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SWEDISH POULTRY PRODUCTION WITHOUT IN-FEED ANTIBIOTICS - A TESTING GROUND OR A MODEL FOR THE FUTURE?

J. INBORR

Summary

Poultry production in Sweden has undergone many changes and faced many challenges during the past two decades. The ban on the non-therapeutic use of antibacterial feed additives appears to have had mainly positive effects on the poultry industry prompting producers and the feed industry to improve management and feed formulation standards. As a result, poultry production is more efficient and safer (from a consumer health viewpoint) than ever before.

I. INTRODUCTION

During the past 10 to 15 years poultry production in Sweden has undergone major changes both with regard to the structure of the industry and the feeding and management of the birds. Whereas the total egg production has not changed very much, the industry has undergone major structural changes resulting in fewer flocks of increased size. For example, in 1980, the number of layer flocks was approximately 24 000 whereas in 1997 the number had decreased to approximately 8000 (Figure 1, SCB, 1998). However, the average head count is still rather low with less than 300 flocks of 5000 layers or more.

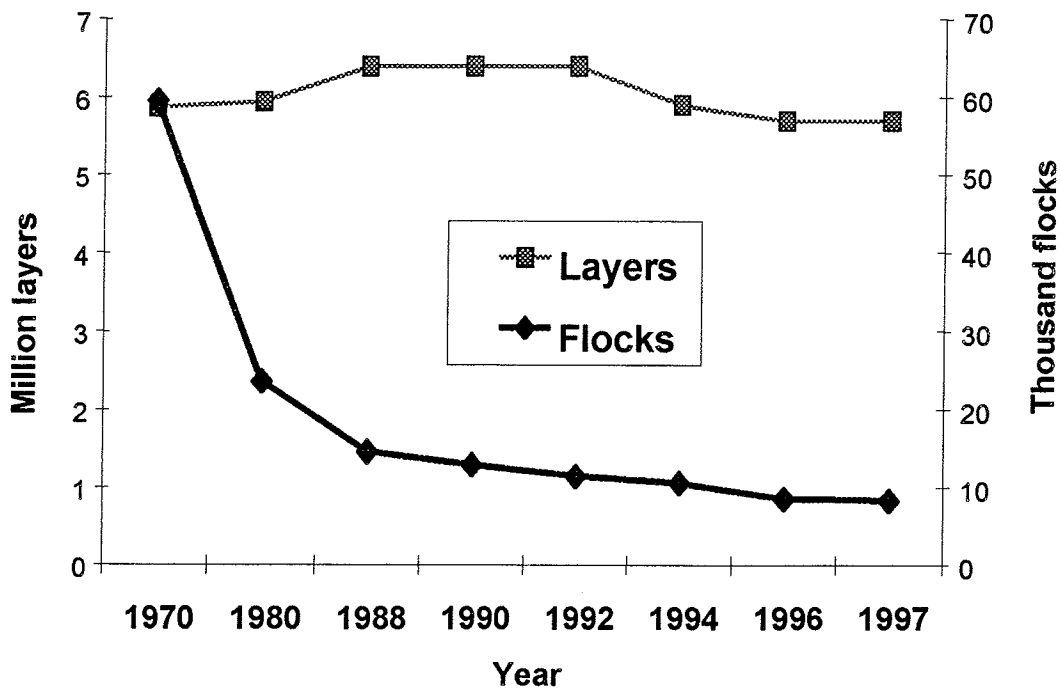


Figure 1. Number of layers and layer flocks in Sweden between 1970 and 1997.

In contrast to egg volumes, chicken meat production has more than doubled during the last ten years (Figure 2, Swedish Poultrymeat Association, 1999). This reflects the increasing popularity of chicken meat and the competitiveness of the industry compared with other kinds of meat production such as pork and beef. Turkey and duck production in Sweden are of little significance.

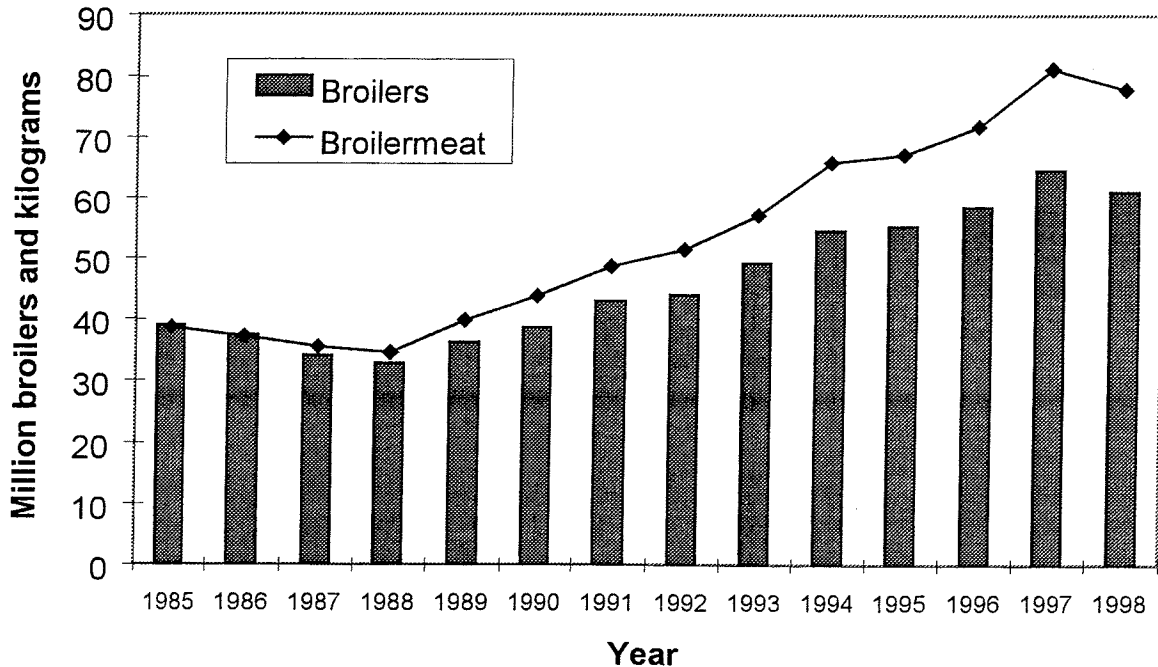


Figure 2. Number of broilers (millions) and amount of broilermeat (thousand tonnes) produced in Sweden between 1985 and 1998.

Another major change and challenge to the broiler industry was the implementation of the ban on in-feed antibiotics in 1986. In this paper, the background of the ban and the consequences of it in terms of bird management, feeding and hygiene status will be discussed.

II. NON-THERAPEUTIC USE OF ANTIBIOTICS BANNED SINCE 1986

The debate in the Swedish press that eventually led to the ban on the non-therapeutic use of antibiotic growth promoters was initiated in the early 1980s. A journalist of the daily *Dagens Nyheter* revealed that 30 tonnes of antibiotic substances were fed to healthy animals every year. In 1981, the Federation of Swedish Farmers and the cooperatives (representing approximately 90 per cent of the farmers) issued a joint policy statement declaring their ambition to reduce the use of antibiotics in animal production. Prophylactic actions, such as improved hygiene standards, better management systems and feeds in combination with increased knowledge were corner stones of the policy. The use of antibiotics needed to be based on actual needs of the animals and, therefore, the justification for a general use for growth promotion was questioned. A proposal in 1981 by the Federation of Swedish Farmers to stop using in-feed antibiotics was not regarded as justifiable at that time by the authorities.

However, the debate was kept alive by both politicians and farmers' organisations. In 1985 a new feed law was formulated. According to this, antibiotic and chemotherapeutic

substances in feeds could only be used on prescription by a veterinarian. Furthermore, their use was only to be allowed with the purpose of preventing, relieving or curing illness. The new legislation came into force on January 1st 1986.

(a) Production without antibiotics

Despite a lively debate and a lot of speculation on the consequences that preceded the ban, the entire farming community, the feed industry and advisers alike seemed to have been taken by surprise. Very little had been done in preparation for this new situation, which without doubt, affected the pig producers more than any other group of famers. Since no antibacterial feed additives had ever been authorised for use in layer feeds in Sweden, the ban had no impact on egg production.

At the time of the ban, virginiamycin was the main antibacterial feed additive use in broiler feeds. It had been used from the early 1980s when it replaced avoparcin. At the beginning, virginiamycin was used at a dose of 10 ppm but in the autumn of 1985 the dosage was increased to 20 ppm following reports of outbreaks of necrotic enteritis (NE) with increasing frequency. This disease in its subclinical and clinical forms was identified as the main problem to tackle as the in-feed antibiotic was withdrawn. The problem was addressed by a committee consisting of representatives from the producers, the feed industry, veterinarians and the meat inspection services. The outcome of the work was an agreement on a transition period during which virginiamycin would be prescribed for use in broiler feeds at 20 ppm (SOU, 1997).

Field trials with non-medicated feeds indicated that a number of factors needed to be corrected to get control over NE. It was concluded that the construction and the climate of the buildings, hygiene management and feed composition all contributed to the occurrence of NE in broilers. Furthermore, ionophore coccidiostats were found to prevent NE. The main emphasis was placed on improving the environment of the birds because many diseases, including NE, have a multifactorial background. A bonus programme was developed for the producers giving an economic incentive to maintain a high hygiene status and good management systems in production.

In 1988, all prophylactic medications were abandoned and, in case of outbreaks of NE, a two-day treatment with phenoxymethyl penicillin in the drinking water was applied. The amount of active substance of antibiotics used for treatment decreased from approximately 2000 kg of virginiamycin in 1987 to 100 kg of phenoxymethyl penicillin in 1988. Today the need for such treatment of NE has almost disappeared.

(b) Maintaining performance

Whilst performance and health status of cattle and poultry farms were only marginally affected by the ban, the situation on pig farms was quite the contrary. As a consequence of the ban, the use of antibiotics in compound feeds was reduced by 2/3 and the number of pigs treated with antibiotics for post weaning diarrhoea fell in 1986 to nearly 1/10 the level used in the previous year. However, this resulted in clinical problems and health disorders, which resulted in an increased demand and use of antibiotics in feeds at therapeutic levels. For example, in 1988 and 1989 the use of Olaquinox (g/pig) increased to levels higher than prior to the ban. In 1989, 75 per cent of the pig population in Sweden were treated with antibiotics (Wierup, 1996).

Most affected were the sow herds with post weaning pig mortality increasing by 1.2 percentage units and age of piglets at 25 kg LW increasing by 5.2 days in the first year of the ban (Robertsson and Lundeheim, 1994).

Broiler performance was not as severely hit as that of pigs. At the time the ban was introduced, broiler production was quite extensive, with low bird densities (25 kg live weight per m²) and good health status. However, the new situation prompted nutritionists within the feed industry and researchers at the universities to search for alternatives and develop new management and housing systems in order to maintain broiler performance and control NE. The years following the ban were a period of intense activity to test and evaluate any non-antibiotic concept such as:

- lactic acid bacteria, yeasts, acidifiers (organic and inorganic acids), energy and protein levels, feed texture, whole grain feeding, feed enzymes.

After a few years of “trial and error” a few concepts emerged having been proven to contribute to a better health status and to improve the performance. With regard to feeds and feeding, nutrient density of feeds, in particular energy and protein levels, were reduced, coarse grinding was introduced, the inclusion levels of soybean meal was reduced and feed enzymes were included in all broiler feeds. All these measures in combination with strict hygiene programmes have, in fact, led to clearly improved broiler performance (Figure 3). Although the average live weight at slaughter has increased by some 20 per cent, feed conversion ratios have decreased by approximately 5 per cent, resulting in a 20 per cent increase in production efficiency (PE) values since 1986.

PE is calculated as follows: $\text{Ave Wt(g)} \cdot (100 - (\% \text{mortality} + \text{culls})) / 10 \cdot \text{FCR} \cdot \text{age(d)}$

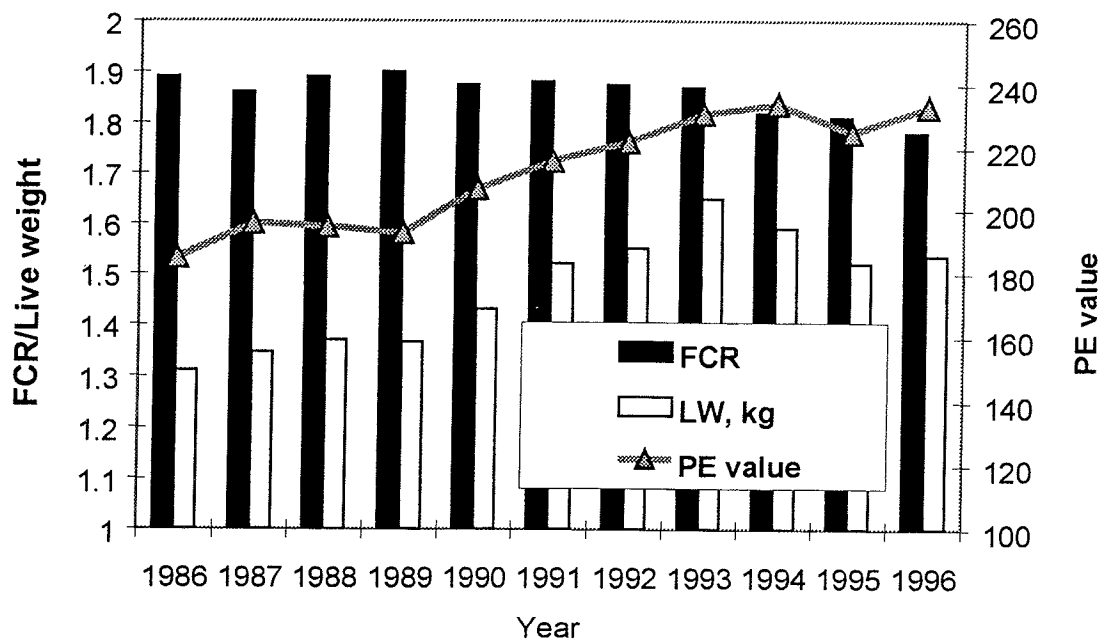


Figure 3. Average live weight, feed conversion ratio and PE-values (all corrected to 35 days of age) of broilers slaughtered in the Kristianstad processing plant between 1986 and 1996 (Lundström, 1999, personal communication).

Today over 95 per cent of broiler producers meet the health and management requirements of the national producer organisation, allowing those producers to keep their birds at a density of 36 kg/m² floor area, the highest bird density allowed today.

(c) Use of in-feed antibiotics

Today the use of in-feed antibiotics (excluding coccidiostats) is approximately 1/5 of that in the years prior to the ban (Figure 4).

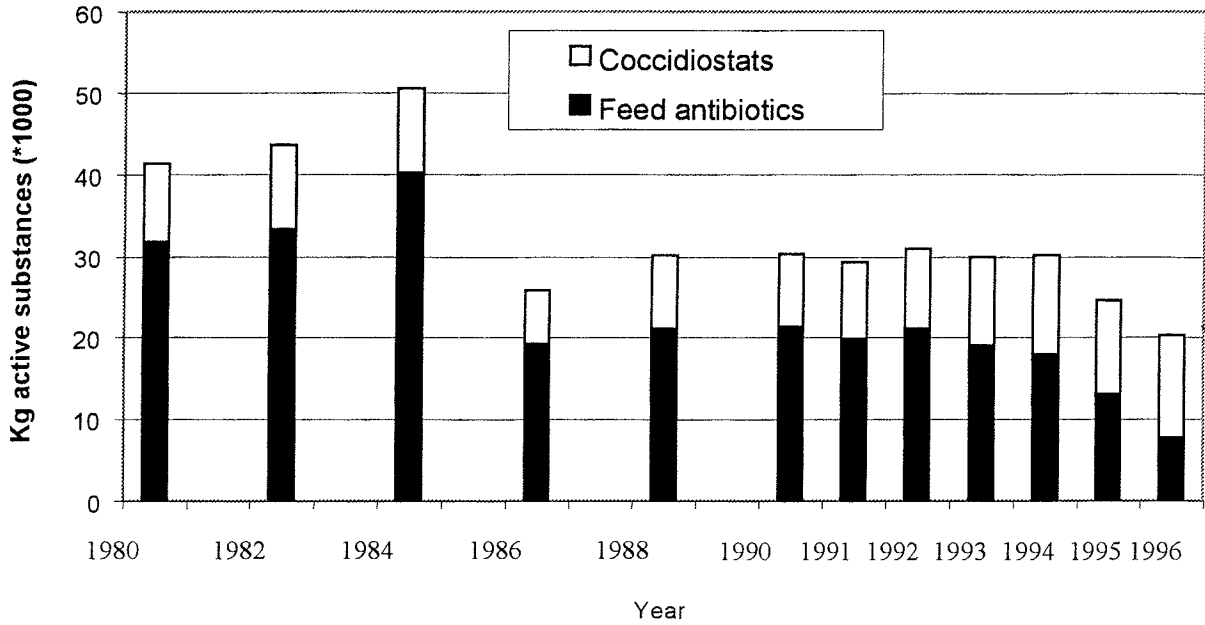


Figure 4. The use of in-feed antibiotics and coccidiostats in Sweden between 1980 and 1996 expressed as kg active substance (SOU, 1997).

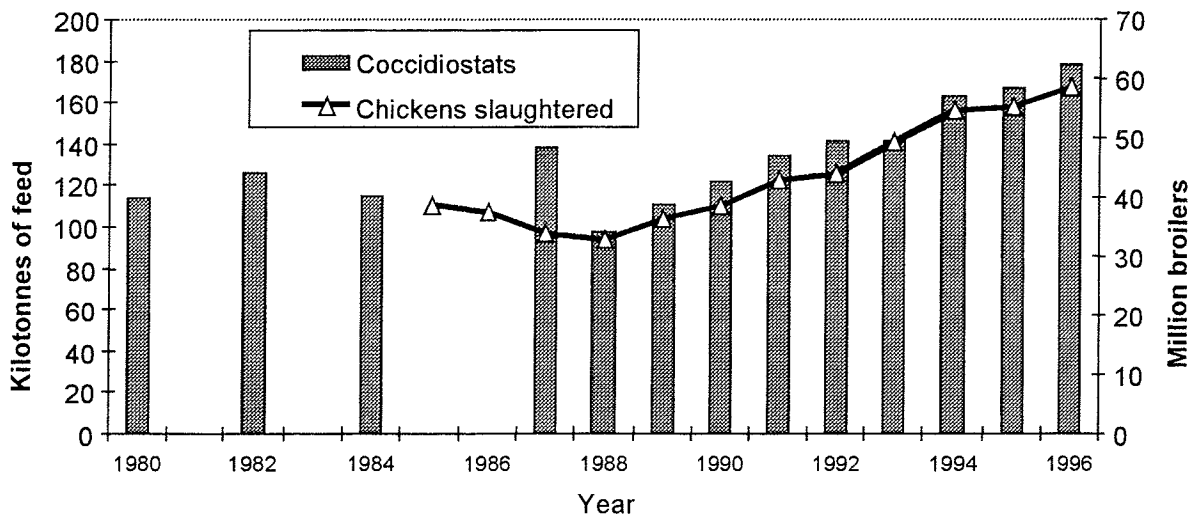


Figure 5. The use of in-feed coccidiostats expressed as kilotonnes of feed and the number of broilers slaughtered between 1980 and 1996 (Greko, 1998).

Coccidiostats are used on prescription in virtually all broiler feeds. The amounts used have increased at the same rate as broiler production (Figure 5). Narasin has been the coccidiostat of choice for the last six to seven years.

III. HYGIENE STATUS OF THE POULTRY INDUSTRY

(a) Salmonella control and outbreaks

A voluntary salmonella programme has been running in Sweden since 1970. Within the programme all generations are sampled for bacterial examination. The sampling procedure has been designed to detect salmonella infection if the prevalence of infected birds in a flock is higher than 5 per cent. Today the programme is compulsory for all egg and meat producing flocks, with a compliance of more than 95 per cent.

When salmonella of any serotype is found in grandparents, parents or meat producing birds the entire flock is destroyed. Layer flocks, however, are only destroyed if invasive serotypes are found.

The entire bird and egg handling chains are today covered by legislation and guidelines to prevent and detect salmonella infection in the poultry industry to prevent infection from reaching the consumers. These measures have proven to be quite effective as can be judged from the number of reported outbreaks during the past decades (Figure 6; Mårtensson *et al.*, 1983; Eld *et al.*, 1991; Malmqvist *et al.*, 1995.).

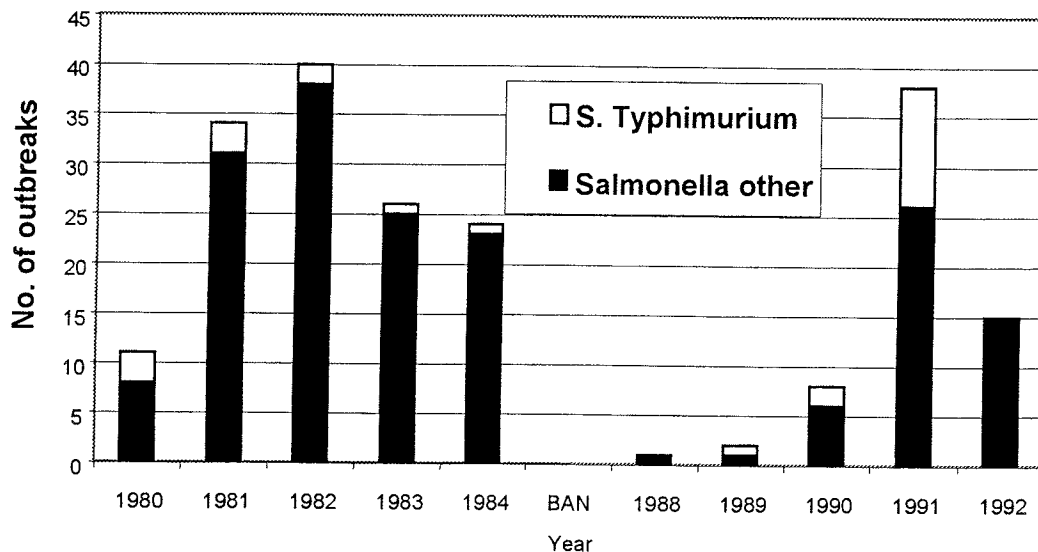


Figure 6. Number of reported salmonella outbreaks in poultry during two five-year periods in Sweden.

In 1992 a nation-wide investigation was carried out in order to examine the prevalence of salmonella bacteria in carcasses of beef, pigs and broilers after slaughter (Wahlström *et al.*, 1993). Approximately 3000 carcasses of each animal species were sampled. Salmonella was found in less than 1 per cent of the samples (Table 1).

Table 1. Prevalence of salmonella in carcasses of cattle, pigs and broilers after slaughter.

Animal species	No of abattoirs	No of animals	No of animals contaminated	Percentage contaminated
Cattle	13	2924	22	0.8
Pig	13	3026	4	0.1
Broiler	10	2730 (910*)	12	0.2-0.7

* three samples pooled to one

Certain salmonella serotypes seem to appear, disappear and then reappear again. There is no data suggesting that any serotype would have completely disappeared. Moreover, there is no evidence of any serotypes that would have been found year after year in poultry, for example. However, it appears that the number of reported outbreaks has decreased during the last decade. This is probably a result of the salmonella control programmes in place covering the entire chain of birds, eggs and feeds.

(b) Salmonella control in the feed industry

Monitoring and control of salmonella in animal feedstuffs has been carried out in Sweden since the late 1940s. Since 1958, data on salmonella of imported raw feed materials of animal origin as well as of domestic meat meals and feedstuffs have been compiled. In that same year, the major compound feed manufacturers formed the Association of Veterinary Feed Control (AVFC). The members agreed to set up and sponsor a laboratory at the National Veterinary Institute (NVI) to focus on feed hygiene. Salmonella soon became an important part of the work at the laboratory. Since this time, the laboratory for feed hygiene at the NVI has been actively involved in the process of developing methods for salmonella analysis and control in the feed industry as well as monitoring the hygiene status of feeds and feedstuffs (Häggbloom, 1995).

Today a hygiene control scheme, which is partly voluntary and partly implemented by legislation, is in place in the feed mills of the AVFC members, representing more than 90 per cent of the compound feed production in Sweden. This scheme is based on a HACCP (Hazard Analysis of Critical Control Points) approach, with the aim of preventing salmonella-contaminated raw materials from entering the feed mills and keeping bacteria counts at a minimum in all stages of the manufacturing process. This scheme includes:

- storage of high risk raw materials in quarantine until declared free of salmonella;
- hygiene control programmes at the feed mills, including cleaning and sampling of critical points (raw materials, dust, feeds) every week (5 samples/feed mill/week) for salmonella analysis;
- inspection of feed mills by authorised people once a year;
- mandatory heat-treatment of poultry feeds (min. 75° C), and of all other feeds manufactured in feed mills with poultry feed production;

Raw materials that frequently have been found contaminated with salmonella have been categorised into three classes based on previous records as follows:

- **S1** - all raw materials of animal origin (e.g. fish meal, meat meal, feather meal). These are put in quarantine and stored by the manufacturer until proven free of salmonella;

- **S2** - high risk raw materials of plant origin (e.g. soybean meal, rapeseed meal, coconut). These are kept in quarantine outside the feed mill until proven free of salmonella;
- **S3** - low risk raw materials of plant origin (e.g. "by-pass" soybean meals). These can enter the feed mill before the results of the salmonella analysis are available.

The number of samples to be drawn is related to the size of each shipment or batch. The sampling procedure has been developed to detect salmonella with a probability of 99 per cent. Sampling is usually carried out by skilled personnel of authorised companies.

In case salmonella is found in a "S2" raw material, it has to be decontaminated either by heat- or chemical treatment (e.g. acids or formaldehyde). The treatment adds USD 13-18/metric tonne to the raw material costs. New samples are taken and have to be free from salmonella before the material is allowed to be delivered to the feed mill.

When salmonella is found in any of the weekly samples from the feed mills different action programmes are carried out depending on where the contamination has been found. In some cases, e.g. if salmonella is found on the "clean" side i.e. after pelleting, manufacturing must stop and no feed must be delivered. The production lines have to be cleaned and disinfected and subsequent samples found salmonella-free before manufacturing can restart and feeds can be delivered again.

Each year a number of shipments of raw materials are found to be contaminated by salmonella. In 1995, on average 1.6 per cent of the samples were salmonella positive. Samples of compound feeds had the same incidence of salmonella positives, i.e. 2 out of 124. Of the two positive ones, one represented dog food and the other mink food. Out of more than 6,400 samples from the feed mills only 75 or 1.2 per cent were salmonella positive. This shows that the hygiene programmes are quite effective for controlling salmonella.

Further proof of the excellent salmonella status in Sweden is the fact that at present, less than 0.05 per cent of the farms holding either cattle, pigs or poultry are in quarantine due to salmonella infections.

The cost of the hygiene control schemes are quite high. In 1995, the cost of analysis amounted to US\$260,000 (SEK 2 million). Additional costs arise from other activities e.g. sampling, cleaning, heat-treatment of feeds, disinfection of equipment and feed mills, decontamination of raw materials and reformulation of feeds. These are estimated to be around US\$950,000 per annum (SEK 7,3 million) adding US\$0.55/metric tonne to the cost of feed based on the annual national tonnage of 2.2 million metric tonnes.

(c) Campylobacter infections

In a small scale investigation of 42 samples of frozen and 13 samples of fresh chickens taken from food stores in the Stockholm area nine were found contaminated with *Campylobacter* (Ekman, 1994). Due to the small number of samples, however, the results are not to be considered as representative for the *Campylobacter* status in Swedish poultry meat.

IV. CONCLUSIONS

The Swedish farmers and farmers' organisations have set themselves the goal of having "the cleanest agriculture in the world". Within the framework of the so called "Swedish model" the means and activities to reach this goal have been described. In short, these are as follows:

- restrictive use of antibiotics in animal production;

- salmonella-free animal production;
- a ban on the use of hormones;
- high animal welfare status;
- environmental care.

The ban on the use of antibacterial feed additives in 1986 together with the strict hygiene regulations in animal and feed production are corner stones of the "model". Meeting the standards has cost Swedish agriculture a lot of money. In return it has gained the confidence of the consumers and created an excellent marketing tool both for the domestic and export markets. It remains to be seen how this tool is being used. However, as the pro-ban forces seem to gain momentum the Swedish experiences appear increasingly valuable and can most likely be applied in many other countries. The Swedish "testing ground" has become a model for the future.

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JETACAR RECOMMENDATIONS – OUTCOMES FOR THE POULTRY INDUSTRY

T. M. GRIMES

Summary

International and domestic concerns by the medical profession and scientific community that essential antibiotics were becoming ineffectual due to microbial resistance resulted in the establishment of the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR) in late 1997. The purpose of JETACAR was to evaluate the Australian situation on the use of antibiotics in food-producing animals and the development of antibiotic-resistant bacteria in humans. JETACAR produced a report in October 1999 containing 22 recommendations for development of appropriate future management plans for antibiotic use, particularly in food-producing animals. While the poultry industry has been developing health programmes to reduce dependency on antibiotic use for many years, necrotic enteritis of meat chickens will require alternative methods of control if currently used antibiotics become unavailable or too expensive.

I. INTRODUCTION

The development and spread of antibiotic-resistant-bacteria in humans and the consequential difficulty in controlling some bacterial infections particularly in hospitals have become increasingly frequent topics for scientific discussion and media reports in recent years. Some segments of the medical profession fear that the situation is so serious that there will be a return to the “pre-antibiotic era” (prior to the 1940s) when bacterial infections commonly resulted in death of humans. While it is likely that antibiotic resistance has occurred mainly due to the use, overuse or improper use of antibiotics in man and the failure by the medical profession to control the spread of antibiotic-resistant bacteria, there are some in the scientific community that have targeted the use of antibiotics in animals, particularly food-producing livestock, as a possible cause of the antibiotic resistance problem in human medicine.

It was in this context that in December 1997 the Federal Minister for Health and Family Services and the Federal Minister for Primary Industries and Energy agreed to establish the Joint Expert Technical Advisory Committee on Antibiotic Resistance on The Use of Antibiotics in Food-Producing Animals: Antibiotic-Resistant Bacteria in Animals and Humans (JETACAR).

II. JETACAR OPERATION

JETACAR consisted of invited experts from public health, human medicine, veterinary medicine, molecular biology and primary industries which guaranteed a diversity of viewpoints.

The Terms of Reference given to the Committee were first, to review the scientific evidence on the link between the use of antibiotics in food-producing animals, the emergence and selection of antibiotic-resistant bacteria and their spread to humans; and secondly to develop evidence-based recommendations for the appropriate future management of antibiotic use in food-producing animals.

The Committee met on seven occasions between April 1998 and June 1999, communicated electronically between meetings, considered numerous recent international

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reviews on the topic, commissioned a review of the scientific literature on antibiotic resistance in four key bacterial pathogens, listed the current regulatory controls on antibiotics in Australia, compiled data on current use patterns in animals and man in Australia, discussed the current status of antibiotic resistance in Australia and addressed some of the benefits of antibiotic use in animals. As part of the information gathering process, 23 of 52 key stakeholders invited to provide scientific data and practical advice made submissions to the Committee and subsequently comments from 35 stakeholders on the draft Report were considered before the Report was finalised.

JETACAR produced a lengthy Report containing 22 recommendations in October 1999 (JETACAR, 1999).

III. JETACAR RECOMMENDATIONS

The JETACAR recommendations can be broadly grouped into seven categories, namely Regulatory Controls (1-9), Monitoring and Surveillance (10 and 11), Infection Prevention Strategies and Hygienic Measures (12-14), Education (15-17), Further Research (18), Communication (19 and 20) and Co-ordination of the Resistance Management Programme (21 and 22).

Below is a precis of recommendations and comments on the possible outcomes for the poultry industry.

(a) Recommendation 1

That antibiotics used as growth promotants or with similar use patterns not be registered unless they are efficacious, are not used as systemic therapeutic antibiotics in humans or animals and are not likely to impair the efficacy of other antibiotics through the development of resistant strains of organisms.

This recommendation reaffirms in general the recommendations of the Swann (1969) and the World Health Organisation (WHO Berlin, 1997) Reports and has been applied since the early 1970s by the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) and the Working Party on Antibiotics of the Therapeutic Goods Administration (WPA) when registering antibiotics, including growth promotants (Table 1), for animal use in Australia.

Table 1. Antibiotics registered as growth promotants in Australia.

Antibiotics Class	Growth Promotant	Registered Animal Species
Arsenical	3-nitro-arsonic acid	Pigs, Poultry
Bambermycin	flavophospholipol	Cattle, Pigs, Poultry
Glycopeptide	avoparcin	Cattle, Pigs, Meat Poultry
Macrolide	tylosin	Pigs
	oleandomycin	Cattle
	kitasamycin	Pigs
	lasalocid	Cattle
Polyether (ionophore)	monensin	Cattle
	narasin	Cattle
	salinomycin	Pigs, Cattle
	bacitracin	Meat Poultry
Polypeptide	olaquinox	Pigs
Quinoxaline	virginiamycin	Pigs, Meat Poultry
Streptogramin		

Source: JETACAR REPORT 1999

(b) Recommendation 2

That antibiotics currently registered as growth promotants, that do not now appear to fulfil the criteria in Recommendation 1, undergo a review by the NRA with a priority on glycopeptides (avoparcin), streptogramins (virginiamycin) and macrolides (tylosin, kitasamycin, oleandomycin).

Avoparcin was already undergoing a review prior to the JETACAR Report because of purported links to the development of bacterial resistance to vancomycin, which is now considered a critical antibiotic for human use. Avoparcin has never been registered in northern America, its use was suspended in Europe in 1998 and the manufacturer (Roche) has recently withdrawn it from sale in Australia.

Virginiamycin was listed because the streptogramin quinupristin/dalfopristin is now considered a likely critical antibiotic for treatment of vancomycin-resistant enterococci (VRE) and multiresistant *Staphylococcus aureus* (MRSA) in humans.

If antibiotics fail a NRA Review, JETACAR accepted the principle that industries must be given a phaseout period in which to develop alternative health programmes.

(c) Recommendation 3 (and 11)

That importers of antibiotics be licensed, that more accurate records of antibiotics imported be compiled and that audit trails be developed from importer to end-user to better define use patterns of antibiotics in animals in Australia.

All antibiotics used in Australia are imported. Currently permits to import antibiotics are issued by the Therapeutic Goods Administration (TGA) and data are collated by both the TGA and the NRA. For example, data collected between 1992 and 1997 indicate that 64% of antibiotics imported were for animal use and that antibiotics with growth promotant claims constituted the majority of the antibiotic active ingredient imported.

The intent of this recommendation is to improve the existing process and to extend data collection past the importer. The poultry, feed and chemical industries could currently provide some components of this information.

(d) Recommendations 4 and 5

That the NRA apply a Risk Analysis of Microbial Resistance Safety (Special Data Requirements for New Antibiotic Applications) for all new applications, major extensions of use and reviews of currently registered antibiotics.

This process will formalise and make the existing process more transparent and accountable. In addition post-registration antibiotic resistance monitoring has been included as a requirement.

It is possible that this upgraded process will result in fewer antibiotics being registered for food-producing animals. Already some antibiotics registered overseas for use in poultry, eg. fluoroquinolones, cephalosporins, nitrofurans, chloramphenicol, gentamicin and colistin, are not registered for use in poultry in Australia. It is also likely that the cost of antibiotics to the poultry industry will increase as a result.

(e) Recommendation 6

That all antibiotics be classified as prescription drugs.

Currently most of the antibiotic active ingredient used in food-producing animals in Australia (particularly for growth promotion and for some prophylactic use patterns) does not require veterinary intervention. This is in contrast to the situation where antibiotics are used in individual animals or humans. In this case, a veterinary or medical prescription is required.

The intent of this recommendation is to make the veterinary profession more responsible for the use of antibiotics in food-producing animals. Since the poultry industry is already well serviced by veterinarians, appropriate availability and cost should not be affected by this recommendation.

(f) Recommendations 7 and 8

That the Agricultural Resource Management Council of Australia and New Zealand (ARMCANZ) harmonise legislation on Control of Use of antibiotics between states.

The current situation where a veterinarian can prescribe some antibiotics for specific uses in some states but not others (Table 2) does not appear to have any rational scientific basis.

Table 2. Legislation to control use of antibiotics in food-producing animals by veterinary script*

	QLD	NSW	VIC	SA	WA
Use of unregistered products	Yes	No	Unscheduled ingredients only	Yes	Scheduled ingredients only
Use of human medicines	Yes	No	Single animals only	No	Yes
Use of products compounded by veterinarian	Yes	No	Unscheduled ingredients only	No	Yes
Varying dose or treatment regime	Yes	Yes	Yes	Yes	Yes
Use on off-label species	Yes	Yes	Yes unless prohibited	Yes	Yes
Use contrary to label prohibitions	Yes	Yes	No	Yes	Yes

* Lee Cook, Personal Communication 1999

(g) Recommendation 10

That a formal antibiotic resistance monitoring system be established in food-producing industries.

The Chicken Meat Committee of the Rural Industries Research and Development Corporation has been proactive in supporting a project entitled "Antibiotic Resistance in Bacteria isolated from Poultry" supervised by Dr. Mary Barton at the University of Adelaide, partly to develop technical methods that could be useful for future monitoring programmes.

The mechanism and funding of the proposed monitoring system will need discussion, but the poultry industry has an existing precedent in the chemical residue monitoring programme undertaken by the National Residue Survey (NRS) of the National Office of Food Safety of the Commonwealth Department of Agriculture, Fisheries and Forestry (AFFA).

It is likely that the cost of antibiotic use by the poultry industry will increase due to this requirement to conduct resistance monitoring.

(h) Recommendation 12

That Hazard Analysis Critical Control Point (HACCP) Programmes be implemented as a means of reducing the contamination of food products with foodborne organisms, including antibiotic-resistant organisms, and that these programmes include on-farm infection control.

The Australian Standard for Hygienic Production of Poultry Meat for Human Consumption (ARMCANZ, 1997), which is based on HACCP principles and which now includes a requirement for microbiological testing, forms the basis for processing of poultry meat in Australia. Poultry companies are being required by retail customers to implement audited food safety programmes, including on-farm HACCP programmes. ARMCANZ has endorsed a poultry national biosecurity plan that includes food safety elements and the Australian Animal Health Council (Turner, 1999) is currently developing this plan in conjunction with industry.

Hence the poultry industry is already undertaking substantial measures to provide safe food, but the chicken meat industry may need to formalise and promulgate a HACCP programme for livestock production. Such a programme will need to address procedures to limit the development and transfer of antibiotic-resistance bacteria.

(i) Recommendation 13 (and 18)

That alternatives to antibiotics be researched and developed to control bacterial diseases and improve feed utilisation.

Long term uses of antibiotics in feed for prevention of disease (prophylaxis) and feed enhancement (growth promotion) are particularly being targeted by the medical profession and microbiologists.

The Australian poultry industry has a proven record of developing vaccines to control bacterial diseases such as fowl cholera (*Pasteurella multocida*), infectious coryza (*Haemophilus paragallinarum*), duck infectious serositis (*Riemerella anatipestifer*), chronic respiratory disease (*Mycoplasma gallisepticum*), infectious synovitis (*Mycoplasma synoviae*), turkey erysipelas (*Erysipelothrix rhusiopathiae*) and paratyphoid (*Salmonella typhimurium*). Necrotic enteritis caused by *Clostridium perfringens* is the only disease of poultry which is still controlled by in-feed antibiotic programmes based on avoparcin, virginiamycin or bacitracin.

The Chicken Meat Committee of the Rural Industries Research and Development Corporation has recently supported four projects related to development of possible alternatives to antibiotics for control of necrotic enteritis, including vaccines.

Alternatives such as organic acids, competitive exclusion products, probiotics, prebiotics, oligosaccharides, nucleotides, essential oils, enzymes, and plant extracts are being trialled in Europe where many in-feed antibiotics can no longer be used. The Australian chicken meat industry may be able to benefit from the experiences of European poultry companies.

(j) Recommendations 15-17

That Prudent Use Guidelines/Codes of Practice be developed, used, updated when required and promulgated by stakeholders of antibiotic use. Principles to control antibiotic resistance should be included.

The Australian Veterinary Poultry Association developed a Code of Practice for the Use of Schedule 4 Restricted Substances in the Poultry Industry in 1987 and this was updated in 1995. A further update, including more emphasis on the control of antibiotic resistance and extracts of the World Veterinary Association Prudent Use Guidelines (WVA *et al.*, 1999), will be needed.

The poultry, feed and chemical industries should be made aware of the key principles in this updated Code.

IV. PERSPECTIVES

Some facts recognised by JETACAR that warrant mention include:

- The use and overuse of antibiotics in human medicine is the major factor contributing to the development of antibiotic resistance in man.
- Australia has one of the highest usage rates (25 defined daily doses per 1000 population per day) of antibiotics in humans in developed countries.
- Antibiotic-resistant bacteria and resistance genes can be spread internationally by people and food movements.
- The predominant VRE isolated to date in Australia are of a type (*vanB Enterococcus faecium*) that has not been associated with avoparcin use in animals.
- While avoparcin use in animals in Australia has remained relatively constant, the use of vancomycin in humans has increased by more than 300% since 1992 (annual import of 299 kg in 1992-94 compared with 868 kg in 1994-97).
- Australia does not have fluoroquinolone-resistant campylobacter or multiresistant *Salmonella typhimurium* DT104, that are of concern overseas, and fluoroquinolones are not registered for use in food-producing animals in Australia.
- Australian poultry flocks are free of *Salmonella enteritidis*, the major salmonella type infecting people in many overseas countries.
- The recorded quantity of antibiotics used in animals in Australia is substantially elevated by the inclusion of some chemicals that are mainly used as antiprotozoals, eg. coccidiostats and histomonostats.
- Australia already has one of the strictest registration processes in the world for the supply and use of antibiotics in animals.
- The results of antibiotic residue monitoring by the NRS in the last decade indicate excellent compliance with antibiotic withdrawal periods by the poultry industry, giving consumers considerable confidence that Australian poultry meat and eggs do not contain antibiotic residues.

V. CONCLUSIONS

The JETACAR recommendations are likely to accelerate a process that has been occurring for some years in the Australian poultry industry, namely less reliance on antibiotics to control bacterial diseases.

Other disease control measures including eradication, vaccination, hygiene, biosecurity, husbandry and nutritional programmes will need to continue to be applied to the

control of bacterial infections. Strategic, short term, scripted antibiotic regimes may be used as an adjunct in the future.

The range of antibiotics available to food-producing industries is likely to continue to constrict, due to registration and post-registration requirements becoming even more rigorous and costly. Less availability of antibiotics internationally due to fewer antibiotics being developed for animals is likely to be a factor in this process.

The poultry industry in Australia has always supported research on topics that are critical to the industry and this is already occurring for the development of alternatives to antibiotics to control necrotic enteritis.

Similarly the industry has always been industrious in transferring relevant technology and research findings from overseas. Once again there could be a need for this approach.

The industry will need to continue to demonstrate a willingness to self regulate the control of antibiotics to convince consumers and public health officials that the industry is adopting a responsible attitude to the control of antibiotic-resistant bacteria and antibiotic resistance genes.

While it is clear that overuse and improper use of antibiotics is the major reason for increasing antibiotic failure in humans, it is also clear that food-producing industries will increasingly need to justify the use of specific antibiotics and particular dosage regimes.

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THE ROLE OF NUTRICINES IN HEALTH AND TOTAL NUTRITION

C. A. ADAMS

Summary

Feed is more than a supply of nutrients. It is also inextricably linked to disease avoidance and health maintenance. Feed is an enormous collection of different molecules which can be classified into two major groups; nutrients and nutricines. Nutrients are the generally recognised components of feed such as carbohydrates, fats, proteins, minerals and vitamins. Nutricines are components of feeds that exert an influence upon health and nutrition, yet are not direct nutrients. Important nutricines are antioxidants, antimicrobial compounds, non-digestible oligosaccharides, enzymes, emulsifiers, flavours and colours. Nutricines are those components of feed that link health and nutrition. Consideration of both nutrients and nutricines in feeds leads to the concept of Total Nutrition where feeds must be designed to supply nutrients, to avoid diseases and to maintain health.

I. INTRODUCTION

The challenge in modern nutrition is to develop diets that not only provide essential nutrients but also contribute to disease avoidance and to health maintenance. Feed must be safe and free from pathogens. It must sustain an efficient immune system, which is not activated unnecessarily, does not lose the property of self-tolerance, and is not unduly suppressed. Feed must protect the animal against the ravages of oxidation and mitigate the development of non-infectious diseases.

In the recent past many of the challenges to health of poultry were overcome by use of antibiotics either as growth promoters or as therapeutic agents (Gustafson and Bowen, 1997). However in the European Union (EU) several antibiotic growth promoters; virginiamycin, spiramycin, zinc bacitracin, tylosin phosphate, avoparcin, carbadox and olaquinox, have now been prohibited. This leaves only avilamycin, flavomycin, salinomycin and monensin for use as growth promoters in animal nutrition. There is also considerable consumer demand to reduce and even eliminate the use of all antibiotics in poultry production. In response to these pressures some major poultry producers and retailers have indicated that they will produce and market poultry without recourse to antibiotics.

As a consequence of various food safety issues in the EU there is also a much greater emphasis now upon production of "Organic Foods." This is officially recognised by the EU in a new document {Council Regulation (EC) No. 1804/1999}, published in July 1999. This document deals with organic production of livestock and strictly defines feedstuffs and practices permitted for the production of organic foods.

All these recent developments in the EU necessitate another approach to poultry nutrition. In the past poultry feed has usually been considered as a source of nutrients and we certainly have accumulated a wealth of information on the nature, necessary amounts and availability of essential nutrients. In practical reality however, animals consume a great diversity of different molecules in feed in addition to the conventional nutrients. Many of these molecules have been regularly consumed for thousands of years and play an important role in animal and human health and welfare.

Feed is now being seen as more than just a collection of nutrients but consisting of an enormous collection of different molecules which can be classified into two major groups; nutrients and nutricines (Figure 1), (Adams, 1999a). Nutrients are the generally recognised

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components of feed such as carbohydrates, proteins, fats, minerals and vitamins. Nutricines are components of feeds that exert a beneficial effect upon health and metabolism yet are not direct nutrients. Important nutricines are; antioxidants, antimicrobial compounds, non-digestible oligosaccharides, enzymes, emulsifiers, flavours and colours. In poultry nutrition, flavours and colours are of little consequence but the concept of nutricines is applicable to both animal feeds and to human foods. The nutricines are those components of feeds and of foods that link health and nutrition.

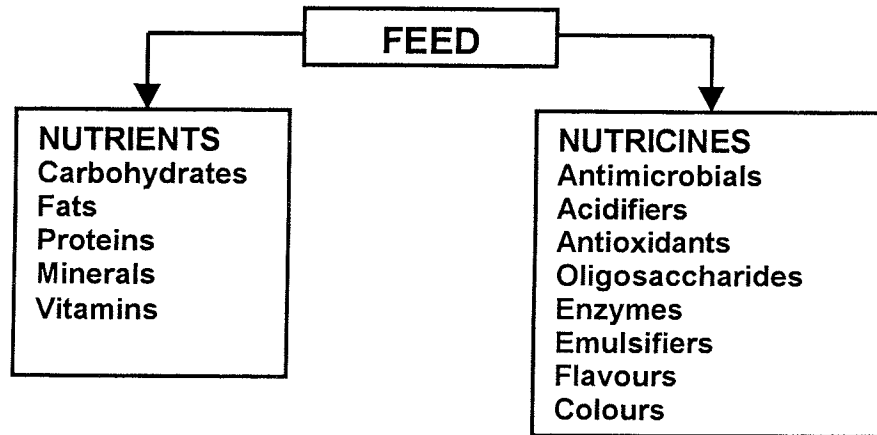


Figure 1. Components of feed: nutrients and nutricines.

Nutricines play an important role in establishing and maintaining health in animals and humans (Figure 2). They may: prevent oxidative damage, control the growth of micro-organisms in feed and food, stimulate appetite, influence the immune system, assist digestion and absorption of nutrients, and modify the microflora in the gastro-intestinal tract.

Large scale raising of poultry, pigs and cattle is necessary to provide us with the required quantities of low cost food and there is increasing consumer pressure to do this without recourse to antibiotics and to other drugs. There is also a requirement to reduce the discharge of nutrients by the animals in the form of manure. Animal production however must remain economically viable and animal health must be maintained to satisfy welfare concerns. The judicious use of a range of nutricines will assist in the maintenance of large scale, low cost production of food of animal origin, which ultimately benefits the human consumer.

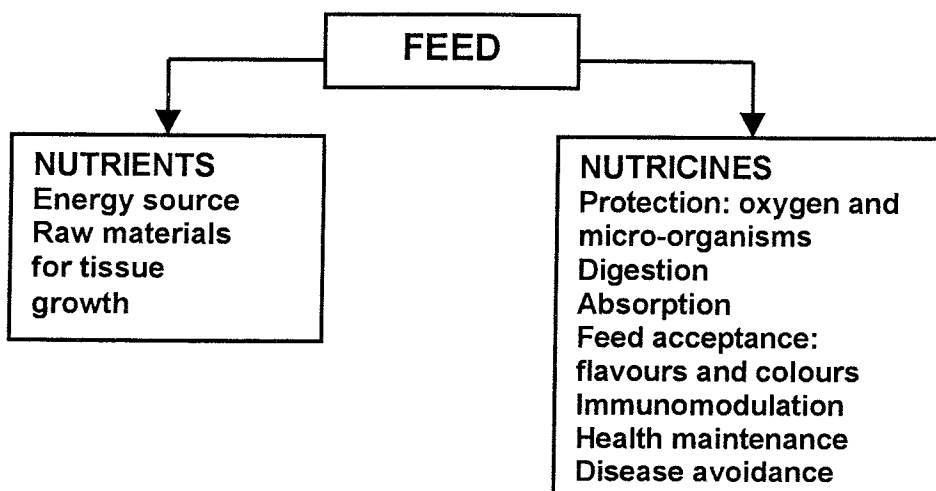


Figure 2. Function of nutrients and nutricines in health and nutrition.

Greater awareness of nutraceutical components of food will extend our knowledge of diet and nutrition and allow us to develop further the connection between health and nutrition. The quantities and type of nutrients necessary for growth of animals and of humans is well defined. It is however less well understood whether the general nutritional requirements for growth and maintenance of body weight are equally suitable for disease avoidance, control of oxidation, development of the immune system and maintenance of health and well-being.

II. MODE OF ACTION OF NUTRACINES

Nutraceuticals are a diverse group of chemical compounds and exert many different effects in nutrition and health. Nutraceuticals are of fundamental importance in the safety of feed raw materials and of stored feeds. They influence acceptance and voluntary consumption of feeds. They improve digestion and absorption of nutrients from the gastro-intestinal tract and modify the micro-flora in the gastro-intestinal tract. Some nutraceuticals also have a systemic effect and influence the metabolism of the body to avoid disease and promote health. They are important in supporting the immune system (Table 1).

(a) Feed safety

Conservation of raw materials of food and feeds for both human and animal nutrition is of fundamental importance. It is also a major challenge, since the very nature of these materials makes them susceptible to contamination and to spoilage by insects, moulds and bacteria. Microbiological safety of feed is a perennial issue and there is an endless search for new and novel agents to control the growth of feed-borne pathogens and spoilage organisms. Many components in feeds are susceptible to oxidation. Oxidised feeds are unpalatable due to rancidity and lose nutritional value if fats and vitamins are destroyed by oxidation. Consumption of oxidised foods is also not desirable and may lead to increases in oxidative stress that is related to the onset of several non-infectious diseases (Aruoma, 1998).

Table 1. Problems in nutrition and health and appropriate nutraceuticals.

Problem	Nutraceutical
Feed safety	Organic acids, phenols, peptides, antioxidants
Voluntary feed intake	Flavours, sweeteners, colours
Nutrient digestion and absorption	Enzymes, emulsifiers
Microflora in GI tract	Non-digestible oligosaccharides, organic acids
Immune system	Antioxidants, peptides, plant extracts
Non-infectious diseases	Antioxidants, phyto-oestrogens
Manure production	Enzymes, emulsifiers

Organic acids are the most widely used nutraceuticals for food and feed preservation. They have a wide range of functions not directly related to nutrition (Tamblyn and Conner, 1997). They provide acidity which contributes to flavour, and retards enzymatic deterioration. They act as chelating agents that bind metals which helps in preventing metal-catalysed oxidations. Organic acids are powerful inhibitors of microbial growth and are used extensively in feed for young animals such as piglets to alleviate scouring and general digestive disorders (Partanen and Mroz, 1999).

There is now increasing interest in the application of organic acids in poultry production following the recent prohibitions of antibiotic growth promoters in the EU

(Adams, 1999b). The various acids used in foods and feeds are listed in Table 2. Generally they are all small molecules with molecular weights less than 200. Lactic, propionic, acetic, formic and phosphoric are all liquids in the pure state. The others are all solids. Phosphoric acid is an inorganic acid but is widely used in feeds and foods together with the other organic acids.

Table 2. Various acids used in foods and feeds.

Acetic	Lactic
Benzoic	Phosphoric
Citric	Propionic
Formic	Sorbic
Fumaric	Tartaric

The mode of action of these various organic acids in controlling the growth of moulds and of pathogenic bacteria is still not completely established. There seems to be a pH effect and also the effect of the anions. Also there is some degree of specificity in that propionic acid is a very powerful mould inhibitor whilst lactic acid is a powerful bacterial inhibitor (Shelef, 1994).

All vertebrates, from amphibians to mammals, seem to have developed a non-specific chemical defence system based on a series of broad-spectrum antimicrobial peptides (Gururaj Rao, 1995). They are able to destroy numerous pathogenic micro-organisms including viruses, bacteria, protozoa, yeasts and fungi (Giacometti *et al*, 1998).

The mechanism of action of antimicrobial peptides is that they form pores in cytoplasmic membranes of micro-organisms which lead to an increase in permeability and impair the energy-generating system. Peptides inhibit microbial growth very rapidly because they are easily able to invade microbial cells. They are also extremely potent and often kill susceptible bacteria at a concentration of less than 4 ppm (4 mg/kg) (Hancock and Lehrer, 1998).

Antimicrobial peptides generally have a high degree of heat stability and this would make them useful preservatives for both animal feeds and human food. Furthermore they could be useful agents to guard against recontamination after pasteurisation. Alternatively lower processing temperatures can be employed. This will improve nutritional value, flavour, texture and appearance of feeds.

The peptide nisin is currently used in food preservation, but is too expensive for use in feeds. It is a peptide of 34 amino acids and is quite effective against Gram positive bacteria, (Delves-Broughton, 1990).

Oxidation of fats and oils in feeds has been extensively studied over many years and numerous antioxidants have been developed (Benzie, 1996). Antioxidants are defined as substances which, when present at much lower concentration than an oxidisable substrate, significantly delay or prevent its oxidation. Antioxidants play two major roles in nutrition. Firstly they protect feed from oxidation and secondly they are important in living tissues to control oxidative stress. Antioxidants may also enhance immunity in animals and consequently improve animal production efficiency (Chew, 1996). Usually different antioxidant molecules contribute to these functions.

As with so many feed and food components today, there is a great interest in the use of natural antioxidants to protect feeds. Many plants contain molecules with antioxidant activity. Some of the better known are the tocopherols from seeds, extracts of rosemary, and catechins from green tea (Chen and Chan, 1996) and there is much active research into new sources of natural antioxidants. At present natural antioxidants, due to their greater expense compared to

synthetic products, are mainly used in petfoods. It may be some time before they are routinely used in poultry feeds.

(b) Voluntary feed intake

Successful nutrition requires feed to be consumed in adequate quantities to support health and development. Flavours, sweeteners and colours play a role in ensuring suitable voluntary food intake in humans and in some animal species. Both humans and animals have developed a flavour sensing system that is related to food or feed quality. It encourages the consumption of energy dense materials such as carbohydrates and fats. Poor quality or spoiled materials frequently have unattractive flavours. Some flavours, particularly herb and spice extracts, also have antimicrobial and antioxidant activities and could be useful in terms of food and feed safety.

Humans and many species of animals have quite positive flavour preferences although poultry in general seem largely unresponsive to flavour. There are however molecules which poultry will reject and not consume such as methylanthranilate and some essential oils but they do not show very pronounced positive responses to flavours (Rose, 1991).

For humans, food colour is of major significance and is frequently perceived as an indication of quality. Colour is not necessarily an indication of quality, but nevertheless, it has a major impact upon our behaviour in terms of which foods we buy and what food we eat.

Many animal species do not have good colour vision and therefore colour of feeds is not a criterion for acceptance.

(c) Nutrient digestion and absorption

Monogastric animals eat many molecules they cannot break down because they do not produce the appropriate digestive enzymes. Pentosans, beta-glucans, fructans, cellulose, lignin, and phytic acid are common examples in feeds. Consequently there has been great interest in recent years in supplementing feeds with enzymes in order to help in the digestion of feed components. These nutraceuticals have been particularly successful in the poultry industry. Furthermore, enzymes are also approved by the EU in Council Regulation (EC) No. 1804/1999 which covers production of organic livestock. Therefore we can expect to see ever-greater use of enzymes in poultry production in the EU.

Absorption of nutrients is assisted by natural emulsifiers in the bile secretions. Phospholipids, in particular, play an important role in nutrient absorption as powerful emulsifiers and by the formation of micelle structures in the gastro-intestinal tract.

A class of phospholipids, the lysophospholipids, are of interest in nutrition as they are more hydrophilic than other phospholipids and spontaneously form micelles with bile salts, fatty acids and monoglycerides. These micelles are small and very stable and consequently lysophospholipids may enhance absorption of nutrients after digestion.

A commercial lysophospholipid mixture has been studied in various animal species (Schwarzer and Adams, 1996) and shows useful benefits in improving nutrient utilisation.

(d) Microflora in the gastro-intestinal tract

The microflora of the gastro-intestinal tract are largely located in the large intestine, and can have positive or negative effects on animal health and performance. There is increasing interest in possible manipulations of the microflora in the gastro-intestinal tract by the use of non-digestible oligosaccharides, (Gibson and McCartney, 1998) and organic acids. Many carbohydrates based on mannose, fructose or pentoses are not digested in the small intestine in

monogastric animals, but are fermented in the large intestine where they may have beneficial effects in encouraging the growth of desirable bacteria such as lactobacilli and bifidobacteria. There is also some evidence for a systemic effect of non-digestible oligosaccharides in lowering blood lipid content by modifying activity of hepatic enzymes (Delzenne and Kok, 1998).

(e) Immune system

The characteristics of a diet can influence susceptibility of the individual to infectious diseases and to the development of allergies and arthritis which are influenced by the status of the immune system. Subtle changes in nutrients and nutraceuticals may at times be of critical importance in disease development or disease avoidance and in regulating the immune status of the animal.

Immune challenges and subsequent physiological events starting from the production of proinflammatory cytokines such as interleukin-1, interleukin-6 and tumour necrosis factor extend well beyond the immune system (Spurlock, 1997). The cytokines produced during periods of immune challenge orchestrate a response in which nutrients are directed away from tissue growth to ensure an adequate supply of nutrients for proliferation of macrophages, antibody production, and hepatic synthesis of acute phase proteins. This is a major obstacle to animals achieving their genetic potential for growth and good economic performance.

Many herb and spice mixtures are currently being marketed on the basis of an immunomodulating effect. A wide range of plant species are being used including oregano, sage, thyme, rosemary and garlic. This is playing a major role in poultry nutrition in the EU now that many antibiotic growth promoters have been banned. Whilst dietary components clearly influence the immune system it is not easy to determine which components are active. There is some evidence that carotenoids enhance immune response. Linoleic acid, usually considered as a nutrient, acts as a nutraceutical in an immune response. Linoleic acid is elongated into arachidonic acid that is ultimately converted into metabolically active molecules such as prostaglandins, leukotrienes, and thromboxanes (Smith, 1985).

(f) Non-infectious diseases

One of the indisputable benefits of our modern society is that diseases caused by infectious organisms have declined dramatically both in the human population and in our animals raised for food. As mortality from infectious diseases decline more people and animals will inevitably succumb to non-infectious diseases such as ascites, liver diseases, cancers, heart diseases, strokes, diabetes, and arthritis. It is a curious paradox that modern diets, and wide availability of food, are now perceived as contributing to the prevalence of these non-infectious diseases in the human population.

Guidelines for health-promoting diets are widely disseminated. These usually recommend a reduction in total and saturated fat consumption, an increase in consumption of complex carbohydrates and an increase in consumption of fruits and vegetables. This, however, is only one strategy to improve health through nutrition. An alternative strategy is to enrich foods with beneficial components, the nutraceuticals, which may be lacking in modern diets. This has already been enthusiastically undertaken by the food manufacturing industry with the production of a wide range of "Functional Foods" and "Nutraceuticals."

There are at least three major areas of active research into nutraceuticals and non-infectious disease today. Dietary fibre and non-digestible oligosaccharides are protective factors against some cancers and problems of cholesterol. An understanding of the adverse effect of free radicals and oxidative stress in the body has encouraged research on antioxidant nutraceuticals in foods. Much attention is now focussed on how nutraceuticals influence the

oxidative potential of tissues. Carotenoids, tocopherols, uric acid, glutathione, and polyphenols are important cellular antioxidants and are implicated in avoidance of cancers, heart diseases and strokes. Glucosinolates found in plants of the *Cruciferae* and phyto-oestrogens that occur in soyabeans and cereals are now actively being studied as potential anticancer agents.

(g) Manure production

Considerable research is under way in many countries to reduce the impact of modern intensive livestock production upon the environment (Jongbloed and Lenis, 1998). The major concerns involve disposal of manure and generation of noxious odours and increases in the population of flies.

Application of nutraceuticals such as phytase and pentosanases has already had some benefits in reducing phosphorus contents of diets. Addition of urease inhibitors and of organic acids to feeds may reduce ammonia emissions. Improvements in digestion and absorption of nutrients is of major importance here and increased use of enzymes and emulsifiers is likely.

Bacteria in the large intestine synthesise amino acids that are available to the host (Fuller and Reeds, 1998). This means that there is a difference between the metabolic requirement for an amino acid and the dietary requirement for the same amino acid. Judicious application of various nutraceuticals may be able to further promote this synthesis of amino acids in the large intestine and require less to be added in the feed. Nitrogen excretion can be substantially decreased if the protein level is lowered by more than 2% in feed (Jongbloed and Lenis, 1998), but this will demand radical reformulation of animal feeds to minimise manure output as well as to maximise animal growth and performance.

III. TOTAL NUTRITION

Feeds and foods must firstly supply essential nutrients to animals and humans. Modern nutrition however is now increasingly concerned with the kinds and amounts of both nutrients and nutraceuticals needed to obtain good growth and performance, and to maintain physiological and mental functions. Our consumer-oriented society is increasingly looking to nutrition to minimise development of various non-infectious diseases, to support the immune system, and to deal with infectious diseases and auto-immune diseases. These requirements are of concern both for human nutrition and for the nutrition of animals raised for food.

There may well be different levels of nutrients and nutraceuticals required to fulfil these various functions. It will be necessary to define the amount of nutrients and nutraceuticals to support basic nutrition, to provide health benefits and the amounts that may lead to health hazards or toxic problems. Perhaps we are looking at a new concept called "Total Nutrition".

The recognition that some feed components, nutraceuticals, might be required in substantial amounts, yet not have a nutrient function, indicates that the concept of nutritional essentiality should be reconsidered. The conventional concept of essentiality was based on observational and experimental findings that nutrients function to prevent deficiency diseases.

In the new concept of "Total Nutrition," the minimal level of any feed or food component, nutrient or nutraceutical, that affects the metabolism and gastro-intestinal function in a manner beneficial to good health must be considered. This less rigid concept of essentiality recognises feed as being much more than a collection of essential nutrients but also as an important source of nutraceuticals which impact upon the health of our commercial animals. It

also suggests that optimum health might be difficult to achieve by simply feeding mixtures of basic nutrients.

Perhaps the future is not to define optimum nutrient intakes but rather to determine the Total Nutrition necessary to give optimum health and nutrient status in the animal or human target. Total Nutrition could be assessed by measurement of various functional indices which should be directly related to disease mechanisms or ill health. Examples of general functional indices useful for indicating optimum nutritional status and thus setting the limits for Total Nutrition are; immune function, antioxidant status, glucose tolerance, bone health, muscle strength, blood pressure, arterial compliance, DNA repair, work capacity, and cognitive performance.

Total Nutrition may be defined when a functional index reaches a certain quantitative value where it is no longer affected by intakes or stores of a particular nutrient or nutrient. Total Nutrition covers the nutritional status associated with the prevention of overt deficiency disease, the nutritional status associated with toxic symptoms and the nutritional status associated with good health. Clearly a lot more work needs to be done so that a cause and effect relationship is established not only between nutritional status and the functional indices but also between nutritional status and health. This will be a daunting task for nutritional research in the future.

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BRITISH SUPERMARKETS: FORGING CHANGES IN POULTRY NUTRITION

J. RATCLIFF

Summary

The pro-active response of British supermarkets to the potential risk of the transfer of antibiotic resistant micro-organisms from animals to humans has resulted in the removal of antibiotic growth promoters (AGPs) from most broiler feeds within the UK. It is likely that AGPs will be banned from all poultry during 2000. Their removal has resulted in the search for "natural" and safe alternatives. Alternatives to AGPs need to be properly evaluated and due consideration given to the quality, safety and efficacy of each product. It is unlikely that a single product will emerge as a direct replacement for the registered effect of AGPs. It is more likely that a combination of products may be considered together with a review of the various stress factors that may affect performance and disease, including nutrition, environment and management practices.

I. INTRODUCTION

The influence of the British supermarkets on poultry nutrition dates from the late eighties with the collapse in confidence in the UK feed industry resulting from the Salmonella and Bovine Spongiform Encephalopathy crises. Since that time animal feed has hardly been out of the press throughout Europe, thanks to a continuous stream of consumer sensitive issues ranging from genetically modified raw materials and antibiotic resistance to Dioxin and sewage waste.

A number of the more influential British supermarkets decided that animal feed production was unaccountable in terms of food safety and did not provide adequate traceability and due diligence. Subsequently the supermarkets started to impose their own restrictions on the range of feed ingredients and additives that could be included in animal feed as well as specific production requirements, such as the implementation of HACCP and the heat treatment of poultry feed.

The leading supermarkets continue to monitor consumer attitudes to sensitive issues and take any steps necessary to maintain the confidence in the quality and safety of their products. It is for this reason that the UK's largest supermarket recently announced the removal of antibiotic growth promoter (AGPs) from broiler feed. At the same time they have challenged pig and poultry producers to review their systems of production to reduce stress and prevent an increase in the therapeutic prescription of antibiotics, such as occurred in 1986 when Sweden imposed a ban on AGPs (Best, 1996).

The response of supermarkets to these issues tends to precede any political legislation. The EU ban on avoparcin in 1997 was a clear signal that politicians were beginning to take the consumer issue seriously despite the conclusions of the SCAN report (SCAN, 1996), which found no evidence of a build-up of antibiotic resistance in humans as a direct result of the use of antibiotic growth promoters in animal feeds. The ban was extended by the EU in 1998 to include tylosin phosphate, zinc bacitracin, spiramycin and virginiamycin, leaving only avilamycin and flavomycin as the two growth promoters registered for use in poultry.

At present two of the antibiotic substances listed by the EU are also approved as coccidiostats (salinomycin and monensin sodium). Any move to add these two substances to the banned list would have serious consequences for the poultry industry both in terms of coccidiosis and clostridial control.

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Although the EU commissioner for consumer affairs, David Byrne, has repeated his concern about the usage of AGPs, and signalled their likely ban within Europe in two years, the British supermarkets have taken their own lead. This came on the back of a number of significant developments within Europe.

Firstly, in 1996, Denmark implemented a voluntary ban. Their experience has been well documented (Jenson, personal communication) and demonstrated that the impact of the ban on animal performance and health has been less than expected. Their strategy not only focused on alternative products but the type of in-feed coccidiostat used (ionophore or chemical), stocking density, ventilation, hygiene standards, chick quality and feed composition.

Secondly, in October 1999, the UK's largest poultry company, Grampian Country Food Group, announced the removal of AGPs from all its broiler and breeder feed. This decision came on the back of extensive trials with alternative products, under commercial conditions, over a two year period in conjunction with a review of management practices. Significantly, there were no detrimental effects with regard to bird health, suggesting that there would be no need for greater use of therapeutic antibiotics to treat flocks. This is particularly important, because there would be a considerable backlash if it were established that the net effect of removing AGPs was an increase in the use of therapeutic antibiotics. The supermarkets, for obvious reasons, are closely monitoring this issue.

II. ANTIBIOTIC RESPONSE

The growth promoting effects of sub therapeutic dietary levels of antibiotics were discovered in the late 1940's when a fermentation extract of *Streptomyces aureofaciens* was included in chicken diets. A very strong body of data on the subsequent use of AGPs has since been accumulated. A recent review of the literature indicates that in 12,153 trials, the addition of AGPs to animal diets increased production 72% of the time (Rosen, 1996).

AGPs are active in the bird's intestine where they create favourable conditions for beneficial bacteria and disadvantage harmful bacteria. This improvement in gut health allows more efficient absorption of nutrients resulting in improvements in growth rate and feed conversion. In addition AGPs are associated with bodyweight uniformity and control of specific disease conditions such as necrotic enteritis. It is the effect on mortality and disease control, particularly necrotic enteritis, that poses the greatest challenge for alternative products (Pritchard, personal communication).

III. ALTERNATIVES TO ANTIBIOTICS

EU legislation requires AGPs, which are termed zootechnical additives, to undergo a registration process that satisfies strict requirements for safety, quality and efficacy. The alternatives to AGPs are registered in other categories, and unfortunately, therefore, many of these additives have not been through the same rigorous registration process and thus tend to be backed by less scientific data. Consumer and retailer requirements will demand that alternative products are safe to animals, consumers and the environment. There are specific concerns with some of the alternatives with particular reference to pharmacological and /or systemic effects, residues and microbial resistance to the product (Ward, J. 1999).

(a) Probiotics

Probiotics are cultures of living organisms designed to manipulate and maintain a beneficial microflora in the gut. They include competitive exclusion, probiotics, bioregulators and pre-biotics.

(i) *Competitive excluders*

Competitive exclusion products are a culture of multi species non-pathogenic microflora, which may be administered as a spray direct on to chicks at day old, applied in the drinking water or a top dressing on feed. Providing young birds with their normal intestinal flora helps prevent colonization of the gut by potentially harmful and pathogenic bacteria. Significant reductions in salmonella have been demonstrated from their use and some effects against clostridial challenge have also been observed. These products have been widely used as a once-only oral dose in broiler breeders.

(ii) *Probiotics*

Probiotics are a culture of specific living organisms (primarily Lactobacillus) that colonise the gut and provide a healthy environment for the establishment of an intestinal population of beneficial organisms, thereby inhibiting the multiplication of pathogenic bacteria. Administration is continuous in the feed or in some cases via the drinking water. The lactobacilli must be host specific for effective colonization to take place. Unfortunately many probiotic preparations are marketed as multi species products. In addition, Lactobacilli are heat sensitive and do not survive standard pelleting temperatures.

(iii) *Bioregulators*

Bioregulators are single Bacillus species products in the form of spores that are heat resistant and sporulate inside the bird releasing beneficial bacteria in the gut. These bacteria are not host specific but their proliferation produces a probiotic effect. Data on poultry is limited. Administration is continuous in the starter feed.

(iv) *Pre-biotics*

Pre-biotics are fermentable sugars that promote the growth of beneficial organisms in the gut of the bird. Administration is continuous in the feed or drinking water. Data on poultry is limited.

(b) Nucleotides

Nucleotides are biological mixtures of nucleotides, RNA and yeast which, it is claimed, have a probiotic effect as well as stimulating the immune response and enhancing the effectiveness of vaccines. They have been used in breeders using continuous administration via the feed.

(c) Oligosaccharides

Oligosaccharides are sugars which are not capable of being broken down by digestive enzymes and which occur naturally in many feed ingredients. There are two main types of

oligosaccharide product, fructo-oligosaccharides (FOS) and mannan-oligosaccharides (MOS). Administration of both products is continuous in the feed.

FOS products claim to feed the beneficial bacteria at the expense of the harmful ones. Whilst there is evidence they do this, there is also data showing that FOS promotes clostridial proliferation (Kaldhusdal, 1999).

MOS is prepared from the yeast cell wall of *Saccharomyces cerevisiae*. Not all cell preparations are the same. The efficacy of the product is dependent upon the process of phosphorylation to produce phosphorylated gluco-mannans. MOS modifies the microbial ecosystem of the intestine by not allowing pathogens to attach on to the intestinal tract. Bacteria have lectins on the cell surface that recognize specific sugars and allow the cell to attach to that sugar. These sugars can be found on the epithelial cell surface. Many enteric pathogens, including *Salmonella typhimurium*, *Salmonella enteritidis* and *Escherichia coli*, use type-1-fimbriae, which bind to D-mannose bearing lectins (Finucane *et al.*, 1999). Because digestive enzymes do not degrade MOS, it passes through the tract with the pathogens attached, thereby preventing colonization.

Further work suggests phosphorylated gluco-mannans stimulate immune response and macrophage activity (Savage *et al.*, 1996). There is an increasing amount of poultry data being published which endorses the efficacy of MOS compared with other additives (Peterson *et al.*, 1999).

(d) Acidification

Organic acids are registered as preservatives in feed legislation and are widely used in the UK for the control/inhibition of salmonella in both raw materials and finished feed. Mixtures based on propionic/formic acids or salts reduce bacterial and mould counts in feed, limiting the stress associated with introduction of these pathogens to the bird. The pH effect of organic acids in the feed potentially encourages a beneficial microbial flora in the upper intestine whilst at the same time reducing the growth rate of pathogenic bacteria, although the effect in poultry has not been as pronounced as in pigs (Huyghebaert, *et al.*, 1999). Organic acids can be applied either within the feed or drinking water.

Formic acid has been shown to be particularly effective against enteric bacteria such as *E. coli*, but there are health and safety problems associated with using straight acid. Salts of the acids are easily handled and release formic acid in the crop of the bird.

Formaldehyde based products have been shown to be very effective at reducing *Salmonella* and *Enterobacteriaceae* in raw materials, feed and birds. There is some commercial evidence that the cleaner feed results in performance benefits, particularly mortality levels in poultry.

(e) Essential oils, herbs and spices

These products are associated with homeopathy and are therefore considered natural and acceptable to the consumer. Commercial results appear to show some of them to be effective but possibly less predictable in terms of performance response, particularly over a sustained period of time (Francesch *et al.*, 1999). Specific essential oils, such as oregano, are known to be antimicrobial and have been associated with enhanced gut function through the development of a beneficial gut flora. Herbs and spices are believed to be antipathogenic and stimulate digestive function. Unfortunately, in many cases, the manufacturers market the product as a mixture and do not specify the active ingredient, making measurement difficult. The objective should be to identify and isolate specific activities.

(f) Plant extracts

A number of products have been used in feed that may indirectly promote the development of a beneficial gut flora. Yucca products have been successfully used to control ammonia emissions in pigs and poultry. Extracts of sanguinaria and garlic have been associated with antimicrobial effect. Commercial evaluations in poultry however have been disappointing.

(g) Gut conditioners

(i) *Enzymes*

Enzymes are now widely used in the poultry industry to overcome the anti-nutritive effect of non-starch polysaccharides (associated with increased gut content viscosity and poor digestibility) and phytin phosphorous. Enzyme preparations containing proteases and lipases are also becoming more widespread. It may be possible to develop specific enzyme preparations that enhance the gut microflora.

(ii) *Betaine*

Work on betaine suggests that protection can be given to the structure of the epithelial cells in the gut, improving nutrient absorption and fluid retention, particularly under stress conditions. At levels in excess of 1kg/tonne its beneficial effect on coccidiosis control is well accepted. It is also very effective for flushing turkeys when scouring is a problem. Unfortunately commercial levels are generally less than 500g/tonne.

(iii) *Particle size*

Physical properties of the feed affect gut function. The use of whole wheat in commercial broiler and turkey diets has been shown to improve gut morphology and function. In addition, it has been shown that coarser particle size in broiler feed pellets can improve physical performance and reduce the incidence of necrotic enteritis.

(iv) *Feed ingredients*

The non-starch polysaccharide fraction of cereals is known to influence the utilization of nutrients in the diet in the bird. The incorporation of carbohydrate hydrolases in diets containing either wheat, barley, oats or rye can significantly improve bird performance and litter quality (Riddell and Kong, 1992). Due to its viscosity characteristics, maize has been known to reduce the incidence of necrotic enteritis when included in the diet at levels in excess of 20% (Branton *et al.*, 1997). In Denmark up to 30% of the wheat content in starter and grower rations for broilers has been substituted with maize, in the absence of AGPs.

Other feed ingredients that are associated with gut condition and necrotic enteritis are animal protein sources, (fishmeal and meat meal), and fat type.

(h) Mineral growth promoters

The possibility of using copper and zinc are limited in the inorganic form because of the UK feedingstuff regulations for poultry diets. "Organic" or "chelated" minerals provide a means of increasing the availability of trace elements at the same inclusion level as the

inorganic salts. Studies with broilers at Missouri University have shown a comparable response to organic copper at 50 ppm compared to copper in the form of copper sulphate at 175 ppm (Carlson personal communication).

(i) Immunity enhancers

Vitamin E, at levels significantly higher than those recommended has been shown to improve the immune status of poultry with subsequent implications for disease control and day old viability. The interaction of selenium and vitamin E is well documented (Combs, 1999). Organic selenium has been shown to significantly improve parameters such as meat quality and feathering compared with sodium selenite (Mahan, 1999). More recently, studies have shown an improvement in the immune status of day old chicks, as well as a vitamin E sparing effect, resulting from the supply of organic selenium in broiler breeder diets (Surai *et al.*, 1999).

IV. CONCLUSION

One of the first alternatives to consider is removal of AGPs without any substitute or replacement strategy. Where this has been tried commercially for broilers, initial results have been comparable to those in AGP treated birds but subsequent batches of birds have shown a steady decline in performance. In addition, certain of the alternatives to AGPs show significant benefits to the physical product and to financial returns compared with untreated birds.

Measuring the effect of any specific product is not easy, particularly when trying to determine the potential changes in gut microflora given that the most important beneficial species of microflora are not yet fully identified. The validity of microbe plate cultures must be questionable. Hopefully, advanced DNA evaluation techniques will help in the future in assessing the effects on gut microflora population. Products that consist of unspecified mixtures should be challenged to identify and isolate specific activities.

Evaluation of products should be carried out in fully controlled and replicated trials. However it should be noted that, if the trials are carried out under hygienic conditions and low levels of stress, the ability to measure the response to some of those products that work through inhibition of harmful bacteria may be significantly reduced. Commercial farm trials are useful but account must be taken of variation associated with differences in sites, houses, environment, chicks, feed and management. Particular care must be taken in the evaluation of data relating to mortality and disease. At the commercial level, results should be assessed over a continuous period, not just for a single batch of birds.

At present, the practical approach may involve targeting different areas of the gut. To reduce the bacterial load in the upper intestine, acidification, either in water or feed, or competitive exclusion products, may be considered. In the small intestine, attention should be given to those factors that can affect the gut condition such as enzymes, raw materials and particle size. To promote and maintain a beneficial bacterial population in the hindgut the use of phosphorylated mannan oligosaccharides (MOS) or essential oils may prove most effective.

The choice of product(s) must be made in conjunction with an appropriate coccidiostat programme and an assessment of stress factors associated with nutrition, environment and management. Evidence from Scandinavia and now the UK suggests that, through appropriate management, it is possible to limit the effect of the total withdrawal of AGPs.

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COMPETITIVE EXCLUSION: PROBIOTIC PREPARATIONS FOR POULTRY

J.M. COX and B.L. CHUNG

Summary

Competitive exclusion, involving the administration of single strains or mixtures of microorganisms to young poultry, is a strategy to minimise or prevent colonisation by enteric pathogens of veterinary and/or public health concern. The best competitive exclusion preparations are undefined microbial consortia, produced through fermentation of caecal or faecal material from adult chickens, or semi-defined microbial consortia prepared from faecal, caecal or mucosal cultures, and contain a diversity of microorganisms, including Gram-negative and Gram-positive, facultative and obligate anaerobes. Competitive exclusion preparations, alone or with amendments, can be applied to poultry in several ways, but early administration is paramount. While not a panacea, competitive exclusion is a useful part of an integrated pathogen control strategy in poultry production.

I. INTRODUCTION

Today, more than ever, food safety, and particularly the threat to public health posed by pathogenic microorganisms, is a major global issue. Worldwide, poultry is considered to be a significant vehicle of transmission of a range of enteric microbial pathogens, most notably *Salmonella* and *Campylobacter*; hence, management of enteropathogens during poultry production is crucial, and various approaches, singly or as integrated strategies, have been proposed. These include use of pathogen-free stock, feed and water, strict biosecurity, a comprehensive cleaning and sanitation program, and the topic of this paper, competitive exclusion (CE).

The concept of probiotics, the administration of microorganisms to animals (including humans) to enhance gastrointestinal balance and resistance to colonisation by enteric pathogens, is not new (Playne, 1999). Last century, Metschnikov promoted the consumption of yoghurt as a means to maintain gut health. More recently and specifically, the use of probiotics in poultry was first demonstrated in the early 1970s, and the term 'competitive exclusion' was coined (Nurmi and Rantala, 1973). The aim of this paper is to provide an overview of competitive exclusion for the control of enteropathogens in poultry.

II. DYNAMICS OF THE GASTROINTESTINAL MICROFLORA

To gain an insight into how probiotic strains or CE mixtures work, it is necessary to consider how enteric pathogens, the normal gut microflora and the host interact, and how the latter two minimise or prevent colonisation by the former. Pathogens have to contend with a hostile environment within the gastrointestinal tract of the healthy adult host, including low pH of the stomach, mucin trapping, peristaltic clearing, villus sweep, immune exclusion, toxic bile acids, secretory antibodies, cell mediated immunity, oxygen tension, and nutrient availability (Stern and Meinersmann, 1989). In addition, the normal gut microflora is believed to enhance exclusion through several mechanisms including, broadly, indirect and direct antagonistic effects (Rolfe, 1991). Indirect effects include modification (deconjugation) of bile salts (Barrow, 1992), induction of immunological processes and stimulation of peristalsis. Direct antagonism involves depletion of or competition for essential nutrients

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(Hume *et al.*, 1997), competition for bacterial receptor sites, creation of a restrictive physiological environment and elaboration of antibiotic-like substances.

Mucosal attachment is a prerequisite for colonisation of the intestinal tract by both the indigenous or beneficial microflora and pathogens (Fuller and Gibson, 1997). Colonisation involves interaction between cell wall polysaccharides of bacteria and heteropolysaccharides on the epithelium, and evidence suggests that the affinity of the former for the latter is generally greater among indigenous gut microorganisms than pathogens (Snoeyenbos *et al.*, 1979; Pivnick and Nurmi, 1982). Physical hindrance through prior occupation of receptor sites is considered a major mechanism of competitive exclusion (Drasar and Barrow, 1985; Stavric *et al.*, 1987).

Creation of a restrictive physiological environment is believed to be associated primarily with the production of weak organic acids, both non-volatile and volatile fatty acids (VFAs), and the consequent reduction in pH and prevalence of undissociated species of the VFAs (Barrow, 1992; Corrier *et al.*, 1995). In this form, VFAs concentrate within the microbial cell until cellular energy is insufficient to drive their efflux, at which point cytoplasmic pH decreases dramatically and the cell dies. In addition, some microorganisms among the indigenous gut microflora and present in CE mixtures are able to produce other potentially inhibitory substances ranging from hydrogen sulphide to bacteriocins (Barrow, 1992). For example, strains of Enterobacteriaceae have been shown to produce substances specifically inhibitory toward *Campylobacter jejuni* (Schoeni and Doyle, 1992; Schoeni and Wong, 1994).

III. APPROACHES TO PREPARATION OF CE PRODUCTS

Different research groups have taken significantly different approaches in attempting to produce efficacious CE preparations. Broadly, these can be classified as use of:

- single microbial strains
- undefined preparations or mixtures
- semi-defined preparations or mixtures
- fully defined preparations or mixtures

Regardless of approach, the efficacy of CE preparations should be assessed using a standard method. To that end, Mead *et al.* (1989) developed a standard *in vivo* assay, involving challenge with an enteropathogen of two groups of day-old chicks, one of which serves as an untreated control, while the other receives the putative CE preparation one day prior to challenge. Five days post-challenge, following sacrifice, the mean average log population, or infection factor (IF) of the pathogen in each group, is determined through culture of caecal material. The ratio of IF values between the control group and the treated group yields the protection factor (PF); the higher the PF, the more protective the CE preparation. A PF of >4 is considered necessary for efficacious application under commercial rearing conditions.

Single or limited mixtures of strains from the bacterial genera *Bacteroides* (Barnes *et al.*, 1979), *Bifidobacterium* (Barnes *et al.*, 1979), *Clostridium* (Rigby and Pettit, 1980), *Enterococcus* (Hinton *et al.*, 1991a,c), *Lactobacillus* (Barnes *et al.*, 1980; Soerjadi *et al.*, 1981b; Impey *et al.*, 1982; Stavric *et al.*, 1992; Jin *et al.*, 1998), *Streptococcus* (Soerjadi *et al.*, 1981b) and *Veillonella* (Hinton *et al.*, 1991a,c), as well as the yeast, *Saccharomyces boulardii* (Line *et al.*, 1997), have been evaluated as CE agents, with little, or at best temporary, exclusion effect.

Undefined CE preparations have been produced from whole caeca, caecal contents, mucosal scrapings from caeca, or faeces, used directly, in suspension or after a cultural process such as continuous-flow fermentation. Unlike single strains or limited mixtures, undefined preparations appear to offer the best protection against colonisation by enteropathogens. However, undefined CE preparations may inadvertently introduce other avian or even human pathogens into stock, unless carefully screened. (Corrier *et al.*, 1993; Methner *et al.*, 1997). From a commercial perspective, the composition often cannot be standardised, although strategies such as passage through gnotobiotic animals should facilitate consistency.

As a compromise between efficacy on the one hand and safety and consistency on the other, 'fully' and semi-defined CE mixtures have been developed, using as source material whole caeca, caecal contents, mucosal scrapings from caeca, or faeces. While some of these preparations are claimed to be fully defined, the lack of identification of some consortium members to species or even genus level suggests 'fully' is a misnomer. Fully defined preparations have also been prepared from known probiotic microorganisms, although the source of the strains is not necessarily poultry.

The microorganisms involved in CE preparations, other than those based on single strains, represent a diversity of genera and species, including a number of organisms that remain incompletely identified (Table 1). The organisms represent Gram-negative and Gram-positive bacteria, both facultative and obligate anaerobes, and most are typical of the types of microorganisms associated with the gut microflora of many animals. Efficacious CE mixtures, whether relatively simple or complex, contain significant numbers of lactic acid bacteria, reflecting the central role of this group of organisms in maintaining gastrointestinal stability.

IV. CURRENT PRODUCTS

In relation to commercial CE products, Nurmi (1983) considered they should have five vital characteristics. The product should: be safe, free from microbes constituting hazards to human or animal health; prevent infection and spread of salmonellae and other pathogens in growers; not impair growth, but rather promote growth and general health; be easy to transport, preserve and apply; and the price should be moderate and in proportion to the benefits conferred.

There are several commercial CE products currently on the market. Broilact® (Finland), Aviguard (UK), CF3/Pre-Empt (USA; DeLoach 29 in Japan), AviFree (USA) and *Lactobacillus reuteri* (USA) are all derived from caecal material of poultry, while Protexin (UK), is a mixture of fully defined organisms derived from and identified as probiotics in a range of non-avian animal hosts.

Apart from *L. reuteri*, which is essentially a probiotic, all products in the first group are undefined to some degree. Aviguard and AviFree are non-selected, mixed cultures derived from the entire caecal contents of an adult chicken, whereas Broilact® undergoes a selection process to screen out any poultry or human pathogens.

Broilact®, based on a mixture of 32 pure cultures of partially to fully identified intestinal bacterial strains isolated from adult chickens (Nurmi, 1983), was the first commercial CE product, developed in Finland. It was launched in Finland and Sweden in 1987 as a liquid product, but since 1994 it has been sold as a lyophilised product. Schneitz *et al.* (1998) found that the bacterial population of Broilact® was able to attach to epithelial cells and protect chicks against *Salmonella* colonisation, improve the degradation of β -glucans and arabinoxylans by supplementing enzyme activity in the feed, resulting in lower

viscosity of ileal contents, improve nutrient digestibility, and increase the propionic acid concentration of caecal contents.

Table 1. Bacterial composition of 'defined' CE treatments, which provide protection against a single challenge of 10 cfu of *Salmonella* (after Stavric *et al.*, 1991b).

Genus	Number of strains in CE treatment									
	2	3	3	5	8	8	10	18	28	50
<i>Escherichia</i>			3				1			6
<i>Streptococcus</i>							1		7	8
<i>Bacteroides</i>	2						3		6	11
<i>Fusobacterium</i>							1		2	2
<i>Lactobacillus</i>					8		3	18	4	10
<i>Eubacterium</i>									3	3
<i>Propionibacterium</i>									1	1
<i>Clostridium</i>										2
<i>Bifidobacterium</i>		3		5		8			1	1
Gram-positive anaerobic rods							1		4	6

Schneitz (1998) assessed the protective efficacy of three CE products, CF3, Aviguard and Broilact[®], finding they yielded Infection Factor (IF) values of 3.0, 2.8 and 0.3, respectively. Although PF values are not mentioned, the last figure suggests Broilact[®] is efficacious. Palmu and Camelin (1997) found Broilact[®] treatment significantly reduced *Salmonella* contamination of birds both on-farm and at the processing plant. The administration of Broilact[®] in the hatchery via a modified vaccination cabinet was shown to be an effective and safe means of treating commercial broilers. In addition, birds pretreated with Broilact[®] were largely protected against strains of *S. Enteritidis* and *S. Typhimurium* at a challenge dose of 10⁴cfu/bird (Methner *et al.*, 1997).

PREEMPT (also known as CF3, Pre-Empt), is the latest commercial product, and has recently been approved for use in the poultry industry by the US FDA (Douglas, 1998). The product is a 'fully' defined culture of 29 facultative and obligate anaerobes that is sprayed on newly hatched chicks to establish an environment in the gut of the chicken to effectively prevent the establishment and growth of *Salmonella*. This product was developed by Dr. John DeLoach and his research team over a period of 10 years, initiated by the USDA/ARS Food Animal Protection Research Laboratory at Texas A & M University. Recent reports report the product to provide excellent protection against *Salmonella* colonisation in commercial layers, as demonstrated by the virtual elimination of *Salmonella* in layers in Japan (Glaser, 1998; Stephenson, 1998).

Avian Pac Plus, a commercial product containing *Lactobacillus acidophilus*, *Streptococcus faecium* and *S. Typhimurium*-specific antibodies, was found to significantly reduce colonisation by *S. Typhimurium* in market-aged broilers when administered at the hatchery and at the farm from day 1 to day 3 (Promsopone *et al.*, 1998). However, more work is needed to determine if *S. Typhimurium*-specific antibodies alone have a beneficial effect to reduce *S. Typhimurium* colonisation.

Despite the reports of successful exclusion of enteropathogens, particularly *Salmonella*, by a range of CE products, Schneitz (1998) stated that it is unlikely that any efficacious CE product originating from caecal material could be wholly defined, and that only complex undefined treatment cultures are likely to provide adequate and consistent

protection to chicks against colonisation by salmonellae. The difficulty in producing an efficacious, defined preparation is made worse by the use of current, inadequate isolation techniques or insufficient knowledge of the normal intestinal microflora.

V. ADMINISTRATION

A number of methods have been assessed for their practicality and relationship to efficacy in administration of CE preparations to poultry. The most common method involves supply in drinking water, although chicks may refuse to drink for significant periods, or experience difficulty in locating drinkers in commercial broiler sheds (Schneitz, 1992; Schneitz *et al.*, 1992). Overarching all methods is the desire to expose poultry to the beneficial microflora as early in life as possible. To this end, while delivery may be achieved through administration to freshly hatched chicks via water, spray or droplet application, *in ovo* inoculation has also been investigated (Schneitz, 1992), ensuring inoculation of the CE microflora, before chickens are exposed to the environment and consequently, potential exposure to enteropathogens. The efficacy of CE decreases significantly when a preparation is administered simultaneously with the challenge pathogen, and is ineffective if the challenge is administered first (Seuna, 1979; Soerjadi *et al.*, 1981b; Hinton *et al.*, 1990). In the commercial context, administration at the hatchery is advocated, but only if the challenge is likely to occur in the broiler shed. If contamination with pathogens occurs through vertical transmission, or at the hatchery, CE is likely to prove ineffective.

VI. AMENDMENTS

Several approaches to enhancing the efficacy of CE preparations have been described. The most common of these is inclusion of carbohydrates, most notably lactose, among others (Corrier *et al.*, 1990, 1997; Bailey *et al.*, 1991; Schoeni and Wong, 1994), in the diet. In the case of lactose, it is likely to be utilised readily by a range of gut microorganisms, most importantly lactic acid bacteria, thereby enhancing the population of these organisms, reducing gut pH, and increasing both production and the proportion of undissociated forms of volatile fatty acids. Other sugars such as mannose may interfere with adherence of pathogens to epithelial tissue (McSweegan *et al.*, 1986). The immune system may also be stimulated, generally or specifically. In the former case, organisms such as *Lactobacillus casei* enhance IgA secretion (Perdigon *et al.*, 1990), while in the latter, administration of attenuated vaccine strains (Methner *et al.*, 1997), antigens derived from specific pathogens (Methner *et al.*, 1997), or direct administration of anti-pathogen antibodies (Stern *et al.*, 1990; Brandt *et al.*, 1997) may enhance the effects of CE. Although these amendments offer promise in laboratory trials, use in the field is problematic, not only in the technical, but in a practical, sense in that any amendment adds to the cost of use, and may not offer a significant benefit.

VII. FACTORS AFFECTING EFFICACY

The source of cultures may have a profound effect on efficacy of CE. While it might seem obvious that the most protective cultures derive from poultry rather than non-poultry sources, the nature of the source poultry and the conditions under which they are reared profoundly influences their microflora. Several studies have shown that the microflora from SPF birds is far less protective than that derived from conventionally reared poultry (Stavric and D'Aoust, 1993). In addition, breed, diet, growth conditions and geographic location may influence the microflora (Salanitro *et al.*, 1974).

A range of stressors, including extremes of temperature, starvation, thirst (Brownell *et al.*, 1969), transport (Bailey, 1988), and other disease conditions such as coccidiosis (Arakawa *et al.*, 1992), have been found to increase colonisation by and shedding of enteropathogens, particularly *Salmonella*. Presumably, the stressors impact on the indigenous gut microflora, and are thus likely to influence the efficacy of any CE treatment.

The therapeutic or prophylactic use of antimicrobial substances has been reported to have a positive, little (Smith and Tucker, 1978; Barrow, 1992), or negative (Impey *et al.*, 1982; Bailey *et al.*, 1988) impact on the efficacy of CE. The type of impact depends very much on the substance used, particularly its effect on any enteropathogen present compared to its effect on the indigenous microflora. Barrow (1992) suggested that the use of certain antimicrobials or growth promotants may lead to resistance among indigenous organisms, facilitating manipulation of the native gut microflora.

Lastly, the target pathogen can be considered to influence efficacy. Many studies (Nurmi and Rantala, 1973; Lloyd *et al.*, 1977; Seuna, 1978; Rigby and Pettit, 1980; Soerjadi *et al.*, 1981a; Stersky *et al.*, 1981; Impey *et al.*, 1982; Stavric *et al.*, 1985, 1987; Impey and Mead, 1988; Hinton *et al.*, 1991b; Stavric *et al.*, 1991a; Bailey, 1993; Blankenship *et al.*, 1993; Nisbet *et al.*, 1994; Corrier *et al.*, 1995; Hume *et al.*, 1996, 1998) have shown that *Salmonella* is excluded by undefined CE preparations, somewhat irrespective of the source material and its treatment. This is not the case for *Campylobacter* (James and Kaplan, 1998), which relates to fundamental differences in the ecology of the two pathogens in the avian gastrointestinal tract. While organisms such as *Salmonella* and pathotypes of *E. coli* colonise through attachment to the epithelium, *Campylobacter* tends to exist in a free-swimming state within mucin in the crypts of the lower gastrointestinal tract (Beery *et al.*, 1988). For any given pathogen, including specific serovars of *Salmonella*, strain differences relating to expression of virulence or colonisation factors may influence the efficacy of CE.

VIII. CONCLUSIONS

Despite the expenditure of substantial time and effort, a highly efficacious CE mixture is yet to be developed, particularly one that proves highly effective in excluding enteropathogens from poultry under commercial production conditions. It appears that current products are best integrated into a holistic pathogen control strategy that also includes stringent biosecurity (exclusion of animate vehicles of pathogen transmission), selection of pathogen-free stock, feed and water, and maintenance of a clean production environment.

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FACTORS LIMITING THE ACCURACY OF AMINO ACID NUTRITION

C. FISHER

Summary

Some possible limits to the accuracy with which amino acids can be delivered to flocks of poultry are discussed. Limits are associated with the description of feeds as amino acid sources, with the description and prediction of flock performance and with the prediction of the expected response to protein and/or amino acids.

I. INTRODUCTION

Accuracy in nutrition may be defined as the delivery of nutrients to meet the needs of each flock of birds. It is not uncommon to hear the argument that it is not worthwhile introducing a specific technical innovation since the benefit may be unrealised because of lack of control elsewhere in the system. However, in general, it seems to be widely accepted that improved accuracy is a good thing and many technical advances in nutrition are seen in this light. In recent years questions of accuracy in protein nutrition have received additional attention because of concerns about pollution.

The idea from the Organising Committee of this Symposium, that the limits to this process might be subject to some form of analysis, seems to be entirely new. It is certainly quite challenging. By trying to analyse such limits, we may increase our understanding of the system that we, as poultry nutritionists, are trying to control, we may get better understanding of how to select amongst competing technologies in our own businesses and we may clarify our thoughts about the priorities for further research.

In very simple terms the limits to nutritional accuracy may be seen as falling into three areas:

- Limits to our ability to describe feeds as sources of nutrients.
- Limits to our ability to describe or to predict variations between flocks of birds.
- Limits to our ability to predict animal response.

This paper discusses some aspects of these limits as they apply to amino acid nutrition.

II. DESCRIBING FEEDS

Other speakers in this session will discuss advances in this field. By way of introduction we might note that the scientific problems involved appear to be mostly solved. The technical problems, and in particular their application in day-to-day feeding, however remain formidable. The chemistry that we are trying to describe is very complex and the associated analytical methods remain a significant source of uncertainty. Data handling resources are virtually unlimited, but there are still great organisational problems in using nutritional information. Finally the application of the technology in feed mills in real-time still presents many challenges.

From a scientific point of view, it appears that description of feed ingredients as sources of amino acids, which are digestible at the terminal ileum, provides an adequate solution for applied nutritionists. If we were able to do this, the following additional factors still seem to challenge our understanding of the system.

- Interactions between dietary components that influence amino acid digestibility: anti-nutritive substances, interactions with (in particular) soluble non-starch polysaccharide components, the effects of exogenous enzymes and other additives might be listed.
- Sources of variation in digestibility: bird factors (breed, age, health etc.), environmental factors (temperature).
- Uncertainty about post-absorptive utilisation, especially of crystalline amino acids when used at high levels.

III. DESCRIBING FLOCKS OF BIRDS

This is perhaps the most challenging part of the problem. We know, in principle, that feeding needs to be adjusted to the circumstances prevailing in each flock and, crudely, we know how to make these adjustments. However, exploitation of this knowledge is difficult because we need to predict what will happen with individual flocks or have systems that can react to changed circumstances in real-time. Even in a well-managed integration, variation in performance of flocks is a familiar experience. For others making feeding decisions e.g. in a feed company, the range of expected conditions is much wider. Should these feeds be designed for the average flock, for the best flock or for what? The theoretical answer (as usual) is that this is an economic question - the answer depending on the costs and returns of different solutions taking account of different types and sources of risk. However such analysis is very complex and has not, so far as I know, been reported. In a sense, all nutritionists will seek some pragmatic solution to this problem in order to defend their continued employment. The uncertainty involved is one of the factors leading to this discussion today.

Nutritional intervention in real-time is already quite important in the poultry industry and may have a potential to be extended further. The administration of soluble vitamins is probably the most common example. Such intervention is usually in response to observations of problems by the stockman, but this may be a technology that could be developed further. Biochemical markers can be used to assess the nutritional status of birds with respect to several vitamins. If the measurement of these could be developed as simple, on-farm, tests then one could envisage using modest levels of vitamins routinely with controlled supplementation for specific flocks based on a real-time assessment of need. This is all rather futuristic but does seem to be feasible in principle.

For amino acid nutrition the situation is different from vitamins but some real-time interventions are possible, and indeed are currently in use. Measurements relevant to amino acid nutrition include food intake and bodyweight. Whether this list can be extended is rather uncertain. Body condition can, in principle, be assessed by ultrasound and probe methods for measuring body fatness have been described. The practical application of these is perhaps rather unlikely.

In current systems, amino acid nutrition is adjusted in response to observations of feed intake and bodyweight by altering the proportion of two feeds offered to the birds. Typically one of the feeds is a whole grain, as in the Flockman system (Filmer, 1991), and this also allows choice feeding to be exploited. In the Flockman system the rule for controlling amino acid nutrition is simply that the mixture is controlled to provide a preset level of lysine intake. However, modeling and control systems are under development which will permit much more complex rules to be developed; research projects with these objectives are under way in the UK (A. Frost, personal communication) and Belgium (G. de Groote, personal communication). The possibility that amino acids, which are water-soluble, could be delivered in the water in response to assessed needs of individual flocks at particular stages, is also worth thinking about.

In discussing nutritional modeling, Emmans and Fisher (1986) argued that a description of the animal was a pre-requisite to the understanding of nutritional response. In the present context we might say that a description of the flock is a pre-requisite for accurate nutrition. Such a description would include the genetic potential of the animals, as used by Emmans and Fisher (1986), but would need to go beyond this to account for environmental factors. Conceptually we might identify the idea of the first-limiting factor as being useful in defining nutritional response. For a given flock, nutrient requirements would then be determined on economic grounds but taking account of the asymptotic limit to production determined by the first-limiting factor. If this can be modified to allow a higher asymptote, then changes to nutrition would be potentially beneficial. If the first-limiting factor cannot be modified then the resulting level of production determines how the birds should be fed.

Apart from genotype, there are perhaps three main causes of variation in flock performance that could be recognized in feeding decisions; these are stocking density, environmental temperature and disease. The last of these, disease, can probably be ignored here because it seems unlikely that the correct strategy will be to feed down to the level of bird performance that is expressed in diseased birds. In these circumstances prevention or curing the disease is likely to be the priority.

Figure 1 shows the effect of stocking density on lysine requirements as estimated by a broiler growth model (EFG Software (Natal)). The magnitude of the response to stocking density in the model is roughly equivalent to published experiments (unpublished comparisons) and the model assumes that the birds' potential to respond to nutrients is effectively constrained. Over the practical range of stocking densities, between 10 and 20 birds per m², lysine requirement seems to be modified by about 0.3g/kg. This is a small but commercially significant amount.

The direct effect of environmental temperature on growth rate is probably the major source of variation in flock performance in many countries. The practical situation will be very complex but there is little doubt that amino acid requirements will be reduced in flocks where growth is limited by temperature. Also, this is one situation in nutrition when feeding excess nutrients may be detrimental as well as wasteful (see below). Therefore some system of calculating the effects on nutrient requirements is needed. At the present time this does not seem to be available. An added complication arises because temperature may also influence the availability of amino acids from feedstuffs (Wallis and Balnave, 1984; Zuprizal et al., 1993).

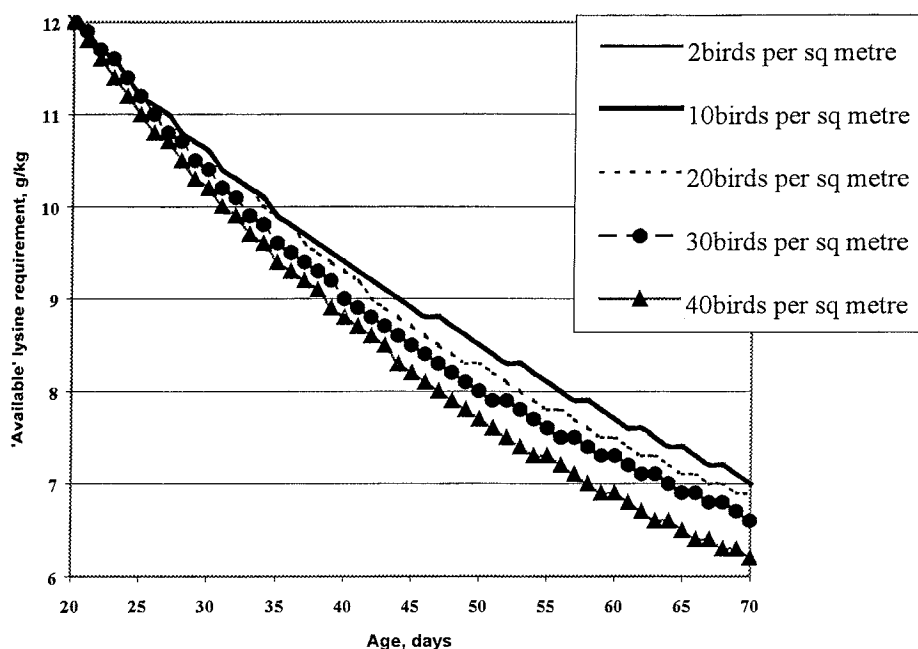


Figure 1. Effect of stocking density of broilers on calculated lysine requirements (EFG Broiler Growth Model). Note that the 2 and 10 birds per m^2 data lie on the same line.

IV. RESPONSE TO DIETARY PROTEIN

Current schemes for controlling amino acid levels in practical poultry feeds will normally take account of 3 to 10 essential amino acids expressed on total or available scales. Crude protein levels will also normally be subject to some control. The idea of determining the correct level of (available) lysine and relating all other amino acids to this, using the idea of an 'ideal' protein, is being increasingly adopted (Baker, 1997). In general, amino acid levels will be defined in the linear program as minimum values but the control of an upper limit to total protein level may have some constraining influence on amino acid balance.

The rules for feed formulation rest heavily on the idea that the 'protein value' of both ingredients and mixtures can be described by the content of the first-limiting amino acid which is presumed to behave additively. Further, bird performance is presumed to be proportional to the level of the first-limiting amino acid. Whilst commercial practice doesn't go much further than this, nutritional science recognizes some other factors that will influence the response to dietary protein.

(a) Interactions amongst amino acids and amino acid balance.

The interactions arising from the conversions of methionine to cystine, phenylalanine to tyrosine and glycine to serine are routinely considered in feed formulation. Specific interactions between lysine-arginine and amongst the branched-chain amino acids (leucine, *iso*-leucine and valine) are recognized. In practice these interactions do not present many problems in practical feed formulation although the effect of excess leucine may be ignored more than it should be.

These specific interactions, usually called amino acid antagonisms, are part of a larger scheme of amino acid balance. The work of Harper et al. (1970) is the most widely accepted analysis of this scheme and a wide range of experiments has been reported to test various ideas. However no general set of rules has evolved for applying these ideas in practical feed formulation. The practical questions that arise might be summarized as follows:

- How should total protein levels be controlled?
- Is it worthwhile spending money on upper limit constraints for individual amino acids?
- Are there important constraints on the extent to which synthetic amino acids can be used to maintain essential amino acid supply whilst limiting excesses?

The answer to none of these is completely clear at present.

The lower limit to crude protein levels will be determined either by essential amino acids which are supplied only as peptides or by total-N or non-essential amino acid (NEAA) requirement. This is discussed below. The use of an upper limit will improve amino acid balance in some general way, although it is difficult to judge how much additional cost to incur to maintain such controls. Similar arguments apply to the application of upper limits to individual amino acids.

All practical attempts to control amino acid balance involve the use of individual crystalline amino acids. Excesses of some amino acids can only be avoided if these products are used to maintain the levels of essential amino acids. Today, lysine, methionine, threonine and tryptophan are available at feed-grade prices and the extent of this list undoubtedly creates an upper limit to the extent to which amino acid balance can be controlled.

Possible upper limits to the use of synthetic amino acids to replace protein have been discussed by Bach Knudsen and Jorgensen (1986). Concern arises mainly from the demonstration by Batterham and Murison (1981) that utilization of lysine in growing pigs was much reduced when feeding was once per day compared to eight times per day. Broiler breeders are typically fed once per day or, in skip-a-day rearing systems even less frequently. It is perhaps in this class of stock that concern about the utilization of crystalline amino acids might arise first. These ideas have been tested in broilers by Baker and Izquierdo (1985) and, indirectly, in laying hens by Shannon (1981).

In general, the experiments of Baker and Izquierdo (1985) show that reduced lysine utilisation is unlikely to occur in broiler production. Feeding periods reduced to 1 hour twice in each day reduced food intake and growth but showed no evidence of diminished lysine utilisation. Synthetic lysine in these experiments supplied between 0 and 20 percent of the total. Similar conclusions were reached with other experimental models, but the authors correctly caution against extrapolation of these results with lysine to all amino acids.

In laying hens, Shannon (1981 and unpublished) used variable levels of methionine, lysine and intact protein in different daily sequences of supplementation. All sequences supplied the same average amino acid or protein levels in the feeds. With both free amino acids and intact protein 24 hour variations in supply gave poorer production than continual levels. Variations over shorter time periods were not tested. These results leave open the question whether broiler breeder hens are likely to show reduced protein or amino acid utilisation, but the question does seem to merit further investigation.

(b) Total protein level and non-essential amino acid supply

The control of total dietary protein is a significant factor in our ability to predict response to amino acids. Protein must be supplied to meet requirements for those amino acids,

which are only supplied as intact peptides, and also to fulfill the birds' need for total nitrogen. In addition we have to consider the effect of protein level *per se* on amino acid requirements and also the energy cost of excreting excess nitrogen. Finally it should be remembered that some important responses appear to be to excess protein as such.

Total protein or NEAA requirements cannot be reviewed here in detail. Usually lysine, methionine, threonine and perhaps tryptophan are available in crystalline form. Thus in Australia, the minimum protein level is likely to be defined by *iso*-leucine or perhaps arginine levels.

Although attempts to define a minimum protein level in the presence of sufficient essential amino acids have been reported many times, the results are rather inconclusive. Bedford and Summers (1985) suggested the ratio of EAA:NEAA for broilers should be 55:45. This was confirmed recently by Lippens et al (1997) who found that a ratio of 54:46 was optimal amongst the levels tested. These ratios do not, however directly determine a minimum nitrogen supply. In broilers an excellent experiment reported briefly by Colnago et al. (1992) suggested that growth performance was reduced when crude protein content of an amino acid supplemented maize-soyabean ration was reduced below 220 g/kg. In laying hens there is reasonable agreement that 16g crude protein (CP)/day will meet the needs of high-producing hens (Fisher, 1994) although this has not been re-examined in recent years.

Morris et al. (1999) have summarized the argument, arising from a number of experiments, that in broilers amino acid requirements should be kept at a constant ratio to crude protein content. The practical levels suggested to maintain growth and feed efficiency were:

$$\begin{aligned} \text{Lysine requirement} &\geq 0.057 \cdot \text{CP} \\ \text{Tryptophan requirement} &\geq 0.012 \cdot \text{CP} \\ \text{Methionine requirement} &\geq 0.025 \cdot \text{CP} \end{aligned}$$

If we assume that a broiler starter feed might vary in crude protein content between 220 and 240 g/kg, then the proportional adjustment to amino acid levels is as shown in Table 1. The magnitude of these adjustments appears to be of considerable practical importance.

Table 1. Adjustment of amino acid levels according to crude protein content using the rules proposed by Morris et al. (1999).

	Crude protein level, g/kg	
	220	240
Lysine	12.5	13.7
Tryptophan	2.6	2.9
Methionine	5.5	6.0

Excess protein, even if it seems economical in feed formulation, does have a cost to the bird. The energy cost of excretion has to be met in some way and might be significant, especially at high temperatures. The magnitude of this effect has been estimated using a broiler growth model (EFG Software (Natal)) in which heat production is based on the effective energy scale described by Emmans (1994). The consequences for bird performance will depend, in detail, on the rules governing heat loss that have been outlined elsewhere (Emmans, 1989). Figure 2 shows the probable maximum magnitude of these effects in practical feeds and the interaction with environmental temperature. In this model study feed intake was depressed by 3 to 5 percent by excess protein, depending on temperature. This led to reductions in

bodyweight between 4 and 6 percent. Again, we seem to have effects which are of potential commercial importance.

V. RESPONSE TO AMINO ACIDS

(a) Individual amino acids

Although the concept of the first-limiting amino acid is clearly useful it has not been very rigorously tested. Bray (1968), working with laying hens, showed that when two amino acids are limiting to a similar extent, a small response to additions of either, made singly, may be obtained. A more striking example in growing turkeys is illustrated in Table 2 (Wylie, 1999). In this experiment very large growth responses were obtained to each of four amino acids (tyrosine, arginine, methionine, valine) when added singly to a low protein diet. The treatments had no effects on feed intake. At first sight these results are a total contradiction of the theory of the first-limiting amino acid. Some possible explanations might include; different animals in the population having different limiting amino acids; that metabolic 'sparing' may occur in some tissues or that some metabolic process which leads to amino acid loss can be 'down-regulated' by the supply of amino nitrogen *per se*. Certainly, if such effects are widespread in growing birds, then these results raise serious questions about our ability to predict and control the commercial response to amino acid supply.

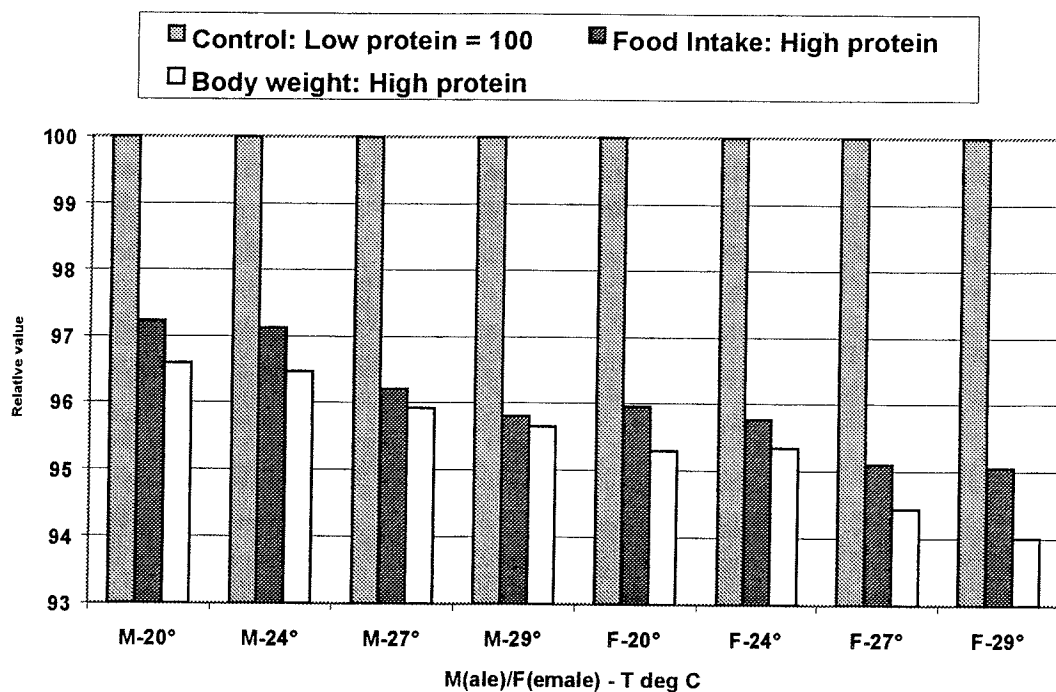


Figure 2. Results of a model broiler experiment (EFG Broiler Growth Model) comparing a low (190, 180 and 170 g/kg) and high (250, 240 and 230 g/kg) crude protein in starter, grower, finisher feed at constant levels of lysine (13.8, 13.0 and 11.0 g/kg total lysine in starter, grower and finisher). All temperature profiles started at 29°C and declined in a conventional way until the temperature shown was reached. Data are for relative food intake (0-49 days) and relative bodyweight at 49 days.

Table 2. Effect of adding single amino acids to a low protein diet on the growth of turkeys (Wylie, 1999).

Diets	Bodyweight at 42 days (g)
1. Crude Protein 180g/kg	1458
2. 1 + tyrosine	1875
3. 1 + arginine	2096
4. 1 + methionine	2042
5. 1 + valine	1996
6. Crude Protein 260g/kg	2458

Control feeds (Diets 1 and 6) were predominantly wheat-soyabean. Amino acid supplements to diet 1 were calculated to bring levels up to those in diet 6. All diets were made iso-nitrogenous by varying glutamic acid. Male turkeys (BUT Big 5) in cages received the diets from 14 to 42 days of age.

Although a rather limited number of amino acids are likely to be limiting in practical feeds it is possible that we need to give more attention to the metabolic role of individual amino acids and their possible effects, even perhaps when they are not limiting in the conventional sense.

The outstanding case appears to be arginine. In his review of 'ideal' protein ratios Baker (1997) suggests that arginine should be 105-108 percent of the lysine level. In recent experiments with 20-40 day old broilers an arginine:lysine ratio of 112 percent was derived (Mack et al., 1999). This is presumably the 'requirement' for protein synthesis. At the other end of the scale Brake et al. (1998) have suggested beneficial effects under heat stress of arginine levels up to 143 percent of the lysine level. In addition to the work on heat stress (Brake et al., 1998, Gorman et al., 1997, Mendes et al., 1997), high arginine levels (relative to lysine) have been implicated in ascites resistance (Wideman et al., 1995), immune response to bacterial infections (Ramirez et al., 1996) and on the relative value of methionine sources (Balnave et al., 1999). Oral dosing with arginine did not modify the course of a coccidiosis infection (Allen, 1999). This is not the place to review this mass of information in detail but it is clearly difficult to specify a minimum arginine requirement for broilers grown under a variety of commercial conditions.

(b) Meeting Amino Acid Requirements

Finally, we need to consider questions that arise in meeting the amino acid requirements of individual flocks, assuming these to be known. Generally, the response to amino acid level is assumed to track a diminishing-response curve up to an asymptotic value (asymptotic curve). In some studies the response appears to be polynomial with depressed, rather than asymptotic, performance at excess levels. This is an important distinction, because the consequences of oversupply are clearly different in the two cases. However no basis for deciding which kind of response will apply in different circumstances can be suggested. The use of a 'broken-stick' or rectilinear model to describe responses to amino acids seems to be indefensible.

In laying hens, Fisher et al. (1973) suggested that the curvilinearity of the response could be reconciled with a rectilinear biological model (simple factorial) by considering the variation amongst birds in output characteristics. This led to the formulation of a simple, but useful, response model (Curnow, 1973). Similar ideas can be applied to growing birds (Clark et al., 1982) but in this case it seems appropriate to consider both variations amongst

individuals and the change in response of a single individual over time. In the case of the growing animal the rectilinear biological model is assumed to apply to one individual at one time. A useful statistical model for describing asymptotic response has been described by Pack and Schutte (1995) and this forms the basis of a useful tool for calculating optimum amino acid doses (Amino Chick, Degussa, AG).

Using this program, and assuming economic conditions typical of the UK, a calculated requirement of 10.9 g/kg total lysine is determined for male broilers in the 28-42 day period. The plot of marginal income against lysine level, leading to this conclusion, is shown in Figure 3. At this level of supply, the predicted body weight gain is about 1319 g/bird against an asymptotic value of 1320 g/bird (data not shown). The predicted FCR is similarly 1.002 times the asymptote and breast meat yield: 0.994 times. For females the optimum total lysine level is 10.7 g/kg and the proportion of the asymptotic values achieved are 0.999, 1.003 and 0.995 for body weight gain, FCR and breast yield respectively.

Thus, the economically optimum lysine levels for broilers are generally very close to the asymptotic responses, a conclusion that also applies to laying hens. Nutritional decisions must be made in areas where the biological responses are very flat. The consequences of being wrong about feeding levels are fairly small in terms of animal performance but may be much larger in terms of economic margin (see Figure 3). Conversely, our ability to discriminate experimentally between different lysine levels is obviously quite limited given normal errors. A danger is that amino acid specifications are continually reduced because 'no significant difference' is detected between current levels and a lower one. It is essential that feeding decisions are based on some sort of response analysis and not on least-significant differences (Morris, 1983). Generally, the fact that we are trying to make decisions on very flat response surfaces is a further limit to accuracy.

If the variations amongst birds create the diminishing response to that amino acid level, a converse question is to consider the differences that occur in nutrient requirements between individuals in a population. Such variations are difficult to study directly and only the experiments of Tolan and Morris (1969) are known. Emmans and Fisher (1992) used calculations to determine the probable variation in lysine requirements of broilers, and found a standard deviation of about 2% of the mean. Interestingly, these studies suggest that it is variation in body composition (fatness), which lead to different requirements, and not differences in growth rate.

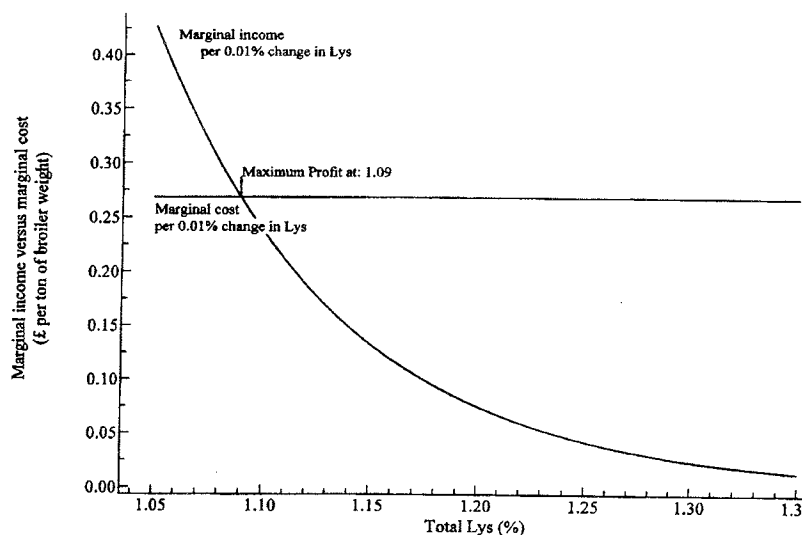


Figure 3. Calculation of optimum lysine level for male broilers 28 to 42 days of age under typical UK economic conditions. Using Amino Calc program (Degussa, AG).

The question of optimum feeding of flocks showing such variation can only be solved in economic terms (what proportion of the flock is it worthwhile feeding fully?). The Reading Model (Fisher et al., 1973) provides a way of doing this for the laying hen, and similar ideas can be applied to growing birds. These analyses assume that over-feeding most of the flock does not affect their performance and, if this assumption is in error, then quite serious errors in marginal analysis probably arise. Kleyn and Gous (1989) and Curnow and Torenbeek (1996) have emphasized the importance of considering all amino acids together in such an analysis and have suggested linear programming (Kleyn and Gous) and theoretical (Curnow and Torenbeek) ways of doing this.

Rearing and feeding the sexes separately is the only practical method of reducing the economic consequences of variable requirements when a single feed is used. If more than one feed is given then, in principle, some choice by individual birds is possible and nutrient intakes can more closely meet nutrient requirements. The most widespread application of this idea is found in the use of whole grain feeding (Filmer, 1991; Forbes and Covasa, 1995). An experiment described by Gous and Swatson (1998) tests most severely the ability of chicks to select diets on the basis of amino acid composition.

VI. CONCLUSIONS

The discussion above reveals many factors that limit the accuracy with which amino acids can be supplied to poultry. From the practical point of view, the description of feeds as sources of amino acids and the differences between flocks of birds are probably the areas of most concern. From an experimental point of view the need for better understanding of the response to protein is perhaps the priority. Amongst the amino acids, it is arginine that most requires further study.

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DIGESTIBLE AMINO ACID VALUES: VARIATION AND APPLICATION

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Summary

Formulation of poultry diets on a digestible amino acid basis has been shown to be superior to formulation on a total amino acid basis when using ingredients with low amino acid digestibility. However, few commercial nutritionists currently formulate diets based solely on digestible amino acids because of wide variations in published digestible amino acid values from different sources, arising from differences in sample variation, type of birds, assay diets and assay methodology. There is also insufficient knowledge of the batch-to-batch variation of amino acid digestibility values for locally grown feedstuffs.

I. INTRODUCTION

The matching of feed quality to amino acid requirements of poultry will depend on a description of the response of a defined genotype (laying hen or broiler chicken strain) to amino acids supplied under defined conditions and of an understanding of the relationship between measured feed quality and amino acid supply from that feed to the bird. As the title of this paper implies it is the second part, namely the quality of dietary protein, that is the focus of this presentation. An important feature of protein quality for the feed industry is knowledge of the availability of amino acids in feedstuffs. Reliable values will permit more efficient formulation of diets. Many attempts have been made to determine amino acid availability (defined as that proportion of dietary amino acids that is in a form suitable for digestion, absorption and utilisation) using *in vitro* (enzymatic and chemical assays), indirect (microbiological or plasma amino acids) or direct (growth and digestibility assays) methods (reviewed by Ravindran and Bryden, 1999a). Discussion in this paper will be confined to digestibility assays.

II. DIGESTIBILITY ASSAYS

The digestibility assay has become the most favoured technique for estimating availability, largely because the values apply directly to the bird and all amino acids can be measured in the one assay. Digestibility assays are applied assuming that the difference between input and output is a valid indicator of bioavailability and that digestibility is likely to be the rate limiting step in amino acid availability. Digestibility assays may be divided into faecal and ileal procedures.

(a) Excreta digestibility

Excreta digestibility has been used by many workers because of its simplicity. Estimates of amino acid absorption made by using excreta of intact birds are in error because avian urine contains some amino acids (Sibbald, 1987). However, the very low concentrations of amino acids in urine mean that the error is likely to be small. Determination of amino acid digestibility in excreta has been widely criticised because intestinal microflora in the hindgut have a substantial affect on the amount of individual amino acids excreted in faeces. Some estimates put this as high as 25% of excreta protein (Parsons *et al.*, 1982). Caecetomised

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birds were developed to overcome the problem of microbial modification of dietary protein and microbial protein synthesis in the hindgut. However, the influence of caecetomy on apparent amino acid digestibility appears from the literature to be quite variable (Ravindran and Bryden, 1999a). Nevertheless, the excreta method using precision-fed roosters has been widely adopted in Canada, the United States and France and in the latter two countries the birds are caecetomised. In this procedure true amino acid digestibility is determined after correction for endogenous amino acid secretion into the gut.

(b) Ileal digestibility

Since microbial activity is concentrated in the hindgut and the main sites of absorption of amino acids are the jejunum and ileum, Payne *et al.* (1968) suggested that the analysis of ileal contents rather than excreta might be a reliable method for assessing protein and amino acid digestibility. Ileal digestibility can be determined in two ways depending on the technique of sample collection. The simplest method for the collection of ileal digesta is to kill the bird and the alternative is to use an ileal cannula. Ileal cannulation has been developed for adult cockerels (Rajaho and Farrell, 1984; Gurnsey and James, 1985). Although ileal cannulation seems to provide some theoretical advantages over the other method it is a sophisticated technique for practical application. Some questions may arise such as the rejection of the cannula, the type and placing of the cannula, the free flow of digesta through the cannula or the use of an appropriate marker (see Sauer *et al.*, 1989). Moreover, for the cannulation technique to be cost effective, it must be undertaken with adult birds and there is always the question that digestibility measured with adults may not reflect digestibility in the rapidly growing broiler chicken. It is for these reasons that at Camden we have developed an ileal digestibility assay with five week old broiler chickens and have published a monograph that contains the digestibility of 92 samples representing 23 feedstuffs (see Ravindran *et al.*, 1998a).

III. VARIATION IN DIGESTIBILITY VALUES

A number of factors influence amino acid digestibility. The nature and digestion of dietary protein will reflect breeding programs, agronomic conditions, presence of antinutritive factors and processing. Variation in digestibility values will also arise from difficulties associated with the conduct of assay procedures and the measurement of endogenous amino acid losses. Surprisingly, there are few instances in the literature where the significance of these sources of variation have been evaluated.

(a) Dietary protein digestion

All dietary sources of protein are heterogenous mixtures of different proteins. It would be anticipated, therefore, that different proteins would be digested at different rates and this in turn would cause a variation in the rate at which different amino acids were taken up from the gut. However, the situation is more complex than this as proteins, although different in their chemical compositions, are not isolated entities but have various linkages with carbohydrate, lipids and other proteins so that these interactions and the composition of the diet may affect the digestibility of dietary protein (Hughes and Choct, 1999). In addition, digestion and absorption may be inhibited by the presence of anti-nutritive factors in the diet. Protease inhibitors, lectins, polyphenolic compounds, saponins and non-starch polysaccharides are examples of anti-nutritive factors that depress protein digestion and utilisation (Bryden, 1996; Hughes and Choct, 1999). Ironically, those feedstuffs (grain

legumes, oil seed meals) which are used extensively as sources of dietary protein also contain the highest concentrations of anti-nutritional factors. For example, soyabean meal contains a range of anti-nutritional factors, many of which are heat labile and destroyed during feedstuff manufacture (Dale, 1996). Heat treatment, essential for inactivation of many anti-nutrients, may reduce protein quality in the presence of carbohydrates by Maillard type reactions.

Processing, especially heat treatment, may contribute to the variability of ingredients such as protein meals and cotton seed meal (Dale, 1996). Lysine is heat sensitive and the low digestibility of lysine in cotton seed meal may reflect heat processing of the meal. The variations in digestibilities of amino acids in meat meals are likely to be due to differences in raw ingredients, time between slaughter and rendering and the duration and temperature of the rendering process (Skurray, 1974). Obviously, optimum processing conditions for all protein meals that do not reduce amino acid digestibilities need to be established. Another aspect of processing, grinding, modifies particle size and shape without causing chemical changes in feedstuffs. It has been shown that grinding improves nutrient digestibility in birds (Hamilton, 1995). This may reflect the increased surface area available for enzyme attack during digestion.

It has been known for some time that the major influence of anti-nutritional factors on protein nutrition has been a reduction in apparent protein digestibility. It is only recently that the actual cause of the reduction on apparent digestibility has been determined with any certainty. The application of new techniques for the measurement of endogenous amino acid excretion has allowed researchers to separate the effects of reduced digestion of both exogenous and endogenous protein and increased endogenous secretion (Angkanaporn *et al.*, 1994). Both factors would reduce apparent digestibility. The relative importance of these two avenues of amino acid loss by the bird will vary with different anti-nutritive factors (Bryden, 1996).

The application of feed enzymes to poultry diets has also demonstrated the impact of anti-nutritive factors on apparent amino acid digestibility. In a series of studies (see Ravindran and Bryden, 1999b) we have shown that the application of xylanases and phytase alone and in combination improves amino acid digestibility by amounts which can be quite significant in terms of overall feed formulation. The positive effect of enzymes on amino acid digestibility again demonstrates the impact of anti-nutritive factors on either reducing protein digestion or increasing endogenous amino acid loss. The net result is a decrease in apparent amino acid digestibility.

(b) Assay procedures

There are now a number of reference sources (see Table 1) of known digestibility values for a range of feedstuffs. However, there is great confusion when one examines these compilations to know how to compare the values obtained by different procedures. It is apparent from Table 1 that there are a number of different assay procedures that vary in terms of the age of the birds used, the collection site of digesta, feeding procedures, basal diet, dietary inclusion level of test ingredients, etc. (see Ravindran and Bryden, 1999a) which all add to the uncertainty of the values obtained. Difficulties associated with amino acid analysis can be a major source of variation which is often overlooked (Ravindran and Bryden, 1999a). Moreover, the application of rapid techniques such as NIR is dependent on the reliability of chemical analysis of amino acids.

Two major areas of contention in digestibility assays are the use of ileal versus excreta collection procedures and correcting digestibility values for endogenous secretions. There have been few direct comparisons of ileal versus excreta digestibility methods and in a

Table 1. Terminology used to describe amino acid digestibility in feedstuffs for poultry - selected samples

Reference	Terminology	Type of bird	Method of feeding	Site of sampling	Endogenous output measurement
Raharjo & Farrell (1984)	Apparent ileal digestibility	10-week-old cockerels	Allowance feeding, 100 g	Ileal digesta	-
Raharjo & Farrell (1984)	Apparent ileal digestibility	Adult cockerels, ileal-cannulated	Allowance feeding, 100 g	Ileal digesta	-
Sibbald (1986)	True digestibility	Adult cockerels, intact	Precision-feeding, 2.5-40 g	Excreta	Fasting
Green (1987)*	True digestibility	Adult cockerels, caececctomised	Precision-feeding, 50 g	Excreta	Protein-free diet
Parsons (1991)*	True digestibility	Adult cockerels, caececctomised/intact	Precision-feeding, 2.5-50 g	Excreta	Fasting/protein-free diet
Rhone-Poulenc (1993)*	True digestibility	Adult cockerels, caececctomised	Precision-feeding, 50 g	Excreta	Protein-free diet
Rhone-Poulenc (1995)*	True ileal digestibility	Adult cockerels, caececctomised	Precision-feeding, 50 g	Excreta	Protein-free diet
Angkanaporn <i>et al.</i> (1996b)	True ileal digestibility	Adult cockerels, caececctomised	Precision-feeding, 30 g	Ileal digesta	Homoarginine labelling
Ravindran <i>et al.</i> (1998a)*	Apparent ileal digestibility	5-week old broilers	<i>Ad libitum</i>	Ileal digesta	-

* Sources of compilation of amino acid digestibility data

series of studies (Ravindran *et al.*, 1999) we have shown that there is greater variation in excreta values than there is in ileal values. Differences observed between ileal and excreta digestibilities in these studies clearly demonstrated that amino acid metabolism by hindgut microflora in chickens may be substantial and that digestibilities determined at the terminal ileum are more accurate estimates of amino acid availability than those determined in excreta. If feed intake is low, as it is in precision-fed assays, endogenous amino acids become a greater proportion of the amino acids measured in digesta and excreta. Apparent digestibility is depressed accordingly. The problems associated with the quantification of endogenous amino acids are discussed below.

(c) Endogenous Amino Acid Losses

As shown in Table 1, most excreta digestibility assays and some ileal digestibility procedures include a correction for endogenous amino acids in an endeavour to provide a more accurate value for comparing different diets or protein sources. Approaches to the estimation of endogenous amino acids in poultry (see Angkanaporn *et al.*, 1996a) have included the measurement of amino acids in excreta either during starvation, when fed a protein free diet, or by determining endogenous output at zero intake by regression analysis. However, the use of these practices, especially the first two, is intrinsically unsound because starvation or the absence of a nutrient, such as protein, profoundly alters metabolism and the bird can no longer be regarded as physiologically normal. Starvation or feeding a protein free diet are the methods used for endogenous correction in the precision-fed rooster excreta digestibility assay which has been adopted in many laboratories. We have used both the protein free diet and the regression analysis method to measure the entry of endogenous amino acids into the lower ileum of broilers and roosters and have shown that the two methods give different results that vary with the maturity of the bird. We have compared these techniques to the homoarginine method and have shown that both techniques significantly underestimate endogenous amino acid secretion when compared with the latter technique (Siriwan *et al.*, 1994). Bryden *et al.* (1996) and Ravindran and Bryden (1999a) have discussed in detail the assumptions that are used when applying the homoarginine technique and these assumptions have been shown to be valid when tested. Interestingly, the values obtained by the homoarginine technique have been reported to be of similar magnitude to those measured using isotope dilution (Roos *et al.*, 1994) and also the peptide elimination ultrafiltration technique (Ravindran and Bryden, 2000). All three techniques have the advantage that they measure endogenous amino acids in birds that can be considered physiologically normal.

IV. APPLICATION OF DIGESTIBILITY VALUES

The major advantage of using digestible amino acids in diet formulation is that it makes it possible to increase the inclusion levels of alternate ingredients (in particular, low quality protein sources) in poultry diets. In effect, it will increase the range of ingredients that can be incorporated, improve the precision of formulation and ensure more predictable bird performance. In a series of studies evaluating canola meal (Ravindran *et al.*, 1998b), cottonseed meal (Ravindran and Bryden, 1999c) and meat and bone meal (Ravindran and Bryden, 1999d), the beneficial effects of using apparent ileal digestible amino acids in broiler diet formulations to increase the inclusion levels of poorly digestible ingredients were demonstrated. In these studies, as expected, increasing dietary levels of canola meal, cottonseed meal and meat and bone meal on a total amino acid basis significantly lowered weight gains and feed efficiency of broilers. The observed depressions were, however,

largely overcome when the diets were balanced on a digestible amino acid basis. This is in accord with previous studies on cottonseed meal (Fernandez *et al.*, 1995) and several byproduct ingredients (Rostagno *et al.*, 1995; Douglas and Parsons, 1999). These results confirm that the inclusion levels of poor quality protein sources in broiler diets can be increased as long as they are based on amino acid digestibility values.

Additivity of digestible amino acids, determined in single feedstuffs, is a crucial consideration in the formulation of complete diets. Studies in our laboratory (Angkanaporn *et al.*, 1996c) found that digestible amino acid supply in a complete diet can be predicted, with reasonable accuracy, based on apparent amino acid digestibilities determined for individual feed ingredients (soybean meal, sunflower meal, meat and bone meal). Investigations with a wider variety of ingredients may be warranted to determine the possibility of associative effects between other feedstuffs.

A question often posed by commercial nutritionists concerns which digestible amino acid system is most appropriate for use in the formulation of poultry diets - apparent or true digestibility values. Apparent digestibility measures the digestibility of amino acids of both dietary and endogenous origins. True digestibility, on the other hand, includes a correction for endogenous amino acid secretions. The relative merits of these two systems have been discussed in detail by Ravindran and Bryden (1999a). It would appear that the choice of the appropriate system of digestible amino acids may depend on the method of formulating diets. If diets are being formulated to least-cost using linear programming, then apparent ileal digestibility values are the most appropriate as they take into account the endogenous cost of digestion. On the other hand, if diets are being formulated in computer simulation models, then true digestibility values will be relevant as the model should correct for the endogenous cost of digestion. It should be appreciated, however, that both digestible amino systems are superior to the total amino acid system currently employed in practical feed formulations and that all current methods of amino acid evaluation have specific applications and shortcomings.

V. CONCLUDING COMMENTS

As growth rates of birds improve, protein requirements increase and as enhanced feed intake is unlikely to be achieved, improved utilisation of dietary protein is required. In this regard, information on the availability of amino acids is therefore becoming increasingly important in poultry feed formulation. Not only will it allow a better match of dietary amino acids with the birds' requirements, but it will also allow a higher inclusion of less digestible and less expensive feed ingredients. However, for industry to adopt the digestible amino acid approach to feed formulation it must be confident in the reliability of the digestible amino acid values. This can only be achieved through continued research. For every ingredient sufficient samples must be assayed to estimate the variance in digestible amino acids, and identify the sources of variation (e.g. variety, location, season, agronomic, processing etc). Allowing for ingredient variability will improve the overall quality of diets. Improving quality ultimately depends on the ability of nutritionists to identify avenues for enhancing the nutritive value of raw ingredients (Dale, 1996). Opportunity to improve utilisation may occur, for example, through plant breeding, the addition of enzymes or changes in processing.

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OPTIMISATION OF THE PROTEIN AND AMINO ACID SUPPLIES TO LAYING HENS

J.V. NOLAN and G.N. HINCH

Summary

This review examines the ability of poultry to self-select diets that meet their nutritional requirements. The issue of whether energy is the dominant factor determining choice is raised and an alternative possibility presented.

The role of learning or training in determining the accuracy of selection to meet nutrient requirements is examined, highlighting the sensitivity of the young chick to "food events" in the first days of life. The potential impact of learning is then reinforced in the mature bird and it is suggested that lack of training about foods may be an explanation for the variability in the success of choice systems to optimise nutrient intakes.

The ability of hens to select appropriately for amino acid and protein from a combination of feeds is examined, highlighting recent studies which show the ability of poultry to rapidly differentiate between feeds differing only in the level of methionine. The potential physiological mechanisms that may allow this to occur are discussed.

Finally the implications of these findings are discussed in the context of optimisation of amino acid intake.

I. MODERN FEEDING SYSTEMS

Birds require fairly precise ratios of energy and all essential nutrients in their diet (i.e. a 'balanced diet') to enable them to express their genetic potential for production. However, these requirements vary with genotype and are altered by factors such as ambient temperature, age of bird, stage of production and presence of disease, and are therefore difficult to determine precisely.

Currently, diets are usually made by mixing a number of feeds together to meet the putative requirements of birds for maintenance and production expressed in terms of daily requirements for metabolisable energy (ME) and individual amino acids (AA), minerals and vitamins. In this context, provision of protein or individual amino acids is probably best considered relative to ME intake or as absolute daily requirements for individual ingredients. Specification of dietary concentrations as percentages of the diet, though common, is more a matter of convenience for personnel involved in mixing the dietary ingredients. Values need to be adjusted in relation to the digestibility or metabolisability of the components and throughout the productive life of the bird.

Expression of the requirements for an amino acid (such as methionine) relative to the requirements for ME is based on a presumption that birds with free access to feed will adjust their intake primarily to meet their energy needs. Thus, nutrients should be provided in 'matching' amounts to meet the animals' current needs as closely as possible while preventing deficiencies or excesses. The view that animals have an over-riding tendency to 'eat for energy' has, however, been challenged. Webster (1993), for example, has pointed out that an obese strain of rats offered diets with widely different ratios of protein:energy chose to adjust their intake in order to maintain a nearly constant pattern of protein deposition during growth while their patterns of fat deposition were more variable. It could be argued that the rats' primary objective was to meet their protein requirement while tolerating over-supplies of energy. Indeed, there are reasonable teleological grounds for this suggestion. Animals undoubtedly have a fundamental requirement for energy to meet their needs for cell

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survival. However, a 'chicken and egg' situation exists when we try to determine the importance of energy relative to other essential dietary ingredients. None of the necessary biochemical reactions by which energy is processed can proceed without enzymes to catalyze them – and enzymes are proteins that require amino acids for their synthesis and will only function if vitamins and minerals are present as co-factors. From this standpoint, it is not easy to see why birds should give priority to gaining just energy.

On the basis of earlier studies, Emmans (1991) has suggested that the adage 'animals eat for energy' should be re-cast to imply that animals eat for the most limiting dietary component to which could be added 'provided this does not involve the simultaneous ingestion of toxic amounts of another dietary component'. Diets formulated against the above background can be presented to birds as a mash or as a pellet or crumble – the latter two having as one of their perceived advantages that each bite is a balanced diet containing all nutrients. Such a perception could be taken to imply that all birds in a flock have identical requirements, that the nutritionist knows best what the birds' current requirements are, and that it is appropriate for all birds to be constrained to ingest exactly the same mixture of ingredients.

II. SELF-SELECTION FEEDING SYSTEMS

Diets for poultry have not always been formulated by nutritionists! To survive in its natural environment any animal must either possess innately, or acquire, the ability to select a nutritionally adequate diet from all of the nutritional and non-nutritional or toxic materials present in their environment. Red Jungle fowl in their natural environment were clearly able to do this by selecting feeds that fell essentially into one of two categories, *viz.* high-energy, low-protein feeds such as grass seeds, and low-energy, high protein ingredients such as snails, worms and vegetable materials (Cumming 1994). Until the 1960s, before cages were in common use, egg producers often kept their layers on the floor and offered them whole wheat, meat meal and shell grit in separate feeders. They were taking advantage of the often-demonstrated ability of modern birds to be able to select an appropriate diet from a range of suitable ingredients. This type of feeding system has been referred to more recently as 'free-choice' or 'self-selection' feeding and interest in this type of feeding system may increase with the current concerns for the welfare of caged layers and the increasing use of barn or 'free range' production systems.

Chah (1972) showed that birds offered calcium, protein and energy sources separately ingested calcium almost exclusively in the afternoon and evening and ingested less calcium than hens fed complete pellets (conventional diet). Energy and protein intakes by the choice-fed birds varied more during the day than for the conventionally fed hens, peaking in the middle of the day and showing a smaller peak in the evening before 'lights-out'. They ingested 8% less ME, 9% less protein and 26% less calcium than their conventionally fed counterparts. Birds given complete diets may, therefore, be in the situation of having to eat more during certain times of the day to meet their changing diurnal needs for calcium or protein and, in doing so, having to ingest an excess of energy or a nutrient such as calcium or methionine at these times. Arguments such as this have led to suggestions that birds might produce better or be more efficient if allowed to self-select from an appropriate array of feeds.

Theoretical advantages of free-choice feeding were outlined by Emmans (1975). He argued that birds have different requirements for maintenance and production. The requirements of individuals will differ from the average for the flock so that no single diet can exactly supply the needs of each individual. He proposed a system of feeding in which each bird would be offered free access to two diets, one intended to meet its maintenance

requirements, and the other to meet its needs for production. The bird would then be able to choose from both diets to produce a mix that would more closely meet its individual needs (Emmans, 1975). This proposal effectively assumed that each bird would be able to choose proportions appropriate to its metabolic requirements.

III. THE MECHANISMS ENABLING SELF-SELECTION

Hogan (1973) showed that newly hatched chicks initially peck equally at food particles or sand. Ingestion of food leads, within about an hour, to increased pecking behaviour whereas ingestion of sand results in decreased pecking. Hogan concluded that ingestion of food, but not sand, produced positive post-ingestive effects that reinforced feeding behaviour. These findings were also found by Hale and Green (1988) with chicks at either 0.5 or 2.5 days of age. If, however, chicks were given some additional feeding experience of foods or sand they learnt to discriminate between the materials and came to prefer the foods.

It is now generally accepted that birds, like other animals, learn to recognise foods by their sensory characteristics and develop conditioned associations between the foods and their post-ingestive nutritional and metabolic effects. They will later exhibit stronger preferences for those foods that lead to more positive effects whereas those producing detrimental effects will subsequently be avoided (Rozin, 1968). Studies in which birds have been given dietary choice leave little doubt that they have the capacity to adjust their intakes of protein, minerals and vitamins as well as energy in order to achieve acceptable levels of production (see, for example, Graham, 1932; Li and Anderson, 1982). Moreover, domestic birds given diets deficient in a particular nutrient, for example calcium (Wood-Gush and Kare, 1966) or sodium (Hughes and Whitehead, 1979) or thiamine (Hughes and Wood-Gush, 1971), exhibit an increase in their searching and pecking behaviour which increases the likelihood that they will increase their knowledge of possible sources of nutrients.

The ability of young chicks to balance their diet from a smorgasbord of food ingredients was demonstrated by Funk (1932) and Dove (1935), but learning and recognition of different foods appears critical to their ability to select an appropriate diet. Newly hatched domestic chicks are very sensitive to particular shapes and colours (e.g. Dawkins, 1968). They peck preferentially at particles with round and regular shapes and brown-red colour. Natural feedstuffs, such as grains, vary in their shape, size and colour and when Adrethausberger and Cumming (1985) exposed day-old commercial layer and broiler chickens to different seeds they found that both breeds exhibited an initial preference for sorghum. In contrast feral chickens selected wheat and maize; grey sunflower, rye, oat, rape, lucerne and barley seeds seemed to be less preferred by all breeds. When the seeds were stuck down and it was impossible to swallow them, the chickens were unable to establish an association between shape/colour and ingestive consequence and they then tended to peck seeds almost at random when tested 24 hours later. This suggests that the very first experience of pecking should be at food that is of a form, size and colour that may relate to later food exposures.

Karunajeewa (1978) evaluated the self-selection approach to feeding birds by giving them either conventional balanced mash diets based on barley or wheat, or a choice between the same ingredients given as a whole grains and a concentrate mixture. The choice-fed birds produced as well as those given the complete diets but they ingested 11% less food. Similarly, Leeson and Summers (1978) found that free choice feeding decreased feed intake and increased production efficiency. They concluded that when birds were provided with a free-choice diet, each bird selected a diet closer to its own dietary needs.

IV. SELF-SELECTION (APPROPRIATE OR INAPPROPRIATE?)

There has been controversy about the ability of 'choice-fed' birds to 'fine tune' their dietary choices and demonstrations that birds may choose ingredients inappropriately are one reason why self-selection systems have not been adopted by commercial producers. Dove (1935) found a wide range of individual differences in the selection abilities of chickens. He attributed this variation to possible genotypic differences. Some chicks which selected a 'balanced' diet and grew at a rapid rate chose a 'formulation' that was remarkably similar to that of conventional starter diets. Other chicks were apparently unable to select a diet that would optimise their growth. These chicks apparently either did not possess innate knowledge about feeds or were unable to learn about the characteristics of the feeds available to them.

The ability of birds to differentiate between feeds depends on a learning process which involves the development of conditioned associations between the stimuli occurring in response to eating certain ingredients (post-ingestive consequences which may be positive or negative) and the stimuli invoked by their position or sensory properties. The latter are the means by which feeds that the animal has experienced are subsequently recognised. It can be argued that birds learn about feeds and exhibit feeding behaviour that will enable them to optimise their genetic potential for growth and production. This idea provides a general theoretical framework for considering feed intake regulation and nutrient selection in the manner just described, although Emmans and Kyriazakis (1995) have recommended caution in the overuse of the idea of optimisation, especially in the absence of evidence for it.

The relevant variables to be considered in the theory of diet selection are the animal (its genotype and current physiological state), the animal's environment and the characteristics of the feeds available to it (Emmans 1991). The feed characteristics can include aspects such as composition, palatability and variety, the last two being more applicable to choice-fed birds. A number of studies have examined the contrast between a situation where one or a variety of foods are offered; in general these were foods that are apparently avoided in a two-choice situation and could be considered 'unpalatable', apparently they become 'palatable' when there is no choice given. Moreover, palatability may not be simply an attribute of a feed – it may reflect a bird's experience/training/learning and its current physiological state and the palatability index of any feed may, therefore, change over time.

There are many other possible complications associated with choice feeding. In a comprehensive study, Farrell *et al.* (1989) compared the hen-day production of two strains of layers when fed a commercial layer crumble or given a choice of low (13%-16%) or high-protein wheat or sorghum (11%) with one of three protein concentrates based on either full-fat soybeans, sweet lupin meal or field peas. The protein concentrates were offered as mash or pellets in troughs that were separate from the grains and there were 6 birds per cage. Intake of the mash concentrate was consistently higher than of the pelleted concentrate and the choice-fed birds generally ingested excessive amounts of protein. Some individual treatments resulted in poor egg production and low gross economic margins relative to the commercial diet treatment. These workers concluded that self-selection programs required considerable 'fine tuning' to allow them to be useful in practical feeding systems. They identified trough design and birds per cage as issues requiring further attention. A further issue may have been the age and experience of birds when allocated to their experimental treatments as this was done in this experiment when the birds were 18 weeks of age after a conventional rearing on pullet starter and pullet grower mash. They had apparently not previously experienced the feed ingredients that were to be offered in the laying period.

In their review Cumming and Mastika (1987) concluded: "If choice feeding does not work efficiently the reason almost certainly lies in the method in which the choice feeding has been applied" and it would seem that training is an issue of practical significance if self-selected feeding is to be successful. A complete discussion of factors that may determine the success or otherwise of a self-selection feeding system is beyond the scope of this review but a number of factors are relevant.

1. A bird must first overcome neophobia associated with the presence of a novel feed at its feeding site.
2. It must then identify the material offered as potential food rather than non-food and peck at the material. Social mimicry plays a role at this stage if other birds are present.
3. The bird may need to practise pecking at the food in order to learn how to successfullyprehend and ingest it, especially if the particle size and shape are new to the bird. Picard *et al.*(1999) have reported that there may be a burst of pecking activity when a bird is offered a novel food but in spite of the pecking activity, feed intake is low because the pecking does not result in successful prehension of feed particles.
4. After successful ingestion of a small portion of the food, the bird must form a conditioned association between the sensory properties (colour, beak feel) or position of this food and its post-ingestive consequences. Such associations may take time to develop, and reinforcement results in the formations of stronger conditioned associations.

Only by proceeding through steps 2, 3 and 4 (training) will the bird be able to recognise the food in the future and predict the potential consequences of ingesting it again.

V. PROTEIN AND AMINO ACID INTAKE

Rozin (1969) undertook a series of studies to determine whether there were separate processes for regulating the intake of energy and protein. Karunajeewa (1978) offered crossbred hens on litter a choice of protein concentrate (31% crude protein) and particulate calcium with either whole wheat or barley. He found their food intake was lower by 7 and 15%, respectively, than that of hens offered complete mash diets consisting of the same ingredients. Rate of lay did not differ between choice and mash diet feeding but choice-fed birds produced heavier eggs during the latter half of the laying cycle. He concluded that there were major benefits for choice-feeding from reductions in feed intake and the reduced energy costs associated with the use of whole grain. He also concluded that poor responses to choice feeding can often be attributed to inadequate intake of ME during the adjustment or learning period and non-separation of the calcium supplement.

In a study reported by Fuller (1962), White Leghorn pullets were allowed to self-select from a 33% protein concentrate, whole maize, whole oats and a mineral mixture from 8 to 21 weeks of age. These birds ate 13% less feed and performed more efficiently during the laying phase than those given a choice of a conventional mash (20.8% protein) and whole maize and whole oats. The energy intake was similar over a wide range of protein intakes which suggested that these growing pullets were eating to satisfy their energy needs with little regard for the protein level achieved. However, the range in protein options was such that it could be argued that there was no need for the birds to differentiate between protein sources.

Hughes (1979) suggested three ways that birds might achieve specific intake of individual dietary components: genetically programmed innate behaviour; learnt behaviour

based on an innate learning protocol; learnt behaviour based on socially acquired or personal experience; unable to learn. The current consensus is that birds can make appropriate choices where such choice will avoid deficiency or toxicity by a process of conditioned association between foods and their post-ingestive consequences (Rozin, 1991).

In recent studies we have confirmed that both broiler and layer cockerels are able to distinguish feeds identical in all but methionine concentrations and a colour (food dye) cue. The feeds offered consisted of mash feed formulated to meet bird requirements in all but methionine, one choice containing added methionine to provide an estimated marginal level of 0.29% and the other having no added methionine and with an estimated level of 0.19%. In one experiment four-week old broiler cockerels, not previously exposed to the foods, consistently selected the higher methionine food after an average 40 hours of choice feeding (Figure 1). In a second experiment 6-week old layer cockerels, exposed to the feeds independently for two days prior to choice feeding, choose within hours the feed containing the higher level of methionine.

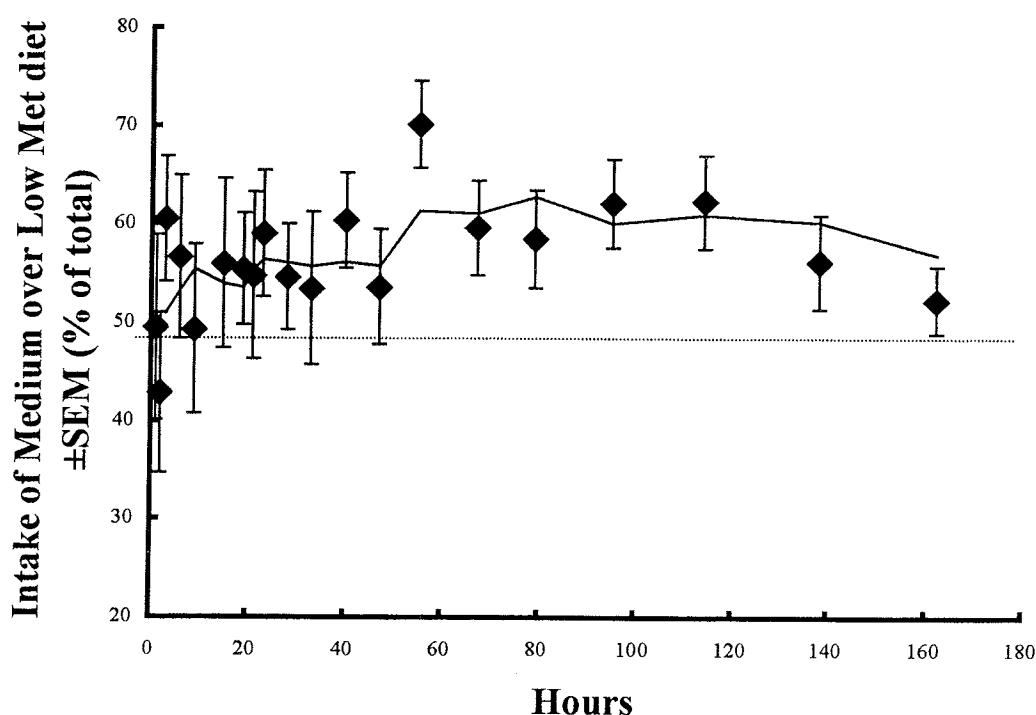


Figure 1 The percentage of the marginal methionine feed chosen by broiler cockerels offered a choice of marginal and deficient methionine feeds (Channon, 1999).

Such findings suggest that training has a major role to play in facilitating the ability to distinguish between feeds and possibly that the learning process is more rapid if the feeds are initially offered separately. However this requires confirmation.

These findings do raise questions about how the post-ingestive consequences of feeds differing in such small amounts of an amino acid are detected and collated? Diets that are amino acid imbalanced are known to induce hypophagic responses in most animals but the physiological mechanisms that enable them to recognise an under- or over-supply of individual amino acids are poorly understood (Leung and Rogers, 1986). Recent studies undertaken in rats by Gietzen *et al.* (1998) have attempted to elucidate the neurochemical

systems that may be involved in the initial recognition of a post-ingestive amino acid imbalance and in the development of conditioned responses to the diet producing the imbalance. They have demonstrated that, before intake of an imbalanced diet falls, there are changes in the activity of neurotransmitter substances (monoamines) in parts of the brain known to have monosynaptic connections to the anterior piriform cortex (APC) which is a chemically sensitive area of the brain. Gietzen *et al.* (1998) believe the APC becomes hyperexcitable in rats fed imbalanced diets when there is a lowering of the concentration of the limiting amino acid in this area. This may allow linkages to the hypothalamus (the traditional feeding behaviour centre) via alteration in noradrenalin or tyrosine concentrations in areas of the hind brain which in turn reduce feeding activity.

After intake had declined significantly, indicators of serotonin (associated with learning) were changed in the limbic system and in areas associated with the taste pathways. They believe that these latter changes which involve increases in serotonin concentration in the parabrachial nucleus and then in threonine concentration in the central nucleus of the amygdala are associated with the development of a conditioned aversion to imbalanced diets. They proposed: 'a circuit for the neural responses in the initial recognition of acute amino acid deprivation that begins with the activation of the APC and includes areas in the hind brain and hypothalamus. After a significant hypophagic response, serotonergic indicators were altered in areas of the taste pathway and the limbic system, suggesting that different circuits mediate the initial recognition and secondary responses to amino acid-imbalanced diets.' Such observations suggest that monogastric animals may be relatively more sensitive to deficiencies than to excesses of amino acids; however, the reality of this needs to be confirmed for poultry before such knowledge can be used to advantage.

IV. PRACTICAL LIMITS TO SUCCESSFUL SELF-SELECTION

Having established that birds can identify differences between feeds differing only in amino acid concentrations, consideration needs to be given to determining whether this ability to distinguish between feeds can be used to optimise intakes of protein or amino acids for individual birds. It appears that there are mechanisms whereby birds can identify differing amino acid levels and establish associations between the physiological outcomes and particular feed cues. If the associations are largely with deficiencies and imbalances then it could be argued that self-selection will only be effective where diets are marginal in an amino acid or protein.

The integration of a series of different physiological responses and cue combinations that would normally be associated with choice feeding is difficult to envisage as we still have no clear picture of the priority established if more than one imbalance occurs. It is possible that the feeding behavioural response to an imbalance is a generalised seeking behaviour, in which case optimisation for any one nutrient is unlikely. However, if training has previously allowed birds to learn about the physiological consequences of eating a particular feed then the accuracy and rapidity of correct/optimal choice may be improved.

Further studies are required to determine the relative importance of training on the ability of birds to make appropriate or optimal selections from a combination of feeds. If it can be shown that training increases the reliability of correct choice then the practical problems of offering choice feed delivery systems (Tauson and Elwinger, 1986) can be reduced.

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THE ECONOMIC DOWNTURN IN ASIA: ITS IMPACT ON THE STOCKFEED AND POULTRY INDUSTRIES

D.J. FARRELL

Summary

This paper attempts to assess the consequences of the economic downturn in Asia on the poultry and feed industries both there and here. Growth of these industries has declined since mid 1997, particularly in countries in Southeast Asia. Currency devaluation made it difficult to import expensive feed ingredients, but importation of poultry meat and eggs at low prices occurred, often undercutting local poultry produce. China, the major producer of poultry and feed, suffered less than other countries in the region and did not devalue the yuan. The impact of the monetary crisis in Asia on the Australian poultry industry has been minimal although exports of egg products declined from A\$1.6 million to A\$0.87 million. Prices of feedstuffs also declined but poultry diets have been substantially cheaper in Australia. The outlook over the next couple of years in Asia is optimistic, but a few countries may take several years to recover from the economic downturn that started in July 1997.

I. INTRODUCTION

The monetary crisis that struck several countries mainly in Southeast Asia in July 1997 has had a domino effect throughout Asia and has been felt by many countries throughout the world. A detailed commentary on the economic upheaval in Asia and its implications for Australia were reviewed by Rodriguez and O'Donnell (1998) and economists have had to revise and update their predictions for economic growth (Table 1).

Table 1. Actual and forecast percent economic growth in selected countries of East and Southeast Asia (1996-2000)

	1997 GNP ¹ (US\$)	Actual			Forecast	
		1996	1997	1998	1999	2000
China	860	9.8	8.5	7.5	7.0	6.5
Taiwan ²	NA	5.8	6.9	4.9	5.0	5.1
Indonesia	440	7.0	5.2	-14.0	-0.2	3.0
Japan	38,160	4.0	0.7	-2.8	0.2	1.0
Malaysia	4,530	7.5	7.4	-6.5	2.5	4.5
Philippines	1,200	5.1	5.0	-0.1	2.0	2.5
Singapore	32,810	6.2	6.1	1.5	3.0	4.2
South Korea	10,550	7.1	5.7	-5.5	3.0	5.1
Thailand	2,740	7.0	-0.2	-8.0	6.0	3.0

¹ Gross national product per head per year (Anon., 1999a)

² Listed as Chinese Taipei

Source: Adapted from Agricultural Economics Section, Australian Bureau of Agricultural Resource Economics (1998) and from Australian Commodities (1999) 6(3), 435-439.

The immediate effect of the economic downturn was a hike in interest rates and a devaluation of currency in several countries. The average decline in five countries in Southeast Asia was

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42%, and in North Asia 19% (Rodriguez and O'Donnell, 1998). For example, the Thai baht increased from 25 to 41 against the US dollar, and the Filipino peso from 24 to 40. China has survived better than most (Table 1) and so far has not had to devalue the yuan but economic growth is slowing down there.

Several of these countries are importers of grain since their poultry feeding is based on 'corn' (maize) and soybean. These ingredients suddenly became expensive and this caused a drastic reduction in production of chicken meat and eggs. Malaysia depends heavily on the importation of feedstuffs to support the high production of chicken meat of over 30kg per head per year (Anon., 1999a), some of which is exported to Singapore.

There has also been an increase in trading of poultry produce. Korea, for example, which has almost fully recovered from the recent economic downturn, is importing increasing amounts of poultry products because local producers are unable to compete. In the first three months of 1999, almost 8,000 metric tonnes (mt) of chicken meat was imported from the US, an increase of 290% compared with the same period in 1998. Korean-grown chicken is about 30-40¢/kg higher than imported chicken (K.H. Nham, pers. com., 1999). Thai eggs are also being imported in large quantities at a price that is considerably less than locally-produced eggs. Korea imports almost all of its feedstuffs.

II. CHICKEN MEAT INDUSTRY

It is difficult to obtain reliable data on this industry in Asia since government official figures are often inaccurate and may have to be revised later. For example, China estimated slaughtering of 6.6 million birds in 1997; this figure has been reduced to 5.9 million (Anon., 1999a). Nevertheless, chicken slaughtering in China in 1990 was only 1.9 million. A very recent report puts China's poultry meat production at 12.9 million mt (Anon., 1999b). This includes almost 1.8 million mt of duck meat.

If the chicken meat output of China is excluded, then in 1998 chicken meat output in the rest of Asia over 1997 was static (Anon., 1999a). Only very few countries showed a decrease in poultry slaughtered between 1997 and 1998 (eg. Indonesia, Korea, Pakistan) and a few showed substantial increase (eg. Vietnam).

Broiler production in the Philippines declined by 15 million birds between 1996 and 1997, while the price has risen from a low of 68 peso/kg dressed to 85 peso in 1998. A recent report by the Philippine Bureau of Agricultural Statistics has predicted a 3.2% increase in poultry production for the first quarter of 1999 (Anon., 1999c). The Philippines has been particularly hard hit, not only due to the financial crisis but to government policies. The agricultural trade balance has gone from plus US\$292 million in 1993 to minus US\$764 million in 1997. Reyes (1999) reported importation of chicken meat from the US and France which was selling at much less than that of locally-produced chicken meat. In 1998, importations of great grandparent stock were infected with myeloid leukosis. This reduced substantially broiler production in the Philippines.

Thailand has benefitted to some extent from the economic downturn. Japan imported in 1998 36% more chicken meat (120,000 mt in 1998) from Thailand compared with 1997, but there was a small decline of about 4,000 mt in the export of further processed chicken meat. However, import price to Japan was down substantially in 1997 and 1998 compared to 1996 (270 vs 240 yen/kg) (Japan Meat Trade Research Centre, Tokyo, 1999).

Malaysia has benefitted from Singapore's decision some years ago to phase out intensive animal production in that country. In 1997, Singapore imported over 41 million birds from Malaysia compared to 3.1 million in 1994 (Anon., 1999c). This is about 11.5% of Malaysia's broiler production. The full impact of the economic downturn was unlikely to be felt until last year (1998), but reliable data are still not yet available. However the recent

outbreak of the Nipah virus in Malaysia, resulting in the slaughtering of 900,000 pigs in 1999, will likely have a positive effect on poultry production there (Anon., 1999c).

Taiwan has not shown the same downturn in broiler production as some other countries in the region. There has been a steady increase in the slaughtering of broiler chickens and 'coloured' chickens between 1988 and 1997 from 58 to 185 million, and 119 to 180 million respectively (Agricultural Economics Division, PDAF, Taiwan).

III. EGG INDUSTRY

World hen egg production (including hatching eggs) increased from 47 million mt in 1997 to 48 million mt in 1998 (Anon. 1999a). Despite the economic downturn in Asia, there was an increase of 2.7% between 1997 and 1998 in this region, dominated by the 17.8 million mt produced in China 1998, an increase of 2.9%. Currently egg prices are depressed there, government-run farms are inefficient and there is no grading system (Hunton, 1999). However, some countries in Southeast Asia showed a downturn. Table eggs produced in Malaysia were 6.6 billion in 1995; in 1998 production was estimated to be 5.9 billion (Federation of Livestock Farmers' Association of Malaysia, 1998). The Philippines have shown a steady increase in egg production of 5% and in price between 1994 and 1997. The egg industry in Thailand has been severely hit by the economic slump. This in part is due to the many small-scale producers (<10,000 layers) who are unable to get extended credit to purchase feed. At the most, credit is for only 7 days from purchase (Farrell, 1998). In 1997, cost of production was greater than market price. Change is anticipated with the possibility of exporting fresh eggs into the Singapore market. There is forecast to be a 7% decrease in egg production in Thailand in 1999 compared with the previous year (Hen-Egg Farmers, Traders and Exporters Association, 1999).

In Taiwan, the numbers of both layer chickens and layer ducks have increased steadily since 1989, now reaching 33 million and 3 million respectively (Agricultural Economics Division, PDAF, Taiwan, 1999).

IV. STOCKFEED

For the first time for 40 years the global manufactured feed industry has experienced a decline from 605 million mt in 1997 to 575 million mt in 1998. This decrease has been attributed largely to the Asian economic downturn where feed production dropped by 24% in the Asia-Pacific region (Gill, 1999a). Indonesian feed output declined in 1998 by more than 40%, Korean by 30% and Thai by almost 25% (Gill, 1999a). Some of these figures have been updated in Table 2. Russia's manufactured feed decreased by 25% to only 13 million mt at the same time. In 1994 it was 60 million mt (Anon., 1996).

Shown in Figure 1 is the distribution of stockfeed in the top 10 countries in the Asia-Pacific region whose total feed usage in 1998 was 128 million mt (Gill, 1999a); of this Australia produced 8 million mt. China is the major player, accounting for 70% of manufactured feed produced in the region. Of this 71% goes to poultry (Gill, 1999b).

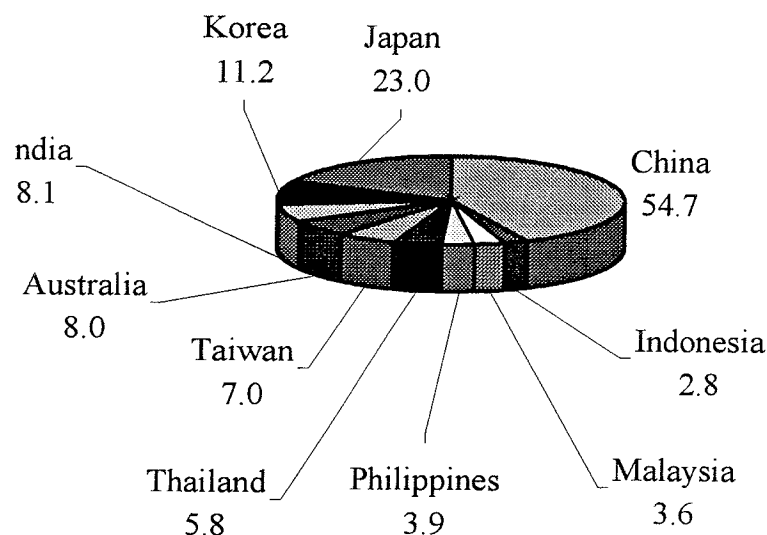
The rapid decline in the once buoyant economies of Asia has been blamed for the very low recent price of maize and soybean meal in the US. In Chicago, in October 1999, soybean meal was forecast to fetch US\$120/mt, the lowest price in 27 years (Welsh, 1999a). Maize was selling at over US\$3.0/bu in 1986; the new crop in December may attract only US\$1.94/bu. The volatility of this market is exemplified by a sudden rise in mid August 1999 in maize to US\$2.25/bu and US\$142/mt for soybean meal (Welsh, 1999b). This has been attributed to the hot, dry weather in the US. The market for feedstuffs remains volatile.

Demand for stockfeed is forecast to decline in Thailand by 15% in 1999 to 8.7 million mt, but this is expected to recover significantly next year (Thai Feedmill Association, March 1999). The Charoen Pokphand Corporation, a very large manufacturer of feed in the region, recorded a loss of 1.6 billion baht in 1997-1998 compared with a 1.37 billion profit in the previous year.

Recent declines in animal stock feed production in some countries in Southeast and East Asia are shown for 1998 in Table 2 (Raghavan, 1999).

Table 2. Decline in livestock feed production in selected countries in 1998.

Country	Decline (%)
Indonesia	70
Malaysia	35
Philippines	15
Thailand	20
Vietnam	10



Source: Gill (1999a)

Figure 1. Asia-Pacific industrial feed production in top 10 countries.

Substantial increase in grain production and a decrease in demand suggests that world stocks of coarse grains will rise by the end of 1999 (IGC, 1998).

V. IMPACT ON AUSTRALIA

The impact of the Asian economic downturn does not appear to have had the severe negative impact on the Australian economy that was anticipated. In terms of poultry meat, there is virtually none exported. The competition from within Asia, their devalued currencies and the ability of the US to dump chicken meat, particularly dark meat portions, on to world markets, give Australia little export opportunity. The US is forecast to export 2.4 million mt of poultry meat and 2,640 million eggs in 1999. The largest importers are China and Russia

for poultry meat, and Japan and Hong Kong for eggs (Anon., 1999a). If the playing field levels out, there is some indication that Brazil could become a threat to the domestic chicken meat market in Australia (Hambly, 1999). Brazil is more than self-sufficient in maize and soybean meal and has a sound poultry infrastructure.

H. McMaster, executive director of the Egg Industry Association (pers. comm., 1999), reported that export of eggs in 1996-97 was just over \$A2 million. Of this about 70% was exported to Asia (Table 3). In 1997-98 this had dropped to only \$A1.25 million. Otherwise the economic downturn has had little impact.

Table 3. Export of egg products from Australia to Asia (A\$000)¹.

Country	Year	
	1996-1997	1997-1998
Singapore	804	334
Malaysia	420	243
Hong Kong	157	172
Indonesia	93	74
Papua New Guinea	0	26
Brunei	167	19
Thailand	0	1
Total	1641	869

¹ Source: H. McMaster, executive director Australian Egg Industry Association Inc., 1999.

Feed ingredient prices, particularly protein concentrates, decreased substantially in September 1999 according to NSW Agriculture and this has been the trend for the past 3 years (Table 4). Recently (mid 1999) synthetic lysine had reached a low of A\$1.80/kg and methionine had dropped to A\$3.50/kg although prices have now increased. These declines have had a significant impact on the cost of manufactured diets in Australia (Table 5).

Table 4. The cost of selected feed ingredients (A\$/tonne) in bulk in the Sydney area during the past 6 years¹.

	1994 (February)	1995 (April)	1996 (February)	1997 (March)	1998 (April)	1999 (September)
Sorghum	180	220	230	169	176	136
Meat meal	443	370	465	437	305	316
Lupins	202	280	255	260	243	206
Cotton seed	320	300	250	257	264	224
Lysine (kg) ²	3.98	4.20	3.50	5.25	4.0	1.80

¹ Source: N.S.W. Agriculture

² In 25 kg bags

Table 5. Changes in the cost (A\$/tonne) of poultry diets between 1996 and 1999 (ex feed mill, Sydney)¹.

	Year			
	1996	1997	1998	1999
Broiler starter	372	363	347	282
Broiler finisher	349	332	313	255
Layer	324	305	293	243

¹ Source: A. Carey, Consultant Animal Feeds, 1999.

These diets are based on a 12 month ingredient average (November to November) with production costs and a profit margin included.

VI. CONCLUDING REMARKS

The economic downturn in Asia has had a dramatic effect in several countries in Southeast Asia, in particular Indonesia (Hutagalung, 2000). The financial crisis has reduced the rapid growth of the poultry and other livestock sectors as well as manufactured feed. However, there are indications that the tide has turned and there is room for optimism, particularly in the last quarter of 1999. November 1999 outlook is for a 4.4% increase in economic growth of 20 countries surveyed in the Asian region, up from 2.6% in 1998 and expected to exceed 5% by the year 2000 (Anon., 1999e). There are already signs of an upturn in the feed industry, particularly in China. Countries such as Thailand and the Philippines are growing alternative feed ingredients to reduce their reliance on maize and soybeans. Wheat, field peas, lupins and tapioca are becoming more popular (Welsh, 1999c).

The impact of the Asian situation on Australia has been minimal. The price of manufactured feed has declined substantially due to low ingredient prices globally. The decline in the Australian dollar has helped to make export of raw ingredients reasonably competitive. There are indications that the situation is changing and changing rapidly but not in all countries in the Asian region (Table 1). A major producer of vitamin and mineral premixes and methionine, with headquarters in Singapore, reports that Asia has almost fully recovered from the economic downturn with Malaysia and Thailand showing extraordinary recovery (K. Hall, Rhone-Poulenc Animal Nutrition, pers. com., 1999). This is encouraging news for Australian exporters. In 1998-99 Asia accounted for 53 per cent of agricultural exports (Anon., 1999f).

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THE MONETARY CRISIS AND ITS IMPACT ON THE DEVELOPMENT OF THE POULTRY INDUSTRY IN INDONESIA

R. I. HUTAGALUNG

Summary

The poultry industry in Indonesia has started to recover from the economic crisis, although the continuing political uncertainty and a lack of confidence of the overseas investors in the current government, together with the low purchasing power of most of the people have markedly affected the industry.

Village chickens continue to play an important role in Indonesia as providers of protein. The small to medium scale poultry farmers have sufficient resources to rear the village chickens, and with the improvements in the production system and disease control, they can make a major contribution to animal protein demand.

Low-protein diets allow the use of large amounts of locally available alternative feedstuffs, thus reducing the need to import feed ingredients. The use of low protein diets for broilers and layers, fortified with essential amino acids, was shown to provide a significant profit margin, even though the production performance was slightly lower than those on the conventional diets that met their nutrient requirements.

I. INTRODUCTION

The impact of the economic crisis was felt in Indonesia since July 1997 when all of the industrial sectors had a sudden setback, with the exception of the agriculture sector.

Indonesia is a country that has had a recent rapid rise in net *per capita* income starting from a relatively low base. Per capita consumption of chicken meat in Indonesia was only 5 kg per year in 1997; in the same year in Malaysia, *per capita* consumption had reached 30 kg. The agriculture sector expanded by 0.2%, an insignificant increase compared to an average of 7% in the previous year. The agricultural sector in 1996 accounted for 16% of gross domestic product and employed 44% of the country's workforce. Gross domestic product and consumption rates should remain flat following the sharp decline in production in 1998 and inflation should drop to 25% from more than 80% recently.

II. POULTRY FEED SITUATION

The government has eliminated the duty on importation of feedstuffs, particularly soybean meal, fish meal, feather meal, meat and bone meal, maize, rape seed meal, maize gluten meal, synthetic amino acids, mineral and vitamin premixes and other additives. A major stumbling block for feedmills was a difficulty in obtaining financing for overseas' purchases of feedstuffs. With Indonesia's banking system in tatters and still struggling to reform itself, local financing was next to impossible. Furthermore, even though the US government offered credit guarantees or soft loan financing, the very unstable rupiah often made it difficult for the feedmills to open letters of credit necessary for the transactions.

The government in 1998 offered indirect subsidy in the form of a lower exchange rate against the US \$ (less than 40% of the actual market currency exchange rate at that time) to allow importation of major feed ingredients. This benefit has not yet reached the farmers, since the National Logistic Board (BULOG) offered this facility only to major feedmillers. No benefits have yet been passed down to the poultry farmers.

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In addition to the transition to a free market (globalization), feedmills have been forced to contend with a precipitous plunge in demand. Feed production in 1996 peaked at 6.5 million tons, declined to 4.96 million tons in 1997 and dived even further to 2.21 million tons in 1998. With an annual installed capacity of feedmills in Indonesia of about 9.5 million tons, output would be only 23% of capacity (Table 1).

Table 1. Feed and poultry production in Indonesia.

Item	1996	1997	1998	1999*	2000 ¹
Feed production (million ton)	6.50	4.96	2.21	2.66	5.00
Poultry population					
Broiler (million)	755.96	641.37	354.00	418.94	431.51
Layer (million)	78.70	70.62	38.86	41.97	45.01
Local (million)	260.71	260.84	253.13	266.00	272.30
Duck (million)	29.96	30.32	25.95	26.28	27.03
Total	1125.34	1003.15	671.94	752.89	775.85
Poultry meat production					
Broiler (x1000 ton)	604.95	515.30	285.00	337.00	348.00
Domestic (x1000 ton)	281.46	314.01	294.00	318.00	320.00
Layer (x1000 ton)	40.13	48.93	26.00	27.00	30.00
Duck (x1000 ton)	20.43	20.39	16.00	18.00	19.00
Total	946.97	898.63	621.00	700.00	717.00
Egg production					
Layer (x1000 ton)	500.61	483.14	267.00	275.00	298.00
Domestic (x1000 ton)	128.79	123.67	126.00	131.00	133.00
Duck (x1000 ton)	150.40	158.22	137.00	140.00	142.00
Total	799.81	765.03	530.00	546.00	573.00

¹ Forecast

With the small economic rebound, Indonesian feed compounders are optimistic that if the economy continues to recover, even slightly, feed production will bounce back to around 2.66 million tons in 1999 and to 5 million tons by 2001/2002. Indeed confidence is high over the long term. The driving factor behind the feed situation was the decimation of the poultry industry. At the end of 1998, poultry operations had contracted to a mere 30% of their pre-crisis levels. The broiler operation suffered the most in that nearly 80% of the farmers ceased production while the egg producers declined by 40%. Even though poultry prospects are improving, few expect the industry to return to its former state. Small to medium independent poultry operators are constrained by high processing cost for chickens, and although they have asked the government to introduce an important program, funding for such an effort remains problematic in light of the current financial conditions.

Compound feed production in Indonesia is mainly for broiler chickens, laying hens, and aquaculture. Production of feed for layer ducks, swine and ruminants (beef and dairy) is small and generally there is on-farm mixing. Production of animal feeds in Indonesia is mainly to sustain these sectors. Of the total feed produced in Indonesia in 1997, 45% was for broiler chickens, 30% for laying hens, and 25% for aquaculture.

III. ALTERNATIVE FEED INGREDIENTS

Farrell (1998) stated that if the poultry industries are to grow at the projected rate there should be a radical rethink of the method and level of feeding by employing available feedstuffs and examining conventional feeding systems. Furthermore he has repeatedly advocated the concept of matching livestock production to available resources.

These alternative ingredients can be divided into energy-based ingredients and protein-based ingredients. Alternative energy ingredients include sorghum grain, cassava root meal, rice bran, and palm oil. Alternative protein ingredients include groundnut meal, coconut meal, kapokseed meal (*Ceiba petandra*), cottonseed meal, palm kernel meal, and rapeseed meal. In Australia, grain legumes like chick peas, field peas, lupins and faba beans, have been successfully fed to poultry (Farrell, 1998).

Addition of appropriate feed enzymes such as phytase, to rice bran-based diets, and enzymes to digest ingredients containing non-starch polysaccharide (NSP) have been shown to improve utilisation and/or digestibility of nutrients in diets comprising high levels of alternative ingredients.

Details of nutrient composition and poultry performance can be found in Farrell (1998) and Hutagalung (1998). With improvements in feed formulation techniques using digestible ingredients *versus* the reduced total nutrient specifications, the low protein diets exhibited comparable results to conventional dietary protein levels indicating excess dietary nutrients in some current published tables of nutrient requirements (Farrell, 1998; NRC, 1994; Ravindran *et al.*, 1998).

IV. PRODUCTION SYSTEMS

Independent poultry farmers with capital, who were able to survive the 1997 economic crash, have managed to regain some profit in 1998/99. Small to medium scale poultry farmers rearing less than 5,000 broilers per cycle joined the contract farmers or so-called integrated partnerships.

Due to the high demand for day-old chicks, the price soared to Rp 2,500 per chick (US\$0.20/chick) from the previous low of US\$0.05-0.10/chick before mid 1997. The government also provided soft term credit cooperative facilities during the economic crisis. However, this soft credit system failed to serve its purpose because the major companies monopolized the entire market operation systems.

(a) Integrated farming systems

The objective of integrated farming systems has been to bridge the gap between two contrasting objectives; these are to allow the major companies to freely accelerate poultry production, and at the same time to give the opportunity for the small scale farmers to rear poultry. However, with a lack of control measures by the government, small farmers have been skeptical of the integrated package offered by the large companies, due to the monopoly practice of the major poultry companies and their ability to create an imbalance in the poultry agribusiness. The recovery of the poultry industry appears to be more for the large-scale operators and not for the small scale producers.

The economic crisis resulted in major changes in the poultry business. Vertical integration is the answer to the present ailing poultry industry in Indonesia (*versus* non-integrated systems). The hybrid chicken, which was mainly owned by the independent poultry farmers, now becomes part of the integrated operation. At present, more than 70% of the broiler operation is under a partnership arrangement. Layer farmers remain as an independent entity, while the majority of broiler farmers operate under a partnership agreement. There are several types of arrangements in the poultry industry such as contract farming, strategic alliances, vertical integration, and cooperative systems. Since 1970, the government has encouraged the contract farming system, which is also known as 'partnership'. Partnership in the poultry industry is a business cooperation between the small-scale farmer and the medium, or large-scale industry operation with the purpose of strengthening the industry's overall system.

(b) Village chickens

Village chickens are locally known as “ayam buras”, meaning “the native chicken”, mixed with a genetic pool of improved strains. Village chickens are those chickens where there is a mixture of various strains, which cannot be genetically detected due to natural crossings. In Indonesia it is likely to be the combination of *Gallus various*, *G. gallus*, *G. sonnerati* and *G. lavayetti*. These various crossings have developed into local, identifiable chickens such as “Kedu”, “Bekisar”, “Nunukan”, and “Pelung”.

Village (native, domestic) chickens continue to play an important role in Indonesia as a source of animal protein. These chickens are normally kept by smallholder farmers in a traditional scavenging system, with minimum inputs and poor performance (Sinurat *et al.*, 1993; Hatmono, 1999). The rearing pattern of the village chickens is mainly traditional (extensive) where the birds are allowed to roam, and to find their own feed with occasional supplementation. Some farmers rear the village chickens under a semi-intensive system from one-day-old chick until marketing at about three months. They are housed and later kept under free-range conditions, or sold. Hence the development of village chickens is not as rapid as hybrid chickens. Lately the demand for village chickens has increased in line with the fast growth of local restaurants and the tendency of people to choose village chicken meat, which they consider tastier, lower in cholesterol, more tender and not containing drug residues or growth promotants. This perception allows good opportunity to intensify the production of the village chickens and to turn it into a modern agribusiness. The demand for the meat and eggs of the village chickens is not seasonal and the price does not fluctuate as in the case of eggs and meat of the hybrid chickens. Apart from the sensitivity to the viral Newcastle Disease and low productivity, village chickens can adapt easily to the poor environment and management; they are not sensitive to high levels of ammonia, can be provided with low quality feed and can survive better under stress compared to the hybrid chickens.

Under the government program entitled “Animal protein self sufficiency in 2001”, out of the total budget of Rp15,898 billion (equivalent to USD2.27 million), the village chicken program represents the largest part (53%) of the total budget, followed by goats (27%), sheep (14.3%), and ducks (5.7%). Currently, native chickens contribute about 30% of meat and 16% of eggs to the total demand. In the development of native chickens, to achieve animal protein self sufficiency in the year 2001, the population of native chickens will increase by 13 million within three years (1998-2001) and produce about 21,882 tons of meat and 8,841 tons of eggs. The development of duck farming for eggs and meat will be carried out at the same time as the village chickens. The population of ducks should increase by 1,334,000 during three years and will supply 2,270 tons of duck meat and 3,000 tons of duck eggs (Hatmono, 1999).

Egg production of village chickens varied from 40 to 150 eggs per year and the body weight of 1.6 kg can be reached at 16 to 24 weeks of age (Sinurat *et al.*, 1993) compared to 5-6 weeks of age for the hybrid broiler. The village chicken is a very convenient source of animal protein for the villagers. Egg production of village Kedu chickens is 79% of hybrid layers, although the eggs are 66% smaller (Table 2).

Village chickens are normally kept by smallholders (10 to 20 birds per family), but their contribution to egg and meat consumption is significant, in many countries (Table 3). During the economic crisis in Indonesia, village chickens contributed more meat than hybrid chickens.

In many countries, meat and eggs of village chickens are in greater demand and fetched a higher price than those of improved breeds. Most of these chickens are reared in a traditional system, although a few have been kept in a semi-intensive system, or almost as

Table 2. Productivity of village layer chicken and ducks in Indonesia.

Breeds	Egg production (eggs/year)	Egg weight (g/egg)	Feed intake (g/bird/day)	Feed efficiency (kg feed/kg eggs)
Chicken:				
Pure native	37-42	35-45	88	4.9
Black Kedu	215	44	93	3.6
White Kedu	197	39	82	3.8
Imported breed	260	55-70	118	2.7
Ducks:				
Bali	150-200	-	150	na ¹
Alabio	250	-	143	na
Indian Runner	140-250	-	155	na
Khaki Campbell	280	-	148	na

¹na = not available. Source: Hatmono (1999)

Table 3. Contribution of village chickens to meat and egg consumption in several countries.

Country	No. of birds (x 1000)	Meat (%)	Eggs (%)	Combined meat & eggs (%)
Burma	27,260	-	-	84.6
Indonesia	199,433	46.5	17.6	-
Malaysia	6,500	10.2	5.2	-
Philippines	59,200	-	-	-
Sri Lanka	2,500	-	-	32.7
Thailand	40,000	-	-	-

Source: Sinurat *et al.* (1993)

intensively as commercial layers. Lack of knowledge, resources and capital have been the main stumbling block for the smallholders to maintain the chickens scavenging. Mortality is high among the village chickens, as they are not vaccinated against various diseases. Newcastle Disease is the major disease contributing to this high mortality rate. The productivity of these village chickens can be improved by supplementation of traditional feeds, or feed by-products such as rice bran, cassava chips, coconut meal etc.

V. POULTRY FEEDING SYSTEMS

The poultry rearing industry in Indonesia started more than 30 years ago and went through a rapid development phase over the last 10 years prior to the economic crisis in July 1997. The poultry industry in Indonesia, however, remains vulnerable mainly due to the large amount of feed ingredients that are still imported. Despite the slow growth of the industry between July 1997 and the end of 1998, feed requirements are projected to increase from 6.5 million tons in 1996 to 7 and 8 million tons in the year 2000 and 2001, respectively. Assuming that 50% of the feed consists of maize, the requirement for maize for poultry is likely to reach 3.5 million tons in 2000. Also, the requirement for soybean meal and fish meal is likely to increase in 2000. Based on the heavy dependency on imported feeds and the fact that feed represents 60-70% of production costs, the major constraint to the poultry industry is a lack of locally-produced feed ingredients to meet feed requirements.

(a) Feeding systems and poultry production

Based on the survey conducted recently on the system of feeding and production pattern for broilers and layers in West and East Java (Sinurat, 1999), the broiler farms used mostly commercial feed, while some layer farms utilized both commercial and home-mixed feeds. Broiler feeds are generally given in a crumbled form, while layer feeds are given either in a mash or crumbled form. Farmers who used entirely commercial feeds were affected more by the price fluctuations of imported raw materials, and the drastic currency devaluation against the US dollar, than those using a combination of imported and local ingredients. Maize and rice bran are the two most important ingredients available to the poultry farmers.

Performance of broilers kept under different types of management is given in Table 4. In general the performance of broilers is slightly inferior to that for standard hybrid broilers, especially growth rate. The broilers were marketed at from 32 to 40 days with a live weight of 1.2 kg to 1.8 kg. These performances can be attained by the commercial broilers at between 28 to 38 days. Also mortality is rather high (5.9%) with wide variation (0-33.3%). This average mortality of 5.9% is nearly the same as the average mortality rate in the tropics, but is higher than that of 4.6% for the sub-tropics (Sinurat, 1998).

Table 4. Performance of broiler chickens in different production systems on Java.

Business Farm Type	Age at marketing (days)	Live weight (kg)	Feed/gain (kg/kg)	Mortality (%)
Integrated	38.4	1.58	1.81	5.77
Poultry shop	35.6	1.38	1.90	5.75
Contract farming	34.2	1.36	1.84	8.28
Rented	32.4	1.33	1.76	6.70
Independent	35.4	1.45	1.71	4.77
Government credit farm	37.3	1.67	1.71	5.10
Standard (Hubbard)	28	1.12	1.64	
	35	1.53	1.71	
	42	1.96	1.90	

Source: Sinurat (1999)

The performance of laying hens of the farmers under partnership arrangements with the poultry shop, and the self sustaining farmers, is still inferior when compared to standard layers (e.g. Isa Brown), notably on the average yearly egg production. The lowest yearly average egg production was observed at the pullet-based poultry shop-layer farmer (70%) and the highest was at day-old chick-based poultry shop-layer farmer (81%) (Table 5).

Table 5. Performance of brown egg layers at the poultry shop-farmer partnership and independent farmers in East Java (Blitar).

Item	Poultry shop & farmer partnership from DOC stage	Poultry shop & farmer partnership from pullet stage	Independent farmer	Standard performance (Isa Brown)
Point of lay (wk)	19.7	18	18	19
Av. production (%/year)	81	70	73	83.8
Culling age (wk)	103	89	101	78
Mortality to:				
Pullet stage (%)	5.13	2.0	3.5	na ¹
Culling stage (%)	6.6	3.0	8.2	6.7
Feed intake:				
Pullet (kg/bird)	13.73	14.10	14.20	na
Production (g/bird/d)	110	116	113	115

¹na = not available; Source: Sinurat (1999)

(b) Low protein diets

Although Indonesia is considered to be among the largest producers of agricultural crops for feed ingredients in the Southeast Asian region, the quantity remains insufficient to meet the demand for compound feed production. Similarly, the production of both animal and plant protein, and of good quality energy-based feedstuffs, is low. In such a situation, steps must be taken to overcome these shortages of quality feed ingredients. There is a need to reduce imported raw materials and to reduce the accepted nutrient requirements for poultry, more specifically the dietary protein and energy contents. The results of various experiments and feed trials on low-protein diets in broilers and layers under hot and humid environments showed that the protein content of layer diets can be reduced from 170 g to 140–150 g/kg without significantly affecting performance, providing that the diets meet the needs for the essential amino acids. Although government regulations specified the minimum protein requirement of 170 g/kg for diets for hens in production, the feedmilling industry established its own standards which do not necessarily comply with government's outdated regulations.

This is also the case for broiler diets, where the protein and energy levels can be reduced without having a significant effect on bird performance. Since 1988, there has been a practice in the feedmilling industry in Indonesia to formulate the broiler starter diets on 190–210 g/kg protein (*versus* the conventional 210–230 g/kg protein) and 12.13–12.55 MJME/kg (*versus* 13.39–13.81 MJME/kg), and for broiler finisher diets at 170–190 g (*versus* the diets of conventional 190–210 g) of protein/kg, and a similar range of energy levels as for broiler starter diets (12.13–12.55 MJ/kg vs 13.39–13.81 MJ/kg). Insufficient essential amino acids are provided by synthetic amino acids (Table 6).

Kompiang and Matondang (1985) compared four protein levels (160, 250, 140, and 130 g/kg) with or without amino acid supplementation in diets of laying hens (Hyline) for 12 weeks. Diets with 150 g protein/kg compared favourably with the 160 g protein/kg diet (control) in egg production, while the quality of those diets containing 160 g protein can be improved by adjusting essential amino acid levels to those on the 160 g protein diet. They claimed that a 130–140 g protein diet could reduce soybean meal requirement by 55% compared to the 160 g protein/kg diet. The use of low protein diets in poultry has the advantage in maximising the dietary protein utilisation and reducing nitrogen excretion (Farrell, 1998). Farrell *et al.* (1998) reported no significant difference in the performance of layers given diets with 120 g and 170 g protein. In a post-moult study of laying hens, improved early post-moult performance is likely to be achieved with 120 g protein/kg diet

when supplemented with various combinations of maize gluten meal, fish meal, methionine and lysine (Koelkebeck *et al.*, 1999).

Table 6. Adapted (A) and conventional (C) nutrient specifications of diets for broilers and brown-egg-laying hens in Indonesia (Sinurat, 1998; Hutagalung, 1998 a,b).

Type of diet	Protein (g/kg)	ME (MJ/kg)	Prot/ME (mg/MJ)	Lys (g/kg)	Met (g/kg)	Thre (g/kg)	Tryp (g/kg)	Ca (g/kg)	P av (g/kg)
<i>Broiler diet²:</i>									
Starter (A)	200-230	12.6-12.9	16.0-17.7	12.5	5.2	8.0	2.1	10.0	5.0
Starter (C)	230	13.4	17.2	11.0	5.0	8.0	2.0	10.0	4.5
Finisher (A)	180-220	12.6-13.4	14.3-17.0	11.5	4.5	7.2	2.0	9.5	4.6
Finisher (C)	200	13.4	15.1	10.0	3.8	7.4	1.8	9.0	3.5
<i>Layer diet³:</i>									
Starter (A)	180-200	11.9-12.6	15.1-16.0	11.0	4.8	7.0	1.9	10.0	4.6
Starter (C)	170	12.1	14.8	8.0	3.8	6.4	1.6	9.0	4.0
Grower (A)	170-190	11.5-12.1	14.8-15.8	8.8	3.0	6.2	1.7	10.0	4.8
Grower (C)	150	12.1	12.4	5.6	2.3	5.3	1.3	8.0	3.5
Developer (A)	140-160	10.9-11.5	12.7-12.9	7.0	3.0	4.7	1.6	10.0	3.8
Developer (C)	140	12.1	9.8	4.2	1.9	3.5	1.0	8.0	3.0
Production (A)	160-180	11.1-12.3	14.3-15.8	8.5	4.0	6.0	1.8	35.0	4.2
Production (C)	160	12.1	11.9	4.9	2.1	4.4	1.1	18.0	3.5

¹ Broiler starter (0-3 wk); broiler finisher (3-6 wk)

² Adapted (A) nutrient recommendations reflect either commercial or economical feed specifications for more use of local ingredients. Conventional (C) nutrient recommendations are recommendations of NRC (1994)

³ Layer starter (0-6 wk); layer grower (6-12 wk); layer developer (12-18 wk); layer production (>18 wk)

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EVALUATION OF DOSE-RESPONSE DATA AND IMPLICATIONS FOR COMMERCIAL FORMULATION OF BROILER DIETS

S. MACK, D. HÖHLER and M. PACK

Summary

The database of this paper consists of nine published dose-response experiments with methionine plus cystine (Met+Cys) and five with lysine (Lys). These data were consolidated with a particular focus on their economic implications for practical feed formulation and bird performance. Minimum feed cost per kg live weight gain and minimum feed cost per kg breast meat were chosen as performance indicators. They are meaningful for the overall profitability of a poultry production enterprise as they can be easily calculated and they combine input and output variables which cover the whole production process. Case scenarios were drafted for the effect of dietary Met+Cys and Lys on economics based on the dose-response data and current prices for feed and supplemented amino acids. The calculations show that the optimum dietary amino acid content is largely influenced by the production goals "live bird" or "breast meat".

I. INTRODUCTION

Most managers of modern poultry enterprises agree that their foremost business objective is profitability. Typically, profitability is defined by criteria like net income and return on investment. The difficulty is that these criteria involve a large number of variables. Therefore, they are not suitable as tools for everyday management decisions in a business which typically consists of different segments like feed mill, hatchery, live production and processing. This conflict often leads company management to focus on performance indicators like feed cost per ton, feed to gain ratio, livability, uniformity, carcass yield etc. They can be directly measured and attributed to the different segments of the operation thereby facilitating the decision making process. Additionally, benchmarking systems implemented in each segment for factors such as "lowest feed cost" or "highest livability", combined with a salary bonus, help to translate business decisions into action. Many of these performance indicators serve to de-link the enterprise into discrete business units. But does this disintegrative way of management really lead to the best overall profit for the operation? Trade-offs between the different criteria exist. For example, lowest feed cost per ton will most likely not lead to the best live production performance or carcass quality. The difficulty for company management using this approach is, firstly, to define the right criteria on which to focus and, secondly, to balance them in such a way that the bottom-line objective of maximizing profit is met. Indicators for overall profitability of livestock production are only meaningful if they comprise the whole production chain, combining key input (feed and supplement cost) and output variables (marketed product). Moreover, they should be easy to calculate, based on current price and animal performance data. The indicators "minimum feed cost per kg live weight gain" or "minimum feed cost per kg breast meat" meet these prerequisites whilst focusing on different production goals. The amino acids lysine (Lys) and methionine plus cystine (Met+Cys) will be used as the variable factors in the present paper. Their concentration in the feed has a large effect on a number of efficiency measures in the various segments of an

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integrated broiler operation (feed cost, live performance, carcass quality). Hence, they may serve as good examples for demonstrating the effect of different decision-making strategies on overall profitability.

II. METHODS

The database of this paper consists of nine published dose-response studies with Met+Cys and five published experiments with Lys. The table gives details of the experimental designs. All experiments were conducted within a similar range of dietary Met+Cys and Lys levels of 0.60 % to 0.96 % and 0.79 % to 1.25 %, respectively. Four to six incremental levels of synthetic DL-methionine or L-lysine were added to a deficient basal diet. Data comprised the grower period of male or mixed-sex broiler chickens of commercial crosses within the range of 7 to 42 days of age. The economically relevant performance criteria weight gain, feed to gain ratio and breast meat yield in g per kg live weight at slaughter were chosen as bases for an economical evaluation. Due to the different experimental conditions and their effect on the general level of performance, the criteria had to be transformed to a relative scale. The performance data were subjected to exponential regression analysis (Schutte and Pack, 1995a) separately for each experiment. The maximum response in performance as described by the asymptote of the regression was set at 100. Then, the performance at each tested amino acid level was expressed as a percentage of the asymptotic value. Thereafter, relative performance values from each experiment were pooled. Each data point represented four to eight replicates of 17 to 50 birds. Subsequently the pooled data were subjected to exponential regression analysis. This gave for each performance criterion one dose-response curve describing the effect of graded dietary amino acid content on relative animal performance.

In order to calculate the economic indicators "feed cost per kg live weight gain" and "feed cost per kg breast meat" assumptions had to be made for actual bird performance as well as for cost of feed and cost of supplemented amino acids. Asymptotic values of the regression curves which represent maximum performance were set at 2000 g live weight gain, 1.8 kg feed per kg of live weight gain and 160 g breast meat yield per kg live weight and reflect commercial practice. Performance at all other points on the curve were calculated from the relative performance at the specific amino acid content in the feed. Cost of basal feed without Met or Lys supplementation, DL-methionine and L-lysine·HCl were set at 0.14 US\$, 2.5 US\$ and 2.0 US\$, respectively. Based on the dose-response curves and the aforementioned price assumptions, the cost per kg feed and the economically relevant performance indicators "feed cost per kg live weight gain" and "feed cost per kg breast meat" were calculated for a wide range of dietary amino acid levels.

Cost / kg feed =

$$\text{Basal feed cost} + ((\text{cost} / \text{unit supplemented test amino acid} - \text{cost} / \text{unit basal feed}) * (\text{supplemented amino acid units}))$$

Feed cost / kg live weight gain =

$$(\text{kg feed} / \text{kg live weight gain}) * \text{cost} / \text{kg feed}$$

Feed cost / kg breast meat =

$$(\text{Feed cost} / \text{kg live weight gain}) / (\text{kg breast meat} / \text{kg live weight gain})$$

Table. Design of the dose-response experiments.

Reference	Sex ¹	Strain ²	Trial period (days of age)	Diet composition ³	Energy content (MJ ME/kg)	Crude protein content (%)
Met+Cys						
# 1	M	Ross	15-35	Sr, Sy	12.6	20.5
# 2	M	Ross	15-35	Sr, Sy	12.6	24.2
# 3	50% M, 50% F	Ross	14-38	M, Sy	13.4	22.7
# 4	M	Cobb	15-33	W, M, Sy	13.2	21.8
# 5	M	Ross	14-35	Sr, M, P, Sy	12.4	23.3
# 6	M	Ross	10-35	Sr, M, P, Sy	13.6	21.7
# 7	M	Ross	7-35	M, Sy	13.2 Grower, 13.6 Finisher	20.9 Grower, 20.2 Finisher
# 8	M	Ross	20-40	M, Sy	13.2	19.0
# 9	50% M, 50% F	Ross	21-42	M, Sy	13.0	20.9
Lys						
# 10	M	Ross	20-40	M, Sy	13.2	19.0
# 11	M	ISA	20-40	M, Sy	13.2	19.0
# 12	M	Ross, Hu x Pe	15-40	M, Sr, Sy	13.4	19.6
# 13	M	Ross	22-42	M, Sr, Sy	13.4	19.5
# 14	M	Ross	22-42	M, Sr, Sy	13.4	19.5

¹ M=male, F=female

² Hu=Hubbard, Pe=Peterson

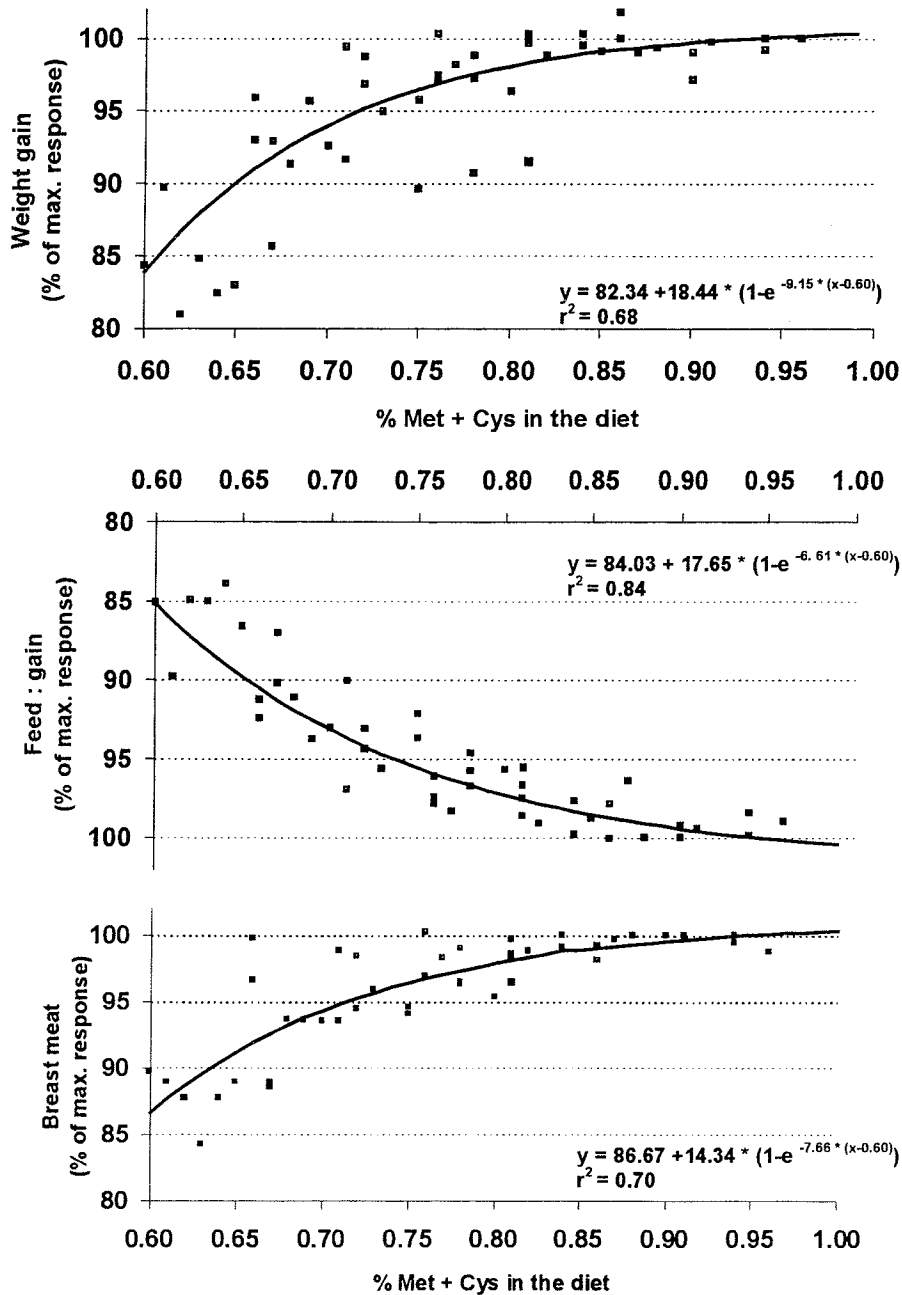
³ W=wheat, Sr =sorghum, M=maize, Sy=soybean meal, P=peas

III. RESULTS

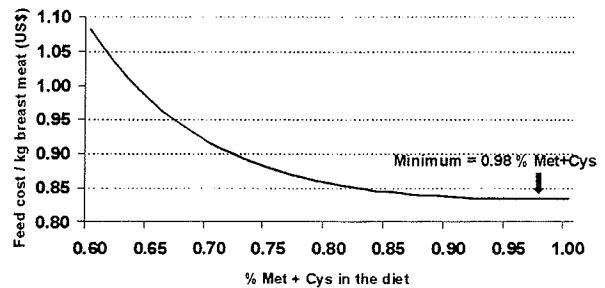
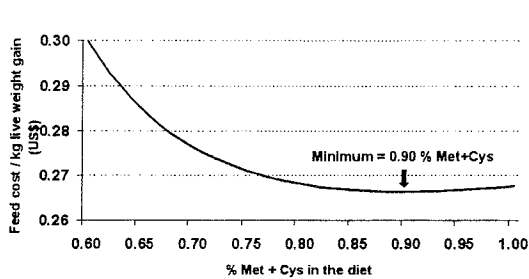
Drawing conclusions on economically optimum amino acid levels in feed requires a solid base of performance data from dose-response experiments carried out over a sensitive and practically relevant range of amino acid concentration in the diet. Pooled data from the included studies which were transformed to a relative scale show a consistent response in bird performance to increasing levels of dietary Met+Cys (Figures 1 to 3) and lysine (Figures 6 to 8). Exponential regression analysis describes these dose-response relations well.

(a) Response to dietary Methionine plus Cystine content

Under the given set of conditions feed cost per kg live weight gain reached a minimum at 0.90 % Met+Cys and increased thereafter (Figure 4). The shape of this response curve was determined, firstly, by the lower feed to gain ratio which approaches an asymptote with rising dietary Met+Cys concentration, and secondly, by the linear, ever increasing feed cost with higher Met+Cys content. The response in feed cost per kg breast meat to a higher dietary Met+Cys content was affected not only by the feed to gain ratio but also by the increasing breast meat portion of the live weight. This extra benefit included in the economic calculation shifted the optimum dietary Met+Cys concentration to 0.98 % (Figure 5).



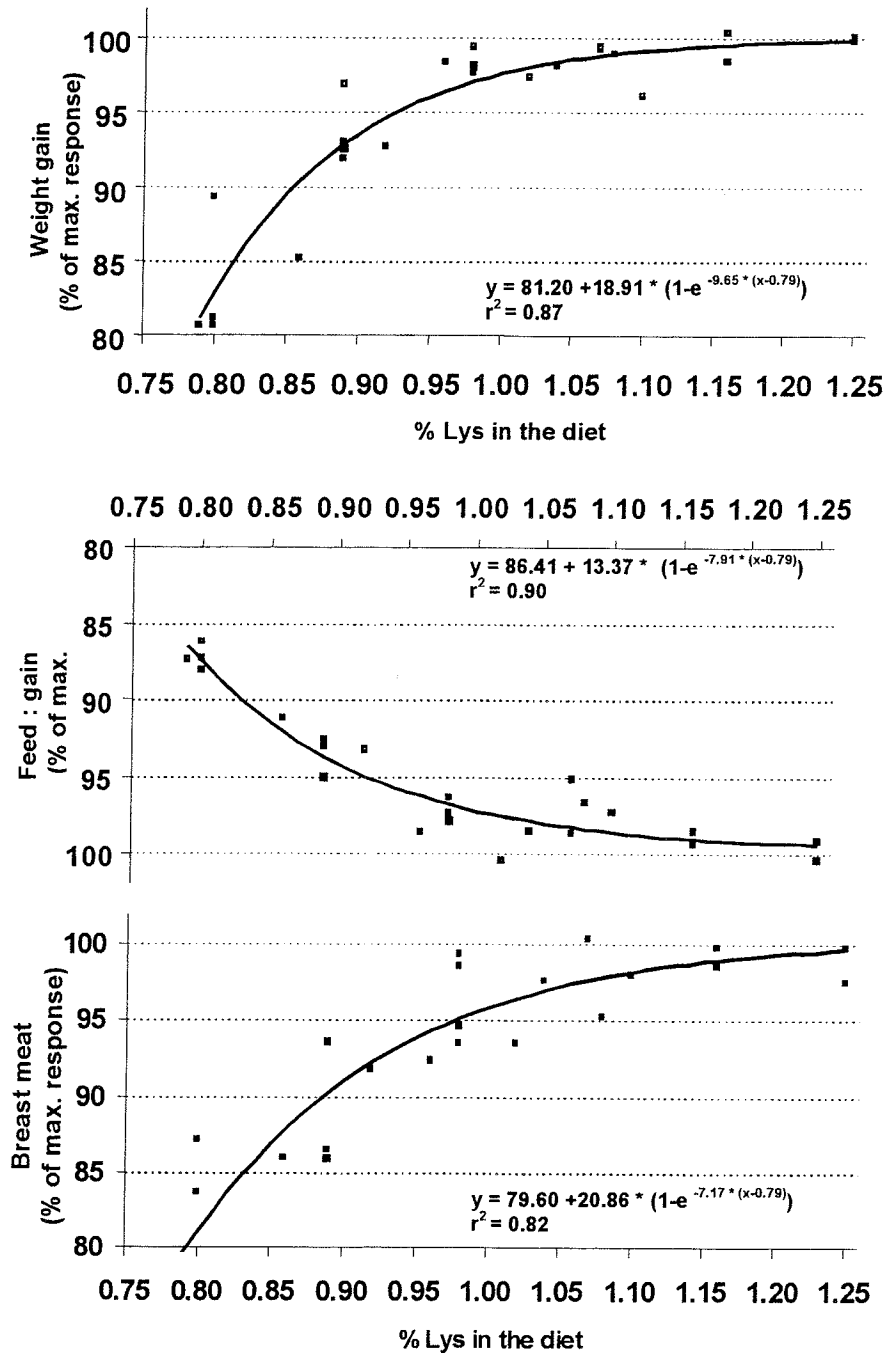
Figures 1 to 3. Relative response in weight gain, feed to gain ratio and breast meat yield (% of live weight) to graded levels of dietary Met+Cys.



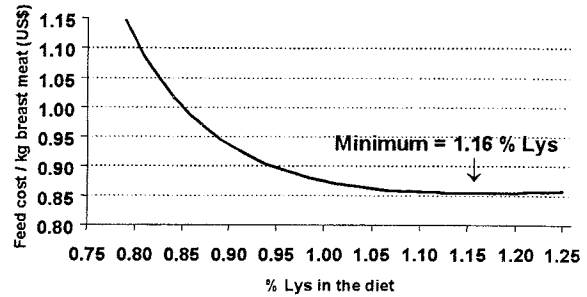
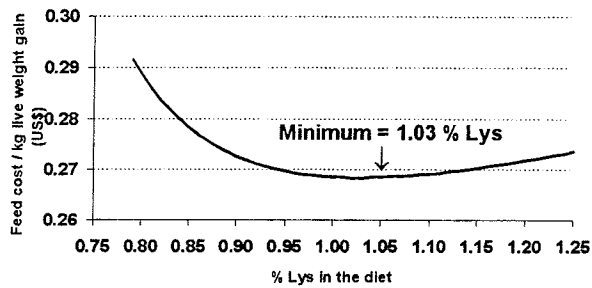
Figures 4 and 5. Effect of dietary Met+Cys content on feed cost per kg live weight gain and feed cost per kg breast meat yield

(b) Response to dietary Lysine content

Although the database for Lys comprised less studies than that for Met+Cys there was a consistent curvilinear response in relative bird performance to increasing dietary lysine concentration (Figures 6 to 8). Under the given set of conditions, feed cost per kg live weight gain and feed cost per kg breast meat were lowest at 1.03 % and 1.16 % dietary Lys, respectively (Figures 9 and 10).



Figures 6 to 8. Relative response in weight gain, feed to gain ratio and breast meat yield (% of live weight) to graded levels of dietary Lys.



Figures 9 and 10. Effect of dietary Lys content on feed cost per kg live weight gain and feed cost per kg breast meat yield.

IV. DISCUSSION AND CONCLUSIONS

Feed represents over 60 percent of the total production cost per bird and is worthy of close scrutiny. However, one has to keep in mind that lowering feed cost per ton does not automatically mean higher profitability.

In general, the process of deciding on raw material quality and nutrient specifications in feed should start with defining the properties of the desired product. The present paper shows large differences in economically optimum dietary amino acid levels depending on the product to be marketed. This approach may well be extended to other nutrients and is meant to serve as a general tool to decide about diet specifications in a meaningful way.

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MODEL FOR ESTIMATION OF AMINO ACID UTILIZATION AND ITS REQUIREMENTS IN GROWING ANIMALS

F. LIEBERT¹, M. RIMBACH¹ and M. PEISKER²

Summary

The model is based on a mathematical description of the pattern of nitrogen balance in growing animals, depending on nitrogen intake and feed protein quality, according to an exponential equation (Gebhardt 1966): $y = PD_{\max}T (1 - e^{-bx})$.

The slope of the curve in this model (b) is directly related to protein quality. Protein quality depends on the concentration (c) and availability of the limiting amino acid (LAA) in the feed protein. Thus the slope is determined by the degree of utilization of the LAA (bc^{-1}). Maximum values for the utilization of the LAA (bc^{-1}) have been identified and used as a reference value to evaluate the efficiency ratio of the LAA (eLAA) in different test proteins. In feed proteins where the amino acid under study is not an LAA, the efficiency factor can be determined using the difference method. The concentration of utilizable amino acids in feed proteins is calculated by multiplying the efficiency ratio of the amino acid under study with its concentration in the feed protein. The above mentioned equation is used to calculate requirements of limiting amino acids (x_{LAA}) after logarithmic transformation. Thus LAA requirements are determined depending on a selected utilization rate of $PD_{\max}T$ and the efficiency of utilization of the LAA.

I. INTRODUCTION

The utilization of dietary amino acids is influenced by a multitude of factors, e.g. the dietary amino acid balance, digestion and absorption patterns, which in themselves are subject to many influencing factors; the level of performance, dietary energy level and others. If the efficiency of utilization for a given amino acid is unknown, rather large safety margins are applied in feed formulation to account for differences in utilization from different ingredients. This is the case when formulating on a total amino acid basis. There have been many approaches in the past to overcome the shortcomings of this procedure. Formulating diets on a true ileal digestible amino acid basis is probably the closest approach to meeting actual requirements. However, in practice there is still some reluctance to adopt this approach for reasons of inadequate databases or questionable techniques when assessing true ileal digestibility. However, a general shortcoming of evaluation systems for amino acids on a digestible basis is that metabolic utilization is not taken into account. Incomplete metabolic utilization can be due to factors such as inevitable post-absorptive catabolism, chemical (metabolic) unavailable amino acids or imbalances in amino acid supply. This applies particularly when using the supplementation technique to a given basal diet with a limiting test amino acid. There is a serious risk that the basal diet may not be fully adequate in amino acids other than the one under test (Morris *et al.* 1999). The proposed approach to evaluate the utilization of limiting amino acids and derivation of requirements accordingly is based on a holistic approach by using N-balance procedures. The technique applied takes advantage of the dilution technique (Fisher and Morris, 1970; Gous and Morris, 1985).

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II. DESCRIPTION OF THE MODEL

(a) Determination of the efficiency of the limiting amino acid (eLAA)

Each animal has a genetic blueprint for its maximum protein deposition, and this is referred to as PD_{max} and includes nitrogen maintenance requirement (NMR). This parameter can be determined in feeding trials with subsequent carcass analysis or in N-balance trials. However, PD_{max} is theoretically somewhat higher for a given genetically homogenous group of animals than can be measured experimentally.

With an appropriate N-utilization model, this theoretical maximum for protein (N) retention ($PD_{max}T$) can be identified and used further for various purposes in growth models and for determination of the efficiency of utilization of amino acid and of amino acid requirements. The model is based on a mathematical description of the pattern of nitrogen retention in growing animals, which depends on nitrogen intake and feed protein quality, according to the following equation (Gebhardt 1966):

$$y = PD_{max}T (1 - e^{-bx}) \quad (1)$$

where:

y	=	actual daily N-balance + NMR / LW kg ^{0.67}	(mg)
$PD_{max}T$	=	max. theoretical capacity for daily N-balance + NMR / LW kg ^{0.67}	(mg)
x	=	daily N-intake / LW kg ^{0.67}	(mg)
b	=	slope of the curve	
e	=	base of natural logarithm	
NMR	=	nitrogen maintenance requirement, and	
LW	=	liveweight.	

Protein quality (b) is linearly related to the concentration of the limiting amino acid (LAA) of the test protein (c). The slope of the regression depends on the efficiency of utilization of the LAA (bc^{-1}) for N-retention. As a result of many N-balance trials with mainly growing pigs of various genotypes and using complex diets, maximum values for the utilization of the LAA (bc^{-1}) have been identified (Liebert and Gebhardt, 1988b; Liebert *et al.*, 1989). These maximum values can be used as a reference value to evaluate the efficiency ratio of the LAA (eLAA) in different test proteins according to equation (2). Figure 1 gives an example for the estimation of a lysine requirement curve in pigs with increasing performance (N-balance) compared with lysine from barley of two different varieties. Obviously there are different efficiency ratios for the utilization of lysine from the two barley varieties.

$$eLAA = \frac{(bc^{-1}) LAA \text{ test protein}}{(bc^{-1}) LAA \text{ standard}} \quad (2)$$

Given that barley is the sole dietary source of lysine, comparison at any given level of lysine intake shows that the efficiency of lysine utilization in barley I is higher than in barley II. Aiming for the same level of N-retention, less lysine is needed from barley I. The same approach can be applied to broilers.

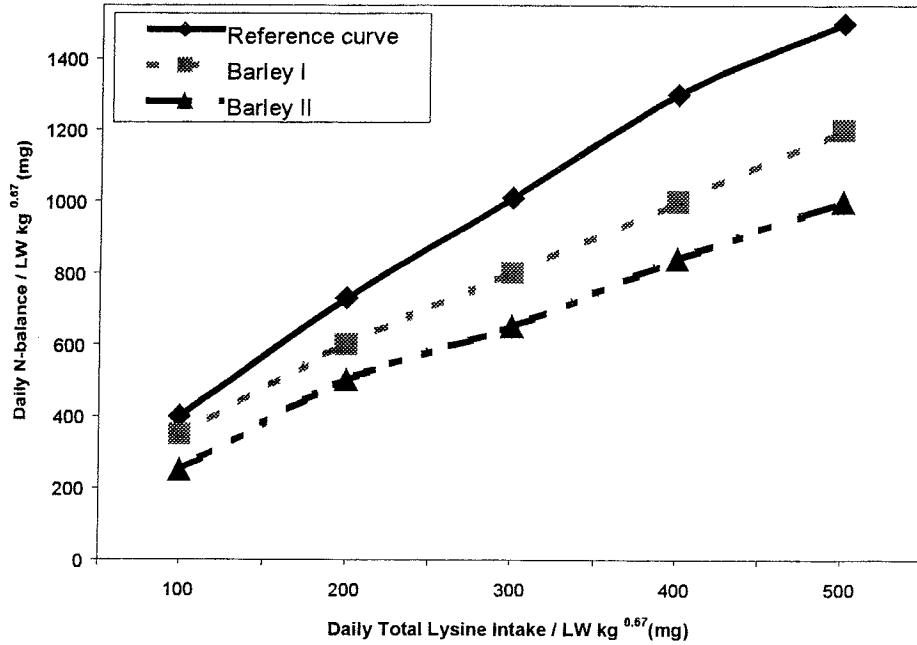


Figure 1 Lysine requirement curve in pigs, relative to different efficiencies of utilization of barley-lysine from different varieties (Liebert *et al.*, 1991).

In order to apply the model, the LAA in the test protein must be identified or set by either supplementation with amino acids or combination with other proteins. Where the amino acid under study is not limiting, the difference method can be applied (Liebert and Gebhardt, 1988b). This is important for feed formulation based on utilizable amino acids. After determining the efficiency ratio of the LAA under study, the concentration of utilizable LAA can be calculated according to equation (3).

$$c_u = c * eLAA \quad (3)$$

(b) Calculation of LAA-requirements

Equation (1) can be adapted to describe the relation between the intake of the LAA and daily N-retention.

$$y = PD_{max}T (1 - e^{-16b/c \cdot xLAA}) \quad (4)$$

c = concentration of the limiting amino acid in the test protein (g/16 g N)

(bc^{-1}) = efficiency of utilization of the LAA

x_{LAA} = daily intake of LAA / LW kg^{0.67} (mg)

After logarithmic transformation of equation (4) it can be used to calculate the requirement of the LAA depending on a selected utilization rate of $PD_{max}T$ and on the efficiency of utilization of the LAA.

$$X_{LAA} = \frac{\ln PD_{max} T - \ln (PD_{max} T - Y)}{16(bc^{-1})} \quad (5)$$

The term $(PD_{\max}T - y)$ in equation (5) represents the actual level of performance (N-balance + NMR) in relation to the theoretical maximum capacity for daily N-balance + NMR ($PD_{\max}T$).

(c) Example for calculation of threonine requirements in growing chicken

The described model was used to evaluate the average threonine requirements of the broiler strains Cobb 500 and Ross 208. In a schematic approach, table 1 shows the calculated threonine requirements for (I) an 80% utilization rate of $PD_{\max}T$ and for (II) a distinct protein deposition rate for both genotypes within the different age periods.

Table 1. Calculated threonine requirements for Cobb 500 and Ross 208 broilers (mean values).

Age period (days)	10 – 15	20 – 25	30 – 35
(I) 80% utilization rate of $PD_{\max}T$			
$PD_{\max}T$ (mgN/d/kg ^{0.67})	3753	3164	2696
Protein deposition (g/d)	6.8	11.3	13.8
Thr-requirement (mg/d)	458	776	991
(II) Distinct protein deposition rate			
Protein deposition (g/d)	7.0	11.0	13.5
Thr-requirement (mg/d)	499	739	952
Thr requirement (g/kg of diet)*	8.9	7.4	6.95

* Approximated on NRC 1994 feed intake figures

However, the rate of utilisation of PD_{\max} is not the same over the entire growing period. Under commercial conditions it will be lower than indicated in table 1, particularly in the first age period. The rate of utilisation that can be achieved under given conditions is, however, the important determinant of requirements

In practice certain levels of daily protein deposition are targeted rather than certain utilization rates of $PD_{\max}T$. The differences for $PD_{\max}T$ for the entire growth period between the tested broiler genotypes were found to be negligible. Thus an average value for $PD_{\max}T$ for both genotypes can be used for calculating requirements.

The model allows the calculation of LAA requirements depending on target amounts of daily protein deposition. It circumvents difficulties arising from conventional requirement studies such as dose-response studies using the supplementation technique. However, in future, feed related bioavailability differences of the LAA should be taken into account. The present data for threonine requirements are based on the bioavailability of threonine in soybean meal. More information about threonine bioavailability in other feed ingredients and its variation can be determined with the model. Based on these data, performance-dependent amino acid requirements can be expressed in terms of utilizable amino acids and considered in feed formulation.

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MAXIMUM PROTEIN DEPOSITION OF GROWING CHICKEN OF DIFFERENT GENOTYPE AND AGE

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Summary

Experiments were carried out to determine the theoretical upper limit for protein deposition ($PD_{max}T$) in two different male broiler genotypes (COBB 500 and ROSS 208) within three different age periods (10 – 15, 20 – 25 and 30 – 35 days). The results indicated insignificant differences with regard to $PD_{max}T$ between the two genotypes, and $PD_{max}T$ decreased with increasing age. The strain-mean values for daily $PD_{max}T$ for Cobb and Ross broiler chicken were determined as 3753 mg N/kg^{0.67} for the age period 10 – 15 days; 3164 mg N/kg^{0.67} for the age period 20 – 25 days and 2696 mg N/kg^{0.67} for the age period 30 – 35 days. The decline in $PD_{max}T$ was more pronounced for the Cobb genotype broilers from the first to the second age period, whereas the Ross genotype broilers showed a continuous decline of $PD_{max}T$. In the first age period (10 – 15 days) the Cobb birds exhibited a numerically higher capacity for N-retention than the Ross birds. In the second age period (15 – 20 days) the Ross birds showed a numerically higher N-retention capacity, whereas in the third age period (30 – 35 days), both genotypes were equal. Over the whole growth period, $PD_{max}T$ of the two genotypes was similar. It is therefore proposed that mean values for $PD_{max}T$ be used within an age period when applying the N-utilization model for calculation of amino acid requirements for these broiler strains.

I. INTRODUCTION

Knowledge of maximum protein deposition (PD_{max}) of growing animals is crucial for establishing amino acid requirements. Several experimental approaches can be used to determine PD_{max} , e.g. carcass analysis or N-balance trials. However, PD_{max} is theoretically somewhat higher for a given genetically homogenous group of animals than can be measured experimentally. With an appropriate N-utilization model, this theoretical maximum can be identified (Liebert et al. 2000). However, $PD_{max}T$ for a given strain is age dependent and declines with increasing age of the animal. To address breeding progress, re-evaluation of $PD_{max}T$ over appropriate time intervals is indicated, e.g. to check on traits connected with protein deposition like breast meat. The protein deposition rate is a key factor influencing amino acid requirements in fattening broilers and pigs. Knowing $PD_{max}T$ for different strains and ages enables nutritionists to derive amino acid requirements and formulate according to a target protein deposition that can be linked to economic factors such as feed cost and market price for broiler meat as well as ecological factors such as N-excretion.

N-balance trials were conducted to determine $PD_{max}T$ for different broiler strains over different age periods.

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II. MATERIALS AND METHODS

(a) The N-utilization model, evaluated parameters and statistical analysis

The model is based on a mathematical description of the pattern of nitrogen retention in growing animals; that depends on nitrogen intake and feed protein quality. The model is described in detail by Liebert et al. (2000). In the present experiment the value for PD_{max} T was obtained with incremental protein intakes of identical quality in N-balance trials. The following parameters were assessed: mean body weight, mean dry matter intake, daily N-intake and daily N-balance. The daily nitrogen maintenance requirement (NMR) was set according to literature data for broilers at $500 \text{ mg N/LW kg}^{0.67}$ (Mueller et al., 1989).

The results of the N-balance trial have been statistically fitted using MATHEMATICA Version 3.0 (Wolfram, 1997). The N-utilization curve was fitted with non-linear fit options for this program, minimising least squares.

(b) Experimental design, housing and diets

Table 1. Ingredient and nutrient composition of experimental diets (g/kg).

	Crude Protein (g / kg)					
	55	110	165	220	275	330
SBM	112	224	336	448	560	672
Soya oil	15	15	15	15	115	150
Cellulose	18	14.5	11	7.5	4	-
Potato starch	802.6	695.6	588.1	480.5	272.9	130.8
Premix	10	10	10	10	10	10
MonoCalcPhos	28.5	25	21.5	18	14.5	11
CaCO ₃	10.3	11.1	11.9	12.7	13.5	14.3
NaCl	0.9	0.9	0.8	0.7	0.6	0.5
MgO	0.7	0.1	-	-	-	-
L-Lysine-HCl	0.5	0.9	1.3	1.8	2.2	2.7
DL-Methionine	1.5	2.9	4.4	5.8	7.3	8.7
	(g/kg DM)					
Crude Protein	66	123	182	247	307	368
Crude Fat	19	19	22	24	137	163
Crude Fibre	24	29	31	33	34	45
NFE	835	769	697	621	441	339
ME (MJ/kg DM)*	14.16	14.39	13.73	13.37	14.85	14.50

* N-corrected metabolizable energy calculated acc. to WPSA (1984) equation.

Male broiler chicken of the genotypes COBB 500 and ROSS 208 were used in a N-balance trial (6 incremental protein levels) within three age periods (10 – 15, 20 – 25 and 30 – 35 days). After hatching, feather sexing and an adaptation period of 4 days, the birds were assigned to the treatments based on average body weight. Each protein level represented one treatment and each treatment had 6 replicates. The birds were housed singly in wire floored cages in a fully climatized room on a 24-h light regime. Temperature settings were according

to the breeder's recommendation. The N-balance experiment comprised a 5-day pre-collection period and a 5-day collection period.

Six diets with incremental protein levels from 55 – 330 g/kg in 55-g increments were fed *ad libitum*. The composition of the experimental diets, which have been pelleted, is shown in Table 1. Varying levels of high protein soybean meal were used to make up the target protein level. L-Lysine-HCl and DL-Methionine have been added at a constant ratio. The amino acid content of the diets was calculated from the analysed data of the soybean meal and the amino acid supplements. The dietary ratio of amino acids was formulated to be constant across diets. On the basis of ideal protein (Baker 1997), threonine was identified as the LAA in all test diets.

III. RESULTS and DISCUSSION

PD_{max}T decreased with increasing age in both genotypes (Table 2). The Cobb genotype showed a stronger decline from the first to the second age period than the Ross genotype. During the first period from 10 – 15 days PD_{max}T for the Cobb birds was 6.2 % higher and for the second age period 8.5 % lower than in the Ross birds. In the last period PD_{max}T was the same for both genotypes.

Table 2. Maximum protein deposition capacity (PD_{max}T) (mg N/LW kg^{0.67})¹ of two broiler strains at different live weights.

Age period (days)	10 – 15		20 – 25		30 – 35	
	Cobb	Ross	Cobb	Ross	Cobb	Ross
Aver. LW (g)	273	268	773	790	1479	1316
PD _{max} T ¹	3865	3640	3034	3293	2696	2695
g CP/d	8.8	8.1	13.4	14.9	17.9	16.5
R ²	0.985	0.973	0.965	0.977	0.952	0.969
Mean PD _{max} T ¹	3753		3164		2696	

Figure 2 gives an example for the pattern of the N-balance curve with increasing N-intake for the two genotypes from 10 – 15 days of age. Each single data point in figure 2 represents an average of six single birds for each genotype. The high R² indicates that the curves were a good fit to the experimental data. Differentiation between genotypes occurs only with higher N-intakes, which in turn has consequences for amino acid requirements.

Based on the figures in Table 2, LAA requirements can be calculated for different rates of utilization of PD_{max}T within an age period for different genotypes. An example for threonine as the LAA is given in the paper of Liebert et al. (2000).

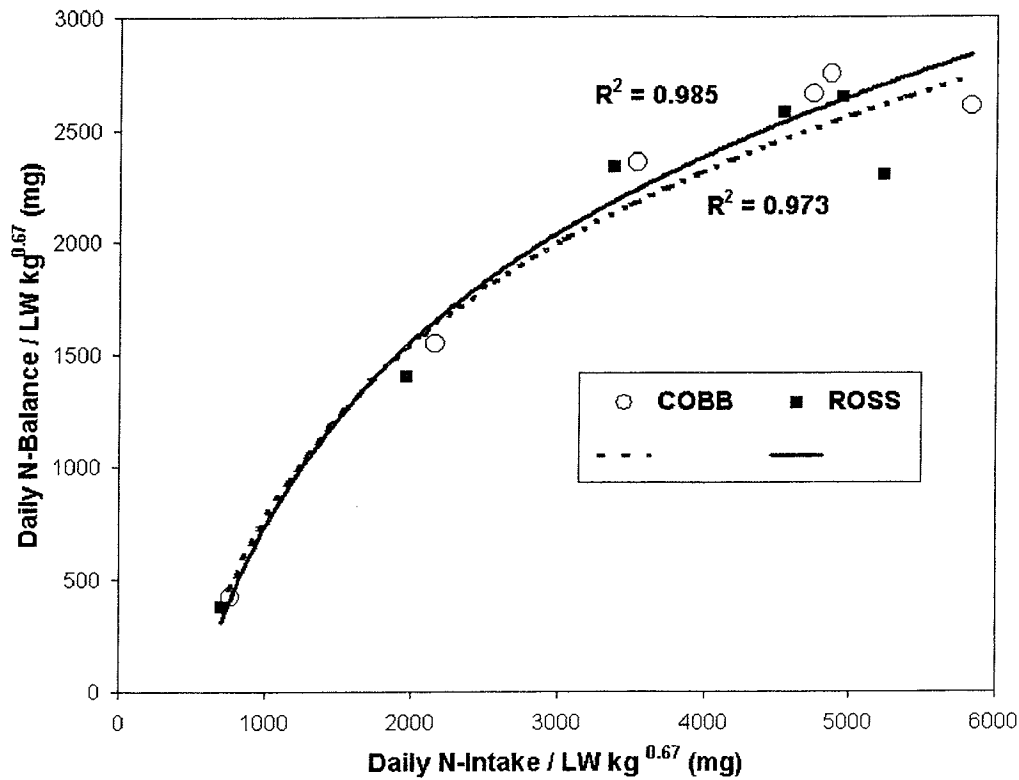


Figure 2 N-balance with incremental N-intake in different broiler strains (10 – 15 days).

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EARLY PRESENTATION OF PRELAYER DIET IN LAYING HENS: EFFECT ON SUBSEQUENT PERFORMANCE

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Summary

Laying performance, faecal moisture and blood electrolytes resulting from feeding pullets three weeks earlier than usual, with prelayer diets, containing 22 g/kg calcium, were studied in ISABrown laying hens. No significant differences were observed in sexual maturity, body weight or egg shell quality between the control and early prelayer groups. However, body weight gain of the early prelayer hens was depressed during the first six months of lay. Consistently, hens fed prelayer diets earlier produced smaller eggs with significantly lower albumen weight and Haugh units, and consumed 6-8% less feed than did the control birds. Neither egg production nor faecal moisture was influenced by prelay treatment. Increased blood ionised calcium was observed in the early prelayer hens during the early laying period followed by decreased levels during the later period of production. It was concluded that early feeding of prelayer diet in pullets adversely affected growth rate, feed consumption, laying performance and plasma calcium balance.

I. INTRODUCTION

Over the past few years, some producers have used a combination of imported layer strains and Australian-bred strains within the same farm. Despite the difference in age at sexual maturity, sometimes all birds have been placed on to prelayer or layer diets at the same time. Australian-bred strains commence lay several weeks later than imported birds (Nolan *et al.*, 1998). Under such conditions, the Australian-bred birds have a high intake of calcium which is not utilised for egg production. Prolonged feeding of high dietary calcium in pullets prior to maturity has been reported to adversely affect body weight gain (Anderson, 1966) and kidney structure (Shane and Young, 1969; Niznik *et al.*, 1985). It was hypothesised that wet droppings may occur due to kidney damage, and production performance could be influenced. Therefore, the current study was carried out to assess the effect of feeding pullets with prelayer ration, earlier than usual, on wet droppings, blood physiology and subsequent performance.

II. MATERIALS AND METHODS

Two hundred ISABrown female chicks were reared in brooders in an isolation laboratory and fed a commercial chick starter diet from day old to 12 weeks of age. At 12 weeks of age, the birds were transferred to rearing cages and provided with a typical commercial grower diet containing approximately 10 g/kg calcium and randomly divided into two treatment groups. The two groups were each represented by sixteen replicates, each consisting of six birds. At 13 weeks of age, the early prelayer group received a prelayer diet containing 22 g calcium/kg until 19 weeks of age whereas the control group continued to receive the commercial grower diet. The control group was placed on the prelayer diet from 16 to 19 weeks of age. Thereafter, all birds were given a commercial layer ration containing 36 g calcium/kg throughout the study, to 60 weeks of age. The composition of the diets is shown in Table 1. At 14 weeks of age, all birds were housed in individual layer cages and exposed to a photoperiod of 12L:12D at 17 weeks of age. One week later, the light period was increased by 30 minutes per week until the birds were receiving 16L:8D at 25 weeks of

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age. Feed and water were available *ad libitum*. Body weight, daily egg production, feed intake and manure moisture were monitored throughout the study. Measurements made every two weeks were: egg weight, shell reflectivity, egg shell breaking strength, shell weight, shell thickness, albumen weight, albumen height and Haugh units, yolk weight, and yolk colour. Every four weeks, from 20 weeks of age, blood samples were collected from a sample of birds from each treatment group. Blood ionised calcium, sodium and potassium concentrations were analysed. Data were subjected to a two factor analysis of variance to determine effects of treatment and age of hen. Differences between means were compared by Fisher's Protected Least Significance Difference (PLSD) Test. Significance was assumed at $P < 0.05$.

III. RESULTS

Table 1. Composition of experimental diets (g/kg).

Diets	CP	Fat	Fibre	ME (MJ/kg)	Ca	P	Na
Prelayer	164	38	47	11.4	22	5.0	1.8
Layer	173	42	40	11.2	36	6.5	1.6

Table 2. Effect of early feeding of prelayer diets on sexual maturity and body weight gain.

Group	Onset of lay		Cumulative body weight gain from 16 wk (g/b/wk)					
	Age (d)	BW(kg)	20 wk	24 wk	28 wk	36 wk	44 wk	60 wk
Ctrl	125.4	2.02	88.60	49.39 ^a	39.48 ^a	24.98 ^a	21.63 ^a	15.19
Treat	125.6	1.98	88.44	42.62 ^b	34.11 ^b	20.96 ^b	18.77 ^b	13.77

^{a-b} Means within columns with different superscripts are significantly different ($P < 0.05$).

Age and body weight at point of lay were not affected by prelay treatment (Table 2). However, a significant reduction of body weight gain was observed in the early prelayer birds from 24 to 44 weeks of age. No significant differences were noticed in hen-day production, shell weight or faecal moisture throughout the trial (Table 3). There were also no significant differences in body weight, feed efficiency or yolk parameters. The feeding treatment had a significant effect on feed intake ($P < 0.05$). Pullets fed prelayer diets earlier consumed 6-8% less feed than did the control birds from 20 to 40 weeks of age. In addition, reduced egg weight, albumen weight and Haugh units were found in the early prelayer birds. Hens fed prelayer diets earlier had a significantly higher concentration of blood ionised calcium at 28 weeks of age than hens given the control diet (1.7 vs 1.5 mmol/L). Conversely, lower blood ionised calcium was observed in the early prelayer birds at 44 and 60 weeks of age (Table 4). Sodium and potassium concentrations followed a similar pattern to the ionised calcium throughout the study.

Table 3. Effect of early feeding of prelayer diet on production parameters of hens.

Group	Age (weeks)						
	20	24	30	34	40	46	58
	Feed intake (g/hen/week)						
Ctrl	746±16 ^a	986±16 ^a	997±16 ^a	949±21	1114±28 ^a	1074±18	1002±24
Treat	690±18 ^b	935±19 ^b	936±16 ^b	903±13	1040±21 ^b	1040±18	1000±18
	Hen-day egg production (%)						
Ctrl	60.1±3.3	92.4±1.1	88.9±1.7	91.6±2.0	89.7±1.6	87.0±2.3	80.3±2.1
Treat	65.9±3.5	93.6±1.6	88.7±1.9	92.9±1.5	89.7±1.4	87.4±1.8	80.1±2.1
	Egg weight (g)						
Ctrl	50.1±0.4	58.2±0.3 ^a	62.0±0.4	62.8±0.5 ^a	65.1±0.5	68.2±0.7	69.5±0.5
Treat	49.7±0.6	56.9±0.5 ^b	61.1±0.4	61.6±0.3 ^b	64.3±0.5	67.6±0.5	69.1±0.7
	Albumen weight (g)						
Ctrl	33.8±0.4	38.2±0.3 ^a	40.1±0.3 ^a	39.9±0.4 ^a	40.9±0.4	44.5±0.8 ^a	43.8±0.4
Treat	33.3±0.5	37.2±0.4 ^b	39.1±0.3 ^b	38.9±0.2 ^b	40.2±0.4	42.4±0.4 ^b	43.6±0.6
	Haugh unit (scores)						
Ctrl	103.8±0.4	98.0±0.6	94.3±0.6 ^a	92.0±0.7	87.2±0.7	85.7±1.3	84.9±0.8 ^a
Treat	103.9±0.5	96.7±0.8	92.5±0.7 ^b	92.0±0.9	86.1±0.9	88.0±0.9	81.5±1.2 ^b
	Shell weight (g)						
Ctrl	5.1±0.1	5.6±0.1	5.9±0.1	6.0±0.1	6.3±0.1	6.2±0.1	5.8±0.1
Treat	5.1±0.1	5.5±0.1	5.8±0.1	5.9±0.04	6.2±0.1	6.3±0.1	5.8±0.1
	Faecal moisture (%)						
Ctrl	76.9±1.2	74.6±1.1	71.6±1.0	75.4±0.7	74.2±1.0	75.7±0.7	76.7±0.8
Treat	75.9±1.2	73.9±1.4	71.1±1.4	76.7±0.9	71.9±0.7	74.9±0.9	75.8±0.7

^{a-b} Means within columns with different superscripts are significantly different (P<0.05).

Table 4. Influence of early feeding of prelayer diet on blood electrolytes of hens.

Treatment	Age (week)						
	20	24	28	36	44	52	60
	Ca ²⁺ (mmol/L)						
Control	1.4±0.1	1.4±0.10	1.5±0.1 ^b	1.3±0.1	1.3±0.04 ^a	1.5±0.02	1.6±0.03 ^a
Treat	1.5±0.1	1.3±0.04	1.7±0.1 ^a	1.3±0.1	1.2±0.04 ^b	1.5±0.03	1.3±0.04 ^b
	K ⁺ (mmol/L)						
Control	4.8±0.1	5.0±0.1 ^a	4.8±0.1 ^b	4.8±0.2	4.4±0.10	4.8±0.1	5.4±0.1 ^a
Treat	4.7±0.2	4.7±0.1 ^b	5.1±0.1 ^a	4.7±0.2	4.5±0.03	4.6±0.2	4.9±0.1 ^b
	Na ⁺ (mmol/L)						
Control	139.4	145.5 ^a	142.9 ^b	139.7	137.6 ^a	150.6	149.3 ^a
Treat	142.4	140.4 ^b	152.0 ^a	142.2	129.3 ^b	146.9	136.7 ^b

^{a-b} Means within columns with different superscripts are significantly different (P<0.05).

IV. DISCUSSION

The bird performance results suggest that reduced growth rate was associated with decreased feed intake, and thus protein intake. Under such conditions, the birds may have been deficient in amino acids necessary for albumen formation. The major characteristic of albumen observed in the early prelayer hens was spreading of the area of thin albumen associated with a less compact thick albumen. Decreased egg weight accompanied by poor albumen quality produced by deficiency of protein in diets has been observed previously (Hussein and Harms, 1994; Leeson and Caston, 1997).

Results from plasma ionised calcium are somewhat difficult to interpret. Early feeding of prelayer diet caused higher plasma ionised calcium at 28 weeks of age. However, lower plasma calcium was found in the early prelayer hens during the latter half of production. Wideman *et al.* (1985) measured blood ionised calcium in nonlaying pullets and found that hypocalcemia was observed in pullets fed high calcium diets. Increased calcium storage in bones resulting from feeding high calcium diets before maturity has been reported (Hurwitz, 1964). In laying hens, negative calcium balance occurs during the early laying period (Morgan and Mitchell, 1938). This status may have caused a greater mobilisation of bone mineral in the early prelayer birds, resulting in higher plasma calcium. Calcium needed for shell formation is mobilised not only from medullary bone but also structural cortical bone (Clunies *et al.*, 1992). Possibly, the birds fed high calcium diets before maturity stored more bone calcium and subsequently mobilised more Ca in response to shell formation during the period of calcium depletion. In addition, it seems that the birds were maintaining plasma electrolytes at a slightly lower level than the control birds during the later production period (Table 4). The results suggest that the level of dietary calcium during the growing period may have a longer term effect on skeletal calcium storage. However, the mechanisms regulating calcium homeostasis and shell calcification are complex since there are many factors involved.

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COMPARISON OF PERFORMANCE OF LAYERS IN A CAGE AND BARN SYSTEM

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Summary

The performance of hens housed in either traditional battery cages or a barn system on a commercial farm was monitored over a three-year period. The report covered a laying cycle of 20 to 71 weeks of age, and included data from 23,205 and 26,356 birds in cage and barn systems, respectively. Hen-day production at 71 weeks of age was higher ($P < 0.05$) in the barn system, whereas hen-housed production to 71 weeks was higher ($P < 0.05$) in cage birds due to the lower mortality in the cage system. Egg weight was unaffected ($P > 0.05$) by the housing system. The average production at 71 weeks of age in the cage and barn systems was 299 and 305 eggs/hen, respectively. Mortality in both systems was within acceptable limits, but was higher ($P < 0.05$) in the barn system. Incidence of floor eggs in the barn was initially high but declined to below 1% by 34 weeks of age.

I. INTRODUCTION

Housing of hens in cages is the current norm in the egg industry because of the space savings and reduced labour and equipment costs. Major criticisms of the cage systems are that they increase the incidence of feather damage, overgrown claws, foot lesions and brittle bones. These welfare issues have attracted considerable attention in recent years regarding cage systems and their effects on the quality of life of laying hens (Barnett and Newman, 1997). Barn systems, on the other hand, are an acceptable alternative to the public since they provide better opportunity for movement and social interaction, and also answer some welfare concerns. The present paper reports data from a long-term monitoring study comparing the effects of cage and barn systems on the performance of laying hens on a commercial farm.

II. METHODS

The study was conducted on a commercial farm in the lower half of the North Island as part of a nationwide monitoring of layer farms. The monitoring focused on production parameters with the intention of improving performance and profits within the New Zealand poultry industry. The system comparison presented in this paper was carried out over a three-year period from 1995 to 1998. The report covers a laying cycle of 20 to 71 weeks of age, since the flocks were culled from week 71 to week 80 to fit with the farm replacement and refurbishment policy.

Prior to the monitoring period the farm was a 1960s vintage cage layer farm. Beginning in 1994 part of the farm was converted to a barn production system. During the monitoring period approximately half the production was from the barn system and half from cages. Seven flocks from cages totalling 23,205 birds, and seven barn flocks totalling 26,356 hens are included in this report. Average flock size was 3,315 hens for the battery and 3,765 for the barn.

All birds were of the same genetic stock (Hy-line Brown). The pullets were reared in flat-deck cages and then transferred to the laying sheds. The birds were transferred to laying cages at 18 weeks of age, and to the barns at 16 to 18 weeks of age. The birds were fed a

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commercial layer feed (Sharpes Feeds, Wellington). Three-phase feeding was practised during the laying period. Phase 1 feeding (2850 kcal AME/kg, 17.6% crude protein) was from housing to peak egg mass, phase two feeding (2780 kcal AME/kg, 16.7% crude protein) until 65 to 70 weeks of age, and a finishing ration (2720 kcal AME/kg, 15.7% crude protein) to the end of lay. Diets have changed over the monitoring period, but the same diets were used in both production systems. The cage birds were fed manually once a day and the barn hens six times daily.

Jansen automatic egg collection systems and Big Dutchman automatic chain feeders were used in the barn. The sheds were approximately 75% plastic slatting and 25% deep litter. Barn sheds were only cleaned on flock turnover. Stocking density was 7 birds /m². The cage layer sheds contained single tier conventional 3-bird cages (30 x 45 cm). Feeding and egg collection was manual. Both sheds were poorly insulated. Accurate records of eggs laid, feed given and mortality were maintained. Egg weight was calculated as the average of the first 300 eggs collected from each flock one morning a week.

The performance data were analysed using the two-tailed *t* test procedure. In all cases, samples were tested for homogeneity of variance. Arc sine transformation was applied to percentage mortality data prior to analysis.

III. RESULTS AND DISCUSSION

Egg production was influenced by the housing system (Table 1). At 25 weeks, hen-day production was higher ($P < 0.05$) in cage birds compared to the barn birds. The reverse trend was observed at week 71 with barn birds producing more ($P < 0.05$) eggs than the cage birds. Hen-housed production to 71 weeks of age, on the other hand, was higher ($P < 0.05$) in cage birds due to the lower mortality in the system. Egg weight was not influenced by housing system.

Hen-day egg production peaked at over 90% in both systems. The birds in the cage system peaked a week earlier and also declined more rapidly towards the end of lay than those in the barns (Figure 1). The lower rate of production by the barns in early lay appears to be the result of the pullets adapting to the barn environment.

Feed intake was influenced by housing system. Cumulative average feed intake from 20 to 71 weeks was higher ($P < 0.05$; 123 vs 115 g/day) in the barn-housed birds compared to cage birds. The cumulative feed conversion ratio (g feed/ g egg) to 71 weeks, however, was not influenced ($P > 0.05$) by housing system. The feed conversion ratio to 71 weeks in the barn and cage systems were 2.36 and 2.25, respectively.

Table 1. Effect of housing on production parameters to 25, 50 and 71 weeks of age.

Parameter	Week 25		Week 50		Week 71	
	Barn	Cage	Barn	Cage	Barn	Cage
Hen-day production, %	91 ^a (1.27) ¹	94 ^b (0.76)	86 (2.31)	83 (2.57)	78 ^a (2.19)	73 ^b (1.91)
Eggs/hen housed ²	28 (8.47)	36 (2.91)	184 ^a (7.14)	192 ^b (3.42)	299 ^a (6.80)	305 ^b (4.65)
Egg weight, g	57.37 (1.41)	57.17 (0.78)	63.36 (1.61)	63.56 (1.28)	65.10 (1.14)	65.06 (1.13)
Egg mass, g/day	52.20 (14.9)	53.73 (6.72)	54.48 (2.61)	52.75 (2.10)	50.78 (1.34)	47.49 (2.20)
Feed intake, g/day ²	110 (4.86)	104 (12.8)	124 ^a (6.60)	135 ^b (4.49)	123 ^a (5.93)	115 ^b (3.56)
Feed efficiency, g/g ²	3.74 (1.70)	2.63 (0.30)	2.40 ^a (0.16)	2.19 ^b (0.09)	2.36 (0.15)	2.25 (0.09)
Mortality, % ²	2 ^a	0 ^b	3 ^a	1 ^b	4 ^a	3 ^b
Floor eggs, %	2.7	-	0.9	-	0.8	-

¹ Standard errors in parenthesis.

² Cumulative data.

^{a,b} Within each age, means in a row bearing different superscripts are significantly different (P<0.05).

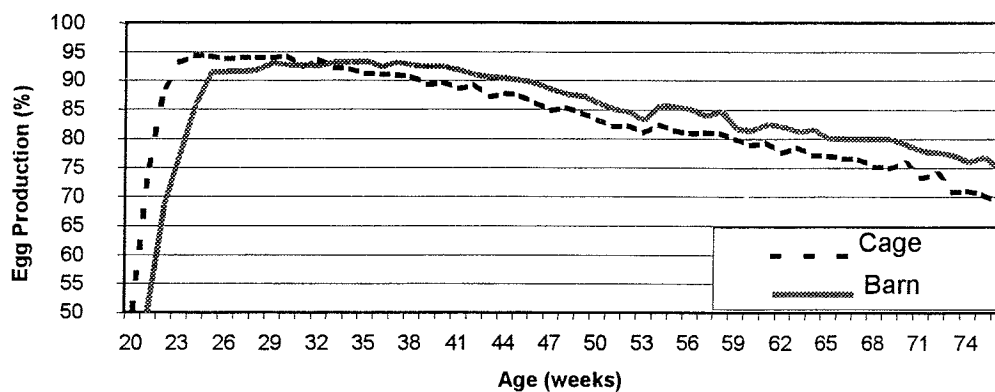


Figure 1. Hen-day egg production (%) in cage and barn systems.

Throughout the laying period, mortality was higher (P<0.05) in the barn system (Figure 2). Mortality within both systems, however, was within acceptable limits. The reasons for the increased barn mortality are unclear, but may have been associated with the adaptation of the cage reared birds to the barn system.

Floor eggs in the barn system were initially high at 13 % but quickly declined to below 2 % by week 29 and to less than 1 % by week 34. Other studies of barn production systems have reported higher levels of floor eggs than those observed here (Barnett, 1999). Correct use of automated nest box systems was considered responsible for the low level of floor eggs on this farm.

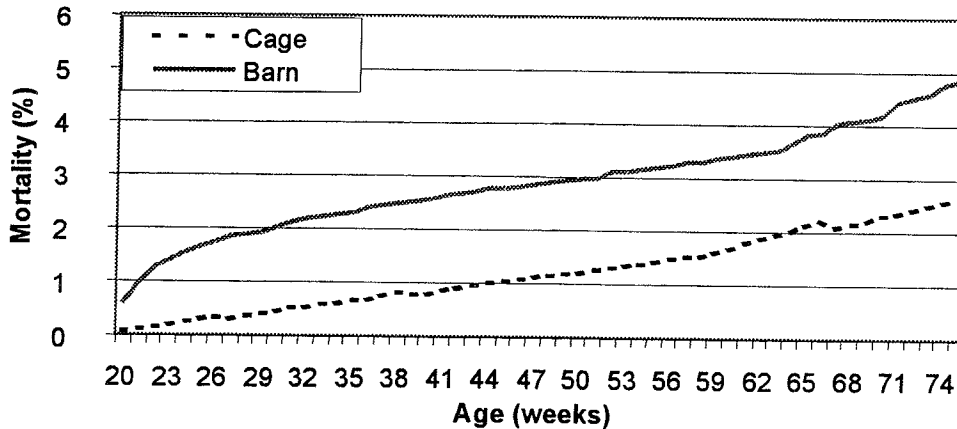


Figure 2. Influence of housing system on percent mortality of hens.

The present data suggest that the relatively higher performance of the barn hens during the latter period of lay may allow for a longer profitable laying cycle than with the cage system. Some caution is required in interpreting the data, however, since this study was conducted in different sheds on the one farm over a three-year period. Recent data from four flocks on this farm (data not presented) indicate that the hen-housed production in the barns is similar to that in the cages by 78 weeks of age. It may be possible to reduce the higher mortality experienced in the barn system through a more appropriate rearing and acclimatisation regime. Recent experience on this farm suggests that mortality can be reduced by transferring the flocks to the barn at six weeks of age. The results suggest that barn egg production systems may provide a suitable alternative to intensive cage systems.

IV. ACKNOWLEDGMENTS

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EFFECTS OF DIETARY ENERGY LEVEL AND CAGE STOCKING DENSITY ON PERFORMANCE OF ISABROWN LAYING HENS

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Summary

Diets containing three metabolisable energy (ME) levels were fed to Isabrown hens housed in single-bird and two-bird cages. The assayed ME values of the diets were 9.78 (L), 11.41 (M) and 12.52 (H) MJ/kg. Over a 64-week period, average egg weight, ME intake, body weight gain and mortality increased and feed efficiency improved with increasing ME level in the diet, while feed intake, efficiency of conversion of energy to eggs and egg specific gravity declined. Average ME intakes of birds on diets L, M and H were respectively 1.234, 1.424 and 1.499 MJ/day. Birds caged in pairs laid more eggs than individually caged birds. The results suggest that the Isabrown bird is rather inefficient at adjusting feed intake to meet energy requirement: ME intake on diet H appeared to be excessive while intake of ME and/or other nutrients on diet L may have been marginally too low to support maximum egg mass output.

I. INTRODUCTION

It is sometimes assumed that laying hens consume approximately the right amount of feed to meet their requirement for energy, regardless of the concentration of metabolisable energy (ME) in the diet. This assumption, which has been widely employed in formulating layer diets, has for long been known to be incorrect (De Groot, 1972). Furthermore, the relationship between feed intake and dietary ME concentration is strain dependent (Morris and Fox, 1963). For commercial strains developed in Australia, overconsumption of energy may occur when dietary ME exceeds 12 MJ/kg (Dillon, 1974). However, the "imported" brown-egg strains that have recently become extremely popular produce considerably more egg mass and generally convert feed to egg mass more efficiently than local strains, so it might be expected that their nutritional requirements are more exacting. Although the breeding companies generally recommend dietary protein concentrations of 175-190 g/kg for these strains in the first half of the laying period, there is some evidence that such high levels are not needed (Balnave *et al*, 1999). The breeders' suggested ME levels are in the region of 11.5-12 MJ/kg, but there is no evidence to support these recommendations under Australian conditions. The aim of the present experiment was to determine the effect of variation in dietary ME concentration on the performance of imported brown egg layers housed in single-bird and two-bird cages in the southeast Queensland environment.

II. METHODS

Five hundred and seventy six Isabrown pullets were housed at eighteen weeks of age in an open-sided flat-deck cage shed. The pullets were distributed at random into 72 experimental groups in a randomised split plot arrangement. There were 24 replicates of each of two cage-types (single-bird and two-bird cages), each of which was subdivided into three diet types (low (L), medium (M) and high (H) energy). Each replicate group contained eight birds, either in eight single-bird cages or in four two-bird cages. The single-bird cages measured approximately 23 cm wide by 46 cm deep (1058 cm²/bird) and the two-bird cages measured 30.5 cm wide by 46 cm deep (701 cm²/bird). The nutrient analyses of the three diets are shown in Table 1. All diets contained sorghum, wheat, soybean meal and meat and Queensland Poultry Research and Development Centre, PO Box 327, Cleveland Q 4163.

bone meal, with the main variable ingredients being millrun, rice hulls, tallow and sunflower oil. The diets were designed to contain nominal ME levels of 10.6 (L), 11.4 (M) and 12.2 (H) MJ/kg, and assayed ME levels using the rapid AME method (Farrell *et al* 1991) were 9.78 ± 0.29 , 11.41 ± 0.25 and 12.52 ± 0.37 MJ/kg respectively. Protein and the limiting amino acids were included in the diets in approximately constant proportion to nominal ME content. Feed and water were available continuously and a constant daily light period of 15.5 hours was provided. The daily average temperature ranges in the shed were approximately 13-24, 19-29 and 10-21°C during the early, middle and late phases of the trial respectively.

Table 1. Nutrient composition of the experimental diets.

Nutrient analysis/kg	Low energy	Medium energy	High energy
ME (nominal, MJ)	10.6	11.4	12.2
ME (determined, MJ)	9.78	11.41	12.52
Density (kg/litre)	0.57	0.77	0.79
Protein (g)	155.0	166.7	175.1
Lysine (g)	7.2	7.80	8.30
Methionine (g)	3.74	4.01	4.43
Met + Cys (g)	6.20	6.70	7.10
Iso-leucine (g)	5.40	5.90	6.30
Threonine (g)	4.82	5.12	5.46
Tryptophan (g)	1.77	1.91	2.06
Linoleic acid (g)	9.2	10.0	10.6
Calcium (g)	34.5	37.0	38.2
Total Phosphorus (g)	5.40	5.70	6.15
Available Phosphorus (g)	3.10	3.21	4.00

*Added micro-nutrients (mg/kg diet): 2.5 retinol, 0.075 cholecalciferol, 5 α -tocopherol acetate, 2 menadione sodium bisulphite, 1 thiamine, 4 riboflavin, 2 pyridoxine, 0.01 cyanocobalamin, 1 folic acid, 10 niacin, 10 calcium pantothenate, 0.03 biotin, 150 choline, 50 Mn, 50 Zn, 50 Fe, 0.6 Mo, 0.5 Co, 0.6 I, 4 Cu, 0.07 Se, 80 Banox (BHA + BHT), yolk pigment.

Commencing at 19 weeks of age, egg numbers were recorded on five days each week and feed intake, egg weights and egg specific gravities were recorded at not less than monthly intervals. Body weights were obtained at 19, 22, 35 and 83 weeks. Maximum and minimum shed temperatures were recorded five days per week. At termination of the trial sixteen birds from each treatment were autopsied and the abdominal fat pad was extracted and weighed. The main effects and interactions of diet and stocking density were assessed by analysis of variance using Statistix[®] programmes.

III. RESULTS

Birds on the high ME diet reached peak production five to seven days earlier ($P < 0.05$) than birds on the other diets but their peak rate of lay was lower (93.3% compared with medium ME 94.4% and low ME 95.4%). Average egg weight, ME intake, body weight gain and mortality over the 64-week experimental period increased and feed efficiency improved with increasing ME level in the diet (Tables 2 and 3), while feed intake, efficiency of conversion of energy to egg mass and egg specific gravity declined with increasing ME level (differences between low and high ME diets $P < 0.001$ for feed and energy intake and conversion, $P < 0.05$ for other parameters). Abdominal fat pad weight (absolute or as a

proportion of body weight) at termination of the trial was lower ($P < 0.01$) for the low ME diet than for the other diets, and this difference was greater in birds that had been caged in pairs than in individually caged birds.

Table 2. Average performance of all treatments from 19 to 83 weeks of age.

ME level	Birds /cage	Eggs/ 100 bird-days	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed/egg mass	Body wt gain (g/d)	Fat pad wt/body wt (g/kg)
Low	1	80.07 ^a	64.66 ^b	51.77 ^a	125.3 ^d	2.421 ^c	0.983 ^{ab}	45.9 ^a
	2	82.63 ^{ab}	63.46 ^a	52.44 ^{ab}	124.7 ^{cd}	2.378 ^c	0.933 ^a	42.4 ^a
Medium	1	81.35 ^{ab}	64.44 ^{ab}	52.42 ^{ab}	122.2 ^c	2.330 ^c	1.004 ^{ab}	51.3 ^{ab}
	2	83.07 ^{ab}	64.25 ^{ab}	53.37 ^{ab}	124.0 ^{cd}	2.323 ^c	1.034 ^{ab}	58.8 ^b
High	1	80.42 ^{ab}	64.97 ^b	52.27 ^{ab}	116.0 ^a	2.219 ^b	1.081 ^{ab}	53.9 ^{ab}
	2	83.82 ^b	65.17 ^b	54.62 ^b	119.1 ^b	2.181 ^a	1.192 ^b	61.8 ^b
LSD ($P < 0.05$)		3.54	1.08	2.14	2.7	0.101	0.163	11.7
Low		81.35	64.05 ^a	52.10	125.0 ^c	2.399 ^c	0.958 ^a	44.2 ^a
Medium		82.21	64.34 ^{ab}	52.90	123.1 ^b	2.327 ^b	1.019 ^a	55.1 ^b
High		82.14	65.07 ^b	53.45	117.6 ^a	2.200 ^a	1.136 ^b	57.9 ^b
LSD ($P < 0.05$)		2.50	0.77	1.51	1.9	0.072	0.115	8.3
1		80.61 ^a	64.69	52.15 ^a	121.2	2.323	1.023	50.4
2		83.17 ^b	64.29	53.48 ^b	122.6	2.294	1.053	54.3
LSD ($P < 0.05$)		1.80	0.67	1.14	1.6	0.053	0.110	7.3

Table 3. Average mortality, egg specific gravity and ME intake of dietary treatments from 19 to 83 weeks of age.

ME level	Mortality (%)	Egg specific gravity	ME intake (MJ/d)	ME/egg mass (MJ/kg)
Low	1.04 ^a	1.0837 ^b	1.223 ^a	23.47 ^a
Medium	4.69 ^{ab}	1.0826 ^{ab}	1.404 ^b	26.54 ^b
High	7.29 ^b	1.0811 ^a	1.472 ^c	27.54 ^c
LSD ($P < 0.05$)		4.25	0.0016	0.021

Individually caged birds came into lay sooner than birds caged in pairs, but achieved a lower peak rate of lay (93.5% c.f. 95.2%). Birds caged in pairs produced more eggs and egg mass than individually caged birds ($P < 0.05$). In the first sixteen weeks of lay individually caged birds ate more feed and converted feed to eggs less efficiently than birds caged in pairs ($P < 0.05$). Within the low ME treatment, individually caged birds laid heavier eggs than those caged in pairs ($P < 0.05$). Within the high ME treatment, individually caged birds ate less feed ($P < 0.05$) than those caged in pairs.

IV. DISCUSSION

The small but significant increase in egg numbers from birds caged in pairs over individually caged birds was most pronounced in the high energy feeding treatment and appeared to be associated with higher feed intake and increased fat deposition. The reasons for these differences are unknown.

Previous trials at this research centre indicate that the characteristic ME intake of Isabrown hens in Queensland is approximately 1.35-1.4 MJ/day. This intake was met by the medium energy diet, exceeded by the high energy diet but unattained by the low energy diet. These differences in energy intake may account for the small but sometimes significant differences in egg weight, egg mass output, body weight gain and abdominal fat proportion between the three treatments. The relatively high mortality in the high energy group may also have been due to overconsumption of energy, which can cause problems such as fatty liver syndrome or an increased susceptibility to heat stress. However there was no evidence of fatty liver syndrome in the experimental birds and most deaths occurred in the cool season.

Birds on the high energy diet consumed 4.35% less feed than those on the medium energy diet while those on the low energy diet consumed 1.28% more feed. Had the ME intakes been the same for all three treatments (and assuming the nominal rather than the determined ME values of the diets are correct), the feed intakes on the low and high energy diets would have been 7% higher and 7% lower respectively. The "efficiency of adjustment" was therefore 62% for the high energy diet and only 18% for the low energy diet. This result is compromised by the fact that the decline in egg mass output with declining energy level may have been caused at least partly by the concomitant reduction in essential amino acid intake. Thus part of the decline in energy intake may have been due to a reduced requirement for productive energy. If efficiency of feed conversion rather than feed intake is taken as the effective criterion, the proportional changes of -5.3% and +3.1% observed for the high and low energy diets compared with the medium energy diet represent efficiencies of adjustment of 76% and 44% respectively. In the first 16 weeks of the trial, however, there was virtually no adjustment of feed intake or feed efficiency to compensate for variation in dietary energy content. This suggests both that the Isabrown bird is rather poor at adjusting feed intake to meet energy requirement and that birds on the diet with the lowest energy content may have been unable to consume sufficient feed to meet energy and/or other nutrient requirements.

The density of the feed may have been a constraint on intake of the low energy diet. The density of this diet was only 0.57 compared with 0.77 and 0.79 kg/litre for the medium and high energy diets respectively. Despite their somewhat lower egg mass output, however, birds on the low ME diet converted energy and protein to egg mass more efficiently than those on the high ME diet. With eggs priced at AUD 2.00/dozen a price difference of less than AUD 40/tonne between the low and high ME diets would be required to make the high ME diet more economical on a hen-day basis. When the effect of mortality is taken into account the high ME diet would be strongly disadvantaged within normal price structures.

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INFLUENCES OF FEEDING AND CALCIUM PRESENTATION METHODS ON TWO LAYER STRAINS I. BONE AND PLASMA CHARACTERISTICS

R. D. TAYLOR¹ and G.P.D. JONES²

Summary

Two strains of layers were fed compound or choice forms of a standard diet and given calcium as either ground limestone or coarse limestone grit fed separately and provided daily or every second day. Plasma alkaline phosphatase level reflected both production and calcium status differences between strains and the influence of feeding method on nutrient intakes. Application of a simple score to sternum deformation was accurate in determining calcium status in laying birds.

I. INTRODUCTION

Despite detailed research into the role of bone in calcium metabolism and its interaction with egg production, losses of laying hens from bone fragility have risen (Riddell, 1992; Roland and Rao, 1992; Whitehead, 1994).

The medullary bone in birds is the only known true metabolic bone, physically separate from skeletal structural bone (Miller, 1992). A sequence of bone calcium turnover rates was established by Hurwitz (1965) with femur medullary bone displaying high turnover, the sternum a lower turnover and humerus cortical bone lower again.

There may be an optimal level (Clunies *et al.*, 1993) or form (Whitehead, 1994) of dietary calcium which maximizes medullary bone content. In young hens, bone mineral reserves may be enriched by manipulating calcium nutrition which may delay the onset of shell degeneration (Cheng and Coon, 1990). Whitehead and Wilson (1992) claimed that transient sub-optimal calcium nutrition may cause structural bone loss leading to bone degeneration.

Newman and Leeson (1997) suggested that bone breakage in aged layers was not likely to be moderated by nutritional manipulation, as genetic selection and changed housing conditions had exacerbated the problem.

However, all calcium feeding methods need to be carefully examined to determine whether they provide the individual bird with the opportunity to maximize bone volume and density for both commercial and welfare reasons. This experiment examined the effect of presenting two strains of hens with different forms of feed and limestone.

II. MATERIALS AND METHODS

At 20 weeks of age (designated point-of-lay (POL)) and on two consecutive evenings, three birds from each of two layer strains (A and B) which had been previously fed by either of two systems (compound and choice) and three methods of calcium provision (ground limestone (Ca 1), limestone grit available *ad libitum* (Ca 2) or limestone grit available every second day (Ca 3)) were bled. Total plasma calcium (Arsenazo Method, Roche Diagnostics) and alkaline phosphatase (ALP) activity (Unimate 5 ALP IFCC, Roche Diagnostics) were measured.

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At 51 weeks of age these measurements were repeated, using six birds which were selected on the basis of consistent, high production (>90 % in all cases), 14-15 hr after their previous oviposition. The morning after each bleed (as above), two groups of 3 birds per treatment were euthanased and the right femurs and humeri removed. The sternum was given a score for deformation being: 1 = straight, normal bone; 2 = slight curvature; 3 = moderate curvature and 4 = gross curvature with or without inward curling. The costo-chondral junction was examined and a score of either 1 (nil deformation) or 2 (exaggerated swelling) was applied.

Breaking strength (N) and bending distance (mm) were measured at the point of bone failure for each cleaned humerus. Each femur was cleaned and its volume (ml) was determined by displacement of a 1% glycerol solution. The medullary bone was removed from the cortical section and the cortical bone volume measured. Medullary bone volume was derived from (total bone - cortical bone) volumes. The cortical and medullary bone sections were then ashed for 24 hr at 600°C. Bone volumes and ash weights were analysed on an actual and per kg body weight basis.

Data were treated by factorial analysis of a 2 x 2 x 3 design within the General Linear Models (GLM) procedure of SAS. The relationships between blood, bone and calcium intake measurements were tested over strain, feed type and calcium method by the reduction in sums of squares technique (Snedecor and Cochran, 1980) using the GLM procedure of Minitab.

III. RESULTS

Plasma Ca and ALP were similar at 20 weeks but at 51 weeks of age plasma ALP level involved a significant ($P<0.05$) feeding system X calcium method interaction (Table 1). The score for sternum deformation mirrored the plasma ALP result (Table 1) such that choice fed birds given the ground limestone (Ca 1) had a higher ($P<0.05$) sternum deformation score and ALP level than those fed the other treatment combinations which were scored similarly. Plasma ALP levels reflected differing Ca intakes influenced by genotype and calcium feeding method (Table 3).

Table 1. Sternum deformation scores and plasma ALP activity (U) at 51 weeks of age of layers on two feeding systems and three methods of calcium presentation.

Feed	Calcium	Sternum deformation score	ALP (U)	SE
Compound	Ca 1	1.5 _a	659 _a	149.8
	Ca 2	1.3 _a	424 _a	157.1
	Ca 3	1.6 _a	623 _a	174.0
Choice	Ca 1	2.6 _b	1530 _b	149.8
	Ca 2	1.5 _a	340 _a	157.1
	Ca 3	1.5 _a	455 _a	164.1
SE		0.21		

Values within a column followed by unlike subscripts are significantly different ($P<0.05$)

Few femoral bone measurements differed across feed or calcium treatments. However, significant differences were found across bird strain (Table 2). Cortical bone volume per kg body weight was lower ($P<0.05$) in the Strain B birds although cortical ash weight per unit volume was lower ($P<0.05$) in the Strain A birds. Medullary bone ash weight, ash weight per kg body weight and ash weight per unit volume were similarly greater

($P < 0.05$) in the B than the A strain. Strain and calcium feeding influenced bone ash parameters (Table 3).

Table 2. Femoral volume and ash measurements (LS Means) from two strains of layers (A and B) fed calcium by three methods of presentation.

Femur measurement	Strain		SE	Calcium			SE
	A	B		Ca 1	Ca 2	Ca 3	
Cortical vol / body wt (ml/kg)	1.87 _a	1.61 _b	0.041	1.75	1.74	1.73	0.051
Cortical ash wt / cortical vol (mg/ml)	578 _b	629 _a	9.1	597	596	618	11.1
Medullary ash wt (g)	0.41 _b	0.63 _a	0.032	0.47	0.52	0.57	0.039
Medullary ash wt / body wt (g/kg)	0.20 _b	0.27 _a	0.013	0.22	0.23	0.25	0.016
Medullary ash wt / med vol (mg/ml)	173 _b	248 _a	13.6	195	206	231	16.7

Unlike subscripts within a row for each factor are significantly different ($P < 0.05$)

Rib scores and humeral breaking strength and deformation distance were not affected ($P > 0.05$) by treatment.

Table 3. Relationships across treatments at 51 weeks of age of two layer strains (A and B) given two feeding systems and three methods of calcium presentation.

Y	Treatment	Regression Equation	R ²	P	SE Slope
ALP (U)	Complete	$Y = 659.00 - 9.60 \text{ Ca intake (g)}$	0.0	0.457	12.71
	Choice	$Y = 2349.36 - 296.98 \text{ Ca intake (g)}$	41.2	0.001	71.82
	Ca 1	$Y = 3940.23 - 778.45 \text{ Ca intake (g)}$	42.5	0.003	217.5
	Ca 2	$Y = 547.92 - 25.57 \text{ Ca intake (g)}$	0.0	0.923	33.22
	Ca 3	$Y = 1277.40 - 116.82 \text{ Ca intake (g)}$	12.7	0.115	68.74
Femoral ash wt. (g)	Ca 1	$Y = 2.018 + 0.00029 \text{ body wt. (g)}$	0.0	0.419	0.0003
	Ca 2	$Y = 0.645 + 0.00097 \text{ body wt. (g)}$	31.0	0.003	0.0003
	Ca 3	$Y = -0.376 + 0.00147 \text{ body wt. (g)}$	51.5	0.001	0.0003
Med. ash wt. (g)	Strain A	$Y = -0.121 + 0.203 \text{ total ash wt. (g)}$	35.1	0.001	0.046
	Strain B	$Y = -0.416 + 0.356 \text{ total ash wt. (g)}$	74.3	0.001	0.035

IV. DISCUSSION

Birds of Strain A may utilize more bone calcium as they produce a larger egg shell mass from a smaller body size while maintaining a similar calcium intake to the birds of Strain B.

This contention is supported by the lower medullary ash weight and lower cortical ash weight per unit volume of cortical bone in Strain A even though this strain has a larger cortical volume per kg body weight than the Strain B bird. Similarly, the high ALP activity in the choice fed hens on the Ca 1 diet indicates that these birds are remodelling more bone; this may be due to less protein concentrate, and hence slightly less calcium, being eaten by these birds which then requires them to utilize proportionally greater bone calcium at the time of egg shell formation. Fleming *et al.* (1998) concluded that limestone particles (2.5-4.0 mm in size) reduced the loss of cancellous bone, particularly to 25 weeks, thereby slightly reducing the severity of osteoporosis in layers. The current experiment, with two strains, highlighted the differential formation of the two bone types (cortical and medullary) between the strains although the birds consumed similar quantities of nutrients per unit body weight.

The body weight of the bird is important in determining total femur ash and medullary ash weight, especially in those birds given limestone grit, or total femur ash to volume in choice fed birds. Birds given the limestone grit daily (Ca 2) were heavier than those given ground limestone (Ca 1); with those on intermittent limestone grit (Ca 3), intermediate. A separate calcium source may allow some birds to optimize body weight and provide for greater skeletal development.

A calcium grit as the sole calcium source allowed individual birds of two strains to select calcium according to their needs for growth, skeletal development and egg production.

Providing layers with their calcium supplement *ad libitum* in the form of limestone grit may allow for the large range of calcium intakes to be catered for without interfering with the intakes of other nutrients.

The results indicate that palpation of the sternum of live birds may provide a good guide as to calcium nutrition in the living bird and the progress of a flock on any one dietary regime.

V. ACKNOWLEDGEMENTS

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INFLUENCES OF FEEDING AND CALCIUM PRESENTATION METHODS ON TWO LAYER STRAINS. II. α -AMYLASE ACTIVITY, INTESTINAL VISCOSITY AND pH

R. D. TAYLOR¹ and G.P.D. JONES²

Summary

Layers of two strains were fed either a compound or whole wheat diet and were provided with either ground or coarse limestone. Amylase activity, pH and digesta viscosity were measured in various gut sections. The provision of calcium grit may have had a physical rather than a chemical effect in the intestine. Grit in the gizzard, with a concomitant influence of feeding whole grain, may have altered grain processing and digestion of the various fractions including the starch granules. A physical rupture of the cell wall and an increased surface area of the starch may have occurred in birds fed coarse limestone grit. Daily feeding of limestone grit may have acted to decrease digesta viscosity hence allowing for more effective α -amylase activity and starch digestion. The grit influence on viscosity may have accrued from pH effects in the upper tract or by the action of the particles in stimulating greater peristaltic function and digesta mixing.

I. INTRODUCTION

The use of exogenous enzymes in broiler diets can improve the utilization of the diet via a reduction in digesta viscosity, which, in turn, is well correlated with feed conversion efficiency (Bedford, 1996) and which may differ between breeds (Bedford, 1997).

There is considerable interest in the application of enzymes to layer diets with enhanced feed conversion being the major benefit (Bird, 1996) and a viscosity rather than cell-wall based degradation mechanism is suggested for this improvement (Bedford, 1996).

The influence of a coarse grit on gizzard function has been implicated as having an influence on the productivity of laying hens. The use of a coarse grit or provision of the cereal component of the diet as a whole grain, offered in the free choice form, may also alter the energy utilization of a diet (Taylor, 1998a). The grit may grind the grain thereby disrupting the cell wall of the starch granule, thence increasing the surface area of the starch content. This may allow the digestive processes of the bird to act more effectively at a higher point in the digestive tract.

The addition of exogenous enzymes may affect the activity of endogenous enzymes (Han, 1996) with the pH of the intestinal contents being important for the activity of enzymes (Marquardt and Bedford, 1996). Calcium carbonate intake in layers may, therefore, also have a profound effect on the activity of fungal, bacterial or cereal α -amylases. The possible buffering capacity of a large carbonate intake, found in many birds given free access to limestone grit (Taylor, 1998b), could be substantial. Similarly, enzyme stability is enhanced with greater Ca^{2+} levels (Anonymous, 1985).

This experiment examines the effect of limestone form on α -amylase activity, digesta viscosity and ileal pH in two strains of layers when fed in conjunction with a wheat based diet presented to the hens in either a compound or whole grain form.

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II. MATERIALS AND METHODS

Two strains of layers were fed compound or choice forms of a diet and given either ground limestone (Ca1) or limestone grit provided daily (Ca2) or every second day (Ca3).

Digesta samples from the duodenum, jejunum and ileum of birds euthanased at 51 weeks of age were centrifuged (9450 x g for 15 min) and the α -amylase activity in the supernatant was determined (AMZ 8/96, Megazyme International Ireland Ltd) as was the pH of the ileal digesta contents. Samples from each gut section were analysed using one of three pH buffers. Buffers A (pH 6.0), B (pH 4.4) and C (pH 7.0) were used to detect the presence and activity of α -amylase derived from cereals, fungi and bacteria respectively. With respect to the pH likely to be experienced in each segment of the intestinal tract (Evans, 1993), the duodenal samples were analysed using Buffer B (pH 4.4) whilst Buffers A (pH 6.0) and C (pH 7.0) were used for jejunal and ileal samples respectively. Jejunal samples were also analysed with Buffer B and ileal samples with Buffers B and A to determine comparative α -amylase activities.

Digesta viscosity was determined using 0.5 ml of supernatant placed in a Brookfield DV III rheometer (Brookfield Engineering Laboratories Inc.) using a CP-40 cone at 25°C. The shear rates used were from 500 - 2 sec⁻¹. The data were treated by factorial analysis of a 2 x 2 x 3 design within the General Linear Models procedure of SAS.

III. RESULTS

Table 1. α -amylase activity (Ceralpha units) from duodenal, jejunal and ileal segments of the intestinal tract at 51 weeks of two layer strains provided with two dietary forms and three calcium feeding methods.

Intestinal segment (buffer pH)	Strain		Feed		Calcium		
	A	B	Complete	Choice	Ca 1	Ca 2	Ca 3
Duodenum (4.4)	1.96	2.17	1.86	2.27	1.73	2.66	1.81
SE (n)	0.340 (36)		0.340 (36)		0.416 (24)		
Jejunum (6.0)	12.12 _b	15.45 _a	12.87	14.69	12.69	13.65	15.02
SE (n)	0.857 (35)		0.857 (35)		1.033 (24)	1.083 (22)	
Jejunum (4.4)	0.94	0.69	0.26	1.37	0.94	0.40	1.11
SE (n)	0.419 (18)	0.377 (21)	0.385 (20)	0.411 (19)	0.477 (13)	0.493 (13)	
Ileum (6.0)	12.12	16.47	12.88	15.71	10.33 _b	19.63 _a	12.92 _b
SE (n)	1.696 (26)	1.762 (26)	1.762 (26)	1.696 (26)	2.320 (15)	1.849 (21)	2.158 (16)
Ileum (4.4)	1.73	2.81	1.56	2.98	2.45	2.93	1.43
SE (n)	0.536 (26)	0.557 (26)	0.557 (26)	0.536 (26)	0.733 (15)	0.584 (21)	0.682 (16)
Ileum (7.0)	9.31	12.09	10.42	10.98	7.92 _b	13.28 _a	10.91 _{ab}
SE (n)	1.019 (25)	1.159 (24)	1.150 (25)	1.030 (24)	1.567 (14)	1.076 (20)	1.322 (15)

Values with different subscripts within a row under each factor are significantly different (P<0.05).

α -amylase activity of the jejunal digesta (Table 1) differed (P<0.05) between strains when this was measured in Buffer A (pH 6.0) with activity being greater in the Strain B birds. The α -amylase activity did not differ (P>0.05) between treatments when measured using the duodenal or jejunal contents. Enzyme activity in the ileal contents was only affected by the calcium method when using Buffers A (pH 6.0) or C (pH 7.0). With Buffer A, the daily provision of limestone grit (Ca2) produced a greater (P<0.05) α -amylase activity than when

the calcium was provided as ground limestone (Ca1) or grit offered every second day (Ca3), which produced similar α -amylase activities. Using Buffer C (pH 7.0), a higher α -amylase activity was found when grit was provided daily (Ca2) compared to the provision of ground limestone (Ca1).

Duodenal digesta viscosity (Table 2) was higher ($P < 0.001$) in Strain A birds and was higher ($P < 0.05$) in birds given grit every second day (Ca3).

Table 2. Digesta viscosities (cP) in intestinal segments and ileal pH at 51 weeks of two layer strains provided with two dietary forms and three calcium methods.

Intestinal segment	Strain		Feed		Calcium		
	A	B	Complete	Choice	Ca 1	Ca 2	Ca 3
Duodenal viscosity	1.7 _a	2.5 _b	1.9	2.3	1.9 _a	1.9 _a	2.4 _b
SE (n)	0.13 (36)		0.13 (36)		0.16 (24)		
Jejunal viscosity	3.1	3.9	3.3	3.7	4.1	3.0	3.4
SE (n)	0.56 (30)	0.61 (23)	0.53 (30)	0.63 (23)	0.66 (20)	0.71 (18)	0.78 (15)
Ileal viscosity	18.6	13.9	11.2	21.2	27.1	8.5	13.1
SE (n)	6.72 (17)	7.08 (18)	7.43 (16)	6.34 (19)	9.31 (11)	8.00 (12)	
Ileal pH	7.1	7.1	6.9	7.3	6.8	7.5	7.1
SE (n)	0.17 (22)	0.17 (21)	0.18 (20)	0.17 (23)	0.23 (14)	0.17 (16)	0.23 (13)

Values with different subscripts within a row under each factor are significantly different ($P < 0.05$).

IV. DISCUSSION

The use of the three buffers to detect the presence and activity of α -amylases from different origins has allowed the treatments imposed in this experiment to be differentiated, in part, on the basis of their effect on energy digestion by the birds.

As might be expected from previous data (Evans, 1993), exogenous α -amylase activity in the duodenum was low, however this does not preclude activity by the bird's pancreatic amylases. That any appreciable activity was not detected by the use of Buffer B does not negate significant release of this enzyme. It may be that endogenous amylase activity increases as pH increases, as Appleby *et al.* (1992) stated that the majority of absorption occurs in the ileum and jejunum, and as such any endogenous amylase activity may be hard to distinguish from that of activity from cereal or bacterial origin. The development of a suitable buffer for use with the Amylazyme method may allow for the activity of the birds' α -amylases, which have pH optima between 7.0 and 8.0 (Gapusan *et al.*, 1990), to be partly differentiated from that of exogenous origin.

The data comparing the two strains of birds used may reinforce this theory. Differing isozymes have been reported between strains of chickens (Yardley *et al.*, 1988) as have responses to wheat α -amylase inhibitors (Gapusan *et al.*, 1990). However, while the presence of differing isozymes has the capacity to seriously influence digestibility trials, it is unlikely that this has happened here. The reported differences in α -amylase activity in the jejunum between the two strains may be more related to pH differences of the digesta. Although not measured for the jejunal samples, the pH of the digesta samples obtained from the Strain B birds may have been higher due to the birds consuming twice as much limestone as Strain A (Taylor, 1998b).

This pH effect is also noticeable when the data from the calcium provision treatments is considered. The birds fed limestone grit on a daily basis maintained a lower ileal pH than birds on the other treatments. Concomitantly, the birds fed grit daily had greater cereal and

bacterial α -amylase activities and lower digesta viscosity values than the birds on the other treatments.

That this effect did not occur in the birds fed the ground limestone may be due to the need for a constant infusion of calcium carbonate into the digestive tract to maintain pH, rather than flushes of calcium carbonate that would occur with the birds fed the ground limestone.

One of the reasons used to promote choice feeding has been that of improved energy utilisation and the data obtained by Taylor (1998a) have shown that choice-fed layers at peak production have significantly greater dietary energy utilisation efficiencies than birds offered a compound feed. The reasons behind this are many but it may be that one result of the choice feeding method employed, with provision of whole cereal grains, improved digesta dynamics which allowed increased cereal and bacterial α -amylase activity to occur in the crop. This will be determined in subsequent experimentation.

Feeding limestone grit daily may produce higher dietary AME values (Taylor, 1998a) due to a constantly higher digesta pH which allows greater cereal and bacterial α -amylase activities to be maintained. The increase in α -amylase activity results in a decrease in digesta viscosity, which in turn, may allow improved digestion and absorption of other dietary fractions.

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THE EFFECT OF TEMPERATURE ON RESPONSES OF LAYING HENS TO CHOICE FEEDING IN A SINGLE FEEDER

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Summary

Two experiments were conducted to examine the ability of hens to meet their nutrient requirements by self-selecting from whole wheat, a protein concentrate pellet and oyster-shell grit on a free-choice basis in a single feeder at environmental temperatures of 20° C or 32° C. Choice fed laying hens kept at 20° C had lower feed intake and better feed efficiency, but no difference in egg production compared with those fed an all-mash composite diet. Choice fed hens kept at 32° C had significantly better egg production compared with those fed the all-mash diet, but there were no differences in feed intake and feed efficiency.

I. INTRODUCTION

There have been several suggestions that the practicality of offering laying hens separate dietary ingredients in a single trough needs to be examined (e.g. Cumming *et al.*, 1987; Forbes and Covasa, 1995). This system, referred to as "choice feeding" or "split-diet" regime, has been a recognised feeding system for many years (e.g. Funk, 1932). There has been a number of studies in which hens have been allowed to exercise dietary self-selection. In most cases two or more separate feed troughs with internal dividers, or small separate containers, have been used (Karunajeewa, 1978; Leeson and Summers, 1978, 1979; Blake *et al.*, 1984; Karunajeewa and Tham, 1984; Scott and Balnave, 1988). From a practical viewpoint, however, such techniques are very expensive (Forbes and Covasa, 1995). Cumming *et al.* (1987) demonstrated that it was unnecessary to supply various feeds in separate feed troughs because hens have the ability to identify and pick up small, individual feed ingredients from a mixture.

Information about the practical application of choice feeding in a single trough for laying hens is limited to one report (Cumming, 1984). The main reason for this lack of information on choice feeding using practical feeding equipment is probably related to technical difficulties in the separation (for measurement purposes) of the feed refusals. Controlled environmental facilities were used in the present study to compare the feed intake and performance of laying hens self-selecting from whole wheat, a protein concentrate pellet and oyster-shell grit on a free-choice basis in a single feeder at environmental temperatures of 20° C (Experiment 1) and 32° C (Experiment 2) with that of hens fed a complete mash diet under the same conditions. A set of sieves with appropriately graded apertures was developed and used in the process of separating dietary components before and after feeding.

II. MATERIALS AND METHODS

Sixty-eight 24-week-old light hybrid pullets of White Leghorn x Australorp genotype (Tegel Tint, Hy-Line 300) were used in Experiment 1. The same birds at 34 weeks old were re-used in Experiment 2. The birds were reared in wire floor multiple-bird cages on a large commercial farm near Tamworth, NSW and received a commercial grower diet from 6 to 18

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weeks of age. From 18 weeks the birds were housed in two environmentally-controlled rooms set at either $20 \pm 1^\circ \text{C}$ (Experiment 1) or $32 \pm 1^\circ \text{C}$ (Experiment 2) and each experiment ran for 8 weeks. All birds, which had been trained for two weeks to choice feeding at 20 weeks of age, were allowed a further two weeks to adapt to the experimental diets. They were kept individually in wire cages and given 16 h photoperiod each day (from 0400 to 2000 h). All cages were fitted with individual feeders and waterers. Each hen was weighed at the commencement of each experiment and at two-week intervals thereafter. At 22 weeks of age, the birds in each room were randomly divided into two treatment groups before being randomly allocated to individual cages within rooms. In each room two replicates of 17 birds were fed *ad libitum* on either a mash diet (170 g CP; 11.6 MJ ME and 35 g Ca/kg, diet A) or on the basis of self-selection of whole wheat (130 g CP, 12.9 MJ ME and 0.6 g Ca/kg) and a layer protein concentrate pellet (332 g CP, 10.5 MJ ME and 34 g Ca/kg, diet B) hand mixed in the ratio 60 : 40 throughout the study (Table 1).

Table 1. Composition of experimental diets (g/kg).

Ingredient	Diet A	Diet B (concentrate)
Wheat meal (130 g CP)	340.0	123.0
Sorghum meal (80 g CP)	340.0	-
Meat meal (500 g CP)	90.0	290.0
Canola meal (370 g CP)	44.0	144.0
Cotton meal (370 g CP)	30.0	97.0
Soyabean meal (450 g CP)	33.0	97.0
Rice pollard (120 g CP)	55.0	236.0
Limestone	65.0	-
Salt	1.0	5.0
Choline chloride	0.3	1.0
DL-methionine	0.5	3.0
Lysine HCl	0.2	0.6
Layer premix	1.0	3.4
Total	1000.0	1000.0

Additional oyster-shell grit was provided in measured by hand amounts *ad libitum*, two times a day, on top of the feed in both feeding regimes to ensure that the birds could satisfy their Ca appetites. The ingredients were presented together in a single feeder for each bird. The individual intake of each ingredient was measured after each fortnight. The procedures for the feedstuff separation using sieves were as described by Henuk (1995). Water intake was measured daily every second week (i.e., d 15-21; 29-35 etc.). Egg production was recorded daily and egg weight was determined by weighing all eggs collected on four consecutive days each week. Specific gravity of eggs was determined on one egg per bird each week. All data were subjected to analysis of variance for a complete factorial experiment according to the procedure described by Burr (1982).

III. RESULTS AND DISCUSSION

The results of both experiments are summarised in Table 2. The results of Experiment 1 at 'normal' temperature (20°C) indicated that there was a large saving of 10.4 % in the mean total feed intake of the choice-fed hens compared to that of the control birds. This figure is higher than the values (6.7, 6.7 and 7.0 %) reported by Blair *et al.* (1973) and

Leeson and Summers (1978, 1979) but is in closer agreement with those of Karunajeewa (1978), in which hens receiving choice feeding consumed 11.0 % less feed overall. The increased efficiency of laying hens given choice feeding at 20° C was presumably because choice-fed birds were able to self-select the appropriate feedstuffs from the range of feed ingredients in the single feeder according to their physiological status and level of egg production. The results agree well with most previous reports in that choice-fed birds were marginally more efficient in converting their diet than those fed the complete diet (e.g., Blair *et al.*, 1973; Karunajeewa, 1978; Cumming, 1984).

Although they consumed less feed in total, birds offered choice feeding gained more body weight than those fed the control diet. This study confirmed previously reported findings that hens given whole grains were able to utilise energy more efficiently and thus gained more body weight than those given ground grain in mash diets (Karunajeewa, 1978). A similar increase in body weight from birds on choice feeding was also reported by Karunajeewa and Tham (1984), who found that laying hens offered whole wheat gained more weight than those offered crushed wheat.

Table 2. Effects of environmental temperature and feeding treatments on individual daily intakes and production parameters of hens.

Parameter	20° C			32° C		
	CF	CD		CF	CD	
Grain intake (g/hen/d)	68.7	83.2	**	64.0	72.7	**
ME intake (kJ/hen/d)	1368	1413	NS	1329	1239	*
Protein sources (g/hen/d)	41.3	39.1	NS	42.4	34.2	**
Protein intake (g/hen/d)	22.6	20.8	NS	22.4	18.2	**
ME : protein intake ratio	60.5	67.9	-	59.3	68.1	-
Shell-grit intake (g/hen/d)	4.6	4.2	NS	4.3	3.0	**
Ca intake (g/hen/d)	3.1	5.8	**	3.1	4.9	**
Feed intake (g/hen/d)	110.0	122.3	**	106.4	106.9	NS
Total feed intake (g/hen/d) (feed + shell-grit)	114.6	126.5	*	110.7	109.9	NS
FCR (g total feed intake/g egg produced)	2.0	2.2	**	1.8	1.8	NS
Water intake (ml/hen/d)	233.7	236.9	NS	308.7	251.9	**
Water : feed intake (ml/g)	2.0	1.9	NS	2.9	2.3	*
Egg production (%)	91.6	91.4	NS	88.1	85.7	*
Egg weight (g)	57.0	56.8	NS	59.1	59.4	NS
Egg specific gravity (g/cc)	1.083	1.084	NS	1.077	1.079	NS
Body weight (g)	1839	1786	**	1969	1912	**

CF = Choice feeding;

CD = Complete diet;

** = (P < 0.01);

* = (P < 0.05);

NS = not significant.

The results of Experiment 2 indicated that at high temperatures (32° C), choice feeding can help in solving the problems of decreasing nutrient intake and poor performance that are commonly experienced by laying hens fed a complete diet in hot climates. This is in agreement with the work of Mastika (1981), who showed that broiler cockerels kept at a constant 30° C coped far better when choice fed than if offered complete diets. The study also confirmed previously reported findings that when feed is limited at high temperatures, hens trained to self-select nutrients from separate energy- and protein-rich feeds are better

able to sustain egg output and body weight than those fed complete diets (Scott and Balnave, 1988).

The results are, however, contrary to those of Blake *et al.* (1984) who found that hens held at high temperatures and offered choice feeding gained less body weight than those fed a complete diet. Our findings do not oppose the suggestion of Forbes and Shariatmadari (1994) that the results obtained by Blake *et al.* (1984) were possibly due to the fact that the hens used by them were not experienced enough in choice feeding. In addition, the birds used in the study of Blake *et al.* did not have the same type of feed choice as the birds in the present study. A likely explanation for the significant difference in protein intake between feeding treatments in Experiment 2 in the present study is that the choice fed hens selectively consumed more protein than energy. Reid and Weber (1973) indicated that ME intake became the first limiting nutrient at high temperatures and that increased protein intakes were likely to only partially overcome the adverse effects of high temperatures. This was not the case in the current work: choice fed birds maintained virtually the same ME intake at 32° C as at 20° C, while those fed the complete diet suffered a decline of 14 % (from 1413 to 1239 kJ/bird/d; Table 2).

In conclusion, choice fed laying hens had a smaller feed intake and improved feed efficiency when kept at a 'normal' temperature (20° C), and maintained an adequate performance at high temperature (32° C), compared with those fed a complete all-mash diet.

IV. ACKNOWLEDGEMENTS

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THE USE OF THE NATURAL ALGAL PIGMENT ASTAXANTHIN AS A YOLK PIGMENT FOR LAYING HENS

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Summary

The use of astaxanthin as a yolk pigment for laying hens was evaluated. Astaxanthin in the form of NatuRose™ was found to be suitable for use as a yolk pigment at a dietary inclusion rate of 6 ppm for commercial hens housed under typical Australian conditions. The inclusion of 6 ppm resulted in a yolk colour of about 11 on the Roche colour scale for both Tegel Tint birds (2.4 kg body weight, consuming 128 grams of feed per day and laying 72 eggs per hen per 100 days) and ISA Brown birds (2.13 kg body weight, consuming 131 grams of feed per day and laying 90 eggs per hen per 100 days).

I. INTRODUCTION

In some countries such as Sweden, where the use of synthetic additives for egg yolk coloration is not permitted, there has been increasing interest in use of natural yolk pigments from different sources. The present study investigated the use of a natural pigment, astaxanthin, which is derived from marine microalgae, *Haematococcus pluvialis*. Astaxanthin is a carotenoid that is widely distributed in nature. The source of pigment used was the proprietary product, NatuRose™ (Cyanotech Corporation, Hawaii, U.S.A.). NatuRose™ was developed initially as a feed additive for aquatic animals raised on commercial aquaculture farms to ensure their flesh had the same pink coloration found in fish under natural conditions. Studies conducted overseas have shown that astaxanthin is a suitable carotenoid for colouring the yolks of laying hens. However, the amount of a carotenoid needed depends on the level of yolk pigmentation required, the nature of the feed ingredients used and the efficiency of incorporation of the dietary pigment into the yolk (Nys, 1999). The present study was the first conducted in Australia, using typical Australian layer diets. Birds were fed a range of levels of NatuRose™ in feed in order to determine the appropriate level of inclusion.

II. MATERIALS AND METHODS

(a) Birds, housing and experimental design

In Experiment 1, Tegel Tint laying hens (mean body weight 2.4 kg and 55 weeks of age at the beginning of the experiment), were housed 2-3 to a cage, in California-style commercial cages, at the University of New England's "Laureldale" farm. Four experimental groups were used, each of 50 birds. The control feed containing no added carotenoid was given to all 200 birds for an initial period of three weeks, and to the control group of birds throughout the experiment. The remaining three experimental groups were given feed containing one of 4, 8 or 12 ppm astaxanthin. At the end of the four week experimental period, it was obvious that the optimal level of astaxanthin lay somewhere between 4 and 8 ppm. Therefore, following the last egg collection, the control group of birds was given feed containing 6 ppm astaxanthin for a period of two weeks. In Experiment 2, feed containing 6

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ppm astaxanthin was given to 200 ISA Brown hens (mean body weight 2.13 kg and 32 weeks of age at the beginning of the experiment) for a period of eight weeks. Yolk colour was measured every two weeks. The experiments were conducted from October to December, 1998, a time of the year when temperatures range from cool to very warm in the New England region.

(b) Diets

The diets were based on a layer crumble, formulated to standard commercial guidelines. All diets were prepared by a commercial feed company and were based on sorghum with a final protein content of 16% and ME of 11.4 MJ/kg. The source of astaxanthin used was NatuRose™, (Cyanotech Corporation, Hawaii, U.S.A.) which was incorporated according to the manufacturer's specifications to achieve the final levels in feed. Feed intake was measured throughout the trial by weighing out and weighing back the feed.

(c) Production and egg quality measurements

In Experiment 1, egg production was recorded throughout the trial. Following the initial three week period on the control diet (0 ppm astaxanthin), 50 eggs were collected from each of the groups of birds, over a two-day period. This was Egg Collection 1. The Control Group remained on the control feed (0 ppm astaxanthin) and the experimental groups received feed containing one of 4, 8 or 12 ppm astaxanthin in the form of NatuRose™. Over the subsequent four week period, eggs were collected every five days. There were six egg collections in total. For Egg Collections 1 and 6, detailed measurements of egg internal quality were made. Eggs were weighed, the colour of the egg shells measured by reflectivity (the lighter the colour of the shells, the more light they reflect and therefore the higher the reflectivity), and breaking strength measured by quasi-static compression (the eggs are compressed at the equator at a constant rate until the shell cracks). The eggs were then broken out to allow for the measurement of albumen height (from which the Haugh Units were calculated), yolk colour and yolk weight. This study used an automated yolk colorimeter (Technical Services and Supplies, U.K.) which gives a completely objective measurement of yolk colour, free from user error and bias. The broken-out shells were washed and dried so that shell weight could be obtained. Shell thickness was measured on an electronic Mitutoyo Dial Comparator Gauge, by recording the average thickness of three pieces of shell taken from the equator of the shell. For Egg Collections 2-5, only egg weight, yolk colour and yolk weight were measured. In Experiment 2, 50 eggs were collected every two weeks over an eight week period and all egg and egg shell quality measurements were made.

(d) Statistical analyses

For measurements of egg production and feed intake, individual birds were treated as replicates, whereas for measurements of egg and egg shell quality, individual eggs were replicates. Data were analysed by ANOVA and means compared using Fishers Protected Least Significant Difference test. Significance was assumed at $P < 0.05$.

III. RESULTS AND DISCUSSION

The mean feed intake throughout Experiment 1 was 128.3 g of feed per bird per day. Percentage production averaged 68.8 eggs per day per 100 hens.

As shown in Figure 1, the yolk colour of the control group remained at about 4 on the Roche colour scale. Addition of NatuRose™ at 4 ppm resulted in yolks with a mean colour score of 9 on the Roche colour scale, by the end of the four-week experimental period. The inclusion of NatuRose™ at 8 ppm or 12 ppm resulted in yolk colour of 11-12 on the Roche scale as soon as 5 days after the diets had been presented to the birds. This depth of colour did not increase significantly from 5 to 28 days of the experimental period. There were no differences between groups and measurement intervals for egg weight (65.1 g), yolk weight (19.2 g) or percentage yolk (29.5%). These findings indicate that 4 ppm NatuRose™ is too low whereas 8 ppm is higher than necessary to achieve a yolk colour of about 11 on the Roche colour scale. There was no effect of level of inclusion of NatuRose™ on the other measures of egg internal quality, albumen height and Haugh Units or on egg shell quality.

As shown in Figure 1, inclusion of NatuRose™ at 6 ppm, in the feed of Tegel Tint birds, resulted in yolk colour scores between 10 and 11, with the mean score increasing from 10.25 on Day 5 to 10.75 on Day 15. When feed containing 6 ppm NatuRose™ was fed to ISA Brown hens for the longer period of 8 weeks, similar results were obtained (Figure 2). The ISA Brown hens consumed an average of 130.5 g of feed per bird per day and percentage production averaged 90.1 eggs per day per 100 hens. There were no differences over the eight-week period for egg weight (62.4 g), yolk weight (17.0 g) or percentage yolk (27.3%).

These findings indicate that inclusion of NatuRose™ at 6 ppm in the feed of laying birds which are consuming approximately 130 grams of feed per day, results in a yolk colour score of between 10 and 11 on the Roche Colour Scale, the preferred colour of egg yolks in Australia.

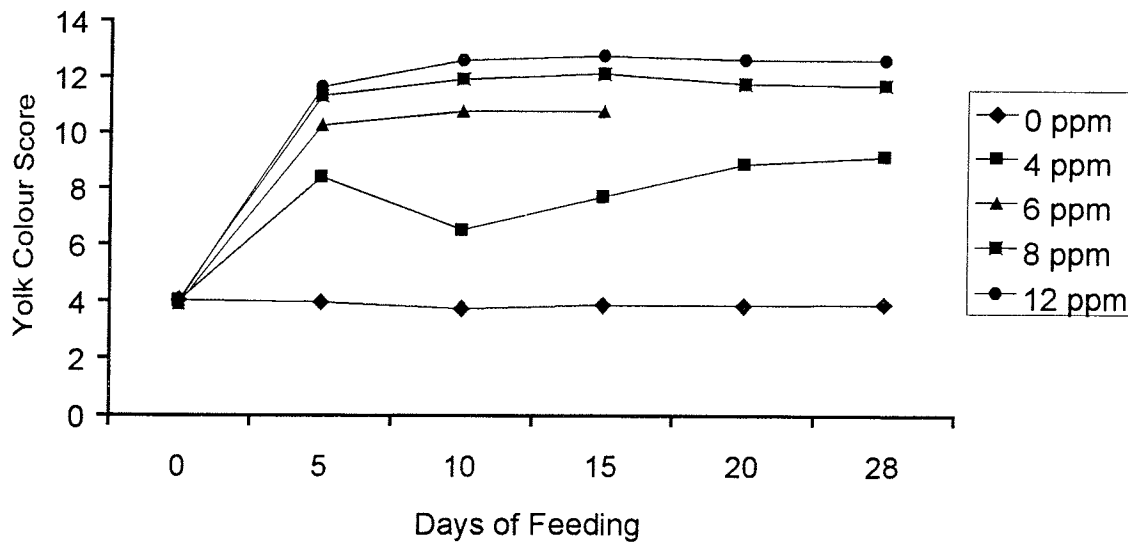


Figure 1. Yolk colour score produced by different levels of Astaxanthin in the feed of Tegel Tint birds.

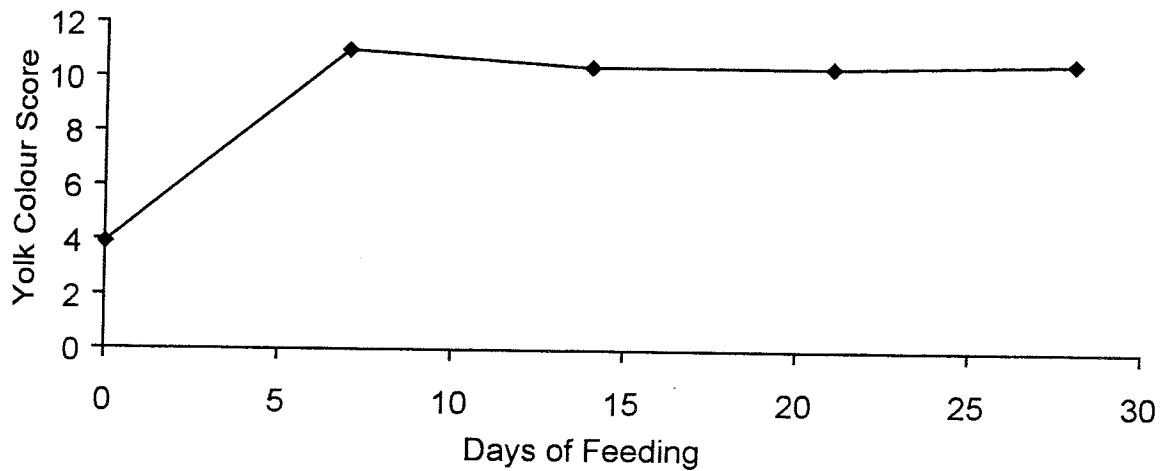


Figure 2. Yolk colour score produced by 6 ppm Astaxanthin in the feed of ISA Brown birds.

The results of the present study could be extrapolated to other strains of birds, with different feed intakes and on different diets, to estimate the amount of astaxanthin that would be required to achieve the desired yolk colour. However, it needs to be borne in mind that the rate of incorporation of any carotenoid into the yolk is not in direct proportion to the levels in feed. In addition, different feed ingredients contain different amounts of naturally occurring carotenoids (Nys, 1999).

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EFFECTS OF SELENIUM SOURCE AND LEVEL ON PERFORMANCE, MORTALITY AND MEAT QUALITY IN MALE BROILERS

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Summary

Selenium (Se) is involved in membrane integrity and numerous selenoproteins, and is required by poultry for the maintenance of optimal health and meat quality. This paper reports data from a semi-commercial study examining the effect of Se source and level on broiler performance and meat quality. The interaction between the level of vitamin E and the source of Se was also investigated. Increased dietary Se levels markedly reduced ($P<0.05$) feed efficiency ratio (FCR) as a result of significantly ($P<0.01$) lower feed intakes of birds while maintaining the same weight gains. Selenium supplementation improved ($P<0.01$) feather scores with organic Se being superior to inorganic Se (sodium selenite). Birds receiving organic Se in their diets had improved eviscerated weight ($P<0.05$), breast yield ($P<0.05$) and reduced ($P<0.01$) drip loss. There were significant ($P<0.05$) level x source interactions on yields of breasts and marylands (thigh plus drumstick), with increased level of organic Se increasing the yields whereas the opposite was true for the inorganic Se.

I. INTRODUCTION

Selenium is an essential micronutrient required for normal growth and maintenance in poultry. The major biological forms of Se are analogues of the sulphur amino acids, e.g. selenomethionine, selenocysteine and selenocystine. Selenium has a number of important biological roles including regulation of glutathione peroxidase (GSH-Px) activity, activation of the thyroid hormones and enhancement of male fertility (Mervyn, 1985). The recommended level for Se in poultry diets is a minimum of 0.1ppm (Mahan, 1995), which varies depending on the form of the mineral supplied and the nature of the diet composition. Interactions with other nutrients in the gastrointestinal tract increase the Se requirements of birds. The current study examined the effects of source and level of Se on bird performance, and meat yield and quality under a commercial setting.

II. MATERIALS AND METHODS

The experiment was conducted at the Research Farm, Bartter Pty. Ltd. at Griffith, NSW, between April and May 1999. A total of 3,600 day-old male broiler chicks (Bartter strain) were divided into 36 floor pens of 100 birds each. There were six starter (to 21d of age) and six finisher diets (to 37d of age). The basal diet was a wheat and soybean meal based diet formulated according to the commercial specifications at Bartter. The treatments differed only in the source and amount of Se (inorganic vs. organic at 0.1ppm or 0.25ppm) and vitamin levels (50 or 100IU/kg diet) as shown in Table 1. Premixes were prepared (Roche Australia) without Se and vitamin E. Thus, the Se and vitamin E were added to the diets at the time of mixing using the sequential dilution technique to make sure an even distribution of these minor ingredients in the diet.

Weekly weights and 21-d (data not shown in current paper) and 38-d feed intakes were recorded. On d 37 feather scores (1 being the poorest and 5 being the best) were

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determined for 10 birds from each pen using the method of Edens (1996). On d 37, feed was withdrawn and the birds fasted overnight prior to processing. On d 38 all birds were weighed and a subset of 50 birds from each diet were leg tagged prior to slaughter. The flock was commercially processed and whole weight and eviscerated weight were measured at this stage. The eviscerated carcasses were then placed in a spin chiller for 30 min at 3° C, and reweighed to obtain "dressed weight", which is the final product for the whole chicken market. The 24-h drip loss was measured by weighing the water lost from the dressed carcass after overnight storage at 3° C. The breasts and marylands (thigh plus drumstick) were dissected and weighed. The data were analysed using Statgraphics Package (Manugistica Software, MD, USA). First a 2 x 2 factorial ANOVA was performed based on two levels and two sources of Se. Then the data were subject to another 2 x 2 factorial ANOVA, but this time based on two levels of vitamin E and two sources of Se. Multiple comparisons were made using the Duncan's test.

Table 1. Selenium sources and levels (ppm) and vitamin E levels (ppm) of each diet.

Diet	Se level	Product	Se source	Vitamin E level
A	0.10	Na ₂ SeO ₃	Inorganic	50
B	0.25	Na ₂ SeO ₃	Inorganic	50
C	0.10	Sel-Plex 50	Organic	50
D	0.25	Sel-Plex 50	Organic	50
E	0.10	Na ₂ SeO ₃	Inorganic	100
F	0.10	Sel-Plex 50	Organic	100

I. RESULTS AND DISCUSSION

The birds grew well on the diets, reaching approximately 2.2kg at 38 d of age (Table 3). Se did not influence growth regardless of the level and source. However, increasing the Se level of the diet reduced ($P<0.01$) feed intake, thus leading to a significant decrease in feed conversion ratio (FCR). The average FCR was reduced from 1.791 (at 0.1ppm) to 1.736 (at 0.25ppm) (Table 2). This improvement was independent of Se source although the organic Se tended ($P=0.084$) to be superior. This result is probably related to the effect of Se on thyroid hormone function, increasing the efficiency of nutrient utilisation (Arthur, 1992). Selenium supplementation has been shown to improve feathering, due possibly to the role of selenocysteine in feather formation (Edens, 1996). In this study, feather scores improved ($P<0.05$) as the dietary Se level increased. Birds fed diets containing the organic Se had a better feather cover at d 37, indicating a better absorption of organic Se.

The data on the effect of Se level and source on liveweight, yields of commercial cuts and meat drip loss are shown in Table 3. Organic Se markedly ($P<0.05$) increased eviscerated weight, suggesting that the effect of Se on FCR was not due to increased feather or viscera weight. There were significant ($P<0.05$) interactions between the source and level of Se on weights of the breast fillets and marylands. Increased levels of organic Se improved them whereas the inorganic Se had the opposite effect. In addition, the organic Se markedly increased ($P<0.05$) the weight of the marylands, a finding previously reported by Edens (1996), although the mechanism involved with this is not known. The ability of muscle proteins to attract water and hold it within the cells is of paramount importance to meat quality. Selenium is a vital part of glutathione peroxidases, which are powerful antioxidants of the body, scavenging free radicals from both within and outside the cells to maintain the

integrity of tissues (Mahan, 1995). Both the level and source of Se seem to influence the antioxidant property of the body. Thus, in the current study, increasing the Se level from 0.1ppm to 0.25ppm reduced ($P < 0.001$) 24-h drip-loss from 1.19% to 0.78%, whilst birds receiving sodium selenite had a higher drip loss (1.12%) than those fed the organic source (0.85%). The inclusion of vitamin E at two levels (50 and 100 IU/kg diet) with a 0.1ppm background Se from both inorganic and organic sources had no significant effects on performance (Table 4) or meat quality (Table 5) although noticeable numerical differences were found, especially when the background Se was organic.

It may be concluded that Se is an essential micronutrient in poultry influencing not only meat yield and quality, but also performance. Organic sources of Se are superior to the commonly used inorganic sources.

Table 2. Influence of source and level (ppm) of Se on feed intake, feed conversion ratio (FCR), mortality and feathering in male broilers.

Se Source	Se level	Intake (g/38d)	38d FCR	Mortality (%)	Feather Score
Inorganic	0.10	3985	1.805	4.5	2.53
Inorganic	0.25	3825	1.759	3.5	2.60
Organic	0.10	3913	1.777	4.5	3.21
Organic	0.25	3730	1.712	3.5	3.47
Pooled SE		0.047	0.021	0.9	0.04
P value					
Se Level (L)		0.001	0.018	0.990	0.001
Se Source (S)		0.094	0.084	0.357	0.000
L x S		0.808	0.611	0.357	0.043

Table 3. Effects of source and level (ppm) of Se on 38-day body weight, eviscerated weight, dressed weight, yields of breasts and marylands (thigh plus drumstick) and 24-h drip loss in male broilers.

Se source	Se level	38d Wt (g)	Eviscer. Wt (g)	Dressed Wt (g)	Breast Wt (g)	Maryland Wt (g)	24-h drip loss
Inorganic	0.10	2208	1525	1569	411	528	1.37
Inorganic	0.25	2174	1509	1542	398	505	0.87
Organic	0.10	2203	1534	1568	401	526	1.01
Organic	0.25	2217	1573	1609	416	534	0.69
Pooled SE			18	19	6.5	6.5	0.07
P Value							
Se Level (L)		0.084	0.525	0.697	0.915	0.245	0.000
Se Source (S)		0.992	0.048	0.080	0.587	0.043	0.000
L x S		0.745	0.137	0.075	0.037	0.015	0.213

Table 4. Influence of Se source and vitamin E level (IU/kg diet) on feed intake, feed conversion ratio, mortality and feather scores in male broilers.

Se Source	Se level	V _E level	Intake g/38d	38d FCR	Mortality (%)	Feather Score
Inorganic	0.1ppm	50	3985	1.805	4.5	2.53
Inorganic	0.1ppm	100	3888	1.767	3.5	2.42
Organic	0.1ppm	50	3913	1.777	4.5	3.21
Organic	0.1ppm	100	3848	1.729	3.5	3.28
Pooled SE			0.058	0.026	0.8	0.05
P value						
V _E Level (L)			0.176	0.115	0.990	0.596
Se Source (S)			0.345	0.224	0.243	0.000
L x S			0.787	0.868	0.990	0.062

Table 5 Effects of selenium source and vitamin E level (IU/kg diet) on 38-day body weight, dressed weight, eviscerated weight, yields of breasts and marylands (thigh plus drumstick) and 24-h drip loss in male broilers.

Selenium Source	Se level	V _E level	38d Wt (g)	Eviscer. Wt (g)	Dressed Wt (g)	Breast Wt (g)	Maryland Wt (g)	24-h drip loss (%)
Inorganic	0.1ppm	50	2208	1525	1569	411	528	1.37
Inorganic	0.1ppm	100	2181	1554	1592	414	537	0.87
Organic	0.1ppm	50	2203	1534	1568	401	526	1.01
Organic	0.1ppm	100	2209	1558	1600	406	523	0.69
Pooled SE			26	20	21	6.5	7.3	0.07
P Value								
V _E Level (L)			0.465	0.197	0.182	0.605	0.683	0.000
Se Source (S)			0.714	0.759	0.860	0.168	0.243	0.000
L x S			0.469	0.888	0.834	0.882	0.426	0.213

V. ACKNOWLEDGEMENTS

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EVALUATION OF A BANTAM CROSS EGG LAYER

G.B. PARKINSON and P.H. CRANSBERG

Summary

This paper reports on a cross between a bantam White Leghorn and commercial Lohmann Brown females. The mean weight of the bantam cross females was 1.64 kg at 45 weeks of age. Production peaked at 96% at 26 weeks of age and averaged just under 90% from weeks 27-45. Mean feed consumption was approximately 11 % lower than the commercial Lohmann Brown egg layers while egg weight was, on average, approximately three grams lighter (5%). The overall production characteristics of this bantam cross suggests the possible commercial use of bantamised layers, if significant improvements in feed conversion efficacy can be demonstrated.

I. INTRODUCTION

Genetic selection over the last 40 years has seen substantial improvements in the performance of laying hens; however the rates of feed efficiency gain are likely to slow as egg production approaches the threshold of 365 eggs per annum. Two genetic approaches to improvement in feed efficacy have been suggested, both of which involve substantial reduction in body weight in commercial laying strains, viz. the introduction of dwarf or bantam genes into commercial lines. Research with the sex linked dwarfing gene demonstrated lower peaks of production and reduced persistency of production (Polkinghorne and Lowe, 1973).

Unlike the introduction of the sex-linked dwarf genes, bantam genes have shown more promise as a mechanism to alter the relationship between body weight and egg weight without affecting ovulation rate. Yoshida and Saito (1983) found that the introduction of Sebright bantam genes into various strains of fowl reduced adult body weight by 8-17%, reduced egg weight by only 3-4% and had no effect on production. Additionally, Stanhope and Parkinson (1988) introduced bantam genes into a commercial White Leghorn x New Hampshire cross with promising results. The bantamised hybrid (F1) had a mature body weight ranging from 1.5-1.8 kg with an average feed consumption of 90 grams/day. The bantamised hybrid egg weight ranged from 56.6 - 61.6 grams, production was 263-305 eggs to 78 weeks of age and egg mass was 14.9-18.9 kg. Clearly these bantamised hybrids had unusually high egg weight to body weight ratios and maintained competitive rates of egg production compared to Australian commercial egg laying stocks.

The experiment reported here assessed the performance of a cross between a commercial Lohmann Brown female and a bantamised White Leghorn male for production characteristics and general viability, and to compare these results with the commercial Lohmann Brown layer.

II. MATERIALS AND METHODS

The strains used in this trial were a commercial Lohmann Brown, and a cross between a bantam White Leghorn male (mature body weight of 1.35 kg) and the commercial Lohmann Brown bird. Both strains were raised in litter based environments on different properties in the same area, were fed similar diets, and were exposed to natural daylength to 13 weeks of

age. In November at 13 weeks of age, 48 birds from each strain were placed into two bird cages at a research facility, where light was constant at 16 hours per day.

The birds were kept in an environmentally controlled room with temperatures varying between 16-26°C with an average between 20-22°C. All birds were fed a commercial grower ration (12.1 MJ ME/kg and 180 g crude protein/kg) until 19 weeks of age, followed by a commercial layer diet (11.6 MJ ME, 179 g crude protein and 35 g calcium/kg) for the remainder of the experimental period. Feed and water were available *ad libitum*.

Birds were weighed at two-week intervals, commencing at 17 weeks of age and continuing through to the end of the experiment at 45 weeks. Egg production was recorded daily and accumulated to provide weekly figures. Egg weight was measured weekly (all eggs from a particular day were weighed) while feed consumption was calculated from 20 to 44 weeks.

Also included in the results and discussion section are references to the Lohmann White-LSL standard. The data provided is obtained from the management guide provided by Lohmann Tierzucht, and is used to provide a comparison for the bantam cross bird.

III. RESULTS

(a) Comparison of bantam cross with commercial Lohmann Brown

Table 1. Summary of production parameters of the bantam cross and the commercial Lohmann Brown to 45 weeks of age.

Parameter	Layer strain	
	Lohmann Brown	Bantam cross
Body weight at 45 weeks (kg)	2.08	1.64**
Ave. feed consumption (gm/bird/day)	120.6	107.5**
Ave. number of eggs / hen housed (weeks 20-44)	157.4	151.5*
Average egg weight (gm)	60.3	57.3*
Egg weight / 45 week body weight ratio (gm/kg)	29.0	34.9
Feed conversion - kg feed / dozen eggs	1.55	1.43
- kg feed / kg eggs	2.08	2.04
Mortality (including culls) (%)	10.5	14.6

* significant difference at $P < 0.05$

** significant difference at $P < 0.001$

(b) Summary of bantam cross characteristics

(i) *Body weight*

At 13 and 45 weeks of age the bantam cross had an average body weight of 991 and 1641 respectively. The bantam cross was significantly smaller ($P < 0.01$) than the Lohmann White-LSL standard and the trial Lohmann Brown (Figure 1).

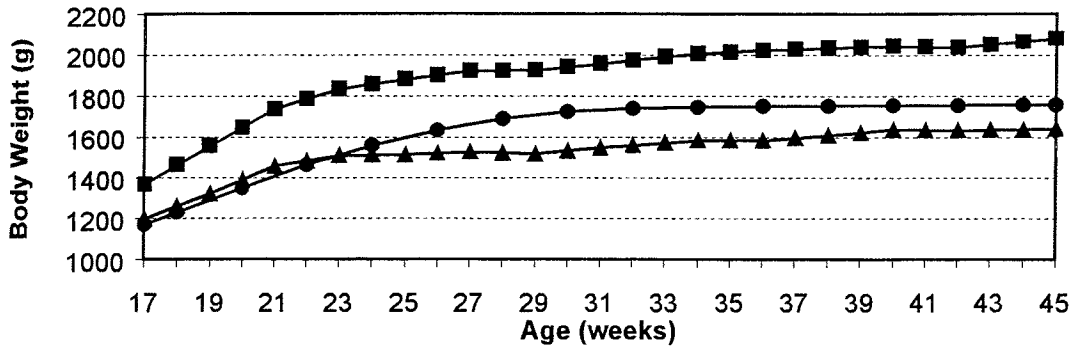


Figure 1. Average body weight of the bantam cross (▲), Lohmann Brown (■) and Lohmann White-LSL standard (●).

(ii) Egg production

The bantam cross had high levels of egg production, peaking at 96% at 26 weeks of age and averaging approximately 90% from week 27-45 (Figure 2).

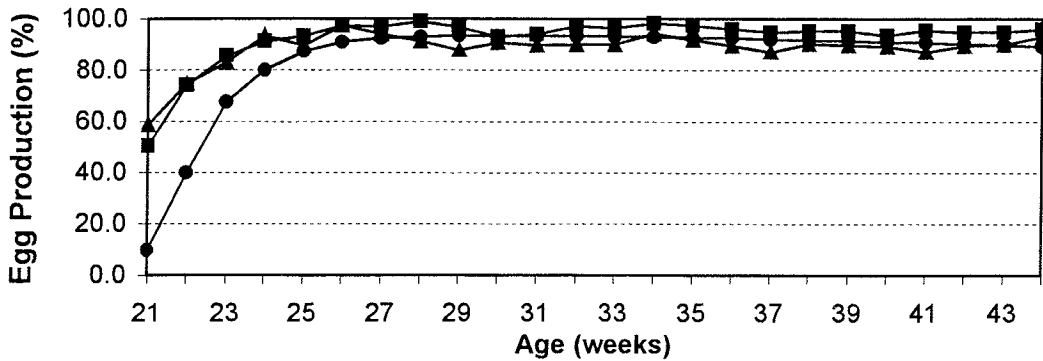


Figure 2. Average egg production of the bantam cross (▲), Lohmann Brown (■) and Lohmann White-LSL standard (●).

(iii) Egg weight

The bantam cross had an average egg weight of 57.3 grams from 21 to 44 weeks, which is almost identical to the Lohmann White-LSL (Figure 3).

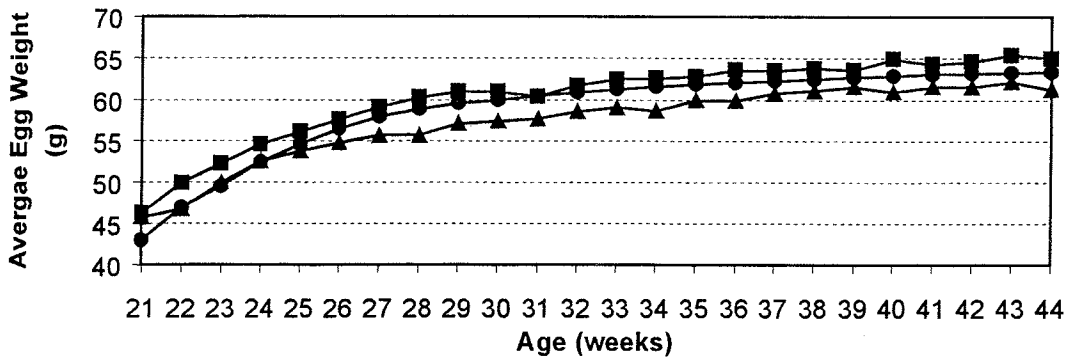


Figure 3. Average egg weight of the bantam cross (▲), Lohmann Brown (■) and Lohmann White-LSL standard (●).

IV. DISCUSSION

The average body weight of the bantam cross at 45 weeks of age was 1.64 kg. Despite the large reduction in body weight (21% at 45 weeks) compared to the Lohmann Brown, the reduction in both egg weight (5%) and production (3.7%) were relatively small. It is anticipated that the production performance can be further improved by backcrossing to the commercial strain whilst retaining selection for the bantam traits, and providing a diet that more closely meets the bird's nutritional requirements.

The lower average egg weight in the bantam cross bred than in the two Lohmann birds was not unexpected as egg weight is generally correlated with bird weight. However, the ratio of egg weight to body weight in the bantam cross (34.9 g/kg as compared to 29.0 g/kg in the commercial brown egg layer) illustrates the large egg size relative to body size of the former.

Feed consumption of the bantam cross was 11% lower than that of the Lohmann Brown and feed conversion was slightly, although not significantly, better. The increased recognition of the importance of feed conversion efficiency in egg production suggests the possibility of commercial use of bantamised layers if such can be demonstrated to be superior in this regard. Additional research will be directed at producing bantam crosses smaller than those produced in this trial. For example, a cross between the bantam White Leghorn male used in this trial and a commercial White Leghorn female (mature body weight of 1.8 kg) would produce a female F1 with a mature weight of approximately 1.4 kg.

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PEARL MILLET (*PENNISETUM AMERICANUM*) – AN ALTERNATIVE FEED GRAIN FOR LAYERS

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Summary

The effects of feeding two varieties of pearl millet (PM) on performance and egg quality was studied in 33-week old laying hens. Diets were based on sorghum/wheat as a control (C) or with graded levels of the varieties Katherine (K) (0, 200, 400 and 600 g/kg) and Siberian (S) at 400g/kg. No significant differences were found in egg production, feed conversion ratio or egg weight during or at the end of the 20-week experimental period. Feed intake of birds fed the S cultivars was significantly higher ($P<0.05$) than that of K or C based diets. The results indicate that feeding the PM has no adverse effect on egg production and could substitute for wheat, sorghum and some soybean meal in layer diets.

I. INTRODUCTION

In the USA pearl millet (PM) is considered to be “the new feed grain crop”. In feeding trials carried out in the USA, PM was at least equivalent to maize and generally superior to sorghum in protein content, protein efficiency and metabolizable energy (ME) levels (Hoseney *et al.*, 1987; Rooney and McDonough 1987, Sullivan *et al.*, 1990; and Bramel-Cox *et al.*, 1992). In comparison to sorghum, PM does not contain any polyphenol compounds such as condensed tannins that can interfere with or slow down digestibility when fed to poultry. In broiler chickens, Sullivan *et al.* (1990) have shown that weight gains and feed/gain ratios obtained in PM based diets are equal to that of maize and some sorghum cultivars. Smith *et al.* (1989) similarly reported that PM could replace maize without adversely affecting live weight gain or feed efficiency. Both the gross energy and ME values of PM tend to be higher than maize and may have been previously underestimated by about 20% (Fancher *et al.*, 1987).

Studies conducted by several research workers elsewhere (Collins *et al.*, 1997; Mohan *et al.*, 1991; Abd-Elrazig and Elzubeir; 1998) have also shown that PM compared favourably with maize in poultry diets. When PM was compared to maize on an isocaloric and isonitrogenous basis it gave the best liveweight gain and feed efficiency with broilers (Abd-Elrazig and Elzubeir, 1998). Collins *et al.* (1997) and Mohan *et al.* (1991) concluded that PM is suitable as a grain in layer diets and could be included at up to 600g kg⁻¹. The objective of the experiment reported herein was to study the effects of feeding two Australian varieties of PM (Katherine and Siberian) on laying hen performance.

II. MATERIALS AND METHODS

The five layer diets used in the study, and formulated to contain varying levels of two varieties of pearl millet, are shown in Table 1.

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Table 1. Ingredient and chemical composition (g/kg) of the experimental diets.

Ingredient					
Sorghum	400	208.0	160	70	145.3
Wheat	289.2	310.0	172.6	75.2	160.0
Sunflower meal	80	80	80	80	37
Soybean	96.1	63.5	51.7	39.1	107.8
Katherine millet	-	200	400	600	-
Siberian millet	-	-	-	-	400
Sunflower oil	1.62	1.52	1.51	1.56	9.36
Meat & bone meal	39.3	39.4	38.6	37.7	47.5
Limestone powder	43.0	44.5	43.3	43.5	42.0
Salt	1.56	1.74	1.75	1.8	1.46
Sodium bicarbonate	0.39	0.44	0.44	0.45	0.36
DL Methionine	1.54	1.17	0.99	0.72	2.79
Lysine	1.79	2.81	3.35	3.87	2.08
Vitamin/mineral premix ¹	2.0	2.0	2.0	2.0	2.0
Choline	0.45	0.45	0.45	0.45	0.45
Yolk pigment	0.04	0.04	0.04	0.04	0.04
Analysis²					
Crude protein	169	163	159	155	171
Lysine	7.8	7.8	7.8	7.8	7.8
Methionine	4.0	4.0	4.1	4.2	4.6
TSAA	6.7	6.7	6.7	6.7	6.7
Calcium	36	37	36	36	36
Total phosphorus	6	6	6	6	6
AME (MJ/Kg)	11.4	11.3	11.3	11.2	11.0

¹The vitamin and mineral premixes added per kg of diet: 2.5 mg retinol, 75 ug D₃, 5 mg α-tocopherol acetate, 2 mg menadione sodium bisulfite, 1 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine, 10 ug B₁₂, 1 mg folic acid, 10 mg niacin, 10 mg Ca pantothenate, 30 ug biotin, 225 mg choline, 50 mg Mn, 50 mg Zn, 50 mg Fe, 600 ug Mo, 500 ug Co, 600 ug I, 4 mg Cu, 70 ug Se, 80 mg Banox.

²calculated values

The diets were formulated to contain similar energy and protein levels and to satisfy the minimum nutrient requirement for maximum egg production (Table 1). PM varieties Katherine (K) and Siberian (S) were grown in Australia at the Biloela Research Station. The variety K was fed at three levels (200, 400 and 600 g/kg) whilst the variety S was fed at 400 (g/kg). A commercial layer diet based on sorghum, wheat and soybean meal was used as the control diet (C). Each of the five experimental diets was fed to 30 Isabrown birds for 20 weeks. These birds were 33 weeks of age at the beginning of the experiment and were housed in individual wire cages with feed and water available *ad libitum*. Egg production was recorded for five consecutive days/week with weekly measurements for feed intake (FI) and egg weight whilst specific gravity was recorded monthly. All birds were weighed at the end of the experimental period. A completely randomised experimental design was used. Data were analysed using an ANOVA and treatment means compared using a protected Least Significant Difference (LSD) test.

III. RESULTS AND DISCUSSION

Diets containing inclusion levels of 200, 400, and 600 g/kg of K and the 400 g/kg of S had no effect on egg production, feed conversion, egg weight, egg mass, specific gravity and final bird body weight (Table 2). The only significant difference ($P=0.009$) found among treatments was for feed intake. Hens consuming S (400 g/kg) had significantly higher intake ($P<0.05$) than birds fed on K (200 and 600 g/kg) and C diets. This could be due to the higher content of crude fibre and NDF (154 and 248 g/kg respectively) found in the S variety when compared with the K variety (75 and 151g/kg of crude fibre and NDF respectively). High fibre content in the diet is usually correlated with dietary AME depression and birds need to increase intake in order to obtain enough protein and energy to maintain production. We have recently determined the AME value of both pearl millet varieties (K and S) (Singh *et al.*, 1999) with broilers using the total collection method and with adult cockerels using a rapid bioassay. These results indicate that PM variety S presented at least 1.5 MJ/Kg DM lower than the K variety.

Differences ($P<0.05$) in feed intake were also found among the birds fed diets containing K. Birds fed 600g/kg consumed less food than birds fed K at 400 g/kg. During the course of the experiment it was observed that some birds consuming K at 600 g/kg regurgitated food.

Table 2. Egg output (%), feed intake (g/d), egg weight (g), egg mass (g/d), feed conversion ratio (FCR), specific gravity (SG) and final body weight (kg) for laying hens fed pearl millet experimental diets during 20 weeks.

Treatment	Inclusion g/kg	Output	Intake	Egg Wt.	Egg mass	FCR	SG	Body Wt.
Control	0	93.3	121.3 ^{bc}	65.0	60.6	2.0	1.09	2.1
Katherine	200	92.7	120.1 ^{bc}	65.0	60.2	2.0	1.111	2.1
Katherine	400	93.7	125.5 ^{ab}	65.1	61.4	2.06	1.09	2.2
Katherine	600	90.4	118.3 ^c	65.8	59.3	1.99	1.088	2.1
Siberian	400	94.2	130.1 ^a	65.9	61.9	2.10	1.091	2.1
P ¹		0.16	0.009	0.81	0.23	0.13	0.410	0.81
LSD		2.93	7.06	1.98	2.39	0.098	0.026	0.13

¹= Probability. LSD= least significant difference at 5%.

IV. CONCLUSIONS

The results suggest that PM varieties grown in Australia are suitable as a feed ingredient for layers. This is in agreement with the studies of Abd-Elrazig and Elzubeir (1998), Collins *et al.* (1997) and Mohan *et al.* (1991) who reported that PM can be incorporated in layer diets at up to 60% of the diet without any adverse effect on layer performance. From this study it can be concluded that PM offers great potential as a feed grain crop.

Many authors believe that the greatest advances in the next decade or so will come from the development of PM as a feed grain crop, adapted to warmer climatic regions. Though the good nutritional status of the grain is already established, opportunities for further improvement in feed value are known to exist; for example, the use of enzymes to improve digestibility of fibre in PM.

V. ACKNOWLEDGEMENTS

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NUTRITIVE VALUE OF WINTER WHEAT FOR BROILER CHICKENS

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Summary

The nutritive value of Australian winter wheat varieties for broiler chickens was assessed in a series of conventional energy balance studies each of 7-days duration. Across all samples, the mean and standard deviation for apparent metabolisable energy (AME, MJ/kg dry matter) were 14.29 ± 0.36 (n=25) and ranged from 13.68 to 15.02. For individual varieties, means and standard deviations were 14.80 ± 0.03 for Declic (n=2), 14.31 ± 0.33 for Lawson (n=16), 13.97 ± 0.34 for More (n=4) and 14.20 ± 0.09 for Paterson (n=3). In conclusion, winter wheats were consistently high in AME but, nevertheless, were responsive to endo-1,4-xylanase added to the diet. Uplift in AME of wheat due to enzyme supplementation averaged 0.7 MJ/kg (4.9%) and ranged from 0.22 MJ/kg (1.5%) to 1.13 MJ/kg (8.2%).

I. INTRODUCTION

In 1995, CSIRO Plant Industries and the Australian Wheat Board introduced Lawson, a leaf and stripe rust resistant winter wheat, which provided an opportunity for livestock producers in cooler, high rainfall areas to diversify into wheat production. There are now ten winter wheat varieties with varying degrees of rust resistance, five of which can be grazed during winter and then recover to provide a high yielding wheat crop in summer. Currently, the most popular varieties are More, Declic and Paterson. Tennant, which is due for release, is stem, stripe and leaf rust resistant. Winter wheats now comprise about 4% of the Australian feed wheat market, the remainder being spring wheat deemed unsuitable for milling.

The apparent metabolisable energy (AME) of Australian wheats is highly variable (10.35 - 15.9 MJ/kg DM) according to studies by Mollah *et al.* (1983) and Rogel *et al.* (1987) with broiler chickens. Recent studies also with broilers (Choct, 1995; Hughes *et al.*, 1996; Hughes and Choct, 1997) have confirmed earlier reports of wide variability. AME of wheat is related to the non-starch polysaccharide (NSP) level, which in turn is affected by climatic conditions such as high temperature or low rainfall during the growth period (Choct *et al.*, 1999). Feed manufacturers routinely add NSP-degrading enzymes to wheat based broiler feeds to enhance the AME of low nutritive value wheat and to minimise variability in AME between wheats. Since winter wheats are grown under mild conditions it is likely that these wheats will be of high nutritive value with low variability compared with spring wheats.

This paper summarises for broiler chickens (1) the nutritive value of four winter wheat varieties determined in ten studies between 1993 and 1999, (2) the nutritive value of two winter wheats from each of two regions with differing environmental conditions, and (3) the effects of dietary addition of a commercial enzyme product with endo-1,4-xylanase activity.

II. MATERIALS AND METHODS

The AME values of wheats were determined in conventional energy balance experiments involving measurements of feed intake and excreta output as described by Mollah *et al.* (1983) with

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minor modifications, and subsequent measurement of gross energy values of feed and excreta by bomb calorimetry. Day-old mixed sexed broiler chickens were raised in floor pens on a commercial broiler diet to 3-4 weeks of age then transferred in groups of five to metabolism cages in controlled temperature rooms. Semi-purified basal diets used prior to 1999 contained (per kg) 820 g sorghum, 134 g casein, 26 g dicalcium phosphate, 11 g limestone, 5 g mineral and vitamin premix, 3.6 g salt and 0.4 g choline chloride (50%). In 1999, semi-purified basal diets contained (per kg) 800 g sorghum, 152 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%). Wheat replaced sorghum in the basal diets as required. In each study, dietary treatments were replicated at least four times. Cold-pressed diets were fed for seven days. The first three days enabled the chickens to adapt to the cages and feeds. During the following four days, all excreta were collected and dried. 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 seven 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).

III. RESULTS AND DISCUSSION

Summarised results from 10 AME studies conducted between 1993 and 1999 are shown in Table 1. There were no indications of any sample of wheat of any variety falling in the "low-AME" category, that is, less than 13MJ/kg dry matter.

Table 1. Effects of variety of winter wheat on AME (MJ/kg dry matter) and feed conversion ratio (FCR, g feed/g gain) measured over a seven-day period commencing when chickens were 3-4 weeks of age. Each dietary treatment was replicated at least four times.

Variety	Number of samples	Mean	Standard deviation	Minimum	Maximum
AME					
Declic	2	14.80	0.14	14.71	14.90
Lawson	16	14.31	0.33	13.83	15.02
More	4	13.97	0.34	13.68	14.51
Paterson	3	14.20	0.09	14.10	14.27
Overall	25	14.29	0.36	13.68	15.02
FCR					
Declic	2	1.94	0.03	1.92	1.95
Lawson	16	2.00	0.14	1.82	2.41
More	4	2.01	0.03	1.98	2.05
Paterson	3	1.93	0.05	1.88	1.97
Overall	25	1.99	0.12	1.82	2.41

The results of an experiment to study the effects of variety, growth site and enzyme addition are summarised in Table 2. There were no significant effects of variety or growth site on feed conversion, AME, dry matter digestibility or excreta moisture content. In contrast,

enzyme addition significantly improved weight gain (373 vs 355 g/bird), feed conversion (1.89 vs 1.98), AME (14.8 vs 14.0 MJ/kg dry matter), and dry matter digestibility (0.74 vs 0.71 g retained/g eaten), and reduced excreta moisture content (670 vs 697 g/kg). The only effects of variety of wheat were on feed intake and weight gain. Chickens given the diet based on Paterson ate significantly less (98 vs 104 g/bird/day) and grew at a slower rate (355 vs 374 g/bird) than those on More.

Table 2. Effects of variety, growth site and enzyme addition (200 g/tonne) on feed intake (FI, g/bird 22-29 days), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of wheat, dry matter digestibility (DMD, g retained/g eaten), and excreta moisture (EM, g/kg). Means (n=6 replicates, each comprising five birds) having a common postscript letter are not significantly different ($P<0.05$).

Variety	Site	Enzyme	FI	GR	FCR	AME	DMD	EM
Paterson	Ballarat	-	97 bc	345 c	1.97 abc	14.10 cd	0.715 bc	682 abc
Paterson	Ballarat	+	99 bc	368 ab	1.89 de	14.70 ab	0.740 ab	672 abc
Paterson	Hamilton	-	96 c	344 c	1.94 bcd	14.24 bcd	0.719 bc	690 abc
Paterson	Hamilton	+	99 bc	361 bc	1.93 bcd	14.46 abc	0.730 abc	664 c
More	Ballarat	-	105 a	370 ab	1.99 ab	13.88 cd	0.705 c	710 a
More	Ballarat	+	103 ab	379 ab	1.91 cde	14.88 a	0.751 a	671 bc
More	Hamilton	-	105 a	362 abc	2.02 a	13.84 d	0.706 c	705 ab
More	Hamilton	+	101 ab	384 a	1.85 e	14.97 a	0.753 a	674 abc
Pooled SEM			2	8	0.02	0.20	0.007	13

The results of a further experiment to examine effects of growth site and enzyme addition are summarised in Table 3. There were no significant differences between growth sites but the beneficial effects of enzyme approached significance for AME (15.2 vs 14.8 MJ/kg dry matter, $P=0.09$) and for dry matter digestibility (0.763 vs 0.741, $P=0.06$).

Table 3. Effect of enzyme addition (200 g/tonne) to variety Declic grown on separate sites in the Ballarat region on feed intake (FI, g/bird 22-29 days), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of wheat, and dry matter digestibility (DMD, g retained/g eaten). Means (n=4 replicates, each comprising five birds) having a common postscript letter are not significantly different ($P<0.05$).

Site	Enzyme	FI	GR	FCR	AME	DMD
Bungaree	-	97 a	349 a	1.95 a	14.90 a	0.746 a
Bungaree	+	98 a	356 a	1.93 a	15.41 a	0.771 a
Bradvale	-	104 a	381 a	1.92 a	14.71 a	0.737 a
Bradvale	+	100 a	369 a	1.90 a	15.08 a	0.754 a
Pooled SEM		4	14	0.03	0.24	0.010

The results of another experiment to examine further any effects of variety and enzyme are shown in Table 4. There were significant differences between the varieties Paterson and More in live weight gain and feed conversion. Chickens given the diet based on More gained less weight (328 vs 363 g/bird) and were less efficient (1.94 vs 1.85) than chickens given Paterson. The beneficial effects of enzyme were clearly evident ($P<0.05$) for live weight gain (362 vs 329 g/bird), feed conversion (1.86 vs 1.93), AME (15.2 vs 14.3 MJ/kg dry matter),

and dry matter digestibility (0.77 vs 0.73 g retained/g eaten). Feed intake and excreta moisture were unaffected by either variety or enzyme addition in this experiment.

Table 4. Effects of variety and enzyme addition (200 g/tonne) on feed intake (FI, g/bird 22-29 days), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of wheat, and dry matter digestibility (DMD, g retained/g eaten). Means (n=6 replicates, each comprising five birds) having a common postscript letter are not significantly different (P<0.05).

Variety	Enzyme	FI	GR	FCR	AME	DMD
Paterson	-	95 a	354 a	1.88 b	14.27 b	0.726 a
Paterson	+	97 a	371 a	1.83 b	15.06 a	0.764 b
More	-	94 a	304 b	1.99 a	14.37 b	0.735 a
More	+	95 a	352 a	1.90 a	15.37 a	0.779 b
Pooled SEM		4	14	0.03	0.20	0.009

IV. CONCLUSIONS

Winter wheats were consistently high in AME but, nevertheless, responded to enzyme supplementation. Further studies are warranted to confirm or refute the notion that winter wheats are consistently high in AME because they are relatively low in soluble NSP content in comparison with spring wheats, as a result of being grown in areas with mild weather conditions. However, there were small but important differences between winter wheat varieties, hence it would be useful to examine other features of grain, such as starch granule size, composition and structure, which can affect nutrient utilisation by monogastric animals.

V. ACKNOWLEDGMENTS

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EFFICACY OF TRITICALE INCLUSION IN BROILER CHICKEN DIETS

G.P.D. JONES¹, R.S. JESSOP² and K.V. COOPER³

Summary

Broiler chickens were fed sorghum/soybean based diets containing 200 g/kg of one of six triticale varieties. Bird performance on each diet was measured by bodyweight response and feed conversion efficiency when compared to a sorghum/soybean meal/meat meal control diet. The effect of exogenous feed enzyme was also investigated with one triticale variety. Mean growth rate to 42 days of age in birds given the triticale diets was no different than those fed the control diet, although there were differences between those given the individual triticale diets. Two triticale diets produced an increase in feed conversion ratio while the addition of an exogenous feed enzyme overcame this effect. It is apparent from this work that the majority of the currently grown triticale cultivars do not negatively affect broiler performance at the level of inclusion examined.

I. INTRODUCTION

Triticale (genus *X Triticosecale*), a relatively new cereal crop developed from crosses between wheat and rye, is increasing in production in Australia as its agronomic benefits are increasingly being recognised. While there have been some reports of triticale being inferior to wheat as a stockfeed, these have been largely based on early cultivar releases or have flawed experimental design. More recent studies, however, have concluded that triticale is better than, or equivalent to, wheat in sugar content (Evers *et al.*, 1999), metabolisable energy content (Wilson and McNab, 1975; Farrell, 1978; Wiseman and McNab, 1995) and protein content (McKenzie and Farrell, 1980; Flores *et al.*, 1994).

Tao and Jones (1997) showed that pioneer cultivars, such as Growquick, which was grown in Australia in the early 1970s, depressed broiler chicken performance at levels of inclusion greater than 100g/kg feed, with a resultant decrease in bodyweight and food conversion efficiency associated with an increase in digesta viscosity. However, the inclusion of Tahara (released in 1988 and currently the most commonly grown cultivar in Australia) at levels up to 600g/kg feed had no effect on bird performance (Tao and Jones, 1997). Digesta viscosity was, however, high but was reduced by the use of exogenous feed enzymes.

Variation in feed quality can be high between samples of grain due to environmental and climatic factors, with this variation being decreased in good seasons (Hughes and Choct, 1999) compared to poor years (Johnson and Eason, 1988). However, it is considered that this variation is of lesser importance than the variation experienced between cultivars (Wiseman and McNab, 1995).

With the rapid emergence of new triticale cultivars in Australia, including some with significantly improved grain morphology and test weight, there is an ongoing need to evaluate the performance of triticale as a stockfeed. This paper provides information on the efficacy of newly released triticale varieties when included in broiler diets.

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II. METHODS

The experiment was a randomised within block design (Snedecor and Cochran, 1980). Eight treatments were replicated six times with each group consisting of eight male broilers allocated to each group by weight. The birds were housed in wire-mesh cages in environmentally controlled rooms with continuous fluorescent lighting and *ad libitum* feed and water from 5 to 42 days of age. The control dietary treatment (Table 1) consisted of a sorghum/soybean meal/meat meal base. One of five triticale cultivars (Abacus, Credit, Everest, Madonna and Tahara) was included at 200 g/kg in the control diet at the expense of the sorghum component. Two sources of Tahara were tested (Yarrawonga, Vic. and Warialda, NSW) with one source (Warialda) included with or without an exogenous enzyme (Avizyme 1302; 0.5 g/kg). The treatments were fed as starter diets (12.35 MJ ME/kg, 220 g/kg crude protein(CP)) from 5 to 21 days of age and as grower diets (12.75 MJ ME/kg, 200 g/kg CP) from 22 to 42 days of age. The diets were commercially formulated and, within each period, were isoenergetic and isonitrogenous.

Table 1. Composition (g/kg) of the sorghum control diets.

Component	Starter (g/kg)	Grower (g/kg)
Sorghum (90 g/kg CP)	617.7	671.7
Soybean meal (460 g/kg CP)	291.0	223.0
Meat meal (500 g/kg CP)	50.0	70.0
Tallow	14.0	16.0
Limestone	8.3	5.0
Vitamin/mineral premix	3.0	3.0
Dicalcium phosphate	7.5	3.8
DL-methionine	2.7	2.4
Salt	2.8	2.0
Sodium bicarbonate	1.5	1.5
L-lysine HCl	0.8	0.9
Choline chloride	0.7	0.7

The birds were weighed at the commencement of the experiment and at 21 and 42 days of age. Feed conversion ratio (FCR) was determined for the starter and grower phases as well as between 5 and 42 days.

III. RESULTS AND DISCUSSION

The replacement of sorghum with the triticale cultivars had no significant ($P > 0.05$) effect on the bodyweight of the broilers compared to the sorghum control (Table 2) when measured at either 21 or 42 days of age. However, there were differences between the individual triticale diets at 42 days of age with the Abacus diet producing lower bodyweights than Credit and Madonna. There was no effect of the dietary enzyme on bodyweight in the groups given the diets containing Tahara (Warialda) triticale.

Feed conversion ratio (FCR) was not affected by diet in the starter phase, although there was a suggestion that the birds fed the Abacus and Tahara based diets may have had poorer FCRs. In the grower phase, the birds fed the Tahara diets, regardless of source had

poorer FCR values ($P < 0.05$). The addition of the exogenous enzyme to the Tahara (Warialda) diet improved FCR to the level of the control sorghum diet (Table 2).

When considered over the entire experiment from 5 to 42 days, it was observed that the birds fed the Abacus and Tahara based diets had poorer FCR values than those fed the control diet or the other triticale based rations. Enzyme addition to the Tahara (Warialda) diet decreased FCR to a similar value to the sorghum diet (Table 2).

Table 2. Performance of male broilers when fed diets containing 200 g/kg triticale.

Diet	Bodyweight (g)		Feed conversion ratio		
	21 days	42 days	5-21 d	22-42 d	5-42 d
Sorghum control	885	2474 ab	1.482	1.909 ab	1.759 a
Abacus	881	2427 b	1.534	1.966 bcd	1.822 b
Credit	900	2575 a	1.517	1.903 a	1.757 a
Everest	904	2492 ab	1.493	1.939 abc	1.758 a
Madonna	898	2567 a	1.500	1.933 abc	1.780 ab
Tahara (Yarrowonga)	904	2492 a	1.524	1.987 cd	1.811 b
Tahara (Warialda)	902	2508 ab	1.509	2.005 d	1.817 b
Tahara (Warialda) + E ¹	894	2456 b	1.498	1.936 abc	1.765 a
SED ²	23.7	40.2	0.034	0.030	0.022

Values within columns followed by unlike letters are significantly ($P < 0.05$) different.

¹ E = 0.5 g/kg Avizyme 1302; ² Standard error of the difference of the means.

Previous experimentation with triticale as a component in broiler and pig diets has resulted in many inconsistent results, with work using older cultivars producing a depression in growth rate and performance of broilers (Ruiz *et al.*, 1987; Proudfoot and Hulan, 1988). In this trial, Abacus and Tahara produced the poorest bird performance in terms of feed conversion although there was no impact on bodyweight gain. The use of an exogenous fed enzyme in the Tahara (Warialda) diets overcame the depression in feed conversion efficiency, indicating that this cultivar in particular may have excessive levels of non-starch polysaccharides. That this depression was not observed with Credit and Everest suggests variation in the levels of non-starch polysaccharides in triticale cultivars.

Tahara produced similar results, regardless of source, indicating that the influence of growing environment may be negligible compared to that of genotype.

The data from this experiment, using a range of cultivars, indicate that currently available triticale cultivars are suitable for inclusion in broiler chicken diets. Further study of the effects of enzyme inclusion with the different cultivars, is indicated.

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THE PERFORMANCE OF MALE BROILER CHICKENS FED A DIET CONTAINING SAGO STARCH

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Summary

An alternative energy source in broiler diets to substitute for expensive cereals gains is needed. A study was conducted to assess the effects of diets containing 10 % sago on the performance of male broiler chickens in PNG. Performance was compared with the normal commercial broiler starter and finisher diets used by the Niu Gini Table Birds Company. A complete randomised design was used with three replicates each of 135 birds for each of the two treatments. Feed intake and live weight were recorded weekly. The production performance of male broiler chickens fed a diet containing 10 % sago was not significantly different from chickens fed the control diets. Sago starch can therefore be included as a substitute for cereal grains in broiler diets at an inclusion rate of 10% without detriment to growth and efficiency.

I. INTRODUCTION

There is a need to find an alternative energy source to grains to include in livestock diets in developing countries because of the high cost of importing grain. The total cereal imports for livestock feed into PNG are estimated at 47000 tonnes per year (DAL, 1992) with the Niu Gini Table Birds Company importing about 9500-10000 tonnes annually. There are several energy sources in PNG that have the potential to be developed, such as cassava, taro and sweet potato. These feedstuffs are low in energy compared to cereal grains, but can replace some of the ingredients in a diet (eg. Springhall, 1965; Mandich, 1989).

Sago (*Metroxylon spp*) starch, which is extracted from the stem tissues of the sago palm, is one of the energy sources that could be developed for livestock feed. There are large areas of swampland covered with sago palms in the southern and northern parts of PNG that could be exploited for sustainable sago starch production. Commercial sago starch production has been developed in countries like Indonesia and Malaysia whereas in PNG the resource is yet to be developed. Successful use of sago in livestock feeding has been reported (Springhall, 1965; Springhall and Ross, 1965a,b; Dunsmore and Ong, 1970; Ong, 1973; Ong, 1976). The aim of this study was to assess the effects of diets containing sago on the performance of broiler chickens in PNG.

II. MATERIALS AND METHODS

As there is no sago processing company in PNG, Niu Gini Table Birds Company imported the sago starch from Malaysia. The control diets were normal commercial broiler starter and finisher diets formulated and mixed by the Niu Gini Table Birds Company. The experimental starter and finisher diets were formulated using sago to replace 10 % of the grain in the commercial ration. The crumbled starter diets contained 220 g CP/kg and the pelleted finisher diets contained 200 g CP/kg.

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A total of 810 day old (Tegel strain, TM80) male broiler chickens were used in the study. A completely randomised design was used with three replicates of 135 birds per treatment. Three pens in a broiler shed at the University farm were partitioned for the trial. The University farm is located in the lowlands of PNG. The relative humidity was high and ambient temperatures range between 25 and 30° C. All birds were weighed weekly. Chickens were fed *ad libitum* and the starter diet was changed to finisher diet at 21 days of age. Daily feed intake was recorded for the 42 d trial period. Live weights were recorded on a weekly basis. Mortalities were recorded as they occurred during the trial period. Data were analysed using the Minitab system (Ryan, *et al.*, 1985).

III. RESULTS AND DISCUSSION

An analysis of feed intake, live weight gain and feed efficiency indicated that there was no significant difference in the starter or grower phase between the groups on the two diets (Table 1). There was no significant difference ($P>0.05$) in overall mortality rate in chickens fed the control and sago diets in the starter (3.7 and 2.7%) and grower (11.6 and 7.9%) phases respectively.

Table1. Response of broiler chickens to diets with and without 10% sago (Mean \pm SE).

Trait	0-21 days		0-42 days	
	Control	Sago	Control	Sago
Feed intake (g/kg)	1.05 ^a \pm 0.03	1.04 ^a \pm 0.01	3.28 ^a \pm 0.02	3.18 ^a \pm 0.08
Weight gain (g)	749 ^a \pm 5.8	742 ^a \pm 3.4	2120 ^a \pm 21	2157 ^a \pm 23
FCR	1.30 ^a \pm 0.03	1.30 ^a \pm 0.06	1.95 ^a \pm 0.03	1.93 ^a \pm 0.03

Mass within the same row and trait with the same superscripts are not significantly different ($P>0.05$).

These results showed no adverse effects of feeding sago at an inclusion rate of 10 percent of the diet, which confirms other findings (Springhall and Ross, 1965a,b; and Dunsmore and Ong, 1970). Further study of the effects of higher rates of dietary inclusion is indicated.

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IMPLICATIONS OF THE USE OF WHOLE GRAIN IN PELLETTED BROILER CHICKEN DIETS

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Summary

Ground or whole triticale were included at 200 g/kg in sorghum based broiler diets to examine the influence of whole v ground grain on bird performance and digestive tract characteristics. There was no significant effect of triticale form or dietary enzyme supplementation on bodyweight gain. The birds fed the whole grain had poorer feed conversion efficiencies in the starter phase, whereas those fed the ground grain had poorer efficiencies in the grower phase. Enzyme addition lowered the FCR of the birds fed the ground triticale such that the FCR was equivalent to that of the birds fed the whole grain. No effect of enzyme supplementation was observed when the birds were fed whole triticale.

I. INTRODUCTION

Previous work has suggested that the use of whole grain (Cumming, 1992) and large fibre particles (Rogel, 1985) allows for greater development of gizzard musculature so that the bird can better digest its feed. However, the influence of whole grain or large fibre particles on dietary utilisation has not been clarified. It may be expected that the resultant improved digestion would be reflected in altered dietary AME values and/or changes in bodyweight and feed conversion efficiency, either through alterations in energy availability *per se*, as Taylor (1998) found an improvement in dietary metabolisable energy when layers were fed large particles of grit, or through altered disease resistance (Cumming, 1992).

If these changes are apparent, then there exist large implications for metabolisable energy determinations. Different methodologies in feed preparation may produce disparate AME values and hence comparison of AME values between laboratories may be compromised. Certainly, AME responses to exogenous feed enzyme addition have not been consistent (Bedford, 1997), and may be related to a number of factors, as Annison (1993) indicated that factors such as upper gastrointestinal tract micro organisms, ionic calcium concentrations and the physical effects of the feed are part of a complex interaction affecting the metabolisable energy of a diet.

The following experiment examines the influence of one of these factors, grain particle size, on broiler performance when either whole or ground triticale is included in a ground dietary base.

II. METHODS

Triticale (cv. Tahara) was substituted (200 g/kg) into sorghum/soybean/meat meal based starter and grower diets described by Jones *et al.* (2000). The experiment was a 2 x 2 factorial, randomised within-blocks design (Snedecor and Cochran, 1980) with each treatment replicated six times. The triticale was included in the diets as either whole or as finely hammermilled grain and an exogenous feed enzyme (Avizyme 1302; 0.5 mg/kg diet) was either added to, or omitted from, each diet. The sorghum component of both diets was also

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finely hammermilled. After mixing, the diets were cold pelleted through a 4 mm die and were offered *ad libitum* to groups of eight male broilers from 5 to 42 days of age, housed in wire mesh cages in environmentally controlled rooms (Jones *et al.*, 2000). The birds were weighed at the commencement of the experiment and at 21 and 42 days. At 30 days of age, 12 birds fed each dietary treatment, but maintained separately from the main trial, were slaughtered and dissected and proventriculus and gizzard weights determined. A segment of intestine, from Meckel's diverticulum to the ileocaecal junction, was removed and measured. At 42 days of age, three broilers, randomly selected from within each replicate group, were slaughtered to similarly determine organ responses to the diets.

III. RESULTS

At the commencement of the experiment at five days of age, there were no differences ($P>0.05$) in mean bird bodyweight (95 g) between the treatments due to the selection methods employed. There was no effect on bird bodyweight at 21 days from either the form of triticale used or from dietary enzyme supplementation (Table 1). However, a triticale form x enzyme interaction was observed, whereby enzyme supplementation of the whole triticale diet improved bodyweight whereas no effect of enzyme supplementation was observed when ground triticale was used (Table 1). No effects on bodyweight were apparent at 42 days.

Table 1. Broiler performance when fed diets containing 200 g/kg whole or ground triticale with or without enzyme addition.

Triticale	Enzyme ¹	Bodyweight (g)		FCR		
		21 days	42 days	5-21 d	22-42 d	5-42 d
Ground	--	902	2508	1.509	2.005	1.817
	+	894	2456	1.498	1.936	1.765
Whole	--	858	2438	1.541	1.900	1.764
	+	917	2519	1.481	1.906	1.736
SED ²	Form (F)	14.5	39.3	0.023	0.025*	0.012*
	Enzyme (E)	14.5	39.3	0.023	0.025	0.012*
	F x E	20.5*	55.6	0.033	0.035	0.018

¹Avizyme 1302 (0.5 mg/kg)

² Standard error of difference of means

Values followed by an asterisk indicate significant statistical differences ($P<0.05$)

Food conversion ratio (FCR) was not influenced by either treatment in the starter phase (5-21 days) although the data tended to reflect the 21 day bodyweight data, with a combination of whole triticale/nil enzyme addition having a poorer FCR than the other treatments (Table 1). The reverse occurred in the grower phase (22-42 days), with the birds fed the ground triticale diet having greater FCR values. When considered over the experimental duration (5-42 days), there was both a form and enzyme effect, with the ground triticale and nil enzyme treatments both producing greater FCR values (Table 1).

There was no influence of treatment on proventricular or gizzard size at 30 days (Table 2). However, at 42 days, the size of both the proventriculus and gizzard were affected by the form of triticale used in the diet. At this age, the proventriculus was decreased in size by

feeding whole rather than ground triticale (Table 2), whereas the gizzard responded by increasing in size when the whole grain was offered. The length of the intestinal segment when measured at either 30 or 42 days of age was not affected by dietary treatment.

Table 2. Influence of feeding diets containing ground or whole triticale and with or without enzyme on organ characteristics (g/kg bodyweight) of 30 and 42 day old male broiler chickens.

Diet	Provent. (g/kg)		Gizzard (g/kg)		Intestine (cm/kg)	
	30 d ²	42 d ²	30 d	42 d	30 d	42 d
Ground	5.14	4.58	17.57	11.08	42.2	27.5
Whole	4.53	3.92	17.52	12.98	44.8	27.3
Ground + enzyme ¹	4.97	4.49	18.41	11.55	45.5	27.7
Whole + enzyme	4.69	3.93	18.25	12.23	43.5	28.2
SED						
Form (F)	0.23	0.24	0.70	0.49	1.44	0.75
Enzyme (E)	0.23	0.24	0.70	0.49	1.44	0.75
F x E	0.32	0.34	0.96	0.68	2.04	1.06

¹Avizyme 1302 (0.5 mg/kg)

²n=12 at 30 d, n=18 at 42 d

Values followed by an asterisk indicate significant statistical differences (P<0.05)

IV DISCUSSION

Bird age influenced the development of the gizzard in response to diet; at 30 days there was no difference in gizzard size as measured nor were there visual differences in the morphology of the gizzard between birds fed on either the whole or ground triticale whereas at 42 days, the gizzard of the birds fed the whole triticale diet was greater in size and had visually greater musculature than that of the birds on the ground triticale diet. Concomitantly, the proventriculus increased in size as the birds aged and when the birds were fed the ground triticale treatment.

The data obtained here, when considered in conjunction with other work, highlight the problem of current AME methodologies and the variable responses obtained with exogenous enzyme addition to broiler diets.

McNab (1996) indicated that the currently observed variation in AME values (the low AME wheat 'phenomenon') may be simply a reflection of substitution techniques. With Wootten *et al.* (1995) indicating a positive correlation between pentosan (fibre) content and AME or nutritive value and Rogel (1985) showing an effect of fibre particle size, it is suggested that the feeding of whole or coarsely cracked grains to chicks, as currently happens in some laboratories in Australia, does not produce a true AME value until the bird's digestive system has adapted to the diet.

The observation by Bedford (1997) that exogenous enzyme additions to broiler diets do not produce consistent responses may be a reflection of this problem. The lack of response to exogenous enzyme addition may be due to a number of factors. The more muscular gizzard observed at 42 days when the whole grain diet was fed may allow greater intestinal and/or pancreatic reflux (Bird, 1971) thereby improving digestion via both the bird's and the grain's natural amylase activities as the feed stays in the upper digestive tract for longer periods

(Taylor, 1998). Petterssen and Åman (1989), working with rye, indicated that both gizzard function, through feed grinding, and bacteria and/or endogenous enzymes in the crop and gizzard, are mainly responsible for fibre degradation, thereby negating the effect of the exogenous enzymes.

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RAW SOYBEANS SELECTED FOR LOW TRYPSIN INHIBITOR ACTIVITY FOR POULTRY DIETS

R. PEREZ-MALDONADO¹, P. MANNION¹, D. FARRELL² and A. JAMES³

Summary

The main objective of these experiments was to provide recommendations to industry on the nutritional value of selected Kunitz trypsin inhibitor (TI) free SB genotypes and hence their commercial attributes in least-cost poultry diets.

It is recommended that Kti SB not be used in broiler starter diets unless first heat-processed to reduce its residual TI activity to levels comparable with commercial SB meals. Steam conditioning and pelleting temperatures below 90° C are inadequate for this purpose. For layers, non-steamed Kti SB can be fed at levels up to at least 110 g/kg without adversely affecting egg production parameters. It is recommended though that the nutrient concentration of the diet be adjusted to account for the reduction in FI of about 6%.

I. INTRODUCTION

Full-fat soybeans (FFSB) are a rich source of crude protein (395 g/kg) and oil (180 g/kg) but their use in poultry diets is limited due to the presence of Kunitz and Bowman-Birk trypsin inhibitors (TI). Fortunately these TI can be denatured by heat before feeding them to monogastric animals but this is expensive. Genetically modified Kunitz trypsin inhibitor-free (Kti) SB have been developed (Bernard and Hymowitz, 1986) containing less TI activity than Raw SB but higher levels than in processed soybean meal (SBM) due to the remaining Bowman-Birk TI. The development by CSIRO of new genotypes of Kti SB that are agronomically suitable for Australian environments presents an excellent opportunity for the poultry industries to better exploit this outstanding feedstuff.

The Kti CSIRO genotypes were evaluated for poultry in two broiler experiments and one layer experiment at the Queensland Poultry Research and Development Centre. The use of steam pelleting as a procedure to reduce residual TI activity allowing higher inclusions of full fat SBM (FFSBM) in diets was investigated.

II. MATERIALS AND METHODS

(a) Bio-assays, chemical analysis, diets and management

Prior to each experiment, the Kti and other commercially available SB samples were analysed for dry matter (DM), ash, calcium (Ca), phosphorous (P), fat, nitrogen (N), amino acids, gross energy, TI activity and apparent metabolisable energy (AME). AME of the SB samples was determined in broilers (21 days of age) at 250 and 400g/kg inclusions in a basal diet and calculated by linear regression. The AME of selected experimental broiler diets (exp. 2) was determined within the second growth experiment while that of the layer diets was determined by the rapid method with adult cockerels (Farrell *et al.*, 1991). All assays employed total excreta collection. In both experiments all groups of chicks were housed in

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wire cages with food and water *ad libitum*. Illumination was for 23 h and hot-air brooders provided heating and ventilation to the bird's requirements. In broiler experiment 1, Kti and Raw SB at 70, 120 and 170 g/kg were compared with a commercial FFSBM and a solvent extracted SBM with added SB oil both at 170 g/kg. Each diet was fed to six replicate groups of eight chickens. In broiler experiment 2, Kti and Raw SB, both non-steamed or steam-pelleted and reground, at 70, 120, and 170 g/kg were compared with a commercial FFSBM at 170 g/kg. An additional five treatments comprising a DL-methionine supplement to the 170 g/kg inclusion level of each SB were also included to test for potentially unavailable cystine in the Kti and Raw SB. Food intake (FI) liveweight gain (LWG) and feed conversion ratio (FCR) were measured after 20 d and 21 d in experiments 1 and 2, respectively. At the completion of the experiments 2 birds from each cage were killed, weighed, and their liver and pancreas weights measured. All diets were fed as mash and formulated to contain similar Ca, P and calculated AME content and meet the minimum amino acids requirements estimated for maximum growth (SCA, 1987). Completely randomised block designs were used.

For the layer trial, production parameters on steam pelleted Kti and Raw SB were compared with non-steamed Kti and Raw SB each at two levels (70 and 110 g/kg) and fed to 30 individually caged Isabrown hens from 29 weeks of age. Food and water were available *ad libitum* and a photoperiod of 15.5 h was maintained for 19 weeks in a completely randomised experimental design. Six hens from each treatment containing 110g/kg level of SB were sampled for organ measurements. Diets were formulated to least-cost nutrient specifications (SCA, 1987) with similar N and AME contents and prepared as mash. Data for all experiments were analysed using an ANOVA and treatment means compared using a protected LSD test.

III. RESULTS AND DISCUSSION

(a) Chemical analysis

The AME of the Kti SB was comparable to that of a commercial FFSBM (14.1 MJ/Kg DM). The AME of the Raw SB meal was 9.47 MJ/Kg DM but after steam-conditioning its value improved to 14.1 MJ/Kg DM. Nutritional analyses showed the crude protein, ether extract, Ca, P and essential amino acid content of Kti SB to be similar to that of Raw SB and commercial FFSBM. The TI activity in Kti SB was about 20% lower than that in Raw SB but greater than in commercial FFSBM.

(b) Broiler experiments

Both broiler experiments gave similar results in respect of the performance of birds fed Kti and Raw SB when compared with the SB controls (Table 1). Kti significantly ($P < 0.05$) reduced LWG by 18%, FI by 9.0% and FCR by 11% whilst Raw SB significantly ($P < 0.05$) reduced LWG by 28%, FI by 16% and FCR by 19% when averaged over the two experiments. Kti significantly ($P < 0.05$) increased pancreas weight by 59% and Raw SB by 102% over both experiments. All parameters deteriorated as the levels of inclusion of Kti or Raw SB increased in the diet. In both experiments, the Kti treatment was superior ($P < 0.05$) to that of the Raw SB treatments. Steam pelleting both the Raw and Kti SB failed to improve growth and FCR over non-steamed SB treatments. In our experiment the residence time of the SB in the steam-conditioner and pellet die was approximately 30 seconds and the maximum temperature did not exceed 85° C. It would appear therefore that Kti SB require less heating than conventional Raw SB to maximise chick performance, the steam-

conditioning parameters used in this experiment were insufficient to improve bird performance despite reducing the measured TI activity from 2.6 to 0.36 mg/g. Pancreas weight was reduced in the steam-pelleted treatments although the difference was significant only ($P < 0.05$) when Raw SB is compared with Kti SB.

Table 1. Main effect of dietary treatments on LWG (g), FI (g), FCR and organ weight (g/100 g live weight) of broiler chickens at 20 d (Exp. 1) and 21 d (Exp. 2) evaluated in two growth experiments.

Main effect	LWG	FI	FCR	Pancreas	Liver
Soybean type					
Kti (1)	588.4 ^a	916.9 ^a	1.567 ^a	0.44 ^a	2.79
Raw (1)	510.6 ^b	847.3 ^b	1.678 ^b	0.54 ^b	2.91
LSD	38.87	40.02	0.059	0.044	NS
<hr/>					
Kti (2)	572.8 ^a	898.6 ^a	1.583 ^a	0.54 ^a	
Raw (2)	499.5 ^b	829.6 ^b	1.703 ^b	0.70 ^b	
LSD	19.6	24.74	0.0306	0.076	
Treatment					
Non-steamed (2)	542.5	872.8	1.635	0.67	
Steamed (2)	529.8	855.4	1.650	0.57	
LSD	NS	NS	NS	NS	
Level (g/Kg)					
70 (1)	605.4 ^a	929.8 ^a	1.545 ^a	0.43 ^a	2.72
120 (1)	548.9 ^b	885.4 ^a	1.622 ^{ab}	0.50 ^a	2.91
170 (1)	494.2 ^c	831.0 ^b	1.700 ^b	0.55 ^b	2.92
LSD	49.46	52.22	0.081	0.059	NS
<hr/>					
70 (2)	624.5 ^a	958.7 ^a	1.543 ^a	ND	
120 (2)	542.6 ^b	881.0 ^b	1.649 ^{bc}	ND	
170 (2)	497.4 ^{cd}	827.0 ^c	1.682 ^{cd}	0.62	
LSD	27.0	34.98	0.0434		
Controls					
FFSBM (1)	696.1	977.0	1.404	0.325	2.56
SBM + oil (1)	695.8	996.4	1.434	0.293	2.66
LSD ($P < 0.05$)	NS	NS	NS	NS	NS
<hr/>					
FFSBM (2)	720.7	1018	1.412	0.305	
FFSBM + methionine (2)	712.0	1018	1.430	ND	
LSD	NS	NS	NS		

Means in the same column within trial not followed by a common letter differ significantly ($P < 0.05$). (1) = Experiment 1. (2) = Experiment 2.

(c) Layer experiment

Treatment means for the main effects of SB genotypes, processing and inclusion level each compared with the FFSBM control diet are shown in Table 2. Raw SB significantly ($P < 0.05$) reduced FI, egg weight, egg mass output, body weight and pancreas enlargement when compared with the FFSBM. A similar reduction in FI occurred in the Kti treatments but egg weight, egg mass, body weight and pancreas size were similar ($P > 0.05$) to that of the

control diet. The contrast in results between layers and broilers suggests that older birds are less susceptible to the effects of TI. Clearly, though, the higher levels of TI in the Raw SB diets was sufficient to increase pancreas weight and depress egg mass output. In the contrast of main effects, the greater egg number and egg mass output associated with the higher level of inclusion of SB is difficult to explain.

Table 2. Main effects of dietary treatments on food intake (g), egg production (%) egg weight (g), egg mass (g), FCR (g/g), specific gravity, body weight (kg), and organ weights (g/100 g BW) of layer hens from 29 to 48 weeks of age.

Main effect	Food intake	Production	Egg wt.	Egg mass	FCR	Specific gravity	Body wt.	Liver	Pancreas
Soybean type									
Raw	116.0 ^a	90.8	62.1 ^a	56.7 ^a	2.06	1.089	1.99 ^a	2.34	0.255 ^a
Kti	117.2 ^a	92.0	63.1 ^b	58.0 ^b	2.03	1.091	2.06 ^b	2.26	0.215 ^b
FFSBM	124.8 ^b	94.0	63.6 ^b	59.8 ^b	2.09	1.089	2.13 ^b	2.27	0.188 ^b
LSD	2.68	NS	0.83	1.34	NS	NS	0.056	NS	0.033
Treatment									
Non-steamed	117.0 ^a	91.5	62.9	57.6	2.04	1.090	2.02	2.41	0.223 ^{ab}
Steamed	116.2 ^a	91.3	62.5	57.2	2.05	1.090	2.02	2.19	0.247 ^a
FFSBM	124.8 ^b	94.0	63.6	59.8	2.09	1.089	2.13	2.27	0.188 ^b
LSD	2.68	NS	NS	NS	NS	NS	NS	NS	0.035
Level (g/kg)									
70	115.1 ^a	90.0 ^a	62.5	56.5 ^a	2.05	1.090	2.03 ^a	ND	ND
110	118.1 ^b	92.8 ^b	62.7	58.2 ^b	2.04	1.090	2.01 ^a	2.28	0.235
FFSBM	124.8 ^c	94.0 ^b	63.6	59.8 ^b	2.09	1.089	2.13 ^b	2.27	0.188
LSD	2.66	2.05	NS	1.33	NS	NS	0.055	ND	ND

Means within the same column with different superscripts are significantly different ($P < 0.05$). NS= not significant. ND= not determined.

IV. ACKNOWLEDGEMENTS

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EFFECTS OF WHEAT AND XYLANASE ON GROWTH PERFORMANCE OF BROILER CHICKENS FROM 0-42 DAYS OF AGE

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Summary

The effects of dietary inclusion levels of wheat and supplementation with exogenous xylanase on the growth performance of broiler chickens from 0 to 42 days of age were determined. In a 3x4 factorial array, three broiler diets were formulated to contain wheat at 250, 450 and 650g/kg and were supplemented with a feed enzyme with predominantly xylanase activity at levels of 0, 25, 50 and 100g/tonne. A total of 2,520 chickens were allocated to the 12 dietary treatments. Weight gains, feed intakes and feed conversion efficiencies were determined at 21 and 42 days of age.

Over the entire feeding period, increasing dietary levels of wheat reduced both weight gain ($P<0.001$) and efficiency of feed conversion ($P<0.001$). Xylanase supplementation increased both weight gain ($P<0.001$) and feed efficiency ($P<0.01$) from day 0 to 42. Neither treatment had significant effects on feed intake, nor were there any significant interactions between the two treatments for any of the three performance parameters.

I. INTRODUCTION

Wheat is one of the major ingredients of poultry diets in many countries. It is well known that the nutritive value of wheat can be highly variable (Huyghebaert and Schöner, 1999), and this may be influenced by the inclusion level (Hughes and Zviedrans, 1999). The precise identity of the factors responsible for these antinutritive effects is still not absolutely clear but it is recognised that water soluble non-starch polysaccharides (NSP) are major contributing factors. Soluble NSP increase intestinal viscosity and depress nutrient digestibility and performance of the birds. Moreover, the insoluble NSP fraction, by acting as a physical barrier to digestive enzymes, may impede the activity of endogenous enzymes. Supplementation of wheat-based broiler diets with NSP-degrading enzymes has been shown to improve their nutritive value, either by enzymatic depolymerisation of soluble NSP decreasing intestinal viscosity, or by disruption of insoluble NSP and intact cell wall material, rendering trapped nutrients available for digestion.

The objective of the present study was to determine the effects of different dietary levels of wheat and xylanase supplementation on the performance of male broiler chickens over a 42 day feeding period.

II. MATERIALS AND METHODS

On an as-is basis, the wheat used in this study had an apparent metabolisable energy (AME) content of 12.72 MJ/kg with 123.1g/kg crude protein (CP). Three basal diets contained either 250, 450 or 650g/kg of wheat (designated as W250, W450 and W650 in table 1). With increasing levels of wheat the inclusion rates of corn were reduced from 380 to 190 and 0g/kg and of soybean meal (478g/kg CP) from 292 to 281 and 267g/kg, respectively. All diets contained animal fat (30g/kg), calcium carbonate (5) dicalcium phosphate (16) a vitamin and mineral premix (3.5), salt (2) methionine (2) and lysine (0.8). The three diets had calculated energy contents of 12.48, 12.40 and 12.26MJ/kg. The analysed crude protein and

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lysine contents were 209, 207, 204g/kg and 10.80, 10.75, 10.98g/kg, respectively. The three wheat diets were supplemented with 0, 25, 50 and 100g/tonne of a granulated feed enzyme with predominantly xylanase activity (Natugrain® Blend), which are designated as E0, E25, E50 and E100 in table 1. The diets were pelleted at 65-70°C.

A total of 2,520 male chickens were fed the 12 dietary treatments from 0-42 days of age with each treatment consisting of seven replications of 30 birds each. Day old birds (Ross) were allotted to floor pens where water and feed were available *ad libitum*. The temperature was maintained at 33°C for the first three days and then gradually reduced to 21°C with continuous lighting over the 42 days feeding period. Growth rates, feed intakes and feed conversion efficiencies were determined at days 21 and 42. Data were analysed using the general linear model (GLM) procedure (SAS Institute Inc., 1987) and Duncan's multiple F-test. The results are presented as means and standard error of means (SEM) calculated by standard procedures.

III. RESULTS AND DISCUSSION

The performance data for the periods from day 0 to 21, day 22 to 42 and for the entire period day 0 to 42 are presented as Table 1. Over the entire feeding period, increasing levels of wheat (from 250 to 650 g/kg) reduced average weight gain ($P<0.001$) by 2.6% from 1936 to 1886g per bird and increased feed conversion ratio (FCR) ($P<0.001$) by 3.8% from 1.86 to 1.93. From day 0 to 21, increasing levels of wheat increased ($P<0.01$) FCR by 3.9% from 1.52 to 1.58 but did not significantly affect growth rates. From day 22 to 42, increasing levels of wheat reduced weight gain by 3.3% ($P<0.01$) from 1311 to 1268g per bird and increased FCR by 4.5% ($P<0.001$) from 2.02 to 2.11, respectively.

During the initial period, xylanase supplementation significantly ($P<0.001$) increased weight gains at each of the wheat inclusion rates. The three different levels of enzyme had a positive effect on growth rates which were increased, on average, by 6.3% (250g/kg wheat), 9.1% (450) and 6.8% (650) when compared to the respective control groups. At each wheat level, xylanase increased ($P<0.001$) feed conversion efficiency in comparison to the control group. However, with the 650g/kg wheat diets only the highest level of xylanase resulted in a significant improvement of feed efficiency. The comparable responses in feed efficiency were 4.5, 5.1 and 3.5% respectively.

For the second period of the trial, from day 22 to 42, the effects of xylanase were less pronounced than in the initial period. In the diets containing 250g/kg wheat, the addition of the lowest and highest enzyme level resulted in significant ($P<0.05$) improvements in weight gain. In the diets containing 450g/kg wheat only the highest enzyme level significantly improved weight gain. In the diets containing 650g/kg wheat, enzyme addition did not improve weight gain in the second period. Similarly, the effects of xylanase on feed conversion efficiency were less in the second period of the trial. The highest enzyme level improved ($P<0.05$) feed conversion efficiency by 4.4% in the 250g/kg wheat diet but in the 450 and 650g/kg diets. There was essentially no effect of xylanase on FCR.

For the overall feeding period, xylanase increased ($P<0.001$) weight gain; the effect was significant at each wheat level and the highest enzyme level was the most effective. Here, weight gain was increased by 5.3% (250g/kg wheat), 7.3% (450) and 3.1% (650), in comparison to the respective control groups. Feed conversion efficiency was increased ($P<0.001$) by xylanase at all wheat levels and again the highest enzyme level was the most effective. Feed efficiency was enhanced by 4.2% (250g/kg wheat) 4.1% (450) and 2.6% (650) by 100g per tonne xylanase in comparison to the respective control groups.

Table 1. Performance of broiler chickens fed diets with increasing levels of wheat and xylanase supplementation¹.

Treatment diet	Feeding period (days)	Weight gain (g)	Feed intake (g)	FCR
W250/E0	0-21	596 ^{def}	932	1.57 ^{abcd}
W250/E25	0-21	632 ^{abc}	940	1.49 ^e
W250/E50	0-21	636 ^{abc}	961	1.51 ^{de}
W250/E100	0-21	638 ^{ab}	953	1.50 ^e
W450/E0	0-21	579 ^f	939	1.62 ^a
W450/E25	0-21	610 ^{cde}	935	1.54 ^{cde}
W450/E50	0-21	647 ^a	989	1.53 ^{cde}
W450/E100	0-21	655 ^a	1005	1.54 ^{cde}
W650/E0	0-21	586 ^{ef}	943	1.61 ^{ab}
W650/E25	0-21	619 ^{bcd}	974	1.58 ^{abc}
W650/E50	0-21	634 ^{abc}	982	1.55 ^{bcd}
W650/E100	0-21	631 ^{abc}	962	1.53 ^{cde}
W250/E0	21-42	1278 ^{bcd}	2625	2.06 ^{abcd}
W250/E25	21-42	1325 ^a	2669	2.02 ^{de}
W250/E50	21-42	1304 ^{abc}	2638	2.02 ^{cde}
W250/E100	21-42	1335 ^a	2628	1.97 ^e
W450/E0	21-42	1272 ^{bcd}	2672	2.10 ^{ab}
W450/E25	21-42	1315 ^{ab}	2704	2.06 ^{abcd}
W450/E50	21-42	1272 ^{bcd}	2663	2.10 ^{abc}
W450/E100	21-42	1330 ^a	2711	2.04 ^{bcde}
W650/E0	21-42	1267 ^{cd}	2683	2.12 ^a
W650/E25	21-42	1252 ^d	2627	2.10 ^{ab}
W650/E50	21-42	1274 ^{bcd}	2681	2.11 ^{ab}
W650/E100	21-42	1279 ^{bcd}	2685	2.10 ^{ab}
W250/E0	0-42	1874 ^{def}	3557	1.90 ^{cde}
W250/E25	0-42	1957 ^{ab}	3608	1.84 ^{fg}
W250/E50	0-42	1941 ^{abc}	3599	1.86 ^{efg}
W250/E100	0-42	1973 ^a	3581	1.82 ^g
W450/E0	0-42	1851 ^f	3612	1.95 ^{ab}
W450/E25	0-42	1925 ^{bc}	3639	1.89 ^{cde}
W450/E50	0-42	1918 ^{bcd}	3652	1.90 ^{cd}
W450/E100	0-42	1986 ^a	3716	1.87 ^{def}
W650/E0	0-42	1853 ^f	3626	1.96 ^a
W650/E25	0-42	1871 ^{ef}	3601	1.93 ^{abc}
W650/E50	0-42	1908 ^{cde}	3662	1.92 ^{abc}
W650/E100	0-42	1910 ^{bcd}	3648	1.91 ^{bcd}
SEM	0-21	6.5	16.1	0.02
	21-42	10.9	28.7	0.02
	0-42	11.5	39.1	0.01
<u>Probabilities</u>				
0-21days	Wheat	NS	NS	0.01
	Enzyme	0.001	NS	0.001
	Wheat x Enzyme	NS	NS	NS
21-42 days	Wheat	0.01	NS	0.001
	Enzyme	0.05	NS	0.02
	Wheat x Enzyme	NS	NS	NS
0-42 days	Wheat	0.001	NS	0.001
	Enzyme	0.001	NS	0.01
	Wheat x Enzyme	NS	NS	NS

¹ Means within each growing period without common superscripts are different (P<0.05).

There were no significant effects of wheat level or enzyme supplementation on feed intake over the trial period. Nor were there any significant interactions between the two treatments for the parameters assessed.

The fact that the weight gain and feed efficiency responses observed following xylanase supplementation at 100g per tonne were more pronounced during the initial phase of the feeding study, from day 0 to 21, was expected. However, it was surprising that, generally, the responses to xylanase with the diets containing 650g/kg wheat were less than those containing lower inclusion levels of wheat. Pentosans present in wheat have been reported to increase viscosity of intestinal contents and depress performance. Depending on the wheat variety and the extent of increased gut viscosity, the level of inclusion of an exogenous enzyme might be of importance. Conceivably, greater levels of xylanase supplementation than evaluated in this study may be warranted in diets with high levels of wheat.

The mode of action of xylanases in enhancing nutrient utilisation and performance from wheat-based diets is not perfectly understood. However, the enzymatic degradation of highly viscous non-starch polysaccharides is a key factor (Hughes and Choct, 1997). Viscous gut contents may deny digestive enzymes access to their substrates, reduce the rate of passage and influence intestinal microflora. The influence of xylanase supplementation on AME and the digestibility of fat and amino acids was determined in this study. The addition of 50 and 100g per tonne xylanase increased ($P < 0.001$) both the AME content of the diets and the digestibility of fat irrespective of the wheat content of the diets. Also, xylanase tended to improve the total tract digestibilities of amino acids. Improvements in energy utilisation and amino acid digestibility following xylanase supplementation of wheat-based broiler diets have been previously reported (Annison and Choct, 1991; Hew *et al.*, 1998).

IV. CONCLUSIONS

The results of this study demonstrate that increasing the dietary levels of wheat, at the expense of corn, in a broiler diet has negative influences on bird performance. However, supplementation with an exogenous xylanase preparation significantly improved performance and the highest inclusion rate of 100g per tonne resulted in the most pronounced improvements of weight gain and feed conversion efficiency.

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ENZYME SUPPLEMENTATION OF DIETS CONTAINING HIGH LEVELS OF LEGUMES – A REVIEW

A. KOCHER and M. CHOCT

Summary

Increased levels of indigestible carbohydrates, such as α -galactoside oligosaccharides and non-starch polysaccharides (NSPs), limit the inclusion rate of grain legumes in diets for pigs and poultry. There is contradictory information available on the anti-nutritive effects of legume NSPs and the effects of feed enzymes on legume NSPs. The addition of carbohydrases to diets containing high levels of soybean meal (SBM) as the main protein source has a significant effect on ileal protein digestibility and ileal NSP concentrations. However, the overall effect on broiler performance remains controversial. Similarly, the addition of carbohydrases to lupin-based diets has been shown to alter the intestinal NSP concentrations in comparison to unsupplemented diets. The overall effects on weight gain, feed intake and FCR, however, varied from study to study.

I. INTRODUCTION

Grain legumes are primarily grown for human consumption; however, soybean meal, whole seed or dehulled lupin, peas, faba bean or chickpeas are used as protein sources in diets for domestic livestock. The occurrence and amount of anti-nutritive factors (ANF) and their effect on protein and energy utilisation limits their inclusion in diets for pigs and poultry. Reduction of ANF through genetic selection or physical treatment such as dehulling or heat processing make grain legumes more suitable for inclusion in diets for pigs and poultry. Legumes included at a high level as the main protein source in broiler diets will result in poor growth and can cause excessive gas production from bacterial fermentation in the hindgut and osmotic diarrhoea. In cereal grains, such as wheat and barley, high levels of soluble NSPs raise digesta viscosity in the intestine of chickens leading to reduced starch, protein and lipid digestion (Choct and Annison, 1990). Although the mechanism by which viscosity affects digestion is not fully understood it is evident that the addition of feed enzymes can counteract these effects. This review will discuss the potential use of exogenous feed enzymes in broiler diets containing high levels of grain legumes. In view of the annual production and therefore economic importance of the various grain legumes, this paper is mainly limited to soybean meal and lupins with some reference to field peas and faba beans.

II. OLIGOSACCHARIDES AND NON-STARCH POLYSACCHARIDES IN LEGUMES

Legumes are rich in galactosyl-sucrose oligosaccharides (OS). These oligosaccharides are part of the raffinose family (raffinose, stachyose, verbacose and ajugose). The distribution and the quantity of OS vary widely among legume species as well as the variety within species. Due to a lack of endogenous α -galactosidase monogastric animals cannot digest α -galactosides. Bacterial degradation of α -galactosides in the hindgut can lead to increased fluid retention, increased hydrogen production, and can impair the utilisation of nutrients (Saini, 1989). However, the nutritive value of legume α -galactosides in broiler diets remains unclear. Coon *et al.* (1990) showed that OS from soybean using ethanol extraction increased nitrogen corrected true metabolisable energy (TME_N). On the other hand, Kocher *et al.* (1999a)

demonstrated that ethanol removal of OS from lupins (*L. angustifolius*) significantly decreased apparent metabolisable energy (AME). The addition of OS extracted from peas (Trevino *et al.*, 1990) or lupins (Brenes *et al.*, 1989) to an OS-free pea/lupin meal based diet had no effects on broiler performance or starch digestibility.

The NSP content of legumes used in poultry diets depends heavily on the degree of processing and subsequently on the hull proportion in the final product. The total NSP content in whole seed legumes ranges from 177g in faba beans to 350 g/kg DM in some lupin varieties (Table 1). The predominant monosaccharide residues of legume NSPs are glucose and xylose in the hull fraction and galactose, arabinose, glucose and uronic acids in the cotyledon. The main NSP structures in legume seeds are cellulose (30-43% of polysaccharide component), and pectic polysaccharides such as rhamnogalacturonans, galactans and arabans (Arora, 1983).

Table 1. Non-starch polysaccharides in grain legumes (g/kg DM).

Legume	OS	Soluble NSP	Insoluble NSP
Beans (<i>V. faba</i>) ¹	59	50	140
<i>L. albus</i> ²	79	14	319
<i>L. angustifolius</i> ²	114	31	336
Pea (<i>P. sativum</i>) ¹	58	52	129
Soybean meal ¹	60	63	154

¹Bach Knudsen (1997), ²Kocher *et al.* (1999b)

Although NSPs cannot be digested by the endogenous enzymes of chickens the water soluble fraction of the NSPs can be almost completely degraded through bacterial fermentation in the intestinal tract of birds (Carré *et al.*, 1990). The major site of bacterial fermentation is the caeca; however, it is known that increased levels of soluble NSPs will also increase fermentation in the upper gut which is detrimental to the overall performance as well as the bird's health (Choct *et al.*, 1996). Insoluble NSPs remain mostly intact through the intestine and will not be fermented, therefore it was suggested that they act as energy diluents and have little or no effect on overall nutrient utilisation.

III. EFFECTS OF EXOGENOUS ENZYME ADDITION ON THE NUTRITIONAL VALUE OF GRAIN LEGUMES

It is well documented that in diets containing high levels of wheat, triticale or rye, added xylanases will degrade soluble arabinoxylans and in diets containing high levels of barley or oats added β -glucanases will depolymerise the soluble β -glucans present in these grains (Annison and Choct, 1993). Recent studies with non-viscous grains such as corn and sorghum reported that the addition of commercial glycanases also improved the nutritive value of these grains (Creswell *et al.*, 1998). Enzymes used in diets containing high levels of grain legumes contain increased levels of polygalacturonase (pectinase) designed to hydrolyse pectin present in legumes. However, enzyme products used in the feed industry contain a range of activities, and therefore will target a range of substrates. When discussing the effects of enzymes on the nutritive value of diets containing high levels of grain legumes, it has to be considered what other ingredients are present in the diet. An overall improvement in performance and energy availability could be the result of improved digestibility of an added cereal grain rather than actual improvement in the digestion of the tested legume.

Addition of enzymes to a corn-SBM broiler diet resulted in a significant improvement in weight gain and FCR as a result of increased ileal digestibility of crude protein (CP), starch and fat (Zanella *et al.*, 1999). Similarly, Marsman *et al.* (1997) reported a significant improvement in ileal digestibility of CP and NSPs in broiler diets with corn-soybean meal. It was concluded from both studies that the added enzyme products not only had cell wall degrading activities but also exhibited protease activity, which explained the improved nutrient digestibility. The bacterial degradation of soluble NSPs from soybean is very high (80-90%) (Carré *et al.*, 1990). It is possible that added enzymes solubilised parts of the insoluble NSPs, which resulted in the improved NSP digestibility. In addition, the release of proteins bound to carbohydrates could explain the improved protein digestibility. On the other hand, Irish and Balnave (1993), found that the addition of two multi-activity enzyme preparations to corn/wheat-SBM diets resulted in a significantly poorer growth compared to an unsupplemented control diet. It was concluded that NSPs were broken down into fragments. The presence of large amounts of indigestible low molecular weight NSPs results in fluid retention in the small intestine and can adversely affect the absorption of nutrients (Wiggins, 1984).

Several authors reported significant improvement in broiler performance and weight gain when commercial enzyme products were added to diets containing 45% dehulled *L. albus* (Brenes *et al.*, 1993a; Roth Maier and Kirchgessner, 1994). A recent study by Kocher *et al.* (1999b) showed a significant improvement in ileal NSP digestibility of broiler chickens fed a diet containing 30% *L. albus* supplemented with an enzyme containing a high level of pectinase activity. The increased digestibility is directly related to increased digestibility of glucose, xylose and arabinose in the insoluble NSP portion. Although the sugar composition of various species of lupin are similar (Al-Kaisey and Wilkie, 1992), no such effects were observed when adding the same enzyme product to a different lupin species (*L. angustifolius*). AME, growth performance and digestibility of NSPs in the ileum and microbial fermentation in the ileum and caeca were not affected when adding the enzyme; however, a significant rise in digesta viscosity and increased concentration of soluble NSPs in all sections of the intestine were observed. Detailed analysis of monosaccharide composition in the ileum and jejunum showed increased concentration of soluble rhamnose and galactose, which indicated solubilisation of galactans and, possibly, rhamnogalacturonan also. The apparent lack of depressed nutrient absorption due to the increased viscosity may be explained by the fact that the addition of the enzyme degraded endosperm walls and subsequently released intracellular encapsulated nutrients, which helped to offset the reduced nutrient digestion.

Studies on the effects of enzyme addition to other grain legumes such as peas are very limited and give confusing results. Brenes *et al.* (1993b) reported that the addition of a pectinase to broiler diets containing 75% whole seed peas had no effect on broiler performance, although the authors reported a significant reduction in feed intake and weight gain. In contrast, Igbasan and Günter (1996) showed a significant improvement in weight gain and a significant increase in feed intake of broilers fed a diet containing 80% peas supplemented with a pectinase. However, the addition of enzymes did not improve the overall feed conversion ratio.

IV. CONCLUSION

It is evident that commercial enzyme products have some effect in diets containing high levels of grain legumes. However, enzymes do not always affect growth and performance of broilers and therefore the consequences of enzyme addition can only be seen after detailed analyses of feed and digesta. In order to develop enzyme products which will improve the nutritive value of grain legumes, further *in vivo* studies of structural changes to cell walls are necessary.

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NEW ENZYME TECHNOLOGIES FOR POULTRY BY-PRODUCTS

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Summary

A new approach to the treatment of poultry by-products, especially of feathers, is to incubate them with a specific enzyme preparation prior to processing. A protease-based enzyme preparation was developed and tested for its ability to break down feathers under large scale, commercial conditions. Use of this enzyme preparation has been shown to reduce the severity of the processing conditions that feathers are exposed to. The *in vivo* digestibility of the resulting feather meal (enzyme-hydrolyzed feather meal) is much higher than conventional feather meal (67.8 of 56.4%). As a result, the scope for incorporating it in animal diets is increased, while savings in the processing cost can be expected due to the reduction in processing temperatures.

I. INTRODUCTION

One of the challenges to the modern poultry industry is the disposal of processing waste. This waste material is proteinaceous in nature and has potential to be used as a feedstuff. However, feather is predominantly made from the structural protein, keratin, which is hard and extremely durable. Currently, the most economical method for breaking down keratin is to hydrolyze feathers under high temperatures and pressures for a set period of time. This produces a fine powder which is easy to manage but there is no doubt that much of the heat-labile components, especially amino acids, are denatured. The resulting feather meal is an extremely good source of protein and sulfur amino acids. However, its inclusion in animal feeds is limited because of the extremely poor availability of amino acids.

Enzymes offer a lot of potential in the breakdown of keratin. However, the physical features of feather poses a number of obstacles. Firstly, the substrate is not defined, thus a number of different enzyme preparations need to be used synergistically; secondly, keratin contains disulfide cross-linkages which are difficult to cleave and finally, feathers are covered in a waxy cuticle which increases their resistance to water. Therefore, a preparation containing a variety of enzyme activities is essential. This paper describes an enzyme preparation that has been developed for the breakdown of feathers, briefly describes how the enzyme is applied in practice and summarizes a number of results that have been obtained around the world.

II. MATERIALS AND METHODS

A new enzyme preparation (Allzyme FD™) was developed by Alltech Inc. to aid in the breakdown of the feather structure in commercial rendering plants. The enzyme preparation consists of a number of enzyme activities but the major activity is a protease of fungal origin, (E.C. 3.4.23.18) with an activity of 12,700 HUT/g. The major activity of this enzyme is the hydrolysis of proteins with a broad specificity (Webb, 1992). The product also contains lipase with an activity of 117 LU/g.

It has proven very difficult to conduct properly controlled tests using the enzyme in commercial rendering plants, due to the nature of the business and variability of materials and

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contaminants in the feather being tested. Table 1 shows how the enzyme was incorporated into an existing feather hydrolyzing process.

Table 1. Comparison of processing conditions used in each experiment.

Test Conditions	Conventional	Enzyme
Process Time	10 min to increase pressure then held for 50 min @ 140°C	45 min at 50°C followed by 15 min @ 125°C
Drying time to 40% moisture	120min	120min
Total time	180min	180min

In all cases, the enzyme was added to the raw feathers and mixed thoroughly while the cooker was filling. The temperature was then raised to 50°C for 45 minutes as this is the optimal temperature for the enzyme to work effectively. The incubation period was followed with a cooking step which completes the breakdown of the feather but the temperatures and pressures used were much lower than normally used. A sample of the new feather meal (referred to as enzyme-hydrolyzed feather meal (EHFM)) was taken. A sample of feather meal processed in the traditional manner was also taken and both samples analyzed. The most appropriate method for comparing EHFM with traditional feather meal is through *in vivo* digestibility studies. This work was carried out at the Roslin Institute in the UK (McNab and Blair, 1998).

III. RESULTS

A summary of the results obtained in approximately 20 trials conducted in commercial rendering plants all over the world is given in Table 2. The improvements observed in *in vivo* digestibility and amino acid content were of the order of 20%. The reason for this is likely due, in part at least, to the less harsh processing conditions.

Table 2. Comparison of conventionally rendered feather meal (n = 12) with enzyme-hydrolyzed feather meal (n = 20) from trials conducted worldwide. Standard errors in parenthesis.

	Conventional feather meal	Enzyme-hydrolyzed feather meal
Crude protein, (g/kg)	850	844
Average <i>in-vivo</i> protein digestibility (%)	56.4 (1.1)	67.8 (2.9)
Cystine content (g/kg)	35.4 (2.0)	49.5 (2.1)
AME (MJ/kg)	13.82 (0.26)	15.64 (0.20)

IV. DISCUSSION

Enzyme technology offers potential in the hydrolysis of feathers. The application of a fungal protease and lipase preparation to raw feathers yields significant benefits on feather meal quality. The digestibility of protein is increased by over 20% on average when the enzyme is used. This is a direct result of the lower temperatures and pressures used when the enzyme is applied and incubated with the feathers. While no negative controls were used in any of these trials, practical experience tells us that it is not possible to achieve the same level

of degradation of feather particles under the conditions used when the enzyme is applied. The enzyme may be facilitating the breakdown of the feather material which can then be completed by lower processing conditions. While the enzyme does not appear to have any direct effect on the di-sulfide bonds in keratin, it may have a role in breaking down the cuticle layer which is itself quite resistant to heating and this will allow the use of lower processing temperatures. The protease component of the enzyme probably initiates the hydrolysis of keratin to smaller peptides, aiding in the breakdown. It also appears to work extremely well under the harsh conditions observed in high-pressure cookers but as the optimal temperature for activity is 50°C, then it is not surprising that it performs well. Such a complex structure as feather requires a combination of enzyme activities to help break it down. Improvements in feather meal quality increase the nutritive value of the product, allowing more to be used in animal rations. Savings in process temperatures and conditions also should lead to reductions in processing costs. On a more practical note, the enzyme has been successfully incorporated into existing rendering plants without the need for any adjustment to the processing equipment.

V. CONCLUSION

The enzyme preparation, Allzyme FD, has been shown to be a very useful tool in the rendering of feathers. Improvements in the nutritive value of feather meal, coupled with savings in processing costs, demonstrate how enzyme technology can be successfully used by the poultry industry.

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GASTRO-INTESTINAL TRACT STRUCTURE AND ENERGY METABOLISM IN BROILERS

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Summary

Relationships between apparent metabolisable energy (AME) of the diet and gut structure were examined in two experiments designed to expose the extent of potential variation in AME by application of treatments involving cleanliness of the rearing environment, sex of chickens and addition of mannan oligosaccharide (MOS) to the diet. It was concluded that large between-bird variation in AME reported in previous studies could not be explained by differences in the weights of duodenum, jejunum and ileum relative to metabolic body weight, or by changes in villus height or crypt depth in these sections of the small intestine. The possibility that variation in AME is associated with functional differences in gut epithelial tissue is worthy of investigation.

I. INTRODUCTION

Hughes and Choct (1997) demonstrated extremes of AME values in a single experiment involving only one sample of wheat containing a high level of soluble arabinoxylan given to broilers hatched and reared under identical conditions. We concluded that the "low-ME" wheat phenomenon was not entirely dependent on the physico-chemical nature of wheat but it was a multi-faceted problem closely linked with the individuality of digestive physiology of broiler chickens. Why some birds in the same flock were not affected to anywhere near the same extent as others in terms of poor nutrient digestibility and excessive output of wet, sticky excreta warrants further investigation.

Recent unpublished studies at the Pig and Poultry Production Institute (PPPI) on probable causes of gastro-intestinal tract dysfunction in commercial flocks strongly point to a highly significant bird component to be the problem. These observations are consistent with the hypothesis that wide between-bird variation in digestive function persists in commercial breeds of broiler chickens despite heavy selection for economically important traits such as lean tissue growth and feed efficiency. To date, the importance of bird-related factors in resolution of nutritional problems in commercial practice has received little attention from researchers.

Other studies at PPPI indicate significant differences in digestive function between male and female chickens, which is contrary to commonly held views in commercial practice. In addition, we have circumstantial evidence that young chickens reared in the presence of older birds, and hence exposed to low levels of poultry pathogens, will exhibit wide variation in liveweight without showing any signs of clinical disease. Iji and Tivey (1998) concluded that chickens exposed to disease agents in this manner could benefit from addition of synthetic oligosaccharides to the diet, particularly mannan-oligosaccharides which are thought to act through their capacity to bind to pathogens and to stimulate the immune system.

This paper summarises the results of two experiments to examine the relationships between energy metabolism and gut structure of broiler chickens. An objective in both experiments was to uncover the extent of potential variation in AME by imposing treatments such as cleanliness of the rearing environment, sex of chicken, and addition of MOS to the diet.

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II. MATERIALS AND METHODS

For experiment 1, day-old sexed broiler chickens were raised on starter crumbles from hatch to 20 days of age in experimental rearing cages. One set of cages was housed in a "clean" environment and the other in a "dirty" environment. The "clean" rearing environment involved isolation of chickens in a rearing room cleaned and fumigated to industry standards. The "dirty" environment involved exposure of chickens to air-borne debris from an older flock of healthy chickens reared in floor pens in the same room. Chickens were transferred to 96 single-bird metabolism cages located in a controlled-temperature room kept at 22 - 25°C and given two experimental diets containing MOS added at 0 or 5 g/kg to coarsely milled finisher pellets which were then cold-pressed. At the end of the 7-day classical AME study, each bird was killed by intravenous injection of pentobarbitone. The gastro-intestinal tract (GIT) from the proventriculus down to the ileo-caecal junction was dissected. The empty proventriculus, gizzard, duodenum, pancreas, jejunum and ileum were rinsed with water, dried by blotting, then weighed. The GIT sections were dried at 40°C to constant weight. Dry matter (DM) contents of pelleted and milled feeds were measured. Gross energy values of dried excreta and milled feeds were measured with a Parr isoperibol bomb calorimeter to determine AME.

For experiment 2, day-old sexed broiler chickens were raised from hatch to 15 days of age in experimental rearing cages in "clean" and "dirty" environments. Birds were given commercial starter crumbles containing MOS at 0 or 5 g/kg. At 15 days of age, the chickens were transferred to 96 single-bird metabolism cages located in a controlled-temperature room kept at 25 - 27°C initially, and given two experimental diets based on wheat and casein, and containing MOS at 0 or 5 g/kg. AME values of the wheat and casein diets with and without MOS were measured over the following 4-day period. The purpose of this part of the study was to enable individual chickens to express natural variation in uptake of energy from a "novel" diet based on wheat and casein. Then chickens were given two commercial finisher diets containing MOS at 0 or 5 g/kg. AME values of these diets were measured over the following 7-day period. A total of 24 chickens was selected on the basis of AME values obtained on the wheat and casein diet. Chickens with lowest, highest or average AME value within each combination of rearing treatment, sex of chicken and dietary addition of MOS were killed by intravenous injection of pentobarbitone. Sections of duodenum, jejunum and ileum were fixed in buffered formalin, embedded in paraffin, sectioned (7 µm longitudinal to the plane of the villi) and stained with Haematoxylin and Eosin. Villus height and crypt depth were measured by image analysis (Leading Edge Pty Ltd, Adelaide, South Australia). A minimum of 15 villi and crypts were measured in each type of tissue from each chicken.

Blood sera samples were collected from chickens seven weeks of age in both experiments. ELISA tested the presence of antibodies against Marek's Disease Virus (MDV), Infectious Bursal Disease (IBDV) and Chick Anaemia Virus (CAV).

III. RESULTS AND DISCUSSION

In experiment 1, AME of diet (13.7 MJ/kg DM) was unaffected ($P > 0.05$) by cleanliness of the rearing environment, sex of chicken, or addition of MOS to the diet of chickens 22-29 days of age. Live-weights of chickens from the clean environment were greater at the start (893 vs 852 g/bird) and end (1301 vs 1271 g/bird) of the 7-day metabolism study in comparison with those from the dirty environment. However, the adjusted rate of gain in live-weight during the study was greater for chickens from the dirty environment (495 vs 464 g gain/kg live-weight). There was a significant interaction between rearing environment and diet which resulted in chickens from the clean environment given MOS being heavier than controls at the end of the 7-day metabolism study (1320 vs 1283 g/bird). In

contrast, there was no difference due to MOS in chickens from the dirty environment (1271 g/bird).

After dried weights of gut sections were adjusted for metabolic body weight ($\text{weight}^{0.75}$), the proventriculus tended to be greater ($P=0.09$) for chickens reared in the clean environment (1.14 vs 1.06 g) compared with dirty. In addition, the pancreas was significantly heavier ($P<0.05$) for females compared with males (0.99 vs 0.90 g), indicating that cleanliness during rearing and sex of chicken can affect gross structure of the gut which in turn could influence growth performance through digestive function. Step-wise regression analysis was used to develop a prediction equation for AME of the diet and dry matter digestibility (DMD) from adjusted weights of gut sections. AME and DMD coefficients ranged from 12.3 to 14.4 MJ/kg DM and 0.59 to 0.68, respectively. Corresponding means and standard deviations for 96 chickens were 13.7 ± 0.3 MJ/kg DM and 0.645 ± 0.014 . In both cases, the weight of the gizzard was the only significant variable ($P<0.05$) in the 6-variable equation. Only 17% of the variation in AME and 24% of the variation in DMD was associated with adjusted gut weights. These results are comparable with unpublished work done in 1997 by R.J. Hughes, A. Kocher, R.B. Cumming and M. Choct who found similarly poor statistical associations between AME and weights of freshly dissected gut sections. These two studies indicate that, at most, variation in gross structure could play a part in the overall functionality of the gut but cannot explain the massive between-bird variation in AME of the nature observed by Hughes and Choct (1997).

In experiment 2, effects of the relative cleanliness of the rearing environment on live-weight were similar to those observed in experiment 1. Live-weights of chickens from the clean environment were greater at the start (626 vs 594 g/bird) and end (833 vs 798 g/bird) of the 4-day metabolism study in comparison with those from the dirty environment, a finding frequently noted under practical conditions. However, in contrast to results from the previous experiment, the adjusted rate of gain (338 g gain/kg live-weight) was not significantly affected ($P>0.05$) by rearing treatment.

AME and DMD coefficient of the wheat and casein diet were significantly affected ($P<0.05$) by an interaction between cleanliness of the rearing environment and sex of chickens. Males had lower AME than females (15.15 vs 15.32 MJ/kg DM) when reared in the dirty environment but there was no difference between males and females (mean value 15.29 MJ/kg DM) from the clean environment. Similarly, DMD was 0.758 and 0.766, respectively, for males and females from the dirty environment, and 0.763 for chickens from the clean environment. In the following 7-day period when chickens were given commercial finisher diet, AME (13.5 MJ/kg DM) and DMD coefficient (0.626) were unaffected ($P>0.05$) by rearing treatment, sex of chickens, and addition of MOS to the diet.

Effects of cleanliness of the rearing environment on villus height and crypt depth in duodenal, jejunal and ileal sections are shown in Figure 1. Villus height in duodenal mucosa was significantly ($P<0.05$) reduced in chickens reared in the dirty environment (1440 vs 1299 μm) compared with chickens from the clean environment. Other morphological features were unaffected ($P>0.05$) by rearing conditions, dietary addition of MOS, or sex of chickens.

Step-wise regression analysis was used to examine relationships between gut morphology and AME of the diet and dry matter digestibility. AME and DMD coefficients ranged from 12.7 to 14.3 MJ/kg RDM and 0.59 to 0.66, respectively. Corresponding means and standard deviations for 24 chickens were 13.46 ± 0.36 MJ/kg DM and 0.621 ± 0.018 . Only 16% of the variation in AME and 23% of the variation in DMD was associated with villus height and crypt depth in gut sections. There was a significant ($P<0.05$) but weak association ($R^2=0.17$) between DMD and crypt depth in ileal tissue, however AME was not related to any single measurement or a combination of morphological features of the small intestine.

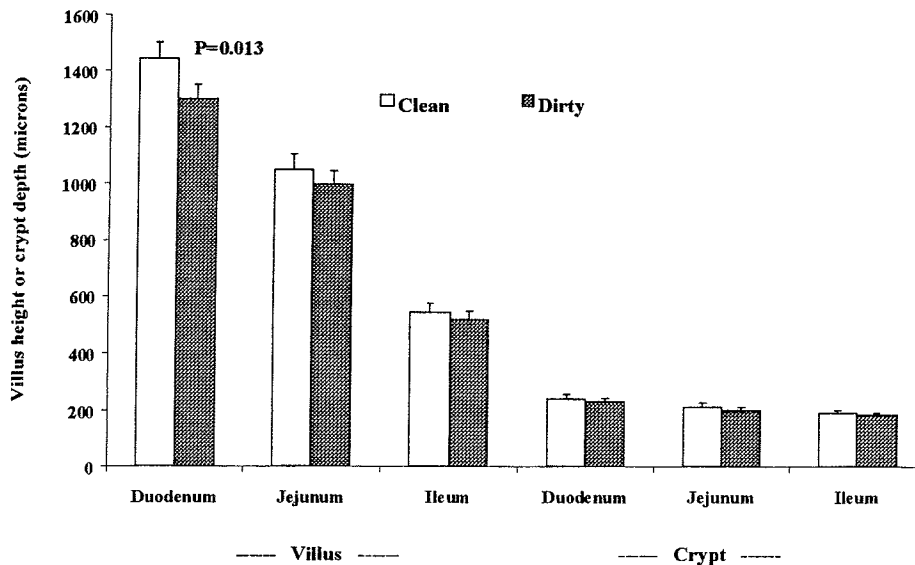


Figure 1. Effects of cleanliness of the rearing environment on villus height (μm) and crypt depth (μm) in duodenum, jejunum and ileum (means \pm SE; $n=12$ chickens).

In both experiments, ELISA tests indicated an absence of challenge from immunosuppressive agents such as MDV and IBDV. Some chickens (3 from 24 in experiment 1 and 8 from 48 in experiment 2) tested positive to CAV, which implies that any challenge is likely to have occurred after each metabolism study. Hence, it seems that effects attributed to rearing treatments would have involved an immunologic stress arising from accumulation of inhaled or ingested non-pathogenic microbes, dust and dander (Klasing *et al.*, 1999).

IV. CONCLUSIONS

Less than 20% of the variation in AME observed in two experiments was associated with either gross structural characteristics such as the weights of duodenum, jejunum or ileum relative to metabolic body weight, or with finer morphological measurement of villus height and crypt depth in these sections of the small intestine. It is possible that between-bird variation in AME could be associated with differences in gut epithelial function not detectable by the histological methods used in this study.

V. ACKNOWLEDGMENTS

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¹³CO₂ BREATH TESTS FOR EVALUATING GASTRO-INTESTINAL FUNCTION IN BROILER CHICKENS

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Summary

Preliminary experiments using simple PVC helmets to collect breath from individual birds provided proof of the concept of using ¹³CO₂ breath tests as non-invasive tools for studying gut physiology in broiler chickens. Individual chickens 3-weeks of age were given a gelatine capsule containing 3.6-3.8 mg ¹³C-octanoic acid dissolved in vegetable oil to examine gastric emptying of liquid, or given 10 g homogenised corn kernel (naturally enriched in ¹³C-starch) via a tube inserted in the oesophagus to examine solid phase gastric emptying and starch digestion. The patterns of recovery of the stable isotope in the form of ¹³CO₂ in breath samples were similar to those seen in humans and other animals, with the possible exception of a more rapid rate of release in chickens. Further studies are required to validate these ¹³CO₂ breath tests for routine use in poultry nutrition experiments, for diagnosis of intestinal disorders in commercial flocks, and for the study of candidate genes and associated traits in quantitative genetics research.

I. INTRODUCTION

Analysis of expired breath is a non-invasive method for diagnosing gastro-intestinal function in humans. Breath tests involving stable isotopes are useful alternatives to radio-scintigraphy, particularly for infants and pregnant women, and when multiple or frequent tests are required (Amarri and Weaver, 1995; Swart and van den Berg, 1998). The test involves ingestion of a ¹³C-enriched substrate of relevance to the particular rate-limiting intestinal process under investigation, for example, ¹³C-triglyceride in the case of pancreatic lipase function (Vantrappen *et al.*, 1989), and lactose ¹³C-ureide for small intestinal transit time (Heine *et al.*, 1995), followed by serial breath sampling. Isotope is released as ¹³CO₂ by a series of metabolic processes following digestion and absorption of labelled feedstuffs, then transported via the blood stream to the lungs for excretion. The breath samples are then analysed with an isotope-ratio mass spectrometer. The ratio of ¹³C and ¹²C isotopes in the breath is directly related to functionality of the gut in terms of release of digestive enzymes, epithelial function or digesta transit time, all of which are measured individually by this technology (Amarri and Weaver, 1995; Swart and van den Berg, 1998).

Tivey and Butler (1999) recently concluded that adaptation of breath tests for use in agricultural species would provide powerful analytical tools for nutrition research and for veterinary diagnostics. In our view, the breath tests likely to be of most benefit for broiler nutrition studies include those which examine activity of digestive enzymes such as lipase and amylase, digesta transit time, and microbial growth in the small intestine in order to address problems such as variation in energy metabolism in broilers (Hughes *et al.*, 2000).

This paper describes experiments (1) to develop a technique for sampling breath from birds, and (2) to determine whether two particular breath tests developed for humans are applicable for broiler chickens. The first test utilised ¹³C-octanoic acid given as a liquid meal to examine gastric emptying of the liquid phase of ingested food. The second test utilised

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corn, naturally enriched in ^{13}C through the metabolic pathway described by Hatch and Slack (1966), to examine solid phase gastric emptying and starch hydrolysis in the small intestine.

II. MATERIALS AND METHODS

Helmets of different dimensions (40 or 50 mm internal diameter and 95 or 100 mm length, respectively) were constructed from capped PVC pipe to suit chickens of different ages and hence size. 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 CO_2 . A sample of breath was drawn through the cap via Luer lock fittings into a 10 mL evacuated tube (Exetainer). Breath samples were analysed by isotope ratio mass spectrometry (ABCA, Europa Scientific, UK).

Following overnight fast, the baseline $^{13}\text{C}/^{12}\text{C}$ isotope ratio for each of four chickens was determined in three breath samples taken at 3-5 minute intervals. Each chicken was given a gelatine capsule containing a weighed amount (approximately 95 mg) of vegetable oil containing ^{13}C -octanoic acid (37.8 $\mu\text{g}/\text{mg}$ vegetable oil) then allowed brief access to feed to ensure that the capsule was not regurgitated. Chickens ate steadily during this period (5-10 minutes) and consumed 4-10 g feed. Thereafter, water, but not feed, was available during the sampling period. Breath samples were taken at frequent intervals initially (at 5 or 10 minutes for 30 minutes) then at increasingly longer intervals (15, 30 and 60 minutes) for up to five hours post-feeding of labelled substrate. Each breath sample was taken 30-45 seconds after the helmet was placed over the head of the chicken. After the sample was taken the helmet was removed and the chicken was returned to the metabolism cage. Care was taken during handling and breath sampling to avoid stress on the chickens in order to minimise any variability associated with physical activity or abnormal breathing patterns as indicated by visual observation of frequency and depth of breathing.

Similar procedures to those described above were used for each of three chickens given 10 g cooked corn kernel (Edgell brand) administered by plastic tube inserted 4 cm into the oesophagus. This proved to be a quick and easy method of application which appeared to be well tolerated by the chickens. Serial breath samples were taken at 15 minute intervals for two hours then at 30 minutes for a further two hours.

III. RESULTS AND DISCUSSION

Helmet size and re-breathing period are based on the need to collect breath containing a concentration of CO_2 greater than 1% but not exceeding 5%. Achievement of these limits was important, firstly, to provide 10 mL of sample containing at least 1% CO_2 for precise and accurate analysis of the isotope ratio by mass spectroscopy. Secondly, it was deemed necessary to avoid disruption to normal respiration by the chicken as a result of excess CO_2 or depleted O_2 in re-breathed air in the helmet. Each expired breath was estimated to be approximately 25 mL and contain 5% CO_2 based on data presented by Freeman (1984). Since leakage of CO_2 by diffusion to atmosphere is inevitable using this type of collection method, a period of re-breathing enabled the build up of total CO_2 in the air space in the helmet from which a 10 mL sample could be taken for analysis. It was assumed that both isotopic forms of CO_2 would diffuse at a similar rate under these circumstances, hence any leakage should not have affected the ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ in the sample. This assumption needs to be verified during further development of breath tests for poultry.

Enrichment of ^{13}C in breath CO_2 (defined as the increase in the ratio of ^{13}C to ^{12}C relative to the baseline determined for each chicken) following ingestion of ^{13}C -octanoic acid is shown in Figure 1. The smooth transition from zero enrichment to a peak within 30 minutes then return to levels near baseline after 4-5 hours indicate that there were no artefacts associated with this method of breath collection. The results are consistent with observations in humans and experimental mammals in which ^{13}C -octanoic acid is rapidly absorbed in the

intestine, metabolised, then excreted by the lungs (Tivey and Butler, 1999). Peak enrichment in chickens between 5 and 30 minutes is comparable with mice (Symonds *et al.*, 1998) and considerably less than the 53 minute delay observed in adult humans given a semi-solid test meal containing sodium [$^{13}\text{C}_1$]-acetate to measure emptying of the liquid phase (Braden *et al.*, 1995). The flattened, delayed peaks for two chickens (Figure 1) might indicate true variation between birds in terms of gastric emptying time. Alternatively, but less likely, it could be due to association of some of the ^{13}C -label with the solid phase of the small amount of feed consumed followed by a delay in release further down the gut.

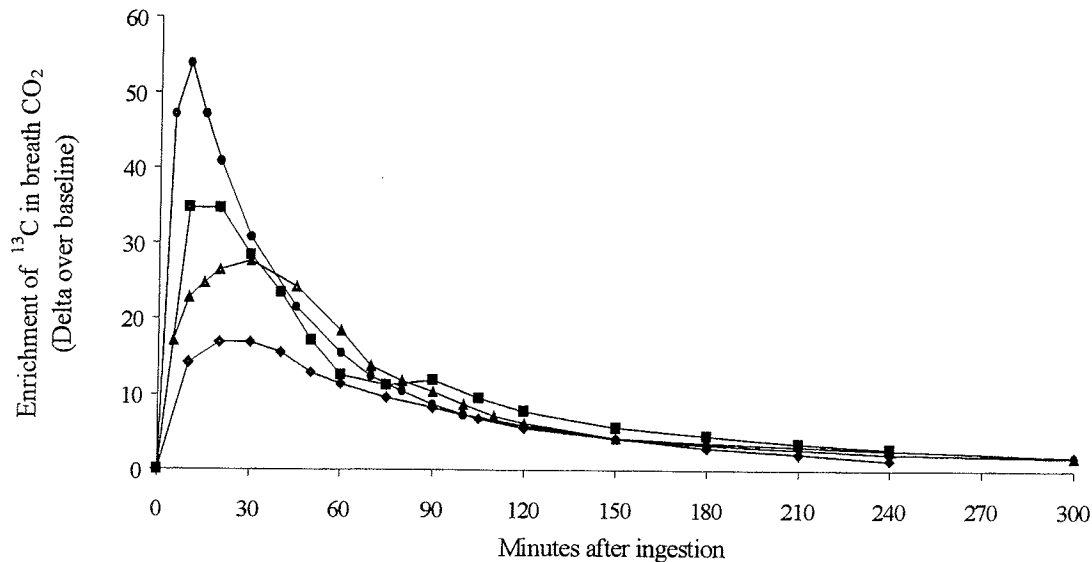


Figure 1. Enrichment of $^{13}\text{CO}_2$ in breath following ingestion of a gelatine capsule containing 3.6-3.8 mg ^{13}C -octanoic acid dissolved in vegetable oil. Each curve represents results from an individual chicken.

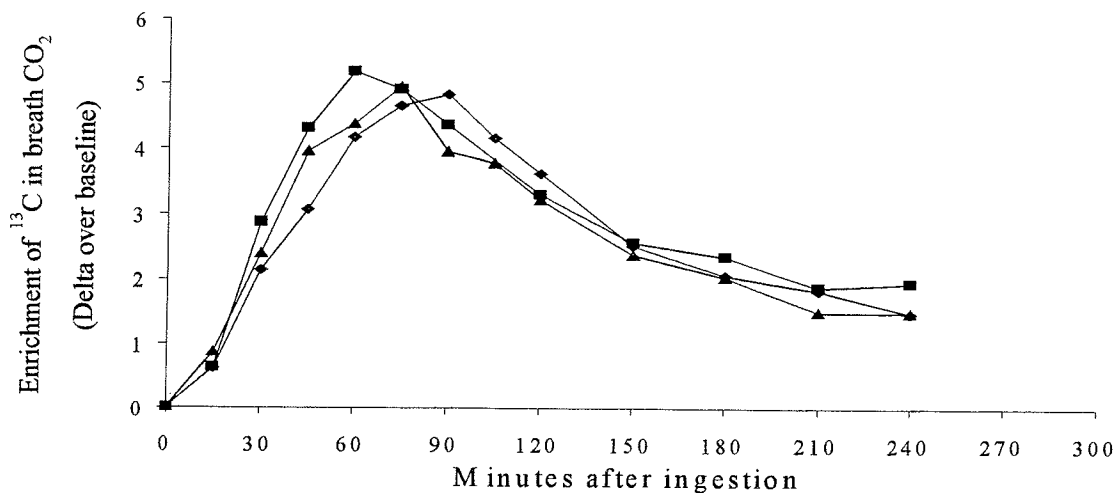


Figure 2. Enrichment of $^{13}\text{CO}_2$ in breath following ingestion of cooked corn kernel naturally enriched with ^{13}C -starch. Each curve represents results from an individual chicken.

The enrichment of ^{13}C in breath following ingestion of ^{13}C -starch in corn is shown in Figure 2. Peaks were observed 60-90 minutes post-ingestion. This is much quicker than the average time of 153 minutes observed in human infants 7-16 months of age with mean weight

of 8.6 kg given a test meal made from maize flour (Weaver *et al.*, 1995). Hiele *et al.* (1989) reported peaks in $^{13}\text{CO}_2$ excretion at three and five hours in healthy volunteers and patients with pancreatic disease, respectively, following consumption of a test meal made from corn starch suspended in water. As with the results in Figure 1, the main difference between chickens and humans is the shorter time to peak enrichment of ^{13}C in breath CO_2 from chickens.

There appear to be no fundamental differences between avian and mammalian species in terms of basic physiology and biochemistry under-pinning these $^{13}\text{CO}_2$ breath tests for gastric emptying and pancreatic function. Hence, it should be possible to develop these non-invasive tests for application in poultry research and commercial production.

IV. CONCLUSIONS

These preliminary experiments indicate that existing breath tests used routinely for clinical diagnosis of gastro-intestinal function in humans can be adapted for studying digestive physiology in chickens. Further work is required to refine and validate $^{13}\text{CO}_2$ breath tests before they can be applied in poultry nutrition experiments and for quantitative genetic research, or used for diagnosis of intestinal disorders in commercial flocks.

V. ACKNOWLEDGMENTS

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EFFECT OF ASTAXANTHIN-RICH ALGAL MEAL (*H. PLUVIALIS*) ON GROWTH PERFORMANCE, CAECAL *CAMPYLOBACTER* AND *CLOSTRIDIUM* COUNTS AND TISSUE ASTAXANTHIN CONCENTRATION OF BROILER CHICKENS

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Summary

Supplementation of the feed of female broiler chickens with natural astaxanthin (in the form of algal meal) slightly reduced feed conversion ratio but had no effect on live weight gain. At higher levels of inclusion, the algal meal reduced the caecal *Clostridium* count, whereas *Campylobacter* counts were unaffected by inclusion level. By increasing the inclusion level of algal meal, tissue astaxanthin concentrations were increased. Adding the algal meal as an oil suspension sprayed on to the feed pellets appeared to give higher tissue astaxanthin concentrations indicating better absorption of the carotenoid than when added as a powder. Tissue astaxanthin concentrations were higher in birds inoculated with *Campylobacter* than in uninoculated birds.

I. INTRODUCTION

Earlier experiments with broiler chickens have shown that algal meal with a high concentration of astaxanthin improved growth performance and increased tissue astaxanthin concentration (Inborr and Lignell, 1997). In experiments with *Helicobacter pylori*-infected mice, treatment with algal meal significantly reduced the bacterial count in the stomach (Wang *et al.*, 1998). *Campylobacter* spp are one of the most common causes of human diarrhoeal illness, and poultry is considered a major source of such infections (Blaser, 1983).

Necrotic enteritis (NE) is an enterotoxaemic disease in poultry, caused by *Clostridium perfringens*. The number of these bacteria in the chicken intestine is negatively correlated to weight gain (Stutz and Lawton, 1984), and numbers are dramatically elevated in birds suffering from NE (Kaldhusdahl and Hofshagen, 1992).

The aim of the experiment was to study the effects of dietary natural astaxanthin (*Haematococcus pluvialis*) on broiler performance during an experimental infection with *Campylobacter jejuni*. Broiler performance (growth, feed intake and feed conversion) and the colonisation of *Campylobacter jejuni* and *Clostridium perfringens* in the chicken caeca was recorded. In addition, tissue astaxanthin concentrations were measured.

II. METHODS

The experiment included 960 female broiler chickens, divided into 48 pens (0.75 x 1.5 m) with initially 20 chickens per pen. The birds had free access to water and the pelleted experimental diets from day-old to slaughter at 35 days of age. Wood shavings were used as bedding.

The experimental diets were supplied with either 0, 350, 1800 and 8950 mg algal meal (NOVASTA®) /kg feed, providing 0, 7, 36, or 179 mg astaxanthin/kg feed, respectively (giving a daily intake of approximately 0, 1, 5 or 25 mg astaxanthin/day at 5 weeks of age). In addition, two different inclusion methods of the astaxanthin were studied, including

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astaxanthin mixed into the pelleted feed or the same amount mixed with oil and sprayed on top of the pellets.

At 10 days of age the birds in half of the groups were inoculated with 1.6×10^8 *Campylobacter jejuni* per group via the drinking water. Control and challenged birds were separated and measures were taken to avoid cross infection. Thus, the experiment included 16 treatments (2 challenge/control x 4 inclusion levels of astaxanthin x 2 algae meal inclusion methods) with three replicates each.

Chicken live weight and feed intake were recorded regularly. Prior to inoculation and once a week after challenge (in total on four occasions) one bird per group was killed and caecal samples were taken for quantitative analyses of *Clostridium perfringens* and *Campylobacter jejuni*. In uninoculated groups random samples were taken for qualitative analysis of *Campylobacter jejuni*. At slaughter, samples of liver, kidney, breast muscle, abdominal subcutaneous fat, and intestines (mid-ileum) of three birds per treatment were collected and analysed for total carotenoid and astaxanthin concentration.

III. RESULTS

Feed conversion of birds given the highest dose of algal meal was significantly lower than that of birds of the control group and those given the lowest dose of astaxanthin. There were no differences in bird performance due to the method of algal meal mixing into the feed. However, up to 24 days of age birds inoculated with *C. jejuni* had lower live weights and feed intake than uninoculated birds. Feed conversion at 17 days was significantly higher ($P < 0.001$) in inoculated birds. At 32 days of age there were no significant differences in performance between the treatment groups.

At 17 and 32 days of age, caecal *Clostridium* counts of birds on the two highest astaxanthin doses (5 and 25 mg/day at 35 days of age) were significantly ($P < 0.05$) lower than that of birds fed 1 mg astaxanthin/day (Table 1).

At 32 days of age, *Clostridium* counts of birds fed 5 mg astaxanthin/day was significantly ($P < 0.05$) lower than that of birds fed 0 and 1 mg astaxanthin/day. There were no difference in *Campylobacter* counts due to astaxanthin inclusion levels. However, spraying the algal meal mixed with oil significantly ($P < 0.03$) reduced the caecal *Campylobacter* counts at 32 days (data not shown).

As shown in Figure 1, kidney tissue astaxanthin concentrations increased significantly with increasing levels of algal meal inclusion rates. Spraying the algal meal mixed with oil on to the pellets resulted in higher tissue astaxanthin concentrations than obtained through incorporation of the powdered algal meal into the diet. Tissue astaxanthin concentrations in the kidney (Figure 1), intestine and breast muscle were significantly ($P < 0.05$) higher in inoculated birds.

Table 1. Effect of astaxanthin inclusion level (intake g/day), method of algal meal inclusion and *Campylobacter* inoculation on caecal *Clostridium* counts (log/g digesta).

Age, days	Level of astaxanthin ¹				Method of inclusion ²		Campylobac inoculation ³		P-values			
	0	1	5	25	Meal	Oil mix	Yes	No	Level of asta.	Meal vs. oil mix	Ino-cul.	CV, %
10	1.70	2.01	2.17	1.30	2.06	1.53	1.74	1.85	0.59	0.27	0.82	92
17 ⁴	1.38 ^{ab}	2.70 ^a	0.70 ^b	1.08 ^b	1.80	1.13	1.79	1.14	0.03	0.18	0.19	115
24	4.02	4.35	3.44	3.41	3.80	3.84	3.42	4.23	0.67	0.99	0.28	60
32 ⁴	5.45 ^{ab}	6.48 ^a	3.59 ^c	4.96 ^{bc}	5.17	5.06	5.22	5.01	0.01	0.84	0.68	35

¹ pooled values for all methods of incorporation and inoculated and non-inoculated values;

² pooled values for inclusion levels and inoculated and non-inoculated;

³ pooled values for inclusion levels and methods of incorporation;

⁴ means in the same row with different superscripts are significantly ($P < 0.05$) different.

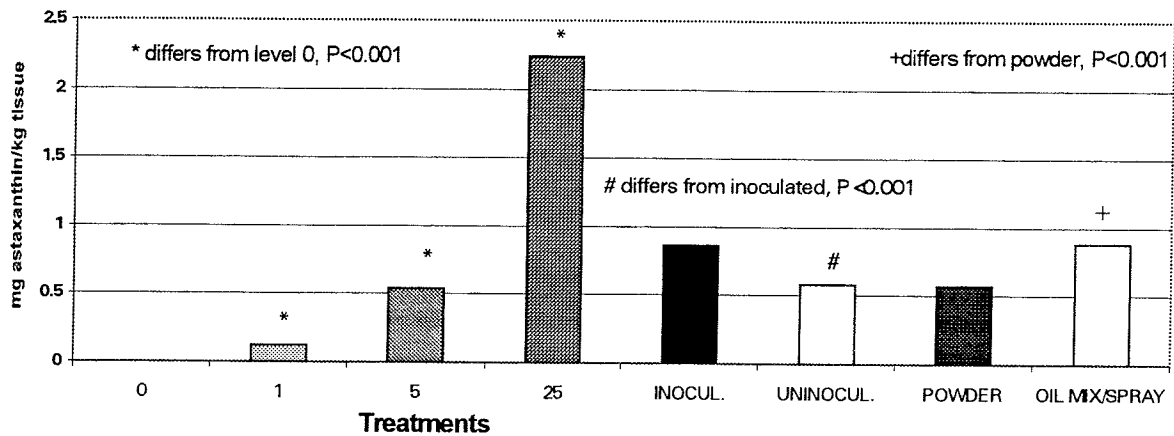


Figure 1. Astaxanthin concentration of kidney tissue for the different treatments.

IV. DISCUSSION

Adding algal meal to the feed did not influence broiler growth performance. Feed conversion ratio was slightly improved at higher inclusion rates. In contrast, inoculation with *Campylobacter* at 10 days of age reduced live weight gain up to 14 days after inoculation and increased FCR at day 17 but not thereafter. *Clostridium* counts appeared to be lower at higher inclusion rates of the algal meal, whereas *Campylobacter* counts were unaffected. These results are difficult to explain, and further studies are required to obtain an understanding of the mechanisms involved. As expected, tissue concentrations increased with increasing levels of inclusion of the algal meal. In general, applying the algal meal as an oil suspension by spraying it on to the pellets increased tissue astaxanthin concentrations. Since astaxanthin is a lipophilic compound, the absorption of astaxanthin in the intestine is likely to be improved by mixing it with oils and fats. Surprisingly, tissue astaxanthin concentrations appeared to be higher in inoculated birds.

V. CONCLUSIONS

The results indicate a possible *Clostridium* reducing effect of the algal meal, whereas *Campylobacter* counts were unaffected. Tissue astaxanthin and carotenoid concentrations increased with increasing levels of algal meal inclusion. Increased tissue astaxanthin levels were obtained in response to inoculation with *Campylobacter jejuni* and higher levels were obtained with the algal meal mixed with oil and sprayed on to the feed than added as a powder.

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DIET-INDUCED CHANGES IN INTESTINAL VISCOSITY AND RESPONSE TO LOW DOSE *EIMERIA MAXIMA* INFECTION IN BROILER CHICKENS

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Summary

This study investigated whether diet-induced changes in intestinal viscosity could influence the response of broiler chickens to *Eimeria maxima* challenge at a single low dose (50 oocysts/bird). Immediately before the challenge, birds fed a low-ME wheat diet showed a higher level of intestinal viscosity than those fed either a control maize diet or a xylanase-supplemented low-ME wheat diet. The birds fed the low-ME wheat diet also had a significantly lower total faecal oocyst output than those fed the control maize and xylanase-supplemented low-ME wheat diets that elicited relatively lower intestinal viscosity. The results suggest that intestinal viscosity may influence the host resistance to low-dose *Eimeria* infection in broilers. The possible impact of this on the efficacy of low-dose live vaccines should not be overlooked.

I. INTRODUCTION

The interaction between specific dietary nutrients and the pathogenicity of coccidiosis has been widely studied in chickens, but little is known about the role of gut environment in the development of coccidiosis. Although reduced intestinal viscosity has been shown to improve nutrient utilisation and growth performance in both healthy broilers and broilers affected by coccidiosis (Bedford and Classen, 1992; Morgan and Bedford, 1995), its direct impact on the pathogenicity of coccidiosis is unclear. With growing interest in using live *Eimeria* oocysts for "low-dose" vaccination (Joyner and Norton, 1976; Pierson *et al.*, 1997; Williams, 1998; Danforth, 1998), it is important to determine whether intestinal viscosity affects the response of birds to a low-dose *Eimeria* infection. In this study, we tested the hypothesis that diet-induced change in intestinal viscosity can affect the host response to a low-dose infection with *E. maxima* in broilers.

II. MATERIALS AND METHODS

(a) Chickens and dietary treatments

A total of ninety Cobb broilers (Baiada Poultry Pty Ltd, Australia), 25 days of age, were divided into three treatment groups (I, II, III). Each treatment group had six replicates of five birds. Treatment I received a control maize-based diet during the whole experiment. Treatment II and III received a low-metabolisable energy (ME) wheat-based diet without and with a xylanase (Biofeed Wheat) at 250g per kg of diet respectively during the pre-infection and pre-patent period of coccidiosis followed by the control maize-based diet for the patent period.

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(b) Coccidial infection and oocyst output

E. maxima oocysts used in this study were kindly supplied by Dr W.K. Jorgensen, Animal Research Institute, DPI, Queensland. Birds, at day 29 of age (four days after being on experimental diets), were each dosed orally with 50 oocysts in 1ml saline. Daily total faecal outputs were collected between day 6 and 12 post-infection and total oocyst output determined.

(c) Collection of intestinal digesta for viscosity test

At day 29 day of age (immediately before *Eimeria* infection), 5 birds per diet group were euthanased by intraperitoneal injections of pentobarbitone sodium and the contents of duodenum, jejunum and ileum collected and centrifuged at 12,000g for 15 min. The supernatant was stored at -20 °C until viscosity was measured using a Brookfield viscometer at 25°C with a CP40 cone and shear rate of 5-500 s⁻¹.

(d) Growth performance measurement

Bird weights and feed intake were recorded during the pre-infection, pre-patent and patent periods. Liveweight gain and feed conversion rate (FCR) were calculated.

(e) Statistical analysis

The data were subjected to two-way and one-way analysis of variance and multiple comparisons using Student-Newman-Keuls method. All values are expressed as mean ± SEM.

III. RESULTS

Regardless of diet treatments the pre-patent and patent periods had higher levels of feed intake and FCR than the pre-infection period ($P<0.05$). For Treatment II, the pre-patent period had a significantly higher daily weight gain than the pre-infection period ($P<0.05$). During the pre-infection period, Treatment III had a significantly higher ($P<0.05$) daily liveweight gain than Treatment II, but the latter had a significantly higher ($P<0.05$) FCR than both Treatments I and III (Table 1).

Figure 1 shows that birds on Treatment II (low-ME wheat diet) had higher jejunal and ileal viscosity than those on Treatments I (control maize) and III (low-ME wheat diet with xylanase) ($P<0.05$). Duodenal viscosity was found to be higher in the birds on Treatment II than those on Treatment I ($P<0.05$). Birds on Treatment III also tended towards ($P=0.054$) a lower level of duodenal viscosity than those on Treatment II. In response to *Eimeria* infection, birds on Treatment II had a significantly lower ($P<0.05$) total faecal oocyst output than those on Treatment I (Figure 2). Birds on Treatment II also tended towards ($P=0.054$) a lower level of faecal oocyst output than those on Treatment III.

Table 1. Effects of diets on liveweight gain, feed intake and FCR of broilers.

Period ¹ (Age)	Treatment (Diet used)	Liveweight gain (g/bird/day)	Feed intake (g/bird/day)	FCR (g/g)
Pre-infection (Day 25-29)	I (Maize control)	56.2 ± 1.4 ^{ab}	108.8 ± 1.1	1.94 ± 0.06 ^a
	II (Low-ME wheat)	47.1 ± 5.0 ^a	105.1 ± 9.4	2.28 ± 0.11 ^b
	III (Low-ME wheat + xylanase)	66.5 ± 2.8 ^b	108.6 ± 3.2	1.64 ± 0.03 ^a
Pre-patent (Day 30-34)	I (Maize control)	55.4 ± 4.8	166.7 ± 8.0	3.23 ± 0.51
	II (Low-ME wheat)	69.6 ± 3.7	183.2 ± 9.9	2.70 ± 0.27
	III (Low-ME wheat + xylanase)	63.2 ± 5.9	173.6 ± 1.4	2.95 ± 0.42
Patent (Day 35-43)	I (Maize control)	63.5 ± 5.4	177.8 ± 5.4	2.86 ± 0.15
	II (Maize control)	59.4 ± 2.5	173.1 ± 3.7	2.93 ± 0.11
	III (Maize control)	60.1 ± 7.7	181.8 ± 2.2	3.27 ± 0.42

^{ab} Values with different superscripts differ significantly within the same column and period at $P < 0.05$. ¹ For effect of period, refer to the text.

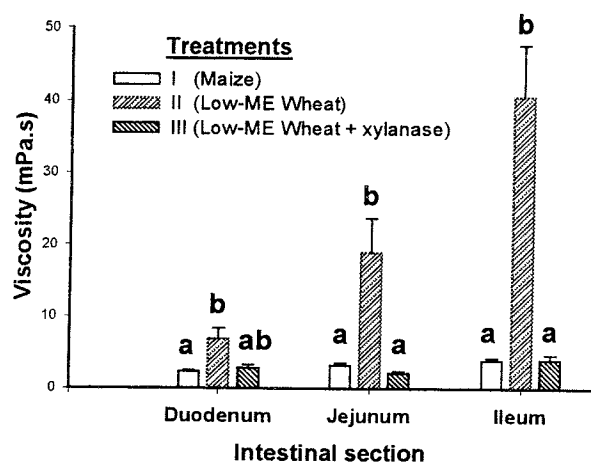


Figure 1. Effect of diet on the viscosity of digesta collected from various intestinal sections in birds on day 29 immediately prior to *E. maxima* infection. ^{ab}Values with different superscripts differ significantly within the same intestinal section at $P < 0.05$

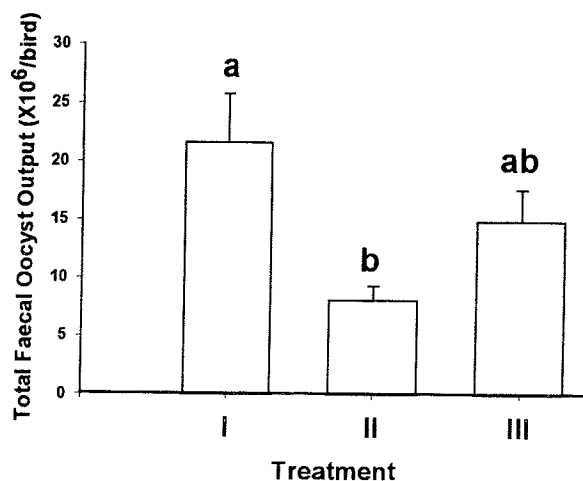


Figure 2. Effect of treatment on the total faecal oocyst output between day 6 and 12 post-infection with *E. maxima*. ^{ab}Values with different superscripts differ significantly at $P < 0.05$

IV. DISCUSSION

In response to a low-dose infection with *E. maxima*, broilers fed a low-ME wheat-based diet that led to a higher level of intestinal viscosity during the pre-infection period showed a markedly reduced level of faecal oocyst output, compared to those fed the other diets with lower intestinal viscosity. We speculate that the high intestinal viscosity might physically prevent sporozoites from invading the gut wall and hence reduce their infectivity. This notion is supported by a recent study showing that increasing dietary fibre level reduced total faecal oocyst output in chickens infected with a high-dose of more pathogenic *Eimeria*

strains (Muir and Bryden, 1998). However, we cannot exclude effects due to differences in the nutritive value of the experimental diets. This might also influence resistance to *E. maxima* during its life cycle within the gut tissue. However, since there was no difference in the growth performance among the three treatment groups during the pre-patent and patent periods, the impact of differences in the nutritive values, if any, between the experimental diets appears to be minimal.

The supplementation of low-ME wheat diet with xylanase was found to significantly improve weight gain and feed conversion of the birds prior to *E. maxima* infection, which was consistent with previous findings (Choct, 1998). The reason why this difference did not continue into the pre-patent period is not clear. Dosing birds with a low number of *Eimeria* oocysts should not have any significant impact on their growth performance (Conway *et al.*, 1993; Richard, 1998). However, coccidiosis itself may reduce intestinal viscosity in broilers (Morgan and Bedford, 1995). Whether *E. maxima* had any impact on the intestinal viscosity in the birds fed the low-ME wheat-based diet and consequently modulated nutrient utilisation, which cancelled out pre-infection differences in the growth performance, remains to be solved. In this regard, our data showing that the birds fed the low-ME wheat diet had an improved liveweight gain following the infection in the pre-patent period, has supported the above explanation.

In conclusion, our findings support the hypothesis that diet-induced change in intestinal viscosity can affect the host response to a low-dose *E. maxima* infection in broilers. The *E. maxima* dose used in this study was similar to those commonly used for low-dose vaccination. Whether the current finding of variation in oocyst output is due to differences in digesta viscosity and/or differences in host immunity remains to be confirmed. As the use of low-dose live vaccines has become an important control measure for coccidiosis in chickens, we should not overlook the possible role of diet formulation and feed enzymes in modulating their efficacy.

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EFFECTS OF INTESTINAL SPIROCHAETE INFECTION ON EGG PRODUCTION IN MEAT BREEDERS

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Summary

This trial examined whether infection with intestinal spirochaetes affected egg production in female Cobb meat breeders. Infection with *Serpulina pilosicoli* led to delayed onset of laying and reduced egg production when compared with the control group. Egg production by birds infected with *S. innocens*, however, was not significantly different from that of the control group. These results suggest that infection with *S. pilosicoli* may be a significant cause of egg production loss in commercial birds.

I. INTRODUCTION

Over the past decade colonisation with intestinal spirochaetes has been recognised as a reason for previously unexplained production losses and/or diarrhoea in layers and meat breeders in Europe, the United States and Western Australia (Davelaar *et al.*, 1986; Griffiths *et al.*, 1987; Dwars *et al.*, 1989; Swayne *et al.*, 1992, 1995; Trampel *et al.*, 1994; McLaren *et al.*, 1996).

In Western Australia (WA), intestinal spirochaetes were isolated from birds in 64% of flocks with signs of intestinal disease, compared with 28% of clinically normal flocks (McLaren *et al.*, 1996). Of the WA isolates identified, 41% were identified as *S. intermedia*, a pathogenic species known to infect pigs (McLaren *et al.*, 1997). An isolate of *S. intermedia* from a WA layer was subsequently used to experimentally infect layer hens, causing increased faecal water content and reduced egg production (Hampson and McLaren, 1999).

In 1998 we conducted a survey of 69 meat breeder, layer or meat chicken flocks in the eastern states of Australia. Overall, birds in 42.9% of meat breeder and 68.2% of layer flocks were colonised with spirochaetes, but no birds in meat chicken flocks were infected. The association between colonisation with spirochaetes and the occurrence of wet litter and/or reduced production was highly significant in the chi-square test with Yates adjustment, with $P < 0.0001$ (Stephens and Hampson, 1999).

A trial was conducted in 1999 at the Queensland Department of Primary Industries Animal Research Institute to evaluate the performance of meat breeder females inoculated with one of two species of intestinal spirochaete. This paper outlines some results of this trial.

II. MATERIALS AND METHODS

Thirty Cobb 500 meat breeder females were obtained at 13 weeks of age. The birds were placed in individual cages with mesh floors, egg roll-out trays and waste trays. Clear plastic sheet was hung between cages to prevent cross-infection. The birds were kept in an air-conditioned room with temperatures varying between 17-23 °C. The daylength was set at eight hours until 19 weeks of age, then gradually increased to 15 hours at 23 weeks of age and thereafter maintained at 16 hours. The birds were fed a commercial pullet developer diet until 19 weeks of age, at which time they were given a pre-breeder ration. When production

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in the control group reached approximately 15% all the birds were given a breeder diet. The feed was restricted, the birds being given 62 grams daily at 13 weeks of age, with this being gradually increased to a maximum of 165 grams per day by 27 weeks of age. Water was provided *ad libitum* by means of individual water bottles with nipple drinkers.

After arrival the birds were acclimatised in the experimental cages for four weeks. Over this period, individual faecal samples from each bird were cultured once a week for spirochaetes. After four weeks each of the birds was weighed and randomly assigned to one of three groups, each of ten birds. Birds in Group A (control group) were inoculated orally with 1 ml of sterile broth. Birds in Group B were inoculated with 1 ml of a broth culture of *S. innocens*. Birds in Group C were inoculated with 1 ml of a broth culture of *S. pilosicoli*. The inocula in each instance contained approximately 10^8 cells. Both these isolates had been obtained from meat breeder flocks with production problems.

During the course of the trial the birds were individually weighed once a week. At the same time, individual faecal samples were collected from each bird. These were cultured for intestinal spirochaetes. Percentage faecal moisture was also determined with weekly differences analysed using least significant difference values. Egg production by each bird in each group was recorded daily and accumulated to provide weekly figures. These were compared using Students *t*-test.

III. RESULTS

(a) Body weight

There was no significant difference in the body weights of the birds in each of the groups. All birds gained weight as expected for the type and age of bird and remained comparable with birds of the same batch in commercial production.

(b) Faecal culture

All faecal cultures carried out prior to inoculation of the birds were negative. All faecal cultures of birds in group A, the control group, following inoculation, were negative for the duration of the trial.

One week following inoculation, one bird in group B yielded a positive culture. One week later, an additional two birds were positive. All three birds remained positive for a further week. Four weeks after inoculation, only one of these three birds was positive on faecal culture. The following week, none of the birds in group B were positive and they remained negative. Faecal samples from three birds in group C were positive for spirochaetes one week following inoculation. These three birds continued to yield positive samples for three weeks. Thereafter all samples from birds in group C were negative for spirochaetes. The same species that was inoculated was recovered from the birds in both groups B and C.

(c) Faecal moisture

For the three weeks following inoculation, mean faecal moisture for group C was consistently higher than that of groups A or B. Faeces of birds in group C were on average 4-6% wetter than those of the other two groups, but overall this difference failed to reach significance. From four weeks post-inoculation, there was no clear difference in the faecal moisture content between the three groups. The mean faecal moisture for this period for birds in group A was 56.43%, for birds in group B it was 56.54% and group C 56.45%.

(d) Rate of lay

Rate of lay (%) was determined for each group each week. There was no significant difference in rate of lay between groups A and B. Both groups maintained production levels comparable for birds of similar type and age in commercial production.

The production levels for group C were however, significantly below that of the other two groups throughout the trial ($P < 0.02$). At 33 weeks of age, rate of lay in groups A and B were 85.71% and 82.86% respectively, whereas that of group C was 65.08%. The difference in egg production between group A, the control group and group C, that inoculated with *S. pilosicoli*, are illustrated in Figure 1.

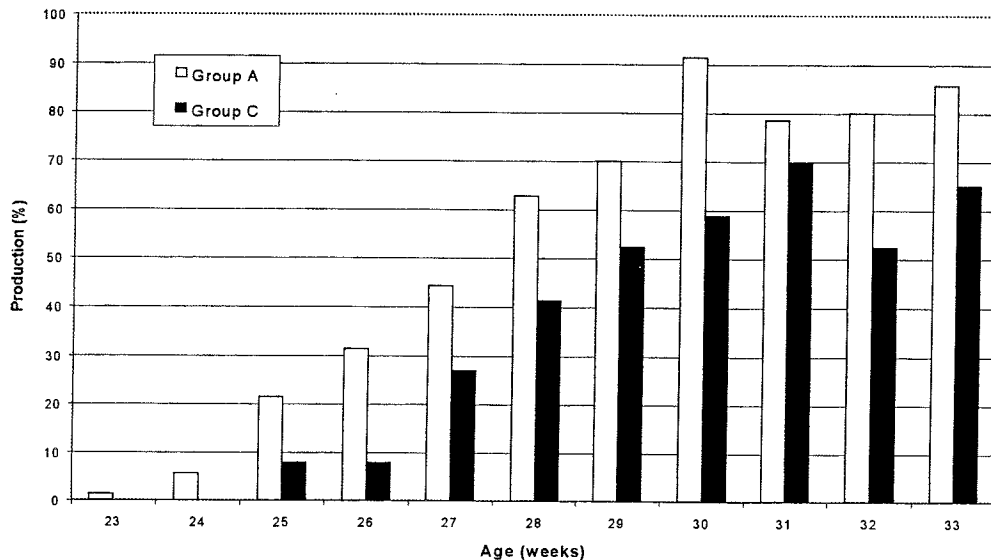


Figure 1. Per cent egg production. Group A (control) and group C (inoculated with *S. pilosicoli*).

These results indicate that infection with *S. pilosicoli* has the capacity both to delay the onset of lay and to cause a sustained and marked reduction in egg production.

IV. DISCUSSION

The reason for the reduced egg production seen in group C is unclear. Spirochaetes are most often found in the caecum of birds and it is possible that their presence in some way affects absorption. This may explain the increased water content of the faeces. Further experimental studies are required to clarify the pathogenesis of intestinal spirochaete infection. Improved methods of diagnosing and controlling the infection in the field are also needed.

V. ACKNOWLEDGMENTS

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LESSONS FROM THIRTY YEARS OF MAREK'S DISEASE CONTROL IN AUSTRALIA FOR 2000 AND BEYOND

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Summary

An analysis of the history of Marek's disease control in Australia over the past 30 years revealed some differences from that experienced by overseas countries. However, the outcomes from short-term approaches to control are likely to lead to similar consequences through the evolution of more virulent strains of Marek's disease virus. Further data required to make sound judgements on the control of the disease in Australia are listed. However, the level of control that can be maintained beyond 2000 is largely in the hands of the poultry industry.

I. INTRODUCTION

Some 30 years of Marek's disease (MD) control in Australia has provided significant economic benefits for the poultry industry and to a lesser extent to the vaccine manufacturers. This period has been marked by two major successes, the initial control of classical and acute strains of Marek's disease virus (MDV) in 1971 and the more recent control from 1998 of very virulent strains in imported genotypes by Rispens and high titred herpesvirus of turkey (HVT) vaccines. However, there have been long periods of only moderate control and some significant vaccine failures. It is the intention of this paper to analyse the history of MD control in Australia and recommend steps to ensure that control beyond 2000 is maintained at a high level.

II. MAREK'S DISEASE IN AUSTRALIA

The incidence and economic impact of MD in Australia has followed what could be described as " a roller-coaster ride" affected by government policy on importation, emergence of more virulent strains of MD virus and the introduction from overseas of strains of chickens that are more susceptible to MD. A chronological history of MD control over the past 30 years in Australia can be summarised as follows:

- 1960 - Widespread and heavy losses from MD in the poultry industry
- 1971 - HVT vaccine introduced resulting in rapid improvements in MD control
- 1973 - Maternal antibody interference problems with cell-free HVT vaccines
- 1974 - Contamination of HVT with reticuloendotheliosis virus (REV)
- 1975 - Improved vaccine manufacturing standards
- 1978 - Introduction of a serotype 2 MD vaccine (Maravac)
- 1983 - Low potency vaccine batches
- 1985 - Recognition of very virulent strains of MDV
- 1986 - Wider adoption of bivalent vaccine and improved hygiene
- 1992 - Importation of poultry genetic material
- 1993 - Increasing incidence of MD problems in layers and broiler breeders
- 1995 - Introduction and failure of a serotype 1 MDV vaccine (CR/6)
- 1996 - MD problems in broiler chickens increasing
- 1997 - Importation of Rispens and HVT vaccine masterseeds from France
- 1998 - Dramatic improvement in MD control using the above vaccines
- 1999 - Adoption of *in ovo* vaccination of broiler chickens

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III. AN ANALYSIS OF THE HISTORY

The pattern of MD history overseas has some similarities to that observed in Australia. However, Witter (1997) makes frequent reference to a step-wise increase in virulence often related to the introduction of new strains of MD vaccines. He has suggested that the use of new vaccines can be expected to generate environmental pressure that will most likely result in the appearance of yet newer and more virulent strains of MD virus. In Australia, it would appear as though this step-wise increase in virulence has been blurred by the existence of more resistant strains of chickens and a greater reliance on hygiene and biosecurity than undertaken overseas. Whereas very virulent strains of MD virus appeared both overseas and in Australia in the mid 1980s, Australia experienced only sporadic outbreaks of disease associated with these more virulent strains (McKimm-Breschkin, *et al* 1990) compared to the heavy losses described overseas. Concurrent problems in the 1980s associated with other infectious agents resulted in the adoption of higher standards of biosecurity and hygiene. Hygiene programs often included the use of high levels of formalin followed by terminal fumigation with formaldehyde gas. These measures are thought to have reduced the challenge from MD on many farms.

The importation of more MD susceptible strains of both layer and meat genotypes and their commercial supply throughout Australia from 1993 saw increasingly more serious outbreaks of MD. Producers adopted increasingly more complex and expensive vaccination programs. The locally manufactured vaccines were inappropriate both in potency and cost to curb the heavy losses. Finally, intense industry lobbying resulted in the importation of masterseeds of MD vaccine strains (Rispen CVI988 and HVT FC 126) that were able to be manufactured with a higher potency and to a quality standard that allowed safe and efficacious vaccination of the imported genotypes (Jackson, 1998).

(a) Recommendations from the analysis

(i) *Vaccine Standards*. To avoid a reoccurrence of the problems associated with maternal antibody interference, contamination with extraneous microorganisms and low potency, it is essential that vaccine manufacturers adhere to standards that are contemporary with current research and the programs adopted by the poultry industry. Standards for MD vaccines in Australia were written in the 1970s and do not meet current usage of those vaccines by the poultry industry. The NRA has promised to develop monographs for each of the poultry vaccines. It should be noted, however, that in the USA where standards are constantly under review, the existing standard on potency for MD vaccine is considerably below industry practice. One area where the US has made significant progress is in the area of standardisation of a challenge model to determine virulence of field isolates (Witter, 1999). Access to this model would be of value to Australian vaccine manufacturers. Despite standards, the poultry industry will often use a product contrary to the label.

(ii) *Vaccine Usage*. Rudd (1996) has warned about the misuse of MD vaccines in the USA. He stated that the history of vaccination in the USA has been one of increasing virus virulence counted by additional vaccine antigens. The new vaccines are often required to be used in the first instance in areas where there is a high intensification of production. The short-term cost benefit approach by the US poultry industry has seen excessive dilution of vaccines and a switch to new vaccine strains where older strains were still highly effective. Companies may choose to vaccinate broiler chickens because they are not prepared to leave them unvaccinated lest managers be considered at fault if a MD problem does arise. The switch to Rispen vaccine in the USA prompted warnings against dispensing with bivalent vaccines (Witter, 1997). Concern was raised that Rispen may be the last effective antigen and that it should be held in reserve and only used where essential (Shane, 1999a). In

Australia it is apparent that Rispens is a very effective vaccine and certain strains are more responsive to it than to the bivalent vaccines. However, we do not know if Rispens is essential in all situations.

(iii) *Vaccine Administration.* Jackson (1999) identified a number of deficiencies in the methods of handling and administration of MD vaccines by Australian hatcheries. He outlined an audit system used by The Marek's Company (TMC) to support the correct administration of that company's vaccines. It is essential that Australian hatcheries maintain high levels of vaccine administration and not resort to cost cutting measures as described in the USA such as excessive chick handling speeds, excessive tubing for vaccine distribution, inclusion of incompatible antibiotics and high vaccine dilution rates (Rudd, 1996). The introduction of *in ovo* vaccination has provided many benefits in terms of reduced labour costs and lowering of stress levels on chickens. However, hatcheries must remain conscious of the need to avoid excessive vaccination speed and to ensure that an early vaccine viraemia is induced in every chicken.

(iv) *Vaccine Evaluation.* The response to vaccination can easily be measured where there is a dramatic reduction in mortality. In the broiler industry the benefits may be more difficult to measure. It is essential for the industry to continue to measure the benefits from MD vaccination. Whilst most failures may be the result of errors in administration, there are recent reports from overseas of biological changes in the virus showing up as variations in the clinical syndrome observed in the field. Witter (1997) has described the appearance of a paralysis syndrome in young chickens aged 1-2 weeks of age associated with more virulent strains of MDV. The appearance of MD in turkeys in France (Witter, 1997) is further evidence of a change in the biology of the virus. MD vaccination failures should be reported and investigated. The causative virus should be isolated and protection tests undertaken with existing vaccines.

(v) *Industry Factors.* In addition to the correct use of vaccines, the future outlook for MD control can be significantly influenced by the approach that the industry takes to the choice of genetic stock, biosecurity and hygiene, and husbandry factors that could act as stressors. The serious outbreaks of MD in Australia that followed the importation of layer and meat strains in 1993 have been attributed to poor responsiveness to the locally manufactured MD vaccines (Jackson, 1996) and to differences in immune competence (Walkden-Brown, *et al.*, 1999). There is also evidence that some strains of chickens respond better to different serotypes of MD vaccine (Bacon and Witter, 1993). Hence the industry can have a significant impact on the future of MD vaccines depending upon the choice of genotypes that it imports into Australia. The level of biosecurity and hygiene in Australia is considered to be significantly higher than in some areas of the US. However, there is an obvious trade-off in the cost of shed clean-out with the cost of MD vaccination. Cost/benefit analysis of MD broiler vaccination should take into account the risk of creating resistant strains of MDV. Shane (1999a) has reported an association of outbreaks in mature birds with poor biosecurity on multi-aged sites. There is also evidence that mature birds may become infected with MDV possibly related to the presence of immuno-depressive factors associated with some form of environmental stress (Witter, 1999). Taylor *et al.* (1999) described an experiment in which birds fed a lower calcium ration had a significantly higher level of MD. Witter (1999) has recommended that the industry needs to adopt a truly integrated approach involving vaccines, biosecurity, genetics and management. He considers that these multiple barriers are needed to reduce the likelihood of evolution of new virus strains. He warns against assuming that new vaccines will always be discovered.

III. CONCLUSIONS

Australia has obtained good control of MD from 1998 following the importation of masterseeds and local manufacture of vaccines from those masterseeds. However, the vaccination programs adopted by the industry, whilst apparently cost effective in the short term, could lead to vaccine failures in the longer term. This opinion is consistent with that of a number of overseas authorities following reviews of the current USA vaccination practices (Shane, 1999a,b; Witter, 1999). The practices of concern are (1) the dependence on Rispens vaccine in layers and breeding flocks, and (2) the dilution of HVT vaccine in broilers to a point where escape mutants of more virulent viruses could be generated.

If the industry adopts a short-term approach to MD control, the evolution of more virulent strains of MDV is a problem that the industry will have to face. The following information should be made available to allow industry to make correct judgements on effective MD control in the future:

1. The range of pathogenicity of isolates of MDV present, particularly those that have resulted in vaccination failures.
2. The clinical and pathological features of the syndromes that those isolates can produce in the field. Tests to differentiate MD from other diseases causing tumours should be available.
3. The level of protection that can be expected from the existing range of MD vaccines in Australia against those isolates. This knowledge could extend to the sending of Australian MDV isolates overseas to determine if more effective vaccine seeds exist.
4. Factors that can contribute to vaccination failures or failure to optimise response to MD vaccination (eg defective administration of vaccine, environmental stress, immunosuppressive agents, husbandry factors, interfering maternal antibody, etc.).
5. Development of alternate types of vaccines or vaccination programs that may replace or augment the existing vaccines. This could include the importation of masterseeds of vaccines that have been demonstrated to be effective in overseas challenge trials.
6. The response of different genotypes of poultry to different serotypes of MD vaccines.

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SITE OF VACCINE DEPOSITION DURING *IN OVO* VACCINATION OF BROILER CHICKENS AGAINST MAREK'S DISEASE INFLUENCES THE TIMING OF POST-VACCINAL VIRAEMIA.

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Summary

In ovo vaccination of broiler chickens against Marek's disease is a relatively recent industry practice. This study was designed to determine whether the timing of post-vaccinal viraemia following vaccination with cell-associated herpesvirus of turkeys (caHVT) is influenced by the site of vaccine deposition or dose of vaccine. Extra-embryonic vaccine deposition resulted in significantly later development of viraemia (~4 weeks) relative to both intra-embryonic vaccination (~2 weeks) and vaccination at hatch (~3 weeks). There were no effects of vaccine dose (4,000 or 8,000pfu) on the timing of post-vaccinal viraemia, irrespective of site of deposition. There were no treatment effects on egg hatchability, mortality, feed intake or growth, but feed conversion ratio was shown to improve with increased dose of vaccine.

I. INTRODUCTION

In ovo vaccination of chickens is an important emerging technology (Ricks *et al.*, 1999) and it is now widely used to vaccinate broiler chickens against Marek's disease (MD). Vaccination by this method deposits vaccine either extra-embryonically (EE) into the tissues surrounding the embryo or intra-embryonically (IE) into the body of the embryo. We have previously demonstrated that the deposition site of vaccine in the embryo by the automated INOVOJECT® method varied considerably with embryo age and egg size (Islam *et al.*, 1998). The present study formed part of our ongoing investigations into the role of site of vaccine deposition on the efficacy of vaccination and was designed to test the following hypotheses:

1. that intra-embryonic (IE) injection with cell associated HVT (caHVT) will result in earlier post-vaccinal HVT viraemia than extra-embryonic injection.
2. that vaccine dose (4,000 or 8,000pfu) will influence the timing of post-vaccinal viraemia when vaccine is deposited EE but not if deposited IE.

II. MATERIALS AND METHODS

Before the main experiment a manual *in ovo* vaccination method was validated by the injection of 0.2ml vegetable food dye into embryos at day 18 of incubation and subsequent determination of the injection site. For IE injection, a sharp 21Gx1½" hypodermic needle was inserted through the pre-made hole in the air cell end of the egg until the embryo was encountered and the vaccine was then inoculated into it. For EE injection, a blunted 21G needle was inserted carefully through a hole and the air cell, into the extra embryonic space to a depth of 2.5-3cm, carefully avoiding the embryo proper before vaccine was deposited. The injection technique was found to be reproducible with an accuracy rate of >95%.

The design of the main experiment was a 2x3 factorial with two injection sites (IE and EE) and three doses of vaccine (0, 4000 and 8000pfu). An external control treatment was also

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included comprising the industry standard of subcutaneous injection with 4000pfu caHVT at hatch. For each of the seven treatment combinations five replicates of seven chickens were used.

The vaccine used was caHVT, strain FC 126 (The Mareks Company, North Ringwood, Victoria, Australia). All vaccine doses were administered in 0.1ml diluent. *In ovo* vaccination was performed manually at day 18 of incubation by a single operator using the previously validated method and aseptic technique.

The birds used in this experiment were Cobb broilers. The parent flock had been vaccinated with a MD virus serotype 1 (MDV1) vaccine (Rispen CVI 988) so the embryos and chickens used would have had maternal antibody directed against MDV1 but not HVT. Immediately after the hatch was taken off and the chicks vaccinated for infectious bronchitis (VicS strain) and MD (external control treatment), they were transferred to enclosed brooding facilities at UNE to limit exposure to natural challenge. Commercial feed and water were provided *ad libitum* throughout the experiment. At three weeks of age the birds were transferred from multi-deck electric brooders to Californian slide cages.

Individual bird weights and group (replicate) feed intakes were recorded weekly throughout the experiment. At weeks one and two of age, two chickens were removed from each replicate (n=10/treatment), stunned, sampled for blood and then euthanased. At weeks three, four and five of age, blood samples were collected from the wing veins of two birds in each replicate. Peripheral blood lymphocytes (PBLs) were separated using Ficoll-paque medium. HVT isolation was performed by inoculating PBLs into a secondary culture of chicken embryo fibroblast cells derived from specific pathogen free chicken embryos (SPAFAS Australia Pty Ltd, Woodend, Victoria, Australia) at day 10-12 of incubation. Cell monolayers were read for cytopathic effects (plaque formation) at 4-day intervals up to 20 days.

Associations between continuous variables were analysed using linear regression. Non-continuous data were analysed using logistic transformation and the generalised linear model method of S-plus 4.5 (Mathsoft Inc. Cambridge, MA, USA).

III. RESULTS

Overall hatchability of eggs containing embryos (determined by candling at day 17 of incubation) was 93%. Treatments had no effect on hatchability.

There were no effects of site of vaccine deposition or dose of vaccine on bird weight, feed intake or mortality. Final means (\pm SEM) for these variables at the end of week five were 1545 \pm 27g/bird, 2471 \pm 14g/bird, and 3.75% respectively. However, within the *in ovo* vaccinated treatments there was a significant negative association between vaccine dose and FCR (feed/gain, $R^2 = 0.15$, $P < 0.05$) with mean FCR (\pm SEM) for the 0, 4000 and 8000pfu vaccine doses being 1.71 \pm 0.09, 1.66 \pm 0.05 and 1.52 \pm 0.02 g feed/g gain respectively.

The timing of post-vaccinal viraemia was significantly influenced by the treatments applied (Figure 1). Amongst *in ovo* vaccinated groups vaccinated with 4,000 or 8,000pfu of HVT there was a significant ($P < 0.05$) effect of site of vaccine deposition with more IE birds being viraemic at weeks two and three (7/13 and 18/19 respectively) than EE birds (2/17 and 3/18 respectively). Vaccine dose (4,000 vs 8,000 pfu) had no effect. Subcutaneous vaccination at hatch produced viraemia in 10/10 birds at three weeks, which was significantly earlier than that in EE but not IE groups. No data were available from week one.

IV. DISCUSSION

Hypothesis 1, but not 2, was supported by the data. The major finding of this experiment was that EE deposition of caHVT at day 18 of incubation delayed post-vaccinal HVT viraemia relative to both, IE deposition at day 18, or subcutaneous injection after hatch. Vaccine dose did not affect this. To our knowledge this is the first published report on differences in the timing of post-vaccinal HVT viraemia following *in ovo* vaccination due to differences in the site of vaccine deposition.

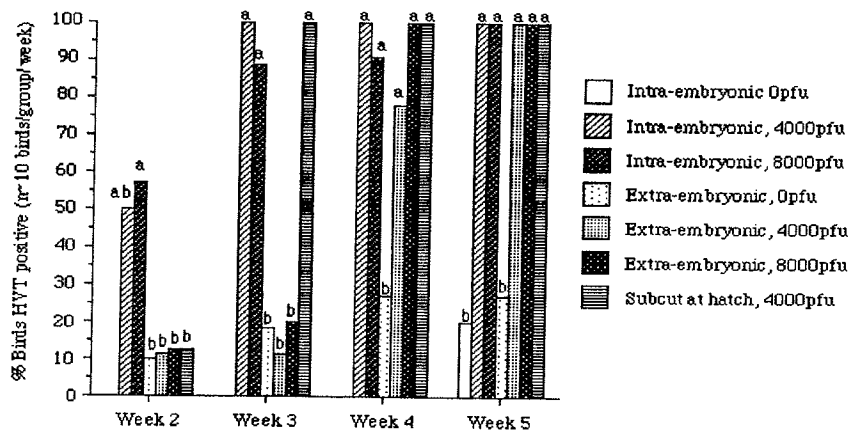


Figure 1. Percentage of chickens showing post-vaccinal HVT viraemia in each treatment during the experiment. Columns within each week not having a common letter are different ($P < 0.05$).

We have already demonstrated that under Australian commercial hatchery conditions there is considerable variation in the ratio of EE to IE vaccine deposition due to egg size and more importantly, the day of incubation when vaccination takes place (Islam *et al.*, 1998). In that work, we found that the proportion of IE vaccination sites when birds were vaccinated *in ovo* at day 18 of incubation was only 15%. Together with those data the present study suggests that a high proportion of chicks vaccinated extra-embryonically *in ovo* with caHVT may have significantly delayed post-vaccinal viraemia relative to those vaccinated IE or subcutaneously at hatch. The extent to which these differences impact on protective immunity to virulent MD virus (MDV) is currently being investigated.

The first report of *in ovo* vaccination against MD stated that vaccine deposition, both IE and EE, resulted in post-vaccinal viraemia within one week (Sharma and Burmester, 1982). However this work did not test the effect of site of deposition and furthermore it was performed on SPF White Leghorn birds, quite different from the industry situation.

The reasons why IE injection should produce a much earlier detectable vaccinal viraemia than EE injection are not known and were not examined in this experiment. Reasons may include one or more of the following: differences in the mode of entry into the embryo; the presence of antiviral activity in the extra-embryonic tissues; or differences in the level of interference from maternal antibody. Sharma *et al.* (1984) claimed that EE vaccine deposition results in infection of the embryo via the respiratory tract with initial replication in the lymphoid tissues associated with the lung, whereas IE deposition probably results in initial viral replication in the spleen and other lymphoid tissues. It is possible that the latter leads to a more rapid spread of infected lymphocytes into the blood. Anti-viral activity has been reported for mammalian amniotic fluid and a similar phenomenon may occur in avian species (discussed by Sharma and Graham, 1982). However, were this so, one could expect the

negative effects to be partially compensated for with an increased dose of vaccine and there was no suggestion of this in the present experiment. The birds used in our experiment came from a serotype 1 vaccinated parent flock (the normal industry situation) and would thus have passed on maternal antibody to MDV to the chicks. This may have contributed to both the delay in viraemia (often detected in less than a week in SPF birds) and possibly the observed difference between IE and EE vaccination. Cell-associated HVT vaccines are far less susceptible to the effects of maternal antibody than cell free HVT vaccines (Sharma and Graham, 1982) but are unlikely to be completely free of effects. In avian species maternal antibody is transferred via the yolk and so the yolk sac would be expected to contain a high concentration of maternal antibody. In an earlier study we found that 20% of EE injection sites involved the yolk sac, possibly contributing to the delay in post-vaccinal viraemia associated with EE vaccine deposition. The isolation of HVT from a small number of control birds was unexpected and may be due to lateral spread of virus although this is generally not considered to occur.

The effects of vaccine dose on FCR are difficult to explain in the context of an experiment where challenge with wild virus was not controlled, but assumed to be low. The improved FCR associated with higher vaccine doses suggests either that challenge with wild MDV occurred during the experiment or that vaccination with caHVT triggered a non-specific immune response that enhanced bird performance in the face of challenge from the normal range of pathogens.

V. CONCLUSION

This experiment has clearly demonstrated that the site of vaccine deposition is an important determinant of the timing of post-vaccinal viraemia following *in ovo* vaccination. Should delay in post-vaccinal viraemia be shown to compromise protective immunity to MD these findings will have important ramifications for the poultry industry. We are currently addressing these questions.

ACKNOWLEDGEMENTS

We are grateful for funding support for this work from the Australian Research Council (ARC) and Baiada Poultry Pty. Limited under ARC (SPIRT) grant 9804599. Thanks are also due to Dr Robert Murison for assistance with statistical analyses and Dr Edla Arzey for advice and help with the virus isolation methods.

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REMOVING THE LYSINE SUPPLEMENT FROM A LOW-PROTEIN FINISHER DIET HAS NO ADVERSE EFFECT ON THE PRODUCTION RESPONSES OF HEAT-STRESSED BROILERS

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Recent studies have shown that the ideal dietary amino acid balance for broilers varies with ambient temperature. In particular, the optimum arginine:lysine (Arg:Lys) ratio increases at high temperatures (Brake *et al.*, 1998). Arg:Lys ratios of approximately 1.35 appear optimal for 3-7 week old broilers at 30°-32°C compared to ratios of 1.10 and 1.18 calculated from National Research Council (1994) recommendations for broilers aged 3-6 and 6-8 weeks, respectively. The benefits of increasing the dietary Arg:Lys ratio are most evident at older ages (42-49 d of age) when broilers are most susceptible to heat stress (Balnave *et al.*, 1999). The ratio can be increased by either increasing the dietary arginine concentration or by decreasing the dietary lysine concentration. The present studies were carried out to determine whether the body weight gain of heat-stressed finishing broilers, held at 30°C, could be maintained in the presence of a more ideal amino acid balance induced by the removal of the lysine supplement from a low-protein finisher diet containing an Arg:Lys ratio of 1.18.

Cobb 500 male broilers were used in all four studies. They were fed grower diets between 3 and 6 weeks of age that varied in Arg:Lys ratio from 0.88 to 1.35 in the four individual studies. The same finisher diet formulation used in each experiment contained 160 g crude protein/kg and included supplements of L-lysine (0.8 g/kg), DL-methionine (0.8 g/kg), L-threonine (1.0 g/kg) and L-arginine (0.5 g/kg). The amino acid composition of this diet met the NRC (1994) amino acid recommendations for 42-49 d old broilers, including lysine (8.5 g/kg), arginine (10.0 g/kg), methionine (3.2 g/kg) and total sulphur amino acids (6.0 g/kg). The removal of lysine from, or the addition of arginine to, this diet was balanced by alterations to the dietary concentration of solka-floc, an inert cellulose supplement. Mash feed and water were supplied *ad libitum* and continuous fluorescent lighting was provided.

Removing the lysine supplement from the low-protein finisher diet had no adverse effects on performance in any experiment. Removal of the lysine supplement gave production responses similar to those obtained from the complete diet when grower diets containing Arg:Lys ratios of 0.88 to 1.05 were fed prior to the introduction of the finisher diet or the lysine-depleted diet at 42 d. However, when grower diets containing Arg:Lys ratios of 1.15 to 1.35 were fed prior to 42 d, improvements in feed intake, body weight gain and feed conversion were obtained as a result of removing the lysine supplement from the finisher diet. Responses obtained by the removal of lysine from the finisher diet mirrored those observed from the addition of arginine in the two experiments where that treatment was employed. This suggested that the observed effects were due to the Arg:Lys ratio *per se*. This conclusion was supported by the observation that removal of the methionine supplement in addition to the lysine supplement resulted in poorer performance in some experiments.

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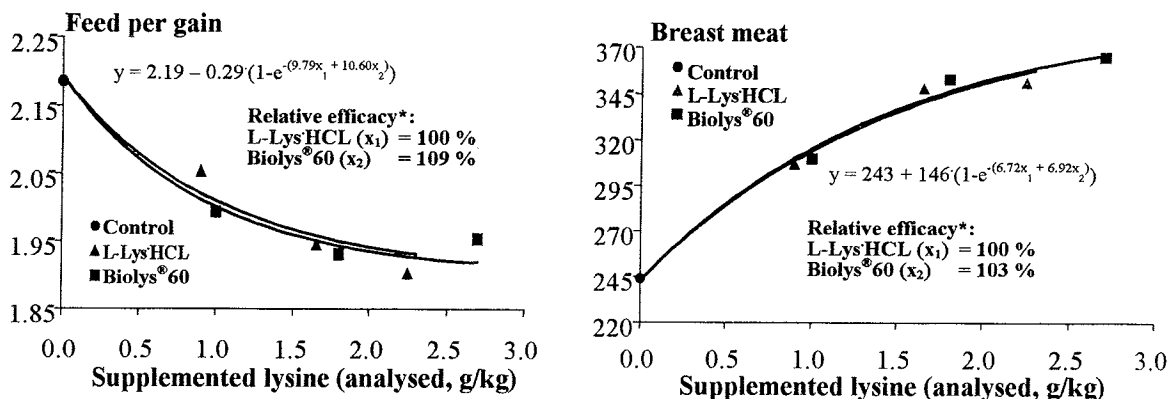
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PERFORMANCE AND CARCASS QUALITY OF BROILER CHICKENS FED DIETS SUPPLEMENTED EITHER WITH L-LYSINE SULPHATE OR L-LYSINE·HCL

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Biolys[®]60 is a new form of lysine containing a minimum of 46.8% L-Lysine in the form of its sulphated salt, plus co-products from the fermentation. Biolys[®]60 is produced by microbial fermentation of *C. glutamicum*, and delivers 60% of the L-lysine contained in Lysine·HCL (78% x 0.60 = 46.8% pure L-lysine). In the present experiment the bioefficacy of Biolys[®]60 and L-Lysine·HCL were compared in broiler chickens. A total of 840 male day-old broiler chicks were assigned to seven different treatment groups, each consisting of six replicates of 20 birds. Treatment I received a basal corn-soybean meal diet adequate in energy and all nutrients except lysine. The lysine contents were 8.5 g/kg (starter, 1-21 days) and 7.9 g/kg (grower, 22-42 days), respectively. Three graded inclusion levels of lysine of 0.8, 1.6 and 2.4 g/kg were added to the treatment diets II, III and IV as well as to the treatment diets V, VI and VII, respectively. These supplement levels represent additions of 1.0, 2.1 and 3.1 g/kg L-Lysine·HCL (diets II to IV), and 1.7, 3.5 and 5.2 g/kg Biolys[®]60 (diets V to VII), respectively. The supplement levels of each lysine source were confirmed by analysis. The birds were housed in floor pens and feed and water were supplied *ad libitum*. Regression analysis of the parameters weight gain, feed conversion and breast meat yield was used to determine the bioefficacy of the two lysine sources as shown in the table.



* Not significant ($P > 0.05$).

The broiler chickens responded significantly to the addition of the two lysine sources used in this experiment. Experimental data were subjected to a non-linear multiexponential statistical analysis. Such a non-linear model allows the evaluation of the complete response curve to graded supplements of a limiting essential nutrient. Therefore, it can be applied to determine the relative biological efficacy of different sources of the same nutrient. Figure 1 shows that the response curves for feed conversion efficiency and breast meat (g) were almost identical for the two lysine sources. The calculated relative efficacy of Biolys[®]60 for weight gain (g) and breast meat yield (% of liveweight) was 104 and 97%, respectively. Hence, it can be concluded that the nutritional efficacy of the lysine source Biolys[®]60 is equivalent to that of L-Lysine·HCL.

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INFLUENCE OF AGE ON ILEAL PROTEIN DIGESTIBILITY OF FEEDSTUFFS IN BROILER CHICKENS

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The influence of age on protein digestion in broiler chickens is not clearly established and conflicting results are found in the literature (Jin *et al.*, 1998). In the present study, the apparent ileal digestibility (AID) of protein in eight feed ingredients for broilers was determined at three ages (14, 28 and 42 days post-hatching). The ingredients were assayed using the procedures described previously (Ravindran *et al.*, 1998). Assay diets contained the test ingredient as the only source of protein. Celite was included in all diets as a digesta marker. Following overnight fasting, each assay diet was fed *ad libitum* to five pens (12 birds/pen at 14 days, 8 birds/pen at 28 days and 6 birds/pen at 42 days) of male broilers (Cobb) for three days. At 14, 28 and 42 d, digesta contents from the terminal ileum were collected and processed. Samples of diets and digesta were analysed for nitrogen and acid-insoluble ash, and the protein (N x 6.25) digestibility was calculated. The protein digestibility values for the three age groups are shown in the table.

Ingredient	14 days	28 days	42 days	Pooled SEM
Maize	0.77 ^{a1}	0.79 ^a	0.82 ^b	0.008
Sorghum	0.79 ^b	0.75 ^a	0.78 ^b	0.008
Wheat	0.77	0.75	0.77	0.016
Millmix	0.67 ^a	0.65 ^a	0.73 ^b	0.018
Soyabean meal	0.83	0.85	0.85	0.007
Canola meal	0.75 ^a	0.77 ^{ab}	0.78 ^b	0.005
Cottonseed meal	0.72	0.73	0.72	0.009
Meat meal	0.73	0.76	0.75	0.007

¹ Means in a row bearing different superscripts are significantly different (P<0.05).

The influence of age on ileal protein digestibility varied with ingredients. Age had no effect (P>0.05) on the protein digestibility of wheat, soyabean meal, cottonseed meal and meat meal. In the case of maize, millmix and canola meal, the digestibility values were similar (P>0.05) in 14-day and 28-day old broilers, but increased (P<0.05) in 42-day old broilers. In the case of sorghum, the digestibility values determined at 28 days were lower (P<0.05) than those determined at 14 and 42 days. This unexpected observation is difficult to explain.

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COMPARISON OF METHODOLOGIES TO ESTIMATE ENDOGENOUS AMINO ACID LOSSES IN POULTRY

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Endogenous amino acid (EAA) losses in poultry have traditionally been determined by the measurement of amino acid excretion in fasted birds or in birds fed protein-free diets. However, these techniques have been criticised because starvation or the absence of protein will cause a reduction of digestive secretions, resulting in the underestimation of EAA losses. Two new methods, namely guanidination and hydrolysed casein, which overcome this limitation, are now available. The aim of the present study was to compare the protein-free diet (PFD), guanidinated casein (GC) and enzymically hydrolysed casein (EHC) methods for determining EAA losses in broilers. The PFD was based on dextrose. The other two diets were based on dextrose and GC or EHC to give a protein level of 200 g/kg, and also contained 20 g/kg celite as a digesta marker. Each test diet was fed *ad libitum* to three pens (6 birds/pen) of male broilers from 35 to 38 days of age. On day 38, terminal ileal contents were collected. The diets and digesta were analysed for amino acids (including homoarginine in the GC treatment) and acid-insoluble ash, and the EAA losses were calculated as previously described (Butts *et al.*, 1992; Siriwan *et al.*, 1994). The endogenous flow (g/kg dry matter intake) of selected amino acids and total amino acids are presented.

Amino acid	PFD	GC	EHC	Pooled SEM
Aspartic acid	0.61 ^a	1.59 ^b	1.43 ^b	0.12
Threonine	0.51 ^a	1.44 ^b	1.09 ^b	0.14
Glutamic acid	0.72 ^a	3.56 ^b	3.87 ^b	0.31
Glycine	0.36 ^a	0.69 ^b	0.49 ^{ab}	0.07
Valine	0.42 ^a	1.06 ^b	0.94 ^b	0.08
Isoleucine	0.29 ^a	0.97 ^b	0.88 ^b	0.07
Leucine	0.44 ^a	1.02 ^b	1.00 ^b	0.06
Lysine	0.21 ^a	0.78 ^b	1.02 ^b	0.10
Arginine	0.28 ^a	0.68 ^b	0.54 ^b	0.05
Total	5.26 ^a	15.92 ^b	14.48 ^b	0.63

^{a,b} Means in a row bearing different superscripts are significantly different ($P < 0.05$).

The EAA losses determined with the use of PFD were considerably lower ($P < 0.05$) than those determined by GC and EHC methods. The total losses of EAA from GC and EHC methods were almost 3-fold greater ($P < 0.05$) than those determined by the PFD. The EAA flow values obtained from GC and EHC methods were similar ($P > 0.05$). These results show that EAA corrections based on the PFD method significantly underestimate true digestibility values.

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**ILEAL PROTEIN DIGESTIBILITY OF EIGHT FEED INGREDIENTS DETERMINED
WITH BROILERS AND LAYERS**

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A compilation of apparent ileal amino acid digestibility of 92 samples representing 23 local feed ingredients for five-week old broilers is available to the feed industry (Ravindran *et al.*, 1998). However, it is unclear whether or not these values can be directly used in layer feed formulations. To our knowledge, published data comparing protein digestion in broilers and layers are not available. In the present study, the apparent ileal digestibility of protein in eight feed ingredients was determined using five-week old male broilers (Cobb) and 60-week old layers (Isa Brown). The ingredients were assayed using the procedures described previously (Ravindran *et al.*, 1998). Assay diets contained the test ingredient as the only source of protein. Celite was included in all diets as a digesta marker. Following overnight fasting, each diet was fed *ad libitum* to five replicate pens (6 birds/pen for broilers and 5 birds/pen for layers) for three days, and digesta contents from the terminal ileum were collected and processed. Samples of diets and digesta were analysed for nitrogen and acid-insoluble ash, and the protein (N x 6.25) digestibility was calculated.

Ingredient	Broilers	Layers	Pooled SEM
Maize	0.82	0.79	0.009
Sorghum	0.78	0.79	0.012
Wheat	0.77 ^{b1}	0.72 ^a	0.004
Millmix	0.73 ^a	0.76 ^b	0.007
Soyabean meal	0.85	0.84	0.007
Canola meal	0.78 ^b	0.74 ^a	0.008
Cottonseed meal	0.72 ^b	0.70 ^a	0.004
Meat meal	0.75	0.76	0.005

¹ Means in a row bearing different superscripts are significantly different (P<0.05).

The estimates for apparent ileal protein digestibility in maize, sorghum, meat meal, soyabean meal for five-week old broilers and layers were similar (P>0.05). However, the protein digestibility values determined with layers for wheat, canola meal and cottonseed meal were lower (P<0.05) and that for millmix were higher (P<0.05) than those determined with five-week old broilers. These results were unexpected since the original hypothesis was that the protein digestibility of ingredients, at least for the poorly digested ones, would be greater in layers compared to broilers. It is possible that the observed differences may reflect differences in endogenous protein secretions between layers and broilers. Future studies are needed to investigate this aspect.

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VARIATION IN THE PROTEIN QUALITY OF NEW ZEALAND MEAT AND BONE MEALS

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Meat and bone meal (MBM) is a common dietary ingredient for poultry, but suffers from considerable variability in nutritive value. Variation in ileal amino acid digestibilities of 20 MBM samples from five processing plants in New Zealand was determined using growing rats. Assay diets contained MBM as the only source of protein, with chromic oxide (0.03%) included as an indigestible marker. Each assay diet was fed for seven days to individually-housed male rats. At the end of the trial, digesta contents from the terminal ileum were collected and processed, and samples of the ingredient, diets and digesta were analysed for amino acids and chromium. True ileal amino acid digestibilities were calculated by correction for endogenous amino acid flow, as determined by the enzymatically hydrolysed casein method (Butts *et al.*, 1992). The MBM samples were also assayed for apparent metabolisable energy (AME) using the rapid cockerel method of Farrell (1978).

	Amino acid content, %		Apparent ileal digestibility, %		True ileal digestibility %	
Arginine	3.63	(3.09-4.12) ¹	65.0	(51.6-80.5) ²	67.2	(53.5-82.6) ²
Cysteine	0.35	(0.25-0.46)	27.6	(10.3-57.2)	48.1	(27.9-74.3)
Histidine	0.92	(0.67-1.14)	47.1	(14.7-71.8) ²	58.1	(26.7-81.5) ²
Isoleucine	1.33	(1.14-1.79) ²	55.3	(38.4-81.4) ²	64.7	(49.1-86.5) ²
Leucine	3.03	(2.54-3.69) ²	57.6	(37.2-80.3) ²	63.5	(42.5-83.8) ²
Lysine	2.50	(2.12-2.81) ²	57.3	(36.5-80.3) ²	64.9	(43.9-84.6) ²
Methionine	0.74	(0.65-0.81) ²	63.5	(47.5-81.8) ²	71.1	(54.0-88.8) ²
Phenylalanine	1.65	(1.39-2.11) ²	59.3	(42.1-80.4) ²	65.2	(47.0-84.1) ²
Threonine	1.56	(1.26-1.86)	40.4	(23.9-71.1)	54.0	(36.0-78.7) ²
Valine	2.12	(1.70-2.56)	54.0	(34.8-76.2)	61.6	(41.9-82.8) ²

¹ Values in parentheses refer to range of values.

² Processing plant effect (P<0.05).

The AME values ranged from 5.75 to 12.33 MJ/kg dry matter. The processing plant had no effect (P> 0.05) on the AME values. Plant differences (P< 0.05) in gross amino acid contents of the MBM samples were observed for isoleucine, leucine, methionine and phenylalanine. In terms of apparent ileal digestibilities, significant plant effects (P<0.05) were observed in all amino acids except cysteine, threonine, and valine. True ileal digestibilities of all amino acids, except cysteine, were influenced (P<0.05) by the processing plant. The present data highlights the wide variation in the protein quality of MBM in terms of the digestibility of the essential amino acids, with gross amino acid concentrations differing to a lesser extent. The observed variation in amino acid digestibility is due largely to differences in processing conditions in the different processing plants.

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AMINO ACID OPTIMISATION OF LAYER DIETS

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Based on the prevailing marginal costs of amino acids (AA) and the marginal revenue from eggs, the Aa-Opt program (EFG Software, Natal) determines the economic optimum egg output of a flock and the optimum AA mixture required to sustain that performance (Mannion and Robinson, 1998). The program was applied in a layer trial in which two strains of layer, differing in bodyweight and performance characteristics, were each divided into three bodyweight (BW) categories. Each of these six classes was fed a diet designed by Aa-Opt specifically for that class. For each strain there was also a control treatment comprising uncategorised birds on a least-cost diet (LCD) formulated to commercial specifications. The experimental diets were fed from 22 weeks of age and were reformulated at 8-week intervals. The Aa-Opt software utilises performance characteristics predicted for each flock and current egg returns and feed prices. Gross margins data (egg revenue less the cost of feeding) were used to evaluate the economics of diets formulated via Aa-Opt or by LCD for the first five periods (40 weeks) of the experiment.

Table 1 shows the actual gross margins for one strain derived from egg revenue and the cost of feeding the BW category birds the Aa-Opt diets or the control birds the LCD diets. Also given are theoretical gross margins derived from the application of the Aa-Opt model to the observed performance of each BW group and control birds.

Table 1. Actual and theoretical gross margins (cents/bird/day).

Group	Gross margin	Period 1	Period 2	Period 3	Period 4	Period 5
Low BW	Actual	2.992	4.154	3.322	3.966	4.135
Low BW	Theoretical	3.186	4.427	3.491	3.926	4.188
Medium BW	Actual	3.748	4.221	3.291	3.971	4.008
Medium BW	Theoretical	3.749	4.341	3.437	4.062	4.069
High BW	Actual	3.892	3.507	3.234	3.845	3.887
High BW	Theoretical	3.991	3.743	3.356	3.946	3.939
Control	Actual	3.873	4.282	3.508	4.836	4.298
Control	Theoretical	3.884	4.167	3.427	4.279	4.080

A comparison of actual gross margins within periods shows the control birds as more profitable than most of the BW groups. The greater theoretical gross margins for the BW groups compared with their actual gross margins suggest that inaccuracies in predicting flock performance characteristics may be impairing the economic outcomes from the model. Within the controls, in all but the first period the actual gross margins exceeded the theoretical gross margins. This comparison is not affected by any predictive element and suggests that the Aa-Opt model is specifying higher levels of amino acids than is customary in commercial layer diets. This observation is confirmed by Table 2.

Table 2. Dietary concentrations (g/kg) of limiting amino acids common to control diets formulated by LCD and by application of Aa-Opt to observed performance.

	Period 1		Period 2		Period 3		Period 4		Period 5	
	LCD	Aa-Opt	LCD	Aa-Opt	LCD	Aa-Opt	LCD	Aa-Opt	LCD	Aa-Opt
Tryptophan	1.8	1.9	1.8	2.2	1.8	2.2	-	-	-	-
Lysine	8.2	8.2	8.2	9.2	8.2	8.9	7.7	9.3	7.7	8.6
TSAA	-	-	-	-	-	-	6.5	7.9	6.5	7.2

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LYSINE DOSE-RESPONSE STUDY WITH ISABROWN LAYING HENS HOUSED IN
SINGLE OR MULTIPLE CAGES

D. BALNAVE, R.J. GILL, XIUHUA LI and W.L. BRYDEN

Previous studies have shown that the feeding of additional calcium in the latter part of growth, in the form of a pre-layer diet, had no significant effect on the production of IsaBrown layers during the subsequent laying period and that feed intake and egg production were similar for hens fed layer diets containing either 160 or 180 g crude protein (CP)/kg (Balnave *et al.*, 1999). The lysine concentration in the diet containing 160 g CP/kg was 7.8 g/kg. The present study was carried out to examine the responses of IsaBrown laying hens to diets varying in lysine concentration with the aim of defining the lysine requirements for these hens under Australian conditions. The diets were similar in all other nutrients.

IsaBrown pullets were obtained from a commercial breeder at 16 weeks of age and housed in either single-bird or multiple five-bird cages in a temperature-controlled laying house. They were fed a grower diet for three weeks and then one of five layer diets similar in all ingredients except that lysine was added in lieu of solka floc, an inert cellulose supplement, to concentrations between 7.35 and 8.95 g/kg diet in increments of 0.4 g/kg. Mean minimum and maximum house temperatures varied between approximately 16° and 23°C during the first half of the experiment and between 22° and 28°C during the second half of the experiment. Corresponding ranges in mean relative humidity were 50 and 80%, and 55 and 80%, respectively. Increasing the dietary lysine concentration significantly reduced food intake and significantly increased lysine intake, but no significant effect of lysine concentration was observed with hen-day egg production, egg weight, egg mass output, feed conversion or body weight gain. Mortality was seven-fold greater ($P < 0.001$) with hens housed in five-bird cages and this resulted in significantly poorer hen-housed egg production compared with hens in single cages. Egg shell breaking strength and albumen height were significantly improved by lysine addition.

There was a significant dietary lysine x cage density interaction with hen-housed egg production (Table). The increased mortality observed in the multiple-caged birds fed the diet with the lowest lysine concentration (7.35 g/kg) resulted in the hen-housed egg production in this treatment group being significantly inferior to that of hens fed the same diet but housed in single cages. In all treatments other than the diet with the maximum lysine concentration, liveability and hen-housed egg production were numerically inferior in the hens housed in multiple-bird cages.

Lysine x cage density interaction for hen housed egg production¹ and mortality².

Birds/cage	Dietary lysine (g/kg)				
	7.35	7.75	8.15	8.55	8.95
1	90.0 ^a (0)	89.0 ^a (0)	88.5 ^a (2)	87.0 ^a (2)	84.7 ^{ab} (2)
5	80.2 ^b (14)	87.1 ^a (4)	84.0 ^{ab} (16)	83.7 ^{ab} (6)	89.3 ^a (2)

¹ SEM = 2.27

² % mortality in parenthesis. SEM of transformed $\sqrt{(\% + 0.5)}$ data = 0.50.

^{ab} Means with different superscripts are significantly different ($P < 0.05$).

Balnave, D., Gill, J., Xiuhua Li and Bryden, W.L. (1999). *Proceedings Australian Poultry Science Symposium*. Ed. D.J. Farrell. 11: 154-157.

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THE EFFECTS OF L-CARNITINE SUPPLEMENTATION ON GROWTH PERFORMANCE AND CARCASS COMPOSITION IN BROILER CHICKENS

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L-Carnitine is a small-molecular-weight quaternary amine which occurs naturally in micro-organisms, plants and animals. Its main function is the translocation of long-chain fatty acids from the extramitochondrial space to the mitochondrial space. L-Carnitine is synthesized by most animals but its supplementation can be beneficial under certain conditions including insufficient carnitine synthetic enzyme activity, metabolic abnormalities, dietary deficiencies or malnutrition (Bieber 1988, Borum 1983, Szilágyi 1998). The effects of L-carnitine supplementation on growth performance and body composition were studied in broiler chickens.

One hundred and twenty, 18-d-old Ross broiler chicks were randomly divided into four experimental groups of three replicates each. A practical grower-finisher diet was formulated and served as the basal (control) diet. The experimental diets were prepared by adding three levels of L-carnitine (50, 100 or 150 mg/kg) in the form of Carniking® (LONZA, Ltd) to the basal diet. Feed and water were provided *ad libitum* during the entire experimental period from 18 to 46 days of age. Individual liveweights of the chicks were recorded at the beginning of experiment (18-day old) and on a weekly basis thereafter.

At 46 d of age a sample of six birds from each experimental group, with body weights near the mean of the group, were taken and killed by decapitation for measurement of breast yield and abdominal fat deposition. The means (\pm SE) for the four groups are shown in the table.

Trait	Level of L-carnitine added (mg/kg)			
	0	50	100	150
Body weight gain (g) 18-25d	365 ^b \pm 7.85	394 ^a \pm 12.7	393 ^a \pm 10.2	403 ^a \pm 7.0
25-32d	377 ^b \pm 8.17	414 ^a \pm 11.2	407 ^a \pm 6.25	408 ^a \pm 9.4
39-46d	404 \pm 10.4	406 \pm 11.7	405 \pm 12.3	408 \pm 12.0
18-46d	1540 ^b \pm 21	1630 ^a \pm 28	1623 ^a \pm 23	1633 ^a \pm 30
39d bodyweight, g	1504 \pm 25.2	1588 \pm 29.4	1584 \pm 22.3	1581 \pm 33.2
Breast yield, g	443 \pm 9.8	455 \pm 12.8	456 \pm 12.3	454 \pm 13.9
Abdominal fat, g	44.8 ^a \pm 1.99	35.8 ^b \pm 2.17	37.0 ^b \pm 1.60	36.6 ^b \pm 2.03
Abdominal fat, g/kg	23.2 ^a \pm 0.50	18.7 ^b \pm 0.80	18.8 ^b \pm 0.60	18.5 ^b \pm 0.70

^{a-b} Means in the same row with different superscripts are significantly different ($P < 0.05$).

Early (18-32d), but not late (39-46d) growth rate was significantly ($P < 0.05$) improved, and abdominal fat deposition reduced, by supplemental carnitine at all three levels of inclusion. There was, however, no significant effect on breast meat yield.

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THE PHYSIOLOGICAL RESPONSE OF BROILERS TO ASCITES

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In recent years, the ascites syndrome has become the most apparent, non-infectious cause of loss in the broiler industry. The aim of this research was to investigate the relationship between some physiological parameters and ascites. Two hundred and forty male Cobb broilers were divided equally into 24 groups, and allocated to one of three treatments. The birds in Treatment 1 (16 groups) were fed pelleted feed in a cool environment. Treatments 2 (4 groups) and 3 (4 groups) were offered pelleted or mash feed respectively in a warm (control) environment. Ambient temperatures in the two groups were the same for weeks 1 and 2, 23° v 25° C in the third week and 14° v 22° C in the fourth week and thereafter in the cool and warm treatment groups respectively. The broilers were fed commercial corn-soybean diets formulated according to NRC (1994) standards. Feed and water were provided *ad libitum* and the birds were grown under continuous lighting. Measures were made of blood parameters and organ weights on a sample of birds at 42 d of age. The results presented in the table showed that cool ambient temperature tended to increase plasma triiodothyronine (T₃) but decrease thyroxine (T₄) concentration, with broilers showing obvious signs of ascites having generally lower T₃ and higher K⁺ values. The ascitic broilers also had lower body weights, higher proportions of heart, lung, and liver weight and higher hematocrit. Although mash feeding reduced the incidence of ascites, it had a negative effect on body weight.

	Ascitic birds		Cold		Pellet		Mash	
		n		n		n		n
Body weight (kg)	2.13±0.57 ^c	19	2.89±0.37 ^b	15	3.32±0.15 ^a	10	2.89±0.20 ^b	10
Organ weight (g/kg bodyweight)								
Heart	7.12±0.39 ^a	19	5.91±0.32 ^b	15	4.40±0.12 ^c	10	4.31±0.11 ^c	10
Lung	5.70±0.32 ^a	19	4.13±0.22 ^b	15	3.81±0.11 ^b	10	4.11±0.10 ^b	10
Liver	26.4 ±1.2 ^a	17	19.6 ±0.54 ^{bc}	15	18.0 ±1.1 ^c	10	21.8 ±1.1 ^b	10
Right ventricle/total heart wt	0.26±0.01 ^a	19	0.21±0.01 ^b	15	0.22±0.01 ^b	10	0.22±0.01 ^b	10
Ascites incidence (%)			33.89		2.5		0.0	
Hematocrit	39.7 ±3.9 ^a	8	34.4 ±0.8 ^b	15	28.5 ±0.7 ^c	10	29.0 ±0.8 ^c	10
K ⁺ (mg/l)	292.0 ±22.0 ^a	8	219.0 ±6.0 ^b	15	231.0 ±17.0 ^b	10	207.0 ±5.3 ^b	11
T ₃ (ng/ml)	1.08±1.11 ^{bc}	6	1.93±0.36 ^a	8	1.46±0.41 ^{ab}	10	0.49±0.49 ^c	6
T ₄ (ng/ml)	1.73±1.91 ^c	6	4.39±0.77 ^b	8	7.21±2.55 ^a	10	8.41±2.25 ^a	9
T ₃ /T ₄	1.09±0.90 ^a	6	0.45±0.11 ^b	8	0.24±0.15 ^b	10	0.07±0.07 ^b	6

Means within each row with no common superscript differ significantly (P<0.05). All the ascitic broilers in table were from treatment 1.

NUTRITIVE VALUE OF PEARL MILLET GROWN IN AUSTRALIA

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Pearl millet (PM) has great potential to be grown in Australia as a cereal grain for poultry; however, data on the nutritive value of Australian varieties of PM is inadequate. The objective of this study was to compare the chemical composition and nutrient digestibilities of two pearl millet varieties Katherine (K) and Siberian (S) and contrast them with published values for sorghum. Ileal amino acid digestibilities (IAA) were determined in broilers and layers as described by Ravindran *et al.* (1999). For broilers, three groups of five (21d old) chicks were used for each millet and for layers, six laying White Leghorn hens were used. The AME values were determined with broiler chickens from 14-21 days of age and with adult cockerels using the rapid assay method described by Farrell *et al.* (1991). Results of the chemical composition, IAA digestibilities and AME are presented in the table.

(g/kg DM)	Chemical composition			Ileal amino acid digestibility coefficient (%)				
	Katherine	Siberian	Sorghum	Katherine Broiler	Siberian Broiler	Katherine Layer	Siberian Layer	Sorghum [*] Broiler
Ash	23.2	43.0	12					
Protein	136.9	118.0	112					
Fat	64.9	54.2	28					
Ca	0.3	0.3	0.4					
P	4.5	3.2	3.1					
CF	51.3	154.0	22					
Amino acids								
Arginine	5.06	3.25	3.8	85	85	80	80	78-86
Cystine	2.35	2.21	1.8	66	67	66	63	
Glycine	3.49	2.79	3.1	68	70	62	67	66-75
Histidine	2.64	1.58	2.4	80	80	77	80	72-80
Iso Leucine	4.81	3.95	4.4	80	80	77	79	78-84
Leucine	11.53	9.58	15.1	84	84	82	85	83-89
Lysine	3.41	1.5	2.3	74	63	68	59	75-79
Methionine	2.66	3.44	1.5	88	87	84	88	80-87
Phenylalanine	5.52	5.9	5.9	81	83	80	83	80-85
Serine	5.46	4.72	4.8	74	77	62	74	74-80
Threonine	4.44	3.33	3.5	67	70	55	69	64-72
Tryptophan	3.15	1.58	1.2	86	83	81	81	72-84
Tyrosine	3.32	3.62	4.2	77	83	73	83	73-80
Valine	5.97	4.85	5.5	79	80	75	79	76-83
AME MJ/kg				15	13.5	14.6	13.7	13.8

* From Ravindran *et al.*, (1999).

PM varieties had higher protein, fat and crude fibre compared to sorghum. The AME content of K was more than 1 MJ higher than that of S and sorghum. IAA digestibility of both varieties of PM was similar to sorghum. However the digestibility of nutrients in K appeared to be higher in broilers than in layers. This difference between broilers and layers was also observed with sorghum (V. Ravindran *pers.com.*). These results suggest that PM can be substituted for sorghum in layer and broiler diets.

Farrell, D. J., Thompson, E., du-Preez, J.J., and Hayes, J.P. (1991). *British Poultry Science*, **32**: 483-499.

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EVALUATION OF UNTREATED GRAIN LEGUME VARIETIES FOR LAYING HENS

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While grain legume production in Australia is increasing rapidly, most of this harvest is grown in the southern states, primarily for human consumption. Some legume varieties, however, are well suited to subtropical regions and show promise as competitive sources of protein for livestock. Although most of the antinutritional factors (ANF) in grain legumes can be reduced by appropriate means such as heat treatment or enzyme additives, this is an extra cost which might be avoidable if varieties with low ANF levels can be obtained. The trials reported here investigated the value for laying hens of some current varieties of legumes, which were grown in Queensland and utilised in untreated form.

Two cultivars of chick pea (*Amethyst* and *Barwon*), two of mung bean (*Delta* and *Emerald*) and one of lab lab (*Koala*) were evaluated for Isabrown laying hens in mash and pelleted diets in two trials. In trial 1 the chick pea cultivars were included in mash diets at levels of 100, 200 and 300g/kg and the mung bean cultivars were included at 150, 300 and 450g/kg. In trial 2 the test materials were included in mash and steam-pelleted diets: *Amethyst* chick pea at 200 and 300g/kg, *Emerald* mung bean at 300 and 450g/kg and *Koala* lab lab at 100 (mash only), 200 and 400g/kg. Each trial included control treatments without grain legumes (mash and pellets in trial 2) and all diets were formulated to similar nutrient specifications. Each treatment was represented by six or seven groups of seven or eight birds. The diets were fed for a four-month period (three months for lab lab). Nutrient and ANF profiles were obtained for each legume cultivar by laboratory analysis.

Varieties of the same legume species were nutritionally similar, except that total sulphur amino acid levels were much lower in *Amethyst* chickpea (1.55 g/kg) than in *Barwon* (5.50 g/kg). Trypsin inhibition activity (mg/g) was higher in chickpea (3.8-7.1) and lab lab (3.8-5.5) than in mung bean (1.9-2.9), and higher in *Amethyst* (6.8-7.1) than in *Barwon* (3.8-4.5) chickpea. However, bird performance appeared to be unrelated to ANF levels.

There was no effect of diet composition on mortality, egg weight or egg specific gravity in either of the two trials. In trial 1, diets containing 450 g/kg mung bean or 300 g/kg *Barwon* chickpea resulted in 7-9% fewer eggs, 4-5 g/d lower egg mass and 9-10% poorer feed conversion than the control diet ($P < 0.05$). Bodyweight gain over the trial period was depressed by 90-150 g ($P < 0.05$) in four of the six chickpea treatments. Trends in the data suggested that both chickpea varieties had a depressing effect on egg mass output when included in the diet at levels above 100 g/kg.

In trial 2, lab lab at 400 g/kg in mash or pelleted diets resulted in markedly lower egg number, egg mass output and feed intake and poorer feed conversion than any other treatment ($P < 0.001$) but did not affect body weight gain. For 200 g/kg lab lab these comparisons were almost significant ($0.10 > P > 0.05$). None of the chickpea and mung bean treatments differed from the mash or pellet control treatment ($P < 0.05$) in respect of egg number, egg mass, feed intake, feed conversion or body weight gain. Egg weight was 0.87 g higher ($P < 0.05$) when birds were fed pelleted instead of mash diets. Mung bean at 450 g/kg in the pelleted diet resulted in 14% more eggs than the control pellets ($P < 0.05$). Although there were no other differences between diet forms and no interactions between diet form and diet composition, the chickpea diets tended to depress performance when fed as mash but not when fed as pellets.

The results overall suggest that safe feeding levels of untreated legumes are: lab lab 100 g/kg, *Barwon* chickpea 100 g/kg, *Amethyst* chickpea 100 g/kg in mash or up to 300g/kg in pelleted diets, and mung bean 300 g/kg in mash or >450 g/kg in pelleted diets.

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IMPORTANT PROPERTIES OF DEHULLED LUPINS IN POULTRY DIETS

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After removing the fibrous hull, the kernel of lupin seed (*L. angustifolius*) becomes an ideal source of protein and energy for poultry, but it still contains some indigestible chemical components and has certain physical properties that may limit its use. We analysed some of these components and properties to quantify them and indicate their potential importance. The cell wall content was high (23.1%) and one third of it comprised pectic substances. The pectin had a molecular weight about 144 kDa and contained 10.3% polygalacturonic acid as its main chain. Each chain had a degree of polymerisation composed of 59 galacturonic acid units. Viscosity, water-holding capacity and filtration rate were measured after dissolving the kernel flour in dionised water for one hour at 38°C, and the supernatant was separated by centrifugation (10000 g for 10 minutes at 20°C). As shown in the table viscosity and water-holding capacity were relatively high and the filtration velocity was slow.

Spectrophotometric and chromatographic analyses of acidic and neutral monosaccharides indicated that the sugars in the cell walls, water-soluble polysaccharides and pectin were mainly galactose, arabinose and galacturonic acid, the main constituents of a pectin structure.

The high level of pectin in cell walls suggests that the addition of a pectic enzyme to dehulled lupins may be beneficial, so future studies will investigate whether pectinase (endo-polygalacturonase) can hydrolyse the glycosidic bonds of polygalacturonic acid. If this can be achieved it may be possible to reduce the water-holding capacity and viscosity of pectin and increase the filtration rate. Ultimately this could improve the feed conversion efficiency and weight gain of broilers, and increase the metabolisable energy of diets based on dehulled lupins.

Physico-chemical properties of dehulled lupins.

	Dehulled lupin	Monosaccharides (%)			
		Sugar	CWM	WSP	PS
Cell wall materials (% DM)	23.1	Rha	–	trace	–
Water-soluble polysaccharides (% DM)	5.08	Fuc	–	–	–
Pectin substances (% DM)	6.88	Ara	12.3	8.06	9.38
Molecular weight of PS (kDa)	144	Man	1.43	3.95	4.91
Polygalacturonic acid (% in pectin)	10.3	Xyl	5.97	4.25	7.88
Polymerisation of polygalacturonic acid	58.6	Gal	45.5	9.01	34.8
Viscosity (m.Pas/sec)	1.89	Glu	7.01	5.23	4.37
Filtration rate (µl/sec)	58.4	GA	7.85	7.99	12.3
Water-holding capacity (g:g)	4.65	Total	80.1	38.5	73.6

CWM: Cell wall materials, WSP: water-soluble polysaccharides, PS: pectic substances, Rha: Rhamnose, Fuc: Fucose, Ara: Arabinose, Xyl: Xylose, Man: Mannose, Gal: Galactose, Glu: Glucose, GA: Galacturonic acid.

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PHYTASE SUPPLEMENTATION OF A LYSINE DEFICIENT BROILER DIET
I. EFFECTS ON GROWTH PERFORMANCE AND TOE ASH CONTENTS

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The effects of adding graded levels of microbial phytase or lysine on the performance of broilers fed on a P-adequate, lysine-deficient diet were examined. The aim was to calculate the equivalency value of phytase for lysine from equations obtained for the performance responses to supplemental phytase and lysine. A wheat- sorghum-soybean meal-based diet that met or exceeded the recommended requirements of all nutrients, except lysine, was formulated. It was designed to supply 80% of the recommended level of lysine (10 g/kg). This basal diet was supplemented with increasing levels of either lysine.HCl (to 10.6, 11.2 and 11.8 g/kg) or Natuphos[®] phytase (0 to 1000 FTU/kg diet). Each diet was fed to six pens (10 birds/pen) of male broiler chicks (Cobb) from 7 to 28 days of age. Growth performance and toe ash data were obtained.

Added phytase (FTU/kg)	Weight gain (g/bird)	Feed intake (g/bird)	Feed : gain (g/g)	Toe ash (%)
0	823 ^{a1}	1475	1.792 ^{ab}	12.93
125	832 ^a	1492	1.793 ^a	13.04
250	847 ^b	1516	1.790 ^{ab}	13.32
375	857 ^{bc}	1509	1.761 ^{bc}	13.00
500	864 ^c	1497	1.733 ^{cd}	13.19
750	867 ^c	1492	1.723 ^d	13.43
1000	861 ^c	1494	1.735 ^{cd}	12.94
SEM	4.52	12.1	0.011	0.181
Probability	< 0.001	NS	< 0.001	NS

¹ Means in a column bearing different superscripts are significantly different (P<0.05).

As expected, the weight gains and feed efficiency of broilers were improved (P<0.001) with added lysine. As shown in the table, addition of phytase to the lysine-deficient diet also resulted in improvements (P<0.001) in weight gains and feed efficiency. Toe ash contents were not influenced by added phytase, confirming that the basal diet was adequate in P and the observed performance responses were independent of the P effects of the enzyme. Linear and non-linear functions that best fitted the data set (weight gain and feed/gain) were derived for levels of lysine and phytase. The non-linear or linear response equations with the higher r² value for added lysine and phytase levels were then set equal and solved. When resolved, performance response from 500 FTU phytase/kg diet was calculated to be equal to 0.70 g lysine/ kg diet. In these calculations, it was assumed that the responses to added phytase were due to the release of lysine. However, the responses also reflect improvements in the digestibility of other amino acids and energy utilisation (Selle *et al.*, 2000), suggesting that an accurate estimation of the lysine equivalency value for phytase is not possible from this study.

Selle, P., Ravindran, V., Kies, A.K., Morel, P.C.H. and Bryden, W. L., (2000). *Proc. Aust. Poult. Sci. Symp.* Ed. R.A.E. Pym, 12: 208.

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PHYTASE SUPPLEMENTATION OF A LYSINE DEFICIENT BROILER DIET
 II. EFFECTS ON APPARENT METABOLISABLE ENERGY AND ILEAL AMINO ACID
 DIGESTIBILITY

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The positive effects of supplemental phytase on the apparent metabolisable energy (AME) of broiler diets have been previously reported (Cabahug *et al.*, 1998). As a part of trial to estimate the lysine equivalency value for phytase (Selle *et al.*, 2000), the effects of graded levels of Natuphos[®] phytase on the AME of a lysine-deficient diet for broilers were determined using the total excreta collection procedure (from day 24 to 27). The influence on the apparent ileal digestibility (AID) of nitrogen and amino acids was also investigated. At the end of the trial (day 28), digesta contents from the terminal ileum were collected and processed. Samples of diets and digesta were analysed for AA and acid-insoluble ash, and the AID values were calculated using acid-insoluble ash as the marker.

Added phytase (FTU/kg)	AME (MJ/kg DM)	AID Protein	AID Lysine	AID Tryptophan
0	14.22 ^{a1}	0.781 ^a	0.794 ^a	0.762 ^a
125	14.24 ^a	0.787 ^{ab}	0.812 ^b	0.766 ^a
250	14.34 ^{ab}	0.789 ^{ab}	0.816 ^b	0.758 ^a
375	14.43 ^{bc}	0.798 ^{bc}	0.825 ^{bc}	0.780 ^b
500	14.55 ^{cd}	0.812 ^{cd}	0.830 ^{cd}	0.794 ^c
750	14.72 ^e	0.810 ^{cd}	0.834 ^{cd}	0.792 ^{bc}
1000	14.58 ^d	0.822 ^d	0.841 ^d	0.797 ^c
SEM	0.043	0.005	0.005	0.005
Probability	< 0.001	< 0.001	< 0.001	< 0.001

¹ Means in a column bearing different superscripts are significantly different (P<0.05).

The AME contents were increased (P<0.001) with increasing levels of supplemental phytase up to 750 FTU phytase/kg which improved the AME value by 3.5%. Addition of increasing levels of supplemental phytase to a lysine-deficient diet caused significant (P<0.05) improvements in the digestibility of protein and all 16 amino acids assayed with the exception of methionine (only selected data are shown in the table). The magnitude of response varied depending on the level of supplementation and the amino acid. In general, the increases in amino acid digestibility were minimal at 125 and 250 FTU/kg enzyme additions and the highest responses were observed at 1000 FTU/kg addition. These results suggest that the enhanced performance with added phytase in broilers fed on lysine deficient diets (Selle *et al.*, 2000) is due to the improved utilisation of both energy and amino acids.

Cabahug, S., Ravindran, V., Bryden, W. L., and Selle, P. H. (1998). *Proc. Aust. Poult. Sci. Symp.*, Ed. D. Balnave, **10**: 203.

Selle, P., Ravindran, V., Kies, A.K. and Bryden, W. L., (2000). *Proc. Aust. Poult. Sci. Symp.*, Ed. R.A.E. Pym, **12**: 207.

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XYLANASE SUPPLEMENTATION AFFECTS THE CAECAL MICROFLORA OF BROILERS

M. SINLAE and M. CHOCT

The gut harbours a highly evolved and complex microbial ecosystem containing a vast number of diverse populations. For example, microbes make up approximately 600 g/kg of the wet weight of poultry excreta. The proper feeding of poultry should therefore consider the provision of "correct" substrates for the microflora to keep it stable. Search for natural alternatives to antibiotics is a major research topic in the feed industry due to the ban of feed antibiotics in some countries. An experiment was conducted to examine the effect of xylanase supplementation of a wheat-based diet on *Clostridium perfringens*, the bacterium responsible for necrotic enteritis, and on the total anaerobes in broilers. Broilers were raised on a commercial starter to d 17 and then were switched to two experimental diets (ME type diet used by Mollah *et al.*, 1983), one of which contained a xylanase (2.5g/kg; supplied by Novo Nordisk). One bird from each treatment was killed every other day to d 39. The total caecal anaerobes and *C. perfringens* were counted (Figures 1 and 2, respectively).

Figure 1. Total counts of bacteria in the caeca of broilers fed wheat with or without a xylanase.

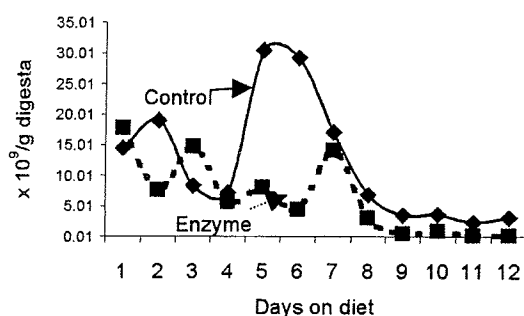
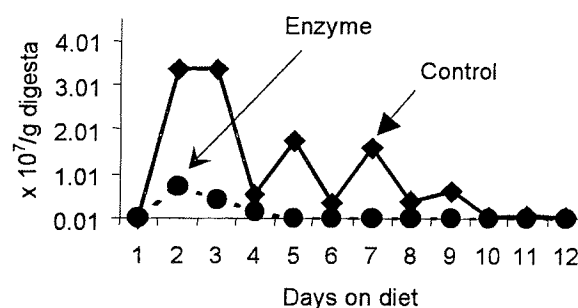


Figure 2. The number of *C. perfringens* in the caeca of broilers fed wheat with or without a xylanase.



The number of bacteria in the caeca of broilers fed wheat with or without a xylanase did not differ significantly ($P < 0.05$), but in the control diet they increased from 7×10^9 to 3×10^{10} five days after introduction of the diet, whereas in birds fed the wheat diet with enzyme there was no such increase. The number of *C. perfringens* increased from about 10^5 to 4×10^7 three days after introduction of the diets with a steady decline thereafter, although levels above 10^5 were maintained in birds fed the control diet. Enzyme inclusion reduced ($P < 0.05$) the number of *C. perfringens* to less than 10^4 after d 5, and remained low to the end of the experiment.

The results show that appropriate enzymes in diets based on viscous grains such as wheat appear to modify gut microflora and suppress the number of undesirable organisms such as *C. perfringens* in the caeca.

We are grateful for Dr Rafat Al Jassim's assistance with microbiology. The work was supported by the Chicken Meat Research and Development Committee of RIRDC.

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TECHNIQUES FOR THE COMPARISON OF XYLANASES FROM DIFFERENT SOURCE MICRO-ORGANISMS

W.D.COWAN

Xylanases are widely used in the feed industry and for the development of new types it is necessary to be able to make comparisons between enzymes. Determination of xylanase activity *in vitro* is not useful when comparing xylanases from different micro-organisms.

Two *in vivo* techniques have been evaluated as to their suitability for the comparison of xylanases. AMEn of a wheat containing diet was determined using 5 replicates of 4 broiler chickens per dietary treatment. The basal diet was composed of sorghum (56%), soya (32%) and animal fat (6%). For determination of AMEn, 50% basal diet was combined with 50% wheat. The diets were supplemented with a xylanase from *Thermomyces lanuginosus* (xylanase A), and from *Humicolor insolens* (xylanase D). AMEn of the diets containing xylanase A was numerically higher than those containing xylanase D.

Feeding studies with the xylanases were carried out using a wheat-based diet, using 48 pens of 30 male broiler chicks, allowing 6 replicates per treatment. Diets were formulated containing 12.0 MJ/kg and 1.26% total lysine, and 12.10 MJ/kg and 1.19% total lysine in the starter and finisher diets respectively. Weight gain and feed consumption were monitored over the growth cycle. FCR values were normalized to a live weight of 2.3 kg using the procedure of Pesti and Rogers (1997). Maximum response of xylanase A was observed at 400 FXU/kg diet but at 600 FXU/kg diet for xylanase D.

The relative biological efficacy of the two xylanases was calculated by comparing the exponential factors for each xylanase in the combined regression equation, using the procedure described by Pack *et al* (1999). In both cases xylanase A was more efficient but the AMEn trial revealed the largest differences. Regression analysis of the response curves provides a method for comparison of xylanases that is superior to analytical determination of enzyme activity as it provides *in vivo* data over a range of dosages.

	Inclusion rate for xylanase (FXU/kg diet)				
Trial I : AMEn MJ/kg	0	100	200	400	600
Xylanase A	12.03	12.24	12.38	12.52	
Xylanase D	12.03		12.23	12.38	
LSD (P<0.05)				0.201	
Trial II:FCR (2,300g)					
Xylanase A	1.821		1.750	1.729	1.727
Xylanase D	1.821		1.756	1.748	1.727
Output from regression equations			AMEn	Growth	
Xylanase D			0.0021	2.75	
Xylanase A			0.0041	4.31	
Ratio (A/D)			1:1.95	1:1.57	

Pack, M, Höhler, D. and Brennan, J.J. (1999). *Proceedings of the 12th European Symposium on Poultry Nutrition*, 342-345.

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EXCRETA VISCOSITY AS AN INDICATOR OF MICROBIAL ENZYME ACTIVITY IN THE HINDGUT AND AS A PREDICTOR OF BETWEEN-BIRD VARIATION IN AME IN BROILERS

M. CHOCT and A. KOCHER

The apparent metabolisable energy (AME) value of an ingredient is not determined solely by the characteristics of the feed; rather it is a parameter reflecting the interaction between the feed and the animal. Thus, highly variable AME values are found between replicates within the same treatment when a low-ME wheat is fed to young broilers are found (Rogel *et al.*, 1987; Hughes and Choct, 1997). The consequences of this variation are: (a) inaccuracy in least-cost diet formulations, and (b) uneven body weight of broilers at slaughter. Differences in gut morphology of the bird do not seem to offer any explanation for this variation (Hughes *et al.*, 2000). The current study examined whether indicators of microbial enzyme activities could be found to explain the between-bird variability in AME when a low-ME wheat diet was fed to three to four week old broilers.

Day-old male broilers were fed a commercial starter diet to 21d. Then 48 healthy birds were chosen and allocated to a wheat-based AME type diet (Hughes and Choct, 1997) with or without a xylanase. Each diet was replicated 24 times in individual cages. The birds were allowed to adapt to the diets for three days followed by quantitative collection of excreta over four days for determination of the AME values. Fresh excreta (5 g) sub samples were also taken on day 4 for excreta viscosity determination. After the last collection, the birds were killed and their ileal and caecal contents collected. Viscosity was determined using a Brookfield viscometer. Ileal and caecal xylanase and β -glucanase activities were determined as follows: to 2 mL of arabinoxylan or β -glucan solution (with a viscosity of approximately 10mPa.s) 0.2mL of digesta supernatant was added and the mixture was incubated at 37°C for 1h with stirring. The viscosity of the mixture was then determined. The drop in viscosity was taken as an indicator of enzyme activity and the relative enzyme activity was expressed as follows: Enzyme activity = (viscosity of control/viscosity of mixture) x 100.

Xylanase supplementation increased ($P < 0.01$) the AME of the wheat from a control value of 12.5 to 13.1 MJ/kg DM and decreased ileal viscosity from 58.6 to 9.5 mPa.s. The current study also measured excreta viscosity as a possible indicator of enzyme activities produced by the gut microflora. Thus, the excreta from the control birds had a viscosity of 14.8 mPa.s, whereas that from birds fed the enzyme-supplemented diet had a viscosity of 4 mPa.s. The significantly lower ($P < 0.01$) viscosity values for excreta compared with ileal digesta suggest that the gut microflora of the chicken produce some enzymes capable of degrading non-starch polysaccharides (NSP). Indeed, xylanase and β -glucanase were clearly detectable in the caeca of birds fed both diets. Furthermore, the caecal xylanase activity was significantly correlated ($r = 0.72$; $P < 0.001$) with AME, but there was no correlation between caecal β -glucanase activity and AME. Neither enzyme was detectable in the ileum of birds fed the control diet. The AME correlated ($r = -0.81$; $P = 0.001$) closely with excreta viscosity, but did not correlate with ileal viscosity in the current experiment.

It is concluded that the high between-bird variation observed when a low-ME wheat diet is fed to young broilers appears to be related to the ability of the hindgut microflora to produce xylanase. The excreta viscosity may be a usable measurement to predict this variation.

Hughes, R.J. and Choct, M. (1997). *Proc. Aust. Poult. Sc. Symp.*, **9**: 138-141.

Hughes, R.J., Choct, J. and Tivey, D.R. (2000). *Proc. Aust. Poult. Sc. Symp.*, **12**: 166-169.

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ORGANIC ACIDS AND NUTRITIONAL MODULATION IN POULTRY

C. A. ADAMS

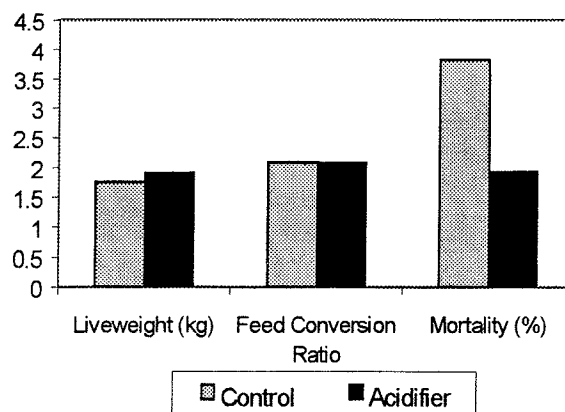
Organic acids are natural constituents of feeds, are synthesised in the gastro-intestinal tract, and are natural metabolites in the body. Mixtures of organic acids as "acidifiers" have been extensively used for performance enhancement in pig diets (Partanen and Mroz, 1999). They have antimicrobial activities and may provide a prophylactic and growth-promoting effect similar to that provided by antibiotics.

A proprietary mixture of organic acids based on lactic acid, ACID LAC, has antimicrobial activity against a wide range of bacteria as shown in the table. A range of naturally occurring *E. coli* strains with resistance to colistin, spiramycin, neomycin, virginiamycin or gentamycin were all found to be sensitive to inhibition by ACID LAC.

Bacteria	Resistance	MIC* values
<i>Escherischeria coli</i> 1201	Tetracyclines	1:2500
<i>E. coli</i> 1212	Ampicillin	1:2500
<i>Salmonella typhimurium</i>	None	1:2500
<i>Staphylococcus aureus</i>	Multiple	1:2500

*MIC is minimum inhibitory concentration

The *in vitro* antibacterial effect of ACID LAC suggests an application as a replacement for antibiotic growth promoters in broilers. In a preliminary trial, broiler performance was compared on a conventional feed containing either an antibiotic growth (avilamycin) or ACID LAC.



There were no significant differences in liveweights, feed conversion ratios or mortality (latter $LSD_{0.05} = 3.2\%$) between the two treatments. These initial results indicate that a mixture of organic acids, with demonstrable antibacterial effect, gives broiler performance comparable to that routinely obtained with antibiotic growth promoters.

Partanen, K. H. and Mroz, Z. (1999). *Nutrition Research Reviews*, 12: 117-145.

A WELFARE AUDIT FOR THE BROILER INDUSTRY

J.L. BARNETT¹ P.C. GLATZ² and A. ALMOND³

A welfare audit has been developed to help fulfil both the chicken meat industry's and the community's expectations of high levels of welfare associated with quality assurance (QA) for chicken meat production. It has been developed specifically focussing on conditions in SE Australia, but would require only minor modifications for use in other climatic zones and for use by the egg industry. QA programmes within industry predominantly focus on animal health and human hygiene and there was a need, coinciding with a more informed and demanding customer base, to expand these programmes to include animal welfare issues. Similarly, there will be a need in the future to include environmental audits into QA programmes. Broiler companies already provide information on animal welfare and growers/unit managers, in their daily tasks, already largely implement this information. The welfare audit documentation has put all this information together and added additional information from the literature and experts in Australia. One purpose of the audit documentation for broiler care is to provide some certainty for all staff involved, so that they, the company, consulting veterinarians, the public and any internal or external auditors are asking the same questions; thus all parties know in advance what questions will be asked.

The documents were developed, based on the HACCP approach, by a management group comprising representatives of commercial companies, farmer groups, animal welfare groups, and teaching and research organizations. To date, documentation has been prepared in separate booklets for the grower, hatchery, rearing and laying sectors of the chicken meat industry. By the end of the year 2000 documentation will have also been prepared for the transport and processing sectors. The completed documentation will represent the first comprehensive welfare audit for an animal industry.

Each booklet is comprised of audit questions and recording sheets, background information on the purpose of the questions and how the questions/practices relate to welfare and recording sheets for growers/unit managers to complete to help demonstrate compliance with the audit process. Although the booklets contain considerable information, every attempt has been made to keep the recording task, for the growers/unit managers, as simple and as short as possible. The audit questions include both critical questions, which are defined as those where "if something goes wrong the welfare of the birds is irrevocably damaged" and good practice questions which reflect the current state of knowledge and its practical implementation in the industry. Compliance can be at the level of the Code of Practice or at a higher standard that has targets higher than those in the Code of Practice; the higher standard is an achievable target for industry over the next five years.

It is anticipated that the audit documentation will fulfil several aims; most importantly, that of demonstrating high standards of animal welfare by providing documented evidence of high quality animal care and by identifying and monitoring equipment, animal problems and human resource issues associated with quality animal care. There are also a number of subsidiary aims, including improved awareness by industry personnel of the interactions between production and welfare and a recognition that welfare must be continually improved.

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