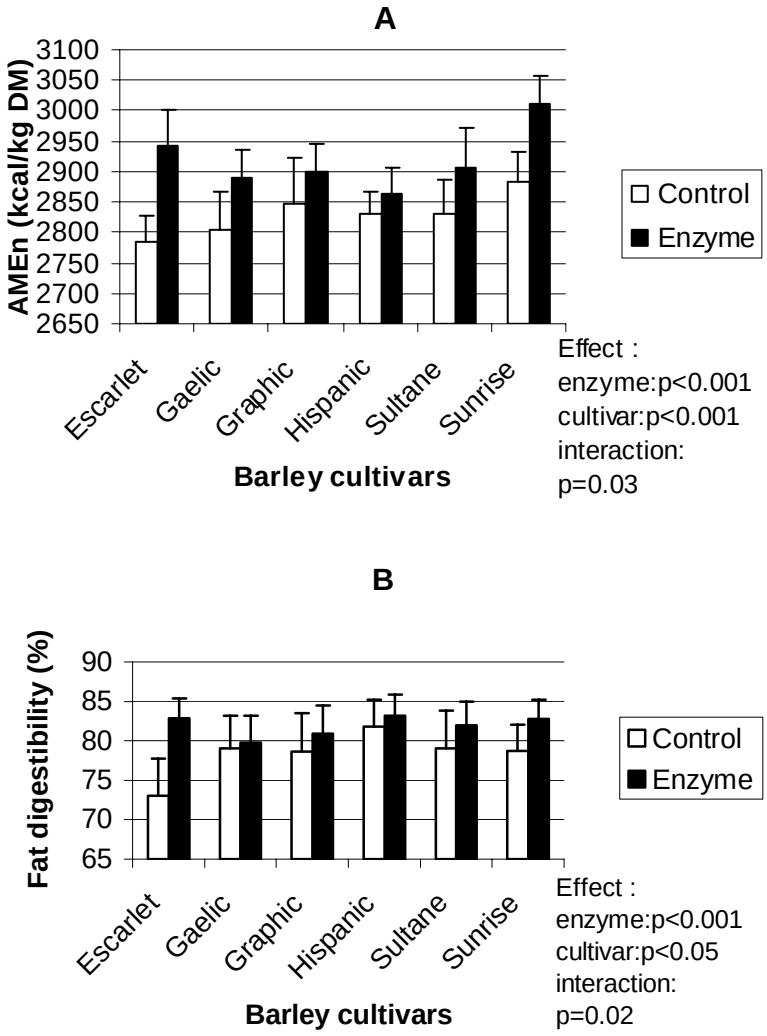

Figure 1. Effect of Rovabio^{TM} Excel on the Apparent Metabolisable Energy corrected for nitrogen balance (AMEn, A) and fat digestibility (B) of barley-soybean meal based diets containing different barley cultivars in broiler chickens (means±SD).

A significant improvement of the AMEn, and fat digestibility of barley based diet with the Rovabio^{TM} Excel supplementation was observed (Figure 1). There was a significant interaction between barley cultivar and enzyme supplementation on AMEn and fat digestibility (Figure 1). Villamide *et al.* (1997) also reported an interaction ($p<0.10$) between barley cultivars and enzyme supplementation on barley AMEn with a trend for a correlation ($p=0.06$, $r^2=0.47$) between AMEn improvement and viscosity value of barley. However, in the present experiment, the AMEn improvement did not appear to be related to the viscosity of barley. Only a low correlation was observed between fat digestibility improvement and the viscosity value of barley ($p=0.08$, $r^2=0.47$). For the AMEn improvement, no correlation was observed between dietary AMEn, fat digestibility or barley characteristics. The only correlation observed was between AMEn improvement and barley dry matter content and water soluble:insoluble β-glucan ratio (AMEn improvement = -164 x dry matter + 15.24 x water soluble:insoluble β-glucan ratio, $p<0.005$, $r^2=0.95$). With wheat, Maisonnier *et al*.

(2004) also reported a significant correlation between dry matter content of the wheat and the response to enzyme addition.

Table 2. Analytical composition of barley cultivars

Barley	Dry matter	Specific gravity	Extract viscosity	β-glucans (%)			
	(%)	(kg/hL)	Cp	Total	Soluble	Insoluble	Soluble/ insoluble
SULTANE	88.23	62.76	3.42	3.24	1.33	1.91	59.0
GRAPHIC	88.99	64.86	4.06	3.87	1.35	2.52	65.1
ESCARLETT	88.15	66.98	4.92	4.43	1.68	2.75	62.1
GAELIC	89.08	59.39	2.34	3.51	1.14	2.37	67.5
HISPANIC	88.98	58.97	2.13	3.02	1.10	1.92	63.6
SUNRISE	88.90	58.44	2.23	3.19	0.96	2.23	69.9

IV. CONCLUSION

As for wheat, the AMEn value of barley as well as the enzyme effect depends on the barley cultivar used. Moreover, barley viscosity did not seem to be responsible for this variability except for the fat digestibility. The barley characteristics measured in the present trial did not explain the variability of the AMEn of barley without enzyme supplementation. However, the AMEn improvement with enzyme can be explained by two barley characteristics: dry matter content and water soluble:insoluble β-glucan ratio.

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RESPONSE OF BIRDS FED INCREASING LEVELS OF PALM KERNEL MEAL SUPPLEMENTED WITH DIFFERENT ENZYMES

B.SUNDU[1], A. KUMAR[1] and J. DINGLE[1]

Summary

The standard recommendation for using palm kernel meal (PKM) in broiler diets is in the range of between 10 to 25 %. However, 40 % PKM in the diet was found satisfactory when balance diets were formulated by inclusion of lysine and methionine and feed efficiency was increased when enzymes were included. Birds were fed diets containing 0, 20, 30 or 40 % PKM with one of five different enzyme products: no enzyme (Nil), Gamanase (G), Hemicell (H), Allzyme SSF (SSF) and combination of G+H+SSF (Comb). Live weight gain of birds was not reduced when PKM were included. DM digestibility and viscosity decreased with increased levels of PKM. Although the use of enzymes increased feed digestibility significantly, they could not increase weight gain to reach the significant level. Feed efficiency improved in Hemicell and SSF supplemented PKM diets.

RESULTS AND DISCUSSION

The standard recommendation for using palm kernel meal (PKM) in broiler diets is in the range of between 10 – 25 %. However, 40 % PKM in the diet was found satisfactory when balance diets were formulated by inclusion of lysine and methionine (Panigrahi and Powell, 1991) and feed efficiency was increased when enzymes were included (Sundu *et al.*, 2004). This present study investigated the effects of increasing levels of PKM in the diet and different enzyme products on production parameters, nutrient digestibility and gut content viscosity of broilers. Eighty male Ross meat chicks were fed diets containing 0, 20, 30 or 40 % PKM with one of five different enzyme products that were selected to digest β-mannan carbohydrate: no enzyme (Nil), Gamanase (G), Hemicell (H), Allzyme SSF (SSF) and combination of G+H+SSF (Comb) for 10 days. The diets were balanced for protein and energy. Faeces and jejunal digesta were collected on d 18-20 and 21 respectively.

Table 1. The effects Palm Kernel Meal (PKM) level on the response of broiler chicks, day 5 – 15

Level of PKM	0 %	20 %	30 %	40 %
Weight gain	297	296	291	296
Feed intake	374^{c}	396^{a}	378^{bc}	393^{ab}
FCR	1.26^{b}	1.34^{a}	1.30^{ab}	1.33a
DM digestibility	81.8^{a}	77.0^{b}	70.6^{c}	65.9^{d}
NDF digestibility	56.5^{a}	56.0^{a}	40.3^{b}	37.2^{c}
Viscosity (cP)	2.67^{a}	2.02^{b}	1.86^{c}	1.81^{c}

^{abcd}: Row means with the same superscript are not significantly different (P<0.05)

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Table 2. The effect of enzyme supplements on the response of broiler chicks, day 5-15

Enzyme	Nil	Gamanase	Hemicell	Allzyme SSF	Comb
Weight gain	290	296	298	294	298
Feed intake	395^{a}	390^{ab}	380^{ab}	372^{b}	390^{ab}
FCR	1.37^{a}	1.32^{ab}	1.28^{b}	1.27^{b}	1.31^{ab}
DM digestibility	73.1^{d}	74.1^{b}	73.4^{cd}	73.6^{c}	74.8^{a}
NDF digestibility	43.2^{d}	48.3^{b}	48.9^{b}	47.3^{c}	49.9^{a}
Viscosity (cP)	2.22^{a}	2.15^{a}	2.14^{a}	2.09^{a}	1.61^{b}

^{abcd}: Row means with the same superscript are not significantly different ($P<0.05$); (Comb, is a combination of the three enzymes)

Live weight gain of birds was not reduced when PKM was included at levels as high as 40%. A decreased DM digestibility of all the PKM diets may be partly due to the high NDF content in the diets. Viscosity was low and decreased with increased levels of PKM, so viscosity was not a problem. Although the use of enzymes increased feed digestibility significantly, they could not increase weight gain to reach the significant level. Feed efficiency improved in Hemicell and SSF supplemented PKM diets. It can be concluded that the use of up to 40 % PKM gave satisfactory results for weight gain, and the use of Hemicell and SSF improved feed efficiency and digestibility.

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THE EFFECT OF A NOVEL ENZYME COMPLEX ON THE PERFORMANCE AND NUTRIENT DIGESTIBILTY IN LAYING HENS FED A CORN-SOY DIET

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Summary

Allzyme® SSF (200g/t) was added to maize and soybean meal based layer diets, in order to determine the effect on laying hens given a low phosphorus and low energy diet. Two hundred and eighty eight birds were assigned to each of the three dietary treatments, which consisted of a positive control, a negative control, and the treatment diet containing the multi-enzyme complex, Allzyme SSF. Egg production, egg weight and mortality were determined daily for each bird from 21 to 41 weeks of age. Feed intake and egg quality parameters were measured weekly. From these measurements, FCR and feed costs were calculated. Dietary treatment had no effect ($P \leq 0.05$) on egg production, egg weight, feed intake, FCR, AME or mortality rate. Excreted P was reduced ($P \leq 0.05$) and AME was improved ($P \leq 0.05$) with Allzyme SSF addition. Allzyme SSF supplementation of the P and energy reduced diet improved phosphorus and energy utilisation to the extent that laying hen performance was equivalent to the control group.

I. INTRODUCTION

Allzyme® SSF is a novel enzyme complex produced by solid-state fermentation (SSF), a process that generates six other enzyme activities in addition to phytase. Solid-state fermentation is a natural method dating back 4000 years and involves the seeding of a solid-state substrate with a bacterium or fungus. Allzyme SSF contains seven enzyme activities derived from *Aspergillus niger:* α-amylase, β-glucanase, cellulase, pectinase, phytase, protease and xylanase.

Allzyme SSF has been proven to be effective in enhancing the utilization of phytate phosphorus (P) and metabolisable energy (ME) in broilers, and has been shown to improve the performance of birds fed wheat-soy diets (Wu *et al*., 2003), corn-soy diets (Brake *et al*., 2003) as well as other less conventional substrates, such as copra meal and palm kernel meal (Sundu & Dingle, 2003). Furthermore, previous studies have shown that the addition of 0.02% Allzyme SSF to a corn soybean diet in broiler breeders can allow a reduction in available P by up to 0.18% without compromising reproductive performance and egg quality (Qiugang *et al*., 2004). The objective of the present study was to examine the effects of Allzyme SSF on the performance, apparent metabolisable energy and nutrient digestibility in laying hens fed a corn-soy diet without animal-sourced protein during summer.

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II. MATERIALS AND METHODS

The experiment was conducted at Huajin Layer Farm in Tianjin, China, from May 25 to September 15, 2003. The enzyme complex, Allzyme SSF, was supplied by Alltech Incorporated (Nicholasville, Kentucky, USA). Three diets were formulated which were based on maize and soybean meal. The ingredient composition and proximate analysis of the experimental diets are shown in Table 1. The positive control diet (PC) was formulated to contain 11.30 MJ/kg metabolisable energy (ME) and 0.35% available P (avP). The ME and avP contents in the negative control (NC) were formulated to be 10.88 MJ/kg and 0.35%, respectively. In the treatment group (TRT), 0.02% Allzyme SSF was added into a low-energy (10.88 MJ/kg) and low P diet (0.25% avP).

Table 1. Percentage ingredient composition and analysis of the experimental diets (g/kg)

Ingredients	PC	NC	TRT
Maize	632.4	624.5	623.0
Wheat bran	0.00	15.2	22.3
Soybean meal	203.0	156.2	154.0
Cottonseed meal	50.0	50.0	50.0
Rapeseed meal	0.00	50.0	50.0
Maize oil	11.1	0.00	0.00
Limestone	82.0	82.8	88.3
Salt	3.0	3.0	3.0
Dicalcium phosphate	13.7	13.0	3.9
Vitamin-mineral premix	2.7	2.7	2.7
Choline chloride	1.0	1.0	1.0
Methionine	1.1	1.1	1.1
Lysine	0.00	0.5	0.5
Allzyme SSF	0.00	0.00	0.2
Calculated Analysis			
ME (MJ/kg)	11.30	10.88	10.88
Crude protein	160.0	160.0	160.0
Calcium	35.0	35.0	35.0
Total phosphorus	6.0	6.0	4.5
Available phosphorus	3.5	3.5	2.0
Crude fibre	28.8	33.5	34.0
Crude fat	36.2	25.4	25.5
Lysine	7.3	7.3	7.3
Methionine + cysteine	6.5	6.6	6.6

PC is the positive control diet, NC is the negative control diet, TRT is the treatment diet

Eight hundred and sixty-four, 20 week-old Hyline brown layers were selected and randomly assigned into 3 treatments with 6 replicates of each. There were 48 birds (12 cages, 4 birds per cage) per replicate. Diets were fed in mash form. Feed and water were given *ad libitum* during the experimental period. Lighting and ventilation were controlled by a programmable automated control system. At the start of the trial, birds received 14.5 hours of light, and this was subsequently increased 30 minutes every week until a total of 16 hours

of light per day was achieved. Room temperature was controlled at 20~28°C, and the relative humidity maintained between 60~70%.

The total number of eggs, broken and soft-shelled eggs and egg weight were determined on a daily basis from 21 to 41 wks of age. Feed intake was measured weekly. Egg quality parameters were measured from 25 weeks of age. For this, all eggs laid over a three day period were collected for each replication and 1/3 of these eggs were sampled to measure shell strength, shell thickness, Haugh unit and yolk colour. Shell strength was measured using the Compression Test Cell in Texture Test Systems (Model T2100C, Food Technology Corp., Rockville, MD). Shell thickness was determined by a mean value of measurements at three locations on the egg (air cell, equator, and sharp end) measured by using a dial pipe gauge (Model 7360, Mitutoyo Corp., Kawasaki, Japan). Haugh unit (HU) was calculated with the HU formula (Eisen et al., 1962), which is based on egg weight and the height of albumen determined by a micrometer (Model S-8400, AMES, Waltham, MA). Yolk colour was measured according to Hoffman-LaRoche Fan (Hoffman-LaRoche, Inc., Nutley, NJ).

The average mass of production for lay, average laying rate, egg weight, average feed intake, broken egg rate, and mortality rate were recorded. Total egg numbers, broken eggs, mortality rate and feed consumption were recorded daily. Feed conversion ratio and feed costs were then calculated.

Apparent metabolisable energy and the excreta (%) of phosphorus, protein and calcium were determined at 25 weeks of age by collection of excreta over three consecutive days from eight birds per replicate. Feed and excreta samples were collected and analysed for proximate components by the AOAC (1990) method. Mineral contents were measured using an inductively coupled plasma emission spectrometer (Model JY-24, Jobin Yvon, Longjumeau, Cedex, France).

Results are expressed as mean values with standard errors. Data were analysed using analysis of variance according to a completely randomised design, and differences between means were determined using the one-way ANOVA. Means were separated by Duncan's multi-comparison range test. Differences were considered significant at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

There were no significant differences among dietary treatments in either the laying rate or total egg mass (Table 2). This may be attributable to the birds producing to their capacity, which, independent of diet nutrient levels, may also be a result of genetics, environment and bird management. Dietary treatment had no effect ($P \leq 0.05$) on egg weight, daily laying egg weight, feed intake, feed conversion ratio or mortality rate over the experimental period (Table 2). The lack of dietary effect on these various parameters may be due to the birds already producing at their optimal output. The addition of Allzyme SSF numerically improved average body weight gain over the whole experimental period. This may be a reflection that the extra energy available from the treatment diet has been partitioned into weight gain. There were no significant differences for the egg quality parameters among the treatment diets (Table 3), again suggesting that eggs were being produced at their optimal output and quality.

Excreta P and calcium content of birds fed the Allzyme SSF supplemented diet were lower ($P \leq 0.05$) than both control groups (Table 4). Dietary treatment had no effect ($P \leq 0.05$) on excreta protein content. The lower P and Ca levels excreted are most likely due to the lower level of avP, limestone and dicalcium phosphate in the treatment diet. However, due to the fact that animal performance (laying performance, egg shell quality, FCR, weight gain) was not compromised suggests that Allzyme SSF may be improving the availability of P and Ca. Specifically, the presence of phytase, which breaks down the phytic acid, would make P more available to the animal. Phytase would also make calcium more available by releasing the cations bound within the phytate molecule, which includes Ca. Lower excreta P in the treatment diet suggests that Allzyme SSF could be useful in reducing environmental P pollution from layer manure.

Table 2. Effect of dietary treatment on laying performance

Parameters	Treatments			
	PC	NC	TRT	SEM
Egg production (% hen-day)	87.67±0.39	88.00±0.74	87.59±0.87	0.99
Egg weight (g)	54.63±0.24	54.44±0.20	54.43±0.28	0.34
Laying production (g/day)	47.67±0.38	47.91±0.46	48.49±0.23	0.52
Feed intake (g)	111.88±1.04	111.22±1.01	110.88±0.93	1.41
FCR	2.31±0.02	2.33±0.03	2.32±0.02	0.03
Weight gain (g)	220.00±25.33	212.08±15.13	263.33±16.93	27.77
Mortality rate (%)	0	0	0	0

[a,b] Mean values within the same row with different superscripts differ significantly ($P \leq 0.05$). Mean±SE. n = 288

Table 3. Effect of dietary treatment on egg quality parameters of laying hens

Parameters	Treatments			
	PC	NC	TRT	SEM
Eggshell strength (kg/cm^2)	3.48±0.28	3.25±0.31	3.28±0.15	0.36
Haugh unit (HU)	4.21±0.29	3.91±0.20	4.35±0.18	0.32
Egg yolk colour (Roche)	6.16±0.14	5.09±0.68	5.80±0.11	0.57
Eggshell thickness (μm)	366.41±9.87	358.31±3.54	365.47±5.79	9.78

[a,b] Mean values within the same row with different superscripts differ significantly ($P \leq 0.05$). Mean±SE. n = 288

Table 4**.** Effect of dietary treatment on excreta calcium, phosphorus and protein contents in laying hens.

Parameters	Treatments			
	PC	NC	TRT	SEM
Excreta P (%)	1.47±0.05^b	1.50±0.05^b	1.18 ± 0.07^a	0.08
Excreta protein (%)	19.40±1.09	22.03±2.04	20.55±0.65	1.96
Excreta Ca (%)	10.18±0.47^b	10.00±0.82^b	7.42 ± 0.26^a	0.80

[a,b] Mean values within the same row with different superscripts differ significantly ($P \leq 0.05$). Mean±SE. n = 288

Table 5. Effect of dietary treatment on the apparent metabolisable energy (AME) (MJ/kg) in laying hens

Parameters	Treatments			
	PC	NC	TRT	Pooled SEM
AME (MJ/kg)	11.96 + 0.09[ab]	11.65 + 0.17[a]	12.42 + 0.29[b]	0.201
AME (MJ/kg DM)	13.75 + 0.10[ab]	13.39 + 0.20[a]	14.28 + 0.33[b]	0.232
AME Digestibility	0.834 + 0.006[ab]	0.806 + 0.012[a]	0.857 + 0.020[b]	0.0138

[a,b] Mean values within the same row with different superscripts differ significantly ($P \leq 0.05$). Mean±SE. n = 288
DM = dry matter

AME (MJ/kg) and AME (digestibility) for the treatment diet were higher ($P \leq 0.05$) compared with the negative control diet (Table 5). This demonstrates that similar available energy was present in the treatment diet compared with the diet formulated with standard energy levels (PC). This effect is most likely due to the presence of the various enzyme activities in Allzyme SSF increasing the energy availability of the feed.

Feed cost of the enzyme supplemented treatment and the negative control was reduced by 6.8% as compared with the control diet. Compared with the positive control, a saving of US$1730 per million eggs (US2.08 cents per dozen eggs) was obtained in the supplemental Allzyme SSF group.

IV. CONCLUSIONS

Supplementing 200g/t of Allzyme SSF into a reduced energy (-0.42 MJ/kg) and available P (-0.15% avP) corn-soy diet gave equivalent production performance and egg quality to a normal ME and avP control diet. Allzyme SSF supplementation was further demonstrated to improve energy, calcium and P digestibility and to reduce P excretion.

Supplementing with Allzyme SSF into a low metabolisable energy and low available phosphorus corn-soy diet has the potential to be commercially significant in reducing P excretion and overall feed cost whilst maintaining production performance.

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AMINO ACID DIGESTIBILITY AND GROWTH PERFORMANCE INTERACTIONS TO PHYTASE AND LYSINE SUPPLEMENTATION OF LYSINE-DEFICIENT BROILER DIETS

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Summary

Supplementation of lysine-deficient, but otherwise nutritionally adequate, diets with exogenous phytase or lysine monohydrochloride (Lysine HCl) increased weight gain and feed efficiency of broilers. However, significant treatment interactions were observed, as the effects of phytase on growth performance were more pronounced in lysine-deficient diets. Phytase increased the apparent ileal digestibility (AID) of amino acids but additional lysine also increased the digestibility of certain amino acids and some significant treatment interactions were observed. It is suggested the increases in digestibility induced by lysine, and interactions with phytase, may be mediated via acid-base homeostasis and intestinal uptake of amino acids.

I. INTRODUCTION

The magnitude of the positive influence of phytase on the utilisation of protein/amino acids in poultry, and the corresponding negative effects of dietary phytate, remains a topic of debate (Augspurger and Baker, 2004). Moreover, the underlying mechanisms have not been completely identified. The present study investigated the effects of phytase supplementation in broiler diets containing two levels of lysine.

II. MATERIALS AND METHODS

The experimental design was a 2x2 factorial where treatments consisted of two levels of dietary lysine (10.0 and 11.8 g/kg), without and with phytase (Natuphos®, 500 FTU/kg). The phosphorus-adequate (4.5 g/kg nonphytate-P) diets were based on a wheat-sorghum blend with monocalcium phosphate (MCP) as the major P source. The basal diet contained Celite as an inert marker and was formulated to contain 13.1 MJ/kg ME, 197.5 g/kg protein, 10.6 g/kg phytate and to meet the requirements of all amino acids except that of lysine. Lysine HCl was included in the lysine-adequate diets and MCP was reduced (500 FTU ≡ 1 g P as MCP) in phytase-supplemented diets. The dietary treatments were offered to male broiler chicks (Cobb) from 7-28 days of age when growth performance was determined. From days 24-27, total excreta were collected to determine apparent metabolisable energy (AME) of the diets. At 28 days the birds were euthanised and samples of ileal digesta and toes taken to determine the AID of amino acids and percentage toe ash by standard procedures (Selle *et al*., 2003). Experimental data was subjected to analyses of variance (SPSS Inc. Chicago, IL).

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III. RESULTS

Both phytase and lysine supplementation enhanced ($P < 0.001$) weight gain and feed efficiency without influencing feed intake (Table 1). Treatment interactions ($P < 0.05$) were observed for weight gain and feed efficiency because the effect of phytase was more pronounced in lysine–deficient diets with increases of 5.3% and 3.4% for weight gain and feed efficiency, respectively. Phytase increased ($P < 0.001$) AME of diets by 0.34 MJ/kg DM, which was independent of dietary lysine, and ileal digestibility of crude protein by 2.8%. Treatments had no influence on toe ash contents (data not shown), which is indicative of the P adequate status of the diets and that the effects of phytase were not related to enhanced P availability.

Table 1. The effects of phytase and lysine supplementation of lysine-deficient diets on growth performance of broilers (7-28 days of age), apparent metabolisable energy (AME) and apparent ileal digestibility (AID) of protein

Treatment		Growth performance				
Phytase (FTU/kg)	Lysine (g/kg)	Weight gain (g/bird)	Feed intake (g/bird)	Feed : Gain (g/g)	AME (MJ/kg DM)	AID of protein (%)
0	10.0	823	1476	1.79	14.22	78.1
500	10.0	867	1497	1.73	14.55	81.2
0	11.8	899	1473	1.64	14.21	79.8
500	11.8	913	1484	1.62	14.56	81.2
SEM		5.73	11.99	0.010	0.048	0.496
Significance (P =)						
Phytase		0.000	0.20	0.001	0.000	0.000
Lysine		0.000	0.52	0.000	0.93	0.12
Treatment interaction		0.02	0.68	0.05	0.80	0.12

Phytase increased ($P < 0.05$-0.001) the apparent ileal digestibility of essential amino acids (Table 2). The percentage increase in AID of threonine (6.1%) was the most, and methionine (0.6%) the least, pronounced following phytase supplementation, which is a typical pattern. Lysine supplementation increased ($P < 0.01$-0.001) the digestibility of some (isoleucine 3.9%, lysine 3.6%, methionine 0.9%, phenylalanine 3.8%, valine 4.0%) essential amino acids. Lysine also significantly increased the digestibility of certain non-essential (aspartic acid, glutamic acid, glycine, tyrosine) amino acids; whereas, phytase increased the digestibility of all non-essential amino acids (data not shown). Significant treatment interactions ($P < 0.05$) were observed for the ileal digestibility of five essential (arginine, lysine, phenylalanine, threonine, tryptophan: and four non-essential (aspartic acid, glutamic acid, glycine, serine) amino acids, where responses to phytase were more pronounced in lysine–deficient diets.

Table 2 The effects of phytase and lysine supplementation of lysine-deficient diets on apparent ileal digestibility (AID) of essential amino acids

Treatment		Apparent ileal digestibility (%)									
Phytase (FTU/kg)	Lysine (g/kg)	Arginine	Histidine	Iso-leucine	Leucine	Lysine	Methio-nine	Phenyl-alanine	Threo-nine	Trypto-phan	Valine
0	10.0	82.2	79.7	76.2	76.4	79.4	91.0	77.1	74.9	76.2	76.9
500	10.0	85.6	82.5	79.5	79.2	83.0	91.7	80.8	80.7	80.0	80.8
0	11.8	83.1	80.5	79.3	77.6	83.7	92.0	81.3	76.0	78.0	80.2
500	11.8	83.8	81.7	82.5	78.3	84.6	92.4	82.7	79.4	79.0	83.9
SEM		0.554	0.658	0.526	0.530	0.514	0.244	0.490	0.431	0.650	0.527
Main effects											
Phytase 0 FTU/kg		82.6	80.1	77.7	77.0	81.6	91.5	79.2	75.4	77.0	78.5
Phytase 500 FTU/kg		84.7	82.1	81.0	78.8	83.8	92.0	81.8	80.0	79.5	82.4
Lysine 10.0 g/kg		83.9	81.0	77.8	77.8	81.2	91.4	79.0	77.8	78.1	78.9
Lysine 11.8 g/kg		83.5	81.0	80.9	77.9	84.2	92.2	82.0	77.7	78.4	82.0
Significance (P =)											
Phytase		0.001	0.01	0.000	0.01	0.000	0.05	0.000	0.000	0.001	0.000
Lysine		0.47	0.91	0.000	0.83	0.000	0.01	0.000	0.82	0.61	0.000
Treatment interaction		0.05	0.24	0.88	0.06	0.05	0.52	0.05	0.05	0.05	0.89

DISCUSSION

Phytase enhanced growth performance of broilers offered lysine-deficient diets, where the 4.5% increase in lysine AID was probably partly responsible. Increased digestibility of other amino acids and AME probably also contributed to this improved performance generated by phytase. It is interesting that AME responses to phytase were independent of dietary lysine levels. However, increases in AID of certain amino acids following the addition of lysine and, moreover, interactions between lysine and phytase in this respect, were unexpected and the underlying mechanisms are not clear.

In the present study, the dietary electrolyte balance (DEB = $Na^+ + K^+ - Cl^-$) of the broiler diets was calculated to be 155 mEq/kg, which is less than the optimum range of 250-300 mEq/kg, recommended by Johnson and Karunajeewa (1985). Basic amino acids, including lysine, are thought to be important regulators of acid-base balances (Austic and Calvert, 1981). Although the effects of DEB on amino acid digestibility has received little attention, dietary electrolyte balance and amino acid metabolism are intimately related (Patience, 1990). Given the low DEB values in the present study, it seems possible that lysine influenced the AID of amino acids via its impact on acid-base balance and the uptake of amino acids from the gut. Na^+-K^+-ATPase activity transfers K^+ into, and Na^+ ions out of, enterocytes, which influences the uptake of nutrients from the gut (Gal-Garber *et al.,* 2003) and a large proportion of amino acids are absorbed by co-transport with Na^+ (Sklan and Noy, 2000). It is noteworthy, therefore, that Cowieson *et al.* (2004) reported that phytate significantly increased Na excretion in broilers to a substantial extent and phytase tended to reduce it. These workers suggested that the increased secretion of Na^+ into the gut is to buffer the polyanionic phytate molecule, which would be countered by phytase. Possibly lysine altered the acid-base balance and absorption of amino acids from the gut and phytase also influenced, or was influenced by, the acid-base balance and this may be responsible for the significant interactions observed in digestibility of amino acids. In conclusion, it is suggested that the influence of lysine on AID of certain amino acids, and its interaction with phytase in this respect, may stem from their effects on acid-base homeostasis in broiler chickens. The determination of the effects of phytase on AID of amino acids and growth performance of broilers offered diets with differing electrolyte balances appears to be justified.

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INFLUENCE OF PHYZYME™ XP PHYTASE ON NUTRIENT UTILIZATION IN BROILERS FED DIETS CONTAINING GRADED LEVELS OF PHYTATE

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Summary

The influence of a new phytase, Phyzyme™ XP, on nutrient digestibility was investigated in broiler chickens fed diets containing different levels of phytate. The results showed that dietary phytate level adversely affected the apparent metabolisable energy (AME) and ileal digestibility of protein and amino acids. Supplemental phytase resulted in consistent improvements in the AME values. The interaction between phytase and level of dietary phytate was not significant for AME, but the improvement was numerically greater in diets containing high phytate levels. Ileal nutrient digestibility data, however, showed that the responses to supplemental phytase for several nutrients, including dry matter, protein and amino acids varied depending on dietary phytate levels. The magnitude of the improvements in digestibility responses, in general, increased as the dietary phytate levels increased. The current study lends support to the thesis that phytase response is dependent on phytate concentrations.

I. INTRODUCTION

The phosphate releasing potential of phytase has been consistently demonstrated and is well accepted in the poultry industry. There is, however, some disagreement concerning the ability of phytase to release other nutrients. These disagreements can be traced to inconsistencies in the scientifically published literature. Some studies have reported consistent effects of phytase on energy and amino acid digestibility, while others have been inconsistent or show no significant effect of phytase. Factors influencing the magnitude of energy and amino acid responses are probably complex, but perhaps the major factor contributing to this variability is the concentration of the substrate, the phytate, in the diet. This hypothesis was examined in the present study by evaluating the influence of supplementation of Phyzyme™ XP phytase on nutrient utilisation parameters of broiler starters fed diets containing varying phytate levels.

II. MATERIALS and METHODS

The study was conducted as a 3 x 4 factorial arrangement of treatments to evaluate the effects of three levels of phytate P (2.8, 3.3 and 3.8 g/kg) and four levels of Phyzyme XP phytase (0,500, 750 and 1000 U/kg of diet). The 'low' phytate diet (2.8 g/kg) was based on corn and soybean meal, and the 'medium' and 'high' phytate diets (3.3 and 3.8 g/kg, respectively) were formulated by the inclusion of increasing amounts of rice bran. The energy and amino acid levels were formulated to be 5-10% lower than the NRC (1994) recommendations for broiler starter diets. The non-phytate P level in all three diets was maintained at 1.6 g/kg below current NRC (1994) recommendations . A total of 600 day-old male broiler (Ross) chicks were equally divided and raised in three groups on floor pens in an environmentally controlled room.

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The chicks were fed one of the three basal diets (low, moderate or high phytate) from day 1 to 14. On day 14, 120 birds from each diet group were selected and distributed into 24 cages of five birds each so that average weights per pen was similar. Each of the 12 dietary treatments was then randomly assigned to six pens. The treatment diets were fed from day 14 to 21. Feed and water were available *ad libitum* during this period.

From days 17 to 20, feed intake and total excreta output were measured quantitatively per pen to determine apparent metabolizable energy (AME). Samples of diets and excreta were analysed for dry matter, gross energy and nitrogen. At the termination of the trial (Day 21), all birds were euthanased by intracardial injection of sodium pentobarbitone and the contents of the lower ileum were collected and pooled within a pen. Samples of diets and ileal digesta were assayed for nitrogen, amino acids (including methionine, cystine and tryptophan) calcium, phosphorus and titanium. Two-way analysis of variance was used to determine the main effects (phytate level and phytase) and their interactions by using the General Linear Models procedure of SAS (1997).

III. RESULTS

The results are summarised in Tables 1 and 2.

Table 1. Effects of graded levels of Phyzyme XP phytase on the AME and apparent ileal digestibility of dry matter, protein, total amino acids (AA), calcium and phosphorus of broiler starter diets containing varying phytate P levels[1].

Phytate P level	Phytase, U/kg diet	AME, MJ/kg DM	Dry matter	Protein	Ileal total AA	Calcium	Phosphorus
Low	0	13.89	71.5	81.7	81.3	34.9	51.6
(2.8 g/kg)	500	14.07	72.0	81.8	82.3	36.3	56.2
	750	14.07	72.7	83.5	84.1	38.1	62.0
	1000	14.10	74.0	84.0	85.3	41.7	64.6
Moderate	0	13.73	71.2	80.6	79.7	38.9	45.7
(3.3 g/kg)	500	13.92	73.7	83.6	83.2	41.0	59.3
	750	13.91	72.9	82.9	82.8	40.1	58.4
	1000	13.97	72.6	83.2	83.0	41.1	66.7
High	0	13.51	69.4	80.7	78.5	32.1	47.8
(3.8 g/kg)	500	13.73	73.8	84.7	84.4	37.2	51.4
	750	13.80	73.5	84.2	84.0	35.7	56.5
	1000	13.90	73.6	83.8	83.7	39.1	64.3
Pooled SEM		0.133	0.4	0.4	0.5	1.8	1.6
Main effects							
Phytate	Low	14.03	72.5	82.7	83.3	37.7	58.6
	Moderate	13.88	72.6	82.5	82.2	40.2	57.5
	High	13.73	72.6	83.3	82.7	36.0	55.0
Phyzyme	0	13.71	70.7	81.0	79.8	35.2	48.4
	500	13.91	73.1	83.4	83.3	38.1	55.6
	750	13.93	73.0	83.5	83.7	38.0	58.9
	1000	13.99	73.4	83.6	84.0	40.6	65.2
Probability, P=							
Phytate		0.01	0.99	0.04	0.01	0.01	0.01
Phytase		0.07	0.001	0.001	0.001	0.01	0.001
Phytate x phytase		0.99	0.001	0.001	0.001	0.69	0.02

1 Each mean value represents an average of six replicate pens (5 birds per pen).

Table 2. Apparent ileal digestibility of selected essential amino acids for broilers as influenced by dietary levels of phytate and Phyzyme™ XP phytase[1].

Phytate-P level	Phytase, U/kg diet	Lys	Met	Thr	Trp	Ile	Leu	Val
Low	0	87.3	89.6	77.3	80.1	82.0	83.4	80.5
(2.8 g/kg)	500	88.0	90.8	78.2	81.1	83.3	84.3	81.6
	750	89.2	91.8	79.2	82.5	84.9	86.0	83.4
	1000	89.9	92.2	80.9	83.4	86.4	87.2	84.2
Moderate	0	85.3	88.8	73.9	78.4	81.1	81.7	79.6
(3.3 g/kg)	500	88.3	91.4	78.2	81.5	84.7	85.6	83.4
	750	87.8	90.6	77.5	81.2	84.2	85.2	82.7
	1000	87.9	91.3	77.7	81.7	84.3	85.1	83.0
High	0	84.5	89.4	73.3	77.7	79.5	81.2	76.9
(3.8 g/kg)	500	88.5	91.9	80.2	83.7	85.2	86.5	83.2
	750	88.6	91.8	80.2	83.2	85.0	86.4	82.9
	1000	88.3	91.7	79.7	83.3	84.6	85.8	82.8
Pooled SEM		0.4	0.4	0.5	0.8	0.5	0.5	0.5
Main effects								
Phytate	Low	88.6	91.1	78.9	81.8	84.2	85.2	82.4
	Moderate	87.3	90.5	76.8	81.1	83.6	84.4	82.2
	High	87.5	91.2	78.3	82.0	83.6	85.0	81.5
Phytase	0	85.7	89.3	74.8	78.7	80.8	82.1	79.0
	500	88.3	91.4	78.9	82.1	84.4	85.5	82.7
	750	88.5	91.4	81.9	82.3	84.7	85.9	83.0
	1000	88.7	91.7	82.4	83.3	85.1	86.1	83.3
Probability, P=								
Phytate		0.001	0.05	0.001	NS	NS	0.05	0.05
Phytase		0.001	0.001	0.001	0.001	0.001	0.001	0.001
Phytate x phytase		0.01	NS	0.001	0.10	0.001	0.001	0.001

[1] Each mean value represents an average of six replicate pens (5 birds per pen).

IV. DISCUSSION

It is logical to assume that nutrient release in responses to phytase supplementation in poultry would be influenced by dietary phytate levels, but published data examining this aspect are limited (Cabahug *et al*., 1999; Ravindran *et al*., 2000). The present results showed that energy utilization and ileal digestibility of protein, amino acids, phosphorus and calcium were adversely affected by dietary phytate level. The negative effects are clearly evident when data from the three unsupplemented basal diets are compared.

Supplemental phytase resulted in consistent improvements in the AME values. Although no interaction was detected between phytase and phytate level for AME response, the improvements were found to be numerically greater in diets containing high phytate levels. Ileal nutrient digestibility data, however, showed that the responses in digestibility to supplemental phytase for several nutrients, including dry matter, protein, amino acids and P, varied depending on dietary phytate levels. The magnitude of digestibility responses, in general, increased as the dietary phytate levels increased.

In the present study, dietary phytate P level was increased by the inclusion of rice bran. This approach may be criticized because rice bran also contains relatively high levels of

fibre. It is known that fibre fractions also negatively influence the availability of minerals and protein. The levels of neutral detergent fibre present in the basal diets used in the present study (ranged from 95.8 to 129.5) are, however, within acceptable standards and are too low to cause any deleterious effects on the performance of broilers. The other option available to vary dietary phytate levels is to use purified forms of phytic acid (phytic acid, calcium phytate or sodium phytate), but this method is also subject to criticism because it is not representative of practical feeding situations.

Overall, the present results, along with those of Ravindran *et al.* (2000), suggest that phytic acid is a potent anti-nutritional factor that can adversely affect energy utilization and the availability of amino acids and minerals in poultry diets. The present data also demostrate that these negative effects can be overcome by phytase supplementation and lends support to the hypothesis that phytase response will be greater when feed formulations contain ingredients with relatively high phytate concentrations.

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A COMPARISON OF TWO PHYTASE SOURCES IN DIETS FOR BROILERS

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Summary

A 3-phytase derived from *Aspergillus niger* and a new 6-phytase derived from *Escherichia coli* were compared in growth trials with broilers offered maize/soybean meal/canola meal-based diets. The products were used according to their commercial recommendations such that the new phytase had approximately 30% lower inclusion levels in feed (FTU/kg). Both products successfully recovered broiler performance to control in a range of diets containing reduced levels of phosphorus and calcium, with or without concomitant reductions in dietary amino acids and energy. The potential cost-saving benefit of phytase addition into vegetable-based diets for broilers was therefore confirmed in this trial. Gross savings of over $5 USD per tonne of feed were achieved, at the time of the trial, in the diets with the highest levels of nutrient reduction.

I. INTRODUCTION

The routine use of the feed enzyme phytase is already well established in broiler nutrition globally. Recent studies have shown that certain new phytase products appear to offer improved bio-efficacy versus their established commercial counterparts (Ausberger *et al*, 2003). This study compared a 3-phytase (derived from and produced in *Aspergillus niger*) against a new 6-phytase (derived from *Escherichia coli* and produced in a yeast *Schizosaccharomyces pombe*). This new 6-phytase showed improved characteristics when compared with the 3-phytase *in vitro* e.g. reduced sensitivity to endogenous proteases (Kumar *et al*, 2003). This could have implications for relative dose rates when using the two products. In line with commercial practice, the two phytases were compared in diets containing reduced total phosphorus (P) and calcium (Ca) levels, with or without reductions in dietary amino acids and energy.

II. METHODS

One-day old Cobb male broiler chicks (3300) were assigned to 11 treatments, each with 6 pen replicates of 50 birds (at 11.6 birds per m^2). Maize/soybean meal/canola meal-based mash diets were fed to 41 days of age (Table 1). The diets contained 0.24-0.25% phytate P, based on measurements on each of the raw materials used in the diet formulation. A positive control (PC) diet in both the starter and grower phases (0-21 days and 22-41 days respectively) was compared against 3 negative control (NC) diets. NC diet 1 (NC1) was reduced in total P (0.12%) and calcium (0.10%) in line with commercial recommendations for the use of the 3-phytase. NC diet 2 (NC2) was reduced in total P (0.12%), calcium (0.10%) and in amino acids and energy (Tables 1 and 2), again in line with commercial recommendations for the 3-phytase product. NC diet 3 was further reduced in total P (0.17%) and calcium (0.15%) and had amino acids and energy reduced to the same levels as NC2. All phosphorus and calcium reductions were made by the removal of dicalcium phosphate (DCP). DCP was reduced by 6.9 kg/t in NC1 and NC2 and by 10.35 kg/t in NC3.

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The two phytase products (both with guaranteed minimum activities of 5000 FTU/g) were added to NC1 and NC2 according to commercial recommendations for each product, where the 6-phytase has a 20% lower inclusion rate recommendation than the 3-phytase for the same P and Ca reduction. All diets were then analysed for phytase activity at two laboratories (Danisco, Brabrand, Denmark and BRI Australia Ltd., North Ryde NSW) and the mean values measured were 646 FTU/kg feed (3-phytase) and 448 FTU/kg feed (6-phytase, see Table 1)

The addition rate of the 3-phytase to NC3 was increased by 50%, under an assumption of linearity in P and Ca response, although this is not claimed commercially at these higher levels of inclusion. The addition rate of the 6-phytase to NC3 was either at the same level as for NC1 and NC2 or increased by 50%. The mean phytase levels measured in the experimental diets containing these higher levels of inclusion were 885 FTU/kg feed (3-phytase) and 625 FTU/kg feed (6-phytase, see Table 1).

Table 1. Diets and treatments (g/kg, PC = Positive control, NC = Negative control)

	Starter phase (0 – 21 days)				Grower phase (22 – 41 days)			
	PC	NC1	NC2	NC3	PC	NC1	NC2	NC3
Reduction in total P/Ca, %	-	0.12 /0.10	0.12 /0.10	0.17 /0.15	-	0.12 /0.10	0.12 /0.10	0.17 /0.15
Reduction in amino acids and energy?	-	No	Yes	Yes	-	No	Yes	Yes
Maize	628	639	656	661	628	641	652	658
Soybean meal	226	225	218	217	218	215	214	213
Canola meal	60	60	60	60	80	80	80	80
Fishmeal	40	40	40	40	-	-	-	-
Soya oil	15.4	11.8	1.7	-	42	38	29	27
L-lysine HCl	1.28	1.29	1.32	1.34	0.79	0.84	0.72	0.74
DL-methionine	1.65	1.63	1.64	1.64	1.18	1.17	1.14	1.13
Limestone	9.7	10.5	10.5	10.9	11.3	12.1	12.1	12.5
Dicalcium phosphate	10.66	3.75	3.79	0.35	12.5	5.62	5.58	2.14
Vitamins and minerals	7.5	7.5	7.5	7.5	6	6	6	6
No phytase or 3-phytase or 6-phytase (FTU/kg feed)	0	0 646 448	0 646 448	0 885 448 or 625	0	0 646 448	0 646 448	0 885 448 or 625
Crude protein	223	223	222	222	201	201	202	202
ME, kcal/kg	3000	3000	2947	2947	3143	3143	3090	3090
ME, MJ/kg	12.55	12.55	12.33	12.33	13.15	13.15	12.93	12.93
Lysine	12.1	12.1	12.0	12.0	10.2	10.2	10.0	10.0
Methionine	5.3	5.3	5.3	5.3	4.3	4.3	4.2	4.2
Calcium	9.2	8.2	8.2	7.7	8.2	7.2	7.2	6.7
Phosphorus (P)	6.8	5.6	5.6	5.1	5.9	4.7	4.7	4.2
Digestible P (CVB, 2003)	3.7	2.8	2.8	2.4	3.0	2.1	2.1	1.7

III. RESULTS

Table 2. Summary of performance, 41 days (PC = Positive control, NC = Negative control)

Treatment	Reduction in total P/Ca, %	Reduction in amino acids and energy[1]?	Phytase product, level of inclusion[2]	Bodyweight gain, g	Feed intake, g per bird	Feed: gain[3] g:g	Mortality %
PC	-	-	-	2176[d]	3782[c]	1.746[a]	3.6
NC1	0.12/0.10	No	-	2025[bc]	3566[b]	1.767[ab]	4.0
			3-phytase 646 FTU	2212[d]	3853[c]	1.745[a]	3.0
			6-phytase 448 FTU	2195[d]	3831[c]	1.749[a]	2.4
NC2	0.12/0.10	Yes	-	1930[b]	3459[b]	1.803[b]	3.6
			3-phytase 646 FTU	2216[d]	3898[c]	1.769[ab]	3.4
			6-phytase 448 FTU	2170[d]	3809[c]	1.767[ab]	2.4
NC3	0.17/0.15	Yes	-	1541[a]	2791[a]	1.886[c]	15.4
			3-phytase 885 FTU	2134[cd]	3761[c]	1.780[ab]	4.0
			6-phytase 448 FTU	2130[cd]	3790[c]	1.782[ab]	2.6
			6-phytase 625 FTU	2228[d]	3913[c]	1.765[ab]	2.6

[a-d] Means with no common superscript in the same column are significantly different (P<0.05)
[1] Formulated reductions in ileal digestible amino acids and metabolisable energy (ME) in the final feed, compared with the positive control: lysine –0.012%; methionine –0.001%; cysteine –0.003%; threonine –0.013%; tryptophan -0.003%; ME –0.22 MJ/kg (-53 kcal/kg)
[2] 3-phytase, derived from and produced in *A. niger*; new 6-phytase, derived from *E. coli* and produced in a yeast *S. pombe*
[3] mortality corrected.

IV. DISCUSSION

Sequential removal of phosphorus and calcium, with or without reductions in amino acids and energy (PC versus NC1, NC2 and NC3) gave significant reductions in bird performance, as anticipated. With the biggest down specification (NC3), bodyweight gain was reduced by 29% and mortality increased from 3.6 to 15.4%. Both phytase products restored bird performance (bodyweight gain, feed intake and feed:gain) in each NC diet to the level of the PC (i.e. no significant differences between these groups at P<0.05, Table 2). Mortality rate was also substantially reduced in NC3 from 15.4% to 2.6-4.0%. The commercial recommendations for use for each product were therefore confirmed in this trial.

The 6-phytase achieved recovery in NC performance to the level of the PC with less added phytase units than the 3-phytase (approximately 30% lower, based on analysed units). In the NC3 group, for example, addition of the 6-phytase at 448 FTU/kg feed gave performance that was not significantly different (P<0.05) to addition of the 3-phytase at 885

FTU/kg feed. This may be indicative of bio-efficacy differences between the two phytase products in line with those reported by Sands *et al* (2004) in a summary of 20 comparative broiler trials.

The economic potential of phytase addition was well illustrated in this trial. For example, at the highest level of addition of the new 6-phytase (625 FTU/kg feed), the gross feed cost savings were over $5 US/tonne of feed at the time of the trial.

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THE BACTERICIDAL EFFECT OF METHIONINE HYDROXY ANALOG

P-A. GERAERT[1], P. GRAULET[1], Y. MERCIER[1], P.M. BECKER[2] and J.D. van der KLIS[2]

Summary

The impact of the Methionine Hydroxy Analog (HMTB) on digestive microflora of broiler was studied in vitro. HMTB reduced digestive fermentations in broilers when added to a corn-soya based diet when compared to non-supplemented diet or a diet supplemented with DL-Met. HMTB also reduced fermentations from jejunum and ileum contents estimated by methanethiol measurements. Finally, HMTB has also a specific bactericidal effect on *Campylobacter jejuni*, the first cause of dietary toxi-infection in humans.

RESULTS AND DISCUSSION

The bactericidal effect of HMTB (2-Hydroxy-4-Methyl ThioButanoic Acid) on bacteria of the digestive tract was tested by comparison of digestive fermentations from growing broilers (42 days) fed a corn-soybean based-diet supplemented with DL-Methionine (DL-Met) or DL-HMTB (0.09 %) vs unsupplemented animals. Digestive tract contents (ileal and caecal) were sampled and microbial activities were estimated by quantification of gas volume production for 24 hours with an *in vitro* fermentation test adapted to poultry gut digesta from the Hohenheimer Futterwert Testen (Menke and Steingass, 1988). Gas volumes were significantly lower with digestive contents (-19 % in ileum and -10 % in caecum, $P<0.05$) from broilers receiving a diet supplemented with HMTB by comparison with control or DL-Met supplemented broilers. This would suggest that HMTB has a bacteriostatic or antimicrobial effect on gut microflora of growing broilers.

In another study, *in vitro* fermentation of DL-Met (5.0 mM) and DL-HMTB (8.6 mM) by total jejuno-ileal chyme harvested from 21-day growing broilers fed a corn-soya based diet was followed by quantification of the production of some volatile sulphur compounds (H_2S and methanethiol) by GC analysis (Derikx et al., 1990).

Table 1. Concentrations (µmol/bottle) of the gases produced in vitro by the jejuno-ileal chyme of 21-days-old growing broilers after 20 h of incubation with the items

	Control	DL-Met	DL-HMTB
H_2S	8.68 ± 0.10	8.32 ± 0.35	8.19 ± 0.53
Methanethiol (CH_3-SH)	0.20 ± 0.18	4.55 ± 2.36	1.15 ± 0.07

No effect was observed on H_2S production, but methanethiol quantities were notably higher with DL-Met by comparison to DL-HMTB or Control values. This indicates that methanethiol concentrations are a good indicator of fermentations of the DL-Met and DL-HMTB sulfur compounds whereas H_2S is more an index of the total activities of fermentations. Thus, HMTB degradation by microorganisms produced extremely low methanethiol concentrations compared to DL-Met, suggesting a different catabolic pathway.
Finally, the bactericidal effect of HMTB was tested on several bacteria species to assess bacteriostatic and antimicrobial effect using the minimal inhibiting concentrations (MIC) and the minimal bactericidal concentrations (MBC) tests, respectively (Holt and Brown, 1989). The results demonstrated a strong bactericidal effect of DL-HMTB on *Campylobacter jejuni*.

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This specific effect of HMTB will thus be highly beneficial for food safety. The different studies reported clearly illustrate a specific effect of dietary HMTB on digestive microflora in broiler.

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USING THE SLOPE RATIO ASSAY TO DETERMINE AVAILABILTY OF LYSINE IN CANOLA AND COTTONSEED MEALS

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Summary

The study was developed to perfect the slope ratio chick bioassay for the determination of available lysine in three canola meals (CM) and one cottonseed meal (CSM) from Australian processors. The lysine bioavailability estimates for the test proteins meals using either LWG or FCR as the criteria for availability were (LWG, FCR): CSM 0.555, 0.609, CM Boree 1.114, 0.919, CM Riverland 0.905, 0.878, CM Melbourne 0.828, 0.876. CM, Boree exhibited the highest lysine availability compared with the other CM sources. Since Boree CM is an extruded extracted meal, this may suggest processing conditions as the main factor affecting availability. The availability of lysine from CM was 49-100% higher than that of CSM. processing conditions for oil extraction and differences in the type of condensed tannins which are present in both meals are responsible for the difference in lysine availability. When compared the availability with the apparent ileal digestibility from a previous study. For CSM, both methods agreed well (slope ratio assay 0.555 c.f. ileal digestibility method 0.515), thus ileal digestibility of lysine appears to provide a reasonable estimate of lysine availability in CSM from Narrabri. The lysine availability of CM from Boree, Riverland and Melbourne were 1.114, 0.905, and 0.828, respectively which were 51, 25, and 20% higher than the lysine ileal digestibilities obtained on similar CM samples using the ileal digestibility method. These results suggest that the two methods compare poorly for CM. The explanation for this outcome is difficult and further investigation is needed.

RESULTS AND DISCUSSION

A slope ratio chick assay was established to determine the availability of lysine in solvent extracted and expeller extracted canola meals (CM) and a solvent extracted cottonseed meal (CSM). These protein meals previously investigated for digestible amino acids (Perez-Maldonado *et al*., 2002) were used to allow comparison between lysine digestibility versus availability. Ingredients were analysed for proximal, amino acids (AA) and apparent metabolisable energy. Twenty one diets were formulated with the basal diet containing wheat, sorghum, wheat gluten, starch, rice hulls and dextrose being a lysine deficient diet (4.0 g/kg) without added lysine (diet 1). Four diets were formulated with synthetic lysine added to determine the chicks' response to standard lysine, and 16 diets for the three CM and one CSM with each of the protein meals added into the basal diet to obtain the four total dietary lysine levels at expense of rice hulls, starch and dextrose. Vitamins, minerals and other essential AA were added to conform to bird requirements. Day-old male chicks (Cobb) were raised in cages for a week then fasted overnight and individually weighed and assigned to treatments producing similar cage mean body weights. At 19 days of age after a three-hour fast, the weight of chickens and feed residues were recorded to assess the chicks' response in terms of live-weight gain and feed conversion ratio (FCR). The completely randomised layout of 92 cages (six birds/cage) with 21 dietary treatments including 12 replicate cages for diet 1 (blanks) and four replicates of each of the 20 diets.

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Test protein meal	LWG Regression equation	LWG Availability	FCR Regression equation	FCR Availability
Basal	Y= - 112.7 + 511.1 X	----------	Y= 3.96 – 3.26 X	---------
CSM Narrabri	Y= - 21.9 + 283.9 X	0.555 (0.046)	Y= 3.45 – 1.98 X	0.609 (0.042)
CM Boree	Y= - 136 + 569.3 X	1.114 (0.058)	Y= 3.85 – 2.99 X	0.919 (0.047)
CM Riverland	Y= - 93.3 + 462.6 X	0.905 (0.052)	Y= 3.80 – 2.86 X	0.878 (0.046)
CM Melbourne	Y= - 77.5 + 423.1 X	0.828 (0.050)	Y= 3.79 – 2.85 X	0.876 (0.046)

The slope ratio assay was conducted within the linear section of the chicks' LWG response to lysine and that the intersecting lines regression model was statistically valid. But for FCR the validity test failed since intersecting lines were significantly different ($P<0.01$) from the blanks means. The difference in the availability of lysine in the test proteins are related to differences in plant processing conditions for oil extraction (particularly heating conditions), and also to differences in the types of antinutritional factors found in CM and CSM. Since condensed tannins (CT) are found in both protein meals, it is possible that differences in CT (structure, molecular weight) may determine the observed differences in lysine availability

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POULTRY CRC PROGRAM 1 - ENHANCED QUALITY AND PRODUCTIVITY USING NOVEL APPROACHES TO DIGESTIVE PHYSIOLOGY AND METABOLISM

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Australian egg and chicken meat producers, along with their counterparts world-wide, are faced with many threats and challenges. Some of these relate to changing consumer perceptions about quality and safety of food, and attitudes on how that food should be produced. Other challenges are to do with a diminishing range of affordable feed ingredients permitted in poultry diets, increasing competition from other animal industries for those feed ingredients, and competition from imported egg and chicken meat products.

Many of the products that have been commonly used in the poultry industry to improve the health and growth of animals will need to be replaced or modified in the near future to keep pace with consumer and industry demands for animal health and welfare, and food safety. Chicken meat producers are now faced with the challenge of how to manage without the prophylactic use of antibiotics. Furthermore, both egg and chicken meat producers will have to contend with a reduction in the supply, and likely ban, of protein-rich feedstuffs such as meat and bone meal and fishmeal that have been traditionally important ingredients in poultry diets. Formulating diets in the near future will become more challenging because, not only are the genetics of the animals rapidly changing, which impacts upon nutrient requirements, but much of our current understanding of nutrient availability has been derived against a background of antibiotic usage in feed. Critical information will have to be re-evaluated under antibiotic-free conditions and this will require a better understanding of the digestive physiology and metabolism of poultry. Recent advances in technologies available in other areas of biological and medical sciences offer significant opportunities for rapid advancement of our understanding of the biochemistry and physiology of digestion and metabolism in domestic poultry. Failure to meet these challenges will reduce the future competitiveness of the Australian poultry industry in regard to products that satisfy consumer demands for home-cooked, dine-out and take-away food. These issues will require a multi-pronged approach involving integration between traditional scientific disciplines and new strategic research.

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POULTRY CRC PROGRAM 2 - SUSTAINABLE POULTRY HEALTH THROUGH DISCOVERY, DEVELOPMENT AND APPLICATION OF EMERGING BIOTECHNOLOGY

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A major problem faced by Australian poultry industries is reduced productivity due to disease. Over the past several decades the two main mechanisms used to control disease have been the use of vaccines and antimicrobials. Vaccines are intended to offer long-term immunity and protection against a particular pathogen following a small number of immunisations. While bacterial, fungal and protozoan diseases of poultry have been successfully controlled with antimicrobials, continued use over many years has led to problems of emerging resistance of pathogens to many antimicrobials. The World Health Organisation has recommended restrictions in the type of antimicrobials used in food production animals and has urged the development and use of alternative, environmentally friendly methods to control disease. Furthermore, public and government concern over these issues have driven the search for alternative health products.

Vaccination strategies provide the second arm of protection from disease, and recent experience has established that effective live vaccines have considerable potential to reduce reliance on antibiotics within the poultry industry. There are, however, concerns over the ability of current live vaccines to protect against emerging hyper-virulent strains of pathogens. There is a need for alternative vaccines for several key diseases of commercial importance to the Poultry industry. These include the need for defined, rationally attenuated live vaccines and also improved killed or recombinant vaccines. However, killed and recombinant subunit vaccines do not usually offer an adequate level of long term protection and often require the use of adjuvants to enhance their activity. Oil-based adjuvants, however, are expensive and induce adverse site reactions resulting in decreased meat quality and animal discomfort and are therefore not recommended. At this time there is a lack of suitable, cost effective adjuvants for use in both the broiler and egg industries. This Program contains a balanced portfolio of projects that are focused on developing and applying new technologies for improved health products. They include projects aimed at developing or enhancing rationally attenuated live vaccines and developing new treatment methods and improved vaccine adjuvants. The discovery component of this program involves studies that undertake longer-term strategic research whereas the application and commercialisation component has a shorter-term focus and would be seen as closer to commercialisation.

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POULTRY CRC PROGRAM 3A - IMPROVING POULTRY WELFARE

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Animal welfare has become a core value in western society. This common concern has its origins in an array of experiences that arouse strong sentiments but disparate individual targets for attention. It is a contributing perspective, strongly influencing views on codes of best practice and the acceptability of various animal management options. Stakeholders in the animal welfare domain thus include the public, generally as consumers, owners or concerned observers, special interest groups, businesses based on the commercial supply of animals and those developing, implementing or auditing compliance with relevant policy at government or community level. Thus, consumer and public attitudes to animal welfare have the potential to dramatically affect the use of domestic animals in society, influencing for better or for worse, medical research, the care of companion animals and the operations of livestock industries, including the poultry industries. There are examples of this community influence already occurring in the egg industry in relation to welfare. For example, the Primary Industries Ministerial Council (formerly ARMCANZ) agreed in 2001 to a number of actions relating to welfare in the egg industry, including the introduction of an industry wide QA program, labelling to identify the production system and further R&D on issues such as furnished cages. All these actions have since been implemented. Similarly, albeit in another industry, some States in the US are stopping all expansion of pig production and in Victoria expansion of poultry farms has been stopped in some areas because of both their environmental and social impacts.

Sustainable livestock production requires economic viability, ecological sustainability and social acceptability. Social acceptability comprises issues such as animal welfare, food safety, genetically modified organisms and quality of life for the farmer/stockperson. Welfare issues are likely to have their impact to varying degrees on future production practices through consumer buying behaviour and consumer and public influence on regulatory legislation, the standards set for the product by processors and retailers and international trade policy. Science has an important role in providing sound knowledge to more clearly define the criteria of fair and humane treatment of animals and promote well-informed debate on attendant issues. Better animal welfare will only occur through the product of clear thinking about one's moral obligations, coupled with factual information about animal treatment, informed by a scientific understanding of animal welfare. However, there is serious disagreement within science on a conceptual framework of animal welfare and in turn the methodology to assess animal welfare. The Australian Poultry CRC is playing an important role in this area to develop both appropriate methodology and to provide factual information. Other CRC-funded projects include alternative housing, beak trimming, stockperson attitudes and behaviour and barriers to adopting welfare into industry QA programs.

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POULTRY CRC PROGRAM 3B - MINIMISING THE IMPACT OF POULTRY PRODUCTION ON THE ENVIRONMENT

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This is a wide reaching research program to minimise the environmental impacts of poultry production on public amenity and health while also providing industry with the knowledge and tools to manage and minimise odour and dust emissions. The program will also investigate ways of adding value to poultry farm (egg and chicken meat) waste products. Initial work will involve an extensive review of Australian and overseas developments and technologies for measuring, modelling and controlling odour and dust emissions. A review of the public health risks identified with the use of poultry farm waste products will also be conducted. The Australian Poultry Industries at a farm production level have received considerable scrutiny with respect to environmental impacts in the past decade. The environmental impacts of most concern are the community amenity issues of dusts and odours, and public health aspects of pathogens in the food chain from the use of poultry manures as organic fertilisers. The Rural Industries Research and Development Corporation Chicken Meat Program and the Australian Egg Corporation Ltd have funded a number of short term projects in environmental management over the past ten years. The farming managers in each state of the national integrated chicken meat companies in recent years have had to become more involved in the management of environmental issues at a state and local government level, within communities and at individual farm levels. The Australian research capability in poultry environmental issues is not well developed with previous project work done on an ad hoc basis and at this stage no industry champions have been identified. There are research groups who have the expertise from other industries to make a significant contribution to the CRC program, these researchers need to be identified and assessed for the program. The development of this Poultry CRC gives the poultry industries the opportunity to develop an environmental research program over a five to six year time period with a multidisciplinary approach. This project is about developing such a program in consultation with poultry farmers, poultry processing companies, planners, industry regulators and a team of multidisciplinary researchers and "change" consultants. The alternative is a program of short term projects of single discipline approach, with little integration and a hit and miss outcome. There is a need to objectively review the previous environmental research projects carried out in Australia and overseas in the areas of odour and dust measurements, odour and dust reduction technologies, odour and dust dispersion modelling, pathogen transfer and risk assessment, issues around adding value to the waste products, and the use of environmental management systems to drive change in the industry and improve environmental performance.

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THE SOCIAL LIFE OF THE AUSTRALIAN CHICKEN

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Summary

The paper outlines the economic, social and cultural significance of the chicken in Australia in the last 50 years, arguing that chicken meat is far more than a cheap source of protein. It reviews the contributions made on the production and distribution sides of the industry to the extraordinary growth in consumption of this particular food. Within a context of the changing requirements of consumers, the paper notes some major obstacles to this growth being sustained.

I. INTRODUCTION

In the mid 1990s, I set about researching why chicken meat was fast becoming Australia's most consumed meat. The humblest of poultry is close to achieving this milestone (ABARE, 2004; estimates that in 2003-04, Australians consumed 37.7 kgs of beef and 35.7 kgs of poultry meat), and will in the next couple of years overtake a national icon, beef. The research raised an unexpected question: the extent to which consumption follows from the esteem with which a food is viewed. Popular foods are not necessarily viewed that highly. Certainly the consumers I spoke to expressed numerous reservations about chicken, and these have only become more pronounced in the intervening years with media stories of human deaths from avian flu and antibiotic resistance linked to the food supply. The concerns are reflected by the preparedness of growing numbers to pay two to three times for organic chooks, and possibly by the recent upturn in lamb consumption (ABARE, 2004).

This paper provides a sociological snapshot of what I have called the social life of the chicken. That commodities, like people, have social lives is a strange idea. Put simply it means that behind every commodity lie the actions of producers, distributors and consumers, governments, unions, professional bodies and those responsible for R&D. Attached to every commodity are stories, myths, accepted ways of using the commodity and symbols conferring worth upon the commodity: often a price signifying monetary value, and other qualities that have been "added" by a producer, government, advertisers, or by consumers who may harken back to childhood memories. Subconsciously we all add qualities, such as goodness, healthiness, or moral worth.

To study the 'social life of things' entails answering questions asked in biographical research (Kopytoff, 1986): Where does this thing come from and who made it? What has been its career so far? How does the thing's use change, and what happens when it reaches the end of its usefulness?

I intend to address these questions to describe how the Australian chicken is becoming the nation's most consumed, but not necessarily best-loved, meat. And it is this ambiguous relationship between consumers and chicken that raises the spectre of whether chicken in its current form will live forever or whether its social career is set for change. The role of poultry scientists who have been fundamental to the industry's success thus far may indeed be pivotal to the next stage of the Australian chook's trajectory within the social life of Australia and further afield. Australian fondness for this particular protein source is being

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matched in many other countries, and it is common to see Australian chicken in the Pacific islands (Dixon and Jamieson, in press).

In the next sections I touch upon the laboratory to mouth story of the broiler. In so doing, I indicate how chicken meat has been fundamental to the restructuring of the food system more generally. It has pointed the way for so many commodity sectors in relation to vertical integration systems, contract farming, supply chain arrangements, marketing and promotion, and conducting trade disputes. This argument is elaborated in my book *The Changing Chicken* (Dixon, 2002). The paper concludes by naming the many challenges faced by the table chicken in consolidating its position as central to Australia's culinary culture.

II. WHERE DOES THIS THING CALLED THE CHICKEN COME FROM, WHO MAKES IT AND WHAT HAS BEEN ITS CAREER SO FAR?

The global poultry industry is generally considered to be multi-domestic, that is most countries produce their own poultry. Compared to other food commodities, like beef, comparatively little is traded between nations although this is starting to change with some aggressive manoeuvring by Thai, Chinese, US and UK companies (Burch, in press). While Australia has begun to import chicken meat, it is fit only for animal consumption, meaning that Australian consumers are fed by Australian producers. Moreover, the lack of international control of the major producing companies since the mid 1980s sets this commodity sector apart from the beef, pork and dairy industries in this country which have seen marked inflows (and outflows) of international capital.

At the heart of the industry are approximately 85 family owned processors dotted around all states, and they contract with about 740 independent farmers (augmented by their own farm labour on company owned farms) to produce 419 million chickens a year (figures for 2002-2003, ABS, 2004). The processing firms with national coverage are Inghams Enterprises, Bartter Enterprises, which includes Steggles Foods, and Baiada. Each state then has its major brands: Marven in Victoria; Cordina in NSW; Golden Cockerel in Queensland. Each of these enterprises has been operating for decades. The relatively few processors together with the size of the three national companies means that the broiler industry is highly concentrated: possibly more so than in any other OECD country[1]. This level of concentration provides 'the big three' and the medium sized firms with incentives to continue to invest in the industry.

Indeed, the industry is remarkable for its stability: based not only on the durability of the processors but on the commitment of the farmers, who have been growing poultry in many instances for several generations. While the contract farming of chickens is often derided, with epithets used such as "he's only a chicken grower", Australian contract farmers are financially comfortable compared to other agricultural producers. This is due to a combination of factors: government regulation of the fees they are paid to rear the birds, the fact that droughts do not affect the farmers (although droughts affect processors who are responsible for paying the bird feed), and the need to be relatively wealthy in the first place. Poultry farming properties, which are sited close to valuable residential lands often on the peri-urban fringe, have high land values and the shedding and equipment are not cheap.

Processor and farmer stability are not sufficient, however, to explain how such a relatively recent and small industry has been termed Australia's most successful agri-food industry of the second half of the twentieth century. *History of the Australian Chicken Meat*

[1] Industry sources say that together the three companies now control about 80% of the national market

Industry 1950-1990 sets out the genesis of the industry with some marvellous portraits of the founders of the oldest firms (Cain 1990). From this and other sources, it is possible to argue that three significant factors contributed to industry growth in the 1950s and 1960s: 1. the entry of stockfeed companies, which meant that farmers could focus on growing birds rather than using mashes as stockfeed; 2. the release of Australia's first scientifically bred chicken meat strain, allowing farmers to diversify from egg production and to profitably engage in chicken meat production; 3. the introduction of chain processing systems transforming a cottage industry to a mass production system. The large capital investments needed by the processors made them attracted to the vertical integration system of coordination of basic inputs. The major firms of the day, endeavoured to control bird breeding, feed mills, processing and the growing of birds via the contract system. The order imposed by vertical integration obviated the need for a marketing board, unlike the egg industry (Dixon and Burgess, 1998).

Following from vertical integration, two critical events consolidated 'the order' that characterises the industry. The first concerns the introduction in the 1960s and 1970s of state-based regulations to set the fees that processors must pay to their contract growers. These regulations arose due to government intervention in the periodic refusal by farmers to raise birds because of the inadequacy of their fees. In my book I outline how Victorian growers became politically organised in the 1960s, a development that rippled across the country, with the result that governments enacted legislation with the explicit purpose of ensuring that the exploitation of growers could not continue. Government appointed committees continue today to make recommendations on the terms and conditions of the contracts offered to growers, to determine disputes between growers and processors, and to determine standard fees for growing birds, and the conditions under which the standard may vary. One feature of the legislation is the right of growers to collectively bargain their fees with the processor, as distinct from a single grower being forced to negotiate fess with a corporation. This feature has been at the heart of attempts by some processors to have the state legislation declared anti-competitive within the context of National Competition Policy introduced in 1995. Since then, governments around the country have reviewed the operations of their legislation and to my knowledge, all states maintain their regulatory oversight of processor-grower relations.

One only has to hear or read of the plight of broiler growers in the US to appreciate the ruling delivered by the ACCC when asked to investigate the arguments being advanced by processors and the SA government for repealing the so-called Shield of Crown in that state in 1996.

A market in which participants have unequal bargaining power is likely to operate less efficiently than one in which bargaining power is equal. The Commission accepts that the present arrangements increase the countervailing power of the growers … Therefore, the Commission considers [this] constitutes a public benefit (quoted in Dixon and Burgess 1998, p.119).

Curiously, when doing the fieldwork I noted considerable ambivalence on behalf of some processors for the removal of collective bargaining. The major reason was that the fee setting arrangements offered a super-efficient mechanism for 'employing' dozens of farmers. I also suspected that over the years the legislation had fostered a culture of a shared destiny. Words like 'transparency', 'accountability', 'looking after the little guy' (as well as the usual expletives) were used when describing the annual negotiation process. No-one wanted the acrimony of the deregulated situation that pertained in the US. And indeed this sense of a shared destiny has worked to the industry's great advantage in fending off the 1994 AQIS recommendation to allow chicken meat imports into Australia. The highly concentrated

processors and the highly organised farmers have proven that together they have countervailing power when it comes to GATT, the WTO, and the USDA.

The second significant event, was a review of the market operations of processors a decade earlier by another Commonwealth statutory body. This review possibly would not have taken place had the growers not had 10 to 15 years experience of collective organisation. When Inghams and Steggles adopted vertical integration systems, they began to use their position in the supply of day-old chicks to exert downward pressure on the prices they would pay farmers. This capacity also had an impact on the ability of the medium sized firms to expand their market share because the ban on the importation of avian stock at the time meant no alternative supplies were available. In 1985 the then Prices Surveillance Authority (PSA) was asked to investigate the market power resulting from the control over this particular input. It found that between 1968-1980, Inghams, Steggles and Amatil (British Tobacco) had been successively increasing their share of the industry through a lack of competition in the supply of day old chickens, and when in 1980 Steggles was sold to Amatil, a near duopoly existed. Further, the PSA added that it was not satisfied that "the major processors related by ownership were operating at arm's length in marketing dressed chicken" (PSA, 1986 p. 2). The PSA's decision to monitor levels of competition among processors saw Amatil and other foreign corporates sell their holdings, setting the scene for a wholly-owned Australian industry.

Behind the processors and farmers is a group that consumers rarely consider: those engaged in R&D, whether in avian, crop, pharmaceutical or veterinary science. Given my audience, I am going to say little about the role of science. Suffice to acknowledge that in all histories of the industry, science and scientists are accorded a special place. And indeed I have read that researchers know more about the genetics of chickens than any other domestic animal, humans included (Boyd and Watts, 1997 p.193).

The long-time executive director of the Australian Chicken Meat Federation has noted that the industry is "technology's child" (Fairbrother, 1988). This statement recognises the fundamental contribution made by the early hatcherymen and bird breeders, and the more classically scientific endeavours of people in this assembled group.

But it is the observation made by Don Blackett, an industry pioneer, that is noteworthy. Blackett (1970) argued that the early and marked success in bird breeding has contributed to the industry being largely in the hands of Australian families, unlike countries where feed suppliers (often being transnational companies) became the dominant party.

Towards the end of my research, some expressed concern that the industry was relying too greatly on the imports of scientific inputs, especially the avian stock and requisite foods and medicines. When I last spoke to someone from the Bartter company (in 2003) I was told that they wanted to move away from the ASA bird because of higher mortality rates in these flocks and to concentrate on Australian-derived stock. Whether this has happened I do not know, but obviously the public's concern with animal suffering (including deformities due to rapid rates of growth and large size) makes the interaction between avian genetics, physiology and feed an ongoing issue.

In concluding with the production side of the industry, it would be remiss to overlook another science, food technology. Food technologists' knowledge about how to make things taste palatable, to have long shelf lives, not to spoil, and to be creative with offcuts make them indispensable to chicken's success. *The Changing Chicken* contains several vignettes of the technologist's role in the development of chicken products: the chicken roll, a lunch-time staple for 30 years now; the "Pocket Rocket", a more recent chicken-like pie; and KFC's rather mixed success to produce a barbecue chicken that customers would buy instead of their fried food offerings.

Developments in the distribution of both the chicken meat product and of the cultural values surrounding the chicken are as important as those in production. Space permits discussion of only two issues here. The first is the way in which the balance of power has shifted away from producers to distributors, particularly supermarkets. The second is the 'nutritionalisation' of the food supply.

Up until the 1960s, chickens were familiar to Australians because they lived in the backyard. Yet they were rarely eaten. Backyard chooks were reserved for eggs and special occasions. They became readily available and cheap due to early enthusiasm by the two major supermarket chains, Woolworths and Coles, to sell chicken meat. These two national chains entered into preferred supplier arrangements with Inghams and Steggles respectively in the 1980s. This streamlined marketing arrangement was far more significant to the volumes being traded than the entry into Australia of Kentucky Fried Chicken in 1968. Corporate decisions by all four parties to accept low profit margins per bird led to consistently low wholesale and retail prices *vis a vis* other meats. In addition, the introduction of the cool chain system to accommodate large volumes of chilled, not frozen, foods allowed chicken meat processors to diversify their product in ways unimaginable in the 1950s.[1] And owing to consumer desire for novelty, a diverse product range is indispensable.

The reasons why supermarkets presently "call the shots" within the industry are many. First, it is a cashed-up sector, with little regulatory oversight and Australian supermarkets are acknowledged as the most concentrated in the OECD (Joint Select Committee on the Retailing Sector, 1999). This situation is conducive to constant investment in new technology in the search for economies of scale and low labour costs. One of their recent innovations has been to reverse the way in which chicken meat orders are placed. A few years ago processors would ring the chains with a schedule of what products had been processed and what volumes they could expect. The cross-docking system means that supermarkets contact processors daily with a list of what they will buy, leaving processors with the problem of unwanted chicken stock. This ability to control the supply chain due to there being many sellers and two buyers, coupled with increasing in-house capacity to further process and value add to their products, means that supermarkets are less reliant on processors for all their items.

The second reason for their power is equally important. Supermarkets have superior market intelligence and they have long been engaged in cultural activities, shaping "the mouth of the consumer". Over many decades, they have been responsible for popularising the 3 C's: cost, convenience and cleanliness. They championed the value of "fresh" produce, and are now doing this with the concept of "home meal replacement". This involves not simply new product development, but helping consumers to rethink "home meals": what they consist of and where they should come from. In this area, supermarket chains and fast food chicken outlets have a common interest.

Chicken has been readily associated with all of the aforementioned values at one time or another: cheap, convenient, clean/safe, fresh, and perfect when cooked by someone else. These values have been enduring. But there has been another quality central to the success of chicken, over which neither the producers nor retailers have had any control: the advent of lipophobia in the 1970s. With links being made between fatty foods (associated with chops, many cuts of beef and pork), cholesterol and coronary heart disease, chicken was waiting in the wings to replace all of them. What is noteworthy is how little industry advertising has taken place extolling the health virtues of chicken. This was done *for* the industry by others, among them dieticians and the National Heart Foundation via Pick The Tick. And as the red

[1] The cool chain was "invented" by UK retailer Marks and Spencer

meat peak body has belatedly come to realise products need be promoted by others, because surveys show that consumers do not trust industry endorsements (CSIRO, 1994; Dixon *et al*., 2004). It is not surprising then to see scientists being asked to research the nutritional benefits of red meat and to publically comment on the results.

Chicken and margarine were the earliest beneficiaries of the nutrionalisation of the food supply. They continue to benefit from a trend, where consumers value the healthful properties of their food above other properties. But their credentials as "natural" foods are being questioned. All mass produced foods are being viewed as too far from nature, and hence less desirable. The rise of organic foods, farmers markets and the return to backyard food growing are all indicators of the back-to-nature movement by middle-class consumers.

III. HOW DOES A THING'S USE CHANGE?

Chickens have always been associated with progress, with successive heads of state promising their citizens "a chicken in every pot". But it was the chicken's rapid transition from special to everyday, thanks to the efforts outlined above, that is so noteworthy. All of this industry development took place within the context of rapidly growing rates of female labour force participation from the 1970s onwards. Serendipitously, chicken meat offered mothers a food that children liked and that provided healthy convenience. The table chicken was crossing the road in the right place at the right time. It was a pretty big crossing too. I have gone so far as to argue that this single commodity has contributed more than any other food to the post War restructuring of the economy, because it became the working family's staple food (Dixon 2002).

However, the social context is changing. Consumers are both more knowledgeable about the food supply and also more fearful of it. And other commodities have come along offering cheap, healthy convenience. These are not the only challenges. Here are some major life events lying ahead for the chicken that may well change its social trajectory.

1. Ongoing pressures from groups, wedded to a particular view of national competition policy, to abandon the Shield of Crown. Will this mean a more strife-riven, and hence unpredictable, industry? In September 2003, Victorian chicken growers upset by deregulatory measures threatened not to accept the delivery of about 400000 chicks (Skulley, 2003:6). Given the Just-In-Time and perishable nature of the commodity, grower strikes do not bode well for daily deliveries of such a staple
2. Ongoing pressures from world trade bodies, governments, and other commodity groups more dependent on exports, like beef, to permit chicken meat imports that are fit for human consumption. Will the Free Trade Agreement with Thailand, for example, falter if that country's behemoth, the CP group, cannot export chickens to Australia? The ease with which consumers could become alarmed at perceived/alleged lower production standards from overseas sources can easily taint the domestic industry because there is no regulation requiring country of origin identification of chicken pieces[1].
3. The siting of farms and processing plants on land that may have greater value to residential developers. Already, there is pressure on some firms and farms to relocate due to neighbour complaints about traffic and odours. Australians do not have much experience of co-location with food growing and manufacturing. Because of animal welfare concerns at the stage of transporting live birds to processing plants and the

[1] I observed this sort of reasoning in the Cook Islands where there was a clear hierarchy of preferred source: New Zealand chicken being at the top, followed by Australia with US chicken deemed too fatty.

practice of daily supplies of fresh product to retail outlets, it makes sense to site farms close to processing plants, which themselves are within close proximity to the majority of customers. But is this sustainable?

4. While chicken has benefited from its perceived nutritional worthiness, the fact that fried foods have become even more suspect in their links with a host of diseases, and the fact that fried and basted, fatty chicken is common could damage the industry's health credentials. Moreover, most beef and lamb producers can claim a health advantage: their products are antibiotic-free. As Shoebridge noted when evaluating the recent MLA campaign: "Getting red meat on Australian tables is about building an emotional connection – and covering yourself on the health issues" (Shoebridge, 2003, p.58). The Sydney Morning Herald series "Fare or Fowl" in March, 2004, may be too little too late as a response to fears that consumers are expressing about poultry.

5. Consumer fears about the production methods associated with chicken, including their feed and feed additives and cruelty in their raising, coupled with the biggest input being crops that are drought-prone, pose ongoing challenges for the scientists assembled here. What is being required is a bird that: is an efficient grower without suffering deformities; does not need pharmaceuticals; relies on reliable supplies of Australian-made inputs; and exhibits all the behavioural characteristics people assume inhere in chickens in the wild. They want a close-to-nature meat that they do not have to kill. And they want it cheap!

The latest figures available at writing show a decline in chicken meat production and a rise in beef production (ABS 2004). I am not able to comment on the reasons. However, it is conceivable that consumer demand for chicken is peaking due to a) media coverage of two food scares - avian flu and the dangers of antibiotics in the food supply - and one animal welfare issue, namely the intensive rearing of animals[1]; b) the successful campaign run by the Meat & Livestock Corporation to get people to eat more red meat, and the increasing numbers who are eschewing all meat; c) consumer perceptions that chicken is too mundane, and other foods are now as cheap and healthily convenient and possibly more natural. Movements such as the Slow Food Movement contribute to a desire for natural foods, as well as unsettling the assumption that food should be cheap and fast.

IV. CONCLUSION

Economically, the abundance of cheap chicken continues to be associated with a particular view of progress: that of efficiency, novelty and value for money. Socially, chicken has met the demands of working women and others caught up in a 24/7 routine of work and family life. Culturally, the chicken has played its part in influencing our ideas that a culinary culture should be principally about 'healthy convenience', and it too has benefited from perceptions that this attribute is what makes chicken special. But views of progress and what is valued in the food supply are changing. The evolving nature of what consumers value in the food supply and the alternative sources of those values coupled with some rather large issues related to government regulation, international trade, the environment and 'nature' are set to provide the table chicken with R & D challenges for many years to come.

[1] The Free Range Party contested the 2004 ACT Government election using the symbol of a chicken.

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CHANGES IN THE ASIAN LIVESTOCK INDUSTRIES: IMPLICATIONS FOR AUSTRALIAN PRODUCERS AND THE FEED INDUSTRY

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Summary

Over the next 20 years and beyond there will be massive changes in the focus of world production and consumption of livestock products. In terms of feed production volume, the Asian region, which currently lies third behind the Americas and Europe, will ascend to become the sustained leader in world feed production. Three main drivers will influence this change:

- Demographic changes and population growth
- Developments in GDP and personal disposable income
- Changes in eating habits both in the developed and developing nations

As a result of these interrelated factors, led by China, Asia will see double-digit growth in its feeds industry year on year for the foreseeable future.

There are strong indications of increasing self-sufficiency in the production of livestock within the region. This will place pressure on meat product exports from other countries but will also tend to reduce the pressure for 'unfair' exports from within Asia.

Japan, Taiwan, and South Korea have the most developed feed markets where production is close to 'market ceiling'. Moderately developed are China, Thailand and Malaysia, whilst The Philippines, Vietnam, India, Indo China and Indonesia are still extensively immature and it will be many years before they attain the same level of development as our Australian market.

The potential for profits for Western feed companies in the Asian stockfeed arena is limited. Cultural alignment, local knowledge and language play crucial roles in success but also remain barriers to entry. Success for European companies has been, at best, poor. Although limited, there are still a few opportunities for feed industry investment in most Asian countries but it must be understood that the 'rich pickings' have long gone!

Commodities, such as feed grains, protein meals, by-products and fats & oils will see intermittent demand depending on price and local supply.

This paper speculates on the future development and size of the feed industry in Asia and how it may relate to the Australian market.

I. INTRODUCTION

The world's population will continue to grow at a steady, but slightly declining, rate of between 0.5 – 1.3% for the next 20 – 50 years (US Census Bureau, 2000). By far the majority of this growth will occur in the Eastern Hemisphere; Asia, the Pacific Rim and the Indian sub-continent.

Population alone cannot stimulate human food consumption. Africa is a clear example of this. However, a combination of economic growth and sound work ethics, offering elevated disposable income amongst the masses, together with population growth, has led to massive developments in the production and consumption of livestock products in the Far East region in recent years. This will continue to grow at an increasing rate for years to come

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providing interesting commercial opportunities for feed and feed related companies from around the world.

Analysis of world population developments and world wide compound feed production, indicate a declining trend, since 1995, in the amount of feed produced per capita of population. Further, according to the International Feed Industry Federation (IFIF), world compound feed production peaked at 605mio MT in 1997 (see Table 1.) and has since fallen by an average of over 1% per annum. This being a real trend, and in consideration of the foregoing comments regarding compound feed developments in the Asian region, one begins to believe that there is a real shift in the focus of feed production from 'west' to 'east'. Is this a sustainable trend and is there a reaction in the 'west' away from meat and other animal products?

Table 1. World Compound Feed Production

Year	Tonnes(mio)	Per Capita Consumption(kg)
1975	290	71
1980	370	82
1985	440	90
1990	537	101
1997	605	104
1999	586	98
2001	591	96

Source: International Feed Industry Federation (IFIF)

This paper will focus much on China, since it is China that will have the greatest impact on livestock and feed production in the region. China already produces more compound feed than the rest of Asia together. However, due to similar cultural origins, developments in dietary demands and therefore, feed production, in other Asian countries will be along the same lines as China but on different scales and at different times, according to size of population and status of economic development, respectively. The Indian sub-continent will be slightly different due to the high level of vegetarianism amongst the people. Foreign investment policy and practice is also not too dissimilar from country to country within the region.

II. CREATING A DEMAND FOR LIVESTOCK PRODUCTION

The livestock industry in Asia is developing in a way and at a rate that has not been seen before at global level. Only in South America has a similar change been experienced but not on the same scale. Compound feed production growth has been forecast to exceed 10% per annum for the foreseeable future. The growth is primarily consumer-based precipitated by a number of interrelated factors:

- Population growth - In terms of absolute numbers, despite the one-child – one family policy, China still leads the Far East. In addition, countries like Vietnam, Philippines and Indonesia all have high forecast population development rates for the coming years. And, if the countries of the sub-continent (India, Pakistan and Bangladesh) are included, there will be a significant increase in the number of mouths to feed in the next 20 – 25 years in the Eastern Hemisphere.
- Economic Development – The Far East, especially China, Thailand and Vietnam, and India have been the focus of substantial foreign investment over recent years. With investment comes increased GDP, internal wealth and particularly, disposable personal income. This is especially relevant in Asia where spending priorities are

quite different from those in the 'west'. Food and dining out have a high priority amongst ordinary people, as do cosmetics, followed by cars but ownership of a 'flash' family home is well down the priority list. These traits are common to most Asian countries and they behave as strong key indicators to social and economic developments in the region.

- Changes in Eating Habits – Japan, South Korea, Hong Kong and Thailand have come through similar economic development pathways and all are now classified as Newly Industrialised Countries. As more disposable income becomes available there are several common cultural trends. Firstly, the basic diet of poor people generally consists of rice or noodles, a small amount of vegetable and an even smaller amount of meat or fish. This kind of diet is still staple in most emerging countries today and often only varying according to flavour or type and amount of spices used. But the Asians love their food and as people become wealthier so an increased amount of income is spent on food; and food of high quality. At this point, rice ceases to be interesting and diets are often dominated by various types of meats, offal, eggs and fish products.

  Asian people also like to entertain for dinner and to 'eat out'. This also comes with wealth and so does a desire to consume more high quality protein foods. In this respect the demand for chicken and eggs will continue to increase for the foreseeable future.

In consideration of the foregoing, it is not difficult to see why the demand for livestock and therefore, animal and aqua feed has increased in recent years and how the forecasts for the future are very positive.

Table 2. Forecast population development to 2005 in the most populated countries

(mio)	2000	2050	Population Change	Change (%)
China	1,261	1,470	209	17
India	1,014	1,620	606	60
USA	276	404	128	47
Indonesia	225	338	113	50
Brazil	173	207	34	20
Russia	146	118	-28	-19
Pakistan	142	268	126	89
Bangladesh	129	205	76	59
Japan	127	101	-25	-20
Nigeria	123	304	180	146
Mexico	100	153	53	53
Philippines	81	154	73	90
Vietnam	79	119	40	51
Egypt	68	113	45	65

Source: International Feed Industry Federation (IFIF)

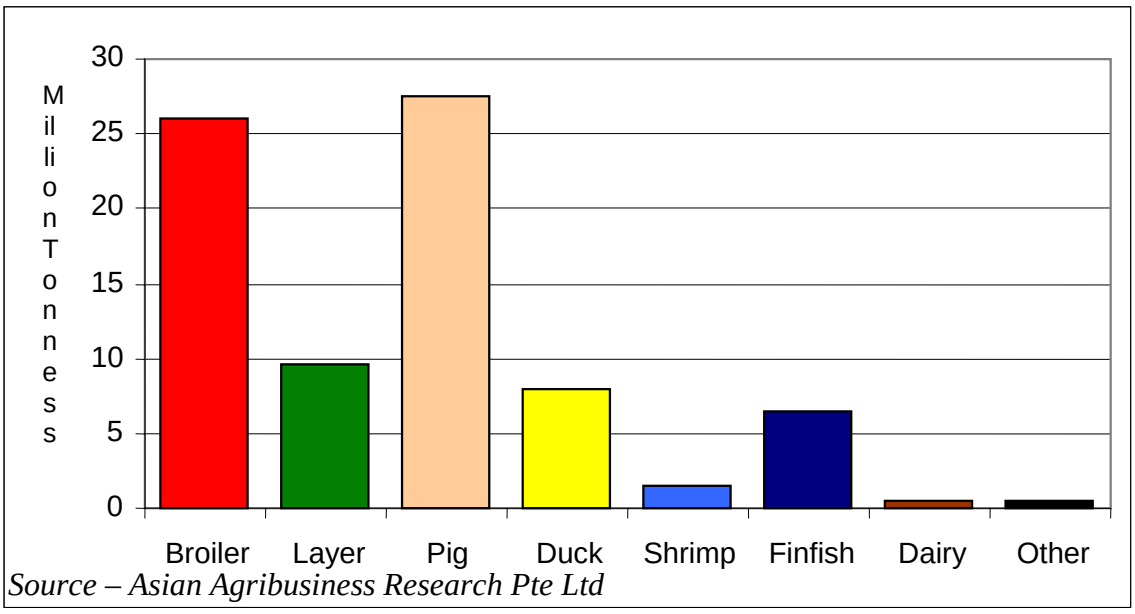
III. DEMAND AND POTENTIAL FOR COMPOUND FEED IN ASIA

Many people have attempted to quantify the demand for animal and aquatic feeds in Asia and there are as many forecasts as reports. The main reasons for the variability are:

- Rapid developments in compound feed production from 'home mix'
- Interpretation of the use of protein concentrates
- Backyard farming is still the norm rather than the exception
- Method of calculation – entrails, offal and adipose tissue, "the fifth quarter" are almost totally consumed as a general practice. Little is wasted.
- Reliability of census information in countries with industrial and geographical diversity.
- Non-conventional farming and feeding – domestic by-products and swill feeding is common practice in most Asian countries
- Variable statistics

Looking at the pig industry in China as an example, the China Statistics Yearbook, 2003 states that there are just over 450mio pigs in China at any one time and that around 600mio are slaughtered annually. The Yearbook also states that the Chinese consumed 33.8kg per capita of pig meat in 2002; a surprising figure when compared with that of western developed countries. These data are supported by Pig International, July 2003 where it was reported that swine meat consumption in China was 44.5mio MT. Assuming that each pig weighs around 75kg at slaughter then, if these pigs consumed compound feed and grew at a feed conversion rate of, say 4:1, pigs in China alone would consume more than 160mio MT of feed each year. In contrast, International Feed Industry Federation (IFIF; 2002) states that total compound feed production in China is still less than 60mio MT! Asian Agribusiness Research Pte Ltd, have indicated that in 2002, 82mio MT of feed were produced in Chinese feed mills of which 28mio MT were pig feed (Figure 1).

If these figures are even broadly true, even in consideration of concentrate feeding, then the amount of 'home mix' and by-product feeding may be grossly underestimated representing a huge potential for the future of compound feeds.



Source – Asian Agribusiness Research Pte Ltd

Figure 1. Compound Feed Production in China by Species – 2002

Tables 3a and 3b give some indication of why the actual and theoretical figures are so different. According to the Chinese Statistics Year Book, 2003 there are 105.4mio registered swine farms in China. Some 95% of these slaughter less than 9 pigs per year. In other words, of the 610mio pigs slaughtered each year, 338mio (55%) come from farms sending fewer than 9 pigs per year for slaughter.

Table 3a. Swine Farm Size Analysis in China

Size of Farm (No. Slaughtered / annum)	Number of Farms	Yearly Slaughtered ('000)
1—9	99,894,369	338,588
10—49	4,438,302	105,344
50—99	790,307	53,637
100—499	212,909	51,651
500—2,999	27,495	29,363
3,000—9,999	3,242	16,432
10,000—49,999	862	12,839
>50,000	28	2,058
Total	105,367,514	609,914

Source: China Statistics Yearbook, 2003

Table 3b. Broiler Farm Size Analysis in China

Size of Farm (No. Slaughtered / annum)	Number of Farms	Yearly Slaughtered ('000)
1—99	51,203,222	864,223
100-1,999	1,284,361	776,501
2,000-9,999	327,836	1,659,800
10,000-49,999	62,956	1,176,358
50,000-99,999	4,232	270,403
100,000 – 499,999	1,317	267,642
500,000 – 999,999	110	77,799
>1,000,000	81	153,003
Total	52,884,115	5,245,728

Source: China Statistics Yearbook, 2003

More significant to this forum, China is a big producer of poultry products. Annually, it slaughters more than 5bio broilers, 3bio assorted water fowl and produces over 20mio MT of eggs (>40% of the world's production)! Figure 1 gives the corresponding compound feed production. By arithmetic progression, one can see a huge gap in theoretical feed consumption from the livestock produced in Tables 3a, 3b and 3c. This gap represents the amount of feed mixed on farm or 'backyard' production.

In a similar, but not quite so spectacular way as pigs, 15% of broilers slaughtered are produced on farms raising less than 99 birds per year and 85% come from farms slaughtering less than 50,000 per year. There is a similar pattern for layers see Table 3c.

Undoubtedly, the industry will change. The smaller farms will get bigger and there will be fewer and fewer farm units. This is a natural progression of economic and social developments and one experienced already in most advanced economies. The burning question is when and what impact can the Asian livestock business have on Australia in a positive and a negative way?

Feed production data for the rest of Asia is equally open to conjecture for similar reasons to those for China but clearly the overall trend is positive growth. Table 4 offers estimates of compound feed production in other Asian countries and speculates about the market 'ceiling' potential. Market 'ceiling' refers to the amount of feed potentially produced when a market is fully developed. Japan, USA and Korea would be examples of this. Japan, and Korea produce 23.5mio MT and 15.6mio MT of compound feed each year, respectively, and since their economies are almost fully developed and per capita GDP's relative to the rest of the developed world are comparable, it is unlikely that there will be much increase in feed

volume in the foreseeable future and therefore they can be said to have reached market "ceiling".

Table 3c. Layer Farm Size Analysis in China

Number of Layers	Number of Farms	Number of Layers (000's)	Egg Production (000' MT)
1-49	56,805,600	714,820	5,343
50-499	2,106,259	403,815	3,885
500-1999	547,775	601,216	5,895
2,000-9,999	139,411	515,997	5,060
10,000-49,999	6,582	108,872	1,336
50,000-99,999	250	15,827	193
100,000-499,999	89	13,366	165
>500,000	2	1,920	29
Total	59,605,968	2,375,833	21,906

Source: China Statistics Yearbook, 2003

Table 4. Current Annual Compound and Total Feed Production – 2003 and Market Potential for Developing Asian Countries

Country	Population (mio)	Main Livestock Produced	Compound and Concentrate (mio MT / y)	Est. Total Feed Consumption (mio MT)	Feed produced percapita (kg)	Market 'Ceiling' Production (mio MT)
Developing						
China	1,261.0	Po / P	82.0	190?	65	275
Thailand	66.0	Po / P	10.2	12.3	154	19
Indonesia	225.0	Po	7.0	15.0	32	49
Philippines	81.6	P	5.6	11.0	69	18
Vietnam	81.7	P	3.8	12?	47	18
Indo China	70.6	P	0.4	10?	75	15
Malaysia	24.2	Po	4.8	9.0	198	6
Sub Total Developing	**1,810.1**		**113.8**			**400**
Developed						
Japan	127.0	All	23.5	N/A	185	25
Korea	46.9	All	15.6	18.5	371	16
Taiwan	23.6	Po / P	7.5	N/A	317	7.5
Sub Total Developed	**197.5**		**46.6**			**48.5**
USA	276		142		514	145

From Asian Agribusiness Research Pte Ltd ;Key: Po – Poultry, P – Pig

Forecasting the development of the food and feed industries in Asia is an important subject. Delgardo *et al*. (1999) and Delgardo (2003) have made similar observations and drawn similar conclusions.

It therefore, can be clearly concluded that despite the already sizeable compound feed market in Asia there is much more to come as the developing nation's economies grow.

IV. IMPLICATIONS FOR AUSTRALIAN PRODUCERS

a) Threats

Logic would indicate that, as internal demand for livestock products increases amongst Asian countries in line with socio-economic growth, threats of cheap exports would decline. This may be the case in the long term but at present logic is not followed. China, Korea and Thailand are big exporters of broilers; Thailand, directly into Europe and China, competing with potential exports from Europe into Japan, Hong Kong and Singapore.

Table 5 clearly shows that 'face value' logic does not always prevail. Regardless of the source of data it would appear that China has a balance of production and consumption of broilers but it also imports and exports between half a million and a million tonnes per year (5 - 8% of production), depending on the source of information.

Table 5. Poultry Meat Supply and Consumption in China in 2002 – Data from two different Sources

000's MT	1999 (WP, 2004)	1999 (CSYB, 2002)	Difference **(%)**	2002 (WP, 2004)	2002 (CSYB, 2002)	Difference **(%)**
Production	8,550	11,150	23%	9,558	12,545	24%
Imports	591	946	38%	435	950	54%
Exports	375	404	7%	438	530	17%
Consumption	8,766	11,692	25%	9,555	12,965	26%

Source: World Poultry, 2004 (WP,2004) & China Statistics Yearbook, 2002 (CSYB,2002)

In relation to exports of poultry meat from Asia to Australia on a large scale, there is currently little volume movement and whist Australian quarantine restrictions remain as they are today this situation is not likely to change in the foreseeable future. For many reasons, especially those of a phyto-sanitary nature, it is also unlikely that eggs and pigs or pig meat will arrive on Australian shores from Asia, although from an economic perspective it may appear to be feasible for processed products due to low cost production and further processing. So, how is this possible?

First and foremost, it should be noted that most Asian countries are net importers of feed grains. These are bought, in general, unsubsidised, at world market prices and they are used in association with locally grown/produced raw materials. The quality of feeds manufactured in modern feed mills is generally good with a high technical input often of western influence. Many Asian students attend our universities and learn from our hard earned experience and that also of Europe.

The key drivers towards low cost exports are low productions costs (especially labour), efficient growth performance (including favourable climatic conditions), low cost processing and finally, but nonetheless significant, lower profit aspirations compared to western counterparts.

State of the art growing units, high quality feeds and 'high-tech' processing plants maintain food chain integrity and as much as worldwide competitors would like to 'fault' Asian imports, hygiene has few weaknesses.

For the future, competition is likely to become weaker rather than stronger. It is unlikely that newly developing economies such as Philippines, Indonesia and Vietnam will focus on exports to Australia. There may still be some movement within the region but local requirements will begin to take priority as demand increases, not forgetting that with economic development come salary increases and higher profits aspirations thereby reducing the incentive to export. But, as mentioned in the foregoing, logic does not always prevail in Asia! Clearly, guarantees on bio-security standards and food chain responsibility must be an integral part of any livestock product exports.

b) Opportunities for compound feed production

Cross-cultural investments in basic industries in Asia are fraught with danger. Some have succeeded quite well but others have failed miserably. Intra-Asian investments have clearly been more successful but even these are not without issues. The fundamental problem is that livestock farming and its associated industries are basic to the very culture of Asian people. The presence of a 'foreigner' can be seen to be a threat, even alien to this culture

It is therefore, concluded that the potential for making profits by western and Australian feed companies in the Asian stock feed arena is limited. However, where the correct approach and flexibility to adaptation are implemented, success is quite possible. Cultural understanding, local knowledge and communication and language proficiency all

play crucial roles in success and if not correctly addressed they remain firm barriers to entry. Many companies from many countries have tried and almost as many have failed. The list of failure is long and alarming!

We now have sufficient experience to be able to classify the type of company that has a high chance of success. Clearly, of those that have been successful, by far the majority come from similar cultural origins ie. Asian companies or have longstanding local experience. Although languages are widely different in most Asian countries, Asians are able to learn other Asian languages faster than their western counterparts.

Another key to success is boldness. Many unsuccessful companies have "had a try" without real financial and personnel commitment. A crucial error made by many 'new entrants' is to try to run an Asian operation from an 'off-shore' headquarters. Certainly, policy can be set at head office but key decision making must be a matter for local management.

The most significant of the successful companies is Thai-based feed giant, Charoen Pokaphand (CP) who, during a period of little over 10 years, established over 100 foreign invested feed mill ventures with feed manufacturing capacity in excess of 12mio MT producing over 8mio MT in 2001, in China, Vietnam, and Indonesia.

Success for western companies over a similar period has been, at best, poor but there are success stories. Gold Coin, a division of FE Zuellig, Switzerland is present in Thailand, China, Malaysia, Indonesia, Vietnam and is run from its Asian base in Singapore. Gold Coin has had market presence for many years by having the vision to enter when risks were high. Through its personnel policy and local management concept, Gold Coin is accepted as a local company by the Asian feed industries. Another example of some success is Associated British Nutrition (ABN) from the UK who invested in China in the early 90's.

Another successful company has been Cargill from USA. There have been no notable investments from Australian feed-related companies. This is somewhat surprising in consideration of the geographical proximity to Asia and the developing economic associations with several Asian countries including Thailand and China.

Investment in the compound feed industries in the mature markets such as Japan, Korea, Taiwan and Thailand would not be easy. Competition is high and cultural barriers have already left a history of carnage.

In the intermediately developed markets such as China, Malaysia and Vietnam, opportunities do still exist but it must be understood that since these markets were 'opened up' in the 80's and early 90's, most of the rich opportunities have already been taken.

The Philippines, Myanmar, Cambodia and Indonesia represent interesting options. Their feed industries are just starting to move. Politics, albeit somewhat turbulent, offer some hope for stability and they are on a similar course of economic development as their Asian predecessors.

Like any investment, choosing the correct vehicle for entry is absolutely critical. Chemistry between partners must fit to achieve enterprise longevity. Partner research and selection is most important. In many cases, financial rewards from operations alone would not be sufficient to satisfy most western shareholders. Synergies need to be sought that will also benefit the off-shore holding company. Technology transfer is a crucial 'attractant' to Asian partners as is size and a strong reputation in the home market. And, remember in Asia, although money counts for a lot it does not mean everything and as one learned Chinese colleague once told me as I was pressing for a joint venture decision after 18 months negotiations, "We have been waiting 5000 years for this, we can wait a little longer"!

The use of cross-cultural mediators who really know the markets, the industry, the culture, personalities and the language is a valuable way to become familiar with potential

partners at a distance that is close enough to make investment decisions. Investments, in general, can be classified into Wholly Owned Foreign Enterprises (WOFE) or Joint Invested Ventures. Each has its own merits. Strong but fair partners, on both sides, are necessary for success and longevity in joint ventures whereas, a sound local knowledge is paramount for WOFE's where success can be at the hands of fickle governments.

c) Broiler production

Compared with other countries in the world, Australia has, over recent years, become one of the lowest cost producers of feed grains. Other feed ingredients are also cost competitive. Accordingly, Australia has a potential to produce feed and, due to its production efficiency, broiler meat as cheaply as any country in the world. What tends to make Australia fall behind international competition is the cost of its labour intensive processing. It is not surprising therefore, that more than one international group has eyed Australia as source of massive broiler production for export to Asia and in turn to import processed product into Australia for local needs. This somewhat controversial initiative holds some merit and may be worthy of deeper consideration.

d) Other feed-related production

Opportunities, however, do still exist for foreign co-operations in the production and sale of specialty feeds, feed additives and advanced technology products, especially where some know-how can be transferred and trade names are well known.

The companion animal market is still quite small in Asia compared with Europe but the rate of growth is alarmingly high and in China the 'one child one family' policy is having a major pull-through effect on growth. Asians in general, are fond of pets and pet food manufacture offers interesting Joint Venture opportunities with good returns.

Asia is one of the leading regions in the world for aquaculture and aquatic feed today is a significant proportion of total feed production (see Figure 1). The main target species are shrimp and fresh water and marine warm-water fish. Technology in this sector of the industry has largely been developed locally and therefore foreign partners are not seen as able to add value to what are highly profitable businesses in a highly profitable industry sector. Several European companies have ventured into this sector. Few have succeeded. Most have been badly hurt!

e) The potential for commodity supply

From the foregoing, it can be seen that meeting the raw materials demand for feed production in the future will require vast quantities of feed ingredients and grains. Most Asian countries have a high population density with preciously small amounts of land available for crop production apart from cash crops and rice. Others are not fertile or have major climatic restrictions to support high yields of feed grains, pasture and crops. It is therefore, likely that for a long time into the future, the Asian feed industry will be a net importer of vast quantities of feed ingredients and grain.

It should also be noted that the Asian eating habit leaves little by-product from slaughter for feeding to animals. Most offal is consumed in one form or another and indeed often brings premium prices from consumers! Spent hens are consumed almost as a delicacy. All kind and sizes of fish are eaten in various forms. There is no fat left for rendering. Consequently, locally-produced meat meals, fish meals and tallows are rarely available in Asia. This is also notable for the pet food industry as a restriction of raw material supply.

Australia is already a major supplier of certain grain and protein raw materials to Asia and the potential for growth and expansion in the future is impressive.

V. CONCLUSIONS

In conclusion, Asia is rapidly becoming the leading feed producing region in the world. Expansion is fuelled by economic and population growth together with changing eating habits. Participation in this business in one form or another by non-Asian feed companies carries risks and a high potential for failure. Cost of entry is high and controversy will never be far away. With the correct approach, success is possible and returns can be high.

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AN UPDATE ON AVIAN INFLUENZA

A. TURNER[1]

Summary

Highly pathogenic avian influenza (HPAI) is a disease of domestic poultry that has reservoir hosts in water fowl and waders. HPAI occurred sporadically before 1995 with 12 outbreaks over 40 years that were eradicated with the destruction and/or culling of about 23 million poultry. Since 1994 there have been 25 outbreaks with more than 200 million poultry destroyed and/or culled to control or eradication infection and human infections are being regularly described. HPAI infection is now established as an endemic infection in the poultry industry of a number of countries of Central America and Asia. This is a dramatic change from what had occurred in the years after 1926 when Newcastle disease was described in 4 sites around the globe and when HPAI became a less common disease. Why AI has again reverted to being a widespread disease for it to become recognised as an emerging zoonotic infection is unclear.

I. INTRODUCTION

Over the last 4 years there have been dramatic changes in the behaviour of avian influenza (AI) with the epidemic in eastern Asia from late 2003 to the present causing more deaths and birds slaughtered to control infection since fowl plague was recognized as being caused by an influenza virus in 1955. Recent outbreaks have not only been in eastern Asia, with major outbreaks of disease in Canada in 2004, Netherlands in 2003 and Italy in 2000 and 2001.

While members of the bird Orders Anseriformes (water fowl including ducks, geese, swans) and Charadriiformes (waders including terns, gulls, sandpipers) are recognized as the birds that are the reservoirs for the 16 haemagglutinin (H) and 9 neuraminidase (N) antigens of AI virus, these birds are also reservoirs for all the other genes that go to make up influenza viruses. Influenza viruses are single stranded RNA viruses. The genome undergoes mutation at a high rate and the 9 separate segments of RNA in the genome undergo reassortment when 2 diffferent viruses infect the one cell. Each H may be combined with any N giving 144 possible combinations. AI viruses in the reservoir hosts have low pathogenicity as assessed from infection in the chicken (Murphy *et al*., 1999).

With respect to disease, chickens and turkeys are more likely to develop clinical signs following infection and the disease will be most serious if the infection is with AI viruses of the H5 or H7 sub-type. Although infection with the H5 or H7 subtypes may start in chickens or turkeys as an unapparent infection, reflecting the situation in the reservoir host, passage through chickens or turkeys is likely to result in the development of a highly pathogenic avian influenza (HPAI). The change from low pathogenicity to high pathogenicity is due to attaining multiple basic amino acids at the cleavage site of the H protein by mutations in the RNA at the cleavage site. This arrangement of the basic amino acids at the cleavage site enables the virus to infect and be released from cells of the internal organs whereas with out this configuration the AI virus can only be released to infect other cells on the external surfaces of the respiratory and alimentary tracts (Swayne and Suarez, 2000). The mutation to pathogenicity has been achieved in the laboratory (Ito *et al.,* 2001).

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HPAI, formerly fowl plague, is a very serious disease of poultry that can spread rapidly in poultry populations and cause very severe losses in domestic chicken and turkey flocks. It was recognised as causing disease in the late 1800s and up until 1926 when Newcastle disease was recorded in 4 sites around the world. With the exception of the 1982-84 HPAI outbreak in Pennsylvania, USA, all outbreaks were recorded in relatively small numbers of chickens until the outbreaks of 1995 in Pakistan and Mexico. Early recognition and action to control and eradicate outbreaks of AI infection have been successful. The virus causing the USA outbreak was allowed to mutate into a more pathogenic virus while discussions were held about whether infection controls should be initiated (Swayne and Suarez, 2000). The Mexico outbreak commenced with a H5 subtype virus of low pathogenicity that developed high pathogenicity; vaccination using a killed vaccine was used to rapidly bring the highly pathogenic virus under control and eradication of HPAI was achieved. However, the low pathogenic virus has continued to infect poultry in Mexico and has spread to other countries in Central America (Villarreal-Chaavez and Rivera-Cruz, 2003; Lee *et al.*, 2004). The situation in Pakistan with a H7 subtype virus has been similar with acute infection breaking out again in 2004. The outbreaks of HPAI in Australia in 1976, 1985, 1992, 1995 and 1997 were all caused by H7 subtype viruses (Selleck *et al.*, 2003).

II. AVIAN INFLUENZA RESERVOIRS.

As outlined above, water fowl and waders are the main reservoirs of AI viruses and the northern American, European and Asian continents appear to be where migratory water birds exchange AI viruses and reassortment occurs for transmission back down the globe along pathways of migratory birds. It is also apparent that there are Eurasian and American families of AI viruses; the Australian H7 subtype viruses are all closely related genetically and are a subfamily of the Eurasian viruses (Suarez, 2000). This would accord with most Australian migratory wader birds migrating to the northern hemisphere through Asia and these birds being the source of new strains of AI viruses for the Australian environment. Australian waterfowl, on the other hand, do not migrate far outside of Australia, Papua-New Guinea and New Zealand so their access to Eurasian AI viruses would have to be from second hand contact with other infected species.

In the waterfowl and wader species, reservoir AI viruses usually cause unapparent infections of particularly the gastrointestinal tract. The infections produce large quantities of virus that in a water environment enables rapid spread of infection when different species share common feeding grounds. This explains why untreated water supplies have been suspected to be a source of virus for outbreaks for infection in domestic poultry.

While it is suspected that AI viruses are disseminated from the northern arctic regions, there is also the possibility that birds returning to these regions may bring with them AI viruses that have been collected on their migration northwards. This raises the possibility that genes from the eastern Asian epidemic will be reassorted with other subtypes and disseminated across a wider region of the globe. Sampling in the Arctic in summer 2004 has not revealed viruses derived from Asia (Swayne, 2004).

III. EVENTS IN EASTERN ASIA.

In 1996, AI viruses of H5N1 subtype were detected in geese in southern China and this virus has been tracked through various avian species. Also circulating in poultry in southern China were H9N2 and H6N1 viruses with genetic mixing of these viruses in domestic waterfowl. In 1997, a virus infected people in Hong Kong that had contact with

poultry in the live bird markets; some 6 of 18 people shown to have contracted AI infection died. Poultry in the markets were slaughtered and the poultry in Hong Kong's small poultry industry were also slaughtered although only one farm was known to be infected. This scenario of infection of live bird markets was repeated in 2002 (Sims *et al.,* 2002) with a different strain of the H5N1 virus. The genetic mixing of the three viruses in domestic waterfowl has lead to the development of H5N1 viruses of greater virulence to ducks and humans (Li *et al.,* 2004). While there were numerous reassorted H5N1 viruses with 4 dominant strains recovered between 1998 and 2002, one strain has become dominant and perhaps there will be less variation seen if no new AI viruses are added to the pool in southern China poultry (Li *et al.,* 2004).

In 2001, H5N1 virus was isolated from duck meat exported from China to South Korea and Taiwan and this virus was shown to be lethal for chickens but in ducks grew to quite high titre in the brains and many other internal organs of apparently healthy ducks (Tumpey *et al.,* 2003). This was an unusual finding as the ability of AI virus to grow in the internal organs was a sign of pathogenicity that usually led to death of the host. Then in 2002, it was reported that H5N1 virus had acquired the capacity to kill wild ducks and other wild waterfowl much as it has killed chickens (Sturm-Ramirez *et al.,* 2004). The clinical disease seen in ducks had more neurological signs in ducks and the clinical course was usually longer.

One thing not made clear, from most writing about the epidemic in eastern Asia outside China, is that all viruses isolated have been highly pathogenic for chickens and that this has been the case for some number of years in Hong Kong. In most outbreaks, both highly and low pathogenic viruses are isolated from time to time but no low pathogenicity viruses have been isolated in the current epidemic.

In the environment of southern China, H5N1 viruses acquired the capacity to infect and kill chickens, ducks and geese and the capacity to infect and kill humans in Vietnam and Thailand when infection spread to those countries in 2003 (Li *et al.,* 2004). The mortality rate in people in Thailand and Vietnam who have developed clinical disease has risen beyond that seen in Hong Kong in 1997. In Hong Kong 6 of 18 clinically affected people died i.e. 33% mortality while in Thailand and Vietnam 32 of 44 cases died i.e. 73% mortality. It has been recorded in Thailand that children have comprised many of the deaths and there has been an association with the keeping of fighting cocks by the family in many cases. Vietnam is also a country with a fighting cock culture. Seromonitoring has not been performed to assess the levels of subclinical infection.

IV. FEARS OF A HUMAN INFLUENZA PANDEMIC.

Influenza viruses are generally adapted to a host species with transmission occurring most frequently between individuals of the same species and transmission most readily occurs between more closely related species (eg chickens and turkeys or waterfowl and turkeys). Interspecies transmission between birds and mammals has occurred only rarely; most of such transfers have produced only single or isolated cases of infection and were eliminated before adaptation or re-assortment occurred and new host-adapted influenza viruses initiated (Swayne and Suarez, 2000). Epidemics of avian influenza occur when a HPAI virus with either a H5 or H7 haemagglutinin is introduced to a naïve poultry population. Severe pandemics in humans occur when major antigenic 'shift' has occurred such as when the haemagglutinin subtype is changed and severe disease epidemics occur when there is 'drift' with significant antigenic change in the haemagglutinin antigen to bypass the acquired immunity in the human (Murphy *et al.,* 1999).

In influenza terminology, the 'shift' in a human virus, as occurred in 1918–19, has been explained by the haemagglutinin of an avian virus developing capacity to grow in pigs through co-infection of pig and avian viruses in cells of the respiratory tract of the pig allowing re-assortment of the genes of the two viruses producing a virus containing the avian haemagglutinin that was capable of spreading in pigs. The new pig virus was postulated to again undergo re-assortment in humans through co-infection of the pig and human viruses in cells of the human respiratory tract, which allowed re-assortment to produce a virus with the pig (formerly avian) haemaggluttinin that was capable of spreading in humans (Murphy *et al.,* 1999). However, this thesis has recently been thrown into doubt, with the demonstration that the 1919 virus had an H gene that had an avian sequence (Stevens *et al.,* 2004).

The occurrence of the human deaths in Hong Kong in 1997 from AI virus infection raised the spectre that H5N1 subtype virus might lead to a human influenza pandemic through either direct or secondary transmission of a new H gene to a human influenza virus. So far there has been no evidence that H5N1 subtype AI virus has been able to establish sustaining transmission in humans (WHO, 2004a). There is limited evidence in eastern Asia that infection has spread from one family member to another and there is more evidence of this limited transmission from studies in the Netherlands following the outbreak in 2003 with H7N7 subtype virus. A small number of relatives in contact with infected workers during the Netherlands H7N7 outbreak became infected (3 of 83 tested; Koopmans *et al.*, 2004). A retrospective serological study in the Netherlands has suggested that an estimated 1000 people, possibly more, became infected with the H7N7 outbreak virus. The serological study showed that about 50% of people who handled infected poultry became infected and antibody was detected in 59% of the members of infected poultry workers' families indicating much more human-to-human transmission than supposed in the initial study (ProMED 2004a). The antiviral drug oseltamivir protected people against infection but mouth and nose masks did not provide protection. Similar human to human spread of H5N1 AI virus was recorded in Hong Kong (Katz *et al.,* 1999). In southern China a small number of mild influenza cases have been associated with infection from H9N2 virus (Peiris *et al.*, 1999; Lin *et al.*, 2000). The low pathogenic H9N2 virus is widespread in the poultry and pig populations of southern China but there has been still no record of major transmission of infection to humans and the start of a pandemic (Peiris *et al.,* 2001). No serious human infections have been recorded in China, Japan, Indonesia, Laos, Cambodia, South Korea and Malaysia.

The World Health Organisation (WHO) has spent a great deal of effort in playing up the possibility that a human pandemic will occur and took a very hard line for slaughter control of HPAI in Asia for the first 8 months of 2004 (WHO, 2004b). Since September, WHO has softened its stance in recognition that the epidemic in eastern Asia is not going to be brought under control using just slaughter or stamping out control alone (WHO, 2004a). In early 2004, WHO called for the elimination of AI reservoirs; this was an unrealistic call and demonstrated a lack of knowledge of the epidemiology of AI infections. The Food and Agriculture Organisation of the United Nations and the Offices des International Epizooties (OIE) have been instrumental in getting this change in stance.

V. INFECTION OF OTHER SPECIES.

Infection of pigs with H7N7 subtype was evidenced by detection of antibody in the sera of pigs during the Netherlands outbreak in 2003 (ProMED 2003); herds with positive serology rates >2.6% were slaughtered. Reports of infection in pigs with H5N1 subtype in China have been confirmed (ProMED 2004b). A small number of virus isolations have been

made but no sustaining transmission of infection has been recorded. There have been much more widespread isolations of H9N2 viruses from pigs in southern China (Peiris *et al.*, 2001).

Infection with H5N1 virus has been recorded in cats that were fed infected chicken meat and snow leopards and tigers in Thai zoos fed infected chicken meat have also contracted infection and died (ProMED, 2004c). The disease has been repeated in experiments following inoculation of domestic cats with cats in close contact also contracting infection (Kuiken *et al.*, 2004) and cats were shown to support the growth of AI viruses by Hinshaw *et al.* (1981).

Pigeons in text books are recorded as being resistant to AI virus infection and were shown resistant to 1997 H5N1 virus (Perkins and Swayne, 2002) but there is one newspaper report of the sudden deaths in significant numbers of pigeons in two nearby towns in Thailand (ProMED, 2004d).

V1. EPIDEMIOLOGY OF AI IN EASTERN ASIA.

Much has been made about wild birds being the cause of AI being disseminated throughout eastern Asia. H5N1 virus infection has been detected in a wide range of wild birds and there have been acute deaths due to AI in a number of bird species. Wild birds can be expected to carry AI viruses for a short or long time as reservoir hosts and they have always provided a convenient excuse for poor biosecurity and lack of control procedures (Swayne and Suarez, 2000). In Hong Kong, South Korea, Japan and Malaysia, outbreaks of HPAI have been controlled successfully by slaughtering out as has been practised in Australia. This has been possible because the outbreak was recognised early and control measures applied immediately. A number of countries were in denial that they had HPAI infection.

The nature of the poultry industry in most Asian countries with poultry species being mixed in live bird markets, significant duck populations and low or no biosecurity measures in place in most of the industry has meant that control by slaughter out was an impractical exercise given slaughtering was based on clinical signs and not on infection. Domestic ducks have only in October 2004 been recognised by WHO (WHO, 2004c) as being a major reservoir for H5N1 virus; ducks were long before recognised by veterinarians as silent spreaders of HPAI infection to chickens in Asia and ducks have the capacity to spread infection through meat as noted previously. Ducks now shed more virus for a longer period than previously recognised and the 2004 H5N1 viruses remain infective for a longer period in the environment than do viruses isolated previously (WHO, 2004b).

Given that H5N1 virus infection has been followed as it evolved in domestic geese and ducks in China, it is surprising that it has taken so long to recognise the significant role that domestic ducks will play in disseminating infection. In Thailand, travelling ducks have been recognised as a likely source of new outbreaks from July 2004. The spread of infection can be well explained by the movements of domestic poultry and persons in a low biosecurity environment and wild birds probably play a minor role in spread that would be diminished if there was good biosecurity and the wild birds were protected against infection from domestic poultry. Control programs cannot be expected to be successful unless all essential control measures are implemented (Swayne and Suarez, 2000).

VII. VACCINATION AGAINST AI.

Vaccination has been a very contentious issue in the control of AI. It is accepted that live virus vaccines should not be used to control infection; this has left inactivated vaccines

as the main vaccines. The experience in humans and mammals generally is that vaccination produces antigenic drift in the circulating viruses so that vaccines have to be engineered from recent isolates if the vaccines are to remain effective in providing protection against infection and clinical signs (Murphy *et al.*, 1999). Human medicine has become obsessed that influenza vaccines do not produce "sterilising immunity" and this has coloured the view on the usefulness of vaccination in controlling influenza infections in animals. The inactivated vaccines for chickens differ from the vaccines for humans in that the vaccine for chickens is killed whole virus incorporated in an oil adjuvant whereas the human vaccine is a split vaccine with alum adjuvant. While whole virus inactivated vaccines have been used in America for many years to successfully control troublesome AI infections, often not H5 or H6 subtype particularly in turkeys (Halvorson, 2002), the Mexico and Pakistan outbreaks of 1995 were the first times that vaccines were used as an adjunct to slaughtering to control HPAI outbreaks (Swayne and Suarez, 2000). Although neither country achieved eradication of infection, the impact of highly pathogenic virus was markedly reduced and even eliminated in both Mexico and Pakistan. Halvorson (2002) has argued that vaccination can play a part in the control and eradication of AI infections if the other measures to control infection spread are carried out strictly.

Studies of the low pathogenic AI viruses circulating in Mexico in 2002 has shown that the protection provided by the vaccine strain chosen in 1995 had diminished and that more regular monitoring of virus strains and modification of vaccines is necessary to achieve better infection control (Lee *et al.*, 2004). Italy explored the use of vaccine to control infection with a low pathogenic virus. The desire to vaccinate against low pathogenic AI emanated from an earlier outbreak where low pathogenic virus had suddenly turned into HPAI, an outbreak that resulted in the death of 16 million poultry before eradication was achieved. Later infection of the poultry industry in north eastern Italy again with a low pathogenic strain resulted in the application of vaccination together with all the other measures necessary to control HPAI infection (Capua and Marangon, 2003). Unvaccinated sentinel birds were clearly identified and maintained in each vaccinated flock so that serology and virus isolation could be used to monitor vaccinated flocks for virus infection. The subtype of AI virus used in the vaccine differed from the outbreak strain by having a different N component so serology could be used to monitor for infection in vaccinated birds. This procedure was called the DIVA principle, differentiating infected from vaccinated animals. Vaccination was only carried out on birds that had a relatively long life such as breeders; vaccination was not carried out on broilers as the vaccine would only be beginning to take effect when the birds were being slaughtered. Italy has twice used this control program to eradicate low pathogenic strains of H7 AI viruses. It is clear that eradication would not have been achieved unless all the other principles of disease eradication measures were carried out including rapid slaughter of infected flocks, strong surveillance to detect infected flocks and active biosecurity was practised on all poultry premises (Swayne and Suarez, 2000; Halvorson, 2002; Capua and Marangon, 2003).

In 2003 there was a large outbreak of HPAI in the Netherlands due to H7N7 virus. Although the Dutch were active in initiating eradication by using slaughtering, the infection spread widely in a crowded industry. The EU with this and the Italian experience have reviewed the no vaccination policy for AI infections and vaccination has been accepted as a valid eradication strategy. Following the 2003 outbreak, the Dutch have a very much strengthened surveillance system that is expected to give earlier warning that influenza infection has started circulating in the vulnerable risk sectors of the poultry industry. The large outbreak of HPAI in the Fraser Valley of Canada in 2004 has initiated a similar review of future policy directions.

Vaccine use has been widespread in China and Indonesia but vaccination has not been taken up by Thailand, Vietnam, Laos and Cambodia. Thailand has not taken up use of the vaccine because there remains the possibility of re-establishing its trade in chicken meat with the EU and the opposition from health authorities. The arguments used by the Thai Committee that investigated vaccine use are available at http://www.fas.usda.gov/gainfiles/200410/146117817.doc. The trade dimension was not a part of the considerations for Vietnam but there was strong opposition from health authorities. The poultry industries of Laos and Cambodia are so dispersed that it was thought that the infection might die out without the use of vaccine. Given the low standard of biosecurity in most of the poultry industry in affected countries, the mostly silent infection in ducks and the presence of mixed poultry species in live bird markets, control and/or eradication of HPAI infection will be a very difficult task and endemicity in China is becoming a real threat to the rest of the affected countries. Vaccination is the only tool available that can modify virus production and dissemination.

VII. FACTORS INHIBITING CONTROL IN EASTERN ASIA.

South Korea, Japan and Malaysia have demonstrated that it is possible to control and eradicate H5N1 HPAI without the need to resort to vaccination programs. The success of the control programs would appear to have been dependent on the sensitivity of the surveillance applied to detect infection early and then mounting a rapid eradication response following detection of infection.

Hong Kong has applied vaccination to its own poultry industry to diminish the possibility of further outbreaks and insists on birds being imported for sale in live bird markets from mainland China being vaccinated; the other measures that are necessary to control/eradicate infection are carried out vigorously. China and Indonesia very early chose to vaccinate and vaccination is not necessarily carried out without much integration with the other measures that are required to control/eradicate HPAI infections (Swayne and Suarez, 2000).

FAO and OIE have organised a number of meetings to discuss regional approaches to control the disease in domestic poultry and country consultancies have been arranged to provide advice on local control measures. WHO initiated a very aggressive campaign with the reports of human infections in Thailand and Vietnam in early 2004 for all reservoir hosts to be slaughtered to eradicate infection (WHO, 2004c). While an aggressive slaughtering program was maintained in some countries, it became obvious that other countries undertaking vaccination were having no more or less influence in controlling HPAI infection. As noted earlier, WHO has softened its stance as it becomes more obvious that H5N1 virus is heading towards becoming endemic in affected countries (WHO, 2004a). The regional meetings and consultancies have identified the following factors as needing additional understanding if a rational approach is to be made in controlling/eradicating HPAI in eastern Asia:

- better and validated tests for the detection of infection, particularly in ducks and pigs;
- a better understanding of the infection processes in ducks and pigs;
- a better understanding of why and how outbreaks occur, including the relative roles of wild birds and domestic poultry; and
- identifying better disease control measures, including validation for the use of vaccine in ducks.

VIII. LESSONS FOR AUSTRALIA FROM AI EVENTS OF THE LAST FEW YEARS.

Australia is probably not at great risk of infection on account of high standard biosecurity operating in the poultry industry and Australian HPAI viruses having a sub-family relationship to the other Eurasian HPAI viruses (Suarez, 2000) suggesting there is not a regular connection with Eurasian viruses. However, it is essential that the Australian poultry industry remains vigilant for any evidence of AI infection to ensure eradication is undertaken as soon as infection is detected. Following the events of early 2004, USA authorities have been carrying out surveillance for infection in at risk sectors of the domestic poultry industry and slaughtering incursions of low pathogenic infections with H5 and H7 subtype viruses.

Australia at this time has an obsession with detecting infection in reservoir hosts; surveillance carried out in the at risk domestic poultry industry sectors will provide more valuable information on AI virus activity in the sector where early action will need to be taken. Free range chickens, ducks and turkeys provide the best surveillance hosts as they have the likelihood of getting infection from wild bird hosts and samples can be regularly taken for testing. However, this exposes any poultry owner that has a flock infected with a H5 or H7 subtype virus at economic risk because low pathogenic infections with these subtype viruses are not part of the listed diseases under the Emergency Animal Disease Response Agreement for cost sharing control activities. In addition, low pathogenic H5 and H7 subtype viruses are not notifiable diseases under the legislation of the states and territories enabling legislated action to be taken when infection is detected.

The experiences of the USA, Mexico, Pakistan, Italy and eastern Asia in dealing with HPAI and their LPAI infections soon after their discovery in the domestic poultry industry should be a warning to the Australian poultry industry and governments that decisive action taken early will be the best choice for controlling infection with AI infections caused by the H5 and H7 subtype viruses.

While vaccination might be useful in certain circumstances in handling a widespread AI infection in Australian poultry flocks the other fundamentals of control will need to be applied rigorously if control and eradication are to be achieved (Swayne and Suarez, 2004).

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THE CHANGING NATURE OF FOOD BORNE PATHOGENS – A BIRD'S EYE VIEW OF HOW THESE MAY IMPACT ON FOOD SAFETY

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Food borne pathogens have always had a major impact on human health, but in the late 19th Century and earlier 20th Century outbreaks of infection due to contaminated foodstuffs tended to be localised. However, with the greater centralisation of food processing there is now a greater opportunity for extensive spread of disease from a single source (Salyers and Whitt, 2002). Food borne diseases, especially those associated with enteric infection, are one of the most important public health issues affecting the developing communities because the net effect is a major loss in productivity.

The expectation of the public is that food products are safe and do not constitute a disease risk. Consequently around the world there are numerous quality assurance projects to ensure food safety. However, as rapidly as food is monitored we are seeing the emergence of "new" pathogens, or the changing nature of "older" pathogens.

From the poultry industry perspective, in the late 1980's/1990's the emergence of *Salmonella* Enteritidis as a food borne pathogen in the USA and Europe had a major impact on the poultry and egg industries. Likewise, the spread of *E. coli* 0157/H7 by undercooked contaminated hamburger meat by a takeaway food chain in the USA, or through contaminated salad products in Japan has impacted significantly on the public perception of safe food.

Perhaps the most significant impact on the changing nature of food borne pathogens has been the development of antibiotic resistance in food borne pathogens.

The development of antibiotic resistance in bacteria is of significant concern in matters pertaining to public health matters. Of particular interest to food animal producers, and the industry in general, is the development of resistance in enteric bacteria. These bacteria, which are normal flora in the intestine of animals and people, are a key resource for the maintenance and spread of resistance to like and related organisms.

There is presently enormous pressure on food producers to limit the use of antibiotics in food production animals. This is because of the perception that the use of growth promoting agents in food production animals has led to the selection of antibiotic resistance (Phillips *et al*, 2003). Where there is an animal reservoir for the maintenance of antibiotic resistant strains the resistance characteristics are even more important.

Since the widespread development and use of antibiotics has occurred only over the past 60 years there is significant information on what resistance levels are like in organisms such as *Escherichia coli* and *Salmonella species* before and after the introduction of antibiotics.

The extent and types of antibiotics that bacteria are exposed to also influence resistance. Antibiotic resistance is determined in two ways in bacteria. The first of these, and the most important, is the characteristic that allows bacteria to resist significantly high levels of an individual antibiotic. This resistance type is due to mutation in, or acquisition of, genes (either plasmid or chromosomal) responsible for antibiotic uptake or destruction. The second characteristic is the development of relatively non-specific genetic resistance to multiple antibiotics (Houndt and Ochman, 2000).

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One key question to be determined in understanding antibiotic resistance is how resistance is acquired and transferred.

For the transfer of high level specific resistance DNA agents such as plasmids, transposons, integrons and gene cassettes are involved. All of these DNA transfer agents have the ability to transfer DNA between strains of the same species or between different species, enhancing the opportunity for acquisition and transfer of antibiotic resistance.

In recent years there has been considerable work undertaken to understand the level of antibiotic resistance in organisms that colonise food animals (Ruiz *et a.l,* 1999). The main driving force of these surveys has been to determine the relationship between animal flora and human pathogens. The key focus of the surveys has been enteric bacteria and in Europe there are a number of countries that routinely survey enteric bacteria from food animals to determine the level of antibiotic resistance.

Bywater *et al.* (2004) reported on slaughter samples from chickens, pigs and cattle from four European countries where 2118 isolates of *E. coli*, 271 isolates of *Salmonella spp.* and 1325 isolates of *Campylobacter spp.* were evaluated for resistance to 11 antibiotics.

In this study the isolation rate of *E. coli* was high. For isolates from chickens the level of antibiotic resistance was relatively low across the spectrum of antibiotics tested with the highest level of resistance being 75% for tetracyclines.

Multiple resistances, represented as resistance to 4 or more antibiotics, in *E. coli* isolates from chickens were 9.5% in chickens but only 2.5% in pigs and 1% in cattle. The most common multi-resistant phenotype in chickens was ampicillin/ streptomycin/ tetracycline/ trimethoprim and sulphamethoxazole.

In the same survey *Salmonella* isolates were also evaluated. The isolation rate for *Salmonella spp.* across the four countries was 4.9% in total, with species prevalence of 7.1% for chickens, 4.5% pigs and 0.6% cattle. (Bywater *et al.*, 2004)

The majority of isolates from chickens were S. Heidelberg, S. Hadar, and S. Typhimurium. For *Salmonella* isolates from chickens there was a relatively high level of resistance to ampicillin and tetracycline. Multiple resistance in chicken isolates was relatively low at 5.8% with the most common resistance phenotype being ampicillin/ chloramphenicol/ streptomycin/ tetracycline/ trimethoprim plus sulphamethoxazole.

For campylobacter isolates the isolation rate varied from 3% (Sweden) to 70% (UK) with *C. jejuni* as the most common chicken isolate. Ciprofloxacin resistance was common in greater than 30% in Holland and France. Multiple antibiotic resistance was low in *Campylobacter spp*, only 1.8%, with the most frequent phenotype being ciprofloxacin/ nalidixic acid/ erythromycin/ tetracycline. Similar results for *Campylobacter spp* in chickens have been reported from Japan (Ishihara *et al.,* 2004) with resistance to oxytetracycline, macrolides and fluoroquinolones the dominant feature.

A recent survey of food borne pathogens isolated from beef, pork and poultry in Austria (Mayrhofer *et al.,* 2004) showed similar results to the work of Bywater *et al.* (2004). However, the Austrian samples had a higher rate of *Salmonella* isolation from chickens at 16.4%, with the variety of serovars indentified including *S.* Enteritidis, *S.* Heidelberg, *S.* Blockley, and *S.* Virchow as the main isolates.

Of these *Salmonella* isolates 57.7% exhibited a resistant phenotype, however the level of multi-resistance was low with only *S.* Blockley isolates and *S.* Hadar showing resistance to four or more antibiotics.

Of great concern internationally is the emergence of the *Salmonella* strain known as multi drug resistant (MDR) *Salmonella enterica* serovar Typhimurium DT104. This strain has been isolated in a number of countries, is commonly known as *S.* Typhimurium DT104, and is resistant to chloramphenicol, streptomycin, sulfonamides, and tetracycline. Some

isolates have been identified that are resistant to fluoroquinolones, trimethoprim and kanamycin (Boyd *et al.*, 2001).

It has been suggested that MDR S. Typhimurium DT104 has increased virulence. This virulence is not necessarily because of enhanced invasion, but it appears to have enhanced invasion independent virulence factors (Boyd *et al.*, 2001).

Recently we have been evaluating virulence characteristics of *Salmonella* and *E. coli* isolates from Australia and Vietnam.

In studies of *Salmonella spp.* and *E. coli* isolates from poultry meat from various retail sources in Vietnam screened against ampicillin, ciprofloxacin, tetracycline, gentamycin, chloramphenicol and sulphamethoxazole 96% of *E. coli* isolates were resistant to one or more antibiotics and 52% of isolates were resistant to four or more antibiotics. For salmonella the percentages were 77% resistant to one or more antibiotics and 17% resistant to four or more antibiotics.

Clearly this is a major concern from a public health perspective.

We have also shown that a wide variety of *Salmonella enterica* isolates have a novel fimbrial gene cluster that plays a key role in attachment and invasion, and which can be used to differentiate salmonella serotypes.

Food borne pathogens are a major concern for the poultry industry. Clearly over time we are seeing an evolution of resistance and virulence characteristics in the major enteric organisms associated with food poisoning that will impact both on disease management and food quality.

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INTESTINAL GOBLET CELLS AND MUCUS PRODUCTION IN THE CHICKEN

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Summary

Histological analysis of duodenal, jejunal and ileal tissues samples stained with PAS/Alcian Blue has shown not only differences in colouration between individual goblets of different dietary groups but also spatial differences outlining changes in mucin composition in both gross intestinal and microscopic villi/crypt regions. These preliminary results provide insight into changes in mucus composition due to external factors such as diet and presence of bacteria. Future experiments involving low pathogen load and conventional chicks will provide information on the potential mechanisms behind these changes and whether or not bacterial interactions play a primary role in goblet cell and mucin cytoarchitecture.

RESULTS AND DISCUSSION

The alimentary tract of the fowl, like that of all animals in a normal environment, is densely populated with microorganisms. Colonisation of the tract by bacteria can be either beneficial (symbiotic) or detrimental (pathogenic) to the host. Enteric infections caused by pathogenic species are responsible for reducing growth rates and/or an increase in mortality, producing consequent economic losses in animal production.

The first line of defence that foreign bacteria encounter when trying to transverse the intestinal mucosa is the overlying mucus-gel layer. The formation of the mucus-gel is through goblet cell packaging and secretion of polymeric mucin glycoproteins (Forstner and Forstner, 1994; van Klinken *et al*., 1995). These glycoproteins bind to heterogeneous oligosaccharide chains on the bacterial wall thus preventing adhesion of bacteria to the epithelial cells and subsequent invasion of the tissue. Contrastingly, the mucus-gel also provides a highly nutritious substrate for microbial populations (Deplancke and Gaskins, 2001), thus the type and quantity of the mucus are considered essential components in the establishment of the intestinal barrier.

Bacteria and/or their metabolic products can affect the production or quantity of mucus secreted either through goblet cell proliferation and morphology or changes in mucin gene expression (Meslin *et al*., 1999). There is, however, little information on whether bacteria can alter the mucin carbohydrate structure itself through changes in its production mechanisms within goblet cells (Smirnova *et al*., 2003) and whether or not changes are beneficial to the host in terms of preventing bacterial adherence or beneficial to the bacteria, for facilitating colonisation and mucin breakdown.

Aims of our research include identifying the determinants of goblet cell number and function and ascertaining the mucus composition in intestine during early development of broiler chickens. Histological analysis of duodenal, jejunal and ileal tissues samples stained with PAS/Alcian Blue has shown not only differences in colouration between individual goblets of different dietary groups but also spatial differences outlining changes in mucin composition in both gross intestinal and microscopic villus/crypt regions.

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These preliminary results provide insight into changes in mucus composition due to external factors such as diet and presence of bacteria. Future experiments involving low pathogen load and conventional chicks will provide information on the potential mechanisms behind these changes and whether or not bacterial interactions play a primary role in goblet cell and mucin cytoarchitecture.

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EFFECTS OF AGE ON THE APPARENT METABOLISABLE ENERGY OF DIETS BASED ON MAIZE, WHEAT OR SORGHUM FOR BROILER CHICKS

D.V. THOMAS[1] and V. RAVINDRAN[1]

Studies on the changes with age in the apparent metabolisable energy (AME) in the young broiler chick are limited. The present study was undertaken to determine the AME of diets based on wheat, sorghum and maize during the first two weeks post-hatch of broilers. Three experimental diets containing wheat, sorghum and maize as the cereal base were formulated. All three diets were formulated to contain similar levels of energy and amino acids. The wheat-based diet was supplemented with a commercial xylanase. Each diet was fed to six replicate groups (8 birds/replicate) from days 1 to 14 post-hatching. On days 5, 7 and 14, classical total excreta collection method was employed to determine the AME values of the diets. The changes in AME values (MJ/kg dry matter) are summarised below.

	Wheat-based diet	Sorghum-based diet	Maize-based diet	Pooled SEM
Day 5	13.12[b]	13.64[b]	13.58	0.33
Day 7	12.24[a]	12.95[a]	13.22	0.30
Day 14	14.15[c]	14.27[b]	13.91	0.16
Pooled SEM	0.26	0.23	0.31	

[a,b] Means in the same column with different superscripts are significantly different (P<0.05).

The results suggest that the changes in AME over the first two weeks of life differed depending on the cereal type. In the case of wheat and sorghum, the AME determined on Day 7 was lower (P<0.05) than those determined on Day 5. In both cereal types, the AME values increased (P<0.05) from Day 7 to Day 14. The age of chick had no effect (P>0.05) on the AME of maize-based diets, with similar AME values determined on Days 5, 7 and 14. The AME values of the diet types were similar (P>0.05) in all three ages. The drop in AME observed on Day 7 for wheat and sorghum-based diets was unexpected and difficult to explain. Clearly further studies are warranted to confirm these results.

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BETWEEN-BIRD VARIATION IN THE CONCENTRATION OF IRON AND OTHER MINERALS IN EGG YOLK ACROSS A LAYING CYCLE

D.K. REVELL[1] and R.J. HUGHES[2]

Summary

Large between-bird variation in the iron and manganese content of eggs was unmatched by variation in the concentration of other minerals. This work indicates that there is potential for producers to identify and segregate a cohort of 'high-iron birds' in their flocks for supply of enriched eggs to niche markets. Alternatively, given the high degree of variation in iron content, breeding programs may be able to select for this trait to increase the iron content of eggs in general. The existence of individual hens with unusually high or low capacity to transfer iron to the egg provides an opportunity for researchers to use the hen as a model for the study of biochemical and physiological processes involved in iron transport.

I. INTRODUCTION

Egg consumption in Australia peaked in the 1970s and, based on estimated consumption in Australian made by the Australian Egg Industry Association, a decline in the order of 10% has occurred since then. Consumption figures have tended to plateau over the last decade, largely because the negative perceptions about the cholesterol content of eggs have been overcome. It is now generally recognised by nutritionists that a low amount of cholesterol in foods will not cause problems if the whole diet is low in saturated fat, and that one egg per day can be consumed as part of a healthy diet. Consumers are more likely to accept eggs as a regular component of their diet if they can see a clear nutritional advantage in doing so.

The nutritional enrichment of eggs may provide the opportunity to encourage consumers to reassess the nutritional value of eggs in a more positive light. Satisfying niche market requirements for consumers who want or need to increase their intake of a particular nutrient is one opportunity to expand the market for eggs. Eggs are a valuable source of dietary minerals, especially the trace elements iron and zinc. Unfortunately, it is not generally understood by health professionals that the bioavailability of iron in eggs is high. In order to make specific nutritional claims about the nutritional value of eggs, which may help develop new markets for eggs and egg products, it is necessary to define the natural variability in mineral concentration and to identify the potential to enrich the concentration of specific minerals.

This report describes natural variation in the mineral content of eggs at different stages of the laying circle for commercial layer strain hens fed a commercial layer diet.

II. MATERIALS AND METHODS

Eighty four Hisex Brown laying hens were housed in individual cages from 17 weeks of age and given a commercial layer diet.. At 27, 37 and 57 weeks of age, eggs were collected for three consecutive days from each hen. Egg weight and yolk weight were determined on all collected eggs. The three yolk samples were pooled within birds at each sampling age and analysed for mineral concentration by inductively coupled plasma mass spectrometry (Waite Analytical Services, The University of Adelaide).

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Table 1. The mean concentration (mg/kg), range and coefficient of variation (CV, %) of minerals in egg yolk collected from birds at 27, 37 or 57 weeks of age during a single laying cycle.

	27 weeks of age			37 weeks of age			57 weeks of age			
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV	Pooled SE
Trace elements										
Fe	73 [b]	53-99	12	66 [a]	44-90	15	69 [ab]	52-118	17	1.6
Zn	48	42-55	6	48	42-54	5	48	41-79	12	0.6
Mn	1.1 [ab]	0.9-1.8	16	1.2 [b]	0.9-1.7	17	1.1 [a]	0.9-1.6	16	0.03
Cu	2.2	1.8-2.5	8	2.0	1.7-2.3	8	2.1	1.7-3.8	18	0.04
Macro minerals										
Ca	1848	1640-2200	6	1806	1580-2000	6	1870	1510-3300	14	28
P	7374 [ab]	6800-7700	3	7146 [a]	6600-7700	3	7614 [b]	6900-12900	12	88
Mg	139 [a]	124-160	6	146 [b]	129-163	5	150 [b]	129-260	13	2
Na	482 [a]	420-560	7	540 [b]	460-630	8	560 [b]	450-930	14	9
K	1374	1290-1630	5	1298	1160-1480	5	1323	1170-2400	15	18
S	2038	1880-2200	4	2063	1940-2200	3	2121	1870-3700	13	23

[ab] Values within rows with different superscripts differ significantly ($P<0.05$)

The mineral concentration of egg yolk, and the total mineral content in yolk (i.e., concentration times yolk weight) were subjected to analysis of variance (GenStat release 5.1, Lawes Agricultural Trust) with time (week 27, 37 or 57) as the main effect.

III. RESULTS

Egg weight increased ($P<0.001$) by 15% (58, 62 and 66 g at 27, 37 and 57 weeks of age, respectively) and yolk weight increased ($P<0.0001$) by 32% (14.0, 16.8 and 18.5 g at 27, 37 and 57 weeks of age, respectively). Consequently, the proportion of yolk to whole egg increased ($P<0.001$) from 24 to 28%.

The iron concentration in yolk was about 10% higher ($P=0.01$) for birds at 27 weeks of age than at 37 weeks of age, and intermediate at 57 weeks of age (Table 1). The iron content of whole eggs was 25% higher ($P<0.001$) at 57 weeks of age than at 27 or 37 weeks of age (1.02, 1.10 and 1.28 mg Fe/egg for 27, 37 and 57 weeks of age, respectively).

The concentrations of zinc, copper, calcium, potassium and sulphur in egg yolk did not differ significantly with age of the birds, but the concentration of magnesium, manganese and phosphorus increased from 27 weeks of age by about 6-8%, and sodium concentration increased by 16% with age of the birds (Table 1).

Iron and manganese concentration showed the highest variation between birds within each stage of the laying cycle (i.e., age), with a coefficient of variation of 12-17%. The highest iron or manganese concentrations were double that of the lowest concentrations. None of the other minerals showed such a wide range between birds at 27 or 37 weeks of age, but by week 57, the between-bird variability had increased for all minerals examined (coefficient of variation for all minerals was 12-18%), with the highest values for individual birds typically about twice that for birds with the lowest values.

IV. DISCUSSION

The variation in concentration of minerals in egg yolk reported here, where coefficients of variation ranged from 4 to 18%, are in general agreement with that reported in a British study (Manson *et al*., 1993), which found coefficients of variation ranged from 4 to 12%. Both studies found iron content of egg yolk to be the most variable between individual birds. Our study has shown that, with the exception of iron and manganese, the concentration of most minerals is relatively constant in egg yolk, at least up to 37 weeks of age, suggesting that the incorporation of these elements is under tight physiological control in laying hens. However, between-bird variability increased later in the laying cycle (57 weeks of age) with little change in the average mineral concentration. This suggests that the regulation of mineral deposition in egg yolk deteriorates as the laying cycle progresses, possibly reflecting changes in mineral transport or cellular uptake, and alterations in the partitioning of minerals between tissues.

The opportunity exists to exploit or manipulate the mineral content of chicken eggs to enhance their nutritional value and marketability. Of particular interest is iron, because worldwide and in Australia, a number of groups of people are at risk of iron-deficient anaemia, for example infants (Wharton, 1999), growing children (Mira *et al*., 1996), adolescent females (Wharton, 1999), women, particularly pregnant women (Viteri, 1994), and elite sportspeople (Haymes and Lammanca, 1989).

Although day-to-day variation in mineral concentration within individual birds was not determined (as yolk samples from each three-day collection period were pooled), the high variability in iron concentration between laying hens throughout their laying cycle suggests three opportunities exist. The first is to develop a biological marker to identify ‘high iron birds’ for use in supplying eggs for niche markets, the second is to develop breeding

programs to exploit the variation in this trait, and the third is to better understand the mechanisms that allow certain birds to deposit twice as much iron in egg yolk as others. This latter opportunity may help identify nutritional or other strategies to manipulate iron transfer to, and deposition in, eggs. If successful, it may allow eggs to be marketed with a nutritional claim, and would provide a useful source of dietary iron for people at risk of deficiency and whose diet precludes sufficient intake of other sources of dietary iron.

The information reported here will help dieticians, nutritionists and other health professionals to evaluate the contribution of egg yolks to the daily requirements of essential minerals.

V. CONCLUSIONS

Large (two-fold) variation between birds exists in the iron content of egg yolk; from 43 to 97 mg/kg. Apart from manganese, none of the other minerals showed such a wide range between birds at 27 or 37 weeks of age, but by week 57, the between-bird variability had increased for all minerals examined. This work has identified an opportunity to meet niche market requirements by using birds that produce eggs with high levels of iron. It may be possible to increase the iron and other mineral content of eggs through genetic selection. In this regard, it would be useful to determine if there are differences in egg mineral concentration between the various commercial strains of laying hens.

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GROWTH PATTERN OF BROILERS FED A PHYSICALLY OR ENZYMATICALLY TREATED COPRA MEAL DIET

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Summary

Large quantities of copra meal are available in South East Asian and Pacific countries. Six different treatment diets (corn-soy, 30 % unmodified copra meal, 30 % ground copra meal, 30 % CM soaked diet, 30 % pelleted and crumbled CM and 30 % CM treated with enzymes) were fed to male broiler chickens for 6 weeks. From day 1 to 35, birds fed the corn-soy diet produced higher feed intake, body weight and live weight gain but they were not significantly different from those of the birds fed the crumbled CM diet. In week 6, body weight of birds fed soaked, enzyme supplemented diet and crumbled CM diet were not significantly different from the controls. The water : feed ratio of birds fed all the copra meal based diets was significantly higher than that of birds fed the corn-soy diets. DM digestibility decreased significantly with the inclusion of CM and increased with age. The AME of the enzyme treated diet and the ground CM diet was higher than the AME of the corn-soy diet.

I. INTRODUCTION

Panigrahi *et al*. (1987) found that 25 % copra meal (CM) in a diet supplemented with methionine and lysine improved body weight to the level of body weight of birds fed a corn-soy diet when the birds were kept for at least 5 weeks of age and that dry matter digestibility increased with the age of birds. It has been found that keeping the birds less than 5 weeks led to poor performance of birds, even when the diets were balanced for nutrients and enzymes are included (Sundu *et al.*, 2004). The poor quality of CM may be partly due to its bulk density and high water holding capacity (Panigrahi, *et al.*, 1987; Sundu *et al.*, 2004) rather than its chemical composition. These two physical properties may lead to a problem of feeding CM diets to young chicks because their gastro-intestinal tract may not be developed well enough either in size or ability to enable sufficient nutrients to be consumed (Panigrahi *et al.*, 1987). Physical treatment can change the bulk density of the diet. Soaking (Yalda and Forbes, 1999), pelleting (Callet, 1965), grinding (Niir *et al.*, 1995) and enzyme treatment (Sundu *et al.*, 2004) have been used to improve the digestibility and performance of broilers. The present study was undertaken to examine the response over a six week period of broilers fed physically and enzymatically treated copra meal in the diet.

II. MATERIALS AND METHODS

168 day-old male broiler chicks of Ross commercial strain were randomly allocated to each treatment in brooder cages and fed from day 1 to 17 with the starter diets. 144 birds were then transferred into the grower cages equipped with a trough feeder and drinker and fed the grower diets from day 17 to 42. Feed and water were given ad-libitum throughout this trial. Soaking the diet was conducted daily at the time of offering the diet with the ratio of 1 kg diet : 1 kg tap water. Temperature was monitored and evaporation was recorded.

Six different treatments were used; a corn-soy based diet {control diet, (CD), 30 % unmodified CM (CM), 30 % fine ground CM diet, passing through a 500 μm screen (FCM),

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30 % soaked CM diet (SCM), 30 % pelleted and crumbled CM diet (CCM) and 30 % unmodified CM diet supplemented with enzymes (Hemicell, supplied by ChemGen Corp, Maryland, USA; and Allzyme SSF, supplied by Alltech Biotechnology Pty. Ltd, Victoria Australia. ECM)}. The bulk densities of the diets were 0.60 for CD, 0.53 for CM and ECM, 0.47 for FCM, 0.67 for CCM and 0.53 for SCM before soaking and 0.86 including the water after soaking. This means that due to the water, the bulk density of the original 30 % CM diet had been increased by 36 % to 0.72 if the density of the water is subtracted. All diets were formulated to meet standard meat chicken nutrient requirements (NRC, 1994). The requirements of amino acids were based on digestible amino acids. The experimental design was a completely randomised design with 6 treatments each with 4 replicate cages each containing six birds.

Table 1. Diet composition (g/kg)

Ingredients	Control diet (CD)		30 % CM diet *		Enzyme diet (ECM)	
	Starter	Grower	Starter	Grower	Starter	Grower
Calculated:						
ME (MJ/kg)	13.4	13.4	13.4	13.4	13.4	13.4
Protein	230	210	230	210	230	210
Dig. Met + Cys	9.3	7.9	9.0	7.2	9.0	7.2
Dig. Lysine	12.0	10.0	11.0	10.0	11.0	10.0
Analysed composition:						
Gross Energy (MJ/kg)	18.0	17.1	19.9	19.3	19.9	19.5
Protein	219	199.	229	203	212	203
NDF	134	151	277	294	282	298

* (CM) : As received CM; (FCM): Fine ground CM; (SCM): diet (CM) + water (ratio 1:1); (CCM) Crumbled CM diet

III. RESULTS AND DISCUSSION

The results of this experiment are shown in Table 2 and 3. Body weight and feed intake of birds fed the corn-soy diet from day one to 35 were significantly higher than those fed other treatments except birds fed the crumbled CM diet. By day 42, birds fed the soaked diet and enzyme treated diet had accelerated their growth to be not significantly different from that of birds fed the corn-soy diet (Figure 1). The water consumption of birds fed the CM based diets was generally higher than that of birds fed the corn-soy diets. The water : feed ratio was higher in birds fed all the CM based diets. The DM digestibility was higher for the corn-soy based diets but increased for all diets, as the birds got older. None of the physical and enzymatic treatments improved DM digestibility. Enzymatic treatment increased the AME of the diet from 13.4 to about 13.8 MJ/kg both in week four and seven.

Data show that the use of 30 % CM in the diets, except crumbled CM diet, during the first 4 weeks impaired the growth of birds even when balanced diets were formulated and enzymes were included. This was similar to previous findings (Sundu *et al.*, 2004). The present finding indicates however that birds have an adaptive capacity to respond to the feed they consume. It took some time for the birds to produce good performance. This was also found by Panigrahi *et al.* (1987). The most considerable improvement in weight gain took place in week 5. Birds fed the plain CM increased LWG from 395 to 633 g in week five (See Table 2) and in this week all the birds had a LWG above 600 g / week except birds fed the ground CM diet (Table 2).

Although the birds fed the unmodified CM had higher weight gain in week 6 than those fed the corn-soy diet due to a higher feed intake in week 6 (Figure 2), but they still did

not reach to the level of the birds fed corn-soy in week 6, while birds fed the soaked and enzyme diet did.

Table 2. Weekly response of broilers fed corn-soy and CM diets, day 1 to 42

Parameters	Week	Diets					
		CD	CM	FCM	SCM	CCM	ECM
Body weight (g)	1	123.9 ^{a}	114.5 ^{ab}	118.7 ^{ab}	111.4 ^{b}	118.5 ^{ab}	114.0 ^{a}
	2	378.3 ^{a}	317.6 ^{bc}	310.8 ^{c}	276.1 ^{c}	346.9 ^{ab}	323.5 ^{bc}
	3	791.7 ^{a}	677.2 ^{bc}	637.3 ^{c}	623.2 ^{cd}	739.1 ^{ab}	683.3 ^{bcd}
	4	1336.7 ^{a}	1072.3 ^{b}	991.0 ^{c}	1143.3 ^{b}	1320.0 ^{a}	1165.1 ^{b}
	5	1974.2 ^{a}	1705.9 ^{b}	1471.2 ^{c}	1782.1 ^{b}	1991.3 ^{a}	1779.8 ^{b}
	6	2592.5 ^{ab}	2331.1 ^{c}	1993.1 ^{d}	2422.7 ^{b}	2674.3 ^{a}	2431.2 ^{b}
Weekly weight gain (g)	1	86.7^{a}	77.3^{ab}	81.5^{ab}	74.3^{b}	81.4^{ab}	76.9^{ab}
	2	254.5^{a}	203.2^{c}	192.1^{c}	164.7^{d}	228.4^{b}	209.5^{bc}
	3	413.4^{a}	359.6^{abc}	326.5^{c}	347.1^{bc}	392.3^{ab}	359.8^{abc}
	4	545.0^{a}	395.1b^{c}	353.7^{c}	520.2^{ab}	580.9^{a}	481.9^{ab}
	5	600.4a	596.5^{a}	443.1^{b}	601.7^{a}	634.1^{a}	577.7^{ab}
	6	581.2^{ab}	588.1^{ab}	484.8^{b}	603.5^{a}	645.9^{a}	614.3^{a}
Feed intake from week 1 (g)	2	294.5 ^{a}	255.8 ^{b}	254.6 ^{b}	217.3 ^{c}	272.2 ^{ab}	289.6 ^{ab}
	3	814.7 ^{a}	706.2 ^{c}	685.5 ^{cd}	660.0 ^{d}	787.5 ^{ab}	753.7 ^{b}
	4	1531.1 ^{a}	1276.9 ^{c}	1205.1 ^{c}	1386.7 ^{b}	1520.1 ^{a}	1374.5 ^{b}
	5	2573.4 ^{a}	2209.0 ^{b}	1990.4 ^{c}	2336.5 ^{b}	2582.7 ^{a}	2322.6 ^{b}
	6	3727.3 ^{ab}	3342.7 ^{bc}	3011.2 ^{c}	3378.9 ^{bc}	3842.8 ^{a}	3442.9 ^{ab}
Weekly Feed intake (g)	2	294.5^{a}	255.8^{b}	254.6^{b}	217.3c	272.2^{ab}	289.6^{ab}
	3	520.2^{a}	450.5^{b}	430.9^{b}	442.8^{b}	515.3^{a}	464.1^{b}
	4	716.4^{ab}	570.6^{c}	519.6^{c}	726.7^{a}	732.7^{a}	620.8^{bc}
	5	1042.3^{a}	932.1^{ab}	785.3^{b}	949.8^{ab}	1062.6^{a}	948.1^{ab}
	6	1154.0^{ab}	1133.7^{ab}	1020.8^{b}	1042.4^{ab}	1260.0^{a}	1120.3^{ab}
Weekly FCR	2	1.19 ^{b}	1.20^{ab}	1.32^{a}	1.36^{a}	1.26^{ab}	1.29^{a}
	3	1.21 ^{a}	1.11 ^{a}	1.38 ^{a}	1.20 ^{a}	1.28 ^{a}	1.33 ^{a}
	4	1.36 ^{a}	2.02 ^{a}	1.40 ^{a}	1.50 ^{a}	1.28 ^{a}	1.24 ^{a}
	5	1.72 ^{a}	1.26 ^{a}	1.77 ^{a}	1.73 ^{a}	1.58 ^{a}	1.67 ^{a}
	6	1.99 ^{a}	1.93 ^{a}	2.13 ^{a}	1.72 ^{a}	1.95 ^{a}	1.83 ^{a}
Water consumption (g/week)	4	1597 ^{c}	1579 ^{c}	1507 ^{c}	1890 ^{ab}	2066 ^{a}	1759 ^{bc}
	5	2210 ^{bc}	2355 ^{b}	2042 ^{c}	2480 ^{ab}	2728 ^{a}	2441 ^{b}
	6	2596 ^{d}	2999 ^{bcd}	2842 ^{b}	3350 ^{ab}	3572 ^{a}	3151 ^{b}
Water : feed ratio	4	2.23 ^{c}	2.76 ^{ab}	2.91 ^{a}	2.60 ^{b}	2.83 ^{ab}	2.83 ^{ab}
	5	2.12 ^{b}	2.53 a	2.60 ^{a}	2.63 ^{a}	2.57 ^{a}	2.58 ^{a}
	6	2.24 ^{c}	2.65 ^{b}	2.80 ^{b}	3.22 ^{a}	2.84 ^{b}	2.82 ^{b}

abcd: Row means with the same superscript are not significantly different (P<0.05)

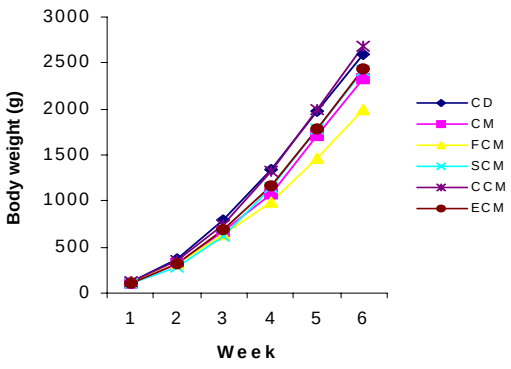

Figure 1. Weekly body weight of birds fed the experimental diets

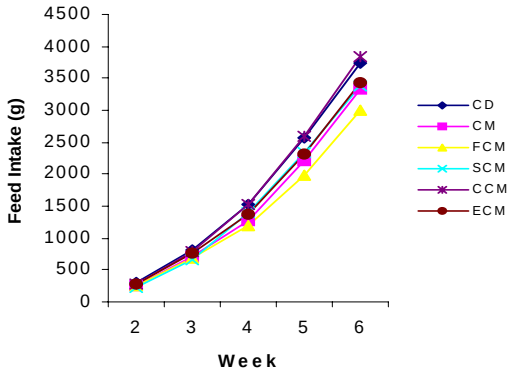

Figure 2. Weekly feed intake of birds fed the experimental diets

Table 3. Digestibility and AME of diet of birds fed corn-soy and CM based diets

Parameters	Week	CD	CM	FCM	SCM	CCM	ECM
DM digestibility (%)	4	81.1 [a]	71.5 [bc]	73.5 [b]	70.3 [c]	71.1 [c]	71.7 [bc]
	7	82.3 [a]	72.3 [b]	73.8 [b]	72.0 [b]	72.3 [b]	73.0 [b]
AME (MJ/kg)	4	13.1 [c]	13.4 [b]	13.7 [ab]	13.4 [b]	13.5 [ab]	13.8 [a]
	7	13.3 [b]	13.4 [b]	13.8 [a]	13.3 [b]	13.3 [b]	13.8 [a]

abcd: Row means with the same superscript are not significantly different ($P<0.05$)

The main problem of using CM thus appears to be its physical properties rather than its chemical characteristics. Bulk density and water holding capacity may be the factors affecting the quality of CM because physical treatment to reduce its bulkiness such as pelleting and crumbling increased the body weight of birds in this study even more than the body weight of birds fed the corn-soy diet. Enzyme treatment and soaked diet seem efficient when the birds were kept longer than 5 weeks.

Although, the body weights of birds fed unmodified CM, ground CM and enzyme supplemented diets were significantly lower than that of birds fed the corn-soy diet, their water consumption was not significantly different in week 4 and 5. Water consumption per kg feed for birds fed the CM diets was higher than for birds fed the corn-soy diets possibly due to the higher water holding capacity of CM. However, the moisture content of faeces was not related to the amount of water consumed. The faeces of chickens fed each diet had the following moisture content in week 4: CD (77.1 %), CM (75.6 %), FCM (75.1 %), SCM (76.8 %), CCM (75.8 %), and ECM (74.8 %). The high water : feed ratio of birds fed the soaked diet was probably due to the fact that they were forced to consume water in the diet.

The lower DM digestibility of CM based diets may be due to their higher fibre content than the corn soy-diet (Table 1). The enzyme diet and the ground CM diet had a slightly increased DM digestibility by 2 %, compared to birds fed the unmodified CM and there was no increase in the digestibility of birds fed the crumbled CM diet. These results indicate that the main factor affecting the high body weight of birds fed the crumbled CM diet was associated with their high feed intake. The capacity of birds to digest the diet increased with age. This is in agreement with Panigrahi *et al.* (1987). A small improvement was found in the DM digestibility of all diets as the birds got older. The birds fed the control diet had a high DM digestibility but it did not attain the calculated level of the dietary AME. Enzyme addition to and grinding the CM increased the AME by about 3 % and these diets had significantly higher AME than the control diet.

The findings demonstrated that copra meal can be successfully used in a broiler diet at the level of 30 % from day one provided a balance diet is formulated and the CM is processed to increase dietary bulk density to over 0.60 g/cm^3 by pelleting and crumbling. Where pelleting and crumbling is not available, for example in CM producing countries, the use of enzymes or soaked diets are able to increase productivity.

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EFFECT OF A MULTI-ENZYME PRODUCT IN CORN-SOY FED LAYERS

S. MAISONNIER-GRENIER[1], P. DALIBARD[1] and P-A. GERAERT[1]

Summary

Rovabio™ Excel improved laying performance in corn-soybean meal fed layers. Feed conversion was reduced by 5.8 % in relation with decrease of feed consumed (-3%). Yolk color was also improved by up to 0.2 points on Roche scale.

RESULTS AND DISCUSSION

NSP-enzymes have long been used in wheat or barley-fed broilers due to the adverse effect of such cereals on growth performance and feed digestibility (Geraert *et al*., 2003). However, recent works, with enzyme-supplemented wheat or barley-based feeds for laying hens, have demonstrated significant advantages on performance, particularly on feed efficiency. With the development of NSP-enzymes use in maize-fed broilers, investigation of their effect in maize-fed layers is also of interest.

The objective of the present study was to determine the effects of Rovabio™ Excel on laying performance of corn-fed layers. Rovabio™ Excel AP was introduced into mash feed at 50 g/ton, and given to ISAbrown laying hens from 29 week-old to 45 week-old, 20 replicates per treatment. Laying performance was individually measured over the whole period. Diet formulation and results are given in Tables 1 and 2.

Table 1. Diet formulation

Ingredient (g/kg)	Experimental diet
Maize	530
Wheat bran	70
Soya full fat extruded	77
Soybean meal 48	199
Palm oil	13
Premix	111

Table 2. Laying performance

	Control	Enzyme	Δ (%)	P
Food consumption (g/hen/day)	133.3 ± 5.36	129.3 ± 6.32	-3.0	=0.06
Mean egg weight (g)	63.1 ± 2.69	64.5 ± 4.97	2.1	NS
Egg mass (g/hen/day)	62.0 ± 3.04	63.1 ± 5.11	1.9	NS
Layer rate (%)	98.2 ± 2.90	98.3 ± 3.00	0.1	NS
Feed efficiency	2.20 ± 0.172	2.07 ± 0.078	-5.8	<0.01
Yolk coloration	11.8 ± 0.60	12.1 ± 0.80	2.5	<0.02

Rovabio™ Excel improved feed conversion (-5.8 %) of laying hens fed with corn-soybean meal based diet due to reduction of feed consumed (3 %) and a slight increase in mean egg weight. This reduced feed intake could be explained by an enhanced energy digestibility which could also support the enhanced egg yolk coloration: + 0.2 points on Roche scale (+2.5%). Corn-soybean fed layers thus also benefits from enzyme addition.

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INFLUENCE OF DIETARY PEPTIDE LEVELS ON ENDOGENOUS NITROGEN FLOW IN BROILER CHICKENS

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Summary

In the present study, the enzymatically hydrolysed casein (EHC) method was used to determine the effect of dietary peptide level on ileal nitrogen flows in broiler chickens. It was found that the endogenous flow of nitrogen was similar (P>0.05) between birds fed the protein-free diet and those fed the 50 g/kg EHC diet. Increasing dietary EHC levels from 50 g/kg to 200 g/kg significantly (P<0.05) increased the endogenous nitrogen flows. These results demonstrate that the presence of peptides causes increased losses of endogenous nitrogen from the small intestine of broiler chickens and that the magnitude of the losses is influenced by the dietary levels of peptides.

RESULTS AND DISCUSSION

The traditional approach of determining endogenous nitrogen and amino acid flows is to feed the bird a diet devoid of protein. This method has been criticised since lack of protein may result in reduced digestive enzyme secretion and rate of protein turnover in the gut. Ravindran *et al.* (2004) reported that dietary protein and peptides exert a positive influence and endogenous amino acid flows. Limited data is available on the effects of dietary concentrations of protein or peptide on endogenous amino acid flows (Hodgkinson *et al*., 2000). In the present study, the enzymatically hydrolysed casein (EHC) method was used to determine the effect of dietary peptide level on ileal nitrogen flows in broiler chickens. Five experimental diets containing EHC at 0, 50, 100, 150 and 200 were formulated. All diets contained 3 g/kg titanium oxide as an indigestible marker. Each diet was fed *ad libitum* to four pens (6 birds/pen) of male broilers (Ross) from 28 to 35 days of age. On day 35, terminal ileal contents were collected and endogenous nitrogen flows were determined as previously described (Moughan *et al*., 1992). The endogenous flows (mg/kg dry matter intake) of nitrogen are shown below.

	Protein-free diet	50 g/kg EHC	100 g/kg EHC	150 g/kg EHC	200 g/kg EHC	Pooled SEM
Nitrogen	1232[a]	1311[a]	1733[b]	2096[b]	2722[b]	58.4

[a,b] Means in a row bearing different superscripts are significantly different (P<0.05).

The endogenous flow of nitrogen was similar (P>0.05) between birds fed the protein-free diet and those fed the 50 g/kg EHC diet. Increasing dietary EHC levels from 50 g/kg to 200 g/kg significantly (P<0.05) increased the endogenous nitrogen flows. These results demonstrate that the presence of peptides causes increased losses of endogenous nitrogen from the small intestine of broiler chickens and that the magnitude of the losses is influenced by the dietary levels of peptides.

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COMPARISON OF IN-OVO AND DAY-OF-HATCH VACCINATION ON THE EARLY IMMUNE RESPONE IN CHICKENS

W. MUIR[1] and M. BARRON[1]

Summary

The humoral and cell-mediated immune response in chicks immunised with tetanus toxoid either in-ovo or orally at day-of-hatch was assessed during the first fifteen days posthatch. There were no significant differences between these two immunization protocols in circulating T-helper and T-suppressor subsets on days 8 and 14 of age, nor in the serum anti-T. toxoid IgM antibody titres on day 9. At day 15, serum anti-T. toxoid IgG antibody titres were notably higher in chicks immunised orally at day-of hatch. However, anti-T. toxoid IgA antibody titres at the intestinal surface were significantly higher in in-ovo immunised chicks at 15 days of age.

RESULTS AND DISCUSSION

Several studies have compared the efficacy of in-ovo immunisation with day-of hatch immunisation in protecting chicks from a homologous pathogen challenge (Sarma et al., 1995; Johnston et al., 1997). Generally the assessment of vaccination efficacy has been based on protection, with limited consideration of the components of the immune system that are activated following antigen delivery. This study was designed to compare the early activation of humoral and cell-mediated immune responses in chicks immunised either in-ovo or orally at day-of hatch.

One hundred and fifty, day 18 embyros (D18E), were obtained from Inghams hatchery, Casula. All eggs were candled, and all fertile eggs (120) were randomly allocated to five treatment groups and set in hatching trays in a Multiplex incubator. The control treatment, group one, did not receive either antigen or placebo throughout the study. On D18E treatment group 2 received 0.5mL tetanus toxoid (T. toxoid) diluted in phosphate buffered saline (PBS) and treatment group three received PBS placebo (0.5 mL), delivered directly into the amniotic fluid. At hatch the chicks were moved into brooders, with feed and water available *ad libitum*. At this time treatment group 4 received 0.5mL T. toxoid and treatment group 5 received the placebo. Both were delivered orally. At 9 days of age each bird in group 2 and 4 received an oral booster dose of T. toxoid, and each bird in groups 3 and 5 received PBS orally (0.5mL). At days 8 and 14 blood was collected from six chicks from each treatment group for assessment of cellular subsets by flow cytometry ($CD4^+$ and $CD8^+$ T cell subsets, and IgM^+, IgG^+, IgA^+ antibody isotypes and Ia^+ cells) (Muir *et al*., 2002). On days 9 and 15 blood was collected from the remaining birds for assessment of total and anti-T. toxoid antibody titres in serum using an ELISA. On day 15 all birds were euthanased and samples of intestinal scrapings (IS) were collected for analysis of anti-T. toxoid IgA antibody titres.

At day 8 there were no significant differences in T and B cell subsets in peripheral blood. Average serum anti-T. toxoid IgM antibody titres determined on day 9 were similar for the chicks receiving the T. toxoid either in-ovo or at day of hatch. Similar to day 8, at day 14 there were no significant differences in T and B cell subsets. Birds receiving T. toxoid at day of hatch had the highest average total IgG antibody and the highest average anti-T. toxoid IgG antibody titres (P=0.07 compared to in-ovo T. toxoid immunised chicks)

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in serum on day 15. Birds receiving T. toxoid in-ovo had the highest average total IgA antibody titre in serum on day 15, and the highest average anti-T. toxoid IgA antibody titre ($P<0.05$ compared to day-of hatch T. toxoid immunised chicks) in the IS. On days 9 and 15 birds which had not been immunised with T. toxoid, that is treatment groups 1, 3 and 5, had negligible T. toxoid-specific antibody titres.

This study has demonstrated the ability of in-ovo and day-of hatch oral immunisation with a protein antigen to generate antigen-specific immunity during the first 2 weeks after hatch. However, when comparing the immune response generated in chicks immunised either in-ovo or orally on the day-of-hatch, the former immunisation protocol generated a significantly higher local, IgA based immune response while oral day-of-hatch immunisation generated a higher average systemic IgG based immune response than in-ovo immunisation. Further studies are required to understand the mechanisms behind these observations and their implications for the industry.

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LASER BEAK TRIMMING

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Summary

An ophthalmic laser was used to beak trim chickens. The laser beam cut through the outer layers of keratin, but the tip of the premaxillary bone could not be fully severed. For a number of birds, incomplete cuts were made on the beak. Subsequently a green laser and CO_2 laser were tested. The CO_2 laser with a 1 sec pulse, 50-micron spot size and power rating of 10W was the most effective laser in cutting a beak sample indicating potential to use lasers for beak trimming.

RESULTS AND DISCUSSION

Given the continuing welfare scrutiny of using a hot blade to cut the beak, attempts have been made to develop alternative methods of beak trimming. Alternative methods include cold blade trimming, arc trimming, robotic trimming, chemical trimming and infrared trimming (Glatz, 2004). Cost of lasers is falling and studies were undertaken to examine the potential of using lasers for beak trimming.

An ophthalmic laser (1.5W, 4 sec pulse, 50-micron spot size) cut through an upper beak sample from a dead chicken. Two passes of the laser beam were required to complete the cut due to insufficient power in the laser beam. Studies with 5-day-old chickens established the spot size to enable coagulation of the tissue and prevent bleeding. When a 50-micron spot size was used for 2 sec there was insufficient energy density in beam to cause coagulation and the beak began to bleed (N=4). When the spot size was increased to 200 microns with a cutting time of 2 sec, no bleeding was observed (N=3) indicating the 200-micron spot size was effective in sealing the wound.

The laser was able to cut through the outer layers of keratin, but could not cut tip of the premaxillary bone of day-old (N=10) or 5 day-old chicks (N=10) with only one exception. The beam was passed across the bony tip numerous times for a number of birds from the dorsal, ventral and lateral position without success. The cuts that were made to the beak looked clean and straight. No problems were observed with healing of the beak stump. The lack of success in being able to cut the tip of the premaxillary bone was considered to be due to the lack of power in the laser. A green and CO_2 laser were tested. The CO_2 laser with a 1 sec pulse, 50-micron spot size and power rating of 10W was the most effective laser in cutting a beak sample.

This work has shown that there is potential to use lasers for beak trimming. The study demonstrated the spot size required to cauterise the beak, and the power to cut the beak. Further work is now required to develop a prototype laser beak-trimming machine with automatic measurement and laser trimming of the beak. The laser beak trimming technology is likely to improve precision of beak trimming, reduce cannibalism and chronic pain in birds.

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AN ECONOMIC EVALUATION OF THREE VARIETIES OF PEARL MILLET COMPARED TO GRAIN SORGHUM IN DIETS FOR LAYING HENS AND BROILERS

M. EVANS[1] AND D.N. SINGH[2]

Summary

Pearl millet (PM) has been identified as a suitable alternative summer crop to sorghum in low rainfall and sandy areas in Queensland (QDPI) where it yields earlier and out yields sorghum (4.8 t/ha by PM hybrids and 1.4 and 3.6 t/ha by Express and Sonic hybrids respectively). This study showed that on an equal protein basis, one can afford to pay more for pearl millet to give the same diet cost and therefore shows the economic and nutritional superiority of PM over sorghum. PM is a viable economic alternative to sorghum as a summer grain crop for inclusion in poultry diets.

RESULTS AND DISCUSSION

Dryland summer cropping in Queensland is currently based on sorghum. Pearl millet (PM) has been identified as a suitable alternative summer crop to sorghum in low rainfall and sandy areas in Queensland (QDPI personal Communication, Cited Singh, 2004) where it yields earlier and out yields sorghum (4.8 t/ha by PM hybrids and 1.4 and 3.6 t/ha by Express and Sonic Sorghum hybrids respectively). PM also has a shorter maturation period (82 days) compared to the quick maturing hybrids (Express and Sonic) of sorghum (107 days). Singh and Perez-Maldonado (2000) and Singh (2004) have evaluated pearl millet in diets for laying hens and broilers and have found it to be a suitable grain for these types of poultry. This paper reports an economic evaluation of three varieties of pearl millet compared to sorghum in commercial diets for laying hens and broilers using linear programming optimisation techniques.

Six diets, three broiler (starter, grower and finisher) and three layer (layer 100, layer 110, layer 120) were formulated using a least cost feed formulation program (Format International, Single-Mix®) based on typical average raw material prices used to formulate commercial poultry diets in August 2004. The raw material nutrient composition data for the Pearl Millet used in the study was from Singh and Perez-Maldonado (2000) and Singh (2004). The nutrient composition data of the sorghum (120g crude protein/kg) used was from the commercial data base of Applied Nutrition. The three varieties of millet were evaluated against sorghum grain having the same crude protein content of 120g/kg as-is. The specifications for the diets were based on apparent metabolisable energy, apparent ileal digestible amino acids, calcium, available phosphorus, sodium, potassium, chloride and choline. For each of the six diets, the sorghum diet was formulated first establishing an unrounded optimal diet cost. The sorghum was replaced by the pearl millet variety under study and its cost adjusted until a diet was formulated with the same diet cost as the sorghum diet. This was repeated for each diet and each pearl millet variety. The results are shown in Table 1.

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Table 1	Equivalent Grain Costs ($/tonne) for Least Cost Diets of the Same Cost		
Broiler	Starter	Grower	Finisher
Sorghum	180.00	180.00	180.00
Pearl Millet 31	193.54	187.60	175.95
Pearl Millet 3	218.87	213.90	197.70
Pearl Millet 4	209.61	203.07	190.42
Layer	100 g/bird/day	110 g/bird/day	120 g/bird/day
Pearl Millet 31	200.51	194.93	187.44
Pearl Millet 3	214.38	215.15	209.06
Pearl Millet 4	214.82	208.97	201.67

This study shows that on an equal protein basis, one can afford to pay more for pearl millet to give the same diet cost and therefore shows the economic and nutritional superiority of PM over sorghum. The price and nutritional advantage of PM is very dependent on the type of diet being formulated and the variety of PM being used. PM has a greater advantage over sorghum in diets that have a higher nutritional density such as in broiler starter or Layer 100 diets. PM3 is economically and nutritionally superior to PM4, which, in turn is superior to PM31. However, Autumn harvest yields show that PM31 (4.1 t/ha) out yields both PM3 (3.4 t/ha) and PM4 (3.0 t/ha). Clearly, PM is a viable economic alternative to sorghum as a summer grain crop for inclusion in poultry diets. This study is a snapshot in time and the economic advantage of one raw material over another will depend upon the cost and ability of a range of raw materials to deliver nutrients to a particular specification.

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FERMENTED FEED FOR BROILERS

A. WHITEHEAD[1] AND T.A. SCOTT[1]

Summary

Wheat-based mash broiler starter diets with or without xylanase were fed *ad libitum* in three forms (dry, wet (1.2g water: 1g feed), and wet plus a commercial silage inoculant (*Lactobacillus plantarum and Enterococcus faecium*) fermented for 24h) to six cages of six male broilers from 0 to 21 d of age. The inoculated wet diet pH decreased from 6.6 to 4.2 following the 24h fermentation period and appeared to be well accepted by broiler chicks. The body weight of the broilers at day 21 fed fermented diets though not significantly greater than those fed wet diets, were 19% greater than those fed the equivalent diets in a dry form. The increase in body weight was matched to an increase in feed intake and the FCR was not significantly different between feed forms, although numerically lower for the birds fed fermented diets. The study confirms that feed intake of dry-fed wheat-based diets is limited by broilers and suggests that it may be possible to incorporate the pro- and pre-biotic value of fermented diets for feeding broilers into wet feeding programmes.

RESULTS AND DISCUSSION

The advantage of wet feeding broiler chickens has been recently reviewed by Forbes (2003); and specifically referenced by Scott (2002) and Scott and Silversides (2003) as being a valuable tool in our understanding of limits in feed intake by broiler chickens fed wheat-based diets. Although merits were observed with wet feeding, there was a strong concern with regards to the potential for microbial proliferation in wet feed, either directly challenging the bird or producing harmful toxins to contaminate the feed. Although preliminary studies (Scott, 2002) did not show an advantage in feeding propionic acid in wet feeds to control microbial growth, the present study was conducted to determine if fermented feed, similar to that used for feeding pigs, would be acceptable to broiler chickens.

A preliminary study was conducted to determine the optimum (low stable pH) fermentation conditions using a commercial silage inoculant (SI-LAC; Genesearch, Australia). Based on several trials, it was found we could decrease the initial pH of wet (1.2g water: 1g feed) diets from 6.6 to a stable pH of 4.2 by using recommended broth applications and maintaining the feed anaerobically (semi-sealed plastic bags) for 24 h at 30^{o}C.

Wheat-based broiler starter diets were prepared, split and one portion remixed with added xylanase (Avizyme 1302; Feedworks Pty Ltd). These two diets were fed *ad libitum* to six cages of six male broilers from 0 to 21 d of age in three forms: a) dry (as is); b) wet (prepared each morning by adding 1.2 g water: 1g dry feed, mixed and fed in plastic-lined feeders with all left over feed weighed back and discarded); and c) wet feed inoculated with SI-LAC (a broth prepared from a commercial ensilage inoculant of *Lactobacillus plantarum and Enterococcus faecium*) that was allowed to anaerobically ferment (30°C) for 24h before feeding as per the wet diets. The feed intake of all birds is expressed on a dry basis. The growth and efficiency of the birds fed the six diet combinations were monitored and four birds / diet were sacrificed at 21d for assessment of digesta pH and gut segment measurement. Based on digesta viscosity measures and performance we concluded that the soluble non-starch polysaccharide levels of the wheat used in this study were not high and

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minimal enzyme response was observed. Therefore, we have only reported the effects of diet form in Table 1.

Table 1. The mean variable response of male broilers (0 to 21 d) to wheat-based mash diets (with or without xylanase) fed in three forms.

Variable	Dry	Wet (1.2 g water: 1g feed)	Fermented wet diets
Feed intake (g/b/d) 0-21d	46.4±2.34[b]	54.3±2.03[a]	53.5±3.22[a]
Body weight (BW) g – 21d	711±44.2[b]	830±39.3[a]	847±54.4[a]
FCR	1.46±0.079[a]	1.43±0.049[a]	1.41±0.067[a]
Crop pH	5.5±1.15[a]	5.2±0.62[a]	4.5±0.08[b]
Gizzard pH	2.8±0.36[a]	2.6±0.28[a]	2.6±0.28[a]
Gizzard wt / BW	2.04±0.28[a]	1.95±0.28[ab]	1.72±0.30[b]
Liver wt / BW	4.34±0.62[a]	3.79±0.65[b]	3.62±0.22[b]

[a,b,c] – mean values with different superscripts indicate significant differences ($P<0.05$).

It is evident from the data that there was no serious consequence of feeding fermented diets with regards to growth and FCR. There was a significant lowering of crop pH, of particular interest is the low std deviation for fermented crop pH, indicating consistency in the crop. We also did not determine the survival of the inoculated microorganisms in the digesta of the gut, however based on work by others this would be expected to exist.

The fermentation process has been credited with changes in nutrient availability; for example, Carlson and Poulsen (2003) demonstrated marked changes in phytate P availability with fermented wheat- and barley-based diets. Some of the benefits of this may relate to activation of endogenous cereal phytases as well as those from the bacteria during fermentation. Heres *et al*. (2003a,b) demonstrated that the pre- and pro-biotic activity of fermented diets was effective in controlling *Salmonella* and *Campylobacter* colonisation of the gut. A component of this was the lowering of the pH of the diet prior to ingestion and halting multiplication of these pathogens, and in some cases destroying them. A second component was the minimisation of cross contamination, due to increased resistance and a reduction in shedding. It is evident that the contribution of wet feeds and the practice of controlling microbial growth by ensilage / fermentation will require further work to determine its practical application for feeding of poultry.

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THE EFFECT OF XYLANASE, PHYTASE AND LIPASE SUPPLEMENTATION ON THE PERFORMANCE OF BROILER CHICKENS FED A DIET WITH A HIGH LEVEL OF RICE BRAN

N.G.A. MULYANTINI[1], M. CHOCT[2], X.LI[1] and U.R LOLE[3]

Summary

Enzyme products did not have a significant effect (P>0.05) on weekly fed intake and weight gain of birds. But feed intake tended to drop and weight gain tended to increase in response to supplementation of the three enzymes. Weight gain of the birds was increased by 0.6% with lipase, 3.7% with phytase and 2.4% with xylanase. Xylanase had a marked effect (P<0.01) on both FCR and AME, decreasing FCR from 1.52 to 1.4 and increasing AME from 12.29 to 12.60 MJ/kg. There was also a highly significant (P<0.01) interaction between the three enzymes on AME. The lipase and phytase both improved FCR but had no effect on AME. It can be concluded that AME of broiler diets containing a high level (30%) of rice bran may be improved by xylanase supplementation.

I. INTRODUCTION

Rice is the principal cereal in many parts of the world and the by-products from its milling are important livestock feed resources. Large quantities of rice bran are produced annually, and approximately 91% of all rice bran is produced in Asia, and this is greater than the production of any other single crop. Hull-free rice bran contains many nutrients, and typically has a composition of 20% oil, 15% protein, 45% nitrogen free extract, plus vitamin and minerals (Sayre *et al*., 1989). It, however, has high level of phytate phosphorus, and high levels of non-starch polysaccharides (NSP), which are not digested and are mostly excreted by poultry. If the NSP could be degraded to their monomeric constituents, the nutritive value of rice bran would be theoretically be improved by 15-20% (Farrell *et al.,* 1993). Another serious drawback of rice bran as a source of oil are the presence of a high level of lipolytic enzymes. Feed enzyme technology may offer possible solutions to these problems of feeding rice bran to poultry by allowing enhanced utilisation of nutrients in rice bran. This study was conducted to examine the effects of xylanase, phytase and lipase and their interactions on energy metabolism and performance of broiler fed a diet containing 30% defatted rice bran.

II. METHODS

One hundred and ninety two mixed sex broiler chickens (21 days of age) were used in a 2 x 2 x 2 factorial design. There were eight treatment groups, replicated six times with four birds per group. The diets were as follows: 1) Control diet containing 30% defatted rice bran, 2) Control + Xylanase, 3) Control + Lipase, 4) Control + Phytase, 5) Control + Xylanase +Lipase, 6) Control + Xylanase +Phytase, 7) Control + Lipase + Phytase, 8) Control + Lipase + Xylanase + Phytase. The diets were isocaloric and isonitrogenous and were cold pelleted. All ingredients used for the control diet were accurately weighed, and thoroughly mixed in a

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rotary mixer. A recommend amount of enzyme A was diluted in 300 mL water and sprayed into the ingredients using a pressure spray while feed mixing continued.

On day 21, the birds were weighed and transferred to ME cages in a temperature-controlled room. The first three days enabled the chickens to adapt to the feeds and served as an adaptation period. During the last four days the excreta were collected daily. Spilled feeds and feathers were manually removed from excreta before drying in a forced-draft oven (80^{o}C) overnight. Dried excreta from quantitative collection were weighed after allowing it to equilibrate with atmospheric conditions for one day, and were then pooled for gross energy determination. The energy metabolised from the gross energy (GE) intake of the birds in a given period was determined using the formula:

$$\text{AME (MJ/kg)} = \frac{\text{[GE diet x feed intake (kg)] -[GE excreta x amount of excreta (kg)]}}{\text{Feed intake (kg)}}$$

III. RESULTS AND DISCUSSION

Table 1 show that none of the enzyme products (xylanase, phytase and lipase) had a significant effect on weekly feed intake and weight gain of the birds. However, feed intake tended to drop and weight gain tended to increase in response to supplementation of all three enzymes. There was also significant ($P<0.01$) interaction between lipase, phytase and xylanase on AME and FCR, showing an additive effect on energy metabolism and feed conversion. The moisture content of the excreta was not affected by any of the enzymes.

Phytase addition increased weight gain by 3.7% and improved FCR by 2.6% compared to control. Since the basal diet contained adequate levels of P (1.16% total P or 0.49% non-phytate P), the observed phytase responses appear to be independent of its effect of P availability. These 'extra-phosphoric' effects of phytase may be attributed to the release of protein and amino acids from phytase protein complexes. The improvement of body weight gain resulting from the addition of phytase was primarily a result of increased feed intake and utilization of dietary phosphorus.

Lipase addition alone was not effective in improving performance of the chickens. Weight gain of the birds increased by 0.6%, and improvement in FCR by lipase was only 2.1%. It is well known that raw rice bran contains an extremely active lipolytic enzyme, lipase, which hydrolyses the triglycerides and releases free fatty acids. Therefore, supplementation of lipase may have been redundant.

Xylanase significantly ($P<0.01$) enhanced AME of the diet from 12.29 to 12.60 MJ/kg and improved feed conversion of the birds by 5.4%. Rice bran contains substantial amounts of NSP that are predominantly insoluble. Also these NSP do not appear to posses any nutritional activity as inclusion of isolated rice bran NSP in broiler diets does not depress AME or bird performance (Annison *et al.*, 1995). Although supplementation of rice bran containing diets with xylanase possible degraded the NSP to an extent, the large improvements in AME and FCR observed in the current experiment were unexpected. The effect of the xylanase on AME and FCR was large and difficult to account for by an increase in energy contribution by fermentation alone. Even though partial depolymerisation of the NSP can lead to increased fermentation in the hindgut of the chicken (Choct *et al*., 1996), the energy contribution from fermentation is estimated to be only 6-8% of the total energy. It is therefore possible that the significant improvement in AME and FCR obtained in this experiment may partially be due to the effect of the xylanase on the wheat and triticale components of the diet.

Table 1. Performance and excreta moisture content (%) of chicks fed a high rice bran diet with or without supplementation of lipase, phytase and xylanase.

Diet		Feed Intake (g/bird)	Weight gain (g/bird)	FCR	AME (MJ/kg)	Excreta moisture content (%)
Lipase						
-		700	473	1.50	12.51	75.3
+		691	470	1.47	12.38	75.5
Phytase						
-		693	463	1.50	12.40	75.4
+		698	480	1.46	12.48	75.4
Xylanase						
-		707	466	1.52	12.29	74.9
+		684	477	1.44	12.60	76.0
Lipase by phytase						
-	+	695	479	1.46	12.52	74.9
-	-	705	467	1.52	12.50	75.6
+	+	702	480	1.47	12.45	75.9
+	-	680	459	1.48	12.31	75.2
Lipase by xylanase						
-	+	696	487	1.43	12.59	76.2
-	-	704	459	1.54	12.43	74.4
+	+	673	466	1.45	12.61	75.8
+	-	710	473	1.50	12.14	75.3
Phytase by xylanase						
-	+	682	469	1.46	12.59	75.5
-	-	703	457	1.54	12.22	75.3
+	+	686	484	1.42	12.61	76.4
+	-	711	475	1.50	12.36	74.4
F values and level of significance						
Main effects:						
-Lipase (A)		0.37	0.09	0.20	1.90	0.13
-Phytase (B)		0.16	2.02	2.40	0.69	0.00
-Xylanase (C)		2.35	0.79	10.12**	10.67**	1.99
Interactions :						
AB		1.28	0.86	0.17	0.42	0.77
AC		1.01	1.31	2.38	2.71	0.77
BC		0.02	0.02	0.01	0.45	1.32
ABC		0.25	1.17	1.53	15.61*	0.43

*Significant at P<0.01.

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DIETARY LIPID SOURCE, LEVEL AND CARCASS FATTY ACID COMPOSITION IN BROILERS

D. ZHANG[1], X. LI[1], M. CAO[1] and W.L. BRYDEN[1]

Summary

The effects of different source and levels of lipid on carcass fatty acid composition in broilers were investigated and reported.

RESULTS AND DISCUSSION

It is a common practice to supplement broilers diets with lipid as a source of energy. The beneficial effects of dietary oil on production performance have been well documented. However, the effects on carcass fatty acid composition with different source and levels of lipid are poorly clarified. The objective of the study was to examine the relationship between dietary lipids, production performance and carcass fatty acid composition in broilers.

Day-old commercial broiler chicks and a corn-soybean meal based diet were used in this study. Experiment 1: two diets were formulated with equal AME and crude protein (CP) levels. Treatment 1: control diet with no oil added; Treatment 2: 2% tallow was added to the grower diet and 2% corn oil in finisher diet. Experiment 2: four diets were formulated with equal AME and CP levels. 2% tallow, 2% corn oil, 4% tallow and 4% corn oil were added respectively to Treatments 1, 2, 3 and 4. In each experiment, five pens of seven chicks were allocated to each treatment. Individual body weight and pen feed intake were recorded weekly and feed efficiency was calculated. At the end of study (day 42), one bird from each pen, close to the mean weight, was selected for breast muscle, thigh and abdominal fat pad measurements. The meat and fat samples were analysed for DM, ether extract, CP, cholesterol, fatty acids and amino acids.

As expected, weight gain and feed efficiency were significantly improved ($P<0.05$) by dietary oil supplementation. Breast muscle, thigh and abdominal fat pad expressed as percentages of body weight did not show significant differences between treatments. There were no significant differences between treatments on the contents of dry matter, ether extract, CP, amino acids and cholesterol of breast muscle, thigh and abdominal fat pad.

Eleven fatty acids were identified in the experimental samples. Among them palmitic acid (16:0), palmitoleate acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) account for more than 96% of total fatty acids content. The carcass fatty acid composition was significantly affected by dietary lipid supplementation. This effect was clearly reflected in the changes of linoleic acid and total unsaturated fatty acids (TUFA) contents in different parts of the carcass.

In Experiment 1, the birds fed treatment 2 diet had significantly higher linoleic acid and total unsaturated fatty acids (TUFA) contents in breast, thigh and abdominal fat pad than the birds fed control diet. In Experiment 2, the birds fed diets with corn oil supplementation had significantly higher linoleic acid and TUFA contents than the birds fed diets with tallow supplementation. The fatty acid profile of the carcass was influenced by the lipid source with the influence being more obvious in abdominal fat than in thigh or breast muscle. It is interesting to notice that the birds fed diets with 2% tallow during growing period and 2%

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corn oil during finishing period had similar linoleic acid and TUFA contents to the birds fed diets with 2% corn oil during the whole experiment period. The results of this study suggest that carcass fatty acid composition in broilers can be manipulated by supplementing diets with different lipid source to favour the requirements of human nutrition. This manipulation is more achievable during the finishing period of broiler production.

(% of total fatty acids, MEAN±SE)

Experiments	Treatments	Linoleic acid			TUFA		
		Breast	Thigh	Abdominal Fat pad	Breast	Thigh	Abdominal Fat pad
1	1	23.4±0.49^{b}	25.3±0.68^{b}	25.7±1.74^{b}	60.6±0.58^{b}	61.6±1.53^{b}	69.3±0.72^{b}
	2	25.8±0.53^{a}	28.8±0.96^{a}	36.1±1.66^{a}	64.1±0.63^{a}	69.1±1.39^{a}	73.9±0.78^{a}
	P value	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01
2	1	22.6±0.96^{b}	22.0±1.37^{b}	23.4±1.69^{c}	59.9±1.16^{b}	64.3±1.68^{a}	67.5±1.68^{b}
	2	26.0±1.13^{a}	30.2±1.49^{a}	35.4±1.38^{b}	66.8±1.45^{a}	66.5±2.55^{a}	75.9±1.82^{a}
	3	19.4±1.13^{b}	19.3±1.45^{b}	19.0±1.16^{c}	59.9±1.54^{b}	65.0±2.64^{a}	68.9±1.81^{b}
	4	27.6±1.27^{a}	32.1±1.84^{a}	40.3±1.98^{a}	62.0±1.22^{b}	66.3±2.59^{a}	77.8±1.60^{a}
	P value	<0.01	<0.01	<0.01	<0.01	>0.05	<0.01

In each experiment, the means within each column with different superscripts differ significantly ($p<0.05$)

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