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**LONG PAPERS**

# EFFECT OF FUMONISIN ON THE METABOLIZABLE ENERGY CONTENT OF AUSTRALIAN MAIZE

M.T. AMBA, G. RAVINDRAN, R.J. GILL and W.L. BRYDEN

Fumonisins are toxic fungal metabolites of some *Fusarium* species, especially *F. moniliforme* and *F. proliferatum* found in maize throughout the world (Marasas, 1995). These mycotoxins cause equine leucoencephalomalacia, porcine pulmonary oedema and various experimental conditions in laboratory and farm animals including liver cancer in rats and immunosuppression in chickens. These toxins have also been linked to human oesophageal cancer in the Transkei region of southern Africa where corn is the main dietary staple (Riley *et al.*, 1993). Six fumonisin analogues have been described at present of which fumonisin $B_1$ is the major compound found in nature and produced in culture (Marasas, 1995). A high incidence of fumonisin contamination of whole kernels and by-products of Australian maize have been demonstrated in a recent study (Bryden *et al.*, 1995). In view of the apparently widespread occurrence of these toxins this study was conducted to determine if fumonisins affect the metabolizable energy of maize.

Maize samples, which were collected from human and animal food manufacturers, appeared normal and were free of visible mould growth. Fumonisin was extracted from ground maize using methanol/water and assayed using a direct competitive ELISA (Veratox®; Neogen) which had a lower detection limit of 0.2 mg/kg. Apparent metabolizable energy (AME) was determined with 28 day-old birds using the total collection method (Mollah *et al.*, 1983).

Out of the 24 samples tested only one did not contain detectable levels of the toxin and fumonisin concentration in the contaminated samples ranged from 0.3 to 14.4 mg/kg with a mean of 2.53 ± 2.87 mg/kg. These values for fumonisin are similar to those reported from other parts of the world in apparently normal maize (Marasas, 1995) although values exceeding 150 mg/kg have been found in Australia in maize associated with horse deaths (Bryden *et al.*, 1995). Dietary concentrations exceeding 5 mg/kg and 20 mg/kg should be avoided for horses and pigs respectively and poultry are less susceptible to fumonisins (Riley *et al.*, 1993).

The range of AME values determined in this study was 15.00 to 16.70 MJ/kg with a mean of 15.66 ± 0.49 MJ/kg. The correlation between AME values and fumonisin levels was not significant ($r$= -0.226) indicating that the presence of fumonisins in maize does not affect its AME content.

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# DIETARY ELECTROLYTE BALANCE IMPROVES THE PERFORMANCE OF BROILERS FED ON CASEIN-BASED PURIFIED DIETS

K.ANGKANAPORN, V. RAVINDRAN and W.L. BRYDEN

Casein is often used as the standard protein in purified diets because of its high amino acid digestibility. During a series of studies conducted to determine the endogenous amino acid secretions in poultry fed on purified diets containing dextrose and casein it was observed that the feed intake and growth were disappointing. Since feed intake and body condition can influence endogenous amino acid output (Nitzan *et al.*, 1974) the aim of the present study was to develop a casein-based purified diet which can support acceptable poultry performance for use in amino acid utilization studies.

Experiment 1 was conducted to examine whether changing the energy base will improve the performance of chicks given casein as the sole source of protein. The two experimental diets were based on either dextrose alone or a mixture of dextrose and maize starch. These purified diets were formulated to meet or exceed the recommendations for nutrients by the National Research Council (1994); they contained 235 g/kg of crude protein, 16.0 MJ of metabolizable energy/kg, 17.6 g/kg of lysine and a lysine/arginine ratio of 1.10. A commercial broiler mash (ground form) served as the positive control. Each diet was fed to three groups of six male broiler chicks from day 1 to day 8 post-hatching. Modifying the energy base failed to improve the performance. Feed intake and weight gain of birds fed on the purified diets were lower ($P < 0.05$) than those given the commercial mash.

The poor performance of chicks given the purified diets in Experiment 1 was associated with watery excreta, suggesting that low electrolyte balance (EB) may be responsible for the poor results. This hypothesis was examined in Experiment 2. The dextrose/casein diet employed in Experiment 1 was used as a negative control. The dietary EB (Na+ K - Cl) of this diet was calculated to be 22 mEq/kg. In the experimental diet, the dietary EB was increased to 250 mEq/kg by the addition of 13 g/kg of $NaHCO_3$ and 13 g/kg of $KH_2PO_4$. The ground commercial mash served as the positive control. Each diet was fed to four pens of eight male broiler chicks from day 1 to day 8 post-hatching. The liveweight gains and feed intakes of chicks were improved ($P<0.05$) and the feed:gain ratio was lowered ($P<0.05$) by increasing the dietary EB. When balanced for electrolytes the performance of chicks was comparable to those fed on ground commercial mash. These results indicate that the manipulation of dietary EB in purified diets based on casein is necessary to optimize poultry performance. It appears that the addition of dietary cations alleviate the adverse effects of excess lysine in casein-based purified diets on the growth of poultry (Scott and Austic, 1978).

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# IMPROVING EGG WEIGHT AND EGG SHELL QUALITY AT HIGH TEMPERATURES

D. BALNAVE and S.K. MUHEEREZA

The hyperventilation and associated respiratory alkalosis which occurs when hens are exposed to high temperatures is reflected in a loss of carbon dioxide and bicarbonate from the blood which, in turn, reduces the availability of bicarbonate in body fluids, including that in the lumen of the shell gland. However, attempts to improve egg shell quality by supplementing the diet or drinking water with sodium bicarbonate have given equivocal results (Hughes, 1988). This may be due to the fact that under a conventional 16 h daily photoperiod the bicarbonate is not consumed during the dark period, the time egg shell formation normally occurs. The present study was carried out to determine whether this problem could be overcome by supplying hens in a continuous lighting regimen with sodium bicarbonate. Additional treatments involving dietary supplementation with either ascorbic acid or zinc methionine were investigated also. These treatments have been shown previously to improve egg shell quality in hens receiving saline drinking water, a procedure known to adversely affect the availability of bicarbonate ions in the lumen of the shell gland (Balnave, 1993).

Tegel Super Brown hens, 67 weeks of age with a mean rate of lay of 73%, were used in this study. Forty-eight hens were selected on the basis of egg production and egg shell breaking strength and 12 individually-caged hens were randomly assigned to each of four treatments in a temperature-controlled room. They were given two weeks to acclimatise to 25°C and a daily photoperiod of 16 h light:8 h dark (16L:8D). The temperature was then increased to 30°C and at the same time the daily photoperiod was increased to 24L and the dietary treatments were introduced. These consisted of controls (basal diet), basal + 10 g $NaHC0_3$/kg, basal + 0.5 g zinc methionine/kg and basal + 0.4 g ascorbic acid/kg. After 15 d at 30°C the temperature was raised to 35°C for 4 weeks.

Increasing the temperature from 25°C to 30°C at the same time as introducing continuous lighting gave a small increase in egg weight which was not significantly reduced by exposure to 35°C. The zinc methionine and ascorbic acid supplements had no significant effect on egg weight or egg shell quality measures. However, compared with controls, egg weight ($P<0.05$) and shell breaking strength ($P<0.10$) were improved by the $NaHC0_3$ supplement with the values observed at 30°C and 35°C also being superior to, or similar to, those obtained at 25°C in the 16L:8D photoperiod. The percent shell weight and shell thickness of eggs from hens receiving the $NaHCO_3$ supplement were similar to controls and to the values observed at 25°C in the 16L:8D photoperiod. The improvement in shell breaking strength at the high temperatures without any concomitant improvements in percent shell or egg shell thickness is in line with the observations of Thomas and Roberts (1995) which indicated that changes in the ultrastructure of egg shells laid by hens during heat stress can offset any thinning of the shells.

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# WELFARE IMPLICATIONS FOR LAYING HENS OF SIMPLE CAGE MODIFICATIONS

J.L. BARNETT* and P.C. GLATZ**

Summary

Three experiments are described on the welfare and production consequences of modifying cages for laying hens with perches and solid sides. The presence of perches increased the strength of the femur, reduced feed intake, egg production, egg weight and shell thickness, and increased the incidence of dirty and cracked eggs. Solid sides resulted in improved feather condition and increased mortality, particularly in hot weather. Thus, modification of laying cages in a naturally ventilated shed has both welfare and production advantages and disadvantages. To overcome the problem of cracked eggs when perches are present, nest sites may need to be provided in the cage environment. Improved cooling and ventilation systems would need to be fitted in conventional laying sheds before the use of solid sides in cages could be considered in Australia.

## I. INTRODUCTION

The housing of poultry in cages rather than in alternative systems is a contentious issue and is likely to remain under intense public scrutiny while the industry houses the majority of hens in cages and while there is a lack of data on the welfare of hens in cages under Australian conditions. Overseas research has demonstrated both welfare and production benefits from cage modifications. For example, solid divisions between cages improved feather cover and concomitantly increased productivity by improving feed conversion efficiency and lowering feed costs (Elwinger, 1981, 1983; Tauson, 1984a). However, the data on production, associated with cage modifications, are equivocal. Some of the earlier work with perches suggested a 2 % improvement in egg production and a 2 % decline in mortality while more recent work has shown no effects on production. A consistent disadvantage of including perches has been an increase in the number of cracked or dirty eggs (Appleby *et al.*, 1992). Most of the assessments of welfare of birds in cages have come from Europe.

From the above, it can be seen that simple cage modifications can affect bird welfare. Considerable research is being carried out on more sophisticated cage modifications, such as the provision of nest boxes and dust baths (Appleby, 1992). However, because of their sophistication they are not yet in commercial production and thus are of less immediate relevance to the Australian industry. If these relatively simple cage modifications are to be introduced into the Australian industry there are two principal barriers. Firstly, it is necessary to determine whether the supposed improvements in welfare occur and, secondly, it is necessary to determine the production benefits/losses of such modifications. Once these data have been provided for Australian conditions where housing, ventilation, temperature, light intensity, strains, diets and performance are different from Europe, it will be possible for soundly-based recommendations to be made to industry. Thus, this paper has two objectives: 1) to determine the effects of including perches and solid sides in cages on

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production of laying hens and 2) to determine the effects of modifying cage design on the welfare of laying hens. While parts of the studies in this paper have been reported previously (Barnett, 1994; Barnett and Glatz, 1995; Glatz and Barnett, 1995), this paper will present an overview of the findings and is the first time that all the data have been available.

## II. METHODS

Three experiments were conducted: 1) to examine the effects of modifying cages with solid sides or perches on production parameters and feather condition, and 2) and 3) to examine the effects of similar cage modifications on the welfare of laying hens. Experiment 1 was conducted at the Parafield Poultry Research Centre and Experiments 2 and 3 were conducted at the Victorian Institute of Animal Science, Attwood, Victoria. Birds for Experiments 1 and 2 were sourced from Parafield, while birds for Experiment 3 were a similar strain (Tegel Tint), but sourced from a Victorian grower.

### (a) Experiment 1 (production parameters)

Birds were housed in cages from 19 weeks of age in 8 treatments involving 4 cage modifications (control, perch, solid side, perch + solid side) and 2 strains (Tegel Brown and Tegel Tint) and were housed at 3 birds/cage with a total floor area of 2025 $cm^2$. In the perch treatment a rectangular wooden perch with a breadth of 4.5 cm and a depth of 3.5 cm was installed 7.5 cm above the cage floor. The perch was placed 24 cm away from the feed trough and parallel to it so that birds could not eat while standing on the perch. Birds were able to reach the water nipples both from the perch and when standing on the cage floor behind it. In the solid side treatment, solid plastic side partitions as described by Tauson (1978) were installed in cages and comprised 1.5 mm rigid plastic (polypropylene) securely fixed to the sides of the cages. A randomised block design was used for allocation of treatments with 20 replicates per treatment. Each replicate comprised 3 consecutive cages, with 3 birds/cage. The experiment was analysed by ANOVA.

Eggs were counted daily and food intake was measured every 28 days. Hens were weighed at 18 and 82 weeks of age. On 3 consecutive days of each 28 day period eggs were weighed. Production was assessed over 5 periods: 19 to 34 weeks, 35 to 50 weeks, 51 to 66 weeks, 67 to 82 weeks and 19-82 weeks. Shell quality measurements were made at 30, 50 and 70 weeks of age. All eggs laid between 0800 and 1030 h on three consecutive days were retained for measurement. Egg weight and shell thickness were measured as described by (Hunton, 1987) At 40 and 70 weeks of age all eggs were candled over a 5 day period to determine the incidence of cracked shells and egg shells with a visibly detectable stain were recorded as dirty. Also, at 40 and 70 weeks of age hens were individually taken out of their cage and examined for feather damage using a scoring system similar to that used by Tauson (1984a). The scoring system was a 4 point score applied to the neck, breast, back, wings, vent, tail, base of tail and legs with a score of 4 being good cover and condition and a score of 1 being for a part of the body with heavily damaged feathers with no or only very small areas being covered with feathers. An average score for each hen was calculated. Thermocouples linked to a Unidata 64K logger were used to monitor temperature to $\pm 0.1$ $^{o}C$ in treatment cages during mid summer and winter.

(b) Experiments 2 and 3 (solid sides and perches)

One hundred and forty four experimental and 60 non-experimental laying hens were used in two factorial + control experiments. The factors were cage modification (solid sides or perches vs standard), tier (upper or lower) and bird numbers (1 or 2 bird cages). Floor pens served as an external control. Thus, each experiment involved 8 cage treatments of conventional and modified cages in 2 tiers and with 1 and 2 birds per cage and a deep litter pen external control treatment. All the cages in Treatments 1-8 measured 32 x 47 x 43 cm (w x d x h) and each treatment had a total of 12 cages in banks of 6 cages with each bank containing 4 treatment cages (the two outer cages on the ends of each bank contained non-experimental hens). Banks of cages were separated by solid steel divisions, protruding into the aisles to minimize visual contact between banks of hens. The solid sides or perches in Treatments 1-4 were either 2 mm thick, grey, rigid plastic sheeting or 3.5 x 1.6 cm timber placed 7.5 cm above the floor and 24 cm from and parallel to the front of the cage. For Treatment 9 there were 6 pens each measuring 2.5 x 2.5 m and separated by solid sides to minimize contact between pens. All the floor pens housed 10 birds, had a deep litter covering of wood shavings, nest boxes and 2 perches providing a total length of 371 cm that were 30 cm off the ground.

Behavioural observations were taken from video recordings commencing at 35 and 60 weeks of age over a 4 day period. At each sampling period 6 treatment cages and 3 treatment pens were used and the data subsequently transcribed for activity and pecking behaviours. Four days (Experiment 2) and seven days (Experiment 3) after the video recordings were completed, blood samples were taken from a wing vein (30 birds/day) from one hen/cage from 6 cages per treatment (Treatments 1-8) and 4 hens/pen from 3 pens in Treatment 9. Sampling commenced at approximately 0900 h and was completed within 1 hour. Plasma was subsequently assayed for corticosterone concentrations using a commercial diagnostic kit (see Barnett *et al.*, 1994). At the time of blood sampling, a blood smear was prepared for subsequent counting of heterophils and neutrophils. Fifteen days after the blood sampling for corticosterone concentrations, adrenal responsiveness was assessed on the basis of the corticosterone response to an intramuscular injection of 25 IU of adrenocorticotrophic hormone (ACTH) (Virbac, Peakhurst, NSW Australia). The same birds previously sampled were given the injection into the thigh muscle and 45 min post-injection a 2 mL blood sample was taken via the other wing vein. Five days after the ACTH challenge the same hens were tested for immunological responsiveness, assessed on the basis of the increase in wattle thickness measured 24 h after an injection of 250 μg of leucoagglutinin (Sigma Chemical Company, USA) in 0.25 mL of saline. On the basis of the belief that if stress increases then welfare decreases, a chronic stress response is evidenced by elevated corticosterone concentrations (from the first blood sample), an increased corticosterone response to ACTH, an increased heterophil/lymphocyte ratio and a reduced cell-mediated immunity. Seven days after blood sampling for corticosterone concentrations and blood smears the birds were assessed for feather condition/cover using a subjective 4 point scoring system applied to the neck, breast, back, wings, and tail (Tauson *et al.*, 1984) as in Experiment 1. At the same time claw length was measured using vernier callipers.

After the above measurements were completed, 12 birds from each of Treatments 1-8 and 4 birds from each of the deep litter pens were euthanased, the birds weighed and the femur, humerus, tibia and the coracoid were removed, wrapped in 'plastic wrap' and frozen. Bone strength was subsequently measured using a Lloyd 1000 K Tensile Testing Machine and a 3 point bending jig that supported the bones at 55 % of their length. The instrumentation parameters were a 5 kN load cell accelerating at 10 mm/min. Although the data were

analysed by ANOVA for main effects of cage modification, tier, bird number, age and any interactions, only the effects for cage modification on physiological, condition and behavioural variables are described in tabulated form although some of the other effects are indicated in the text.

## III. RESULTS

### (a) Experiment 1 (production parameters)

The results are shown in Table 1. In the treatments with perches, egg production was lower ($P<0.05$) over the full laying year. Hens in the perch treatments had lower ($P<0.05$) food intake (20 g/day early in lay; 7 g/day for the rest of lay) and superior food conversion efficiency. The body weight of the perch treatment hens was lower ($P<0.05$) at the end of lay. Egg weight from hens in perch cages was lower ($P<0.05$) and feather cover in the solid side treatment was better than in the controls ($P<0.05$). The provision of perches resulted in lower shell thickness and an increase in cracked and dirty eggs ($P < 0.05$). Overall, the mortality of hens in cages with solid sides was greater ($P<0.05$) than in the other treatments, especially during heat waves. The temperature of the solid side treatments was 1 °C warmer than other treatments when monitored over a 7 day period in mid winter (16.3 °C) and mid summer (28.8 °C). Production performance of hens in the solid side treatments was not different to other treatments.

Table 1. Effects of cage modifications on production parameters of laying hens.

| | CAGE MODIFICATIONS | | | | |
|---|---|---|---|---|---|
| Variable | Perch | Side + Perch | Side | Control | $LSD_{(P=0.05)}$ |
| Egg production (/100 bd)[1] | 64.5[a] | 65.2[ab] | 66.7[b] | 66.9[b] | 1.7 |
| Food intake (g/day)[1] | 116.0[a] | 115.4[a] | 122.8[b] | 122.5[b] | 2.3 |
| Food efficiency (kg/dozen) | 2.16[ab] | 2.13[b] | 2.21[a] | 2.20[a] | 0.05 |
| Body weight (kg)[2] | 2.34[a] | 2.36[a] | 2.47[b] | 2.45[b] | 0.09 |
| Mortalities [1,3] | 10.1[a] | 11.0[a] | 17.7[b] | 14.2[ab] | 4.5 |
| Egg weight (g)[1] | 60.0[ab] | 59.6[b] | 60.7[a] | 60.7[a] | 0.7 |
| Shell thickness (μm)[4] | 361[a] | 364[ab] | 365[b] | 365[b] | 3.0 |
| Cracked eggs (/100 eggs)[5] | 13.0[b] | 12.6[b] | 5.4[a] | 6.0[a] | 2.1 |
| Dirty eggs (/100 eggs)[5] | 14.2[b] | 14.2[b] | 11.6[ab] | 10.1[a] | 2.8 |
| Feather cover[5] | 2.59[ab] | 2.55[ab] | 2.67[a] | 2.46[b] | 0.18 |

[1]From 19-82 weeks of age; [2]at 82 weeks of age; [3]includes culls; [4]measured at 30, 50 and 70 weeks of age; [5]measured over 5 days at 40 and 70 weeks of age.
[ab]Within rows values without a common superscript are significantly different at $P< 0.05$.

(b) Experiments 2 and 3 (solid sides and perches)

The results are shown in Table 2. In the solid sides experiment, solid sides in cages resulted in lower corticosterone concentrations ('at rest') than both conventional cages ($P<0.01$) and floor pens ($P<0.001$). The mean values were also lower in the 2-bird than 1-bird cages ($P<0.05$; mean values ($\log_e$) were -0.32 and -0.09 for 1- and 2-bird cages, respectively; $LSD_{(P=0.05)}=1.067$). The corticosterone response to ACTH was greater in the pen treatment than both cage treatments ($P<0.05$). The heterophil to lymphocyte (H/L) ratio

Table 2. Summary of main effects of cage modification (Solid = solid sides, Std. = conventional cage, Pen = floor pen) on physiological, behavioural and condition parameters measured at 30 and 65 weeks of age.

| | Experiment 2 | | | Experiment 3 | | |
|---|---|---|---|---|---|---|
| Variable | Solid | Std. | Pen | Perch | Std. | Pen |
| **Physiological** | | | | | | |
| Corticosterone (nmol $L^{-1}$)[1,2] | $2.22^{px}$ | $3.38^{q}$ | $4.38^{y}$ | 0.65 | 0.64 | 0.89 |
| Corticosterone response to ACTH (nmol $L^{-1}$) | $72.74^{a}$ | $65.78^{p}$ | $97.18^{bq}$ | 50.1 | 49.1 | 45.2 |
| H/L ratio | $1.36^{a}$ | $1.66^{b}$ | $1.66^{b}$ | 1.71 | 1.63 | 1.97 |
| Immunological response (% increase) | 183.1 | 166.5 | 198.7 | 220.8 | 219.4 | 228.3 |
| **Condition** | | | | | | |
| Total feather condition | $3.33^{b}$ | $3.18^{a}$ | $3.14^{ab}$ | $3.27^{x}$ | $3.32^{x}$ | $3.61^{y}$ |
| Claw length (mm) | $25.1^{y}$ | $26.0^{y}$ | $19.4^{x}$ | $23.9^{ay}$ | $26.0^{by}$ | $17.0^{x}$ |
| Femur strength (N) | $293.0^{a}$ | $280.0^{p}$ | $412.0^{qb}$ | $249.2^{a}$ | $209.3^{bp}$ | $304.2^{bq}$ |
| **Behaviour** | | | | | | |
| Total pecking (%)[1] | $4.2^{a}$ | $13.2^{b}$ | $7.2^{ab}$ | 7.0 | 3.5 | 0.6 |
| Between cage pecking (%)[1] | $2.8^{a}$ | $9.4^{b}$ | - | 5.8 | 4.4 | - |
| Perching (%) | - | - | - | $46.5^{q}$ | - | $5.1^{p}$ |

[1] Statistics performed on transformed data with untransformed values presented; [2] 'at rest' concentration measured from a 'spot' sample.

[ab, pq, xy] Different letters denote a significant difference within experiments at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

was lower in the solid side treatment than in both conventional cages ($P<0.05$) and floor pens ($P<0.05$). While immunological responsiveness was lower in the conventional cages than the other treatments, the differences were not statistically significant ($P>0.05$). Overall feather score was improved by modifying the cage with solid sides ($P<0.05$). Total plumage

score was better in 1-bird than 2-bird cages ($P<0.001$; mean values were 3.35 vs 3.16 $LSD_{(P=0.05)}=0.109$) and in the younger birds ($P<0.001$; mean values at 35 and 60 weeks of age were 3.46 vs 3.05). The feather condition/cover of some areas of the bird was better in pens than cages (i.e. neck, breast and tail) while other areas was better in cages (i.e. tail, base of tail and around the vent). Within cages solid sides improved the score of the tail, base of tail and vent areas ($P<0.05$). Claw length was reduced ($P<0.001$) in floor pens compared to cages. Solid sides reduced the incidence of total pecking in cages ($P<0.05$) as did a reduction in the number of birds in the cage from 2 to 1 ($P<0.05$; mean values were 4.7 and 1.98, respectively). Between and within cage pecking was also greater in conventional cages, although the difference was only statistically different for between cage pecking ($P<0.05$).

In the perches experiment there were no effects of treatment on any of the physiological variables (Table 2). The only improvement in feather condition within cages due to the presence of a perch was the condition of the tail feathers ($P<0.05$; mean values were 3.04 and 2.91 in the modified and standard cages, respectively). These feathers were still not in as good a condition as the tail feathers in the Pen treatment ($P<0.001$; mean value = 3.71; $LSD_{(P=0.05)}=0.216$). Overall, feather condition was better in the Pen treatment ($P<0.001$) and similar in the two cage treatments ($P>0.05$). Feather condition was generally better in 1-bird cages than in 2-bird cages ($P<0.05$; mean values were 3.41 vs 3.22), particularly due to the better condition of the feathers of the back and base of the tail ($P<0.01$). Feather condition declined with age ($P<0.05$), particularly in the regions of the breast ($P<0.001$), base of the tail ($P<0.05$) and the vent ($P<0.001$). The presence of a perch increased the length of claws ($P<0.05$) while the length of the claws was shorter in the Pen treatment than in the two cage treatments ($P<0.001$). Claw length increased with age ($P<0.05$; mean values were 23.2 and 25.8 cm at 30 and 65 weeks of age, respectively).

The presence of a perch in a cage resulted in significant perching activity which was at a higher frequency than in the floor pens ($P<0.01$; Table 2). There were more bird/bird interactions in 2-bird than in 1-bird cages both for pecking and being pecked ($P<0.05$). There was less pecking with age for bird/bird pecking interactions and for pecking between cages. Inclusion of a perch in cages only increased the strength of the femur ($P<0.05$; Table 2), whereas bone strength was significantly higher in the pen treatment than in the cage treatments for all bones, except the tibia in Experiment 3 ($P<0.05$). Tier and the number of birds per cage had no effects on bone strength ($P>0.05$).

## IV. DISCUSSION

The production experiment demonstrated both advantages and disadvantages of cage modification. Where perches were present in cages, there were detrimental effects on production and egg quality parameters. Egg production was reduced by 2 % and egg weight and shell thickness decreased and the percentage of dirty and cracked eggs increased. Associated with the reduced egg production was a reduction in feed intake which also resulted in lighter body weight birds. Mortalities were reduced and there were no effects on feather cover. Incorporating solid sides into cages (without perches) resulted in improved feather cover and increased mortalities, particularly during hot weather. During a heat wave in the middle of summer, when outside temperatures reached 42 °C and hot northerly winds buffeted the shed during the day, the hens housed in solid sided cages showed considerable apparent discomfort as the house temperature rose to 36 °C. Although temperature within the solid sided cages was only 1 °C warmer, an increase in

mortality was measured with hens in the solid sided cages despite use of a fine water spray to cool the hens. The hens with the perch in the solid sided cage were able to project their heads and necks from the cage and appeared to be more comfortable than the hens in solid sided cages without a perch. On the basis of the increased heat-stressed mortalities the use of solid sided cages in a naturally ventilated shed cannot be recommended under hot Australian conditions.

The two welfare experiments showed that solid sides in cages reduced the level of stress, particularly in 1-bird cages and, in association with reduced total pecking and between cage pecking in cages with solid sides, these data suggest that social behaviour was having an impact on the level of stress in 1-bird cages, even though these birds had twice the space of birds in the 2-bird cages. Solid sides in cages improved feather condition, particularly the tail feathers. The major improvement from perches was an increased femur bone strength. However, there were no effects of perches in cages on the strength of other bones while the pen treatment resulted in stronger bones than in both the conventional and modified cages for all bones measured.

## V. CONCLUSIONS

Modification of laying cages has both welfare and production advantages and disadvantages for laying hens housed in naturally ventilated sheds. Solid sides reduce the level of stress and suggest social behaviour may be important to bird welfare. Perches increase the strength of leg bones. However, to overcome the problem of cracked eggs associated with perches, nest sites may have to be considered in the cage environment. The increased susceptibility to heat stress implies that improved cooling and ventilation systems would need to be fitted in conventional laying sheds before use of solid sides in cages could be considered in Australia.

## ACKNOWLEDGEMENTS

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# COMPARATIVE STUDY OF COMMERCIAL XYLANASE PRODUCTS FOR WHEAT-BASED BROILER FEEDS

N.L. BEASLEY*, R.J. HUGHES**, M. CHOCT***, G. ANNISON**** and R.J. JOHNSON*

Improvements in the nutritive value of Australian wheats by the addition of xylanase enzymes were initially reported by Johnson and Campbell (1990). The present study evaluated the performance of broilers receiving wheat- or wheat/rye-based diets supplemented with three commercial xylanase preparations from d-old to d 42 (Enzyme R - Roviabio Xylanase TR L, Rhône-Poulenc Animal Nutrition; Enzyme A - Avizyme 1300, Finfeeds International Ltd, UK; Enzyme B - Biofeed Plus, Novo Nordisk, Denmark). The study examined the relationship between dietary non-starch polysaccharides (NSP) and the viscosity of intestinal contents by randomly allocating 4320 unsexed IM98 day-old broiler chicks to 9 diets with 4 replicates of 120 birds. Birds were housed in controlled-environment deep litter floor pens. Starter crumbles were fed from 0-3 weeks followed by finisher pellets from 3-6 weeks. Diets in both periods were composed of similar feed ingredients. The NSP contents of the experimental finisher diets were determined by the alditol acetate method. Chickens were weighed in groups of 120 immediately prior to placement in pens, in groups of 5 at d 21 and in pairs at d 42. Two chicks from each pen were killed by intravenous pentobarbitone injection on d 24. Jejunal digesta viscosities were measured by the method of Choct *et al.* (1995). Results are shown below.

| Treatment | Growth 0-21 days (g/bird) | Growth 0-42 days (g/bird) | FCR 0-21 days (g:g) | FCR to 2.1kg LW (g:g) | Jejunal Viscosity (CP) |
|---|---|---|---|---|---|
| Control Sorghum | 805$^{abc}$ | 2445$^{a}$ | 1.321$^{a}$ | 1.566$^{ab}$ | 2.4$^{c}$ |
| Wheat/rye (W/R) | 784$^{c}$ | 2395$^{b}$ | 1.304$^{ab}$ | 1.601$^{a}$ | 16.6$^{a}$ |
| W/R + Enzyme 1 | 821$^{a}$ | 2437$^{ab}$ | 1.270$^{bc}$ | 1.572$^{ab}$ | 6.0$^{c}$ |
| W/R + Enzyme 2 | 801$^{abc}$ | 2476$^{a}$ | 1.284$^{bc}$ | 1.543$^{bc}$ | 4.2$^{c}$ |
| W/R + Enzyme 3 | 77$^{bc}$ | 2440$^{ab}$ | 1.292$^{abc}$ | 1.570$^{ab}$ | 12.5$^{b}$ |
| Wheat (w) | 783$^{c}$ | 2431$^{ab}$ | 1.277$^{bc}$ | 1.565$^{ab}$ | 5.0$^{c}$ |
| W + Enzyme 1 | 813$^{abc}$ | 2461$^{a}$ | 1.265$^{c}$ | 1.527$^{c}$ | 2.7$^{c}$ |
| W + Enzyme 2 | 818$^{ab}$ | 2476$^{a}$ | 1.274$^{bc}$ | 1.516$^{c}$ | 2.7$^{c}$ |
| W + Enzyme 3 | 796$^{abc}$ | 2438$^{ab}$ | 1.272$^{bc}$ | 1.507$^{c}$ | 2.8$^{c}$ |

*Means without a common superscript are significantly different at $P<0.05$.

Total NSP were 82.7 g/kg for the wheat finisher diet and 90.9 g/kg for the wheat/rye finisher diet. The viscosity of jejunal digesta was increased by rye and reduced to varying degrees by all enzymes. The enzyme-induced reductions in jejunal viscosity gave improvements in growth and feed conversion, the effects being more evident with the wheat/rye diet.

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# BROILER PERFORMANCE IS INFLUENCED BY FAT TYPE IN HIGH VISCOSITY DIETS

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The role of high digesta viscosity, resulting from the presence of certain soluble fibre polysaccharides, in reducing nutrient absorption in the chick is well established. As would be expected from the processes of absorption, fat is particularly affected. On a similar basis, hydrophobic fats could be expected to be more influenced, and this has been supported by indications that higher digesta viscosity has a greater negative effect on longer chain, more saturated fats. The present trial was designed to test this hypothesis in a broiler production trial with a high viscosity diet containing animal or vegetable fats fed without or with enzymes.

Isonitrogenous diets based on 610 g rye/kg were supplemented with 100g/kg of either tallow (28.5 MJ/kg) or soya oil (36.8 MJ/kg). These diets, adjusted to be isoenergetic by the addition of cellulose and maize starch, were fed to broilers from 1-28 days of age without (controls) and with a xylanase-based enzyme product (Avizyme 1300). Feed intake, liveweight, jejunal digesta viscosity and nutrient digestibilities were recorded.

| | Tallow diets | | Soya oil diets | |
|---|---|---|---|---|
| | Control | +Enzyme | Control | +Enzyme |
| **14 days** | | | | |
| Weight (g) | 105[a] | 365[b] | 372[c] | 392[d] |
| Digesta viscosity (mPas) | 311 | 139 | 438 | 32 |
| **28 days** | | | | |
| Weight (g) | 145[a] | 1059[b] | 1128[c] | 1254[d] |
| Digesta viscosity (mPas) | 176 | 31 | 84 | 43 |

[a-d] Means with different superscripts differ significantly ($P < 0.05$).

Liveweight and viscosity results indicated that the diets induced a high digesta viscosity, which was substantially reduced by enzyme supplementation. Liveweight was exceptionally low in the unsupplemented tallow diet, and at 28 days of age was improved seven fold by viscosity reduction resulting from enzyme supplementation. Liveweight on the soya oil diet was also improved by viscosity reduction, but by a relatively small 10%. This trial indicates that animal fats are more affected by high digesta viscosity, which has implications for the formulation of diets including viscosity-inducing cereals and enzymes.

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# NATURAL LIQUID AND SYNTHESIZED POWDER PIGMENT BLENDS FOR EGG YOLK PIGMENTATION

J. N. BIRD

Summary

The efficacy of a natural liquid paprika / marigold blend (capsanthin, lutein and zeaxanthin) and a synthesized powder pigment blend (ß-apo-8'-carotenoic acid ethyl ester (Apo-ester), canthaxanthin) were compared. The efficacy of the synthesized powder blend was found to be two times higher than the liquid blend based on red pigment content (1g canthaxanthin equivalent to 2g capsanthin).

## I. INTRODUCTION

The dominance of wheat in layer feeds in Australia necessitates the inclusion of pigment supplements to ensure consumer preferences for yolk colour are met. In Australia this typically involves a yolk colour target of between 9 and 12 on the Roche Colour Fan (RCF). The inclusion of pigment supplements is costly and there is great commercial benefit in refining inclusion levels of those available. The basic principle of egg yolk pigmentation is to first build a yellow pigment base in the yolk then to add red pigments to alter the colour hue toward golden orange. As the yolk colour increases the yellow base required remains relatively constant while the red component increases. i.e. the ratio between yellow:red alters (Bird, 1994). For easier application, blends of red and yellow carotenoids are used in the Australian market which fixes the ratio of yellow:red in feed, so that any one blend is most cost effective over a relatively small colour range. Two major sources of pigments are available to the egg producer. Liquid and powder forms of paprika / marigold blends or a range of powder blends of apo-ester / canthaxanthin. Due to the different efficiencies of utilization of the active pigments in these blends comparative dose response trials are relevant for defining the most cost effective pigmentation strategies. The present experiment describes an evaluation of the most commonly used synthesized preparation in Australia (apo-ester: canthaxanthin ratio of 1.28:1) and a natural liquid pigment preparation.

## II. METHODS

Nine diets were prepared and fed to groups of 20 individually-caged Isa Brown layers. Batches of a wheat control diet (100 kg) had various levels of carotenoid pigments added as a 350g/100kg premix. Inclusion levels were based on the assumption the blends were correctly formulated to supply sufficient yellow pigment base across the range of yolk colours required for the Australian market (RCF 9-12) and red efficiencies were compared. Four of the diets contained 1.0, 1.5, 2.0 or 2.5g canthaxanthin per 1000 kg supplied by a commercial synthesized preparation (Roche Products Pty, 1.28:1) and four rations contained 1.7, 2.6, 3.5 and 4.4g capsanthin per 1000 kg supplied from a natural liquid pigment blend (Lynteq Pty Ltd, Bio-Orange). Table 1 shows the corresponding red and yellow pigment contents of the experimental diets. All birds were fed a pigment-free diet

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for 10 days prior to the experiment. Three individuals evaluated yolk colours with the RCF for three consecutive days commencing on day 20 of the feeding experiment.

Table 1. Inclusion (g/1000 kg) of synthesized (canthaxanthin and apo-ester) and natural (capsanthin and lutein + zeaxanthin) pigments in a wheat-based control diet.

| Treatment | Canthaxanthin | Apo-ester | Capsanthin | Lutein + Zeaxanthin |
|---|---|---|---|---|
| 1 (control) | - | - | - | (0.5)* |
| 2 | - | - | 1.7 | 1.5 |
| 3 | - | - | 2.6 | 2.3 |
| 4 | - | - | 3.5 | 3.0 |
| 5 | - | - | 4.4 | 3.8 |
| 6 | 1.00 | 1.28 | - | - |
| 7 | 1.50 | 1.92 | - | - |
| 8 | 2.00 | 2.56 | | |
| 9 | 2.50 | 3.20 | - | - |

*Natural content from feed ingredients.

## III. RESULTS

An average RCF value of 3.0 was maintained on the wheat-based control diet. All treatment groups recorded significantly ($P<0.01$) higher yolk colour compared with control values after 20 days of supplementation. A dose-related increase in yolk colour was observed for the groups supplemented with either synthesized or natural pigments. The yolks of the groups fed synthesized pigments showed a higher colour intensity ($P<0.01$) at low inclusion levels when compared to the natural liquid blend. Dose responses were linear up to RCF 12 for both pigment sources (Figure 1). The slope of the synthesized pigment line was twice that of the natural pigment suggesting that, for the given blend configurations, 1 g of canthaxanthin was equivalent to 2 g of capsanthin. Some deviation from the colour hues of the RCF was observed with low inclusion levels of the natural blend.

## IV. DISCUSSION

The commonly recommended efficiency ratios of 2 - 2.5 g capsanthin being equivalent to 1 g canthaxanthin were confirmed. Hannasch and Nelson (1992) reported that 2.2 mg capsanthin/kg in combination with 8.8 mg marigold xanthophyll/kg produced a RCF value of 7.5. In the current experiment a colour of RCF 5.7 was achieved with 1.7 mg capsanthin/kg. However, this blend only provided 1.5 mg/kg of yellow xanthophyll in the diet. The negative effect of a possible deficiency in the level of yellow pigment in the yolk with the addition of low dietary inclusion of the liquid blend cannot be ignored. Some deviation from the RCF was observed, particularly at low inclusion levels of the natural blend. The same does not appear to apply to groups supplemented with low dietary levels of the synthesized powder blend. Apo-ester has been found to be deposited into the yolk with 2-3 times the efficiency of marigold xanthophyll (Roche, Unpublished data).

Therefore, although the dietary levels of yellow pigment were comparable in the current experiment it is likely the apo-ester content of the yolk was up to 2-3 times higher between comparable treatment groups. This suggests that the yellow pigment base is achieved with relatively low inclusions of the currently tested 1.28:1 powder blend and a blend containing a lower yellow:red ratio may be appropriate for higher yolk colours to avoid excess yellow pigment addition at higher blend inclusion levels.

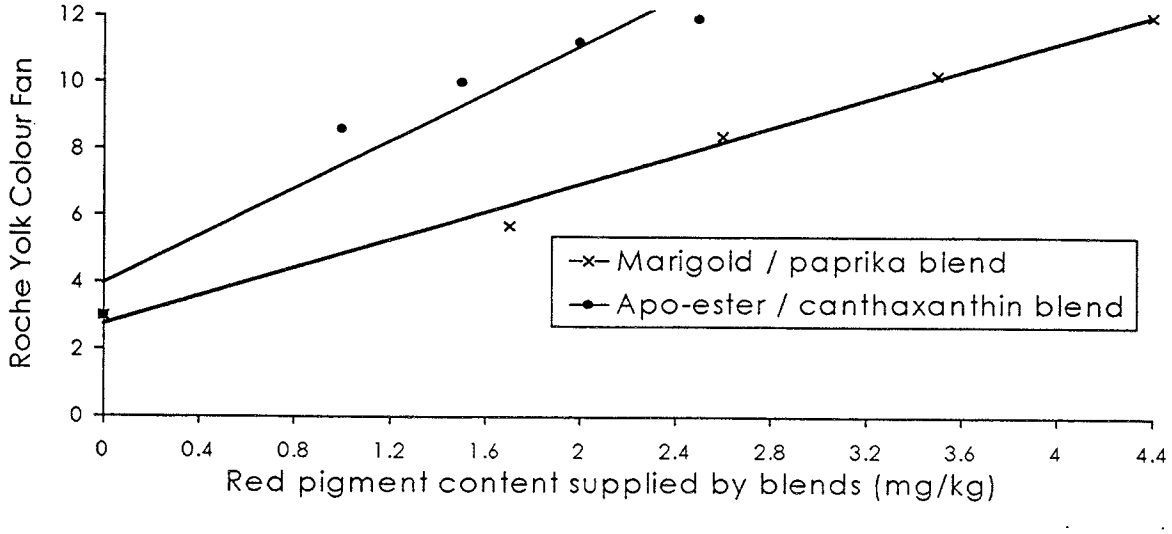


Figure 1. Yolk colour response to dietary inclusion of a natural liquid pigment blend or a synthesized powder pigment blend based on red pigment content. Marigold/paprika blend (RCF = 2.91x + 2.73, $r^2$ = 0.990), synthesized powder blend (RCF = 5.60x + 3.92, $r^2$ = 0.935). Average yolk color of 20 hens/treatment on 3 consecutive days with 3 observers.

## V. CONCLUSION

Both the natural liquid and synthesized powder blends tested can achieve yolk colours in the range required for the Australian market. One gram of canthaxanthin can replace approximately 2 g capsanthin given the blend configurations tested. A limitation of using one single blend across a range of yolk colours is that to satisfy the red pigment requirement the yellow pigment base may be either exceeded at high blend inclusion levels or not reached at low blend inclusion levels. Two blends (low colours of 9/10 and high colours of 11/12) may be more appropriate to most cost effectively achieve the required level of yellow pigment.

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# IMPROVEMENT OF CHICKEN DISEASE RESISTANCE BY HAEMOPOIETIC CYTOKINES

R. BOYD and C. SIATSKAS

Summary

Chickens are particularly susceptible to disease in the newly-hatched period because they do not have prolonged maternal protection and their immune defence mechanisms have not yet reached maturity. As a direct consequence of this susceptibility to opportunistic infection there is frequently a loss in weight or delay in weight gain. Our studies in recent years have addressed the fundamental issue of how immune defence mechanisms arise in chickens. While the adaptive immune response provides long-term protection it is the innate or "natural" immune system which provides immediate protection. This is the domain of bone marrow-derived myeloid cells such as granulocytes and macrophages which develop from precursors under the influence of soluble factors termed haemopoietic'colony stimulating factors. These factors have revolutionised clinical management of many human immunodeficiency states. In chickens two such factors, Stem Cell Factor (SCF) and chicken myelomonocytic growth factor (cMGF) have been identified and the relevant genes cloned. In addition, two other novel factors have been identified; Embryonic Spleen Cell Line (SSL-1) and Avian Haemopoietic Growth Factor (AHGF), derived from embryonic spleen and bursa cell lines respectively. These factors are potent inducers of myeloid cells *in vitro* and act in synergy with SCF. Initial experiments demonstrate that these factors also act *in vivo*, providing a rational basis to minimising the incidence of disease in newly hatched chickens and for boosting the effectiveness of immunization and immune responsiveness in older birds.

## I. RATIONALE

In mammals, the *in vivo* application of well defined cytokines has been extremely successful in boosting the immune-haemopoietic cell systems (Metcalf, 1991). Unfortunately, such mammalian factors are non-functional in chickens. Despite the evolutionary distance between the avian and mammalian species, however, there is a remarkable degree of conservation in the nature, organisation and function of their haemopoietic and immune systems. This is undoubtedly a reflection of the selection pressures which have caused these two fundamentally important cellular systems to evolve in forms optimal for the defence of the host against infection and for the maintenance of normal homeostasis in blood cell formation. It can thus be confidently predicted that the mechanisms regulating chicken immunity and haemopoiesis will be similar to that of mammals. In terms of cell surface receptors, lymphoid tissue architecture and immune responsiveness, this has been clearly shown to be the case. This study seeks to utilize the proven technology and experimental approaches so successfully used to identify and characterize mammalian haemopoietic cytokines, to define cytokines which boost the defence of chickens against infection. The *in vivo* application of such factors provides not only an avenue to enhance the effectiveness of immunization schedules in adult chickens but also a means of promoting the development of the defence mechanisms in the newly-

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hatched period when chickens are most vulnerable to infection because their normal immune system has not yet developed and they are not afforded the protection which mammals receive through maternal milk. Chickens do receive immunoglobulin through the egg yolk which is derived from the hens blood stream but this is often insufficient or of the wrong specificity to defend against infections in the immediate environment at the time of hatching. The establishment of a rapid and effective means of eliminating microorgansims will have an enormous effect on the well-being of chickens with direct consequences on growth and weight gain, particularly in the first three weeks of life when their own defence mechanisms are still developing.

## II. INTRODUCTION

The general health of an individual has a direct bearing on food intake and appropriate deposition of nutrients as muscle protein as opposed to fat. As a corollary, a direct consequence of infection is loss of weight. For animals which form part of the food-chain defence against infection thus has enormous economic consequences. An important focus of farm management of many commercially important species is, therefore, at the level of protection against disease.

When microorganisms such as bacteria or viruses invade a host there are two potential means of defence against the onset of infection. In the vast majority of cases the first line of resistance is through cells of the innate (or "natural" ) immune system. The importance of the innate defence is well illustrated in immunodeficient SCID mice which lack functional T or B lymphocytes yet show considerable resistance to infection (Dorshkind *et al.*, 1984). The innate immune system is comprised predominantly of cells of the myeloid cell lineage (macrophages, neutrophils, eosinophils and basophils) which develop from bone marrow-derived precursors. Neutrophilic granulocytes in particular are the major blood cell involved in ridding the body of bacteria but they have a short life span which means that enormous numbers must be generated each day to circulate in the blood to provide a constant surveillance (in humans the bone marrow produces approximately $1x10^{10}$ blood cells per hour). Granulocytes can be rapidly deployed to sites of infection where they exit from the blood stream to attack the foreign microorganisms. Under normal circumstances the granulocytes and macrophages are produced within the bone marrow through stromal cell induced differentiation of precursor cells. The stromal cells form an interconnected matrix in the bone marrow, not unlike a sponge, through which the developing blood cells migrate as they mature. Thus, the stromal cells normally provide all the necessary haemopoietic growth factors. These are not only stimulatory, however, but also inhibitory to prevent over production and leukaemia formation (Mayani *et al.*, 1992; Arai *et al.*, 1990). A striking functionally important feature of the granulocytes is that they do not require pre-activation. They are able to directly bind the microorganisms, engulfing them into their cytoplasm and, thereby, destroying them by degradative enzymes. When the rate of growth of the microorganism is too great, however, the normal basal levels of these myeloid cells can be too low, with infection and disease developing. Enhancing the number and functional efficiency of these myeloid cells is thus a logical target for immunotherapy.

While the myeloid cells are an extremely important host-defence mechanism they do not have specificity nor do they provide long-term protection against subsequent infection by the same organism. This long-term specific immunity is mediated by cells (lymphocytes) of the adaptive immune system which respond or adapt to infection, eventually removing the microorganism. These are thymic-derived T lymphocytes and bone

marrow-derived B lymphocytes. The former cells either kill targets (e.g. viral infected cells) directly or release cytokines which stimulate either an inflammatory response or B cells to release immunoglobulin. The immunoglobulin can bind to the bacteria, virus or soluble toxins and either enhance their uptake by myeloid cells or detoxify in association with a series of enzymes called complement. While lymphocytes are essential for the maintenance of immune protection the major problem with them from a practical viewpoint is that they require specific activation by a third-party cell (antigen-presenting cell) which must not only take-up the antigen but also break it down into small antigenic peptides which are fitted into the groove in surface major histocompatibility (MHC) molecules for presentation to the T lymphocytes. The T lymphocytes specific for the antigen engages the peptide-MHC complex and becomes activated. A functional response can take several days by which time there can be severe infection.

The design of strategies to improve animal health must therefore take into consideration the need for immediate responsiveness, long-term vaccination or both. There are clearly arguments for both approaches but in most instances, to avoid the need for targeting specific pathogens, stimulating the non-specific innate immune system provides an efficient broad-based protection against infection. The essence to this form of therapy is that the molecules regulating the development and function of myeloid cells have been clearly identified. Collectively they are termed cytokines. In the past decade there has been an enormous increase in our knowledge of cytokines and their receptors, with over 50 now described (Aggarwal and Puri, 1995). The most important of these for the general health and well being of the individual are the haemopoietic cytokines which influence the growth of blood cells, and the interleukins (for example IL 1 and IL 2) and gamma-interferon which affect cells of the immune system. The latter are extremely important in the induction of immune responses but invariably there are many clinical side effects when they are given systemically. The most severe of these are autoimmune diseases where the immune system, which normally only responds to foreign antigens, becomes "overactivated" and actually starts to destroy normal healthy tissues. These side-effects mean they are not ideal therapeutic candidates and they will not be discussed further.

## III. WHAT ARE THE HAEMOPOIETIC CYTOKINES?

Haemopoietic cell growth factors are a class of soluble regulatory glycoproteins required for survival, proliferation and differentiation of haemopoietic stem cells (Metcalf, 1991). They have similar functions and range in size from approximately 18 to 90 kDa. As mentioned above, quite a number of polypeptides have been identified as having a stimulatory effect on mammalian haemopoiesis. Four of these polypeptides that play key regulatory roles are the colony stimulating factors (CSF's), named for their ability to stimulate progenitor cells to proliferate into clonal colonies in semi-solid media such as agar (Metcalf, 1984). These factors are:

Macrophage CSF (M-CSF), which stimulates macrophage formation.

Granulocyte CSF (G-CSF), which stimulates granulocytes formation.

Granulocyte-Macrophage CSF (GM-CSF), which stimulate both granulocyte and macrophage formation.

Multi-CSF (or IL-3), which stimulate mixed colonies of cells consisting of granulocytes, macrophages, erythrocytes, megakaryocytes. Multi-CSF also induces the self renewal of the multipotential precursors.

The discovery of these molecules required ,in essence, two fundamental experimental systems. These were, as mentioned above, the development of an *in vitro*

colony forming assay where precursor cells grown in semi-solid medium (to prevent their migration) are acted upon by the cytokines to cause differentiation and proliferation which are manifested as colonies. The second was the establishment of cell lines which either produced the factors or responded to them. Of major practical importance, these cell lines enabled sufficient quantities of the factors to be purified for biochemical and functional analysis, this being impossible to achieve by simple purification of the molecules from biological fluids. In earlier mammalian studies the lungs from over 100,000 mice were needed to identify and isolate G-CSF.

More recently a new, important cytokine has been identified. This is Stem Cell Factor (SCF) (Martin *et al.*, 1990) which has the unique property of maintaining stem cell viability rather than inducing their differentiation into mature cells. When used in combination with the other CSF's, SCF provides a larger pool of precursor cells which the CSF's can induce to become mature. Hence the synergism between these cytokines is an important means of increasing the innate immune system cells in the blood and throughout the body.

The *in vivo* efficacy of these CSF's has been best exemplified with G-CSF. When injected into mice before and following treatment with the immunosuppressive drug cyclophosphamide there was a dramatic increase in circulating neutrophils and a concomitant increase in resistance to infection against the microorganisms *P. aeruginosa* and *S.aureus* (Nicola, 1989). A wide variety of experimental and clinical applications have now revealed just how pleiotrophic and fundamentally important these CSF's are. Furthermore, recent clinical trials with G-CSF, given as post-chemotherapy to cancer patients or immunosuppressed transplant recipients, have dramatically enhanced recovery by rapidly boosting both the levels and functional activity of granulocytes, thereby minimising or even preventing the chance of bacterial or fungal infections (Nicola, 1989; Metcalf, 1991; Gough, 1989). The CSF's are also important stimulators of immune responses and can, thus, be used as adjuvants (molecules which potentiate immunizations). As a consequence, these CSF's represent a billion dollar world-wide market.

## IV. WHY THE CSF's ARE IMPORTANT TO CHICKENS

Chickens represent a major agricultural commodity. They are the most rapid source of protein generation and the most efficient feed converters of any member of the food chain. They are, however, very susceptible to infection in the newly-hatched period because their immune system is poorly developed and they do not have the maternal protection characteristic of mammals. The common consequence of this is loss of weight and/or failure to put on normal weight gain during the first 2-4 weeks post-hatching (that is up until the immune defence mechanisms are developed). Such disease susceptibility causes approximately 10% stock loss annually which translates to hundreds of millions of dollars annually world-wide. Hence, there is clearly a need to overcome this problem. In view of the remarkable clinical experience with humans the most logical approach is through selective application of haemopoietic cytokines. Despite the great phylogenetic distance between humans and chickens there is a remarkable degree of similarity in the basic adaptive (ie responsiveness to antigens) and innate immune systems. Surprisingly little research, however, has been directed to the elucidation of the cytokines regulating haemopoiesis and lymphopoiesis in chickens. To date only two avian specific haemopoietic cytokines have been characterised, these being chicken myelomonocytic growth factor (cMGF) and SCF.

(a) Chicken monomyelocytic growth factor (cMGF)

cMGF is the best characterised avian haemopoietic cytokine. It is a 24-29kDa glycoprotein which stimulates the development of macrophages. This factor was originally identified in conditioned media derived from concanavalin A-stimulated chicken spleen T cells which was able to induce differentiation of myelomonocytic cells transformed by the myboncogene-containing E26 virus and avian myeloblast virus (AMV). cMGF was subsequently found in LPS-stimulated HD11 macrophage cell-line supernatant. The latter supernatant was also capable of inducing macrophage differentiation in normal bone marrow cells, suggesting it was the avian equivalent to the mammalian myeloid CSF's (Leutz *et al.*,1984). The gene sequence for cMGF shares approximately 50% homology with both human and mouse G-CSF and IL-6 both of which induce myeloid cell development (Leutz *et al.*, 1989). In fact, cMGF is likely to be an ancestral gene from which the mammalian counterparts were derived. Importantly, the cloning of cMGF facilitates the production of large quantities of this cytokine in a variety of expression systems for not only *in vitro* analysis but also for injection. We have produced this cytokine by transfecting COS cells with a cMGF construct and shown that the supernatant from these cells secrete active cMGF. Although, by analogy with the mammalian CSF's, cMGF is obviously potentially very important for treating chickens, surprisingly limited data have been published on its functional activities.

To address this issue an *in vitro* haemopoietic colony forming assay was established for the chicken system. This involved determining an appropriate source of precursor cells (bone marrow or embryonic spleen) and optimising many experimental parameters including serum supplements, kinetics and cell concentrations (Siatskas *et al.*, 1996). This has proved invaluable for the analysis of not only the functional properties of cMGF but also for identifying new haemopoietic cytokines. Extensive use of this system has been made to show, using bone marrow or embryonic spleen precursors, that cMGF is a potent inducer of macrophage-like cells identified by functional ability (phagocytosis) and expression of specific surface markers (as assessed by flow cytometric analysis using a panel of monoclonal antibodies which detect either immature or mature myeloid cells).

(b) Stem cell factor (SCF)

Recently, chicken SCF has also been cloned and has been shown to share approximately 50% homology with mammalian SCF (Zhou *et al.*, 1993). SCF binds to c-kit and has the specific ability to act on stem cells; that is, it does not induce their differentiation but rather promotes their self renewal. The result of this is to markedly expand the pool of precursor cells capable of responding to other maturation-inducing cytokines. The combination of SCF with other cytokines, particularly G-CSF, has thus revolutionized an already highly progressive therapy regimen, and represents an imperative approach to the problem of inducing defence against disease in newborn chickens.

(c) New chicken cytokines

Since mammalian haemopoietic cytokines are not functional on chicken cells there is an obvious need to identify the factors which stimulate the chicken innate and adaptive immune systems. Our studies have addressed the hypothesis that the stromal cells of the lymphoid and haemopoietic tissues provide the factors necessary for this. Following the success of studies in mammals long-term cell lines were made from 11 day embryonic

chicken bursa (the organ which produces B lymphocytes and, in the embryo, granulocytes) and day 16 embryonic spleen (which is one of the major organs in the embryo involved in establishing the haemopoietic system). A chicken cytokine has been identified and partially isolated which has been termed Avian Haemopoietic Growth Factor (AHGF). This has a molecular weight of approximately 90kDa and a P.I of 2.5 (Obranovich and Boyd, 1996). AHGF was identified in the conditioned media obtained from the stromal cell line BSL.2 which was created from day 11 embryonic bursal stromal cells transformed with the Fujinama-P isolate of Rous sarcoma virus. Extensive phenotypic analysis of BSL.2 with our panel of 32 mAbs to chicken lymphoid and stromal cells typed it as deriving from the surface epithelium, a site proposed to be the origin of bursal follicle development and stem cell attraction. AHGF stimulates the proliferation and differentiation of MHC class II-bearing precursors from both the bursa and bone marrow into macrophages. These macrophages are strongly adherent *in vitro* and avidly phagocyte latex beads which mimics uptake and degradation of particulate matter e.g. bacteria. A critical feature to establish the functional value of cytokines is to determine whether they are effective *in vivo*. When injected *in ovo*, AHGF (or at least the cell line supernatant) caused a selective and substantial increase in bursal cell number. This molecule is functionally distinct from cMGF (the latter does not affect embryonic bursal stem cells whereas AHGF causes strong proliferation and differentiation of these cells). Furthermore, the BSL.2 stromal cell line does not express message for cMGF as assessed by Northern blot analysis or reverse transcribed-polymerase chain reaction (RT-PCR). No equivalent molecule has yet been described for the mammalian system. This could be achieved using DNA probes once AHGF is cloned or when sufficient amino acid sequence data has been obtained.

The embryonic spleen cell line (SSL-1), characterized as macrophage in origin, produces another novel haemopoietic cytokine. Using precursor cells from various tissues (adherent cell depleted E16, newly hatched and D7 bone marrow or E16 spleen), it has been shown that the supernatants from SSL-1 induce precursor proliferation followed by differentiation into macrophages (Siatskas *et al.*, 1996). This was confirmed by flow-cytometry using an extensive panel of monoclonal antibodies (mAbs) to chicken lymphoid and myeloid cells, and analysis (morphological, histological, immunohistological and enzyme assays) of colonies grown in the semi-solid medium. While these properties resembled those of cMGF in parallel assays the two factors were shown to be distinct because there were some functional differences and probing the SSL-1 line mRNA with cMGF gene probes either by northern blots or RT-PCR, confirmed that the SSL-1 line did not produce cMGF. One of the most important functional differences between the two cytokines was that SSL-1, but not cMGF, synergized with SCF (stem cell factor). By extensive use of the semi-solid agar colony forming assay it was confirmed that the factors induced myeloid colonies and were thus equivalent to the mammalian CSF's. Currently, work is in progress in this laboratory to try to clone these factors by recombinant DNA technology, a prerequisite for gaining sufficient quantities of the factors for *in vivo* application.

## V. COMPARATIVE ANALYSIS OF SCF, SSL-1 and cMGF

Before any *in vivo* applications can be made with these cytokines it is important to show whether they synergize to produce more haemopoietic cells than, for example, cMGF alone. This was examined in two *in vitro* assays - the proliferation of bone marrow precursor cells and the production of myeloid/macrophage colonies in the semi-solid agar culture system. As a prelude to *in ovo*/*in vivo* experiments, and to determine the functional

significance of SSL-1 in comparison to cMGF, the following experiments have been completed (Table 1).
(Note: unless indicated all experiments involve cMGF, SCF, SSL-1 and mixtures of SCF plus cMGF and SCF plus SSL-1). E = embryonic age; D = days post-hatch; BM = bone marrow.

Table 1. Summary of the *in vitro* effects of SSL-1 on haemopoiesis.

| | |
|---|---|
| **Haemopoietic colony formation** | Using day 7 bone marrow, the majority of developing colonies are macrophages, with some granulocytes also appearing. This was confirmed by cytological, immunohistological staining, enzyme assays and functional analysis of developing colonies. |
| **Multiparameter flow cytometry** | Induction of cells with a mature phenotype (macrophage characteristics) with a concomitant loss of immature cells. |
| **Proliferative characteristics (Haemopoietic)** | Induction of precursor proliferation over an initial 72 hour time course. Repeated stimulation results in the commencement of proliferative activity. |
| **Proliferative characteristics (Lymphopoietic)** | Induction of E15 bursal cell proliferation. |
| **Synergistic effects** | Actively synergises with avian SCF to impart additive/supra-additive effects on stimulated precursors. |
| **Comparative analysis to other known avian cytokine** | No IL-1, IL-2 or interferon activities were detected. No message for cMGF was detected. Using RT-PCR, message for SCF was detected. |
| **Cross reactivity on mammalian precursors** | Human stem cells proliferated and differentiated into mammalian haemopoietic granulocytes and macrophages. Proliferation of the mouse BaF3 mouse cell line engineered to express LIF receptors. |

1. Precursor proliferation assays for dose-response and time-course analysis using E16 spleen, E16 BM, D1 BM and D7 BM. E15 bursal cells were also tested in some assays. This showed AHGF to be distinct to SSL-1 and cMGF.

2. Colony forming assays using E16 spleen, E16 BM, D1 BM and D7 BM.

3. Multiparameter flow cytometric analysis of stimulated E16 spleen and E16 BM using a panel of mAbs to precisely define the types of cells induced by the cytokines.

4. FACS sorting of precursor cells to determine the identity of the responding cell types.

5. Use of human precursor cells - this showed that SSL-1 but none of the other cytokines could cause some activation and differentiation of human cells. This represents a potentially important finding since it is uncommon for avian cytokines to act cross-species.

6. Confirmation by dot-blots, PCR and Northern transfers to demonstrate that SSL-1 does not produce cMGF.

7. Functional analysis (phagocytosis and enzyme content) of E16 spleen, E16 BM, D1 BM and D7 BM stimulated with SSL-1 and cMGF.

8. Investigation of known cytokines (IL-1, IL-2, Interferon) produced by SSL-1 either cultured normally or following stimulation with LPS or PMA.

9. *In ovo* and *in vivo* testing with cMGF transmitted using the fowl pox vector system. Post-hatched chickens treated with this recombinant cMGF had increased levels of macrophages within the blood and decreased levels of immature cells in the bone marrow.

Collectively these studies demonstrated that both SSL-1 and BSL-2 (AHGF) produce cytokines which stimulate myeloid lineage cells - particularly macrophages which are an important part of disease resistance and immune response initiation. They act on precursor cells which is enhanced by SCF whereas cMGF acts only on more mature cells and does not synergize with SCF. AHGF uniquely stimulates MHC class II cells in the bursa. The mapping of the appearance of precursor cells responsive to various cytokines has provided a framework for the timing of *in ovo* application.

On the basis of these successful findings attempts have been made to promote the development of haemopoietic cells *in vivo*, focussing on macrophages which are a major cell type involved in combating infection, particularly with bacteria, fungi and viruses (Table 2).

Table 2. Summary of the *in vivo* effects of cMGF.

| | |
|---|---|
| **Differential blood counts** | Increase in blood monocytes (approximately 40%)<br>Control counts approximated at 10% of total cell number. |
| **Phenotypic analysis of haemopoietic elements** | Increase in macrophage number in blood, spleen and bone marrow.<br>Proliferation and differentiation of stem cells along macrophage lineage with concomitant decrease<br>in precursor cell number seen in the blood and<br>bone marrow. |

## VI. HOW CAN HAEMOPOIETIC CYTOKINES BE APPLIED TO IMPROVE CHICKEN DISEASE RESISTANCE?

There are basically three different phases in the lifespan of chickens which are appropriate for cytokine intervention to improve resistance to disease. As outlined above chickens are most susceptible to disease in the first few weeks after hatching because the immune defence mechanisms are not fully developed. The basic aim is thus to promote the status of the immune system, particularly the innate arm since it is these cells which respond immediately to infection. This can be achieved by injecting mid-term embryos (e.g. day 16 of incubation) with a single dose of a combination of pure, recombinant SCF together with cMGF. The data above would suggest that this should substantially increase the number of myeloid cells in the circulation of newly hatched chickens. If, from a purely practical viewpoint, injection of day old chicks is more acceptable, the same approach could be applied - combinations of purified, recombinant SCF with cMGF injected intraperitoneally. Our preliminary data are consistent with a single injection causing an increase in myeloid cells with a corresponding decrease in precursor cells.

The third potential use of the cytokines is as adjuvants to increase the efficacy of deliberate immunization programs in adult (mature) chickens. It is widely known that the initiation of an immune response is dependent on macrophage/myeloid lineage antigen presenting cells. Boosting the levels of such cells at the time of immunization will lead to a more rapid and more effective immune response (Figure 1).

## VII. CONCLUSIONS

In summary, using the success of human cytokines as a model a similar strategy has been adopted for boosting the immune defence mechanisms of chickens through application of haemopoietic cytokines. However, an absolute requirement of such a project is the initial identification and extensive characterization of the chicken haemopoietic growth factors. Their function, alone and in combination, must first be evaluated using *in vitro* systems, prior to *in vivo* injection. These issues have been addressed and studies have now developed to the point where it is possible to inject developing eggs, and newly-hatched and adult chickens with a combination of SCF and cMGF to promote defence against infection. In the near future it is hoped to complement these studies with recombinant SSL-1 and BSL-2 derived cytokines. Thus the ultimate aims of this project are to better understand the mechanisms of immune system function as a basis for formulating a more rational approach to immunization of adult chickens and to enhance the development and maturation of immune defence mechanisms in embryos and newly hatched chickens, thereby reducing any weight loss resulting from opportunistic infection.

The ability to manipulate the chicken haemopoietic and lymphoid systems by precise application of highly purified cytokines should facilitate a marked improvement in health status immediately after hatch. It should also provide a rational approach to defence against disease. Given the world-wide potential for this technology, substantial financial benefits should also be derived from international sales. Sequence data from the chicken cytokines will also be used to identify the mammalian equivalent molecules. It is worth emphasising that the annual predicted market for mammalian granulocyte-colony stimulating factor is $A20 million for Australia and New Zealand for 1995, with world-wide market sales expected to exceed $A1 billion annually. Such figures reflect the importance of haemopoietic growth factors in disease resistance.

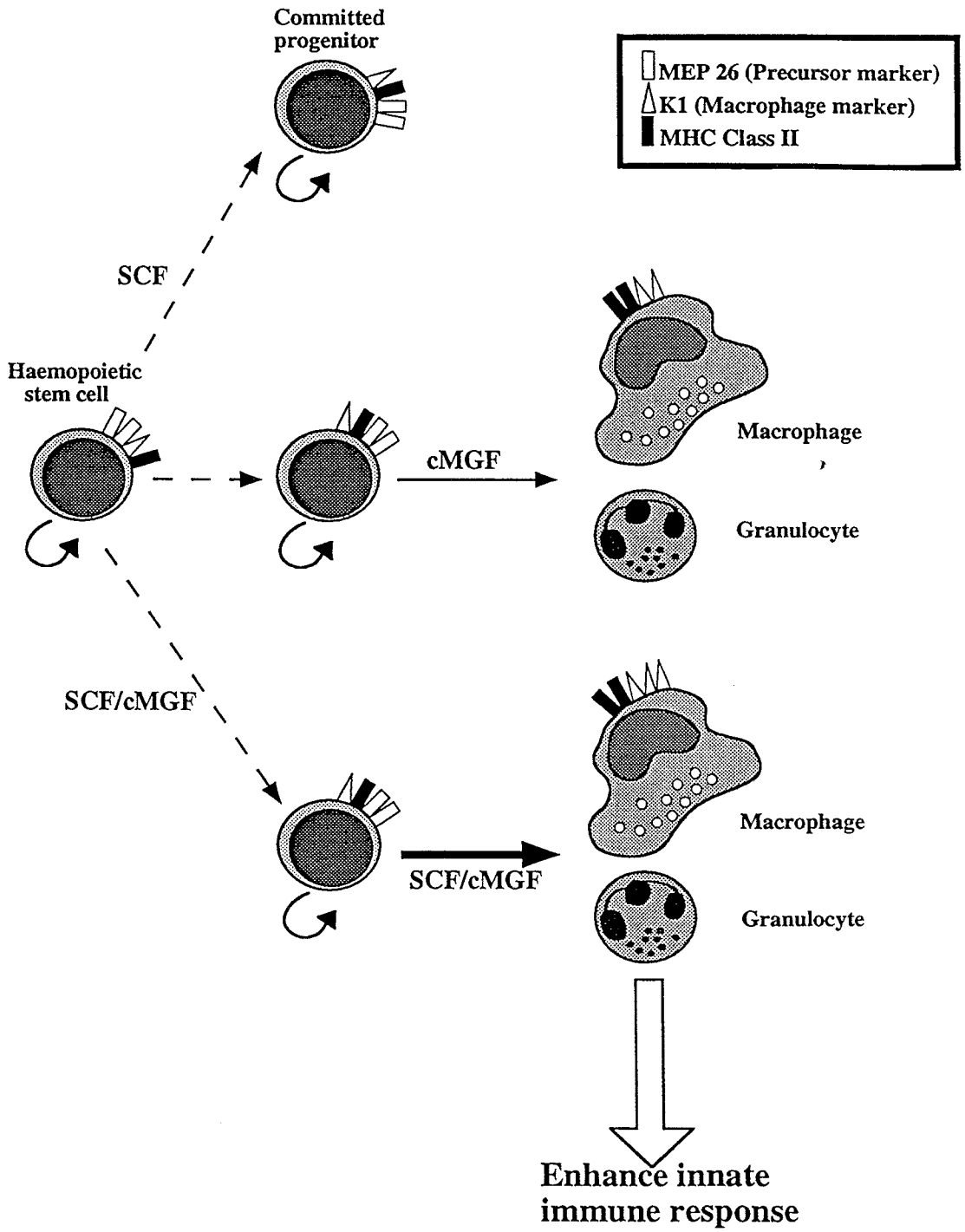


Figure 1. Speculative model of the effects of cMGF and SCF and their combinations on avian haemopoiesis. This diagram illustrates the hypothesis that both cMGF and SCF induce the self-renewal of precursors (curved arrows). However, SCF acts on an earlier precursor while cMGF acts on a committed GM precursor. Thick solid arrows indicate high degree of differentiation, the thin arrows a lower degree of differentiation. A dashed line assumes a hypothetical pathway in which a pluripotential stem cell can give rise to the various precursor subsets.

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# EGG SHELL QUALITY AND ULTRASTRUCTURE RESPONSES TO DIFFERENT CAGE DENSITIES AND CAGE MODIFICATIONS

C.E. BRACKPOOL

The construction of the egg shell and, in particular, the ultrastructure of the mammillary layer of the egg shell, provides an indirect indicator of egg shell quality.

Egg shell quality and egg shell ultrastructure were examined in egg shells collected from laying hens housed at various cage densities or in different forms of a modified commercial cage system. The egg shells from the cage density experiment were provided by Mr. R.J. Bishop, West Australian Department of Agriculture, and Mr. R.J. Hughes, South Australian Department of Agriculture. In this experiment, Tegel Queen and Tegel Brown laying hens were housed at three birds per cage at densities of 450, 525, 650 and 750 $cm^2$ per bird. The egg shells from the modified cage experiment were provided by Mr. R.J. Hughes and Dr. P.C. Glatz, South Australian Department of Agriculture, and Dr. J. Barnett, Victorian Department of Agriculture. Tegel Brown and Tegel Tint laying hens were housed either in a standard commercial cage system or in one of three types of modified cages. The modified cages had either solid side partitions, the inclusion of a perch, or both (solid side partitions and a perch). Measurements of egg shell quality and egg shell ultrastructure were conducted according to Brackpool *et al.* (1994).

The responses to differences in cage densities differed between the two strains examined. In the Tegel Brown hens, reducing the density from 450 $cm^2$ generally resulted in thicker shells with a greater specific gravity, but had no effect on the ultrastructure of the egg shells. In contrast, the reduction in cage density with the Tegel Queen hens had no effect on the traditional parameters of egg shell quality, but affected the ultrastructure of the egg shell. For the hens at the highest stocking density the egg shells exhibited more type B bodies. Type B bodies have been reported previously in poor quality egg shells (Bunk and Balloun, 1978; Koga *et al.*, 1992; Solomon, 1991).

Similarly, the effect of modified cages had a variable effect on the two strains examined. For the Tegel Brown hens, the eggs were smaller and the shells lighter with hens housed in the modified cages. In this strain of laying hen, the egg shell of hens housed in the cages with solid side partitions and perches had the highest incidence of type B bodies, whereas the egg shells of hens housed in cages with only the solid side partitions had the lowest incidence of type B bodies. The eggs were significantly larger with the Tegel Tint hens housed in the cage with a perch and these egg shells had a greater incidence of aragonite. Tegel Tint hens housed in the cages with the perch and with solid side partitions had the poorest mammillary cap quality, while the egg shells of the hens housed in the standard commercial cage had the best mammillary cap quality.

The effect of modified cages and different cage densities on egg shell quality and ultrastructure varied depending on the genotype.

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# ENVIRONMENTAL FACTORS INFLUENCING THE NUTRITIVE QUALITY OF WHEAT FOR POULTRY

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The apparent metabolizable energy (AME) of wheat varies greatly, with some values as low as 9.2 MJ/kg dry matter (DM). Low AME wheats cause production losses due not only to their physiological effect in poultry but also to the unpredictable nature of their occurrence which makes least cost formulation difficult.

To establish the factors affecting the nutritive quality of wheat for poultry, a total of 81 samples from across Australia over three harvest seasons (1991, 1992 and 1993) were surveyed for their AME and non-starch polysaccharide (NSP) contents. The AME was determined by total collection and the NSP were measured by gas chromatography using the alditol acetate technique. The AME and NSP values differed widely, with the former ranging from 9.2 MJ to 15.0 MJ/kg DM and the latter from 81.3 g to 156.8 g/kg DM.

Table Summary of the apparent metabolizable energy (AME; MJ/kg DM) and non-starch polysaccharide (NSP; g/kg DM) contents of wheats.

| Variables | Year | n | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|---|
| AME | 1991 | 27 | 12.1 | 1.4 | 9.2 | 14.3 |
| | 1992 | 38 | 13.4 | 0.9 | 11.1 | 15.0 |
| | 1993 | 16 | 13.4 | 1.0 | 11.6 | 14.5 |
| NSP | 1991 | 27 | 121.2 | 13.4 | 97.0 | 156.8 |
| | 1992 | 38 | 96.0 | 11.9 | 81.3 | 129.3 |
| | 1993 | 16 | 98.3 | 6.2 | 88.9 | 108.3 |

For the 27 samples obtained from the 1991 harvest, the average AME value was markedly lower and the NSP content significantly higher than that of the 38 samples obtained from the 1992 harvest. This coincided with the fact that 1991 was dry during harvest whereas 1992 was wet. The AME and NSP values for the 1993 harvest were similar to those of 1992, and it is worth noting that all 16 samples came from South Australia, West Australia and Victoria, and these regions had a normal harvest in 1993.

High temperature and low rainfall during grain filling can affect the enzymes in the developing endosperm in wheat (Hawker and Jenner, 1993). Also, a wet condition during grain maturation dramatically decreases the extract viscosity of barley whereas a dry condition elevates it (Aastrup, 1979). An elevated extract viscosity indicates a high content of NSP in the cereal, which is inversely related to the nutritive value (Choct and Annison, 1990). It is envisaged that under heat stress an increased amount of the carbon is used for cell wall polysaccharides as opposed to starch, which may be the manifestation of the natural defence system of the plant. Therefore, climatic conditions during grain filling appear to be an important factor influencing the nutritive quality of wheat for poultry.

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# A LONG-CHAIN HYDROCARBON MARKER FOR DIGESTIBILITY STUDIES IN POULTRY

M. CHOCT* and R. J. HUGHES**

Summary

The suitability of a long-chain hydrocarbon, hexatriacontane ($C_{36}H_{74}$), as a digestibility marker in poultry has been investigated. The concentrations of the acid-insoluble ash and hydrocarbon markers in the ileal and excreta samples were closely correlated and the estimations of ileal and faecal dry matter digestibilities were similar. Dry matter digestibility values estimated by the marker technique were identical with those determined by the classical total collection method.

## I. INTRODUCTION

Estimation of nutrient digestibilities, either in digesta or in excreta, by indirect measurement has long been of great interest to animal nutritionists. It is usually achieved by including a marker in the test diet. The markers must be inert with no metabolic and physiological consequences in the alimentary tract of the animal, and preferably: 1) mix intimately with the feed and remain uniformly distributed in the digesta, and 2) be easily and precisely measured. The most frequently used markers are solid-phase makers such as chromic oxide and acid-insoluble ash (AIA), but liquid-phase markers such as Cr-EDTA and polyethylene glycol (PEG) have also been used when the digestibility of a specific soluble nutrient is needed.

Chromic oxide has been used widely as a digestibility marker in both ruminant and monogastric species. Problems associated with using chromic oxide include, incomplete recovery (Vohra and Kratzer, 1967), difficulty in homogenous mixing and in colorimetric determination (Vohra, 1972). The other commonly used marker is acid-insoluble ash (AIA). This technique was first introduced by Vogtmann *et al.* (1975). The recovery of AIA has been reported to be 99.7% (Mollah, 1982). Acid-insoluble ash, although easy to determine, requires relatively large amounts of sample (with a 2% inclusion in the diets, 1 g for excreta and 2-3 g for diets) for a precise measurement of the marker levels. It is also worth noting that chromic oxide is regarded as chemical waste.

Hydrocarbons are naturally present in plants and are not utilized or metabolized in the digestive tract of animals (Dove and Mayes, 1991). Long, even-chain hydrocarbons, such as hexatriacontane ($C_{36}H_{74}$) and tetratriacontane ($C_{34}H_{70}$), are readily available commercially and can be used as digestibility markers in pig and poultry diets. As these compounds are soluble in warm oil they can be mixed easily into the diet and the inclusion of as little as 0.2 g/kg is adequate for precise measurement of the marker.

This paper presents results of an experiment assessing the suitability of a long-chain alkane, hexatriacontane ($C_{36}H_{74}$), as a digestibility marker in poultry.

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## II. MATERIALS AND METHODS

### (a) Diet and bird trial

A wheat-based diet was formulated as outlined in Table 1. Hexatriacontane and celite (as a source of AIA) were added to the diets at 0.2 g/kg and 20 g/kg, respectively. The hydrocarbon was first added to the tallow and then mixed with the other ingredients. The diets were cold pelleted. A total of 48 three-week old broilers were divided into two random groups and kept in individual cages. The birds were fed the trial diets for 7 d. Feed intakes and excreta outputs were quantitatively recorded during the last 4 d of the experiment. After the last collection of excreta, the birds were killed by intravenous injection of Nembutal. The contents of the ileum (from Meckel's diverticulum to 4 cm above the ileo-caecal junction) were collected and freeze-dried for marker determinations.

Table 1. Composition of Diet

| Ingredient | g/kg |
|---|---|
| Wheat | 680 |
| Meat & bone meal | 76 |
| Soybean meal | 170 |
| Fat | 40 |
| NaCl | 2.5 |
| L-lysine | 2.5 |
| DL-methionine | 3.0 |
| Vit. Min premix | 5.0 |
| Choline chloride(50%) | 0.8 |
| Celite | 20 |
| Hexatriacontane | 0.2 |

### (b) Marker determinations

*Acid-insoluble ash*: Diet (2-3 g) and digesta (1 g) samples were dried, accurately weighed into sintered glass crucibles (porosity 4), ashed (480°C, 8 h) and then immersed in boiling 4 M HCl for 15 min. The residue was washed, dried (105°C, 6 h), treated with 4 M HCL once more as described above and collected as the acid-insoluble ash.

*Hydrocarbon*: To 100-500 mg freeze-dried samples, appropriate amounts (50-200 mg) of internal standard ($C_{34}H_{70}$ in dodecane) were added. The samples were then subjected to 1.5M ethanolic KOH in a heating-block at 90°C for 1h with stirring. After cooling, the hydrocarbons were extracted in n-hexane several times, filtered, purified and quantified by gas chromatography.

## III. RESULTS

To assess the validity of using hexatriacontane as a digestibility marker, it was compared with an established marker, AIA, in both ileal digesta and excreta from broilers. There were close correlations between the AIA and hydrocarbon levels both in ileal digesta ($r=0.80$; $r^2=0.63$; $P<0.01$) and excreta ($r=0.95$; $r^2=0.91$; $P<0.01$) (Figure 1). When the ileal and faecal digestibilities of dry matter were calculated using the AIA and

hydrocarbon markers, the results were identical. The digestibility values also agreed closely with the value obtained using the total collection technique (Table 2).

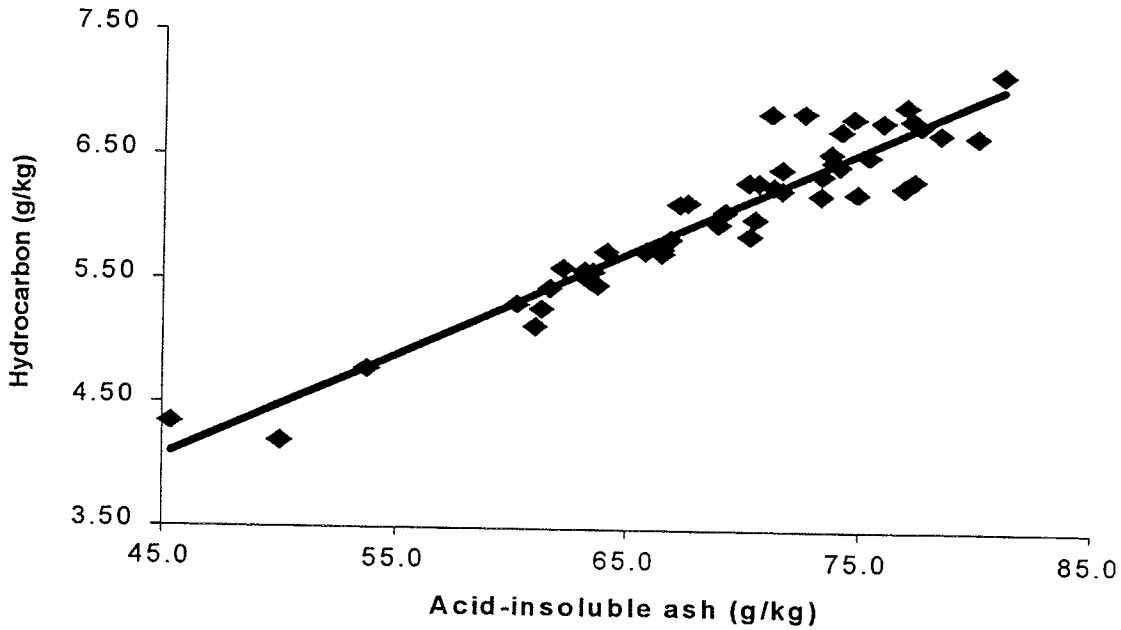


Figure 1. The correlation between acid-insoluble ash and hydrocarbon (hexatriacontane, $C_{36}H_{74}$) levels in the excreta of broilers.

Table 2. Dry matter (DM) digestibility coefficients calculated using the total collection technique, acid-insoluble ash and hydrocarbon markers (n=48; means±SD).

| Technique | Ileal | | Faecal | |
|---|---|---|---|---|
| | Diet 1 | Diet 2 | Diet 1 | Diet 2 |
| Total collection | | | 0.68±0.04 | 0.72±0.02 |
| Acid-insoluble ash | 0.66±0.06 | 0.68±0.07 | 0.68±0.04 | 0.70±0.01 |
| Hydrocarbon marker | 0.66±0.08 | 0.71±0.04 | 0.67±0.04 | 0.71±0.01 |

## IV. DISCUSSION

Hydrocarbons have been used to estimate the intakes of grazing animals (Mayes *et al.*, 1986; Dove and Mayes, 1991). They are normal constituents of plants and are not utilized in the gastrointestinal tract of animals. The faecal recoveries of the long-chain alkanes $C_{35}H_{72}$ and $C_{36}H_{74}$ have been reported to be 94.8% and 94.7% in sheep, respectively (Dove and Mayes, 1991). Experiments in pigs and poultry have shown that the long-chain hydrocarbons are almost totally recoverable (Choct, van Barneveld and Hughes, unpublished data) but further confirmation is required.

The current results indicate that long-chain hydrocarbons can be used as digestibility markers in poultry. In comparison with a well-established marker, AIA, hexatriacontane worked well in measuring dry matter digestibility in both ileal and faecal samples although the correlation with AIA in the ileum was lower than in the excreta. This was perhaps due to the different physicochemical properties of the two markers. Like AIA the hydrocarbons are basically a solid phase marker. There is, however, a subtle difference between them: ie, the hydrocarbons may also associate with the lipid phase of the digesta. A slight separation of the solids and the lipid micelles in the digestive tract may upset the correlation between the two markers in the ileal samples, especially when the difference in the digestibility values are small. The digestibility values obtained using the AIA and the hydrocarbon marker, however, were identical in both the ileum and the excreta. Furthermore, the values for the excreta samples agreed perfectly with those calculated using the total collection technique.

The long chain hydrocarbons offer an attractive alternative to AIA and chromic oxide as a digestibility marker.

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# CHANGES OCCURRING IN AVIAN KIDNEYS IN RESPONSE TO OCCLUSION OF A URETER

I. COULON and J.R. ROBERTS

Wideman *et al.* (1983) studied laying hens during a urolithiasis outbreak and found macroscopic kidney lesions. In many cases, uroliths (kidney stones) had blocked the ureters completely and this had led to atrophy of some sections of kidney tissue and hypertrophy of other parts of the kidneys. These authors hypothesised that the observed kidney pathology was due to an initial blockage of a ureter by a urolith followed by atrophy of the kidney upstream from the blockage and hypertrophy of remaining kidney tissue. This response is similar to that which is seen in mammals in response to a reduction in functional renal mass. Wideman and Laverty (1986) were able to reproduce these effects by ligating the ureters of chicks. Subsequent studies by Wideman and coworkers have shown that a similar response can be obtained by simply severing the ureter.

The aim of the present study was to investigate the changes which occur in the kidneys of birds following the severance of the ureter of one kidney. Day-old male chicks (White Leghorn x New Hampshire) were obtained from a commercial hatchery and transferred to a brooder at the University of New England. Surgery was performed at 2-3 weeks of age. Birds were anaesthetised with halothane gas, a small incision was made in the right flank, the organs of the digestive system were retracted and the right ureter severed at the level of the ischiadic artery (between the medial and caudal kidney divisions) using a fine-tipped pair of forceps and a stereo microscope. The incision was closed with haemostatic wound clips and Terramycin was administered. The sham-operated controls received the same treatment except that the ureter was not severed.

Birds were allowed to recover and then returned to the brooder. Birds were then sampled at 1, 2, 4, 6, 10 and 14 weeks following the initial surgery. At each of these times, 5 birds were selected for glomerular counts and 5 for histological examination of the kidney tissue. For estimates of numbers and sizes of glomeruli, birds were anaesthetised with diallyl barbituric acid, a wing vein was cannulated and a 0.25% Alcian blue solution in 2.5% mannitol was infused for at least 30-60 min. Birds were sacrificed and kidneys removed and placed in ethanol with ammonium hydroxide. At a later time kidneys were digested in 20% hydrochloric acid, suspended in water and aliquots placed in a Sedgewick-Rafter cell for counting under a microscope with a calibrated eyepiece. Tissue taken for histological examination was processed using standard techniques.

The results indicated that new nephrons are formed in this strain up to 5 weeks of age, with subsequent kidney growth accomplished by enlargement of existing nephrons. In the chickens with the severed right ureter, the formation of new nephrons was arrested in both kidneys during the first 2 weeks post-surgery. There was some loss of existing nephrons in the kidney tissue upstream from the ureteral severance (atrophy) with these nephrons being replaced by connective tissue and fat. Two weeks after the surgery the kidney tissue downstream from the severed ureter, and the contralateral kidney, underwent hyperplasia followed by hypertrophy to compensate for the loss of functional renal mass.

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# ENZYMES FOR LAYER DIETS

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Summary

Enzymes are not normally added to layer diets because adult animals are not expected to respond positively to enzyme supplementation. To investigate the validity of this assumption a study of energy availability was carried out using laying hens. This demonstrated similar, but lower, increases compared to those seen with broilers. Diets formulated using the increased energy factors were tested against positive and negative controls to validate the findings of the enery balance trials.

## I. INTRODUCTION

There is a considerable body of literature dealing with the energy requirements of laying hens and, to a lesser extent, that of meat-type breeders. Leclercq (1987) summarised nine studies of the effect of dietary metabolizability energy (DME) on daily egg output and mean egg weight. He concluded from an analysis of the trials that there was no significant effect of DME on mean egg output. However, mean egg weight was significantly enhanced by increased DME (0.5 % increase in egg weight per 1 MJ/kg). He concluded that, in these studies, the leghorn type layers did not regulate energy intake as had previously been supposed. He also concluded that a reduction in DME, although reducing excessive body weight gain, will always have a negative effect on egg numbers and possibly egg weight.

Enzyme supplementation of laying hen diets is not widely practised due possibly to the observation that adult birds are able to extract more nutrients from feed than immature birds. Practical experience with laying hens has yielded conflicting results. Only in some cases has a better feed conversion been observed. Secondary effects such as reduced numbers of dirty eggs (when wheat was incorporated in the diet) have indicated that the enzymes have been active and that the lack of clear results is due to another mechanism.

It was, therefore, decided to study energy availability of raw materials in laying hens and to design an evaluation experiment which would be sufficiently rigorous to demonstrate the effects of any extra energy liberated by the action of exogenous enzymes. It was hypothesised that in diets already optimised for energy, generation of additional energy (through enzyme addition) would not be evident and this is why previous attempts to employ enzymes have not given clear results.

## II. METHODS

### (a) Determination of nitrogen-corrected apparent ME (AMEn) in laying hens

The AMEn of different feed raw materials was determined following the European Reference Method (Bourdillon *et al.*, 1990) using 8 replicates of individually-housed laying hens of approximately 40 weeks of age per dietary treatment. The MEn content of the experimental diets was calculated from their respective E(excreta)/F(feed) ratios (i.e. the ratio between the dry matter (DM) fed and the DM recovered in the faeces) as well as their corresponding gross energy (GE) contents (Huyghebaert, 1993a). No correction was made

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for endogenous secretions or metabolic losses. The basal diet was composed of sorghum (56 %), soyabean meal (32 %) and animal fat (6 %). For determination of layer energy availability with wheat, the basal diet was combined with wheat (50:50 w/w). For peas and soya bean meal the ratio was 75 % basal diet and 25 % soyabean meal or peas.

Previous studies with broilers (Huyghebaert 1993a,b; 1994 a,b) had shown that the basal diet would not react to xylanase supplementation and so all enzyme effects were assumed to be exerted on the added material and the AMEn was calculated accordingly. To determine the effect of added microbial enzymes on energy availability, Bio-Feed Plus CT (an endoxylanase) was added to wheat at 1 g/kg and Energex MG (a pectinase complex) or

Table 1. Diet composition (g/kg) of layer diets used in the feeding trial.

| Raw Materials | Control | Modified Diet | Modified diet + Enzyme |
|---|---|---|---|
| Oats | - | 100 | 100 |
| Maize | 341 | 200 | 200 |
| Wheat | 250 | 290 | 290 |
| Fish meal | 53 | 45 | 45 |
| Meat meal | 35 | 50 | 50 |
| Sunflower seed | 23 | - | - |
| Soy bean meal | 87.5 | 83.5 | 83.5 |
| Grass meal | 50 | 50 | 50 |
| Animal fat | 23 | 27 | 27 |
| Calcium carbonate | 85 | 84 | 84 |
| Mineral Premix | 3.0 | 17.5 | 17.5 |
| Sodium bicarbonate | 2.5 | 2.5 | 2.5 |
| Premix | 17.5 | 16.5 | 16.5 |
| Lysine premix | 1.0 | 3.0 | 3.0 |
| Methionine premix | 18.5 | 21.0 | 21.0 |
| Threonine | - | 0.20 | 0.20 |
| Enzyme | - | - | 0.45 |
| Energy (MJ/kg) | 11.9 | 11.44 | 11.44 |
| Lysine (g/kg) | 8.8 | 8.8 | 8.8 |
| M + C (g/kg) | 7.7 | 7.7 | 7.7 |
| Phosphorus (g/kg) | 6.1 | 6.0 | 6.0 |
| Sodium (g/kg) | 1.63 | 1.63 | 1.63 |
| Chloride(g/kg) | 1.39 | 1.39 | 1.39 |

Bio-Feed Plus CT were added at 2 g/kg to soyabean meal or peas. Statistical analyses of the results from the balance trial were made using a one-factorial analysis of variance according to Statgraphics Version 5 (1991).

(b) Layer Trial

The energy values for the raw materials determined by balance trials were then used in the formulation of layer diets using a least cost formulation programme (Agrosoft, Denmark). A commercial layer diet was used as a positive control (Table 1).

Using the digestibility improvement factor (DIF) system for energy upgrading (Cowan *et al.*, 1994), in which the new energy values are used as a basis for the formulation, a revised diet was formulated. This diet was prepared with and without the addition of a xylanase (Bio-Feed Plus CT) at a dose rate of 1.2g/kg of wheat and oats in the formulation.

The trial was carried out at the Schweizerische Geflügelzuchtschule using 2 pens of 180 layers for the positive control diet and 3 pens of 180 layers for each of the modified diets. As previous experiments had indicated little direct effects of enzyme supplementation to existing diets, this was omitted from the trial plan.

## III. RESULTS

The results for energy and nitrogen retention for peas, soya bean meal and wheat are shown in Table 2.

Table 2. Influence of enzyme supplementation on raw material AMEn in layer hens.

| Raw Material | Enzyme addition | Nitrogen Retention (kJ/kg) | AMEn (MJ/kg) |
|---|---|---|---|
| Peas | - | 384 ± 35 | 11.227 |
| Peas | Energex MG | 481 ± 36 | 11.740 |
| Peas | Bio-Feed Plus CT | 430 ± 72 | 11.648 |
| Soya bean meal | - | 410 ± 52 | 9.212 |
| Soya bean meal | Energex MG | 412 ± 51 | 9.904 |
| Soya bean meal | Bio-Feed Plus CT | 420 ± 64 | 9.668 |
| Wheat | - | 305 ± 29 | 11.519 |
| Wheat | Bio-Feed Plus CT | 343 ± 23 | 11.870 |

The average results from the laying hens receiving the three feeds after 13 months of laying are shown in Table 3.

Table 3. Response of laying hens to dietary modifications.

| Treatment | Food intake (g/d) | Egg weight (g) | FCR (g:g) | Rate of lay (%) | Downgrades (%) |
|---|---|---|---|---|---|
| +ve Control | 116.3 | 67.9 | 2.27 | 75.5 | 9.0 |
| Modified Diet | 117.6 | 67.9 | 2.31 | 75 | 11.1 |
| Modified Diet + Xylanase | 118.9 | 68.5 | 2.26 | 76.0 | 8.5 |

## IV. CONCLUSIONS

The addition of enzymes to raw materials commonly used in layer diets resulted in an increased availability of energy. This energy increase was similar in percentage terms (but slightly lower) to that seen when the same enzymes were tested in broiler chickens (Huyghebaert and de Groot, 1995). The age dependence previously supposed to exist for enzyme effects was not as pronounced in these trials. In addition, the energy values recorded were not always similar to those presented in official reference publications. This indicates that there is considerable year to year and type to type variation in raw material quality.

When the new energy figures were used to formulate layer diets, a reduction in efficiency was seen in the negative control diet in which feed intake was increased to maintain egg weight and laying percentage. The modified diet plus enzyme resulted also in a slightly higher daily intake but this was accompanied by an increased egg weight and laying percentage. While these results are still preliminary and further studies need to made we can conclude that enzymes have a place in improving nutrient availability in layer as well as broiler diets.

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# *SACCHAROMYCES CERVISIAE* AND MANNAN OLIGOSACCHARIDES TO COUNTERACT AFLATOXICOSIS IN BROILERS

G. DEVEGOWDA, B.I.R. ARAVIND and M.G. MORTON

Summary

Two broiler trials and two *in vitro* studies were conducted to evaluate the effect of *Saccharomyces cerevisiae* (SC) and mannan oligosaccharides (MOS) in counteracting the adverse effects of aflatoxins (AF). The two broiler trials involved supplementation of SC at three levels to diets containing AF which effectively reversed the toxicity symptoms elicited. *In vitro* trials to examine the AF binding ability of SC and MOS were conducted separately. The results showed binding of AF to the extent of 88% by SC and 80% by MOS. These results demonstrate that aflatoxicosis in broilers can be effectively counteracted by dietary supplementation with SC and MOS. The effect observed with SC is possibly due to the ability of SC to bind AF on the surface of the yeast cell wall.

## I. INTRODUCTION

The adverse biological and economical effects of AF on poultry and other livestock are well documented (Hamilton, 1987). Numerous strategies for the detoxification of AF in feeds have been tried including chemical methods, physical separation, irradiation and thermal inactivation (Devegowda, 1989; Barmase and Devegowda, 1990). Biological detoxification *in vitro* by *Flavobacterium aurantiacum* on AF (Ciegler *et al.*, 1966) and binding of zearalenone *in vitro* by MOS has been reported (Trenholm *et al.*, 1994). The current study examined the effect of SC supplementation to broiler diets containing AF and *in vitro* studies on the ability of SC and MOS to bind AF.

## II. METHODS

The first experiment involved three levels of SC (0, 1 and 2 g/kg) added to broiler diets containing 500 and 1000 μg AF/kg. The second experiment was undertaken to confirm the results of the first experiment using a larger number of birds. The treatments consisted of a negative control, 500 μg AF/kg and 500 μg AF/ kg + I g SC/kg in the diet. The third experiment, was an *in vitro* study using three levels of SC (0, 0.5 and 1.0 g/kg) and two levels of AF (250 and 500 μg/kg) in Sabouraud's broth at three different incubation periods (48, 72 and 96 h). In the fourth experiment, an *in vitro* study, AF B1 was prepared in aqueous solution at 250 and 500 μg/L concentrations, each in two sets of pH (4.5 and 5.8 respectively) to simulate *in situ* gastrointestinal tract pH levels. MOS was added as a suspension of 500 mg and 1000 mg respectively with controls maintained for both concentrations of AF B1 and pH levels.

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## III. RESULTS

In the broiler trials, toxicity due to AF was characterised by significant reductions in body weight, feed efficiency, relative weight of Bursa of Fabricius, serum protein and haemagluttination inhibition titre against Newcastle disease and by significant increases in mortality and relative liver weights. The trend was reversed upon SC supplementation to the diets containing AF (Table 1).

Table 1. Effect of *Saccharomyces cerevisiae* supplemented to diets containing aflatoxin.

| Aflatoxin ( μg/kg) | 0 | 500 | 500 |
|---|---|---|---|
| *Saccharomyces cerevisiae* (g/kg) | 0 | 0.0 | 1.0 |
| Body weight (g) | 1049[a] | 912[b] | 1308[c] |
| Feed : Gain (g:g) | 2.27[a] | 3.00[b] | 2.36[a] |
| Mortality (/100 birds) | 3.87[a] | 38.40[b] | 3.30[a] |
| Liver (g/100 g body weight) | 2.65[a] | 3.40[b] | 2.87[a] |
| Bursa of Fabricius (g/100 g body weight) | 0.32[a] | 0.20[b] | 0.29[a] |
| Total serum protein (g/dL) | 2.68[a] | 2.12[b] | 2.56[a] |
| Haemagluttination inhibition titre (Log2 value) | 2.46[a] | 1.74[b] | 2.62[a] |

[a-c] Values with different superscripts are significantly different at $P < 0.05$.

In both the *in vitro* experiments AF was extracted and quantified using the CB method (AOAC, 1990). In the experiment using SC, regardless of AF levels, the pattern of binding was uniform with only minor variations. There was a gradual increase in the amount of AF bound as the time of incubation increased, from 50 % after 48 h to 88 % after 96 h (Figure 1). In the experiment using MOS, regardless of the AF B1 concentration, the binding ability of MOS to AF B1 was more or less the same at pH 4.5. However, at pH 6.8, MOS bound AF B1 at higher levels. Further, there was a gradual increase in the amount of AF B1 bound to MOS at higher levels of MOS inclusion (Figure 2).

## IV. DISCUSSION

The effects of AF on body weight, mortality and feed efficiency in broilers agree with the findings of Huff *et al.* (1986), Umesh and Devegowda (1990) and Kubena *et al.* (1993). The relative liver weight was significantly increased by feeding diets containing AF. Similar findings were reported by Phillips *et al.* (1988) and Umesh and Devegowda (1990). Supplementation with SC had a protective effect on the liver, the target organ for AF toxicity.

The Bursa of Fabricius is the target organ for AF in young birds, leading to lowered production of B-lymphocytes. In the present study SC had a protective effect on this organ. Total serum protein was reduced when AF was in the diet due presumably to inhibition of DNA and protein synthesis (Thaxton *et al.*, 1974). SC supplementation of AF-contaminated diets produced a significant rise in serum protein.

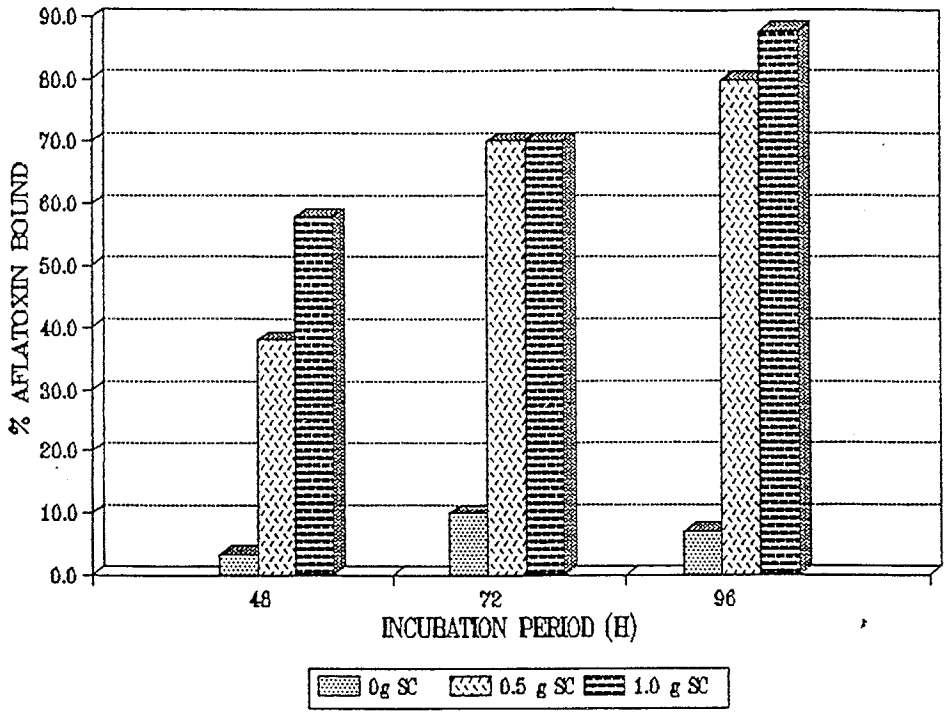


Figure 1. Percentage degradation of aflatoxin (500 ug/kg) by Saccharomyces cerevisiae at different periods of incubation.

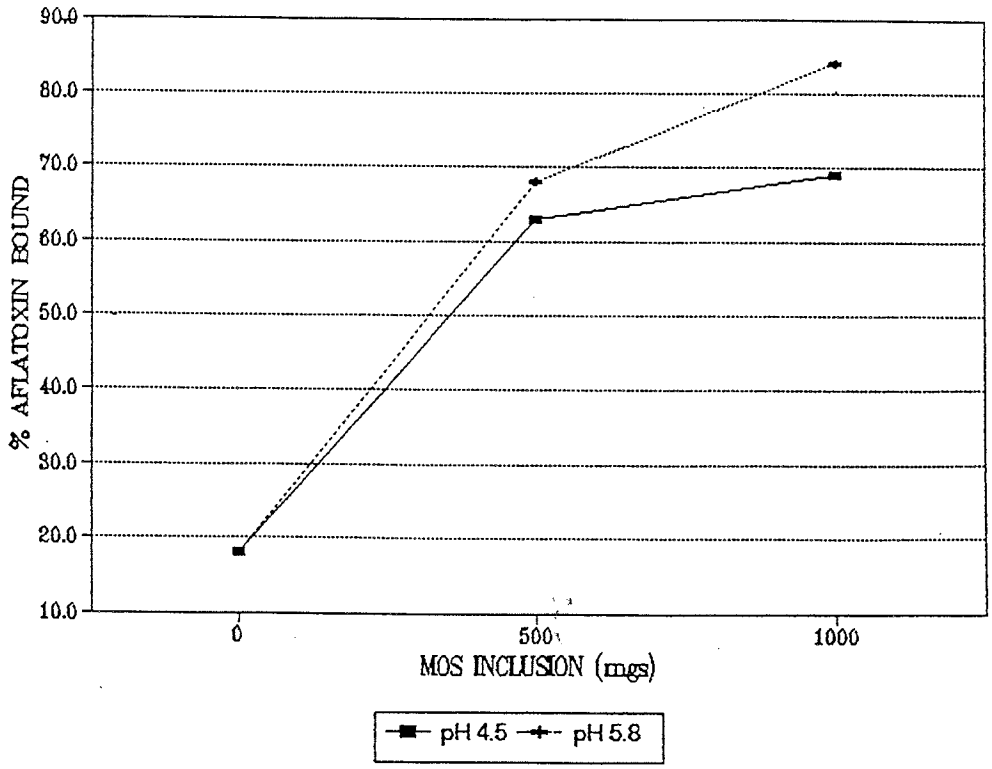


Figure 2. Effect of mannan oligosaccharides (MOS) on the percentage of aflatoxin B1 bound (500 ug/kg) at different pH levels.

Immunosuppression caused by AF has been demonstrated in broiler chickens (Thaxton *et al.,* 1974). SC supplementation of diets containing AF showed a protective effect on haemagluttination inhibition titre values against Newcastle disease which may be due to optimum production of B-lymphocytes since the Bursa of Fabricius was near normal in size.

Additionally, *in vitro* studies revealed binding of AF to the extent of 88 % by SC and 80 % by MOS.

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# ENVIRONMENTAL IMMUNE MANAGEMENT AND SUSTAINABLE POULTRY PRODUCTION

R.R. DIETERT and T.E. MILLER

Summary

This review article focuses on poultry immunotoxicology and the opportunities to enhance the immune performance of poultry in the agricultural setting. The range of environmental factors known to influence the status of the avian immune system is introduced. A consideration of both physical and chemical immunomodulators is included. The paper further discusses the potential benefits of optimized immunity during the management of poultry populations and the need to approach immune assessment in some standardized and predictive manner. Finally, potential strategies for the standardized assessment of poultry immunity are discussed.

## I. INTRODUCTION

Recent information concerning the multiple levels of interaction of the immune system with the neurological and endocrine systems (Marsh and Kendall, 1995) supports the premise that immune assessment can be used to evaluate the overall well-being of humans and animals, including production species. Because the immune system can be influenced by hormonal and neurological factors, and, in turn, controls the health of the animal, knowledge of the immune status of poultry flocks under various environmental management strategies provides a means to simultaneously optimize both sheer productivity and health/well-being. A paradigm shift of poultry management is necessary to achieve integrative productivity in which sheer growth or egg production per hen is optimized in conjunction with health/reduced mortality and animal welfare during production. Such an integrative approach to include animal health in conjunction with productivity would enable the industrial sector to progress in achieving overall environmental sustainability. The recognition that industrial sectors must find management strategies to enable environmental sustainable development has been embraced on a world-wide basis (Schmidheiny, 1992).

## II. ENVIRONMENTAL IMMUNOMODULATION

A wealth of literature describes the numerous environmental factors that are known to influence the avian immune system. The range of factors suggests that virtually all aspects of poultry management afford an opportunity to examine the parameters needed for immune optimization. For example, physical parameters of management such as temperature (Regnier and Kelley, 1981), air quality (McFarland and Curtis, 1989), and housing (Gross, 1985) are all known to influence immune system parameters and to cause immunomodulation.

Within the chemical exposure arena, both formulated poultry diets and potential dietary contaminants represent major sources of poultry immunomodulators. Among dietary factors, vitamins (Sklan *et al.*, 1994; Tengerdy and Brown, 1977), minerals (Pimentel *et al.*, 1991; Marsh *et al.*, 1986), amino acids (Taylor *et al.*, 1992) and dietary fat (Fritsche *et al.*,

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1991) can all influence immune status. Feed contaminants such as aflatoxin-B1 (Dietert *et al.*, 1985), fuminison-B1 (Qureshi and Hagler, 1992), ochratoxin A (Dwivedi and Burns, 1984), and T-2 toxin (Neiger *et al.*, 1994) are of immunological concern. Additional environmental contaminants that are likely to pose a risk to poultry health include lead (Miller and Qureshi, 1992) and the polychlorinated biphenyls (PCBs) (Andersson *et al.*, 1991).

A consideration of environmental immunomodulators also includes infectious agents as a major category. The control of infectious agents offers another opportunity for optimization of poultry immunity. Many viruses, bacteria and parasites produce profound immunomodulation usually altering host resistance to other disease challenges. Examples include Marek's disease virus (Fadley and Witter, 1993), chicken anemia virus (McConnell *et al.*, 1993), infectious bursal disease virus (Giambrone *et al.*, 1997), reovirus (Sharma *et al.*, 1994), *Escherichia coli* (Nakamura *et al.*, 1986) and *Eimeria* (Tellez *et al.*, 1994). However, it is also important to recognize that at least some of the vaccines used to control these infectious diseases can also result in poultry immunomodulation (Montgomery *et al.*, 1991; Friedman *et al.*, 1992). Furthermore, both drugs (Suresh and Sharma, 1992) and vaccine adjuvants (Zacek *et al.*, 1992) can produce immunomodulation. Therefore, it can be argued that a comprehensive approach to poultry disease control needs to include a standardized method of comprehensive immune assessment. This would permit a benchmark methodology for the relative evaluation of various health management options.

Figure 1 summarizes various intervention points in which poultry management can be evaluated relative to the optimization of immune performance and health. The additive benefit of optimization of immunity across the range of intervention points is apparent given the profound immunomodulation that can occur at any single management level.

## III. IMMUNE ASSESSMENT

A significant amount of literature exists concerning the value of immune assessment and the strategies that have been used to standardize immune comparisons. Many of the prior investigations have been oriented to rodent animal models of human health (Dean *et al.*, 1982a,b; Luster *et al.*, 1988; Vos *et al.*, 1989; National Research Council (NRC), 1992; Hinton, 1992, 1995). However, standardized procedures for direct human evaluation have also been recommended (NRC, 1992). Attention to the immune status of animals has also been directed to the agriculture and wildlife arenas. For example, strategies for immune assessment have been introduced for fish (Anderson and Zeeman, 1995; Zelikoff, 1994), poultry (Baecher-Steppan *et al.*, 1989; Dietert *et al.*, 1993, 1995) and for one avian wildlife species (Braune *et al.*, 1993).

The common feature of all immune assessment panels is the inclusion of parameters reflecting both acquired and innate immune functions. This is necessary to ensure that environmental conditions enhancing one immune sector (e.g. acquired immune responses) do not produce significant negative effects in other areas of host defense (e.g. innate immunity). Many of the panels require a large number of assays that could be problematic from a cost-benefit perspective in agriculture (Luster *et al.*, 1988; Hinton, 1992, 1995). However, a recent landmark immunotoxicological risk assessment study (Luster *et al.*, 1992) suggested that a limited number of assays (e.g. 3-5 assays) would be highly predictive for chemically-induced immunomodulation. As a result, the agricultural application of immunotoxicological assessment as it pertains to poultry management should be highly cost-effective. This has resulted in the sample immune assessment panel presented within this paper.

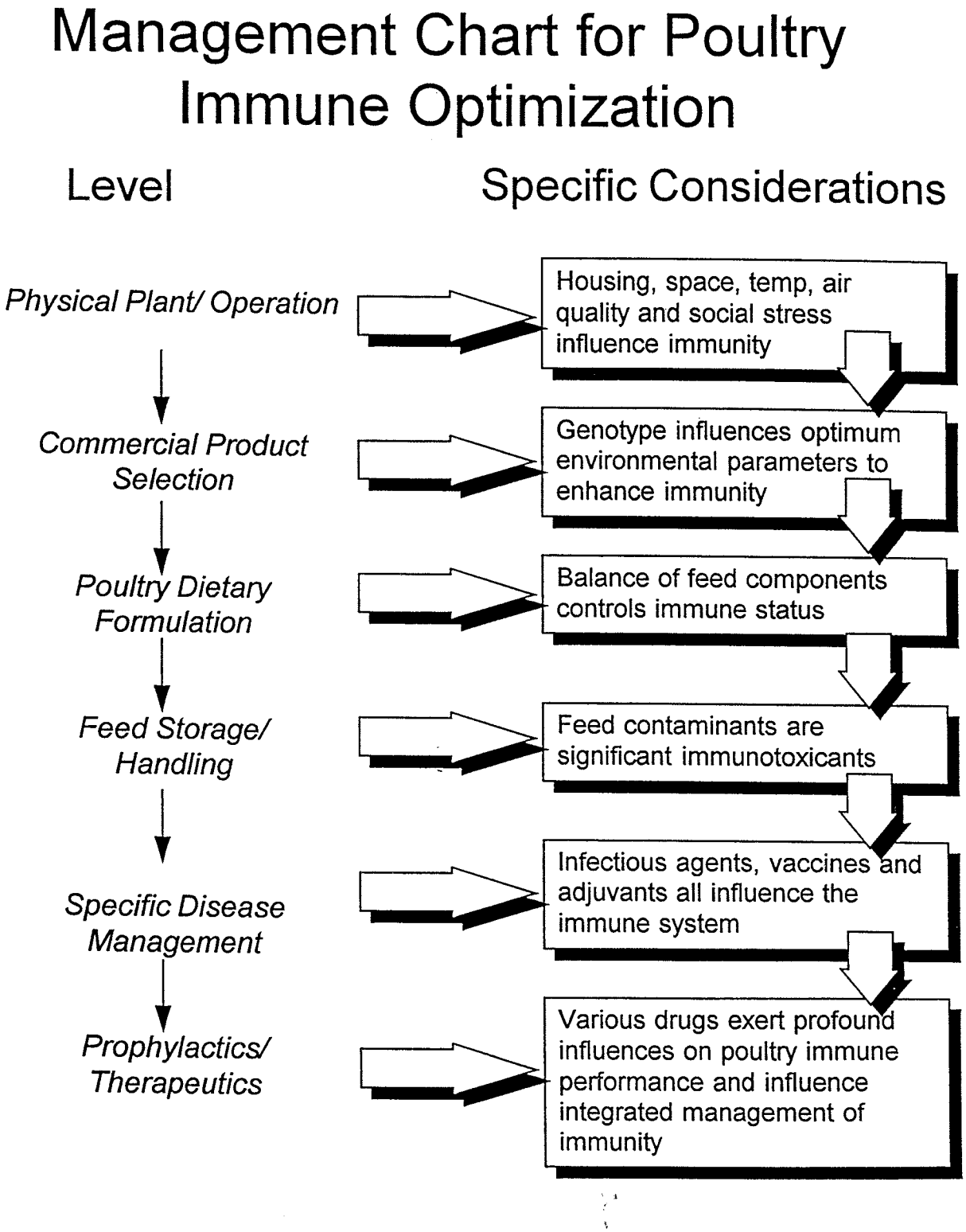


Figure 1. This figure shows a management flow chart in which the various intervention points to control poultry immunity are illustrated. The multiple intervention levels emphasize the need for standardized immune assessment that can be used across the management levels. The information also dramatizes the need for an integrative approach to poultry immune management.

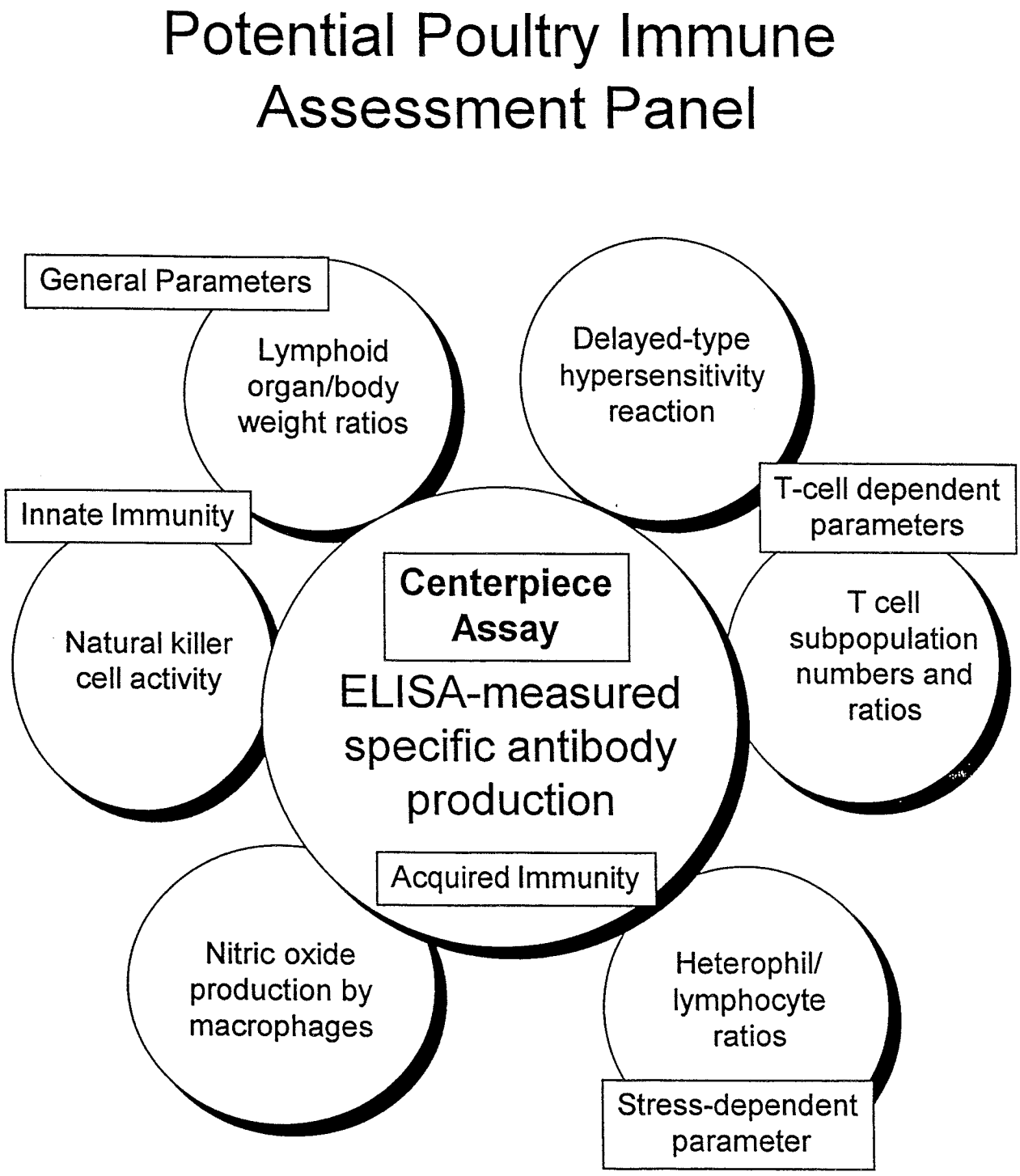


Figure 2. This figure illustrates a sample poultry immune assessment panel that balances economy with predictive assays across the immune system. The centerpiece assay requires multiple immune cell cooperation in a model acquired immune response. Other assays measure different acquired or innate immune functions. Finally, both general measures and neuroendocrine-immune (stress)-influenced measures are included. The information is modified from Dietert *et al.* (1995).

Figure 2 illustrates a potential poultry immune assessment panel that includes assays which combine elements of acquired immunity, innate immunity, general lymphoid organ status and stress-immune interactions.

## IV. SUSTAINABLE AGRICULTURE AND ANIMAL WELFARE

The long-term value of poultry immune assessment lies in the contribution of this decision-making methodology to sustainable agriculture and enhanced animal welfare. For example, the prophylactic rather than therapeutic use of antibiotics in animal feed is recognized as major issue in the public safety arena (Young, 1994; Levy, 1987). It is becoming clear that this element of poultry management does not contribute constructively to sustainable agriculture (D'Aoust *et al.*, 1994; Vasquez-Moreno *et al.*, 1992). However, to shift away from the indiscriminate use of antibiotics to a more integrative approach to poultry health and productivity will require decision-making programs such as comprehensive immunological assessment. Under such a program, the flock-specific selection of physical conditions, feed, vaccines, adjuvants, and therapeutics can be driven by assessment data that cover the range of immunological defense processes.

A second long-term benefit of poultry immune assessment involves the optimization of animal well-being during poultry production. Since the inter-regulatory linkages between the neuroendocrine and immune systems are well established (Blaylock, 1994; Marsh and Kendall, 1995), poultry immune assessment should provide an important measure of the overall well-being of the animals. Recent reviews (Dohms and Metz, 1991; Marsh and Scanes, 1994) have detailed the specific neuroendocrine-immune interactions that would serve as the basis for the immune assessment-animal welfare connection.

Once an immune data base is generated across the management levels shown in Figure 1, the information should facilitate informed decision making by the poultry breeder, feed formulator, engineer, and producer. Conversely, in the absence of information it will be difficult for the poultry industry to achieve an integrated health and welfare approach to poultry production.

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# SIGNIFICANT IMPROVEMENT IN EGG PRODUCTION FROM ENZYME-SUPPLEMENTATION OF HIGH-BARLEY DIETS IDENTIFIED BY INCREASING THE NUMBER OF REPLICATES

J.G. DINGLE and A. KUMAR

Barley (*Hordeum vulgare*) has not been favoured for use in poultry diets as its metabolizable energy (ME) is lower than that of maize and wheat and it tends to cause wet, sticky droppings. These effects have been found to be greater in broilers than in layers and the effects tend to be overcome by adding feed enzymes containing β-glucanases to the diet. Since the effects of β-glucanases in layer diets are small there is doubt about the significance of their effects. In this report two trials are compared, one with six replicates per treatment and the other with 42 replicates per treatment. The results are shown in the Table.

| | 6 REPLICATES | | 42 REPLICATES | |
|---|---|---|---|---|
| Diet | Egg production (/100 bd) | Egg mass (g / d) | Egg production (/100 bd) | Egg mass (g / d) |
| Barley | 76.7 | 45.3 | 71.1 | 43.8 |
| Barley + Enzyme | 83.2 | 49.4 | 78.1 | 47.6 |
| Absolute increase | 6.5 | 4.1 | 7.0 | 3.8 |
| % Increase | 8.4 | 9.0 | 9.8 | 8.4 |
| Significance | $P>0.05$ | $P>0.05$ | $P<0.01$ | $P<0.01$ |

In both trials barley was present in the diet as the sole cereal at over 700 g/kg inclusion. There were differences between the trials in the genotype of the bird and in the enzyme used but the absolute results were similar and allow for the effects of the increases in production from enzyme supplementation to be compared.

Neither the increased egg production nor egg mass output were statistically significant in the trial which used six replicates but the increased egg production and egg mass output were highly significant in the trial which used 42 replicates. In the case of egg mass this was true even though the numerical and percentage increases in the trial using 42 replicates were less than in the other trial.

Therefore, it is recommended that experiments investigating the effects of enzyme supplementation of layer diets ensure that the experimental designs incorporate an adequate number of replicates for each treatment.

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# HOW EFFECTIVE IS 'EFFECTIVE ENERGY' FOR POULTRY?

D.J. FARRELL

Summary

Effective energy is a much more complicated system than the metabolizable energy system. There is a need for more detailed information on ingredient composition and genotypes. This paper examines the components of the prediction equations published recently by Emmans (1994) and attempts to grapple with their bases and underlying principles. It is concluded that, although the new system is a step forward and is a central component of a broiler growth model, the need for much detailed and sometimes new information is likely to preclude its wide use in the near future.

## I. INTRODUCTION

Ever since Davidson *et al.* (1957) and Hill and Anderson (1958) published results showing that the apparent metabolizable energy (AME) of feedingstuffs was less variable than the productive energy (Fraps, 1944) of the same feeds, there has been a preoccupation with methodology of measurement rather than with examining factors that contribute to the utilisation of AME by poultry (Farrell, 1978; Sibbald, 1986).

Attempts have been made to introduce various net energy systems (Nehring and Haenlein, 1973; De Groote, 1974) and it is only recently that complete details of a different system have been published (Emmans, 1994). Much of the theory underpinning Emmans' effective energy system was presented earlier by Emmans and Fisher (1984).

The effective energy system recognises that AME is still the starting point in defining the energy of a feedstuff and that only the organic matter (dry matter - ash component) yields energy. The system also recognises that the AME of a feedingstuff which contains protein should be adjusted to provide energy only and is measured at maintenance. The classical $AME_C$ value is, therefore, corrected to zero protein (nitrogen) retention (NR) by using the equation:

$$AME_n \text{ (kJ/g)} = AME_C - a\ (6.25\ NR) \qquad (1)$$

On the basis that the energy content of nitrogen in urine is 35.2 kJ/gN, the constant for protein 'a' becomes 5.63 kJ. Thus, the end point of the current AME system for poultry is the starting point for the effective energy system. The AME is partitioned into the heat of combustion (energy content) of fat and protein retained in growth and the heat produced as a result of fueling the necessary biochemical processes that 'drive' the system and maintain the animal. Much of Emmans' paper dissects the individual components that contribute to the energy requirement of an animal. These are: heat production and the heat increment of feeding, the fasting animal as a starting point for measuring maintenance, maintenance itself and the requirement for growth and fattening.

It is outside the scope of this paper to examine in detail the theory behind the effective energy system. Instead, the purpose is to describe only the equations used to determine both energy requirements and the energy content of a feedingstuff for broiler growth. The

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effective energy system requires a detailed knowledge of the broiler genotype in relation to rate of lean deposition and a detailed knowledge of the critical components of feedstuffs.

## II. EFFECTIVE ENERGY CONTENT OF A FEEDSTUFF OR DIET (EEF)

### (a) The components

$$\text{Effective energy (kJ/g)} = AME_n - W_d\,FOM - 0.16\,W_u\,DCP + 12\,Z\,DCL \qquad (2)$$

As explained, $AME_n$ (kJ/g) is the starting point of the system. The other three components are faecal organic matter (FOM), or undigested feed organic matter (g/g), digestible crude protein (DCP, g/g), and digestible crude fat (DCL, g/g). All are less than 1.0.

The constants $W_d$, $W_u$ and 12 Z were derived by Emmans from measurements made in careful but limited suitable research work in order to make the necessary calculations.

### (b) Faecal organic matter (FOM)

$W_d$ (= 3.8 kJ) is the energy required to process 1 g of excreta organic matter and represents only the components of a feed that yield energy. It follows that the higher the digestibility of a feed, the smaller the amount of excreta voided and hence the lower will be the cost of processing one kg of that feed. The actual $W_d$ value that Emmans (1994) obtained from analysis of poultry data was 3.27 kJ/kg but this had sufficient variation to be not significantly different from 3.8 found for ruminants.

### (c) Digestible crude protein (DCP)

The assumption here is that all of the protein digested is used for energy purposes only and that all of the N is, therefore, excreted in urine. This is clearly not the case for growing chickens. Thus $W_u$ (29.2 kJ/g) is the heat increment (work) of excreting 1 g N and the factor 0.16 converts the constant to 4.7 kJ/g protein. Adjustment will be made later to N for protein retention when calculating effective energy requirements. There is a need to distinguish between N voided in faeces and that in urine in order to calculate DCP.

### (d) Digestible crude fat (DCL)

In this equation, DCL does not incur a heat increment but there is a positive value added to $AME_n$. The work required to deposit lipid is much less if it comes directly from feed fat (4.4 kJ/g) rather than from protein or carbohydrate (16.4 kJ/g); a difference of 12 kJ. Under normal circumstances the coefficient (Z) is about one third. Hence the heat increment is 0.33 x 12 = 4 kJ. In practice Z will depend on stage of growth and the fat content of the diet.

## III. EFFECTIVE ENERGY REQUIREMENTS (EERQ)

The effective energy requirements of an animal and the effective energy content of a feed ingredient or diet are intimately related. There are three components: the requirement for maintenance (MH), the retention of tissue energy in the form of protein (PR) and fat or lipid (LR) and work (heat increment) associated with these retentions:

$$EERQ\ (kJ/d) = MH + PR\ (h_p - a) + (W_p - 0.16\ W_u) + LR\ (h_l + W_l) \quad (3)$$

(a) Maintenance heat (MH)

Maintenance heat was calculated from detailed chicken slaughter experiments by Hakasson (see Emmans, 1994 for references). Regression equations were then constructed from which the coefficients for PR and LR were determined. These agreed well with coefficients determined for other domestic animals. The intercept value (MH) was the remainder of the heat produced after PR and LR had been accounted for and this ranged from 1.53 to 1.55 MJ/kg body protein. Thus calculation of MH requires a precise knowledge of the feather-free protein content of the chicken at any given time and the protein content of the mature bird of the same genotype at weight stasis. Since body protein (BP) is about 0.20 of bodyweight (Emmans, 1994), this means that a mature broiler chicken (6.0 kgW) will produce MH of 6.0 x 0.20 x 1.53 = 1.84 MJ/d. Since maintenance energy requirement is more closely related to bodyweight scaled to 0.73, MH (MJ/d) is calculated as:

$$1.53BP_m^{0.73}\ u \quad (4)$$

where u is the proportion of BP of the broiler chicken at any weight relative to the mature body protein ($BP_m$) of that genotype

(b) Protein retention (PR)

This component is the work of depositing protein or its heat increment and has two parts:

$$PR = (h_p - a) + (W_p - 0.16\ W_u) \quad (5)$$

where $h_p$ is the heat of combustion of protein tissue (23.8 kJ/g) from which is subtracted the energy content of urinary protein (a = 5.63 kJ/g). $W_p$ is the energy required to deposit 1 g of tissue protein (36.5 kJ/g) and $W_u$, as already noted, is the energy required (29.2 kJ) to excrete 1 g of urinary nitrogen. The term Wp, calculated from a regression equation based on Hakasson's data, is reduced by subtracting the work associated with catabolism of all the urinary nitrogen and balances the correction made previously to DCP (Equation 2). Emmans (1994) uses this correction 'to avoid double counting'. In an earlier report Emmans and Fisher (1986) stated that 'the simplest procedure is to subtract from both the diet and the protein retained the urinary energy that would have resulted if all of the dietary protein was catabolized'. When multiplied the total cost of 1 g of tissue protein gain, which includes both the work needed and the product energy content, is 50 kJ/g. The effective energetic efficiency of the process is 0.48 but in terms of AME it is 0.40 (Emmans, 1994).

(c) Fat retention (LR)

An adjustment has already been made to the effective energy of a diet or ingredient for the proportion of lipid (Z) that is transformed into tissue lipid (0.33). Thus, $W_l$ = 16.4 kJ/g and is a constant. The heat of combustion of tissue lipid (39.6 kJ/g) gives the LR

value of 56 kJ/g. Thus,

$$EERQ\ (kJ/d) = MH + 50\ PR + 56\ LR. \qquad (6)$$

The effective energetic efficiency of lipid deposition is therefore 0.71.

## IV. DISCUSSION

The effective energy system as proposed by Emmans (1994) is a positive step towards the more accurate prediction of bird performance and feed composition than the current AME system. However, it does require detailed knowledge of the feed and the chemical composition of the different genotypes, but this is only to be expected.

There are aspects of the system that need to be tested. First the calculation of the effective energy of an ingredient or diet requires information on chemical composition and the apparent digestibility of some components. These data are difficult to obtain. In practice values are calculated using tables (Anomymous, 1986). The digestibility coefficients for protein are not normally measured directly but from chemical determination of uric acid N in excreta. This is not the only source of N in poultry urine. There is a need to compare calculated values with those determined for a variety of feedingstuffs. Once this has been done the values would be used to compute a diet suitable for a broiler feeding experiment. Although this would be a time-consuming exercise, it needs to be done to validate the use of tables. Furthermore, tables used to calculate $AME_n$ and effective energy are normally based on data determined using adult cockerels (Anomymous,1986). It is known that these values are lower when broiler chickens are used in the assay (Johnson, 1987).

A paper by Hancock *et al.* (1995) illustrates the detailed information required for the effective energy system in order that a growth model can predict accurately broiler performance. Using the Gompertz growth function based on three parameters, Hancock *et al.* (1995) showed that the strains of male birds were growing fastest at 42 days of age and females at 40 days. These are the usual slaughter ages used in commercial practice.

There is no doubt that the metabolizable energy system is still relevant and because of its simplicity and the large bank of information available it will continue to be used by industry. Because of the complexity of the effective energy system and the need for detailed information on both the feed ingredients and strain of bird, it is unlikely that this system will be widely adopted in the near future. It will most likely be used as part of a sophisticated broiler growth model such as that mentioned above.

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# VALUING VARIANCE REDUCTION

R. FAWCETT* and M. WEBSTER**

Summary

Compounded animal feeds offer unintentional variation in the analysis of the final product. This paper explores the major sources of variation and presents a new model for the prediction of variation. It explores the likely effect of feed variation on the animal using growth prediction models and uses models to determine profit-maximising diet specifications. A new method of diet formulation using probability constraints to contain variance is presented.

## 1. INTRODUCTION

In animal production systems the diet specified may not be the diet fed. Unintentional variation in the diet is a consequence of variation in raw materials and errors in formulation, weighing and mixing.

Investigation of the nature, magnitude and financial consequences of variation was the purpose of a project initiated by Format International Ltd. The objective was to evaluate the possible use of quality control techniques and formulation software to exert some control over the variability of the diet.

Commercial specifications of pig and poultry diets are used as examples to study the impact of diet variation on the performance of the animal. Variation in feed composition is only one of the sources of variation experienced by the animal, and it is difficult with feeding trials to isolate the effect of feed variation alone. Biological models have been used in preference to feeding trials, thereby excluding all other sources of variation.

The study commences with the reported variation in raw materials. Selected ingredients are formulated to a given specification on a least-cost basis, manufactured, delivered and "fed".

### (a) Sources of variation

Variation in the finished feed is a consequence of real variation in the component raw materials coupled with errors in analysis, weighing, milling and mixing. The distinction between error and variability is worth maintaining.

Raw materials are not homogeneous and will exhibit sampling variation greater than the analytical error and hence true raw material variation may be identified by difference.

Formulation errors result when the true mean values of nutrient composition are different from the values entered in the linear program matrix. The formula may be rounded after optimization, again introducing error. The rounded formula is then subject to weighing errors. Mixing errors will follow and perhaps partial separation, depending on density, particle size and mill configuration. Pelleting is a method of limiting separation after mixing up to the point of consumption.

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Analytical errors in ingredient contents do not physically add to real variation in the experience of the animal but do lead to uncertainty in the location of the mean values of nutrient composition which, in turn, leads to formulation errors. Analytical error, therefore, contributes to the magnitude of feed variation in an additive manner.

In the analysis of the final product we have analytical error plus true product variation (Alderman, 1985). Analytical error is a function of equipment and test design and is beyond control other than by the choice of laboratory equipment and methods.

Weighing and mixing errors are again largely a function of equipment and process design. Control is exerted through choice of equipment and good monitoring and maintenance practice.

(b) Estimating product variation

A measure of the degree by which analyses vary from the mean value is given by the standard deviation. These statistics are compiled by most feed manufacturers for individual raw materials and for finished products. Once obtained, they are mostly used for historical reporting and rarely used in active quality control.

It is known that the standard deviation in a mixture can be predicted if the standard deviation of the individual components are known. For protein, for example, this is calculated accordingly to the formula shown below:-

$$\text{Protein sd} = \sqrt{\left( (RM_1 \times SD_1)^2 + (RM_2 \times SD_2)^2 + \ldots\ldots (RM_n \times SD_n)^2 \right)}$$

where RM is the protein nutrient contribution from an individual ingredient, SD is the standard deviation of the protein nutrient in an individual ingredient, and n is the number of raw materials in the formula (Chung and Pfost, 1964).

This method predicts the variability in the finished food as a result of the variability in the component raw materials. It does not take account of other sources of "variation" or of "error".

It has been reported (Burdett and Laws, 1979; Duncan, 1988) that the variation from raw materials accounts for only 30% of the variation in finished product analysis and that the rest is explained by milling errors. Specifically, these workers have ignored weighing errors believing them to be a small part of milling errors. They defined milling variation by difference, which inherently treated the "milling" error as additive, as though raw material variations are independent of the weighing system. This treatment suggests that raw material variation constitutes a relatively small proportion of the total (30 %) and, thus, that the benefits derived from controlling it during formulation are small. This simplification would be valid if weighing errors were very small. However it has been demonstrated that weighing errors in the context of dynamic accuracy cannot be ignored.

In fact, weighing errors and raw material variation are multiplicative and a substantial proportion of what is called "milling" error is probably due to the multiplicative effects of analytical and weighing variation. Consequently, the interaction of weighing error with ingredient variation (net of any analytical error) together with a third variable, batch size, will account for a significantly greater proportion of the variability of the product than has been believed. The application of the correct statistical treatment (Goodman, 1960; Bohrnstedt and Goldberger, 1969) to the variance calculation results in an explosion of the potential variation and may account for up to as much as 85% of the final product variation. In this situation reduction of raw material variation will play a significant role in reducing variation in the product.

This realization led to a detailed examination of weighing errors and their interaction with ingredient variation in a commercial feed plant (Fawcett and Webster, 1991). Logically, weighing errors have a multiplicative effect on raw material variation which cannot be ignored in any purposeful examination of finished product variation. From the results of these investigations material weighing errors were shown to be not simply related to scale size but to be a property of the individual material characteristics.

A statistical model incorporating multiplicative errors has been included in commercially available software and is used to obtain a prediction of final product variation, from knowledge of ingredient variation, weighing and analytical errors. In the test mill the model was used to predict the final product variation from a knowledge of the ingredient variation and the weighing and analytical errors. The predicted variation was compared with actual results determined both by near infra-red reflectance (NIR) analysis of complete feeds and conventional (and approved methodology) wet chemistry.

Table 1. Predicted versus measured nutrient composition and variability.

| Method | CP% | | EE% | | |
|---|---|---|---|---|---|
| | Broiler Feed (n=9) | | | | Range EE%7.45-9.62 |
| Model Prediction | 21.66 | (0.545) | 9.28 | (0.313) | |
| Wet Chemistry | 20.94 | (0.386) | 8.69 | (0.751) | |
| NIR Analysis | 21.60 | (0.542) | 9.53 | (0.433) | |
| Rmsd | | (0.320) | | (0.190) | |
| | Turkey Feed (n=14) | | | | Deficit in Oil 1.4% |
| Model Prediction | 24.43 | (0.523) | 7.56 | (0.311) | |
| Wet Chemistry | 23.99 | (0.833) | 6.13 | (0.268) | |
| NIR Analysis | 24.35 | (0.421) | 7.47 | (0.120) | |
| Rmsd | | (0.290) | | (0.170) | |

The model was able to predict at least 90 % of product variation in the case of protein and ash, but underestimated the variation actually found in oil levels (Fawcett and Webster, 1992). This was later explained as a failure of the fat spraying equipment to adequately and evenly distribute the correct amount of liquid fat.

(c) Variation and the animal

Given that it is possible to predict the expected variation in a finished feed it is then interesting to consider whether variation has an impact on animal performance and, if so, to attempt to quantify it. Biological growth models for pigs and poultry were used for this work since these were able to isolate the impact of nutritional changes on performance without any influence from uncontrollable environmental or health effects. The models were driven by the parameters crude protein (and hence amino acids) and energy.

(d) Nutritional value of compound feeds

Formulae were generated using conventional least-cost optimization to an appropriate commercial specification. For the purposes of describing the nutritional value of a compound feed the principle components are energy and protein (amino acid) content. Techniques of analysis for crude protein are sufficiently accurate and well established to

provide reliable estimates of the parameters. The choice of energy units and method for the formulation exercise was a difficult one. *In vivo* estimation of the energy value of ingredients or feeds is both expensive and time consuming. Prediction of energy values from regression equations based on chemically analysed constituents is cost effective for describing, regulating and monitoring the quality of compound feed. Within the European Economic Community suitable regression equations have been agreed for the calculation and declaration of the digestible energy (DE) content of pig feeds and the apparant metabolizable energy (AME) of poultry feeds and these predictions were used for the formulation of the "test" diets. A brief explanation of the calculation of energy content is given below.

AME(MJ/Kg)= 0.1551%CP + 0.3431%EE + 0.1669%STA + 0.1301%SUG (s=0.315)
(Alderman, 1985: p9).

For pigs a similar equation without constant terms can be derived from the publication of Henry *et al.* (1988).

DE(MJ/Kg)= 0.1875%CP + 0.3281%EE - 0.1552%CF + 0.1698%NFE (cv 3.8%)
where %NFE= %DM - (%CP + %EE + %CF + %ASH)

| | | | |
|---|---|---|---|
| NFE | Nitrogen free extractives | EE | Ether extract (oil) |
| CF | Crude fibre | STA | Starch |
| SUG | Total sugars | DM | Dry matter |
| CP | Crude protein | | |

There is generally a dearth of suitable published analysis of starch and sugars particularly relating to the typical variation seen in ingredients. Also missing is data on the variance covariance properties of the elements of the regression equation.

With meaningful estimates of energy and protein, and information on the expected variation in the product recipe, a bivariate probability distribution can be drawn as a series of nested ellipses which describe iso-probability contours in the bivariate diet space. An example is shown in Figure 1.

The covariance properties between protein and other components of the energy prediction equations are responsible for the slope of the axes of the ellipse. With complete independence of the diet parameters the covariance is zero and the axes of the ellipse are parallel to the axes of the diet space.

(e) Response prediction

The Edinburgh Model Pig and the Poultry Growth Models have been used to simulate the effects of variability in the macro nutrient factors DE and CP%. Given the operational characteristics of a commercial fattening unit and current price information it is possible to generate three dimensional financial response surfaces from factorial combinations of energy and protein consumed in a prescribed dietary regime and this response surface is shown in Figure 2. There are in fact no limits to the number of dimensions which can be considered but three is the limit which can be effectively studied visually.

A contour map of this response surface may be drawn and this is shown in Figure 3. This contour map can be readily used to identify the profit maximising dietary specification which occurs at the intersection of ridge lines drawn across the contours

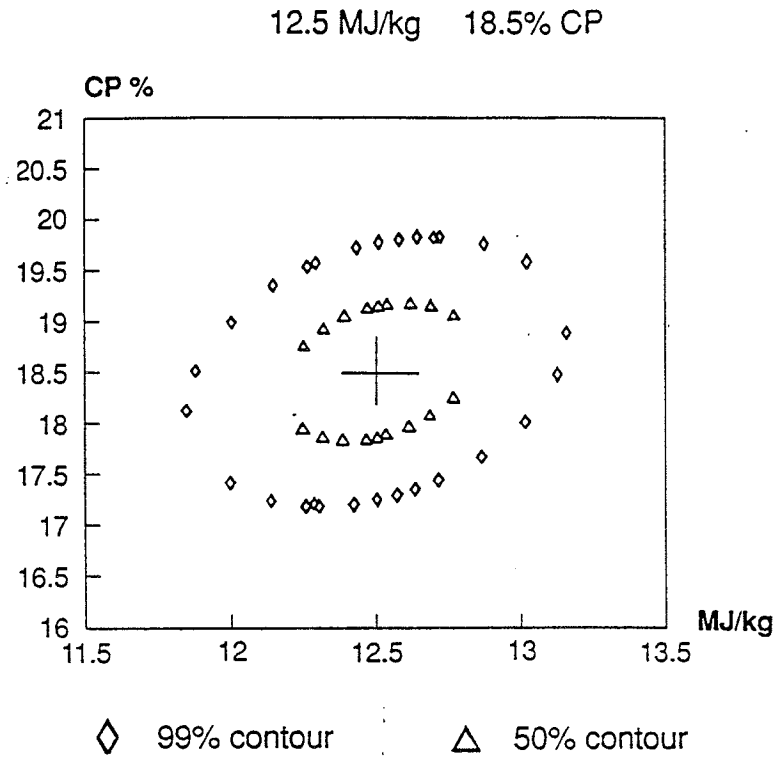



Figure 1. Probability Contours Pig Feed

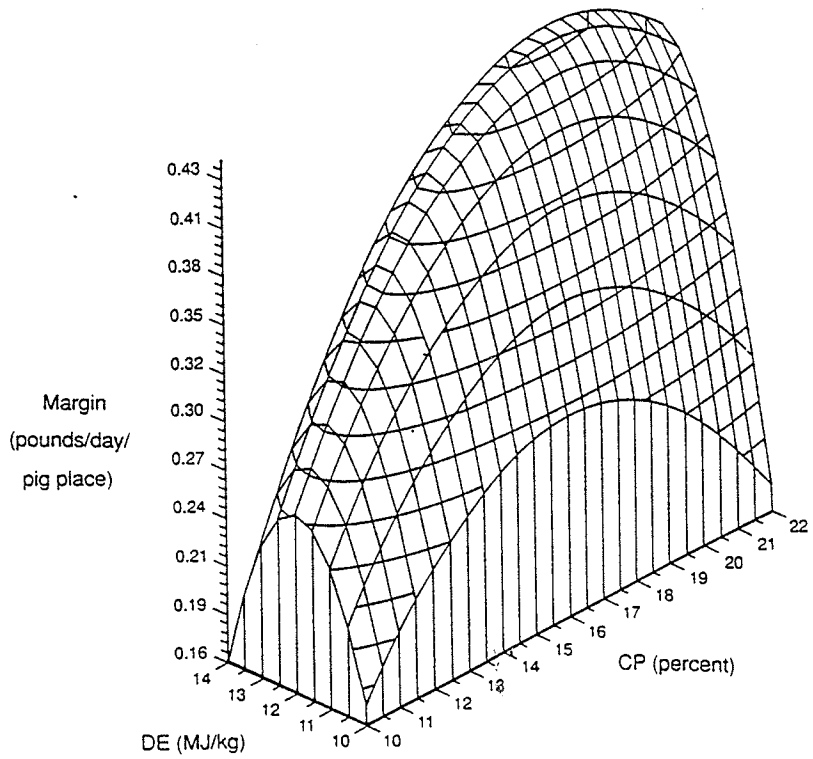



Figure 2. Response Surface Variable Dietary Cost

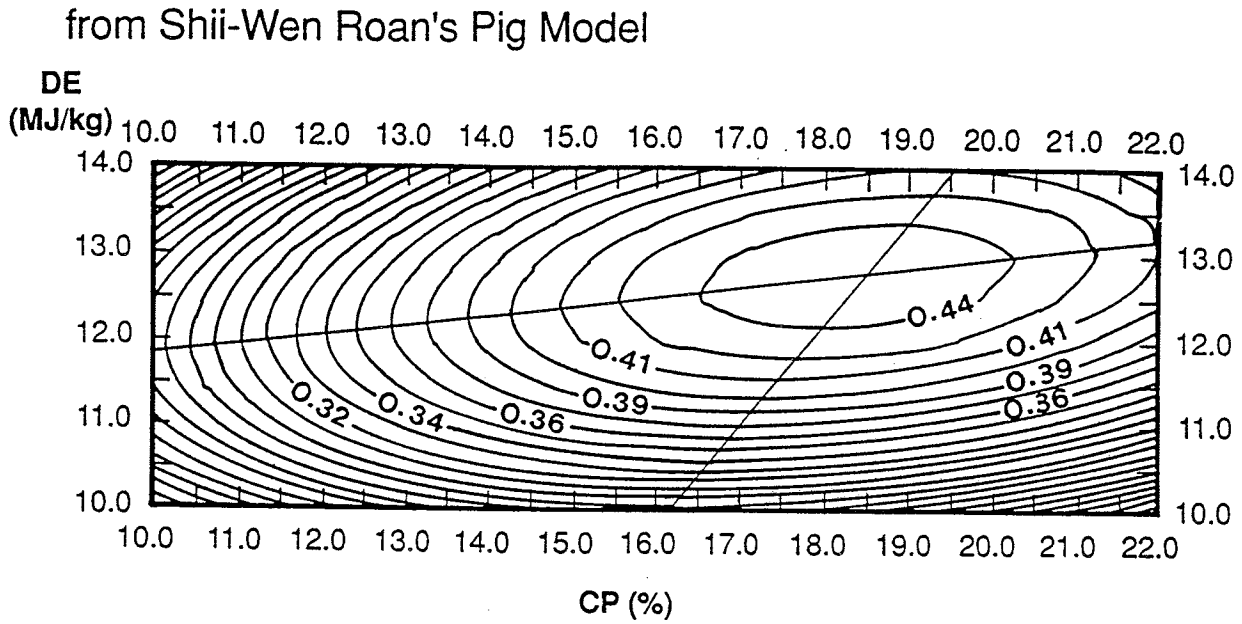



Figure 3. Response Surface Variable Dietary Cost

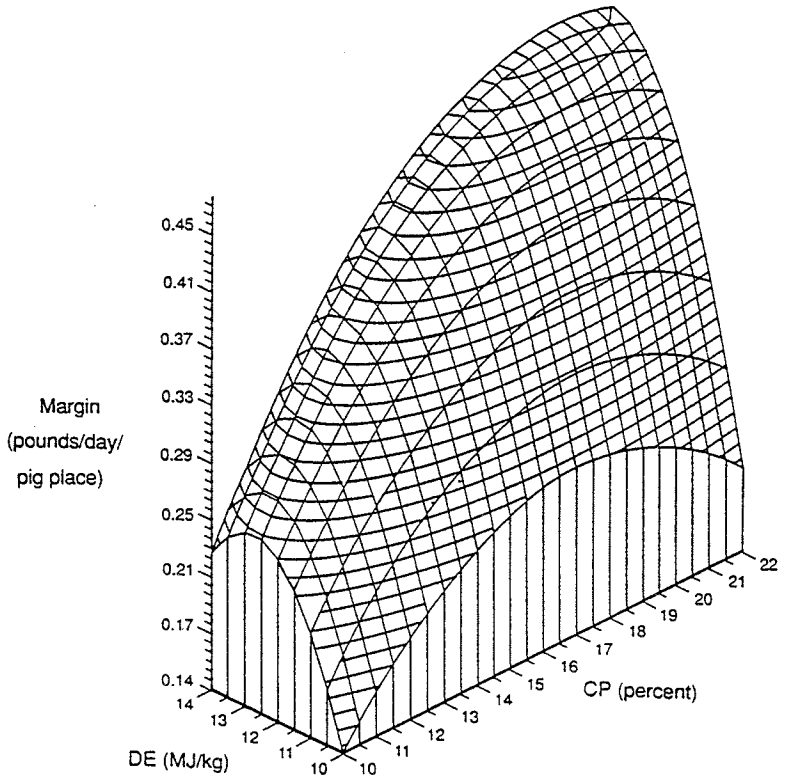



Figure 4. Response Surface Constant Dietary Cost

where they are parallel to the energy and protein axes. This pinpoints 12.8 MJ/kg and 18.5 % CP as the profit maximising diet in this example. The surprisingly low energy level may be a result of the strict carcass classification scheme used in the model and the average genetic potential of the pigs. It is however the technique and not the precise result which is of interest.

Normally, natural variation in the diet will occur and this will be without cost to the producer since he has purchased at a specified price per tonne of feed. Given the cost of producing the profit maximising diet located in Figure 2, it is possible to generate a response surface with constant feed cost. This surface is shown in Figure 4 and simply reflects the physical productivity of energy and protein. A contour map may be drawn for this constant-cost surface and this is shown in Figure 5. Note that if more energy and protein were available at zero cost, profit would continue to increase with increasing nutrient density up to 13.8 MJ/kg and 20% crude protein as shown in Figure 4.

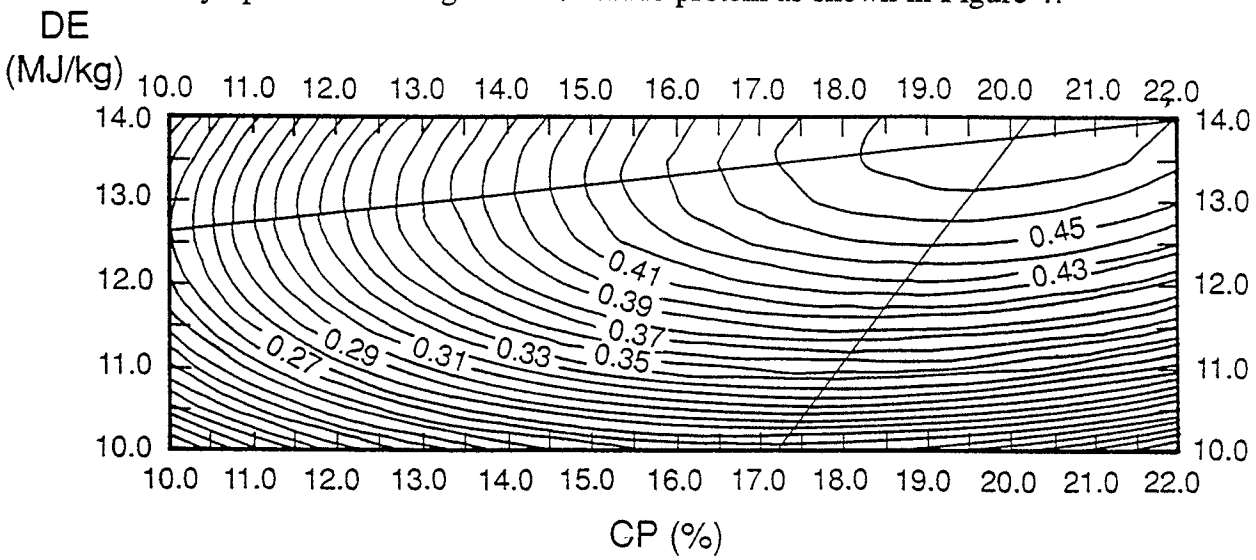



Figure 5. Response Surface Constant Dietary Cost

If natural variation in the diet is withut cost the expected profit from feeding the target specification is obtained by integrating the bivariate probability distribution describing the natural variability of the diet (Figure 1) over the constant-cost response surface.

The variance characteristics of the diet can be changed by reformulation to produce a more consistent (or less variable) product. The value of variance reduction can be estimated by modifying the bivariate probability distribution to reflect a new diet and integrating over the constant feed cost response surface again. The difference in the expected value is an estimate of the net benefit deriving from any change in the parameters of the bivariate probability distribution. Pure variance reduction may be envisaged as the contraction of the probability contours towards the centre of the ellipse.

The shape of the response surface reflects diminishing returns so that the financial consequences of negative deviations are not balanced by the financial consequences of positive deviations. In other words, the penalty suffered by feeding a diet which falls below specification are not fully offset by the benefits of feeding a diet which rises above specification, because of the plateau usually found in the response of animals to increasing nutrient supply.

In this example the expected profit is less than the profit achieved if the target specification is fed. In this situation variance reduction increases expectation.

Preliminary results from fattening pigs indicate that the expected margin per pig place per day will be increased with variance reduction being worth the equivalent to US $ 6/tonne of feed consumed.

The variable price response surface is not necessarily unimodal or smooth. If there are frequent changes in the basis of the LP solution over the response grid surface this can lead to discontinuities in price changes. This is the situation with poultry diets where marked discontinuities can be seen in the response surface (Figure 6a) requiring special mathematical treatment. The corresponding contour map (Figure 6b) can be divided into sections which then isolate profit maximising unimodal features of the irregular solid. Multivariate statistical forms can be moulded to fit the surface and to remove random variation. The smoothed, fitted response surface (Figure 7) can then be transformed to produce the constant-cost surface (Figure 8) for valuation of variance reduction in the same way.

The cost effective way of increasing the probability of exceeding a target specification is by increasing the mean and decreasing the standard deviation, or a combination of both, possibly by introducing variance-reduced materials into the formula.

Clearly the integrated feeding organisation will be looking to define the optimal level of variability in the diet since attempts to reduce variability are bound to run into rapidly diminishing returns, particularly since so much variation is outside the control of the feed manufacturer.

### (f) Formulation with probability constraints

Of interest to the nutritionist, it is now possible, once the variance of a nutrient parameter is known, to calculate the probability of producing a formula within a given tolerance.

Commercial formulation software is available which allows the nutritionist to place a probability target against a nutrient constraint and to formulate to given levels of security. Hence, it is now possible to obtain at least cost a formula which has an 85% probability of containing 20% or more protein. The desired probability levels may be specified by individual nutrient.

Table 2. Results from probability optimization.

| Nutrient | Mean | Standard deviation | Coefficient of variation | Target probability | Actual probability | Linear program limit |
|---|---|---|---|---|---|---|
| Crude protein, % | 20.4734 | 0.4571 | 2.2324 | 85.0000 | 85.0830> | 20.0000 |
| Ether extract, % | 6.0357 | 0.3516 | 5.8259 | -90.0000 | -80.2337> | 5.0000 |

In this example, shown in Table 2, a specification was formulated which required an 85% probability of meeting a protein minimum of 20% and an 80% probability of meeting an oil minimum of 5%.

Probability constrained solutions are different from traditional formulations. The technique provides a least-cost solution by manipulating the mean nutrient analysis, the standard deviation and the number of ingredients in the formula and finding the optimum balance between these. In the above example the mean was raised from 20 to 20.4734 and

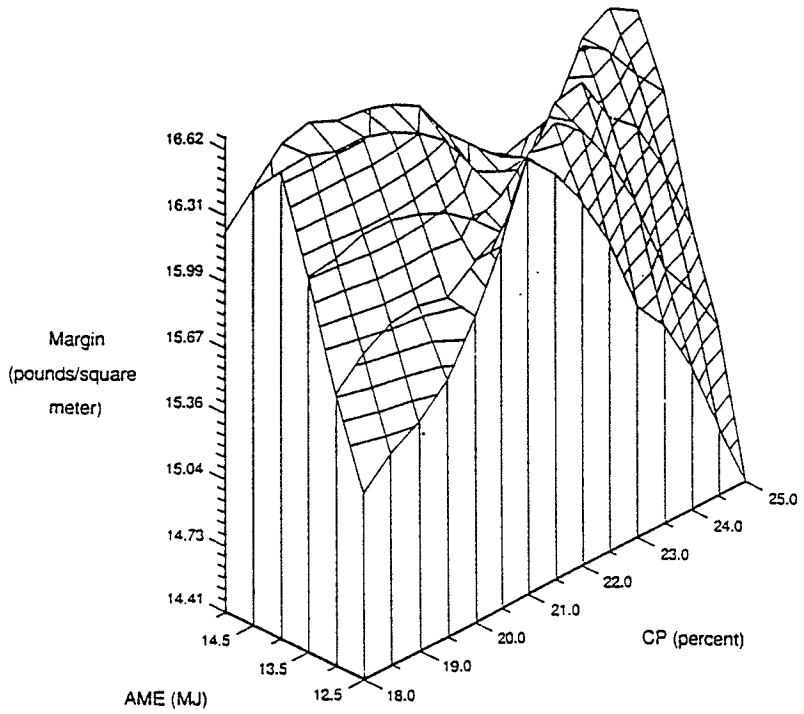



Figure 6a. Response Surface Variable Price

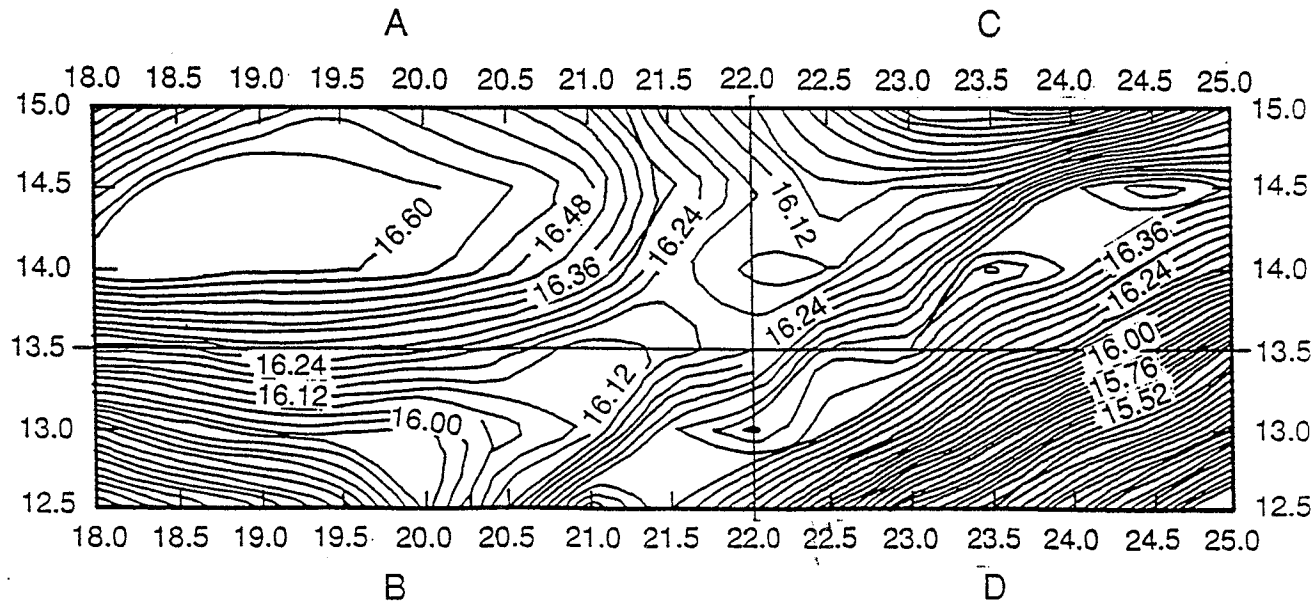
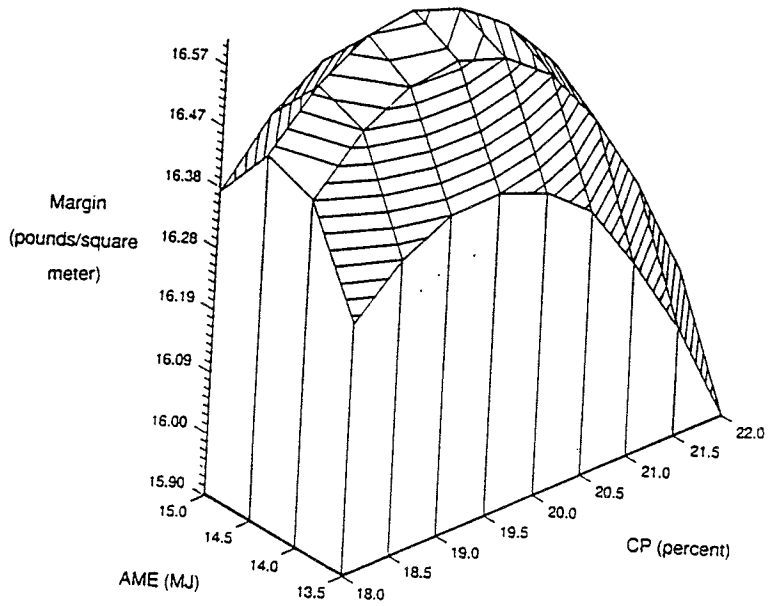



Figure 6b. Response Surface Variable Price



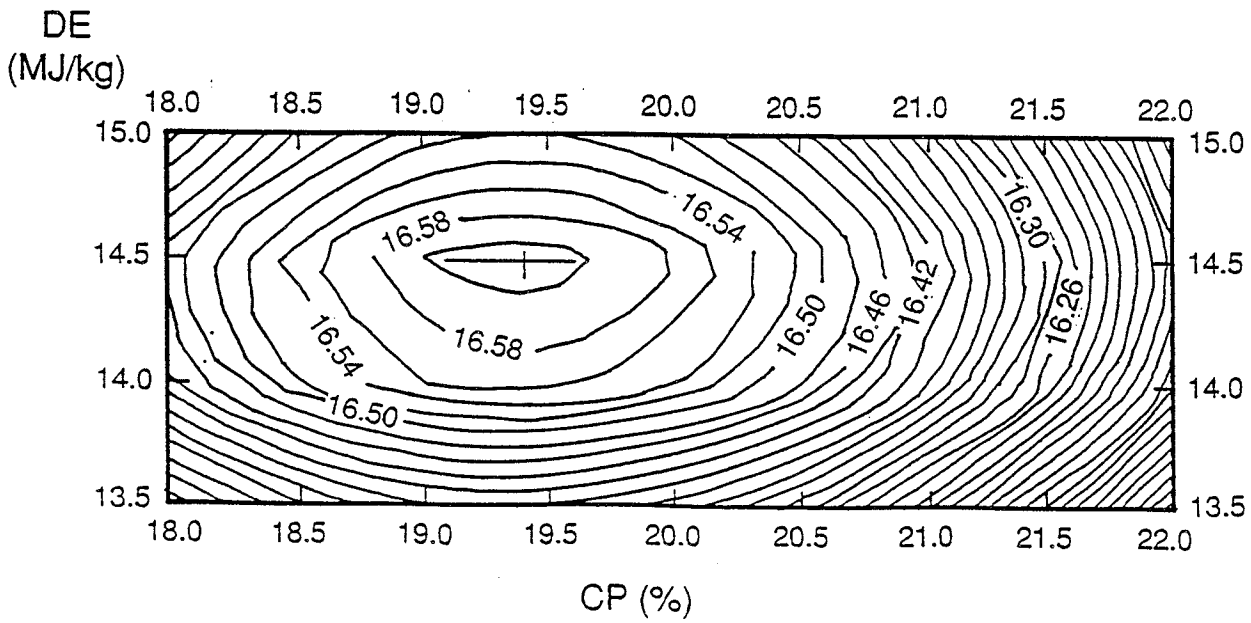
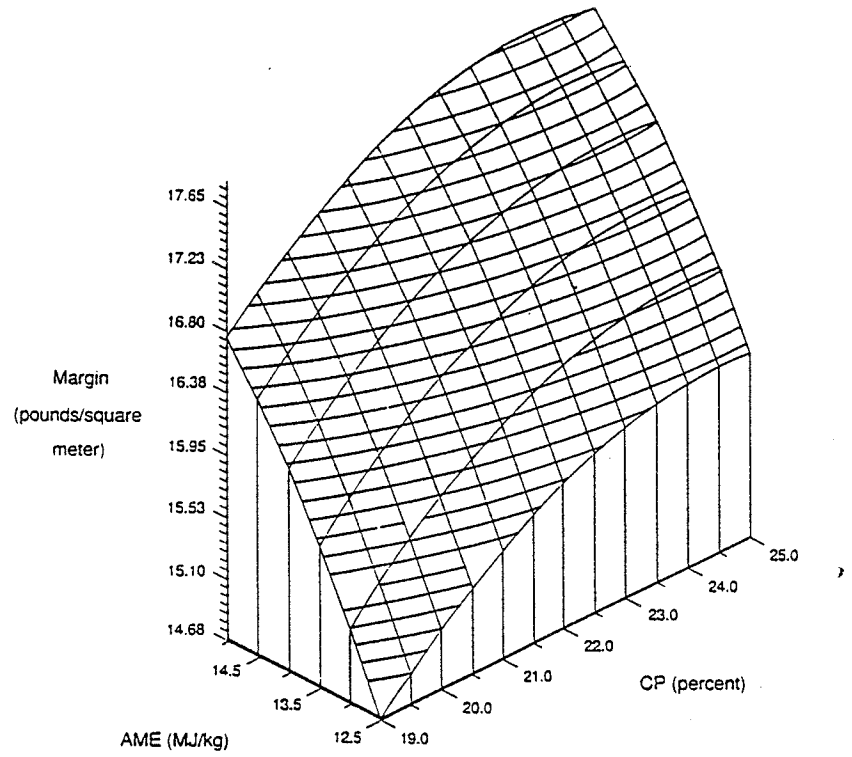




Figure 7. Isolated Response Surface Variable Price



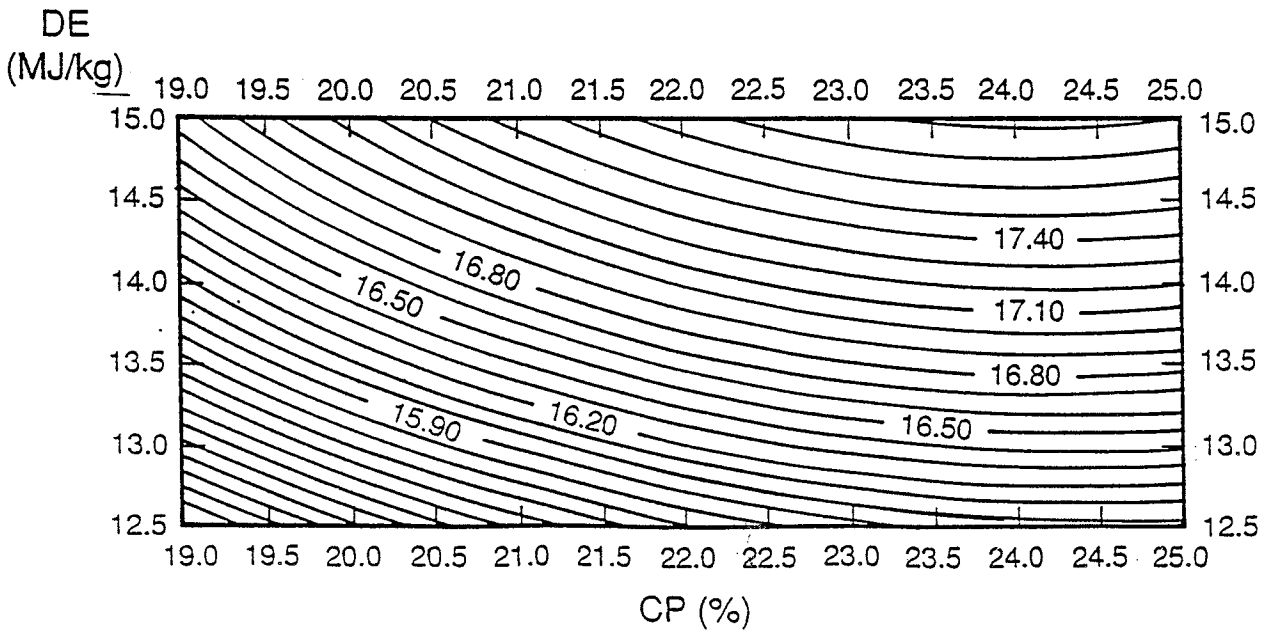




Figure 8. Isolated Response Surface Constant Price

the standard deviation was lowered slightly from 0.4639 to 0.4571, and the number of ingredients was reduced from ten to nine.

This new technique provides a method of achieving the potential financial benefits in animal performance which are the rewards of controlling the consistency of the finished product. Feed manufacturers now have a model to explore the limits that they may wish to impose on the variability of raw materials and products.

## II. CONCLUSIONS

Animal performance is affected by unintentional variation in commercial feeds. Traditional methods employed by nutritionists to attempt to minimise the variability of their products have shortcomings and may mislead if sources of error other than from ingredients is not considered. A new algorithm, available in commercial software, may be used to properly assess the impact of material variation and its interaction with weighing errors, and to control variation at the time of formulation. There are benefits in terms of improved animal performance to be derived from the control of variability in the finished feed.

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# BENEFITS OF COOL DRINKING WATER FOR ISA BROWN LAYERS IN SUMMER

P.C. GLATZ


## Summary

Food intake, egg weight, rate of lay and shell quality characteristics were measured in heat stressed (30°C) Isa Brown laying hens (59-66 weeks of age) provided with drinking water at 5, 10, 15 and 30°C. Hens given cool drinking water (5, 10 and 15°C) consumed more food and produced significantly thicker and heavier shells than hens given drinking water at ambient temperature (30°C). Results from these studies suggest that there is potential for using cool drinking water to improve food intake, egg weight and shell quality of heat stressed Isa Brown hens.


## I. INTRODUCTION

Egg farmers can suffer considerable financial losses as a result of decreased egg production during heat waves in summer. Farmers are also well aware of the decline in shell quality that can occur during these heat wave conditions (Deaton, 1983). Hens are highly susceptible to heat stress and in hot conditions they will reduce their heat production by decreasing food intake (Macleod, 1984). The result of insufficient food intake is decreased egg production and poorer shell quality.

During hot weather egg farmers rightly focus their attention on minimising the heat stress on hens by cooling layer sheds. However, little regard is given to ensuring drinking water is kept cool. Cool drinking water reduces body temperature and helps to dissipate metabolic heat (Van Kampen, 1988). In summer, drinking water temperatures in some laying houses in Australia can exceed 30°C. The combination of high drinking water temperature and elevated environmental temperatures greatly increases the risk of laying hens consuming insufficient food in an attempt to lower body heat.

In some trials cool drinking water has stimulated heat stressed hens to consume more food (Leeson and Summers, 1975; Janssen, 1985; Glatz, 1993) and improve shell thickness (Glatz, 1993). In other work no benefits of cool drinking water have been observed (Damron,1991; Degen *et al.*, 1992). Chilled drinking water for heat stressed hens can result in both beneficial and adverse characteristics in shell ultrastructure (Brackpool and Roberts, 1995).

Anecdotal reports from the Egg Industry in Australia indicate that imported laying strains have difficulty acclimatising to heat wave conditions and do not consume adequate amounts of drinking water and feed during hot weather. The aim of this experiment was to examine if cool drinking water could improve the food intake and other production parameters of Isa Brown hens during hot weather.

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## II. MATERIALS AND METHODS

### (a) Hens

This experiment was carried out using Isa Brown laying hens during the late laying phase (59-66 weeks). A total of 168 birds were housed in single-bird cages (30x45x45cm) in temperature controlled sheds which were set to an air temperature of 30°C at the commencement of the experiment (59 weeks).

### (b) Treatments

There were four treatments (drinking water at 5, 10, 15 and 30°C) with each drinking water treatment provided to 6 replicates of 7 hens. Drinking water was provided to hens via nipple lines in which water was constantly circulated from water tanks chilled or heated to the required temperature.

### (c) Measurements

Eggs were counted daily and food intake measured weekly. On 2 days each week eggs were weighed. At 60 and 65 weeks of age all eggs produced from Monday to Friday from 0800 to 1200 h were weighed (to 0.1 g) in air. The equator (widest plane perpendicular to the long axis) was marked with a pencil. All pieces of shell (including membrane) were washed in tepid running water. Shell pieces were dried at $70^0$C in a still-air oven. The following day, shells were weighed (to 0.01g) and shell thickness was measured (to 0.0001 mm) using a thickness gauge fitted with rounded jaws.

### (d) Statistical analyses

Rate of lay, food intake, egg weight and shell quality characteristics were analysed using the general linear models test using PC-SAS software over the period 59-66 weeks and are presented in Tables 1 and 2. Least significant differences ($P=0.05$) were calculated to compare treatments.

## III. RESULTS AND DISCUSSION

Over the periods 59-62 weeks and 63-66 weeks hens provided with drinking water at 5, 10 and $15^0$C consumed more food, produced heavier eggs and thicker shells (Tables 1 and 2) than birds consuming water at ambient temperature ($30^0$C).

Significant changes in the rate of lay of hens as a result of providing cool water were difficult to detect due to limitations in the size and number of replicates possible in this experiment, although the numerical reduction in rate of lay of the birds consuming $30^0$C drinking water was apparent in both periods. In a previous study (Glatz, 1993) commercial Australian strains acclimatised to the mild heat stress within four weeks and there was a decline in the effectiveness of chilled water to stimulate increases in food intake, in contrast to the findings of this current trial. While there is some danger in comparing current findings with the previous trial (Glatz, 1993) it is speculated that imported strains find it more difficult to acclimatise to heat stress than the local strains and continue to derive a thermoregulatory benefit from drinking cool water.

Table 1. Effect of drinking water temperature ($^0$C) on food intake, egg weight and rate of lay for hens from 59-62 and 63-66 weeks of age.

| Drinking water temperature ($^0$C) | Food intake (g/day) | Egg weight (g) | Rate of lay (%) |
|---|---|---|---|
| | Period 1 (59-62 weeks) | | |
| 5 | 113$^a$ | 69.4$^a$ | 72 |
| 10 | 108$^{ab}$ | 67.8$^b$ | 71 |
| 15 | 103$^b$ | 66.6$^b$ | 75 |
| 30 | 96$^c$ | 64.6$^c$ | 68 |
| | Period 2 (63-66 weeks) | | |
| 5 | 109$^a$ | 69.4$^a$ | 74 |
| 10 | 109$^a$ | 68.1$^a$ | 76 |
| 15 | 102$^b$ | 65.8$^b$ | 76 |
| 30 | 95$^c$ | 64.0$^c$ | 69 |

Means within a column within a comparison with different superscripts are significantly different ($P<0.05$).

Table 2. Effect of drinking water temperature ($^0$C) on egg weight, shell weight and shell thickness of hens at 60 and 65 weeks of age.

| Drinking water temperature ($^0$C) | Egg weight (g) | Shell weight (g) | Shell thickness (um) |
|---|---|---|---|
| | Age (60 weeks) | | |
| 5 | 70.4$^a$ | 6.3$^a$ | 376$^a$ |
| 10 | 67.2$^{ab}$ | 6.0$^{ab}$ | 369$^{ab}$ |
| 15 | 66.7$^b$ | 5.7$^{bc}$ | 358$^c$ |
| 30 | 65.6$^c$ | 5.6$^c$ | 354$^c$ |
| | Age (65 weeks) | | |
| 5 | 69.2$^a$ | 6.3$^a$ | 370$^a$ |
| 10 | 67.7$^a$ | 5.9$^{ab}$ | 366$^{ab}$ |
| 15 | 66.8$^b$ | 5.8$^{bc}$ | 360$^b$ |
| 30 | 64.9$^c$ | 5.5$^c$ | 345$^c$ |

Means within a column within a comparison with different superscripts are significantly different ($P<0.05$).

These studies have demonstrated that Isa Brown hens exposed to mild heat stress and provided with warm drinking water (a common occurrence under Australian summer housing conditions) may not perform to their true potential. If hens were exposed to hotter temperatures it is likely that cool drinking water would have an even greater value in maintaining production parameters and improving bird comfort. It was not possible in this study to examine the underlying mechanisms which contributed to the increase in food intake and egg weight of hens consuming chilled water. The measurement of water turnover, drinking and feeding behaviour and body temperature of hens is required if further studies with chilled drinking water are undertaken. Furthermore, there is a need to examine the role of thermal receptors in the response of different strains of hens to cool drinking water.

From a practical point of view Australian egg farmers should monitor drinking water temperature in their sheds in addition to ambient temperature and consider providing cooled water to hens, particularly during heat waves. Relatively inexpensive ways to assist in keeping drinking water cool are to regularly flush drinking water lines, keep incoming water lines out of direct sunlight, insulate water lines, use ice in header tanks and ensure water storage tanks are well shaded. A more expensive option is the installation of an external water cooling unit.

## IV. ACKNOWLEDGEMENTS

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# THE EFFECT OF TEMPERATURE AND SODIUM BICARBONATE SUPPLEMENTATION ON THE MINERAL EXCRETION OF BROILERS

I. GORMAN*, D. BALNAVE* and J.R. ROBERTS**

Summary

A non-surgical technique was developed for the collection of urine samples free from faecal contamination. This technique was used to study the effects of ambient temperature and dietary sodium bicarbonate ($NaHCO_3$) supplementation on the retention and urinary excretion of mineral ions in broilers. High ambient temperatures resulted in increases in the absolute retentions of sodium, potassium, calcium, magnesium, phosphorus and sulphur. The lack of any temperature effect on the urinary excretion of the non-monovalent ions indicated that the changes in retentions of these ions were due to changes in absorption. The increase in potassium retention was related to a decrease in urinary excretion, as would be expected for an ion regulated predominantly by the kidney. However, no effect of temperature was observed on the urinary excretion of sodium. Sodium bicarbonate supplementation resulted in an increase in both the absolute retention and urinary excretion of sodium. Other minerals were unaffected by $NaHCO_3$ supplementation other than a marginally significant ($P=0.05$) increase in the absolute retention of sulphur.

## I. INTRODUCTION

Previous studies in this Department have shown that broiler performance at high ambient temperatures can be improved by dietary supplementation with $NaHCO_3$ (Gorman and Balnave, 1994). Furthermore, evidence has been reported in the literature demonstrating that heat stress can result in modification of the extent and pattern of mineral excretion in poultry (Belay *et al.*, 1992). The following studies were designed to investigate the effect of temperature and dietary $NaHCO_3$ supplementation on the mineral retentions and the partitioning of mineral excretion in broiler chickens.

Attempts to identify the patterns of either urinary or faecal excretion in poultry usually rely on surgical modification of the bird. Surgical techniques are expensive and traumatic procedures. Furthermore, the use of anaesthetics and the insertion of ureteral cannulae can modify the composition of the urine (Sykes, 1971). Therefore, a non-surgical technique was developed for the collection of uncontaminated urine samples from conscious broiler chickens. The technique used was a modification of the method reported by Benoff and Buss (1976).

## II. MATERIALS AND METHODS

Preliminary studies were conducted to refine a non-surgical urine collection technique. The technique involved restraining individual chickens in a cloth harness and inserting a lubricated cotton plug into the cloaca, followed by a collection tube consisting of a rigid plastic test-tube (12 mm diameter for a 5- to 6-week-old broiler) with a 'window' cut into the side that was large enough to be positioned over the openings of both ureters. This test-

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tube was connected to a 5 mL centrifuge tube via a flexible rubber joint. It was found in a preliminary study that the cotton plug was insufficient to prevent the contamination of urine samples with faeces in the *ad libitum* fed bird. Consequently, birds were starved for approximately 16 h prior to urine collection. Drinking water was replaced with a solution containing glucose and casein (1.5 g casein + 3 g glucose/L for birds housed at 22°C and 1 g casein + 2 g glucose/L for birds housed at 33°C) during this period to reduce any metabolic disturbance caused by the withdrawal of food. Including chromic oxide as a colour marker in the food given after the fasting period showed that digesta reached the lower ileum 1.5 h after feeding. Evidence suggests that most mineral absorption has taken place by the time digesta reaches the lower ileum (Van der Klis *et al.*, 1990). The appearance of faeces containing chromic oxide occurred 3 h after the commencement of feeding. Consequently, urine samples were collected 1.5 to 2 h after feeding.

For the major experiment, day-old male broiler chicks were obtained from a commercial hatchery and brooded in heated cages with *ad libitum* access to commercial starter crumbles (12.3 MJ of ME/kg; 217 g of CP/kg) and town-water until 3 weeks of age. At 3 weeks of age broilers were allocated by weight into grower cages in one of two controlled-temperature rooms (constant 22°C or 33°C) so that average weights and weight ranges were similar for all pens. Four pens in each room were given a mash diet formulated to represent typical commercial rations (13.1 MJ of ME/kg; 175 g of CP/kg) apart from the inclusion of an inert silicate dietary marker (Celite 545), and an indigestible cellulose filler (Solka floc) to enable the inclusion of $NaHCO_3$ without influencing the concentrations of other nutrients. The remaining four pens were given the same diet with 17.8 g $NaHCO_3$ included at the expense of Solka floc.

Excreta samples were collected from each cage for a 24 h period in the week prior to urine collections. The inclusion of Celite in the diet prevented the need for total collection. Excreta samples were dried at 80°C for 24-36 h, equilibrated with atmospheric moisture for 24 h and then finely ground.

In order to keep food and water intakes consistent between birds after the fasting period, 25 g of food and 20 mL of town-water were force-fed to each bird 1.5 h prior to urine collection, using the technique described by Sibbald (1976). Urine samples were collected for 2 to 5 minutes in order to collect approximately 2 mL of urine. Urine samples were weighed, frozen and freeze-dried.

The acid insoluble ash concentrations in food and excreta samples were measured using the method of Mollah (1980). Minerals other than chloride were measured on all samples following a wet digestion (AOAC, 1984) using a Labtam Plasmalab inductively coupled plasma emission spectrometer (Labtam, Melbourne, Australia). Chloride concentrations in food and excreta samples were measured by potentiometric titration using an Activon silver billet combination electrode (Activon Scientific Products Company, Pty Ltd. Australia).

The experimental design was a 2 x 2 factorial with diet and ambient temperature as factors. Each factor had two levels with 8 replicates each. Consequently SEM values were identical for both diet and temperature means. Analyses of variance were carried out using Minitab Data Analysis Software. The SEM values were calculated using the procedure of Steel and Torrie (1981).

## III. RESULTS AND DISCUSSION

The absolute mineral retentions and the mineral composition of the urine are presented in Table 1.

Table 1. Mineral retentions (Retentions: g retained/kg weight gain) and urinary mineral content (Urine: mg mineral/g dry matter) from broilers fed the control diet (Diet 1) or the control diet supplemented with 17.8 g $NaHCO_3$/kg (Diet 2).

| Ion | Diet | Retentions | | Diet Mean | Urine | | Diet Mean |
|---|---|---|---|---|---|---|---|
| | | 22°C | 33°C | | 22°C | 33°C | |
| *Sodium* | 1 | 0.04 | 1.51 | 1.29 [a] | 17.7 | 20.7 | 19.2 [a] |
| | 2 | 2.13 | 4.46 | 3.34 [b] | 48.0 | 37.8 | 42.9 [b] |
| Temp Mean | | 1.58 [A] | 3.03 [B] | | 32.9 | 29.2 | |
| SEM | | 0.313 | | | 4.00 | | |
| *Potassium* | 1 | 3.72 | 5.74 | 4.73 | 79.7 | 64.2 | 72.0 |
| | 2 | 3.55 | 4.50 | 4.03 | 85.7 | 54.9 | 70.3 |
| Temp Mean | | 3.64 [A] | 5.12 [B] | | 82.7 [B] | 59.6 [A] | |
| SEM | | 0.375 | | | 3.69 | | |
| *Calcium* | 1 | 7.12 | 9.86 | 8.53 | 0.47 | 0.52 | 0.50 |
| | 2 | 7.87 | 10.35 | 9.11 | 0.61 | 0.73 | 0.67 |
| Temp Mean | | 7.54 [A] | 10.10 [B] | | 0.54 | 0.63 | |
| SEM | | 0.503 | | | 0.14 | | |
| *Magnesium* | 1 | 0.59 | 1.20 | 0.89 | 2.23 | 2.50 | 2.36 |
| | 2 | 0.73 | 1.25 | 0.99 | 1.79 | 2.13 | 1.96 |
| Temp Mean | | 0.66 [A] | 1.22 [B] | | 2.01 | 2.31 | |
| SEM | | 0.123 | | | 0.19 | | |
| *Chloride* | 1 | 1.26 | 1.52 | 1.39 | | | |
| | 2 | 1.55 | 2.19 | 1.87 | | | |
| Temp Mean | | 1.40 | 1.85 | | | | |
| SEM | | 0.367 | | | | | |
| *Phosphorus* | 1 | 4.76 | 5.99 | 5.37 | 20.6 | 21.8 | 21.2 |
| | 2 | 5.01 | 5.99 | 5.50 | 26.6 | 19.9 | 23.3 |
| Temp Mean | | 4.88 [A] | 5.99 [B] | | 23.6 | 20.9 | |
| SEM | | 0.259 | | | 1.42 | | |
| *Sulphur* | 1 | 2.45 | 3.08 | 2.77 [b] | 7.2 | 8.8 | 8.0 |
| | 2 | 2.37 | 2.59 | 2.48 [a] | 8.0 | 5.0 | 6.5 |
| Temp Mean | | 2.41 [A] | 2.83 [B] | | 7.6 | 6.9 | |
| SEM | | 0.093 | | | 0.70 | | |

* [ab] For each mineral diet, means for Retentions or Urine with different superscripts are significantly different at $P<0.05$.

* [AB] For each mineral temperature, means for Retentions or Urine with different superscripts are significantly different at $P<0.05$.

The high temperature regimen resulted in increases in the retentions of all minerals, with the increases being significant in all cases except chloride. There was a marginally

significant ($P=0.047$) interaction between diet and temperature for sodium retention due to the effect of $NaHCO_3$ supplementation being more pronounced at the higher temperature, indicating that the effects of dietary sodium supplementation and high ambient temperature on the retention of sodium were additive. The increase in mineral retentions observed in this study in response to heat stress are in contradiction with the results reported by Teeter and co-workers (Teeter, 1986; Belay *et al.*, 1992). However, reports in the literature on the effects of heat stress on nutrient utilization in broilers, as evidenced by changes in feed efficiency, also have been found to be inconsistent. For example, Teeter and co-workers report decreases in the efficiency of feed utilization by heat-stressed broilers in feeding trials reported in conjunction with studies investigating the effects of heat stress on mineral retentions. Whiting *et al.* (1991) reported that although acute heat stress reduced weight gains, feed efficiency was improved in one experiment but was unaffected in a second experiment. Dale and Fuller (1980) found that cyclic heat stress reduced feed efficiency when compared to that obtained with a cyclic cool temperature regimen, yet feed efficiency was improved when the heat stress was constant. This inconsistency may be related to such variables as the degree and type of thermal stress and diet composition, particularly the energy content and heat increment of the diet. As it is clear that heat stress can result in either an increase, no change or a decrease in nutrient utilization, it is conceivable that the effects of heat stress on mineral retentions may also be influenced in this manner.

Supplementation of the diet with $NaHCO_3$ gave a highly significant ($P<0.001$) increase in the retention of sodium and a marginally significant ($P=0.05$) decrease in the retention of sulphur. The only significant effect of diet on the urinary mineral concentrations was the increased concentration of sodium. The urinary excretion of potassium was significantly reduced at the high temperature while the concentrations of all other minerals in the urine were unaffected by temperature. A significant interaction occurred between treatments for sulphur excretion due to differing responses to $NaHCO_3$ supplementation in the two temperature regimens. The reason for this interaction is not apparent

The urinary excretion and absolute retentions of sodium were both increased with $NaHCO_3$ supplementation. Without supplementation with the ion in question, it would be expected that an increase in the retention of an ion that is excreted predominantly via the kidneys would be related to a decrease in the urinary excretion. This was the case observed with the temperature-related increase in the retention of potassium. However, the effect of supplementation on the relationship between the retention and urinary excretion of the supplemented ion will depend on the level of supplementation. The level of sodium supplementation used in this study was sufficient to cause an increase in both the retention and urinary excretion of sodium. The effects of ambient temperature on the retentions of the non-monovalent ions were not associated with changes in their urinary excretion, indicating that the changes in retention were due to changes in absorption. However, the lack of any significant effect of temperature on the urinary excretion of sodium was unexpected.

The non-surgical urine collection technique developed in these trials was simple to perform and resulted in minimal stress to the birds. This technique allows multiple collections from individual birds over a reasonable period of time and does not require the birds to be anaesthetised or sacrificed.

## ACKNOWLEDGEMENTS

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# AN EVALUATION OF THE PARAMETERS OF THE GOMPERTZ GROWTH EQUATION THAT DESCRIBE THE GROWTH OF EIGHT STRAINS OF BROILER

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Summary

The study reported here was designed to characterise the broiler strains available to the poultry industry in Australia as well as to determine the growth and carcass characteristics of a wide range of broiler genotypes. Four strains available commercially and four selection lines from the University of Queensland were grown in conditions that would encourage them to grow at, or close to, their potential to 105d of age. Growth and carcass characteristics were measured by sampling the birds frequently during the growth period. Wide differences existed between strains and sexes in growth rate, feed conversion efficiency and feather growth, and these differences can be characterised sufficiently accurately to enable their dissimilar feed and environmental requirements to be modelled by simulation.

## I. INTRODUCTION

Almost twenty years ago Wilson (1977) suggested that useful information could be gleaned from a knowledge of the potential growth rates of different strains of broiler chickens. With the advent of simulation models that describe the relationship between food intake and growth of broilers, a description of the growth potential of different genotypes is seen to be of even greater relevance now than in the past, as the mechanism now exists for making use of such information in day-to-day nutritional and environmental management of commercial broiler operations.

No such study has yet been undertaken to characterise the wide diversity of broiler strains that have been developed in Australia, where selection for food conversion efficiency and leanness has been practised for decades, largely independently of the genotypes used in the rest of the world. Now that strains of broilers from the UK and North America are becoming available in Australia it is valuable to be able to compare the growth characteristics of the local strains with those from overseas.

The methodology used here to describe the growth of broiler genotypes has been briefly described by Emmans and Fisher (1986), and recently by Hancock *et al.* (1995).

## II. MATERIALS AND METHODS

Eight strains of broiler were used, of which four were commercially available to the broiler industry in Australia (Ingham, Steggles, Bartter and Cobb) and four were selection lines developed at the University of Queensland (lines E and C are described in Pym and Nicholls, 1979; the W (weight) and I (index) lines are described in Pym, 1992). At the request of the companies supplying the commercial strains these have been coded in the remainder of this article and are, therefore, not identifiable.

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The experiment commenced on the 28 February 1995 and continued for 105 days (15 weeks). Chickens, obtained either from commercial hatcheries or from eggs incubated at the University specifically for this trial, were sexed at day old, wingbanded, vaccinated against Mareks disease and then allocated by strain and sex among 32 pens measuring 3.2 x 2.7m. Up to 75 chicks, depending on the numbers available, were placed in each pen, there being two replications of each strain x sex. Each pen was equipped with four tube feeders (25 kg capacity) and nine dry-cup drinkers, these being in one line along the side of the pen.

A convection house was used, having upper and lower side openings, an insulated roof, concrete floors on which wood shavings were placed, and infra-red space heaters to provide warmth for the first few weeks, when necessary. Temperature in the house was automatically controlled where possible, the desired temperatures being set at 31°C for the first two days, then decreasing linearly to 24°C by 21d of age, then linearly to approximately 18°C by 49d of age, where it was held thereafter.

Stocking density was reduced throughout the duration of the trial as a result of the sampling of birds at frequent intervals, and at six weeks of age half of the remaining birds in each pen were removed in order to ensure that stocking density did not impede growth within each pen.

Two feeds were used in the trial. The first, a starter feed (14.2 g available lysine/kg), was offered from day old and throughout the trial. The second, a finisher feed containing less protein (9.2 g available lysine/kg) than the starter but equal concentrations of all other nutrients (12.7 MJ of ME/kg) was introduced into two of the four feeders at three weeks of age. Both of these feeds were in a crumbled form for the first six weeks and, thereafter, they were pelleted. From six weeks of age only one feeder in each pen was filled with starter feed, reflecting the reduced proportion of this feed selected by the birds as they grew.

All birds were group-weighed at day old and again at 3, 8, 12, 17, 21, 28, 35, 42, 56, 70, 84 and 105 d of age. Food remaining in each of the troughs was measured at weekly intervals for the first six weeks and then corresponding with the bird weighing times thereafter. All food allocations were recorded, and these, together with the measurements of food remaining, were used to calculate food intake.

At each of the above weighing times, two birds representative of the group were removed from each pen (four per strain x sex) for further analysis. These birds were killed by cervical dislocation without loss of blood. The further analysis consisted of removing the feathers, to determine their weight (accomplished by weighing each bird before and after plucking), dissecting and weighing four parts of the body (wings, thighs, drums and breast) representing the most important physical parts, after which all the components of the body, except for the feathers, were minced together for the chemical analysis of water, protein, lipid and ash. Data concerning the growth and analysis of the physical parts of the body are to be presented elsewhere.

## III. RESULTS AND DISCUSSION

As an illustration of the differences in growth and food conversion efficiency in the various strains of broiler used in Australia, body weight at six weeks of age and the food consumed to that age are presented in Table 1. The differences that existed between commercial breeds (Strains 1-4) appear substantial, with a differential of 200 g/bird between strains 3 and 4. The selection lines from the University of Queensland (Strains 5-8) were considerably smaller than the commercial strains, but the object of the project was to determine the growth characteristics of as wide a range of genotypes as possible. Food conversion efficiencies are impressively high in the commercial strains.

Feather weights appeared to differ between the different genotypes. These differences can be seen in Table 1, where the weight of feathers at 105 d of age is given as a proportion of the body weight at that time. It would suggest that Strains 2 and 3 (lower proportion of feathers) may be more adapted to hot weather than the other strains. Two of the selection lines (Strains 5 and 6) were considerably more covered with feathers than the other strains and they were the smallest genotypes in the study.

Table 1. Body weight at six weeks of age and food consumed to that age, together with the weight of feathers as a proportion of the body weight at 105 days of age, of four commercial strains (1-4) and four selection lines (5-8) of broiler chickens.

| Strain | Body weight at six weeks (g) | | Food intake to six weeks (g) | | Feather weight as proportion of body weight at 105d | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| 1 | 2517 | 2086 | 4045 | 3709 | 6.93 | 5.80 |
| 2 | 2532 | 2135 | 3972 | 3532 | 5.64 | 4.79 |
| 3 | 2622 | 2267 | 4130 | 3716 | 5.41 | 5.01 |
| 4 | 2450 | 1951 | 3787 | 3152 | 6.72 | 5.63 |
| 5 | 795 | 700 | 1742 | 1315 | 10.15 | 10.38 |
| 6 | 843 | 692 | 1544 | 1282 | 10.08 | 10.37 |
| 7 | 2135 | 1805 | 3560 | 3030 | 7.23 | 5.86 |
| 8 | 2105 | 1776 | 3244 | 2895 | 6.23 | 6.69 |

The Gompertz growth equation: $W = A. \exp(-\exp(-B(t-t^*)))$, where the weight of the animal (W) is expressed in terms of A, the weight at which the growth rate becomes zero, i.e. the mature weight of the animal; B, the rate parameter, or rate of maturing; $t^*$, the time at which the growth rate is at its maximum; and t, which is time, was fitted to the growth data from each genotype, and in all cases the fit produced a $R^2$ value in excess of 0.993. In Table 2 the growth characteristics are given for the 16 genotypes in the study, together with the maximum daily gain observed during the growth period. Interestingly, the fastest growing strain to six weeks of age does not have the largest predicted mature size, but has the highest rate of maturing. The male of this strain also has the lowest weight of feathers as a proportion of body weight, which would have important consequences in relation to the methionine and cysteine requirements of this genotype. The consequences of these different degrees of maturity on carcass composition, both chemical and physical, and on nutrient requirements is an important part of the present study, but only the effects on breast meat yield will be presented here.

Where the natural log (*ln*) of breast meat yield (BMY) was regressed against *ln* body weight (BW), a common equation for all the genotypes in this study accounted for 0.988 of the variance in breast meat yield. The equation is *ln* BMY = -4.295 + 1.3466 *ln* BW. Minor, statistically non-significant, differences existed between the different genotypes when the slopes and constant terms of the equations for each genotype were compared one with the other. It appears, therefore, that if differential selection for BMY has either not been applied among these genotypes, or if it has, that it has been unsuccessful in changing the allometric relationship that exists between BMY and BW.

Differences that exist in rate of maturing, rate of feather growth in relation to body growth and the fatness in the gain (not reported here) are of considerable importance to

nutritionists responsible for the feeding of these different genotypes. In particular, the very rapid early growth rates seen in these genotypes, reflected in the rapid rates of maturing, would indicate that the dietary concentrations of amino acids required for body protein growth would need to be higher than those used conventionally in the broiler industry if the benefit of this potential early growth rate is to be realized.

Table 2. Growth characteristics of four commercial strains (1-4) and four selection lines (5-8) of broilers, predicted by the Gompertz growth equation, together with the maximum daily gain achieved by these strains during the period to 105 days of age. (The coefficients of variation of mature size and rate of maturing were 0.022 and 0.024 respectively).

| Strain | Mature size (g) | | Rate of maturing (/d) | | Maximum growth rate (g/d) | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| 1 | 5803 | 4433 | 0.0442 | 0.0461 | 80.8 | 65.1 |
| 2 | 5563 | 4585 | 0.0468 | 0.0453 | 83.6 | 62.8 |
| 3 | 5422 | 4553 | 0.0483 | 0.0472 | 82.8 | 69.5 |
| 4 | 5871 | 4367 | 0.0434 | 0.0434 | 78.5 | 60.7 |
| 5 | 3178 | 2346 | 0.0284 | 0.0306 | 33.7 | 25.9 |
| 6 | 3133 | 2323 | 0.0294 | 0.0306 | 34.7 | 25.9 |
| 7 | 5072 | 4187 | 0.0433 | 0.0423 | 70.1 | 55.8 |
| 8 | 5245 | 4037 | 0.0424 | 0.0421 | 73.2 | 56.3 |

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# THE RESPONSE IN BODY AND FEATHER GROWTH OF EIGHT STRAINS OF BROILERS TO DIETARY METHIONINE IN THE PERIOD ONE TO THREE WEEKS OF AGE

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Summary

The wide diversity of broiler strains that have been developed in Australia provide an opportunity of determining whether differences exist in the efficiency of utilization of amino acids for the growth of body and feather protein. Eight strains of broiler, four commercial and four selection lines, were subjected to a series of five feeds limiting in methionine during the period seven to 21d of age. Nett efficiency of utilization of methionine was the same in all strains and in both sexes, and the rate of feather growth remained a constant proportion of the growth of the body irrespective of genotype or dietary methionine concentration.

## I. INTRODUCTION

Selection pressure applied by poultry geneticists to a range of heritable traits have resulted in differences between strains of broilers in the growth of body and feather protein which is reflected in differences in their mature size and rate of maturing, and in the amount of fatness in the gain (Hancock *et al.*, 1995). The nutrient requirements of these selected genotypes would be expected to differ because of the differential growth of body and feather protein, each with its unique amino acid composition. These requirements could be calculated from a knowledge of the rates of gain of the different components of the body but the question arises as to whether differences exist in the efficiency with which the different strains utilize limiting amino acids for the growth of protein.

In modelling the growth of broilers it has been assumed (Emmans and Fisher, 1986), because no definitive experiments have investigated this issue, that the nett efficiency of utilization of amino acids is fixed between strains and, indeed, it can be argued that this applies between species.

The wide diversity of broiler strains that have been developed in Australia, largely independently from the rest of the world, provide an opportunity for determining whether differences do exist in the efficiency of utilization of amino acids for the growth of body and feather protein.

## II. MATERIALS AND METHODS

Eight strains of broiler were used, of which four were commercially available to the broiler industry in Australia (Ingham, Steggles, Bartter and Cobb) and four were selection lines developed at the University of Queensland (Lines E and C are described in Pym and Nicholls, 1979; the W (weight) and I (index) lines are described in Pym, 1992). At the

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request of the companies supplying the commercial strains these have been coded in the remainder of this article and are, therefore, not identifiable.

The response to dietary methionine was measured by growing the broilers from one to three weeks of age on one of five feeds designed to be limiting in methionine, in a dilution series. The summit dilution technique, as applied to broiler chickens, has been described elsewhere (Gous and Morris, 1985). A summit feed, high in protein but limiting in methionine was formulated to supply all amino acids at, or above, 1.4 of their requirement, with methionine at only 1.2 of requirement. A simulation model developed by the senior author, based on the theory of growth and food intake of Emmans (1989) was used to calculate the amino acid requirements of one week-old broiler chickens.

A non-protein dilution feed was designed to contain the same amounts of energy, calcium, available phosphorus, vitamins and other non-protein containing nutrients as the summit feed. This was blended with the summit feed in the ratios 1.0 summit: 0 dilution; 0.8:0.2; 0.6:0.4; 0.4:0.6 and 0.2:0.8, to provide methionine concentrations of 7.5, 6.0, 4.5, 3.0 and 1.5g/kg. These feeds were offered *ad libitum* to the chickens between one and three weeks of age.

In a preliminary trial the most diluted feed was fed alone and with supplemental methionine to broiler chickens of one of the strains used in this experiment, and a significant response in growth and food conversion efficiency (FCE) was obtained from the supplementary methionine, indicating that the summit feed was indeed limiting in methionine.

Chickens of each strain were sexed at day-old, wingbanded, and then placed in tier brooders and fed a commercial starter feed containing 12.5 MJ of ME and 230 g crude protein (CP)/kg, to one week of age. Fifty chickens of each strain x sex were then removed from this facility and housed in a room in which the temperature was maintained by means of a gas-fired heater such that all areas of the room were kept at the same temperature, rather than having individual heaters in each brooder.

On transfer at one week of age, the fifty chickens of each strain x sex were weighed in groups of ten, after which they were placed in five of 80 pens. In addition, two chickens of each strain x sex were sacrificed for analysis of body composition. Since only 80 pens were available for this experiment, replications of each treatment were obtained by repeating the above procedure on three separate occasions.

An amount of food (approximately 12kg) was measured into a marked plastic bucket for each pen from which the birds in that pen were fed during the trial. Birds in each pen were group weighed at 21 d of age, after 14 d on experiment. All food remaining in the trough was returned to the bucket at the end of the trial, which was then weighed, from which the amount of food consumed/bird/d was calculated. Appropriate adjustments were made to account for mortality which was recorded throughout the trial.

Two chickens representative of each pen were removed at the end of the trial for further analysis. These birds, as well as those sampled at the start of the trial, were killed by cervical dislocation, weighed, plucked, and then weighed again to determine the weight and growth rate of feathers.

## III. RESULTS AND DISCUSSION

Daily growth rates, food intakes and feather growth rates are given in Table 1 for the eight strains used. In most cases the greatest gains were achieved not on the summit feed but on the second feed (0.8 of summit). The most satisfactory explanation for this phenomenon, which has been observed previously with the dilution technique, is that insufficient energy is

Table 1. Growth rate (g/d), food intake (g/d) and feather gain (g/d) of females and males of eight strains of broilers from 7 to 21 d of age given isoenergetic diets decreasing in methionine concentration from 7.5 g/kg (Diet 1) to 1.5 g/kg (Diet 5).

| Strain | Diet | Weight gain | | Food intake | | Feather gain | |
|---|---|---|---|---|---|---|---|
| | | F | M | F | M | F | M |
| 1 | 1 | 37.0 | 38.9 | 53.5 | 57.7 | 1.86 | 1.73 |
| | 2 | 41.0 | 44.7 | 61.1 | 62.4 | 2.18 | 2.05 |
| | 3 | 39.8 | 39.0 | 65.0 | 65.7 | 1.83 | 1.87 |
| | 4 | 27.0 | 27.8 | 60.3 | 59.9 | 1.28 | 0.96 |
| | 5 | 11.3 | 8.6 | 37.0 | 37.4 | 0.30 | 0.34 |
| 2 | 1 | 39.7 | 42.1 | 55.7 | 58.2 | 2.42 | 1.65 |
| | 2 | 37.2 | 43.6 | 55.8 | 64.1 | 2.39 | 2.03 |
| | 3 | 35.4 | 42.1 | 59.0 | 66.8 | 1.98 | 1.66 |
| | 4 | 26.8 | 26.5 | 58.4 | 56.9 | 1.07 | 1.56 |
| | 5 | 17.4 | 9.5 | 37.5 | 38.1 | 0.53 | 0.42 |
| 3 | 1 | 41.5 | 44.6 | 61.3 | 60.8 | 2.21 | 1.93 |
| | 2 | 43.7 | 38.0 | 63.0 | 66.0 | 2.49 | 1.66 |
| | 3 | 41.4 | 44.5 | 67.8 | 69.1 | 2.00 | 1.84 |
| | 4 | 25.1 | 30.7 | 55.9 | 65.4 | 1.21 | 0.90 |
| | 5 | 10.9 | 9.7 | 42.3 | 40.4 | 0.44 | 0.45 |
| 4 | 1 | 38.4 | 41.6 | 56.4 | 59.9 | 2.76 | 1.33 |
| | 2 | 40.2 | 45.1 | 58.7 | 63.5 | 2.27 | 1.96 |
| | 3 | 38.4 | 42.1 | 62.8 | 66.3 | 2.27 | 1.58 |
| | 4 | 26.9 | 28.7 | 59.2 | 62.6 | 1.29 | 1.64 |
| | 5 | 8.6 | 12.0 | 40.0 | 44.8 | 0.45 | 0.38 |
| 5 | 1 | 11.1 | 12.4 | 21.2 | 21.4 | 0.67 | 0.58 |
| | 2 | 15.3 | 13.6 | 24.2 | 24.5 | 0.81 | 0.87 |
| | 3 | 11.1 | 13.3 | 21.3 | 27.5 | 0.37 | 0.74 |
| | 4 | 6.8 | 8.2 | 16.3 | 20.4 | 0.27 | 0.58 |
| | 5 | 1.9 | 1.8 | 12.9 | 14.1 | 0.79 | 0.17 |
| 6 | 1 | 11.8 | 14.0 | 21.4 | 25.1 | 0.32 | 0.83 |
| | 2 | 12.0 | 12.8 | 24.8 | 26.3 | 1.27 | 0.80 |
| | 3 | 12.5 | 12.8 | 28.8 | 26.7 | 0.62 | 0.66 |
| | 4 | 6.0 | 10.8 | 23.9 | 19.7 | 0.40 | 0.28 |
| | 5 | 2.0 | 1.7 | 16.0 | 13.4 | 0.18 | 0.00 |
| 7 | 1 | 31.5 | 34.7 | 49.7 | 54.1 | 1.86 | 2.19 |
| | 2 | 34.4 | 37.6 | 53.0 | 54.1 | 2.20 | 1.64 |
| | 3 | 31.5 | 34.6 | 56.0 | 59.0 | 2.20 | 1.59 |
| | 4 | 21.4 | 23.8 | 48.8 | 50.9 | 0.90 | 0.58 |
| | 5 | 8.6 | 9.7 | 36.6 | 37.6 | 0.54 | 0.30 |
| 8 | 1 | 30.7 | 36.4 | 45.4 | 52.5 | 2.53 | 2.22 |
| | 2 | 36.1 | 38.6 | 53.9 | 57.2 | 2.81 | 2.29 |
| | 3 | 32.2 | 34.4 | 49.0 | 59.8 | 1.81 | 0.81 |
| | 4 | 21.7 | 24.3 | 48.5 | 51.1 | 1.18 | 0.81 |
| | 5 | 8.3 | 6.1 | 35.2 | 31.5 | 0.30 | 0.32 |

available to the bird to enable it to utilize fully the large amount of protein in the summit feed, i.e. the protein:energy ratio of the feed is too high (Kyriazakis and Emmans, 1992).

Food intake increased with the dilution of the summit food up to the third dilution, after which it decreased, being lowest on the most dilute food. The increase in intake as the food is diluted confirms previous observations (Gous and Morris, 1985) and can be explained as an (unsuccessful) attempt by the bird to consume the required amount of the most limiting nutrient in the feed.

Efficiency of utilization of methionine for growth, which is calculated by determining the amount of methionine required per g of gain in treatments in which methionine is limiting (e.g. Treatment 4), appeared to be remarkably similar among all the genotypes indicating that selection for feed efficiency, which has been successfully applied in Australia, has apparently not altered the nett efficiency of utilization of amino acids. Analyses of carcass composition presently underway will determine whether differences exist in the efficiency with which methionine is deposited as body protein.

Feather weight gain appeared to be correlated with body weight gain, with the highest gains being achieved on the second feed treatment (0.8 of summit). Feather weight gain as a proportion of body weight gain remained at approximately 0.053 (±0.006) in all treatments. It appears that these broilers did not adjust feather growth, at least over the short term, in an attempt to allow more heat to be lost from the body, a strategy which, if applied successfully, would allow birds fed a low quality food (e.g. lower protein:energy ratio than required) to consume more of such a food and hence grow faster.

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# INFLUENCE OF EXOGENOUS ENZYMES ON THE METABOLIZABLE ENERGY AND AMINO ACID DIGESTIBILITY VALUES OF WHEAT-BASED DIETS FED TO THREE BROILER STRAINS

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Several studies have demonstrated that the viscous carbohydrates in wheat lower nutrient digestibility and, therefore, chick performance and that these anti-nutritive effects of wheat can be overcome by the addition of exogenous enzymes. Although the efficacy of exogenous enzymes in improving growth performance and apparent metabolizable energy (AME) is established, limited information is available on the influence of added enzymes on the amino acid utilization of wheat-based diets. Hew *et al.* (1995) reported that the addition of two commercial enzymes to diets containing 918 g/kg of wheat improved the apparent amino acid digestibility (AAAD) by chickens. The present study was undertaken to investigate the effect of a commercial enzyme preparation (Avizyme 1300®; Finnfeeds International Ltd, U.K.) on the AME and AAAD of practical diets containing 408 g/kg of wheat when fed to three strains of broiler chickens.

Male broiler chicks from three commercial hatcheries, designated as strains A, B and C, were obtained at 1-day of age and reared under similar management conditions. On day 21, 50 birds from each strain were selected and groups of five were randomly assigned to each of 30 pens. Enzyme treatment (unsupplemented or supplemented) was then assigned within strain to five pens. The unsupplemented basal diet contained 408 g/kg of wheat, and celite (20 g/kg) was added as an indigestible marker. Experimental diets were fed from day 21 to day 42 and total collection of excreta was carried out during the last three days to determine the AME values. At the end of the trial all birds were killed, ileal contents were obtained and apparent ileal amino acid digestibilities were calculated using acid insoluble ash as the marker. In addition, 20 birds within each strain were fed the experimental diets (10/diet) from day 21 to day 28. On day 28, ileal contents were obtained from all birds for viscosity measurements using a Brookfield viscometer.

Strain x diet type interactions were not significant for any of the parameters evaluated. Enzyme supplementation resulted in 2.8% improvement ($P < 0.05$) in the AME of wheat-based diets. The AME contents of the basal and enzyme supplemented diets were 13.20 and 13.57 MJ/kg, respectively. Strain A (13.49 MJ/kg) and Strain C (13.57 MJ/kg) tended to have higher ($P<0.09$) AME values than Strain B (13.10 MJ/kg). The digestibility values of all amino acids were 1-2 percentage units higher in enzyme supplemented diets, but the differences were significant ($P < 0.05$ to 0.001) only for aspartic acid, serine, glutamic acid, methionine, isoleucine, histidine and arginine. Significant ($P < 0.001$) strain effects were observed for all AAAD values, with Strain A recording the highest (80.2 - 94.5%) and Strain B the lowest (70.9 - 91.1%). Viscosity of ileal digesta, however, was not influenced by enzyme supplementation or genotype of bird.

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# APPARENT ILEAL DIGESTIBILITIES OF AMINO ACIDS IN CEREALS FOR BROILER CHICKENS

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Cereals constitute approximately 50-70% of poultry feed formulations and their contribution to the protein supply in diets is nutritionally important. Typically, about 25-40% of the amino acid requirements may be supplied by cereals (Ravindran and Blair, 1992). Wheat, maize and sorghum are the major cereals used in poultry feed formulations. However, these cereals are known to vary in total protein and in amino acid concentrations and, possibly, digestibility. Therefore, an accurate knowledge of amino acid digestibility is of practical importance for proper supplementation of cereal-based diets with amino acids of synthetic or protein origin. The aim of the present study was to determine the variations in the apparent ileal digestibility of amino acids in two cultivars of wheat, five cultivars of maize and four cultivars of sorghum.

Assay diets contained 918 g/kg of the test cereal, 20 g/kg of soyabean oil and 42 g/kg of vitamin and mineral supplements. Celite (20 g/kg) was added to diets as an indigestible marker. Each assay diet was fed ad libitum to three pens (4 birds/pen) of male broilers from 35 to 42 days of age. At the end of the trial, ileal contents were collected and processed as described previously (Siriwan *et al.*, 1993). Samples of diets and digesta were analysed for amino acids and acid-insoluble ash, and the apparent ileal amino acid digestibility values were calculated.

The mean apparent ileal digestibility values of the amino acids in the cereals were remarkably similar. The overall means (%) were: maize, 78.2 (76.0-81.3); sorghum, 78.7 (75.4-82.5) and wheat, 79.0 (77.9-80.1). However, variations were observed in individual amino acid digestibilities among cultivars within cereal type. In the five maize cultivars, threonine and lysine digestibility values varied by 5.1 and 2.1 percentage units, respectively. For the four sorghum cultivars, corresponding figures were 12.6 and 15.1, and for the two wheat cultivars, 5.3 and 3.8, respectively. The high variation observed in sorghum was due to the low digestibilities in two cultivars. Significant cultivar effects on amino acid digestibilities in sorghum have been reported previously (Stephenson *et al.*, 1971). The apparent ileal digestibilities determined for maize and wheat in the present study are higher than the apparent excreta digestibility values reported by Green *et al.* (1987) using precision-fed adult cockerels.

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# IMMUNITY AS A HOMEOSTATIC MECHANISM INFLUENCING GROWTH AND PRODUCTION

A.J. HUSBAND

Summary

The immune system is usually viewed as a defence mechanism responding to environmental pathogens to eliminate microbes and return to a resting state. However, the immune system is part of a broader homeostatic network continually responding to both environmental and endogenous signals. The endocrine and immunological outputs to combined microbial and other ambient stressors are a cross-regulating component of the homeostatic response to environmental change and have a profound influence on non-immune metabolic events such as energy and nutrient partitioning, feed conversion efficiency, growth and development. In intensive animal systems there are considerable potential benefits to be gained if strategies can be developed to manage the adverse effects of these adaptive responses.

## I. INTRODUCTION

Homeostasis has traditionally been viewed as a process in which physiological systems such as fluid dynamics and composition, tissue generation and repair, energy generation and utilization, and body temperature are maintained in balance. This is mediated by biofeedback mechanisms involving central nervous system control responding to data feedback from a variety of organs via effector-affector pathways.

The immune system has not normally been seen as part of this homeostatic network but rather as an effector mechanism reacting to environmental antigenic challenge with defensive responses designed to eliminate "foreign" substances as efficiently as possible and then return to a resting state. However, there is now substantial evidence to suggest that immunity is not only an effector response to environmental challenge but also can be affected by these events. Moreover, the ongoing perturbations in baseline immune output resulting from its reaction to environmental change have profound effects on other physiological processes. Thus, the immune system, in addition to its operation as a defence mechanism, is in part a sensory organ and a regulator of metabolism, working together with the neuroendocrine responses to achieve homeostasis. The implications for animal production will be discussed in this paper, including the apparent involvement of the immune responses to both clinical and sub-clinical pathogen challenge, as well as other environmental stressors, in modification of non-immune metabolic events such as energy and nutrient partitioning, feed conversion efficiency, growth and development.

## II. CNS-IMMUNE PATHWAYS

These physiological interactions occur via a complex set of pathways connecting the immune system and the central nervous sytem (CNS). Although the CNS is the communications processor in the homeostasis network, communication pathways exist between the immune system and the CNS to support bi-directional information flow enabling

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immune output to regulate or influence other physiological adjustments to environmental change.

There is abundant evidence for pathways by which the immune system and the CNS may interact. Lymphoid tissues have extensive autonomic innervation which have marked local effects on immune function (Felten *et al.*,1985). This is partly a result of changes in haemodynamics as a result of neural control of vascular networks serving lymphoid tissue (Anderson and Anderson, 1975). However, the close anatomical association between noradrenergic fibres and T lymphocytes in the spleen has led to speculation that synapse-like communication may occur with cells of the immune system (Felten *et al.*,1988) but whether true synapses exist between nerves and immune cells remains controversial.

The second type of pathway involves neuropeptide transmitters released from the central and peripheral nervous system which have immunomodulating properties. It is well documented that lymphocytes have surface receptors for a wide range of neurotransmitters which have powerful direct effects on both lymphocyte function and migration (Ottaway and Husband, 1992; Ottaway and Husband, 1994). For instance the enteric neuropeptides vasoactive intestinal peptide and substance P have profound effects on lymphocyte migration in the gut (Ottaway, 1991; Stanisz *et al.*,1986).

The third type of communication involves neuroendocrine pathways. Selye's 'general adaption syndrome', in which exposure to acute environmental stressors results in lymphoid tissue involution, is well known (Selye, 1936). This involves increased pituitary output of adrenocorticotrophic hormone (ACTH) in response to stressors perceived from the environment and this, in turn, activates the production from the adrenal cortex of corticosteroid hormones which have powerful suppressive effects on immunity (Axelrod and Reisine, 1984; Cupps and Fauci, 1982). Many of the other pituitary hormones also have immunomodulatory effects - growth hormone release has been reported to promote lymphocyte activation (Bland and Warren, 1986); a-melanocyte stimulating hormone has anti-inflammatory effects (Cannon *et al.*,1986); arginine vasopressin acts synergistically with ACTH to modulate immunosuppression (Gibbs, 1986); and prolactin is immunostimulatory and maintains immunological functions (Reber, 1993; Rovensky *et al.*,1991). In addition, both testosterone and oestrogen have been shown to alter the peripheral distribution of both B and T lymphocyte subsets (McDermott *et al.*,1980; Hann *et al.*,1988; Gudmundsson *et al.*,1988).

The acute sensitivity of the immune system to endocrine influence is such that the diurnal variation in pituitary-adrenal output is reflected inversely in circadian changes in circulating lymphocytes (Kawate *et al.*,1981) and there is a heightened sensitivity to immune stimulation in the afternoon and evening relative to morning (Levi *et al.*,1985). Nevertheless, bioperiodicity in the immune system extends beyond 24 h cycles and there is evidence for 12 h, 7 day, 28 day (in ovulating females) and even seasonal rhythms (Knapp, 1992).

## III. IMMUNE ACTIVATION: EFFECTS ON CNS RESPONSES

While it is clear that the CNS influences immunity, to what extent can immune function influence central nervous output? Whereas hormones and neurotransmitters are the messenger molecules of the neuroendocrine system, cytokines perform the same function for the immune system. Cytokine output during an immune response not only regulates immune function but affects CNS function by stimulating endocrine output. Endotoxin challenge has been shown to result in TNF-a release which stimulates growth hormone, ACTH, cortisol and prolactin secretion (Coleman *et al.*,1993; Kenison *et al.*,1991). The cytokines IL-1 and

IL-2 have been shown to enhance proopiomelanocortin gene expression in the pituitary and to augment ACTH and cortisol production (Brown *et al.*, 1987). A link between immune activation and CNS output is also supported by the observation of a change in the catecholamine content of lymphoid tissues and the hypothalamus in response to antigen challenge in primed animals (Besedovsky *et al.*,1979; Besedovsky *et al.*,1983) and electroencephalographic studies have detected changes in firing rates of hypothalamic neurons in rats following antigenic challenge (Besedovsky *et al.*,1985).

Activated lymphocytes also are capable of endogenous production of many of the endocrine factors associated with the central nervous and immune interactions. Cells of the immune system have been shown to contain either peptides or mRNA for over 20 different neuroendocrine substances, including ACTH, corticotrophin-releasing hormone, growth hormone, prolactin and endorphins (Blalock, 1994). Thus, just as the CNS through sensory input in response to environmental change relays information to the immune system via hormones and neurotransmitters, the immune system also has sensory receptors which detect the presence of antigens (which are not recognised by the central nervous system), resulting in information relay to the CNS via cytokine release. Blalock (1994) has proposed that since cytokines, peptide hormones and neurotransmitters are a set of soluble messengers common to both the immune and central nervous systems, interacting in a bidirectional fashion, the immune system may be considered as both a sensory and effector organ.

The functional significance of endocrine-immune interactions is highlighted by observed differences in disease patterns between different genetic lines of pigs and poultry. Studies in rodents, as reviewed by Mason (1991), have revealed that this phenomenon may be explained on the basis of relative differences in cytokine and corticosteroid responses to microbial and environmental stress. For example, Balb/c mice are highly susceptible to the intracellular protozoan parasite *Leishmania major*, whereas C57/Bl mice are highly resistant (Heinzel *et al.*, 989). Resistance to *L. major* requires a cell mediated effector response but corticosteroid hormones have been shown to promote antibody responses while at the same time depressing cell-mediated immunity (Akahoshi *et al.*, 1988). However, the stress of infection in Balb/c mice results in corticosteroid production sufficient to tip the cytokine profile balance in favour of antibody production, thereby inhibiting the development of cellular immune defences. These animals are then unable to eliminate the organism. On the other hand C57/Bl mice, a low corticosterone responder strain, mount a successful cell-mediated response despite the stress of infection. This pattern of susceptibility is reversed with respect to autoimmune disease. In experimentally induced allergic encephalomyelitis (EAE), PVG strain rats, which like Balb/c mice are high corticosterone responders, are highly resistant (Mason *et al.*, 1990) whereas Lewis rats, a low responder strain, are highly susceptible (MacPhee *et al.*, 1989). The corticosteroid response induced by the stress of disease in this case is an advantage in inhibiting the development, dissemination and effector activities of autoreactive clones (MacPhee *et al.*, 1989).

An even more intriguing manifestation of a functional bi-directional link between the brain and the immune system is the demonstration that immunity can be modulated through learning. Although this concept was first reported by Russian scientists as early as the 1920's (Metal'nikov and Choring, 1926) it did not gain prominence in western scientific literature until the 1970's following the re-examination of the phenomenon by Ader and colleagues (Ader and Cohen, 1975). These studies demonstrated that behavioural (Pavlovian) conditioning could be used not only to achieve predictable physiological responses to sensory stimuli but, if the conditoned stimulus was linked to immune effects, these immune effects could be re-enlisted after stimulus re-exposure. This technique can be used to achieve either

immunosuppression or immunostimulation in both animal and human subjects (Husband, 1993).

## IV. IMMUNE ACTIVATION: EFFECTS ON OTHER PHYSIOLOGICAL PROCESSES

Immune activation also provides feedback with functional effects on other physiological systems. The so-called 'acute phase response' following infection or antigenic challenge is the immunological component of the homeostatic response to infection, tissue injury or other immunological disturbance and is characterised by the production of IL-1, IL-6, TNF-a and interferons. Many of these cytokines produce CNS-mediated effects on behaviour. For instance IL-1 induces fever (Fontana *et al.,* 1984), reduces social exploration and appetite (Dantzer et al.,1993), and impairs spatial navigation learning (Oitzl *et al.,* 1993); IL-6 is somnogenic (Krueger *et al.,* 1984) and induces lethargy, depression, anorexia and fever (LeMay *et al.,* 1990; Dantzer and Kelley, 1989); interferons produce fever, lethargy, anorexia, vomiting and general malaise (Atzpodien *et al.,* 1995).

A less obvious manifestation of immune system feedback on physiological regulation is the effect of chronic immune activation on growth and development (Solomons *et al.,* 1993). In humans, early growth failure and short stature may result from recurrent respiratory and gastrointestinal tract infections (Black, 1991). In limited human epidemiological studies prophylactic use of antibiotics in subjects exposed to poor environments has resulted in improved weight gain compared to controls with the same dietary intake (Robinson, 1952). These effects are more apparent, however, in intensive animal production systems where it is an established management principle that, even in the absence of infectious disease outbreaks, animals grow faster if antibiotics are added as a feed supplement (Coates *et al.,* 1963).

Thus, the effect of microbial load together with other environmental stressors such as ambient, nutritional, or psychological factors, combine to provoke an integrated homeostatic response, described by Elsasser (1993) as an 'endocrine-immune gradient', the sum of homeostatic responses to all environmental stressors whether perceived by the CNS or the immune system. Elevation in the gradient leads not only to growth retardation but also to qualitative changes in nutrient partitioning. Under optimal conditions, at a low plane of nutrition the nutrient input into muscle development exceeds that for fat deposition but, as the plane of nutrition rises, fat deposition takes priority over muscle development (Roura *et al.,* 1992). However, the point at which switchover from muscle to fat deposition occurs is reduced in the face of a high endocrine-immune gradient. This redistribution of resources within the body in response to changes in the gradient explains the large differences observed in certain physical traits such as fatness, feed conversion efficiency and protein accretion in genetically identical animals maintained on a similar nutritional regimen, but exposed to different levels of microbial contamination (Williams *et al.,* 1993).

The contribution to these effects by immunologically mediated feedback on metabolic processes is orchestrated by output from the immune system of the acute phase response proteins and inflammatory cytokines, especially IL-1, IL-6 and TNF-a. In addition to their behavioural effects such as inappetance and reduced feed intake, these cytokines alter metabolic processes causing diversion of nutrients away from normal metabolism to support the host defence responses (Klasing et al., 1991). Indeed in studies with chickens, simple administration of bacterial endotoxin or polydextrins caused a significant reduction in weight gain but only 70% of this effect could be attributed to reduced feed intake, the remainder being accounted for by reduced feed conversion efficiency (Klasing *et al.,* 1987). The cytokine mediation of this effect is supported by the observation that feeding antibiotics to chickens in an environment with heavy microbial contamination decreases the amount of

circulating IL-1 to levels more similar to those of birds raised in a clean environment (Roura *et al.*, 1992).

## V. INTERVENTION STRATEGIES TO MAXIMISE ANIMAL PRODUCTION

Differences in growth patterns and body composition in response to environmental stressors (Klasing et al., 1991) and differences in disease susceptibility (Manuck *et al.*, 1990) may well be explained on the basis of genetically determined variation in the integrated homeostatic response involving immune, endocrine and CNS pathways. This is reflected in the relative slope of the endocrine-immune gradients and manipulation of hormone and cytokine responses represent new opportunities to manage the gradient effects.

Modification of hormonal responses by pharmacological methods in animals grown for human consumption has met with intense consumer resistance. A novel alternative approach has been developed using vaccination techniques to achieve the same result. If appropriate vaccine formulations are used it is possible to achieve sufficient levels of anti-hormone antibodies, or antibodies to hormone receptors which block hormone action, to intercept signal transduction via the pituitary-adrenal axis. Immunization against ACTH in a number of farm animal species has not only resulted in improved weight gain and feed conversion efficiency but has improved carcass quality by reducing fat deposition (Wynn *et al.*, 1994). Similarly, immunization against somatostatin production, to release the biological brakes on endogenous growth hormone activity, has been suggested as an alternative to promoting growth potential by growth hormone administration (Westbrook *et al.*, 1993; Spencer, 1986).

Manipulation of cytokine profiles to optimize immune responses is also possible by the use of anti-cytokine antibodies or gene therapy approaches (Ramsay *et al.*, 1994). Routine therapeutic control of cytokine output by these approaches in production animals is now an imminent possibility for managing the adverse effects of immune activation on growth and production. A more speculative approach is emerging from antisense technologies, in which selective gene transcription can be transiently suppressed by delivery to target cells of genetic material with a complimentary nucleotide sequence which binds to messenger RNA blocking its transcription (Jun *et al.*, 1995). Alternatively the "gene shears" or ribozyme technology may be a useful approach involving delivery of genetic material which inserts itself into mRNA to cause a break in transcription adjacent to the target gene (Sioud *et al.*, 1992). In animal production where economic reality is forcing increased dependency on intensive systems optimizing feed conversion efficiency and carcass quality will depend increasingly on managing the endocrine-immune gradient. These approaches to control the adverse effects of a maladaptive cytokine response to 'immunologic stress' are currently under investigation in this laboratory.

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# SHORT-TERM FEEDING OF NON-STARCH POLYSACCHARIDES TO BROILER CHICKENS: EFFECTS ON VILLUS STRUCTURE AND BRUSH BORDER PEPTIDASE EXPRESSION

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The search for alternatives to replace traditional feed ingredients has meant an increased use of novel sources of energy and protein. Poultry performance on the alternative feeds is often poor (Friesen *et al.,* 1992). This has been attributed to the presence of anti-nutritive factors, notably the non-starch polysaccharides (NSP) which alter the viscosity of digesta and the attendant uptake of nutrients in broilers (Annison *et al.,* 1995). However, whether this is due solely to limiting nutrient access or to changes in villus surface area and the expression of digestive enzymes in the brush border membrane of enterocytes in broiler chickens is not known. This paper addresses the effect of short-term NSP feeding on villus structure and brush border enzyme expression.

Thirty-two broiler chicks three weeks of age (712 ± 8g bodyweight) were randomly allocated to individual cages. A basal diet containing rye (63%), identical in other major ingredients was supplemented with 50 g/kg of one of cellulose, oat hulls, wheat NSP or rice NSP. Birds were maintained on these diets for 7 days. At slaughter, 1 cm samples of duodenum and ileum were either fixed in formalin or snap frozen for histology and histochemical studies, respectively. Data are expressed as the mean ± SE. Significance of difference was determined by analysis of variance.

The non-cellulose supplements depressed metabolizable energy and increased feed intake. Relative to cellulose, the other supplements except wheat NSP reduced weight gain and feed conversion efficiency. However, these differences between diets were non-signficant. For the cellulose supplement the duodenal villi (1020±148 μm) were 40% longer ($P<0.05$) than for ileal tissue. This trend between regions was observed for all diets. However, for any given region, diet had no signficant effect. In the duodenum, mean enterocyte aminopeptidase N (APN) activity was 37±6 arbitary units (AU) whereas dipeptidylpeptidase IV (DPPIV) was not detected. Ileal tissue expressed twice the activty of APN as duodenal tissue and 36±10 AU of DPPIV. In addition, supplementing with cellulose significantly ($P<0.05$) sustained 18-33% higher activity of APN in the ileum than did the other supplements.

Short-term feeding of non-cellulose dietary supplements did not affect the spatial distribution of the major brush border peptidases nor the regional difference in intestinal morphometry. Generally, cellulose and the NSP manifested similar effects on gut structure and brush border peptidase expression in the short term. The long-term effect of these supplements requires investigation.

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# EFFECTS OF LUPINS (*L ALBUS* AND *L ANGUSTIFOLIUS*) ON VILLUS STRUCTURE AND BRUSH BORDER PEPTIDASE EXPRESSION IN BROILER CHICKENS

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New varieties of White Lupins (*L. albus*) and Australian Sweet Lupin (*L. angustifolius*) are currently being evaluated as feed ingredients for poultry. Previous research evaluating lupins has been directed at native varieties with a paucity of information available on the feeding value of new types of lupin (Brenes *et al.*, 1993). Feeding diets of different quality can profoundly affect gut structure (Jin *et al.*, 1994) which may influence digestive capacity. This paper addresses the effect of short-term feeding of low-alkaloid lupins on animal growth, villus structure and brush border enzyme expression.

Broiler chicks three weeks of age (830 ± 3.5g body weight) were randomly allocated 6 per cage with 5 cage replicates per diet. The diets were cold-pelleted and similar in major ingredients, in addition to 300 g/kg supplements of lupins, *L. angustifolius* cv. Gungurru (LAG) or cv. Danja (LAD) or *L. albus* cv. Kiev (LAK). They were compared with a control diet (SC) containing sorghum and casein. Birds were maintained on these diets for 7 days. One bird from each replicate was slaughtered, after which 1 cm samples of duodenum and ileum were either fixed in formalin or snap frozen for histology and histochemical studies, respectively. Data are expressed as the mean ± SE. Significance of difference was determined by analysis of variance.

Final body weight (g), weight gain (g) and food intake (g/day) for all groups were respectively 1207±34, 378±25 and 128±6. No significant effect of lupins as an alternative protein and energy source on these parameters was observed. For SC (control diet), the duodenal villi (1200±208 μm) were 38% longer ($P<0.05$) than for ileal tissue. This trend between regions was observed for all diets. The lupin diets gave a 20% increase in villus length in the duodenum, but not the ileum. The duodenal crypts were significantly ($P<0.05$) deeper on SC and LAD than on LAG and LAK. In the duodenum, mean enterocyte aminopeptidase N (APN) activity was 29±4 arbitrary units (AU) whereas dipeptidylpeptidase IV (DPPIV) was not detected. Lupin supplementation caused small, but non-significant, increases in peptidase expression in the duodenum and ileum.

Inclusion of the tested lupins at up to 300 g/kg posed no detrimental effects in the short-term. Structural changes in the duodenum of birds fed lupin diets may relate to the higher fibre content of these diets. The significance of the structural change in the duodenum to feed utilisation over longer periods needs to be evaluated.

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# DETERMINATION OF TOTAL AND DIGESTIBLE AMINO ACIDS BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

D.A. JACKSON, J.C. BODIN and R. MAILLARD

Summary

The content of this paper discusses an application of near infrared reflectance spectroscopy (NIRS) for the prediction of both total and true ileal digestible lysine, methionine, cystine, threonine and tryptophan in feed raw materials. The work summarises global and specific raw material calibrations of the following categories. (1) Animal proteins, consisting of meat and bone meal, fishmeal and poultry by-product meal. (2) Vegetal proteins, consisting primarily of soyabean, canola and sunflower meals. (3) Cereals, consisting of maize, wheat and barley. Specific calibrations were developed for meat and bone meal, fishmeal and soyabean meal. Calibrations demonstrated good performance as described by coefficients of calibration ($R^2$) greater than 0.90, with the exception of several expressions relating to cystine. The standard errors of cross validation (SECV) were acceptable for this type of prediction at between 5 and 10% of the mean. It is concluded that NIRS can provide an alternative method for the prediction of total and digestible amino acids, allowing alternatives to 'book' values and to regular measurement by the time-consuming and costly *in vivo* method.

## I. INTRODUCTION

Feed formulation for poultry based on ileal digestibility of amino acids provides an accurate method of providing the correct amino acid profile for the production of meat or eggs. Although it differs from the availability of amino acids for tissue growth in poultry (Parsons, 1994), it is the nearest practical measurable estimate of the amino acid value of a foodstuff. The technique involves the precision feeding of a number of caecectomised cockerels and the measurement of the amounts of amino acids absorbed. The technique also includes the measurement of endogenous losses of amino acids to enable the calculation of "true" ileal amino acid digestibility. The technique is expensive to undertake and is very time consuming, thus the data produced is normally of a historical nature and cannot be regarded as being interactive with feed formulation.

Such studies have resulted in the publication of a number of data bases, such as the Rhône Poulenc Nutrition Guide (Rhone Poulenc, 1993) containing considerable information on the digestibility of many raw materials. Whilst these tables are of great application, the information exists as mean values and little or no account can be made for the variability in amino acid digestibility within individual raw materials. It is the application of NIRS that can take the utilisation of digestible amino acids one step further as an interactive tool in feed formulation and help overcome the limitations of using mean tabular values.

The technique of near infrared reflectance was first developed by Norris

Rhône-Poulenc Animal Nutrition, 42, Avenue Aristide Briand, BP 100, 92164 Antony, France.

(1964) as a method of measuring the water content in grain. Its acceptance as an analytical tool was due to the speed of analysis, low operating cost and safety. This early work established the basics of modern NIRS technology and from this has arisen applications to measure protein and fat as well as structural and non structural carboydrate fractions. The major advancements in the applications of NIRS technology have been a result of two factors. Firstly, the recent availability of high speed computing power and, secondly, the availability of affordable scanning NIRS instruments. These developments have taken the limitations beyond the original spectral interpretation of single or multiple absorption peaks to the concept of mathematical modelling of spectra to any analytical parameter. The parameter need not necessarily be a classical chemical determination but an *in vivo* nutritional value.

To the knowledge of the author very little work has been undertaken using NIRS to predict the digestibility of amino acids in feed ingredients. A report on peanut meal has appeared (Wei Ruilan Zhang Ziryi, 1993) for lysine, arginine, histidine, valine, leucine, phenylalanine, threonine and isoleucine. He reported that successful calibrations were developed except for lysine.

## II. MATERIALS AND METHODS

The methodology describes the development of NIRS equations to predict the true ileal digestibility of amino acids in feed raw materials for poultry. The methodology for the *in vivo* technique has been published by Green *et al.* (1987a,b). A second set of calibrations were developed to predict total amino acids in the same feed ingredients.

### (a) Animals and housing

The birds used for these measurements were caecectomised cockerels of the ISA Brown strain. All birds were ceacectomised to remove the main sites of intestinal microflora. The surgery was performed at approximately 18 weeks of age and the birds were used in feeding trials for a period of 9 months. Birds were placed in individual cages to allow total collection of excreta. When not on test birds were fed *ad libitum* a standard poultry feed. Water was constantly available and room temperature and humidity were controlled at 20-22°C and 60-70% respectively.

### (b) Experimental feeds and test schedule

The raw materials used in these analyses were of various types. Animal proteins consisted of meat and bone meal, fishmeal and poultry by-product meal. Vegetal proteins consisted primarily of soybean, canola and sunflower meals. Cereals consisted of maize, wheat and barley. Raw materials were first analysed for crude protein. The raw material was then mixed with purified maize starch, minerals and vitamins to provide a complete feed containing 180 g crude protein/kg. Raw materials containing less than 180 g crude protein/kg (i.e. cereals) were prepared by grinding with the addition of minerals and vitamins. To calibrate for endogenous amino acid losses, the birds were fed a protein-free diet containing 480 g maize starch, 480 g sucrose and 40 g minerals plus vitamins/kg.

A group of 24 birds was used for the measurement of each raw material sample. The birds were fasted for 48 h before being fed the experimental feed.

During this preliminary period 50 g of glucose was fed via the drinking water to limit amino acid catabolism for energy purposes. At the end of the fasting period birds were precision-fed 50g of the experimental feed. Excreta were collected for a period of 48 h after feeding, this time period being necessary to allow complete transit of feed. Excreta were collected twice a day and stored at 4°C. At the end of the collection period the excreta were weighed and a composite sample freeze-dried and then ground and sieved to segregate contamination by feathers and skin desquamations.

(c) Determination of endogenous excretion

The calculation of true digestibility requires estimation of the endogenous losses resulting from digestive enzyme secretions, mucus and gut wall desquamations. The endogenous amino acid values were determined by precision feeding the cockerels as described previously using 50 g of a protein -free diet.

(d) Analysis of amino acids

Samples of protein feedstuffs and excreta were analysed for their amino acid contents by Rhône Poulenc standard methodology (Rhône Poulenc, 1995). The methods are based on either acidic or basic hydrolysis followed by ion exchange chromatography for all amino acids except tryptophan for which high pressure liquid chromatography is employed.

(e) Near Infrared Reflectance Instrumentation and Calibration Development

The NIRS instrument used was a scanning monochromator (Perstorp NIR Systems 6500, 12101 Tech Road, Silver Spring, MD 20904, USA). Samples were scanned using the large rectangular cell in transport mode and a total of 24 scans were recorded as the cell descended in the path of the beam of radiation. The mean of these 24 scans was recorded as the spectra from each sample.

Calibrations were developed using the software NIRS II version 3.0 (Infrasoft International, Port Matilda, PA16870, USA). The spectra were first analysed by a principal component analysis, using the algorithm "Centre" to structure and define the population of samples. Spectral data were then transformed to reduce the effect of differential particle size using the process of "Standard normal variate detrend". Calibrations were developed using the method of modified partial least squares using either first or second order derivatisation.

## III. RESULTS AND DISCUSSION

The NIRS calibrations and their respective performance parameters are given in Tables 1 to 6. The calibrations are described as either being global or specific. A global calibration is composed of several types of feed raw material all having similar spectral characteristics. A specific calibration is composed from only one type of feed raw material. The ability to select spectral populations of similar characteristics is based on the software routine symmetry, (Infrasoft International). This routine describes a population in terms of a principle components analysis and can derive in qualitative terms how similar or dissimilar the spectra of a sample is from the mean of

a population. Obviously, a single raw material calibration is preferable to a global calibration but the number of samples analysed for digestible amino acids is the limiting factor.

The calibration performance parameters for the global animal products are given in Table 1. All the selected amino acids calibrations were successfully developed with the exception of cystine. The calibrations for total amino acids were of greater accuracy than for digestible amino acids but the relative difference remained small and did not detract from the application of the digestible calibrations.

The global animal protein calibrations (Table 1) were then divided into two separate calibration data sets, meat and bone meals (Table 2) and fishmeals (Table 3). The calibrations for meat and bone meals also demonstrated good performance, again with the exception of cystine. Calibrations of cystine were unsuccessful having $R^2$ values below 0.4 and are not presented in the results.

Table 1. Near infrared reflectance spectroscopy calibration performance parameters for total and digestible amino acids of animal proteins (meat meals, fish meals and poultry by-products).

| | N | Mean | $R^2$ | SEC | SECV |
|---|---|---|---|---|---|
| Crude protein | 73 | 59.35 | 0.97 | 1.69 | 2.52 |
| Total Amino acids (% fresh weight) | | | | | |
| Lysine | 77 | 3.42 | 0.98 | 0.14 | 0.22 |
| Methionine | 79 | 1.07 | 0.97 | 0.07 | 0.10 |
| Cystine | 73 | 0.70 | 0.71 | 0.18 | 0.22 |
| Methionine+Cystine | 69 | 1.76 | 0.83 | 0.21 | 0.26 |
| Threonine | 74 | 2.18 | 0.93 | 0.14 | 0.16 |
| Tryptophan | 66 | 0.47 | 0.96 | 0.04 | 0.05 |
| True ileal digestible amino acids (% fresh weight) | | | | | |
| Lysine | 50 | 2.49 | 0.94 | 0.18 | 0.36 |
| Methionine | 50 | 0.84 | 0.95 | 0.08 | 0.13 |
| Cystine | 28 | 0.33 | 0.51 | 0.08 | 0.12 |
| Methionine+Cystine | 48 | 1.18 | 0.78 | 0.19 | 0.27 |
| Threonine | 43 | 1.62 | 0.94 | 0.10 | 0.13 |
| Tryptophan | 37 | 0.29 | 0.92 | 0.03 | 0.06 |

N - number of samples
Mean - mean of population (% fresh weight)
$R^2$ - Coefficient of calibration
S.E.C - Standard error of calibration
S.E.C.V. - Standard error of cross validation (% fresh weight)

The calibrations for fishmeal must be regarded as being in the development stage due to the low number of replicates in the data sets. This is especially true for the digestible amino acid calibrations. However, their potential is evident from the good calibration performance parameters and this justifies their inclusion for discussion in this paper.

Table 2. Near infrared reflectance spectroscopy calibration performance parameters for total and digestible amino acids for meat and bone meals.

| | N | Mean | $R^2$ | SEC | SECV |
|---|---|---|---|---|---|
| Crude protein | 29 | 54.98 | 0.91 | 2.67 | 3.04 |
| Total Amino acids 2.91(% fresh weight) | | | | | |
| Lysine | 30 | 2.91 | 0.97 | 0.08 | 0.19 |
| Methionine | 39 | 0.88 | 0.89 | 0.08 | 0.11 |
| Threonine | 28 | 1.88 | 0.93 | 0.07 | 0.10 |
| Tryptophan | 29 | 0.36 | 0.86 | 0.04 | 0.05 |
| True ileal digestible amino acids (% fresh weight) | | | | | |
| Lysine | 30 | 2.40 | 0.90 | 0.17 | 0.29 |
| Methionine | 30 | 0.77 | 0.85 | 0.08 | 0.11 |
| Threonine | 21 | 1.55 | 0.91 | 0.09 | 0.12 |
| Tryptophan | 20 | 0.26 | 0.96 | 0.02 | 0.03 |

Table 3. Near infrared reflectance spectroscopy calibration performance parameters for total and digestible amino acids for fishmeals.

| | N | Mean | $R^2$ | SEC | SECV |
|---|---|---|---|---|---|
| Crude protein | 19 | 63.89 | 0.92 | 1.80 | 4.18 |
| Total Amino acids (% fresh weight) | | | | | |
| Lysine | 19 | 4.46 | 0.96 | 0.16 | 0.32 |
| Methionine | 19 | 1.67 | 0.93 | 0.10 | 0.17 |
| Threonine | 19 | 2.65 | 0.93 | 0.10 | 0.17 |
| Tryptophan | 19 | 2.26 | 0.99 | 0.04 | 0.19 |
| True ileal digestible amino acids (% fresh weight) | | | | | |
| Lysine | 7 | 3.86 | 0.99 | 0.05 | 0.48 |
| Methionine | 7 | 1.83 | 0.99 | 0.04 | 1.13 |
| Threonine | 7 | 2.16 | 0.98 | 0.06 | 0.40 |

Table 4 represents the calibration performance parameters of a data set of vegetal proteins. Although its application can be regarded as limited, due to accepted book value differences for digestible and total amino acids of each raw material class, its development is a worthwhile first approach to developing single raw material calibrations for vegetal proteins. An example of this is given in Table 5, a set of calibrations for soybean meal. These calibrations demonstrate good performance, with the exception of cystine.

Table 6 gives the results for the preliminary stages of cereal calibrations. This application is accepted as being the most difficult of the calibrations presented due to the low quantities of amino acids present. The performance parameters demonstrate that this application is also possible, but the addition of further samples and cereal-specific calibrations need to be progressed.

As a general overview, these calibrations are very encouraging and they will benefit by an increase in the number of replicates. Predictions were sucessful for total and digestible lysine, methionine, threonine and tryptophan in all raw material classes studied. However, calibrations were least sucessful for cystine. The cause can

Table 4. Near infrared reflectance spectroscopy calibration performance parameters for total and digestible amino acids for vegetal proteins, (soy, canola and sunflower meal).

| | N | Mean | $R^2$ | SEC | SECV |
|---|---|---|---|---|---|
| Crude protein | 47 | 41.9 | 0.98 | 0.65 | 1.06 |
| Total Amino acids (% fresh weight) | | | | | |
| Lysine | 55 | 2.14 | 0.95 | 0.15 | 0.17 |
| Methionine | 52 | 0.63 | 0.84 | 0.04 | 0.07 |
| Threonine | 50 | 1.55 | 0.93 | 0.06 | 0.12 |
| Tryptophan | 34 | 0.52 | 0.87 | 0.04 | 0.06 |
| True ileal digestible amino acids (% fresh weight) | | | | | |
| Lysine | 27 | 1.56 | 0.98 | 0.10 | 0.13 |
| Methionine | 30 | 0.52 | 0.84 | 0.04 | 0.09 |
| Threonine | 32 | 1.17 | 0.90 | 0.09 | 0.16 |
| Tryptophan | 21 | 0.14 | 0.53 | 0.06 | 0.07 |

Table 5. Near infrared reflectance spectroscopy calibration performance parameters for total and digestible amino acids for soybean meal.

| | N | Mean | $R^2$ | SEC | SECV |
|---|---|---|---|---|---|
| Crude protein | 51 | 45.82 | 0.94 | 0.79 | 0.85 |
| Total Amino acids (% fresh weight) | | | | | |
| Lysine | 50 | 2.72 | 0.97 | 0.03 | 0.06 |
| Methionine | 55 | 0.62 | 0.95 | 0.01 | 0.02 |
| Cystine | 55 | 0.62 | 0.78 | 0.02 | 0.04 |
| Methionine+Cystine | 56 | 1.25 | 0.93 | 0.03 | 0.06 |
| Threonine | 54 | 1.73 | 0.98 | 0.02 | 0.03 |
| Tryptophan | 41 | 0.60 | 0.99 | 0.01 | 0.02 |
| True ileal digestible amino acids (% fresh weight) | | | | | |
| Lysine | 27 | 2.43 | 0.98 | 0.03 | 0.07 |
| Methionine | 29 | 0.57 | 0.92 | 0.02 | 0.02 |
| Cystine | 26 | 0.50 | 0.45 | 0.07 | 0.09 |
| Methionine+Cystine | 26 | 1.06 | 0.97 | 0.03 | 0.07 |
| Threonine | 32 | 1.44 | 0.97 | 0.03 | 0.09 |
| Tryptophan | 28 | 0.52 | 0.98 | 0.01 | 0.05 |

either be the inaccuracy of the chemical analysis method for cystine or that the NIRS technique is not suitable. Additionally, the possibility of excreta contamination with feathers and skin desquamations during the *in vivo* study is another possibility as greater sucess was achieved with total rather than digestible amino acid predictions. For these reasons it is likely that it will be only possible to predict cystine as the sum of cystine and methionine using NIRS.

The assessment of the accuracy of a calibration is given by the standard error of cross validation (SECV). These were acceptable for this type of prediction at between 5 and 10% of the mean. An additional point to consider regarding calibration accuracy is the need to compare these calibrations with alternative methods of obtaining the same data. The next stage of calibration development is validation by

statistical comparison with an independent data set. This is limited at present by the number of *in vivo* digestibility tests available.

Table 6. Near infrared reflectance spectroscopy calibration performance parameters for total and digestible amino acids for cereals (maize, wheat and barley).

| | N | Mean | $R^2$ | SEC | SECV |
|---|---|---|---|---|---|
| Crude protein | 23 | 9.64 | 0.99 | 0.23 | 0.58 |
| Total Amino acids (% fresh weight) | | | | | |
| Lysine | 21 | 0.29 | 0.86 | 0.23 | 0.58 |
| Methionine | 22 | 0.16 | 0.88 | 0.02 | 0.03 |
| Threonine | 20 | 0.30 | 0.95 | 0.01 | 0.02 |
| Tryptophan | 19 | 0.09 | 0.88 | 0.01 | 0.02 |
| True ileal digestible amino acids (% fresh weight) | | | | | |
| Lysine | 19 | 0.23 | 0.56 | 0.03 | 0.04 |
| Methionine | 19 | 0.15 | 0.96 | 0.01 | 0.02 |
| Threonine | 19 | 0.24 | 0.91 | 0.01 | 0.02 |

## IV. CONCLUSIONS

It is concluded that, compared with existing methods, the use of NIRS to measure ileal digestible amino acids offers considerable benefits to the feed industry. The technique offers a 'real time' analytical tool for obtaining information on feed ingredients which at present can only beobtained by an expensive bioassay or from mean tabular values. The ability to select and value a protein feed on the basis of greater knowledge and the ability to more efficiently utilise feed ingredients offers the possibility of reducing dietary costs or obtaining more consistent flock performance. The next stage requires the development of NIRS digestible amino acid calibrations for narrow ranges of ingredient types and the extension of this technology to all major feed raw materials.

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# THE IMPORTATION AND DEVELOPMENT OF A SEROTYPE 1 MAREK'S DISEASE VACCINE BASED ON THE CLONE C/R6 OF RISPENS CVI 988 MAREK'S DISEASE VIRUS

C A W JACKSON* and P GROVES**

Summary

The importation of the masterseed of the C/R6 clone of Rispens CVI 988 Marek's disease virus (MDV) was the first live poultry vaccine virus introduced into Australia in over 50 years. The Australian Quarantine Inspection Service (AQIS) gave permission for its importation under an Emergency Permit because of the serious outbreaks of Marek's disease (MD) that were not being effectively controlled by existing Australian manufactured vaccines. Following clearance through the Australian Animal Health Laboratory (AAHL) and extensive testing by Cyanamid Websters for safety and extraneous micro-organisms, a series of laboratory and field trials have been established to prove the value of this vaccine in controlling MD. This paper outlines the development of the new vaccine and the results on safety and efficacy testing to date.

## I. BACKGROUND

Following serious MD problems in 1992 in southern Queensland, Cyanamid Websters applied to AQIS to import an attenuated serotype 1 MD vaccine. AQIS were not prepared to make a decision on this application without widespread industry support. Despite strong representation from a limited part of the industry that was experiencing heavy MD losses, it was not until a general meeting of the Australian Veterinary Poultry Association (AVPA) at the time of the 1994 PIX Conference, that more general support was forthcoming. Following identification of a definitive supplier of vaccine seed, Cyanamid Websters finally secured AQIS permission in December 1994 to import the Rispens C/R6 clone of CVI 988 from Dr G de Boer, Serendip B V, The Netherlands. Permission to import a vaccine seed of C/R6 was granted by the NRA under an Emergency Permit that required clearance by AAHL for safety and freedom from avian pathogens of which imported eggs from domestic hens must be free. In addition, Cyanamid Websters undertook to test the imported seed material for freedom from the 17 avian pathogens specified for SPF flocks in Australia. Cyanamid Websters also undertook to conduct safety and efficacy testing to provide data for registration of the vaccine.

## II. CURRENT MD PROBLEM IN AUSTRALIA

Despite the recognition of very virulent (vv) MDV strains in Australia in the mid 1980s, failure of existing Australian MD vaccines was not reported frequently until 1992 in southern Queensland. Up to 50% mortality was reported in locally bred meat parents and high mortality has continued despite improvements in biosecurity and vaccination programs. Similar losses continue to be reported in imported laying strains but not in locally bred laying strains. The field situation has been described by Jenner (1992,1994) and by Jackson (1995). In contrast to the previous history of MD in Australia, losses were more marked in the

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northern regions and were commonly observed after transfer and during peak production. All breeding companies have undertaken vaccination programs, reviewed vaccine handling and recommended upgrading of biosecurity and hygiene. Vaccination programs using combined Herpesvirus of turkeys (HVT) and a serotype 2 MD vaccine ("Maravac") have reduced losses in imported breeding stock but appear to have compromised vaccination of commercial laying chickens. A second vaccination at 1-3 weeks of age has been found to improve the response to vaccination.

## III. REASONS FOR VACCINATION FAILURES

Whilst the common causes of MD vaccine failure have been well recognised by Australian breeding companies from past experience, the factors associated with the current wave of MD losses have not been well understood. Some of the factors that could be involved include

(a) higher virulence of some Australian MDVs than in the past and their ability to overcome vaccinal immunity especially that induced by serotype 2 vaccine ("Maravac"),

(b) the existence of layer farms that have contributing factors still present that favour early infection by MDV. These factors would include lack of biosecurity, poor brooding area hygiene, close proximity of brooding areas to older laying stock on multi-aged farms,

(c) higher susceptibility of imported layer and meat strains to local virulent MDVs or lack of responsiveness to Australian MD vaccines,

(d) use of bivalent MD vaccines (ie HVT + Maravac) on parents that have then experienced an MD problem thus producing chicks with a very broad spectrum of maternal antibody against all serotypes that can interfere with chick vaccination, and

(e) use of the incorrect vaccination combination or dose for more susceptible strains or farms that may be faced with a combination of some of the above factors.

## IV. RISPENS C/R6 AS THE VACCINE OF CHOICE

As serotype 2 and 3 vaccines have provided good protection to Australian poultry for over 20 years, there has never been a need to develop a serotype 1 vaccine into a commercial product. The original Rispens CVI 988 strain has been widely used throughout Europe for over 20 years and is still widely used often in combination with HVT. When von Bulow (1977) described MD nerve lesions due to the Rispens CVI 988 strain in a susceptible line of chicken, a search was made for a safer serotype 1 vaccine. Consequently, the clone C (de Boer *et al.*, 1986) and its derivative, the clone C/R6 (de Boer *et al.*, 1988) were developed and have slowly replaced the original CVI 988 vaccine. However, in the USA, the clone C gave poor protection when chicks with maternal antibody to bivalent vaccines were vaccinated and has lost market share. More recently, the clone C/R6 has gained market share in Japan and in the USA (Kreager, 1993).

The clone C/R6 was developed to remove any residual pathogenicity and to increase its rate of multiplication in chickens so as to improve its ability to protect against vv MDVs. It was confirmed by de Boer *et al.* (1988) that the protective dose was only 1/3 of that of clone C which had been shown in other studies (de Boer *et al.*, 1986) to be superior to that of

the original Rispens CVI 988. The C/R6 vaccine was also shown to perform better than the clone C vaccine in the face of bivalent maternal antibodies.

In 1994, Cyanamid Websters contracted the Central Veterinary Laboratory, UK on behalf of the Chicken Meat and Egg Industry Research Councils to compare Australian and overseas manufactured vaccines against challenge with the Australian Woodlands vv MDV strain in a challenge trial. The trial (Table 1) found that the C/R6 vaccine gave the highest protection of 90%, whereas the original Rispens CVI 988 gave 67% protection.

TABLE 1. Marek's disease challenge Trial, Central Veterinary Laboratory, UK - Summary of lesions and protection in each vaccine group

| Vaccine group | Number showing specific MD lesions | | | Died without visible MD lesions | Killed at end. No visible MD lesions | % protected |
|---|---|---|---|---|---|---|
| | Tumours | Kidney failure | % | | | |
| Challenge controls | 21 | 9 | 100 | 0 | 0 | 0 |
| CVI 988 | 8 | 2 | 33 | 0 | 20 | 67 |
| C/R6 | 3 | 0 | 10 | 0 | 27 | 90 |
| Maravac | 18 | 1 | 63 | 1 | 10 | 37 |
| HVT | 8 | 1 | 30 | 2 | 19 | 70 |
| Maravac/ HVT together | 5 | 0 | 17 | 0 | 25 | 83 |

Propriety rights and access to the clone C/R6 masterseed was also an important factor in choosing that vaccine over the original Rispens. The C/R6 vaccine had been seed lotted and cleared from contamination by the VRI, The Netherlands and Plum Island Laboratory, USA before being deposited in the American Type Culture Collection repository. A license fee and royalty payments were clearly documented that allowed manufacturing and distribution rights. Access to the original Rispens could only be sourced through competitors of our new international owners. In addition, it was considered that the residual virulence of the original CVI 988 could be of concern to AQIS and importation could be prevented on those grounds.

## V. SAFETY TESTING OF C/R6

The C/R6 seed culture has been shown to be free of extraneous micro-organisms at more than six passage levels. The original seed material developed by de Boer *et al.* (1988) was found to induce a moderate nerve lesion in 1 of 24 MD susceptible laboratory-type chickens compared to 7 of 8 similar chickens vaccinated with the original Rispens CVI 988

(Pol *et al.*, 1986). Subsequent testing of seed material supplied to the American Type Culture Collection via Plum Island, at AAHL and finally at Masterseed level at Cyanamid Websters has failed to demonstrate any lack of safety or presence of extraneous micro-organisms. Cyanamid Websters have also completed safety testing at Working Seed level and produced two trial batches to be used in field trials in Australia. No gross or microscopic lesions have been observed in any inoculated chickens and no extraneous micro-organisms have been detected. All future production batches will undergo Chick Inoculation Tests (CIT) for evidence of safety and absence of extraneous micro-organisms.

## VI. POTENCY TESTING

Cyanamid Websters have developed a plaque assay method under agarose following recommendations from Dr G de Boer. The method has been found to give consistent assay results using one of the trial batches as reference material. The assay methods used in Japan and the USA are more sensitive and give a higher plaque count. However, as it is the relationship of the plaque count to the dose required to protect chickens that is critical, a number of dose-response trials have been set-up to determine this relationship. It has been established that the dose required to protect 50% of chickens is about 12 plaque forming units (pfu) (de Boer *et al* 1988). Vaccine manufacturers commonly extrapolate from this figure to set the dose in their vaccine. In the UK, 100 times the dose required to protect 50% of chickens is the mandatory lower limit. Cyanamid Websters propose to adopt a dose that provides high levels of protection based on dose-response trials currently in progress.

## VII. EFFICACY TESTING

As described above, the efficacy of the C/R6 clone was found to be superior to that of the original Rispens CVI 988 and of clone C. The 50% protective dose has been widely used overseas to compare efficacy of vaccines. It has been found that this parameter is clearly affected by maternal antibody status, amount and type of challenge virus and strain of chicken used for the test. This accounts for different results between laboratories and can cause considerable argument as to which is the best vaccine. Testing should be undertaken using a standard model where possible using both SPF and commercial chickens. Cyanamid Websters have co-operated in industry-sponsored challenge trials at the Animal Research Institute, Yeerongpilly. These trials have been conducted in a commercial strain of chicken which appears to be highly susceptible to the vvMDVs present on some farms in Australia. In addition, the chicks have maternal antibody to at least two MDV serotypes. Unfortunately, the challenge virus has not yet been standardised, so that variable results could be anticipated. Despite this, the ARI trials, together with the efficacy tests being conducted by Cyanamid Websters should go a long way to assessing the value of the C/R6 vaccine in local and imported strains.

Cyanamid Websters have organised extensive field trials of the C/R6 vaccine with each of the major breeding companies commencing from late September. A trial Protocol has been followed and data are being provided to Cyanamid Websters for consolidation into a report to support registration of the vaccine. Indicative data as to efficacy in the field should commence to flow through by early 1996.

## VIII. USE OF C/R6 IN VACCINATION PROGRAMS

The use of C/R6 in existing vaccination programs will depend on pricing, efficacy in the different strains, maternal antibody status of the chicks and the generation to be vaccinated. Pricing is expected to relate to development costs of an imported vaccine including the final dose, the production method adopted and the royalty fee to be paid. Being cell-associated, production costs will be higher than cell-free vaccines such as HVT. Efficacy in local and Australian bred strains is largely unknown but will become clearer as field trial data are accumulated. Some overseas data on maternal antibody effects suggest that the vaccine would be best used where serotype 1 maternal antibody is not high and where chicks are not carrying high levels of maternal antibody to bivalent vaccines. Ideally, C/R6 should be used in chicks whose parents have been well vaccinated with a monovalent vaccine that has MD under good control. C/R6 is also the vaccine of choice for parent flocks to effect good control at that level and to allow a wide range of choices for vaccination of the final commercial generation.

## IX. FUTURE CONSIDERATIONS

Witter (1989) has raised the prospect of the likely continuing creation of new mutants of MDVs in the future. He considers that the current selection pressures in the poultry industry that have already induced two major mutations in the past 80 years, are likely to continue. Therefore, there will always be a continuing need to develop new vaccines. Possibly a more urgent need is a return to some of the other well recognised measures of controlling Marek's disease, i.e. through improved biosecurity and selection for genetic resistance. Recent findings by Bacon and Witter (1992) of the need to optimise selection for resistance in pure lines by undertaking MD vaccination, demonstrates some of the alternate measures that could be undertaken.

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# BINDING OF ENTERIC PATHOGENS AND REDUCTION OF CAECAL COLONIZATION OF SALMONELLA WITH MANNAN OLIGOSACCHARIDES

K. JACQUES, K.E. NEWMAN and P. SPRING

Summary

The ability of mannan oligosaccharides (MOS) derived from yeast cell wall material to adsorb bacteria was demonstrated in experiments with several intestinal pathogens including several strains of salmonella and *E. coli*. In most cases agglutination by MOS of particular species could be attributed to the presence of mannose-specific fimbria. However, additional studies indicated that various species had receptors for other sugars in addition to those for mannose. Addition of MOS to the diet significantly reduced colonization of the caeca by salmonella in broiler chicks challenged with *S. typhimurium* strain 29E. Coliform populations were numerically lower while enterococci and lactobacilli populations were unaffected.

## I. INTRODUCTION

Certain carbohydrates play a role in the attachment of pathogens to the intestinal epithelium and in immune response because of the function of carbohydrate projections on cell walls in cell recognition. This has led researchers to explore new ways of manipulating certain disease processes through the diet. Initial work in this area showed that mannose addition to broiler diets reduced colonization of the digestive tract by salmonella (Oyofo *et al.*, 1989). This occurs because certain salmonella species attach to intestinal epithelial cells via fimbria (Type 1) containing mannose-bearing lectins and in this way dietary mannose blocks attachment and reduces colonization.

An extension of this work involves the use of mannan oligosaccharides (MOS) derived from yeast cell walls. In addition to manipulating intestinal microbial attachment MOS have the potential to strengthen non-specific immunity through activation of the complement cascade which enhances effectiveness of phagocytic processes. The following experiments were conducted to investigate the ability of Bio-Mos (BMOS), a commercially-available feed additive comprised of MOS from a strain of *Saccharomycese cerevisiae*, to agglutinate various enteric pathogens, to demonstrate whether this agglutination was due to the mannose component, and to test the effects of BMOS on the colonization of salmonella *in vivo*.

## II. METHODS

Bacterial strains were grown for 24h on media for the enrichment of Type 1 fimbrae. After incubation, 1 mL of sterile PBS pH 7.2 was added to bacterial slants to suspend the bacteria and 0.1 mL of this preparation was placed in a series of 1.5 mL Eppendorf tubes. Adsorption of pathogens to BMOS was tested by first suspending 10 $\mu$L of BMOS in PBS on a series of microscope slides. Ten $\mu$L of PBS was mixed for 20 seconds with BMOS (negative control). Ten $\mu$L of each bacterial suspension was added to

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subsequent slides. Agglutination was observed under a microscope (100 to 1000x magnifications). In order to determine whether agglutination of pathogens by BMOS was due to mannose or to some other sugar the adsorption experiment was repeated following pre-treatment of the suspensions of some of the *E. coli* and salmonella strains with 100mM solutions of glucose, mannose and fructose prepared in PBS at pH 7.2. Other strains were tested by pre-treatment with mannose only.

Three challenge trials were conducted to examine the effects of BMOS on caecal colonization of salmonella. Forty day old broiler chicks (two groups of 10 birds per treatment) were used in each trial. Treatments consisted of maize/soy-based diets containing either 0 or 2 kg BMOS/tonne. Oral doses of *S. typhimurium* strain 29E (10,000 CFU/bird) were given on day 3. Birds were euthanized on day 10 and the caecal populations of salmonella, coliform, enterococci and lactobacillus were examined and total bacterial numbers determined.

## III. RESULTS

With the exception of strain 4157 all of the *E. coli* strains tested and most of the *S. enteritidis* and *S. typhimurium* strains were agglutinated by BMOS (Table 1). *S. cholerasuis* was unaffected. The pre-treatment with mannose of strains which had demonstrated agglutination previously inhibited subsequent agglutination by BMOS. In some cases agglutination with BMOS but lack of inhibition by mannose indicated that other fractions of the yeast cell wall material may be responsible for attachment. Fructose partially inhibited agglutination of salmonella strains by BMOS.

Addition of BMOS to the diet significantly reduced salmonella colonization of the caeca of challenged broiler chicks (Table 2). Caecal coliforms were also consistently lower in the treatment group. However, the difference was not significant. Total bacterial numbers and enterococci and lactobacilli populations in the caeca were unaffected.

Table 2. Effect of dietary mannan oligosaccharides on caecal populations of salmonella, coliforms, enterococci and lactobacilli[1].

| | Control | Bio-Mos | P>F |
|---|---|---|---|
| | Log CFU/g caecal content | | |
| Salmonella | 5.61 | 4.01 | P<0.05 |
| Coliforms | 8.71 | 8.47 | NS |
| Total bacteria | 9.26 | 9.05 | NS |
| Enterococci | 8.13 | 8.26 | NS |
| Lactobacilli | 6.6 | 6.72 | NS |

[1]Values are means of data from three trials.
CFU = colony forming units.

## IV. DISCUSSION

The adhesive fimbriae with which enterobacteria bind to the intestinal epithelium are lectin-like structures which recognize and bind specific carbohydrates projected from epithelial cells. The "Type" of fimbriae is based on the haemagglutinating properties of the bacteria. Type I fimbriae, such as those on *E. coli*, agglutinate red blood cells, and this agglutination is inhibited by mannose. *Salmonella spp.* have both Type I and Type 2 fimbriae, but Type 1 is more prevalent (Duguid *et al.*, 1966). A number of other pathogenic strains have been

Table 1. Agglutination of bacterial pathogens by Bio-Mos and effects on agglutination of pre-treatment with various sugars.

| | Agglutination by Bio-Mos | Sugar pre-treatment to test fimbrial sugar specificity | | | |
|---|---|---|---|---|---|
| | | Glucose | Galactose | Mannose | Fructose |
| *E. coli* 15R | + | No effect | No effect | Inhibited | Partial |
| *E. coli* K99 | + | No effect | No effect | Partial | No effect |
| *E. coli* K99 O101 | +/- | No effect | No effect | No effect | No effect |
| *E. coli* 4157 | - | | | No effect | |
| *E. coli* K-12 | + | Partial | No effect | Partial | No effect |
| *E. coli* O157:H7 | + | | | No effect | |
| *E. coli* O78:H11 | + | | | Inhibited | |
| *E. coli* O6:K13:H31 | + | | | Inhibited | |
| *E. coli* O6:K13:H1 | + | | | Inhibited | |
| *E. coli* O6:K23:H1 | + | | | Inhibited | |
| *E. coli* O2:O133K:H4 | + | | | Inhibited | |
| *E. coli* O6:K13 | + | | | Inhibited | |
| *E. coli* O6:K23:H1 | + | | | Inhibited | |
| *S. enteritidis* (calf) 13A | + | No effect | No effect | Inhibited | Partial |
| *S. enteritidis* (calf) 371 | + | No effect | No effect | Inhibited | Partial |
| *S. enteritidis*(dairy) | + | | | Inhibited | |
| *S. typhimurium* 13311 | + | No effect | No effect | Inhibited | Partial |
| *S. typhimurium* (chick) 29E | + | No effect | No effect | Inhibited | Partial |
| *S. typhimurium* 14028 | + | No effect | No effect | Inhibited | Partial |
| *S. typhimurium* (chick) 27A | - | | | No effect | |
| *S. typhimurium* 29630 | - | | | No effect | |
| *S. pullorum* 9120 | - | | | No effect | |
| *S. pullorum* 19945 | - | | | No effect | |
| *S. dublin* 15480 | + | No effect | No effect | Inhibited | Inhibited |
| *S. cholerasuis* 13314 | - | | | No effect | |
| *S. cholerasuis* 13312 | - | | | No effect | |
| *S. cholerasuis* 9150 | - | | | No effect | |

+ Denotes adsorption to MOS, - Denotes no adsorption, +/- Denotes weak adsorption. 'Inhibition' indicates pretreatment with the sugar inhibited agglutination by BMOS.

characterized in this manner. One goal of adding MOS to the diet is to provide the lectin on enteropathogens with a mannose residue to block an adhesion site. The experiments described demonstrate that MOS from the yeast cell wall can provide the mannose residue necessary to adsorb or agglutinate a number of intestinal pathogens. However, the presence of receptors recognizing other components of the cell wall material was indicated in species which adsorbed to BMOS even after pre-treatment with mannose. Likewise, inhibition of adsorption by sugars other than mannose also indicated the presence of other receptors.

## V. CONCLUSIONS

Mannan oligosaccharides added to broiler chick diets reduced salmonella in the caecal contents of broiler chicks subjected to oral challenge. This can be explained, at least in part, by the ability of yeast cell wall MOS to agglutinate certain bacterial species and thereby prevent attachment to intestinal epithelial cells.

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# EFFECT OF *YUCCA SCHIDIGERA* EXTRACT ON ASCITES MORTALITY AND BLOOD CHEMISTRY OF BROILERS

K. A. JACQUES* AND J. ARCE**

Summary

The effect of *Yucca schidigera* extract level on performance, total and ascites-related mortality was investigated at a poultry research facility in Michoacan in Mexico. Performance was unaffected by treatment. However, ascites-related mortality was significantly reduced at 21, 42 and 56 days by addition of the extract to the diet. A 90 mg/kg addition resulted in the greatest decrease at 42 and 56 days. Associated with the reduction in mortality were trends toward improved blood oxygen status which were reflected in higher total and partial pressure of oxygen, and oxygen saturation.

## I. INTRODUCTION

The extract of the *Yucca schidigera* plant is frequently used to reduce ammonia ($NH_3$) in the atmosphere of confinement poultry and pig facilities for health and performance reasons. Ammonia is among the many factors that indirectly reduce oxygen uptake and result in the hypoxia that causes ascites syndrome in broilers. In both commercial units in this high-altitude region and in a previous trial at this facility the addition of Yucca extract has resulted in lower ascites-related mortality. The following study investigated the effects of Yucca extract dosage on ascites mortality and blood gas measurements of broilers reared to 56 days.

## II. METHODS

Two thousand eight hundred day-old mixed-sex broiler chicks (Avian Farms) were distributed in a completely randomized design to 28 cement floor pens bedded with wheat straw. The birds were housed at Integracion and Desarrollo Agropecuario Experimental Farm located in Charo, Michoaca in Mexico. Treatments consisted of 0, 60, 90 and 120 mg/kg of dietary *Yucca schidigera* extract (De-Odorase, Alltech Inc.). The pelleted sorghum/soy-based diets were manufactured by Purina for the 0 - 3 and 3 - 8 week stages of growth. Birds were weighed at 0, 3, 6, and 8 weeks of age. Measurements included feed consumption, feed efficiency, general and ascites mortality. At 33 and 53 days of age blood samples from 28 birds (seven per treatment) were taken for the analysis of blood gases. Data were subjected to analysis of variance and means were compared using Tukey's Test.

## III. RESULTS

Liveweight, feed intake and feed efficiency did not differ among treatments at 21, 42 or 56 days ($P > 0.05$, Table 1). Though total mortalities were similar at 21 days, that due to ascites was numerically lowest for the group given 120 mg/kg Yucca extract (Table 2).

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At 42 and 56 days total and ascites mortality were significantly lower only for the group given 90 mg/kg Yucca extract.

Blood gas measurements did not differ at 33 days. However, there was a tendency for birds given 120 mg/kg Yucca extract to have lower blood $NH_3$ (Table 3). In contrast, blood $NH_3$ was remarkably, but unexplainably, significantly lower for the group given 60 mg/kg at 53 days (Table 4). Though other blood gas measurements did not statistically differ at 53 days, certain trends were observed (Table 4 ). Birds given either 90 or 120 mg/kg Yucca extract tended to have lower partial pressure (pp) $CO_2$, higher $ppO_2$, lower total $CO_2$, higher $O_2$ saturation and total $O_2$ and a generally better arterial/alveolar $O_2$ status.

Table 1. Effect of dietary *Yucca schidigera* extract level on performance of broilers at 56 days.

| | *Yucca schidigera* extract, mg/kg | | | |
|---|---|---|---|---|
| | 0 | 60 | 90 | 120 |
| Body weight (g) | 2810 | 2789 | 2818 | 2862 |
| Total intake (g) | 5606 | 5636 | 5644 | 5638 |
| Feed conversion (g:g) | 2.02 | 2.04 | 2.02 | 1.99 |

Table 2. Effect of *Yucca schidigera* extract level on total and ascites mortality at 21, 42 and 56 days.

| | *Yucca schidigera* extract, mg/kg | | | |
|---|---|---|---|---|
| | 0 | 60 | 90 | 120 |
| 21 days | | | | |
| Total mortality (/100 birds) | 3.3 | 4.7 | 3 | 2.6 |
| Ascites mortality (/100 birds) | $2.0^{ab}$ | $3.7^{a}$ | $1.4^{ab}$ | $1.1^{b}$ |
| 42 days | | | | |
| Total mortality (/100 birds) | $15.4^{a}$ | $17.0^{a}$ | $11.0^{c}$ | $13.4^{b}$ |
| Ascites mortality (/100 birds) | $12.9^{a}$ | $14.4^{a}$ | $7.3^{b}$ | $11.3^{a}$ |
| 56 days | | | | |
| Total mortality (/100 birds) | $24.0^{a}$ | $24.7^{a}$ | $17.4^{b}$ | $20.9^{a}$ |
| Ascites mortality (/100 birds) | $20.8^{a}$ | $21.0^{a}$ | $12.6^{b}$ | $16.8^{a}$ |

$^{ab}$Means with a similar superscript are not significantly different at $P < 0.05$.

## IV. DISCUSSION

Environmental factors that produce hypoxia include increased oxygen requirement due to low temperature, lung tissue damage due to infection, chemical and toxic factors that diminish oxygen uptake, carbon monoxide and carbon dioxide levels that stimulate an increase in plasma carboxyhaemoglobin and decrease the ability to capture oxygen, or a low partial pressure of oxygen because of high altitude or deficient ventilation. The trend towards improved oxygen status in birds given the Yucca extract may suggest an explanation for the reduced ascites mortality. However, more research in this area is needed.

Atmospheric $NH_3$ has been shown to increase ascites mortality, possibly as a result of respiratory tract lesions which reduce oxygen uptake. Although $NH_3$ in this facility were relatively low (<20 mg/kg) wide diurnal variation made impact difficult to evaluate. In a previous study of the combined effects of temperature, humidity and $NH_3$ on ascites at

this facility, $NH_3$ levels above 13 mg/kg significantly increased ascites mortality (Arce *et al.*, 1986) while yucca extract reduced $NH_3$ from 13 to 11 mg/kg (Arce, 1995).

Table 3. Effect of *Yucca schidigera* extract level on blood gas measurements at 33 days.

| | *Yucca schidigera* extract, mg/kg | | | |
|---|---|---|---|---|
| | 0 | 60 | 90 | 120 |
| pH | 7.26 | 7.23 | 7.23 | 7.22 |
| $ppCO_2$, mmHg | 50.2 | 54.0 | 53.4 | 56.4 |
| $ppO_2$, mmHg | 2.6 | 2.3 | 2.6 | 2.6 |
| $NaHCO_3$, mmol/L | 18.9 | 18.6 | 18.3 | 18.4 |
| $HCO_3$, mmol/L | 22.7 | 23 | 24.2 | 24.8 |
| $CO_2$, total mmol | 24.3 | 24.7 | 24.2 | 24.8 |
| $O_2$, % saturation | 1.6 | 1.3 | 1.5 | 1.4 |
| Total $O_2$, mL/dL | 3 | .2 | .3 | .3 |
| Venous:alveolar $O_2$ ratio | .03 | .01 | .03 | .06 |
| Arterial/alveolar $O_2$ gradient, mmHg | 50.2 | 50.4 | 50.8 | 47.2 |
| Alveolar $O_2$, mmHg | 57.1 | 52.8 | 53 | 49.9 |
| Blood $NH_3$, mg/kg | 13.6 | 12.3 | 12.1 | 10.7 |

Table 4. Effect of *Yucca schidigera* extract level on blood gas measurements at 53 days.

| | *Yucca schidigera* extract, mg/kg | | | |
|---|---|---|---|---|
| | 0 | 60 | 90 | 120 |
| pH | 7.33 | 7.34 | 7.32 | 7.33 |
| $ppCO_2$, mmHg | 46.6 | 44.6 | 42.4 | 44.1 |
| $ppO_2$, mmHg | 2.97 | 3.5 | 4.8 | 5.2 |
| $NaHCO_3$, mmol/L | 21.2 | 21.3 | 19.8 | 20.6 |
| $HCO_3$, mmol/L | 24.6 | 24.3 | 22.2 | 23.4 |
| $CO_2$, total mmol | 26.1 | 25.7 | 23.6 | 24.7 |
| $O_2$, % saturation | 2.2 | 2.6 | 5.1 | 4.8 |
| Total $O_2$, mL/dL | 0.4 | 0.5 | 0.9 | 0.9 |
| Venous/alveolar $O_2$ ratio | 0.03 | 0.03 | 0.04 | 0.07 |
| Arterial/alveolar $O_2$ gradient, mmHg | 58.6 | 60 | 61.7 | 59.3 |
| Alveolar $O_2$, mmHg | 61.6 | 64 | 66.6 | 64.5 |
| Blood $NH_3$, mg/kg | $13.6^a$ | $5.1^b$ | $12.5^a$ | $11.2^a$ |

[ab]Means with different superscripts are significantly different at $P<0.05$.

## V. CONCLUSIONS

*Yucca schidigera* extract reduced ascites-related mortality as in a previous experiment at this facility. The 90 mg/kg addition level significantly reduced ascites and total mortality at 42 and 56 days. Associated with this reduction in mortality was a tendency toward improved blood oxygen status.

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# INTERACTIONS BETWEEN NUTRITION AND THE IMMUNE SYSTEM

K. KLASING

Summary

When macrophages recognize foreign organisms, they orchestrate a homeorhetic response which alters the partitioning of dietary nutrients away from growth, skeletal muscle accretion or reproduction in favour of metabolic processes which support the immune response and disease resistance. Modulation of nutrient requirements due to an infectious challenge has at least two components: those changes during the challenge when growth is slowed and those changes that occur following elimination of the challenging organism when pathologic insults are repaired and growth is accelerated. During the disease challenge the requirements for amino acids and most trace nutrients are decreased but following the challenge the requirements are increased. Increasing the energy density of the diet with carbohydrate partially compensates for decreased appetite and improves the performance of the immune-stressed chick.

## I. INTRODUCTION

There are extremely important interactions, synergisms and antagonisms between nutrition and immunity that markedly affect productivity. First, nutrition can impact the immunocompetence of an animal and, thus, its disease resistance (Cook , 1991; Latshaw , 1991). Second, immune responses due to infectious challenges impact growth, metabolism and nutrient requirements. Pathology from infectious organisms can impact the absorption and metabolism of nutrients. Further, these important interactions can feed on themselves resulting in a malnutrition-infectious cycle. Malnutrition results in increased incidence, duration or lethality of infectious diseases and infections cause anorexia and malnutrition. Regardless of whether the cycle begins because of poor nutrition or by inadequate control of disease the immune system and nutrient status can simultaneously deteriorate resulting in opportunistic infections and poor production.

In chickens, there are over forty five required nutrients and dozens of economically important infectious diseases, resulting in many hundreds of specific nutrient-disease combinations. Even if the list is limited to the nutrients and pathogens most likely to cause problems, the number of combinations is too great to allow for systematic investigation. In the following review the interactions between nutrition and immunity are reduced to the underlying mechanisms and principles, emphasizing the general problems caused by poor nutrition or by poor disease control.

## II. EFFECT OF DISEASE ON PRODUCTIVITY AND NUTRIENT NEEDS

When a potential pathogen gains entry to the body it elicits several responses from the immune system. Some of the responses are very specific for the antigens on that pathogen and are mediated mostly by lymphocytes. Other response are not specific for a particular pathogen and are mediated mostly by macrophages and neutrophils. These are the inflammatory and acute phase responses. It is this latter response that has the largest impact

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on growth and nutrition-related physiology of the bird. When monocytes and macrophages recognize foreign organisms they orchestrate metabolic changes that underlie the classic acute-phase symptoms of anorexia, lethargy, fever, increased blood heterophil counts, muscle and bone aches. The ensuing metabolic changes impair growth, deposition of skeletal muscle and impact nutrient requirements. It is important to note that it is the responding immune system that mediates these changes. Thus, these symptoms are common to any infectious challenge that evokes phagocytic cells and is referred to as immunologic stress. The intensity of the stress response is proportional to the intensity of the challenge. The duration of the response is dependent upon the length of time it takes the immune system to eliminate the challenging organisms. Each disease organism has its own pathology which modifies the generic response. If there is tissue destruction, such as occurs to the villi of the intestine with coccidiosis, it will affect the absorption of nutrients. If a pathogen settles in the liver it will affect hepatic functions. Many pathogens have their own sets of toxins with associated pathology. Consequently, the total impact of an infectious challenge on metabolism and nutrition is the sum of generalized effects of the immune system responding to the presence of the pathogen plus specific effects due to the destructive actions of the pathogen itself.

### (a) Influence of an Immune Response on Productivity

Immunologic stress has been studied under controlled laboratory conditions by giving chicks frequent injections of different purified immunogens that do not illicit destruction of host tissues (Klasing *et al.*, 1991). Regardless of the immunogen, stimulation of the immune system causes decreased feed intake, weight gain, and the efficiency of feed utilization. The decreased feed intake accounts for about 70% of the decreased growth, while the remainder is due to metabolic inefficiencies caused by the immune response. The metabolic changes caused by immunogens such as pathogenic bacteria that result in extreme morbidity, and by purified proteins that do not cause tissue damage or direct pathology, induce many similar host responses that differ mostly in magnitude. Therefore, stimulation of the immune system results in specific metabolic alterations which are modified depending upon the strength and duration of the immune response. They are brought about by endogenous mediators, collectively known as leukocytic cytokines. These hormone-like peptides are released by macrophages and by lymphocytes. Those released by macrophages and monocytes are referred to as monokines. Interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF), and interleukin-6 (IL-6) are the primary monokines involved in decreased productivity (Table 1). Each of these monokines has a specific role in the regulation of the immune response by acting in the local area of challenge. They also act systemically on target tissues such as skeletal muscle, adipose, liver and bone. Lastly, they can have systemic effects indirectly by altering levels of hormones such as insulin, glucagon and corticosterone (Klasing, 1988; Marsh and Scanes, 1994).

The metabolic changes represent a homeorhetic response which alters the partitioning of dietary nutrients away from growth, skeletal muscle accretion or reproduction in favor of metabolic processes which support the immune response and disease resistance. They form the basis for impaired growth, feed utilization and altered nutritional requirements in chicks. The decreased growth rate is tissue specific with skeletal muscle being sacrificed more than adipose tissue. The rate of growth of the liver and the gastrointestinal tract are reduced the least. Thus, lean meat yields are compromised.

## (b) Changes in Nutrient Requirements

Nutrient recommendations are usually based on the needs of healthy animals raised under excellent management. The established requirements often do not include a "margin of safety" for deviations from the ideal situation. The use of these requirement values requires adjustments for stressors experienced in practical production situations. Modulation of nutrient requirements due to an infectious challenge can be broken into at least two components: those changes during the challenge when growth is slowed and the immune system is responding vigorously and those changes that occur following elimination of the challenging organism when pathologic insults are repaired and growth is accelerated. Thus, the normal animal is fed for normal growth, the infected animal is fed for the immune response and the convalescing bird is fed for accelerated growth and tissue repair. There is a plethora of information on nutrition in the healthy bird and little information on the immunologically-stressed or the convalescing animal (Klasing, 1988). In fact, most University scientists are reluctant to publish the results of experiments designed to determine nutrient requirements if a disease situation develops.

Table 1. Overlapping roles of leukocytic cytokines in the regulation of metabolism[1]

| Response | Cytokines Responsible |
|---|---|
| General | |
| decreased voluntary food intake | IL-1, TNF |
| increased resting energy expenditure | IL-1, TNF |
| increased body temperature | IL-1, IL-6 |
| Glucose metabolism | |
| increased glucose oxidation | IL-1, TNF |
| increased gluconeogenesis | IL-1 |
| Lipid metabolism | |
| decreased lipoprotein lipase activity | IL-1, TNF |
| increased lipolysis in adipocytes | IL-1, TNF |
| increased hepatic triglyceride synthesis | TNF |
| Protein metabolism | |
| increased acute phase protein synthesis | IL-1, TNF, IL-6 |
| increased muscle protein degradation | IL-1 |
| Mineral metabolism | |
| increased metallothionein synthesis | IL-6 |
| increased hepatic ceruloplasmin synthesis | IL-1, IL-6 |
| Hormone release | |
| increased corticosteroid release | IL-1, IL-6 |
| decreased thyroxin release | IL-1 |
| increased insulin and glucagon release | IL-1, TNF |

[1]Abbreviations: IL-1: interleukin-1; TNF: tumor necrosis factor; IL-6: interleukin-6.

Since the most deleterious aspect of immunologic stress on growth is due to decreased feed intake manipulations of the dietary nutrient density would appear prudent. Increasing the

energy density of a ration while keeping required nutrients at a constant proportion of the energy improves energy intake and the rate of gain of immunologically-stressed chicks (Benson *et al.*, 1993). Conversely, the negative impact of immunologic stress on food intake and rate of gain is more evident at lower dietary energy densities. Ingredients containing high levels of fat should not be used to increase energy density as they are not well tolerated during an immune response.

The requirement for lysine and methionine is decreased during immunologic stress, probably as a result of slower growth rates and decreased skeletal muscle accretion (Klasing and Barnes, 1988). Following the disease process, compensatory growth induces an increase in amino acid requirements. In practice, if only one diet is to be fed, the amino acid fortification that supports maximal growth across the different physiological states may be greater than that commonly recommended. Though additional amino acid fortification may be necessary to support intermittent periods of compensatory growth this may not always be a least cost level because there is a surfeit of amino acids during periods when birds are actually being challenged and when they are healthy. Thus, in the case of amino acids a margin of safety may be needed to permit accelerated growth following disease challenges but is not needed during the course of the disease unless pathology specific to the disease (i.e. malabsorption) adds to the requirement.

Quantitative changes in the requirements of trace minerals as the result of immunologic stress have not been subjected to detailed study in poultry but can be surmised from known changes in metabolism, absorption and excretion. Low circulating levels of iron and zinc are an integral part of the immune response and do not, in themselves, indicate higher requirements. The increased use of zinc, manganese and copper for hepatic acute phase protein synthesis indicates a several-fold increase in requirements at the tissue level. This increase in tissue requirements is met largely by a redistribution within the body. Increased fortification of trace minerals over the requirement of non-stressed animals does not augment these redistributions. Preventing the decrease in circulating iron concentrations by very high dietary levels impairs immunity. Increased excretion of zinc and copper and decreased absorption of iron indicate a net loss of mineral during the immune response. Thus, good mineral stores prior to the disease challenge are important and an increase in the requirement for trace minerals is indicated following, but not necessarily during, an infectious challenge unless there is disease-specific pathology that changes the retention of the minerals.

Changes in the need for vitamins to satisfy an immune response are not well understood. In the case of fat soluble vitamins (A, D, E and K) and xanthophylls absorption is impaired due to poorer fat absorption. Needs at the tissue level for antioxidants are also increased because of the higher burden of reactive oxygen intermediates emanating from the immune system. Together these changes indicate increased dietary needs during a disease challenge (Nockels, 1986).

The nutritional modifications discussed above represent best guesses to maximize gain and feed efficiency during a generalized immune response. Additionally, each specific disease state, because of it's unique pathology, will result in additional changes in the optimal diet. This is especially true for diseases that affect the gastrointestinal tract and impair nutrient absorption, or increase the endogenous loss of nutrients (Sell and Angel, 1990). In the case of especially pathogenic disease organisms it is not known if diets that give maximal gain and feed efficiency during the course of an infectious challenge result in maximal livability and minimal condemnation losses.

### (c) Subclinical Challenges Versus Productivity

There is little doubt that most infectious diseases decrease the productivity of animals. What is often not appreciated is that decreased performance is also associated with sub-clinical challenges, even from organisms not usually considered to be pathogenic. Chicks housed in germ-free environments grow 15% faster than those raised in conventional environments where they are continuously exposed to microflora. Chicks housed in disinfected quarters grow faster and convert a higher proportion of their feed into body mass than chicks housed in less sanitary conditions, even in the absence of infectious diseases and pathogenic agents. The response becomes larger and more devastating as sanitary conditions worsen.

### (d) Mitigating Factors

Good sanitation and ventilation is the best way to maximize rates of growth and reproduction. Feeding antibiotics, competitive exclusion cultures or other probiotics improve growth and minimize disease when sanitation is imperfect. Chicks raised in environments with poor sanitation have markedly higher levels of circulating IL-1 than chicks raised with excellent sanitation. Presumably, the high burden of microbes, dust and dander chronically stimulate the immune system and induce the release of monokines such as IL-1. As described above, high circulating levels of monokines result in slower growth. Feeding antibiotics to chicks in a dirty environment decreases the amount of circulating IL-1 to levels more similar to chicks raised in a clean environment. Feeding antibiotics results in little or no improvements in growth rate or changes in circulating IL-1 levels in clean environments. Thus, it appears that antibiotics may act by limiting the number of times, and the vigour with which, the immune system must respond to dispose of frequent microbial challenges along the intestines (Roura *et al.*, 1992).

A major portion of the immune system is localized along the intestinal tract and is responsible for intestinal defense. Bacteria, continually gain access to the body through the intestinal epithelia as a result of small rips and tears that occur due to feed passage and cell turnover. Most of these bacteria are merely opportunistic and are unable to replicate to an appreciable extent in the host's tissues. However, some bacteria can proliferate and these require a relatively vigorous immune response to eliminate them. Still other bacteria that occasionally gain entrance are pathogenic and result in destructive pathology along the intestinal tract. Like good sanitation, dietary antibiotics decrease the frequency that opportunistic and deleterious bacteria gain entrance through the intestinal epithelia. Additionally, antibiotics may inhibit the capability of bacteria to multiply after they have gained entrance. Thus, antibiotics would be expected to subdue the state of activity of the gut-associated lymphoid tissue and this is manifested as improved growth, deceased intestinal thickness, lymphoid mass and numbers of lymphocytes (Cook *et al.*, 1984).

Another approach to decreasing the impact of microbial stimulation of the immune system is through the use of competitive exclusion cultures. In theory, competitive exclusion arises from inoculation of the gastrointestinal tract with microflora that become established in niches that could be occupied by potential pathogens. The introduced microbe should not elicit an immune response but prevents the establishment of pathogens that would invoke the immune system and cause pathology.

An alternative approach to limiting the colonization of the gastrointestinal tract is to feed carbohydrates to block the attachment of pathogens to enterocytes (Bailey *et al.*, 1991). Also, feeding various carbohydrates such as lactose, lactosucrose, fructooligosaccharide or

some types of fermentation products cause a shift in the ecology of the intestinal microflora that may exclude pathogens.

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# IMPROVED BIOAVAILABILITY OF ENERGY AND GROWTH PERFORMANCE FROM ADDING ALPHA-GALACTOSIDASE (from *Aspergillus* sp.) TO SOYBEAN MEAL-BASED DIETS

I.H. KNAP,* A. OHMANN* and N. DALE**

Summary

The effects of adding α-galactosidase (Novozym®696) to diets containing dehulled soybean meal (SBM) to improve bioavailability of energy and growth performance were studied in poultry. In Experiment 1, SBM with α-galactosidase (0, 250, 1000 and 1500 α-galactosidase units (GALU[a)]/kg SBM) was precision-fed to adult Leghorn roosters to determine nitrogen-corrected true metabolizable energy (TMEn). A significant improvement in bioavailability of energy was achieved resulting in increases of 4.4% and plus 8.8% with 1000 and 1500 GALU/kg SBM, respectively. In Experiment 2, a maize-SBM diet was fed *ad libitum* to chickens of the Arbor Acres breed. The diets were supplemented with four levels of α-galactosidase (0, 500, 1000 and 1500 GALU/kg SBM). After 21 days of age the feed conversion ratio was improved by 2.8% with 1000 GALU/kg SBM. After 42 days of age the improvement was slightly smaller -1.7% with 1500 GALU/kg SBM. These results indicate a positive effect on soybean meal digestibility through supplementation with the α-galactosidase.

## I. INTRODUCTION

The oligosaccharides named α-galactosides (eg. verbascose, stachyose and raffinose) are found in vegetable protein sources such as soybean, lupins and rapeseed. The content and spectrum vary within species and varieties of legumes (Saini, 1988). For example, soybeans contain 0.5-1.5% raffinose and 3.5-5.5% stachyose whereas verbascose is absent. Lupins are almost comparable with soybeans except for the verbascose content at approximately 1%. The α-galactosides cannot be metabolized by monogastric animals due to the lack of α-galactosidase activity in the intestinal mucosa (Gitzelmann and Auricchio, 1965), and their presence in the animal tract has been shown to cause flatulence (Rackis, 1975) and reduce TME of legumes (Coon *et al.*, 1990; Leske *et al.*, 1993). Coon *et al.* (1990) also reported that oligosaccharides accelerate intestinal transit time and, thereby, decrease dry-matter digestibility.

By adding α-galactosidase to feed containing vegetable protein sources, the α-galactosides will be degraded (hydrolysis of α-1,6 linkages) into sucrose and galactose, and consequently their detrimental effects will be removed and energy availability improved (Brenes *et al.*, 1993). The objective of the present studies were to evaluate if the commercial α-galactosidase, Novozym®696, improves the bioavailability of energy and the growth performance of poultry fed SBM-based diets.

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## II. MATERIALS AND METHODS

### (a) Experiment 1. True Metabolizable Energy studies

The TME studies were conducted as described by Sibbald (1976) and Dale and Fuller (1984). White Leghorn roosters (55 weeks of age) were used as test animals with 10 replicates per treatment. Dehulled soybean meal (30 g) was fed to each rooster and a 48 h collection period was employed. Four levels of α-galactosidase (Novozym® 696) were added: 0, 250, 1000 and 1500 GALU/ kg soybean meal (SBM).

### (b) Experiment 2. Growth study

Chickens of the Arbor Acres breed were used as test animals randomized in pens each holding 50 birds (25 males / 25 females) with 12 replicates per treatment. A two phase dietary regimen containing 36% / 31% SBM and 58% / 64% maize was fed *ad libitum* during the experimental period. Determination of weights and feed conversions were done at 21 and 42 days of age. Four levels of α-galactosidase (Novozym® 696) were added to the diet: 0, 500, 1000 and 1500 GALU/ kg SBM.

### (c) Statistical Analysis

Data were subjected to general linear model procedures of SAS.

## III. RESULTS

In the TMEn-study supplements of α-galactosidase gave significant improvements in the bioavailability of the energy from SBM. These corresponded to 4.4 % with 1000 GALU/kg SBM and 8.8% with 1500 GALU/kg SBM. In Figure 1 the bioavailable energy from SBM is presented without and with the addition of α-galactosidase. In the growth study an overall analysis on weight gain to 42 days showed no significant differences between treatments. However, on closer examination increasing the dose of α-galactosidase was found to be related to a decreased feed intake. When feed consumption was taken into account as a covariate, a significant ($P=0.014$) difference in weight gain between treatments was found at 21 days of age with a clear dose response. At 42 days of age the improvement was slightly smaller and non-significant (Table 1). This phenomenon is reflected in the feed conversion ratio (FCR) where decreases were noted with increased dosage of α-galactosidase at both 21 and 42 days of age with the former being significant. After 21 days of age an improvement in FCR of 2.8% with 1000 GALU/kg SBM was assessed. After 42 days the improvement was slightly smaller being 1.7% with 1500 GALU/kg SBM (Table 1).

## IV. DISCUSSION

The TMEn value of SBM found in this study is similar to that reported by Coon *et al.* (1990) where removal of the oligosacharides from SBM by alcohol-extraction resulted in an increase in the TMEn of 20%. By adding 5.36% stachyose to a soy protein concentrate Leske *et al.* (1993) reported a decrease in the TMEn value of 17% compared to the TMEn value of the soy concentrate. In contrast to these findings Slominski *et al.* (1994) reported a decrease in the TMEn of canola meal as a result of removing the oligosacarides by ethanol-

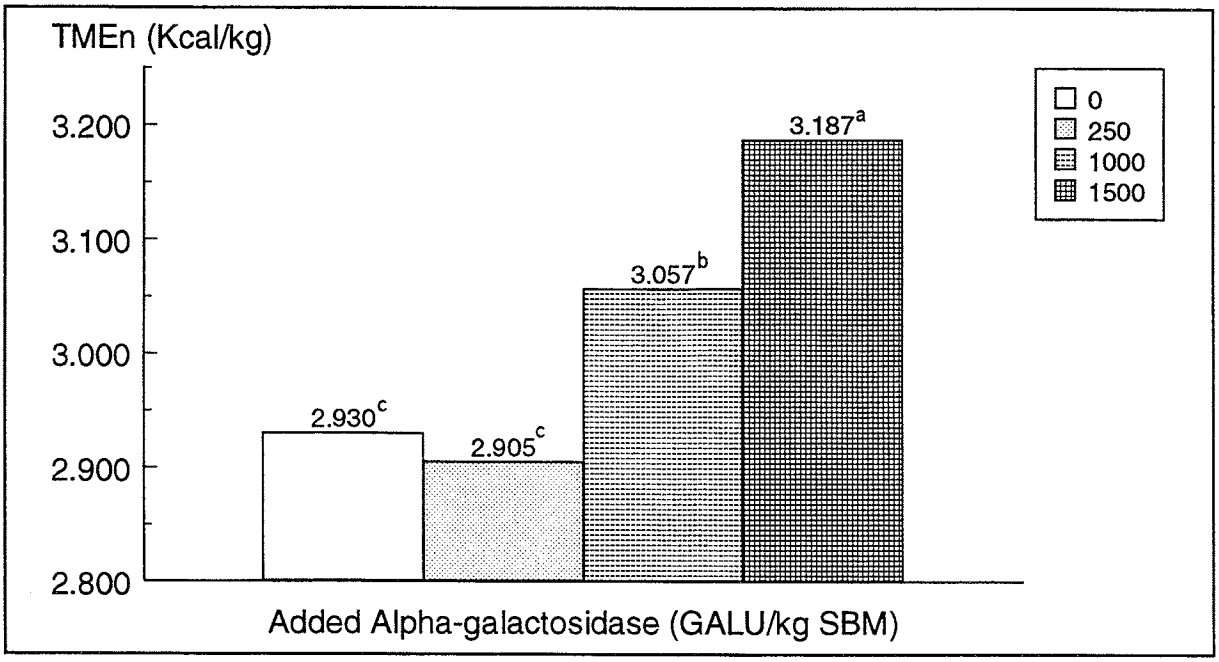


Figure 1. Nitrogen-corrected true metabolizable energy (TMEn) from roosters fed soybean meal (SBM) with varying levels of supplementation with α-galactosidase.
Values with different subscripts are significantly different ($P<0.05$).

Table 1. Weight gain and feed conversion ratio of chickens (1-42 days) fed α-galactosidase-supplemented soybean meal (SBM) diets.

| α-galactosidase (GALU/kg SBM) | Weight gain (LS-means1)[1] 1-21days (g) | 1-42days (g) | Feed conversion ratio 1-21 days (g:g) | 1-42 days (g:g) |
|---|---|---|---|---|
| 0 | 592[a] | 1809[a] | 1.480[a] | 1.903[a] |
| 500 | 599[ab] | 1814[a] | 1.467[a] | 1.885[a] |
| 1000 | 609[bc] | 1820[a] | 1.439[b] | 1.879[a] |
| 1500 | 612[c] | 1832[a] | 1.438[b] | 1.870[a] |

Values without a common superscript are significantly different at $P<0.05$.
[1] Feed consumption is taken as covariate.

extraction. These contradictory results may be explained by differences in substrates and extraction procedures. The improvement in TMEn of 8.8% in our studies is lower than expected when compared with the data obtained by Coon *et al.* (1990) and Leske *et al.* (1993). Further experiments are needed to clarify if an enhanced concentration of α-galactosidase will result in an even greater effect. The correlation between the TMEn studies and the growth study seems satisfactory with an increase of approximately 2-3% in FCR from a diet containing 30% SBM. Brenes *et al.* (1993) found similar results in a broiler

growth study using 70% lupins in the diet. Our results are in contrast to the report by Irish *et al.* (1995), where no beneficial effect from a crude α-galactosidase extract was recorded. The dosages used in this latter study cannot be compared with those used in the current study and the lack of effect may be explained either by a low dosage rate or by poor *in vivo* hydrolysis of the oligosaccharides due to lack of stability of the enzyme in the animals. It has been reported that a combination of invertase and α-galactosidase improves the hydrolysis of α-galactosides compared to the use of α-galactosidase alone (Slominski, 1994; Irish *et al.*, 1995). *In vitro* data from our laboratory has shown no additional effect on galactose production from raffinose through the use of invertase with Novozym®696 compared to Novozym®696 alone (unpublished). It is concluded that the α-galactosidase product used in this study exerts a positive effect of the bio-availability of energy from dehulled soybean meal.

[a] One GALU (**GAL**actosidase **U**nit) is the amount of enzyme which hydrolyses 1 mmol p-Nitrophenyl-a-D-galactopyranoside/minute under standard conditions (pH 5.5; 37°C; 15 min.; $OD_{405}$).

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# THE EFFECT OF RELOCATION STRESS ON EGG SHELL QUALITY IN AN IMPORTED STRAIN OF LAYING HEN

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There is some evidence that disruption of the laying hen's normal environment can lead to the production of abnormal shells (Hughes and Black, 1976; Hughes *et al.*, 1986). Hughes and coworkers (1986) suggested that the formation of abnormal eggs resulted from the release of adrenaline in response to stress and Solomon *et al.* (1987) showed that a single injection of adrenaline had an adverse effect on overall shell quality and shell ultrastructure for a number of days. This experiment was conducted to assess the response of IsaBrown layers to the stress of translocation.

During the experiment 48 IsaBrown hens were moved from floor pens at Laureldale, the farm at the University of New England, to single cages at the university campus, a journey of approximately 3 kilometres. The result of this translocation was quite dramatic. There was an initial decrease in egg production and percentage of good quality egg shells, the incidence of cracked, misshapen and soft-shelled egg shells increased and there were more light-coloured eggs and eggs with abnormal calcium deposits (including white banding).

Calcium coated eggs occur when the hen retains the completely formed egg in the shell gland pouch to such a degree that extra calcium is laid down on the cuticle. White banded eggs are also caused by an egg being retained, although white banded eggs are retained until a second egg enters the shell gland pouch. The characteristic white band of these eggs is formed when the two eggs touch each other and extra calcium is laid down on the first egg except the point of contact with the second egg. The second egg will be slab-sided. Stress is thought to be the usual cause of this egg retention (Solomon, 1991).

The production of white banded and calcium coated eggs continued for up to ten days after the birds were relocated, suggesting that the birds were chronically stressed and the manifestation of that stress was the retention of eggs and consequential extraneous calcium deposits. Several weeks after the first translocation, another group of IsaBrown hens was moved to ascertain if these results were repeatable. The hens responded in the same way as was seen in the initial relocation.

The flock from which the experimental birds were derived was maintained on the Laureldale farm. The farm records show that there was a decrease in production in this flock in response to changes in personnel attending to the birds. These results suggest that the IsaBrown layers are sensitive to changes in their environment and that the effects of these responses are manifest in the shells of the eggs laid.

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# THE PREDICTION OF METABOLIZABLE ENERGY BY NEAR INFRA RED ANALYSIS AND *IN VITRO* TECHNIQUES

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Summary

Determination of nitrogen-corrected metabolizable energy ($AME_n$) using a classical bioassay is time consuming and costly, and consequently is not done routinely for either diets or ingredients. This is of concern to the feed and poultry industries because energy is a major contributor to diet costs and poultry are very responsive to changes in diet energy level. Near Infra Red Analysis (NIRA) offers potential for rapid and inexpensive assay of $AME_n$. By measuring light energy absorption (reflectance) in the 700-2500 nm range, it is possible to correlate results with $AME_n$ and/or other nutrients in feeds and diets. Using 45 diets to develop calibrations, and 35 diets (both of determined $AME_n$) to test these, we have shown good prediction with a correlation of 0.91. Calibrations are also described for individual ingredients. An alternative rapid assay is an *in vitro* technique that simulates gastric and duodenal/ileal digestion. Preliminary studies involved 4 h pepsin digestion followed by 4 h incubation with porcine digestive fluid. Resultant *in vitro* digestible energy (IVDE) correlated well with determined $AME_n$. In more recent studies attempts have been made to replace the porcine duodenal fluid with a mixture of synthetic enzymes. Results are encouraging, although the current system seems to grossly overestimate the $AME_n$ of soybean meal, and to slightly undervalue the energy in maize. These results are discussed in terms of particle size, lipid digestion and general solubility of components in an aqueous medium.

## I. INTRODUCTION

Describing the nutrient needs of various types of poultry is best achieved by taking into account so called "availability" of nutrients. Ideally we would like to describe both ingredients and diets on the basis of nutrient availability at the tissue level for end-products, such as eggs, or lean meat deposition. While such data are being developed in terms of model building, in many instances this is far removed from practical formulation of poultry diets. However, it is also obvious that assessment of diets and the needs of poultry in terms of total nutrient levels in ingredients, is not an ideal solution. For most nutrients the major factors affecting differences between total nutrients in feed and those available at tissue levels are digestibility, absorbability and so-called metabolizability. Of these factors, digestibility is by far the largest and most variable factor. For example, the availability at the tissue level for nutrients such as energy and, for example, lysine is about 80% influenced by digestibility *per se*. In reality it is very difficult to separate the effect of digestibility from that of absorbability and so in reality the two are considered together and loosely termed "digestibility." The effect of absorbability on "digestibility" is probably quite small but, as will be discussed later, does pose a limitation on application of so-called *in vitro* digestibility values, when solubility is assumed to be equal to digestibility (therefore, absorption is assumed to be 100%).

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Availability of energy has been considered as a standard for many years with the industry accepting metabolizable energy (ME) to describe both ingredients and diets. Unfortunately, ME is difficult and time consuming to measure and even so-called rapid bioassays take at least 7d for turn-around and are still expensive. There is obviously a need for a rapid, inexpensive assay for ME and the time element means that consideration must be given to systems other than those involving live animals. There are currently three systems available for assessment of dietary energy, namely correlation with chemical components, near infra red, and *in vitro* assays.

### (a) Relationship With Gross Chemical Analyses

It should be possible to predict ME from a knowledge of chemical constituents. Carpenter and Clegg (1956) were one of the first to correlate ME with proximate components developing a prediction equation based on protein, fat, starch and sugar contents. This type of equation attempts to incorporate some knowledge of the availability of nutrients as they affect ME in that fat is ascribed 2.25 and starch 1.1 times more energy than protein and sugar components. Sibbald *et al.* (1963) showed that the equation of Carpenter and Clegg (1956) accurately predicted classical ME values with an overall standard deviation of 0.586 kJ/g and subsequently derived an equation from data for the chick for the prediction of $AME_n$. Due to the differences in digestive and metabolic processes between different species however, such equations are only of use for the species from which they have been derived. It has been suggested that greater accuracy may be developed in prediction equations for cereal grains if various carbohydrate fractions other than sugar and starch, such as pectin, hemicellulose and cellulose, are also taken into account. If all energy-yielding components provide consistent amounts of energy, then simple equations with no intercept seem logical and attractive. However, this is unlikely to be the case because of nutrient synergism and interaction. Fibre is a good example of this situation. Fibre *per se* is likely to provide little energy, yet incorporation of fibre components into equations invariably increases the accuracy of prediction. It is likely that fibre components influence the digestibility of other nutrients and this may be an age-dependent effect.

As suggested by Fisher (1983), the major question to be resolved about chemical prediction equations is whether different equations are required to predict ME values for different types and ages of bird or whether a single equation is sufficient. In reviewing such single equations reported for complete feed, Fisher (1983) showed residual standard deviation (rsd) of predicted means ranged from 314 to 1966 kJ/kg. The accuracy of prediction for individual ingredients is substantially less. In developing many hundreds of equations to describe ME Fisher (1983) concludes that the so-called best equation has a rsd of just less than 250 kJ/kg, or about 2% for most diets. However, this equation is based on analyses for fat, protein, neutral detergent fibre, starch and fatty acid saturation. The latter analyses are not routinely conducted and hence cost-benefit and speed of assay may be questioned. The European approach of applying "logical" nutrient digestibility coefficients to the various chemical components seems a natural development in attempting to use prediction equations for ME, although again the question arises as to universality of such digestion coefficients.

### (b) *In Vitro* Systems

Unlike the situation with ruminants few attempts have been made at developing *in vitro* techniques for poultry. A somewhat rapid two-stage *in vitro* technique that uses pepsin digestion followed by incubation with pig intestinal fluid was developed by Furuya *et al.*

(1979). While this original method was designed to predict digestible dry matter (DDM) and digestible crude protein (DCP) with pigs, Clunies and Leeson (1984) showed that with certain modifications, the system can be applied for use with poultry diets.

The system uses two stages of incubation, namely pepsin digestion followed by incubation of the residue with porcine intestinal fluid. The method essentially measures dry matter (DM) digestibility (solubility in the system) and so ME is derived from estimates of gross energy of the original sample and that of the undigested (insoluble) residue. The premise for this work is based upon reports by Nelson *et al.* (1975) that the digestibility of DM correlates well with $AME_n$ for sorghum grains and that the $AME_n$ of ingredients have been predicted from metabolizable DM (Han *et al.*, 1976). Clunies and Leeson (1984) tested this technique with a number of poultry diets formulated to provide a range of $AME_n$ values. All diets were assayed for *in vitro* ME and also by classical $AME_n$ bioassay using adult birds. Results in Table 1 indicate good agreement between $AME_n$ and what effectively is *in vitro* digestible energy. Diet 1 (Table 1) was not predicted too accurately, and interestingly this diet contained most supplemental fat, at 6.5%. Most other diets were predicted within $\pm$ 418 kJ ME/kg.

Table 1. Comparison of $AME_n$ and predicted *in vitro* digestible energy values (DE) (MJ/kg)

| | Diet | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| $AME_n$ | 12.55 | 13.56 | 13.10 | 10.29 | 10.96 | 10.63 | 10.13 | 13.68 | 14.06 | 13.47 |
| *In vitro* DE | 11.30 | 13.18 | 12.97 | 10.79 | 11.30 | 10.92 | 10.13 | 13.64 | 14.06 | 13.89 |

The assay takes some 6-8 h to complete but with determinations of DM and gross energy also being necessary, turn-around time for results is at least 48 h. Another limitation of the assay is the necessity to cannulate pigs in order to provide duodenal fluid, although large quantities can be collected from older sows, and the product frozen prior to use. Valdes and Leeson (1991) attempted to replace this porcine digestion fluid with a synthetic mixture of pancreatin, bile salts and enterokinase. Seventy diets ranging in calculated $AME_n$ from 9.20 to 14.23 MJ/kg were formulated from a range of ingredients. Each diet was then assayed for *in vitro* DE and $AME_n$ using a classical bioassay with adult roosters. Almost 50% of the diets were assayed with an accuracy of $\pm$ 418 kJ/kg which seems a reasonable estimate of ME. However there were some major discrepancies and these seemed to relate to use of certain common ingredients. While $AME_n$ of maize was underestimated the values for soybean meal and barley were much larger than expected (Table 2).

Obviously an *in vitro* system of feed evaluation will be of limited usefulness if maize and soybean meal are not accurately predicted. Because maize is underestimated, and soybean meal is overestimated, *a priori* one assumes a general underestimation of starch, and concomitant overestimation of protein by this enzyme system. In attempting to correct these anomalies numerous modifications have been tried including the addition of amylase, etc., generally without much success. In more recent studies it has been shown that the energy value of maize can be more accurately predicted by grinding more finely, and by adding more bile to the digestion medium (Table 3).

Table 2. *In vitro* digestible energy (IVDE) and $AME_n$ values (MJ/kg).

| | $AME_n$ | IVDE |
|---|---|---|
| Maize #1 | 14.14 | 12.55 |
| Maize #2 | 14.43 | 12.64 |
| Wheat | 13.68 | 13.18 |
| Barley | 10.88 | 12.76 |
| Soybean meal | 10.46 | 14.02 |
| Corn gluten | 15.77 | 17.24 |

Table 3. Effect of fine grinding and bile addition to *in vitro* digestible energy (IVDE) of maize and soybean meal.

| Treatment | IVDE (MJ/kg) |
|---|---|
| Maize, 1 mm particle size | 12.05 |
| 0.4 mm particle size | 13.22 |
| 0.4 mm + bile | 13.68 |
| SBM, 1 mm particle size | 13.44 |
| 0.4 mm particle size | 14.39 |
| 0.4 mm + bile | 14.33 |

A finer particle size and the addition of bile to the digestion medium resulted in a significant improvement in the estimation of the ME value of maize (12.05 to 13.68 MJ/kg). However, the same treatment for soybean meal resulted in even greater overestimation of ME. It seems likely that it will be necessary to match more closely the enzyme levels and/or sources to those of the young bird or to develop separate assay procedures for maize and soybean-type products.

(c) Near Infra red Analysis

The use of near infra red reflectance analysis (NIRA) for determining protein, fat, and moisture in grain and oilseed, has been applied routinely for some time now. The principle of NIRA was developed by Dr. F. Norris, USDA, Beltsville, in the early 1960's and eventually was established as a new branch of agricultural chemistry. Some 10 years later Norris applied the technique for the first time in the evaluation of animal feeds. The advantages of NIRA are that it is very fast, requires no chemical reagents and is inexpensive. Similarly there is no sample preparation except grinding, the system does not need trained people after the calibrations are developed and, because costs of running the tests are low, several constituents in a sample can be analyzed simultaneously. On the other hand, initial capital cost of equipment is very high.

NIRA relies on the application of mathematics to analytical chemistry, being an integration of spectroscopy, statistics, and computer sciences. Mathematical models are constructed that relate chemically active groups to energy changes in the near infra red region of the spectrum (700-2500 nm). In this region vibrations of chemical bonds in which hydrogen is attached to atoms, such as nitrogen, oxygen, or carbon are measured. The intensities of absorption in the near infra red region are weak and much lower than those seen in the mid and far infra red region of 2500-1500 nm. Because most feedstuffs are opaque NIRA uses reflectance characteristics of light instead of transmittance. The reflected light from a sample (diffuse reflectance) is used to indirectly quantitate the amount of energy absorbed by the sample. NIRA measures the absorption of infra red radiation by sample components, such as peptide bonds at specific wavelengths in the near infra red spectrum.

The usefulness of NIRA depends entirely on careful and conscientious calibration of the equipment. To some extent this exercise has been simplified through introduction of so called scanning machines that cover a wide band of NIR. Prior to this technology fixed filter (wavelength) equipment only was available, and so *a priori* knowledge of likely absorption bands or tedious testing of numerous wavelengths was essential in order to develop useful calibrations. Over the last few years at Guelph a library of diets and ingredients has been assayed for *in vivo* $AME_n$ using conventional total collection with adult roosters. These diets were formulated to provide a range of anticipated ME levels, as well as variance in protein, calcium, fibre, etc. These, almost 100, diets contained some 40 different ingredients.

Table 4 shows NIRA prediction of $AME_n$ of selected commercial diets and ingredients. Ingredient $AME_n$ was predicted with a calibration developed specifically for ingredients (Valdes and Leeson, 1992). For the commercial diets only calculated $AME_n$ values were available and, as shown in Table 4, predicted values were of the same order.

Table 4. NIRA prediction of *in vivo* $AME_n$ of ingredients and calculated $AME_n$ of commercial diets (MJ/kg)

| Ingredient | *In vivo* $AME_n$ | NIRA $AME_n$ |
|---|---|---|
| Maize #1 | 14.14 | 14.10 |
| Maize #2 | 14.43 | 14.35 |
| Soybean meal | 9.79 | 9.71 |
| Wheat | 13.68 | 13.51 |
| Barley | 11.38 | 11.17 |
| Commercial diet | Calculated $AME_n$ | NIRA $AME_n$ |
| Broiler starter | 13.39 | 13.89 |
| Broiler starter | 13.81 | 13.72 |
| Broiler grower | 14.23 | 14.10 |
| Layer | 11.30 | 12.13 |
| Developer | 11.09 | 11.97 |
| Breeder | 12.01 | 12.22 |
| Breeder | 11.30 | 11.38 |

Data used in developing the ingredient calibration are shown in Table 5.

Table 5. Wavelength, wavelength assignments statistics and coefficients of NIRA calibrations of poultry feeds using a scanner spectrophotometer using Log 1/R (Valdes, 1993).

| Wavelength (nm) | Coefficient | Bond vibration | Structure |
|---|---|---|---|
| 2160 | 127 | 2x amide 1+amide | CONHR |
| 2244 | -101 | N-H str+$NH_3$+def | Amino acids |
| 2308 | -114 | C-H str+C-H def | $CH_2$ |
| 2344 | 442 | C-H str. CH def | $CH_2$, $CH_3$ |
| 2356 | 356 | C-H str. CH def | $CH_2$, $CH_3$ |

In this particular equation there were five active wavelengths, three of which had positive coefficients and two which had negative coefficients. As with any NIRA calibration such data (Table 5) can be continually updated as new samples of known $AME_n$ are added to the data base.

NIRA may also be useful in predicting the energy content of fat. Supplemental fats pose a major problem for $AME_n$ bioassays. Fats are concentrated sources of ME, and it is obviously essential to know their energy contribution with as much accuracy as possible. Because of limitations with diet mixing and handling it is not possible to include much more than 10-15% in a diet, and these low inclusion levels pose a problem in conventional bioassays with extrapolation to 100% fat inclusion, necessary for calculation of $AME_n$. On the other hand NIRA estimates of fats should be fairly easy because they provide a relatively homogenous mixture compared to complete diets or other ingredients. NIRA analysis of fats does require some specialized equipment and care in analysis, but accurate estimates of nutrient components are possible. Valdes and Leeson (1994) carried out bioassay and NIRA estimates of $AME_n$ of a range of fats and fat blends. Results for some of the more common fats and oils are shown in Table 6.

Table 6. NIRA prediction of the $AME_n$ of fats and oils (MJ/kg).

| Fat blend | Determined $AME_n$ | NIRA prediction |
|---|---|---|
| Canola oil | 39.54 | 39.54 |
| Tallow/coconut | 38.87 | 37.66 |
| Poultry/tallow | 39.79 | 38.62 |
| Coconut/corn oil | 36.82 | 34.81 |
| Coconut/poultry | 35.56 | 34.60 |
| Tallow/coconut | 32.55 | 34.31 |
| Poultry/soapstock | 32.64 | 34.06 |
| Tallow/coconut/soya | 35.98 | 36.40 |
| Tallow/soy/safflower | 40.17 | 39.41 |

It also seems likely that calibrations will be developed to determine moisture in fats, as well as oxidation by-products and, perhaps individual fatty acids.

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# BEHAVIOURAL AND ANATOMICAL ASPECTS OF POOR FEATHER COVER IN CAGED HENS

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Summary

The long-term behaviour and histopathology of hens (70 weeks of age) with poor feather cover were compared with those of hens with good feather cover. No significant differences in sitting, preening, hen pecking, cage pecking, eating and drinking behaviours or in the incidence of head scratching, dust bathing, feather ruffling could be detected. There was a trend for hens with poor feather cover to engage in less preening. The thickness and number of mitotic figures in the epidermis were similar in well-feathered and poor-feathered skin, suggesting that featherless skin is not being excessively abraded. The lack of inflammatory response supports other histological findings that feather abrasion does not necessarily induce injury to the skin. These observations indicate that the well-being of hens with poor feather cover is not compromised compared to hens with good feather cover.

## I. INTRODUCTION

The keeping of hens in cages and the use of various husbandry practices have attracted severe criticism from welfare groups and the general public. The main criticism of the caged bird is that wire cages cause discomfort and injury as the feathers are rubbed off and the skin is abraded by the wire. Skin normally covered with feathers is very thin. Consequently an important function of feathers is to protect the underlying skin from abrasion and invasion by parasites and pathogens (Lucas and Stettenheim, 1972). Protection of the skin is essential to the well-being of the bird as the skin contains blood vessels, sensory receptors and nerves. Gentle and Hunter (1990) reported feather removal is likely to be painful, as they observed changes in blood pressure, heart rate and brain wave patterns following feather removal. However, long-term effects on behaviours of hens with poor feather cover have not been examined.

The aim of this study was to assess the longer-term effects of feather abrasion on the welfare of caged hens. In this study, the histopathology of the skin of the neck region was correlated with the behaviours of the hens. Behavioural studies were done on the basis of overall body scores. Hens with different feather conditions were examined. These ranged from complete feather cover to almost complete feather loss. No discrimination was made between the removal of feathers from either cage abrasion or by pecking from other hens.

## II. MATERIALS AND METHODS

### (a) Hens

Forty hens (70 weeks of age) were selected from a caged flock of 2000

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commercial laying hens and allocated in pairs to single tier laying cages (45 x 45 x 40 cm) and maintained on a layer ration. The hens were segregated for behavioural studies into 2 groups (n=20 per group) on the basis of overall body feather cover. Histological data are reported here for the neck region only. Feather cover was scored in the range of 0 for almost total loss of feathers to 5 for full feather cover. A grade of 0-3 was considered as poor feather cover and skin condition (Group 1) whereas a grade of 4 or 5 was considered as good feather cover and skin condition (Group 2).

(b) Video recording of behaviour; viewing video tapes; analyses

A video recording was made for each pair of hens in each treatment post-lay from 1300h-1600h with food and water available *ad libitum*. Data on behaviour were obtained from watching video records and manually keying observations into a hand held micro-computer. The activities recorded were time and bouts of pecking at food, drinking, preening, sitting and number of pecks made at the cage and other birds. Two separate bouts of behaviour were recorded if they were separated by a pause of at least 5 sec duration. Incidence of dust bathing, feather ruffling and head scratches were also recorded. SAS linear modelling procedures were used to analyse the effect of feather cover and skin condition on the behaviour of the hens.

(c) Histological assessment

Immediately after killing by cervical dislocation samples of skin were taken from either the lower or mid neck of 4 hens from each of Groups 1 and 2. The tissue was fixed by immersion in Zamboni's fixative (Stefanini *et al.*, 1967) for several days at 4°C. A small piece of skin was processed by routine wax-embedding and 5μm-thick transverse sections stained with either haematoxylin and eosin, or Verhoeff and van Gieson stains for visualisation of tissue types and any inflammatory response. A second piece of skin was processed for the identification of nerve fibres using a triple silver impregnation stain (Gilbert, 1965) on frozen transverse-sections of 40 μm-thickness.

## III. RESULTS

(a) Behaviours

Hens with poor feather cover showed no significant difference in sitting bouts, preening bouts, hen peck bouts, cage peck bouts, eating bouts and drinking bouts compared to hens with good feather cover. The only variables to approach significance between the treatments (Table 1) were preening bouts (P=0.09) and drinking bouts (P=0.12).

Table 1. Effects of poor feather cover (PFC) and good feather cover (GFC) on the number of bouts of sitting (SB), preening (PB), hen pecking (HPB), cage pecking (CPB), eating (EB) and drinking (DB) averaged over 1 h. P= probability value in analysis of variance.

| Treatment | SB | PB | HPB | CPB | EB | DB |
|---|---|---|---|---|---|---|
| PFC | 4.4 | 20.3 | 6.7 | 2.1 | 22.4 | 12.6 |
| GFC | 3.6 | 25.8 | 6.1 | 2.6 | 23.6 | 9.2 |
| P | 0.39 | 0.09 | 0.65 | 0.55 | 0.64 | 0.12 |

Similarly, hens with poor feather cover showed no significant difference in time spent sitting, preening, eating and drinking or in the incidence of feather ruffling, head scratching and dust bathing compared with hens with good feather cover. The only variables which approached significance between the treatments (Table 2) was time spent preening (P=0.09) and the incidence of head scratching (P=0.13).

Table 2. Effects of poor feather cover (PFC) and good feather cover (GFC) on time (sec) spent sitting (ST), preening (PT), eating (ET) and drinking (DT) and in the incidence of feather ruffling (FR), head scratching (HS) and dust bathing (DB) averaged over 1h. P= probability in analyses of variance.

| Treatment | ST | PT | ET | DT | FR | HS | DB |
|---|---|---|---|---|---|---|---|
| PFC | 705 | 546 | 733 | 266 | 0.4 | 6.6 | 1.0 |
| GFC | 807 | 654 | 749 | 247 | 0.4 | 5.3 | 0.4 |
| P | 0.53 | 0.10 | 0.85 | 0.70 | 0.94 | 0.13 | 0.29 |

(b) Histopathology

There were no observable differences in the histology of skin taken from either the mid or lower regions of the ventral surface of the neck from either goup. Other than the difference in numbers of feather follicles and the associated muscle and elastic fibres the morphology of skin in which the feathers had been almost totally removed could not be discerned from skin with total feather cover using either haematoxylin and eosin, or Verhoeff and van Gieson stains.

The skin of the neck was extremely thin, the keratinised epidermis consisting of three to four layers of cells. The epidermis was intact in all sections and showed no evidence of excessive abrasion. Similar numbers of mitotic figures were present in the stratum germinativum of the epidermis of all hens examined. A thick dense band of collagen, 15 to 40 μm in width, was observed running immediately beneath and parallel to the epidermis. Beneath the band of collagen the dermis consisted of dense irregular collagen bundles supporting numerous blood capillaries and venules. Macrophages and small aggregations of lymphocytes were observed in all tissues examined. Eosinophils were numerous in the dermis of two hens with poor feather cover. Beneath the dense collagen bundles adipose tissue surrounded large blood vessels and nerve bundles. Associated with the feather follicles were smooth muscle bundles that inserted via elastic tendons into both the adipose and dense connective tissue and the collagenous sheath surrounding the follicles.

Silver impregnation revealed numerous nerve bundles in the dermis and subdermal connective tissue. The nerves were frequently associated with blood vessels and feather follicles. No differences were observed in either the distribution or numbers of nerve bundles in skin having poor feather cover compared to skin with good feather cover.

## IV. DISCUSSION

Hens with poor feather cover demonstrated no changes in behaviour compared to hens with full feather cover. These results indicate that considerable feather loss does not compromise the long-term well-being of caged hens. Although no statistically significant differences in behaviour were observed between the two groups of hens, hens with poor

feather cover showed a reduction in preening which approached significance, $p=0.09$. Indeed, hens with less covering feathers would be expected to reduce the time spent grooming.

The lack of behavioural differences between the two groups of hens is in agreement with the histological findings. The thickness of the epidermis as well as the number of mitotic figures were similar in well-feathered and poor-feathered skin. These findings suggest that the featherless skin is not being excessively abraded by the wire. If this was the case, the epidermal cells would be expected to undergo an increased rate of mitosis, causing an increase in the thickness of the skin in an attempt to replace excessive sloughing of the damaged epidermal cells. In addition, with the exception of a few sections from skin with very poor feather cover, the number of macrophages and eosinophils were similar between the two groups. The lack of inflammatory response supports the other histological findings that feather abrasion does not induce injury to the skin. However, the analyses have to date been confined to the neck region of four hens from each group.

Although it is well known that sympathetic nerves supply the feather muscles, the *mm. pennarum,* little is known regarding the sensory innervation of the skin. Silver staining revealed an abundance of nerves fibres in the dermis and subdermal tissue of the neck skin of all hens examined. No nerve fibres, however, penetrated beyond the dermis into the epidermis. Currently immunohistochemistry is being used to distinguish the types of sensory nerves innervating the skin. It is predicted that some of these nerves will have the histological features indicating they are nociceptors that transmit painful stimuli.

In summary, the histological and behavioural data to date strongly indicate that feather-abrasion of the neck region does not compromise the well-being of caged hens.

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# THE ANATOMY OF THE EMU TOE: CONSEQUENCES OF DE-CLAWING

C.A. LUNAM*, P.C. GLATZ** and P. O'MALLEY***

Summary

The microscopic structure of the toe was determined in three 70-week-old emus that had been de-clawed at hatch. The structure and distribution of scales, dermal papillae, blood vessels, herbst corpuscles, phalangeal bone, nerves and chondroid tissue was similar to that of the intact emu toe which had not been de-clawed. Nerve bundles mostly accompanied the larger blood vessels. Neuromas were not observed in any of the de-clawed toes. These preliminary results indicate that conservative de-clawing at hatch induces minimal perturbation of the anatomical structures developing within the emu toe and reduces the risk of traumatic neuroma formation.

## I. INTRODUCTION

De-clawing is commonly practised in commercial emu farming. This procedure is performed to reduce damage to the skin inflicted by the toe-nails during natural bouts of aggression and to decrease the risk of injury to personnel handling the emus. De-clawing is approved by the Standing Committee on Agriculture, in the Australian Model Code of Practice for the Welfare of Domestic Poultry.

De-clawing, or permanent removal of the nail, involves partial amputation of the toes. In chickens, severing of nerves during partial amputation of either the toes (Gentle and Hunter, 1988), or beak (Gentle, 1986; Lunam and Glatz, 1995) can result in the formation of neuromas. As neuromas are a known cause of chronic pain (Devor and Rappaport, 1990), the potential presence of persistent neuromas in the de-clawed toe stump of emus would be an important welfare concern.

The aim of this work was to examine de-clawed toes of commercially farmed emus for the presence of persistent neuromas.

## II. METHODS

Three emu chicks were de-clawed on the day of hatch. De-clawing involved amputation at the distal phalangeal joint using a hot blade-debeaker, angled to retain the ventral aspect of the distal phalanx within the foot pad. An emu chick, which had not been de-clawed served as a control. Chicks were intensively reared on sawdust litter during initial brooding and then given free access to large open paddocks. The chicks were hatched and maintained until slaughter on a commercial West Australian emu farm with food and water provided *ad libitum.*

At 70 weeks of age the emus were slaughtered by electrical stunning and the toes immediately excised and immersed in Zamboni's fixative (Stefani *et al.*, 1967) for several

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weeks. After fixation each toe stump was cut into 1cm lengths, most of the hard keratin peeled from the toes and the central core of the phalangeal bone excised. The tissue segments were decalcified in ethylenediaminetetra-acetic acid for 10-14 days. Some segments were processed by routine wax-embedding and 10μm-thick transverse sections stained with either haematoxylin and eosin (H & E), or Verhoeff and van Gieson (V & VG) for microscopic visualisation of the structures within the toe stump. Most segments were processed for the identification of nerve fibres using a triple silver impregnation stain (Gilbert, 1965) on frozen transverse-sections of 40 μm-thickness. Sections were collected at 500 μm intervals through each segment.

## III. RESULTS

Tissue types, sensory receptors, blood vessels and nerve bundles were clearly identified using H & E and V & VG stains. The epidermis consisted of two distinct layers, a thick stratum germinativum and a dense corneum. Differences were observed in the dermal-epidermal junction between the dorsal and ventral margins of the toes. Dermal papillae, though numerous at the ventral region of the toe were absent at the dorsal margin where the keratin had become dense to form scales. The dermis consisted of dense irregular collagen and elastic fibres that encapsulated the bone of the distal phalanx. Herbst corpuscles were observed in the dermis close to the lateral margins of the dorsal scales of all toes. The dermis was well supplied with blood vessels. These were particularly numerous in the ventral dermis.

An extensive mass of chondroid-like tissue was present immediately ventral to the tendon of the flexor digitorum longus muscle. This cartilage-like tissue contained abundant elastic fibres.

Silver staining confirmed the distribution and size of nerve bundles observed with conventional staining. The distribution of nerves in the de-clawed toe was comparable to that in the intact toe. Nerve bundles mostly accompanied the larger blood vessels. Within nerve bundles fibres were aligned parallel to one another. Nerves were rarely observed near the dermal-epidermal junction. Intra-medullary nerves were present in the bone of the distal phalanx.

Neuromas were not observed in any histological sections taken through the extent of the distal 4 cm of each de-clawed toe.

## IV. DISCUSSION

The microscopic structure of the emu toe was found to be similar to the toe of the domestic fowl (Lucas and Stettenheim, 1972), both species having similar distributions of scales, dermal papillae, herbst corpuscles, blood vessels, chondroid tissue and nerves. These similarities suggest that the structures within the avian toe have been highly conserved during evolution.

An interesting feature is the chondroid-like tissue immediately beneath the tendon of the flexor muscle. This is also present as a thin band of tissue in toes of the domestic fowl (Lucas and Stettenheim, 1972). The function of this cartilage-like tissue is unknown. Numerous elastic fibres within its matrix suggest that it may function as elastic cartilage, providing both flexibility and support to the emu toe, the extensive development of this tissue in the emu being an adaptation to the extreme weight bearing of the toe.

No neuromas were detected in any sections taken through the distal 4 cm of the de-clawed toes. The absence of neuromas is likely to result from conservative removal of

tissue on the day of hatch. As is the case with beak-trimming, the development of neuromas in emus may be dependent on the age at which the chick is de-clawed and the amount of tissue amputated. In support of this, the risk of persistent traumatic neuromas developing after partial amputation of the beak of domestic fowl is significantly reduced if a conservative amount of beak is removed at hatch (Lunam and Glatz, 1995; Lunam, Glatz and Hsu, personal observations). In contrast to the lack of neuromas in the de-clawed emu toes, neuromas have been described in the toe-stumps of chickens 60 days after de-clawing on the day of hatch (Gentle and Hunter, 1988). However, in this latter study a considerable amount of the toe was removed, greater than 50% of the hallux being amputated in some chicks.

These preliminary results indicate that conservative amputation, leaving part of the distal phalanx intact, conducted at hatch using a hot-blade debeaker, will reduce the risk of neuroma formation after de-clawing of emu chicks.

## ACKNOWLEDGEMENT

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# EFFECTIVENESS OF ZINC BACITRACIN IN HEAT-STRESSED POULTRY

K. MÄNNER

Few studies have utilized feed additives to ameliorate the adverse effects of high temperatures on poultry production. The present study evaluated whether supplementation with zinc - bacitracin (ZBA) induced positive effects on the performance, energy metabolism, and upper critical temperature (UCT) of growing broilers (1-49 d of age) and laying hens (22-52 wk of age) kept at constant temperatures of 20° or 34°C. Also, the effect of ZBA on heat tolerance was measured using 4 to 12 wk old pullets. Mash diets were either unsupplemented or supplemented with 50 mg (broiler) or 100 mg (pullets, laying hens and broilers) ZBA kg/diet. Indirect calorimetry with open air circulation was used for measuring gas exchange. Heat production was calculated from the formula of Romijin and Lokhorst (1961). The maintenance ME requirements and the efficiencies of utilization of ME for energy deposition in body and eggs were calculated by regressions (Chwalibog, 1985). The UCT was calculated by regressing environmental temperature on heat production starting at 10°C and raising the temperature by 5° steps to 30°C and then to 34° and 38°C. The procedure used for heat tolerance measurements was similar to that described by Washburn *et al.* (1980).

ZBA induced a more pronounced increase in performance in heat-stressed hens than in hens kept at 20°C. Egg numbers, egg mass, and feed efficiency of ZBA-treated heat-stressed hens were significantly improved by 10.9, 16.7, and 9.9%, respectively, compared to untreated hens. ZBA supplementation of hens acclimatized to 20° and 34°C reduced the ME requirements for maintenance by 9.6 and 13.1%, respectively. The efficiency of ME utilization for energy deposition in body and eggs was improved. The energy retentions at 20° and 34°C were, respectively, 19.3 and 16.5% higher than in untreated hens. Heat tolerances of chickens fed ZBA and acclimatized to either 20° or 34°C were significantly improved (23.7% at 20 ° and 51.2% at 34 °C). Also, the UCT of the ZBA-treated hens was increased significantly. Weight gain and feed efficiency of heat-stressed broilers supplemented with 50 mg ZBA/kg were significantly improved by 10.8 and 6.1%, respectively. This advantage was due to a higher efficiency of ME utilization which was attributed to the significantly reduced heat production of heat-stressed, treated broilers by 9.3% relative to untreated broilers. The addition of 100 mg ZBA/kg to broiler feed gave no further improvements relative to 50 mg ZBA/kg.

The results indicated ZBA improved the performance of hens and broilers acclimatized to 34°C by reducing overall heat production. Therefore, treated, heat-stressed hens and broilers were able to maintain a higher feed intake than untreated, heat-stressed birds. However, the fact that the performance of ZBA-treated hens during heat stress was reduced relative to the moderate temperature also shows that this effect is of limited value. The better heat tolerance of ZBA-treated hens suggests that the mortality rate, which is often due to extremely high temperature peaks of short duration, can possibly be reduced by the addition of 100 mg ZBA/kg feed.

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# PROBLEMS IN THE MEASUREMENT OF β-XYLANASE, β–GLUCANASE AND α–AMYLASE IN FEED ENZYMES AND ANIMAL FEEDS

B. V. McCLEARY


## Summary

Measurement of *endo*-acting enzyme activities in commercial enzyme preparations is complicated by the presence of interfering enzymatic activities and by high concentrations of interfering substances such as reducing sugars. These problems can be overcome using dye-labelled substrates which specifically measure only the *endo*-activity of interest, (ie. *exo*-acting enzymes are not measured with the assay and they do not interfere with the sensitivity of the assay for the *endo*-activity).

Separate from the problems mentioned above, the measurement of these enzyme activities in animal feeds introduces a host of new problems. These include the sensitivity of the assay, the extraction of the enzyme from the feed, problems associated with the selective extraction of particular forms of a given enzyme and, finally, the presence of compounds in the extract which interfere with the assay either by acting as alternative substrates or as inhibitors of enzyme activity.

The advantages and limitations of specific soluble and insoluble chromogenic substrates for the assay of β-xylanase, β-glucanase and α-amylase will be discussed as will the use of a soluble colourimetric substrate for α-amylase. Particular attention will be given to the assay of β-xylanase in feed samples.


## I. INTRODUCTION

Beta–glucanase, *endo*-β–xylanase and α–amylase are added to animal feeds to catalyse depolymerisation of β–glucan, arabinoxylan and starch. β–Glucan and arabinoxylan are the major endosperm, cell-wall polysaccharides of cereal grains. These water-soluble, or water hydrateable polysaccharides form solutions, or slurries, of high viscosity and they reduce the rate of nutrient absorption from the gut. The viscosity building properties of β–glucan and arabinoxylan are rapidly destroyed by β–glucanases and xylanases. In fact, it can be demonstrated that the viscosity properties of these polymers can essentially be destroyed by cleavage of just one bond per thousand by the appropriate *endo*-hydrolase.

In the current paper, methods which have been developed for the specific measurement of α–amylase, β–glucanase and β–xylanase in industrial enzyme preparations, will be described. Problems faced in the measurement of trace levels of enzymes, particularly β–xylanase, in chicken feeds will be discussed in detail.

Assay procedures which specifically measure *endo*-acting enzymes in the presence of *exo*-acting enzymes and glycosidases can be summarised as follows:

1. Viscometric procedures using pure polysaccharides.
2. Assays based on soluble dyed polysaccharides.
3. Assays employing insoluble dyed polysaccharides.

Megazyme (Aust) Pty Ltd, 2/11 Ponderosa Parade, Warriewood, NSW 2102.

4. Assays which measure decreased interaction of a native polysaccharide with a second compound eg. starch with iodine; β–glucan with congo red or calcofluor.
5. Assays using well defined oligosaccharides with a chromophore attached e.g. *p*-nitrophenyl maltosaccharides.

## II. ALPHA–AMYLASE

There is some, although limited, interest in the addition of α–amylase to feeds to improve digestibility. Several good methods are available for the measurement of this activity and these include those employing dyed-crosslinked starch or amylose (eg. Phadebas$^R$ and Amylazyme tablets) and those using blocked *p*-nitrophenyl maltosaccharides (McCleary and Sheehan, 1987; McCleary, 1991).

Assays based on the use of *p*-nitrophenyl maltosaccharides generally employ an "end-blocked" nitrophenyl maltosaccharide in the presence of excess quantities of the *exo*-acting enzymes, amyloglucosidase and α–glucosidase (e.g. the Ceralpha method; McCleary and Sheehan, 1987). The blocking-group prevents hydrolysis of the substrate by these enzymes. When *endo*-acting α–amylase cleaves the maltosaccharide, the *exo*-acting enzymes instantaneously hydrolyse the nitrophenyl maltosaccharide reaction-product to glucose and free *p*-nitrophenol. The reaction is terminated and the colour is developed by the addition of a weak alkaline solution. This is a simple and very specific assay for the measurement of α–amylase, and can be used to specifically measure this enzyme in the presence of other starch degrading enzymes such as amyloglucosidase and β–amylase. The assay is ideal for the measurement of α–amylase in industrial microbial preparations, and can also potentially be used to measure this enzyme in feed preparations. The only possible limitation may be sensitivity. If the levels of activity are too low to be accurately measured with the above method, then assay formats employing dyed, crosslinked substrates in tablet form should be employed.

The sensitivity of assays for trace levels of α–amylase in cereal flours or feed materials can be increased 10-20-fold by performing assays on whole flour slurries. In a typical format, flour sample (0.5 g) is suspended in acetate buffer at 50°C, and reaction is initiated by the addition of an Amylazyme tablet (McCleary, 1991). After a set incubation time (2 min), the reaction is terminated by the addition of a weak alkaline solution (e.g. Trizma Base; pH ~9.0). The reaction mixture is filtered through glass fibre filter paper, and the absorbance of the filtrate is measured at 590nm. In assays such as this, starch in the sample being analysed will act as an alternative substrate, but the effect of this can be determined by constructing a standard curve in the presence and absence of the flour. Typical results are shown in Figure 1, where curves have been constructed in the presence and absence of wheat flour. It is evident that the interference by the flour starch is significant (about 30%) but is easily allowed for. A similar degree of interference has been experienced with a range of wheat flours, even with "starch-damage" values ranging from 6 to 15%. This degree of interference is likely to vary depending on the source of the α–amylase being measured e.g. for fungal α–amylase, the degree of interference was found to be less than 10%.

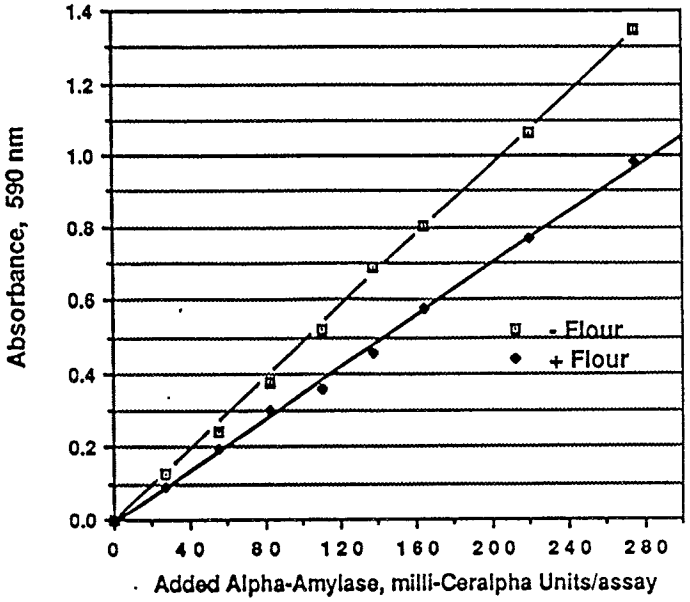


Figure 1. Standard curves relating the level of α–amylase (Ceralpha, milliUnits per assay) to absorbance at 590nm using Amylazyme tablets (Lot 50504) under standard assay conditions with purified wheat α–amylase in the presence or absence of sound wheat flour.

## III. BACTERIAL BETA–GLUCANASES AND CELLULASES

*endo*-Beta–glucanases (including *endo*-1,4-β–D-glucanase i.e. cellulase) in industrial enzyme preparations can be assayed using a pure polysaccharide substrate such as barley β–glucan or carboxymethyl cellulose (CM-cellulose), with measurement of reducing sugar release with the Nelson/Somogyi (Somogyi, 1952) or DNS (Bailey, 1988) methods. The major problem with these assays is that they may not be specific, i.e. β–glucan will also be hydrolyzed by *exo*-acting cellobiohydrolase, and oligosaccharide fragments by β–glucosidase. It is essential to use an assay which is specific for the *endo*-acting activity.

In the brewing industry, a viscometric assay is commonly used for the measurement of β–glucanase in malt (Bathgate, 1979). This method employs highly purified β–glucan of defined viscosity properties. The assay is absolutely specific, but extremely tedious.

Alternative assays for *endo*-glucanases employ soluble or insoluble, dyed β–glucan, or dyed cellulose derivatives. The soluble substrate, Azo-barley glucan (McCleary, 1987) is now widely used in the malting, brewing and animal feed industries. In principle, this type of assay involves the incubation of enzyme with a high molecular-weight dyed polysaccharide under defined conditions of time, temperature and pH. The reaction is terminated and non-hydrolysed material is precipitated by the addition of an alcoholic solution, and the slurry is centrifuged. The absorbance of the supernatant solution (containing low molecular-weight dyed fragments) is measured at 590nm, and activity is determined by reference to a standard curve. Alternatively, insoluble, dyed polysaccharides in tablet form can be used. These tablets can contain barley β–glucan or cellulose as the primary substrate. Barley β–glucan is susceptible to hydrolysis by fungal cellulases, bacterial β–glucanases and malt β–glucanases. The latter two enzymes are classified as *endo*-1,3:1,4-β–D-glucanases and have no action on cellulose derivatives, whereas fungal cellulases attack both β–glucan and cellulose.

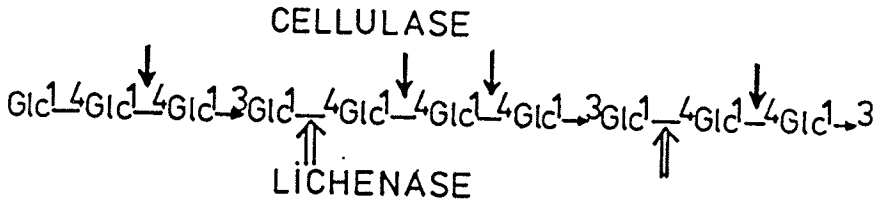


Figure 2. Cleavage of 1,3:1,4-β–D-glucan by cellulase and 1,3:1,4-β–D-glucanases.

Many of the problems likely to be experienced in the measurement of β–glucanases in animal feeds are the same as those experienced in the measurement of xylanase, i.e. extraction of the enzyme (binding to feed components) and inhibition by soluble components from the feed.

## IV. MICROBIAL XYLANASES

Of the carbohydrase enzyme used as feed supplements, one of the most difficult to measure has been *endo*-1,4-β–D-xylanase (xylanase). These problems are attributed to several factors, including the low levels added to the feed, inactivation during pelleting, binding to feed components, and inhibition by soluble feed components.

In our experience the only biochemical assays with sufficient sensitivity to measure the trace levels of xylanase added to chicken feeds are viscometric assays and those employing dyed, crosslinked wheat arabinoxylan (Xylazyme AX tablets; Megazyme Pty. Ltd). These two assays have similar degrees of sensitivity. With the viscometric assay it is essential to employ a high-viscosity substrate such as wheat arabinoxylan. Good linearity between enzyme activity (Nelson/Somogyi reducing sugar method; Somogyi, 1952) and viscosity change (Inverse Reciprocal Viscosity Units; Megazyme Technical Booklet, 1995) can be obtained over a wide range of enzyme concentrations (10-fold).

In the preparation of dyed, crosslinked xylans or arabinoxylans, several parameters are important, including the purity of the polysaccharide, the degree of natural substitution (i.e. with arabinosyl-, or 4-0-methyl glucuronosyl- residues) and the molecular size of the polysaccharide. Initially, Xylazyme tablets were prepared from birchwood xylan. However, the quality of commercially available birchwood xylan (particularly molecular size) fluctuates significantly. With low-molecular-weight material it is not possible to produce stable, dyed and crosslinked gel particles. Consequently, to obtain a consistent quality substrate (purity and molecular size), it became essential to find a source of xylan, which could be purified in-house. This source was wheat flour, and the substrate is arabinoxylan. This is now used in both Xylazyme (100 mg) and Xylazyme AX (60 mg) tablets.

As mentioned, viscometric assays are extremely tedious, and only a limited number of assays can be performed concurrently, or in a given period of time. In contrast, assays employing Xylazyme AX tablets, which have the same sensitivity and specificity as

viscometric assays, have the added advantages of simplicity and rapidity. The standard curve for pure Trichoderma longibrachiatum xylanase (pI 9.0 form), relating reducing-sugar units (Nelson/Somogyi method; Somogyi, 1952) to colour release on hydrolysis of Xylazyme AX tablets, is shown in Figure 3.

Since xylanases from different microbial sources, and even different forms of the enzyme from the same source, have different action patterns, it is to be expected that standard curves relating enzyme activity (by a method which measures bonds broken e.g. reducing-sugar methods) to colour released on hydrolysis of dyed, crosslinked arabinoxylan (a measure of depolymerisation), will vary. However, this is generally not a problem in standard practical applications, because in most situations the behaviour of a single preparation is being evaluated, either in fermentation situations or in feed applications.

It is generally accepted that xylanase enzymes which are best suited to feed applications have optimal activity at pH 6.0. Consequently, these enzymes are generally assayed at this pH in 0.1 M sodium phosphate buffer. For this reason enzyme extractions were initially performed in this buffer. However, because the level of activity recovered was quite low (< 20% of the amount of enzyme activity added in spiking experiments), a range of other extraction buffer mixtures and conditions were evaluated and the final conditions of extraction and assay are detailed below in the section "Extraction and Assay of Xylanase in Feed Samples". Of all the extraction and assay mixtures and conditions evaluated, the best recovery and activity was obtained using 0.1 M acetic acid at room

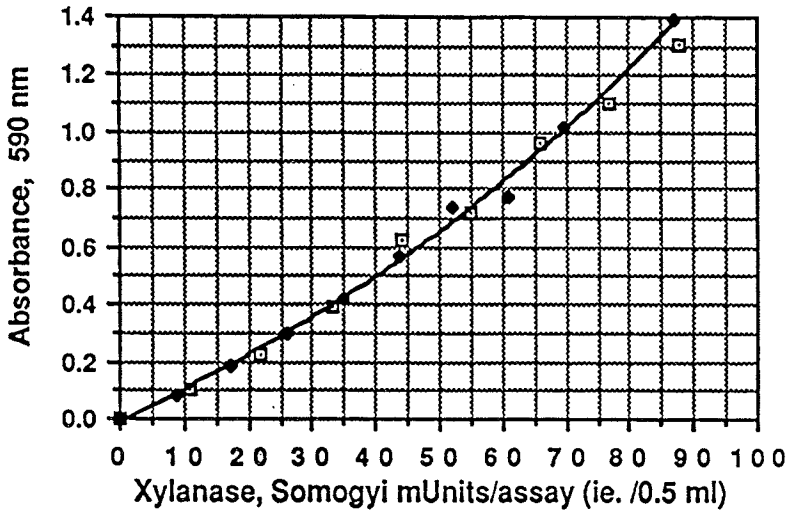


Figure 3. Standard curve relating the action of *T. longibrachiatum* xylanase (pI 9.0) on Xylazyme AX (Lot 40602) to enzyme units on wheat arabinoxylan (Nelson/Somogyi method). The tablet was added to 0.5 mL of enzyme preparation in 25 mM sodium phosphate buffer (pH 6.0) at 40°, and incubated for 10 min. Reaction was terminated by the addition of Trizma Base (2%, pH 9.0) with stirring. The slurry was filtered, and the absorbance of the filtrate was measured at 590nm.

temperature. Information on the percentage recovery of xylanases added to feeds was obtained by performing incubations and assays under the standard conditions for feeds (refer below), with four levels of added enzyme, with and without feed in the mixture. The pelleted feed used was milled ( < 0.5 mm) before use. From the results shown in

activity. Unfortunately, the curve obtained for "enzyme without added feed" is more curved than the line for enzyme recovered from "enzyme/flour mixtures". However, this problem can be resolved, in part, by performing assays on feeds with and without added enzyme (i.e. spiking experiments).

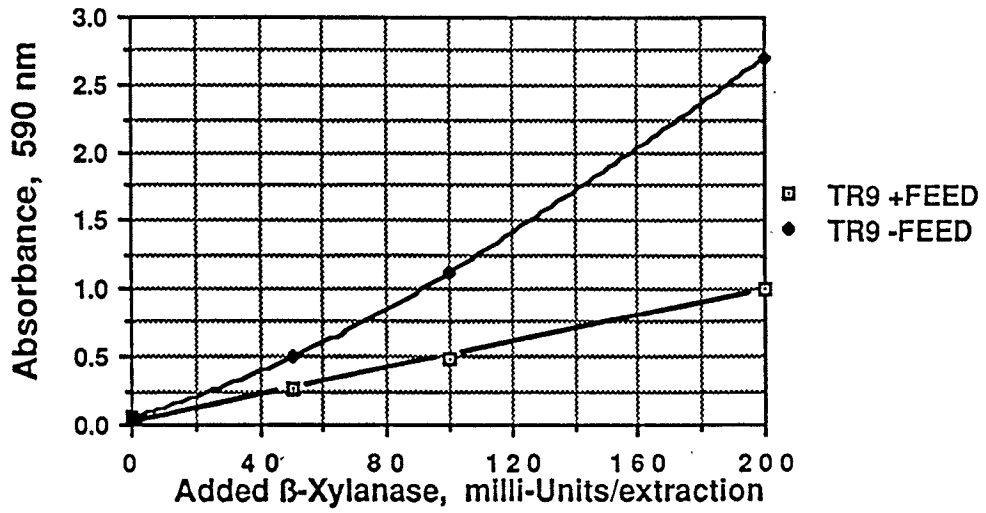


Figure 4. Curves showing the recovery of *T. longibrachiatum* (pI 9.0) xylanase (as absorbance 590nm on hydrolysis of Xylazyme AX) from flour/enzyme mixtures in comparison to measured activity in preparations free of added flour.

The observation that the best extractant for *T. longibrachiatum* xylanase was 0.1 M acetic acid was quite surprising. Under these extraction conditions (0.5g flour/5mL of 0.1 M acetic acid), a final extract pH of 4.0 + 0.1 is obtained, whereas the optimal pH for activity of this enzyme preparation is 6.0 (with only 70% of maximal activity at pH 4.0; refer to Figure 5). This result suggested that the extractant selectively solubilises a particular form of the enzyme (i.e. the pI 5.5 form which has a pH optima of about 4.0). However, this theory was discounted based on the fact that 1) the pI 5.5 form of the enzyme represents only a small percentage of the total xylanase in the mixture being evaluated, and 2) when the pI 5.5 and 9.0 forms of *T. longibrachiatum* xylanases were separated, purified and evaluated in binding studies it was found that the pI 5.5 form binds to the feed more strongly (lower recoveries; see Figure 6) than does the pI 9.0 form.

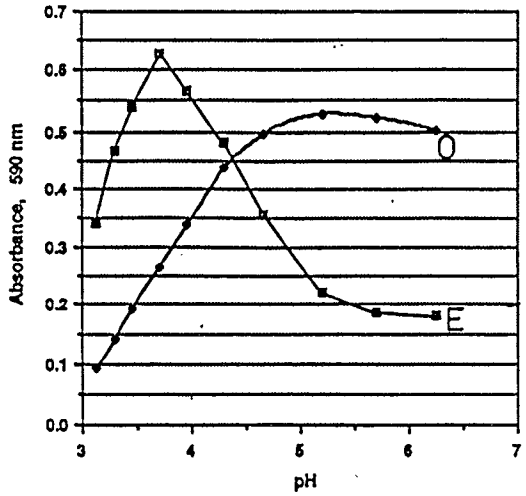


Figure 5. pH activity curves for original enzyme preparation (O) and for the xylanase fraction extracted (E) from the feed sample.

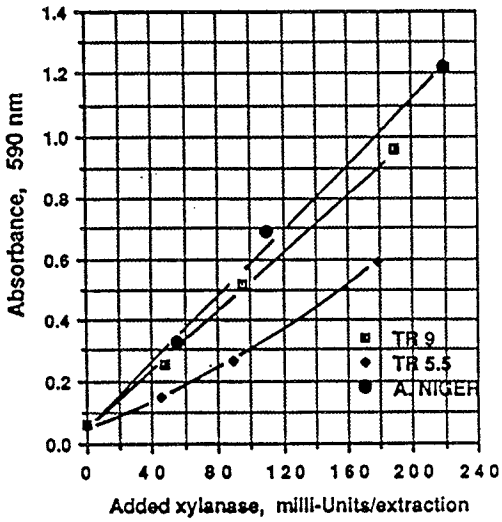


Figure 6. Binding of different highly purified xylanase enzymes to feed components. Extraction and assay conditions are as described in the relevant section below.

## V. EXTRACTION AND ASSAY OF XYLANASE IN FEED SAMPLES

Extraction:

Feed sample (milled to < 0.5 mm, 0.5 g) is suspended in 5 mL of 0.1 M acetic acid and stirred on a vortex mixer. The slurry is then treated either with water (0.2 mL) or with control xylanase solution (0.2 mL; in 50% glycerol plus stabilisers), and then stirred several times over 10 min. The slurry is then centrifuged (3,000 rpm, 10 min) and the supernatant is used directly in assays.

Assay:

To an aliquot (0.5 mL) of supernatant solution (with or without added enzyme), at room temperature, is added a Xylazyme AX tablet, and the tube is immediately placed in a water bath set at 50°C +0.1°C. The tube contents **are not stirred**. After exactly 30 min

at 50°C, the tubes are treated with Trizma Base solution (2%, 5 mL) and stored at **room temperature** for 5 min. The tubes are then stirred again, and the slurry is filtered through a Whatman No. 1 (9 cm) filter circle. The absorbance of the filtrate is measured at 590 nm.

Calculations:

The level of xylanase activity in the flour sample is calculated as follows:

$$\text{Activity in feed sample (0.5 g)} = \text{Added Activity} \times \frac{SA}{TA - SA} .$$

where:

Added activity = the amount of xylanase added to the feed slurry at the time of assay (in control xylanase solution , 0.2 mL)

SA = absorbance of extracts of the sample assayed by the standard format.

TA = total absorbance; i.e. the absorbance of extracts of the sample to which the additional xylanase was added.

thus:

Xylanase/Kg feed = Activity in the feed sample x 2000
(2000 converts from 0.5g as assayed to 1 Kg).

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# CONCANAVALIN A, FED AS A PART OF RAW JACKBEAN SEEDS, DOES NOT AFFECT ANTIBODY PRODUCTION OF BROILER CHICKENS

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An experiment was conducted to determine the effect of a lectin, concanavalin A (Con A), contained in raw jackbean seeds, on the immunological response of broilers. A maize-soyabean meal basal diet was prepared to which was added either 25, 50 or 100 g/kg of ground raw jackbean (RJB) seeds. The RJB seeds contained 24 g Con A/kg on a dry matter basis, as measured by a Rocket immunoelectrophoresis technique. Similar diets were prepared by using the same levels of JB after toasting at 190°C for 16 min. In addition, the basal diet was pair-fed to groups of chicks at the level of feed intake of chicks fed the 10% RJB-containing diet. Each diet was fed to six groups of 6 chicks for 6 weeks. At 5 weeks, three groups of chicks on each diet were immunized against Brucella abortus (BA) and the anti-BA antibody titers were determined one week later by ELISA. Also, antibody production against Con A was also measured by the same method. Con A binding to intestinal villi and subsequent endocytosis were confirmed by microscopic examination using a specific PAP-staining technique. Performance was recorded weekly.

Feed intake and weight gain were reduced ($P<0.05$) only by the diet containing 10% RJB indicating that broiler chicks can tolerate intakes of approximately 5 g of Con A (1.2 g/kg diet) over 6 weeks without affecting performance. The antibody response to BA did not differ with dietary treatment. Serum from chicks fed RJB contained antibodies against Con A. The bursa, thymus, spleen and pancreas dry weights, as a percent of dry body weight, were not affected by the experimental diets. The data indicate that Con A binds to the cells of the gastrointestinal tract, passes into the general circulation and eventually elicits an immunological response against itself without affecting the production of antibodies against other antigens.

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# FEED MICRO-IMAGING - A NEW TOOL FOR NUTRITIONAL RESEARCH

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Developments in analysis techniques over the past few years have improved understanding of feedstuff composition, particularly of the fibre fraction. Coupled with appropriate animal models, this has led to advances in knowledge of how various feed components are digested in the avian intestine. These techniques have highlighted the role of soluble fibres in wheat- and barley-based diets in increasing digesta viscosity in broilers, and affecting the digestion of nutrients such as fats. However, although informative, such studies give only an overall picture and no information on what is happening at the structural level in the feed.

Recently, staining and microscopic techniques used in other areas of research have been applied to nutritional studies. This involves using colour stains to differentiate between chemical entities within the feed, including fibre, protein and starch. In animal studies, the intestinal microflora can also be identified. Sections of feedstuffs are then photographed under a microscope. This technique has been used to investigate the structure and digestion of feedstuffs such as wheat, soyabean and rapeseed using both an *in vitro* poultry digestion model and *in vivo* samples. Specific cell wall degrading exogenous enzymes were used to throw further light on the role of this component in digestion.

These studies have confirmed the known structure of feedstuffs, with cell walls surrounding and protecting the nutrients. There were also indications of structural differences in the cell walls between low and high ME wheats. Milling led to a general disintegration of the starchy endosperm in wheat, but the aleurone remained relatively intact with protein enclosed within the thick-walled cell. These intact aleurone cells, with the enclosed protein, were basically unaffected by digestion *in vitro* or *in vivo*. Supplementation with appropriate enzymes disrupted the intact cell wall, leading to a release of the protein. *In vivo* studies with wheat-based diets demonstrated that microbial proliferation in the foregut of the broiler can led to a considerable destruction of feed structure and loss of nutrients. Supplementation with a xylanase-based enzyme product (Avizyme 1300) reduced digesta viscosity and rather surprisingly helped maintain feed integrity into the duodenum. Treatment of soyabean and rapeseed with appropriate enzymes led to a disruption of the cell wall, releasing enclosed nutrients. These studies demonstrate that micro-imaging, coupled with animal models and analytical data, is a useful tool to develop an understanding of the composition of feeds and the processes of digestion.

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# COMPARISON OF PERFORMANCE OF ONE IMPORTED AND TWO AUSTRALIAN LAYER STRAINS

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Summary

Production responses from one imported and two Australian strains of commercially available laying hens were compared under industry conditions at the University of New England's poultry farm 'Laureldale' using two diets. SIRO-CB hens had very low mortality and the highest hen-housed production, but had lower feed conversion efficiency than ISABrown and Hy-line hens. The latter two strains had high mortality and lower hen-housed egg production. Compared with the other two strains the imported ISABrown hens seemed more sensitive to disease and management stress and produced an undesirably high proportion of eggs in excess of 67 g after about 20 weeks into lay. However, ISABrown hens were earliest into lay and had the highest efficiency of feed conversion into egg mass.

## I. INTRODUCTION

The importation into Australia of new strains of laying hen raises new questions for the Australian poultry industry. Dietary recommendations for the imported strains differ from those currently accepted for Australian layers whose diets, commonly made up largely of wheat and meat meal, differ in nature from those in common use in Europe and North America. Moreover, in Australia, hens are more likely to be housed in sheds that have little or no environmental control and to be held in cages under conditions that differ from those in use overseas. The imported strains may be more susceptible to disease challenges in Australia. It is pertinent to ask whether the 'premium' diets recommended for some imported strains are economically justified under Australian conditions. This report provides results of a comparison of an imported layer (ISABrown) with two Australian layers (Hy-line Tint - White Leghorn x New Hampshire) and SIRO-CB - Black Australorp x New Hampshire).

## II. METHODS

All strains were hatched on 13 October, 1993 and reared in wire-floored brooders on a commercial farm near Tamworth. At 5 weeks of age they were moved to the University farm 'Laureldale', and reared in groups of about 85 birds in deep litter pens until 18 weeks of age on a pullet grower diet. The birds were then moved into single-deck, Californian-type laying cages in a saw-tooth roofed shed (3 birds/cage). Each strain was represented by 336 birds distributed in 8 blocks (each of 42 birds in 14 adjacent cages) distributed at random in the 12 rows of cages in the shed. Four blocks of each strain were given one of two commercially produced diets (Australian standard layer or ISABrown 'premium' diet) provided in the form of a loose mix. The diets were reformulated and supplied on 4 occasions, in March, July, October and December, 1994. The amounts of protein and mineral/vitamin mix in the formulations were varied by the supplier to allow for the changing feed intake of the birds according to seasonal conditions (Table 1).

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Table 1. Calculated dietary specifications.

| | Australian layer diet | 'ISA-premium' diet |
|---|---|---|
| ME (MJ/kg DM) | 11.0 | 11.5 |
| Crude protein (g/kg) | 163-174 | 164-180 |
| Calcium (g/kg) | 37.7-38.4 | 36.9-38.5 |
| Total P (g/kg) | 7.7-7.9 | 6.2-8.1 |
| Available P (g/kg) | 5.3-5.9 | 3.7-6.0 |
| Methionine, (g/kg) | 3.6-3.8 | 3.6-3.9 |
| Methionine + Cysteine (g/kg) | 6.2-6.6 | 6.3-6.9 |
| Lysine (g/kg) | 7.6-8.1 | 7.7-8.4 |

The two Australian strains were found to be slightly overweight at 18 weeks and a time-feed restriction was applied until they were 23 weeks of age. The ISABrown birds were introduced to their layer diet from 18 weeks and the two Australian strains from 20 weeks of age.

The birds were allowed natural lighting from hatching until 24 weeks of age (April, 1994) at which time natural day length was decreasing rapidly. They were then given supplementary artificial lighting to increment daylength, by 20 min at weekly intervals, until the birds were exposed to 16 h of light. This daylength was then maintained until the end of the trial.. The trial was stopped when the birds were 59 weeks of age. The ISABrown strain commenced laying at about 18 weeks of age and the other two strains at 20 weeks of age.

Feeders were topped up three times per week and the amounts of feed added were recorded. Egg numbers and egg weights were recorded each day from Monday to Friday, and weekly egg mass was estimated. All mortalities were also recorded. Results were analysed by analysis of variance.

## III. RESULTS AND DISCUSSION

There were no significant between-diet differences in feed intake, egg weight, hen-day production, egg mass output or feed conversion ratio. Results for each strain have, therefore, been combined across diets. Differences in nutrient concentration among the 4 batches of feed may have reduced the likelihood of detection of effects of diet on egg production.

Mean feed intake differed significantly ($P<0.001$) between strains, being highest in the ISABrown and SIRO-CB and lowest in the Hy-line Tint hens (Figure 1). Intake changed over time being higher in the colder winter months.

Mean egg weight across the 3 strains changed ($P<0.05$) over time from 57.5 g early in lay to about 63.5 g after 40 weeks in lay, with the ISABrown eggs being about 6 g heavier at all times than those of the other two strains (Figure 2). Some eggs from the ISABrown hens were in excess of 75 g.

As hen-day production declined during the laying period, on average from 89% to 77 %, egg mass production also declined (Figure 3).

The ISABrown hens commenced laying about 2 weeks before the Australian strains and had the highest overall egg-mass production per bird. However, mortalities during the laying period were substantial in the ISABrown (24 %) and in the Hy-line Tint (31%) hens compared with the SIRO-CB (2.2%) hens.

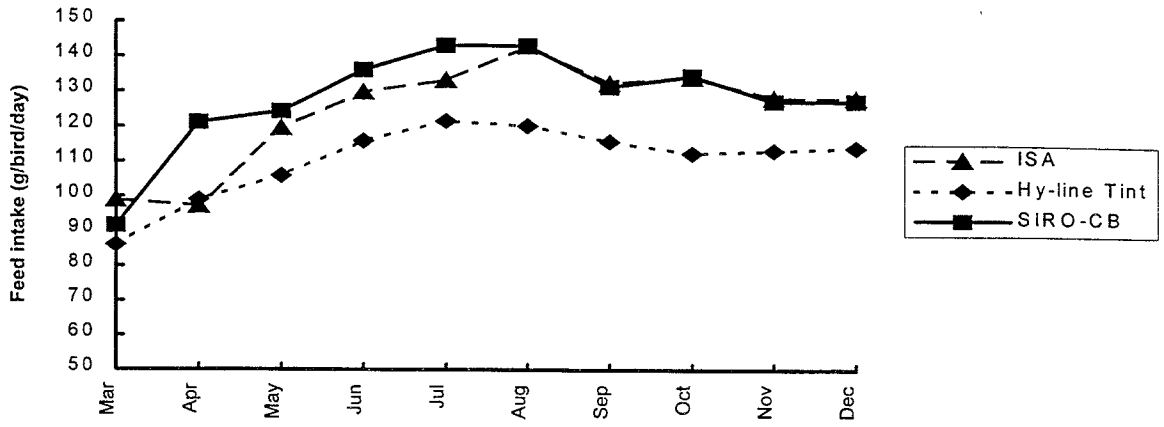


Figure 1. Feed intake of hens from 3 strains over a 40-week period of lay.

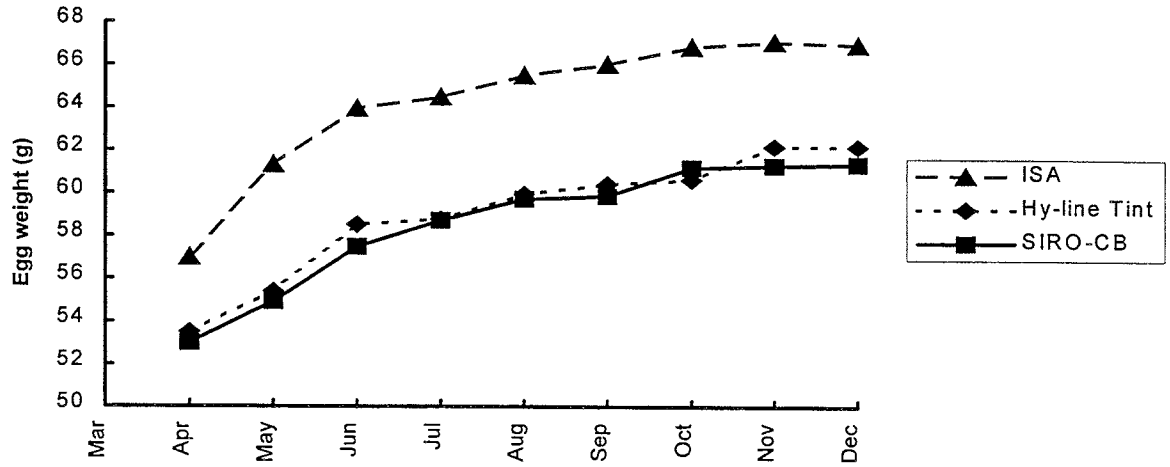


Figure 2. Mean egg weight (g) of hens from 3 strains over a 40-week period of lay.

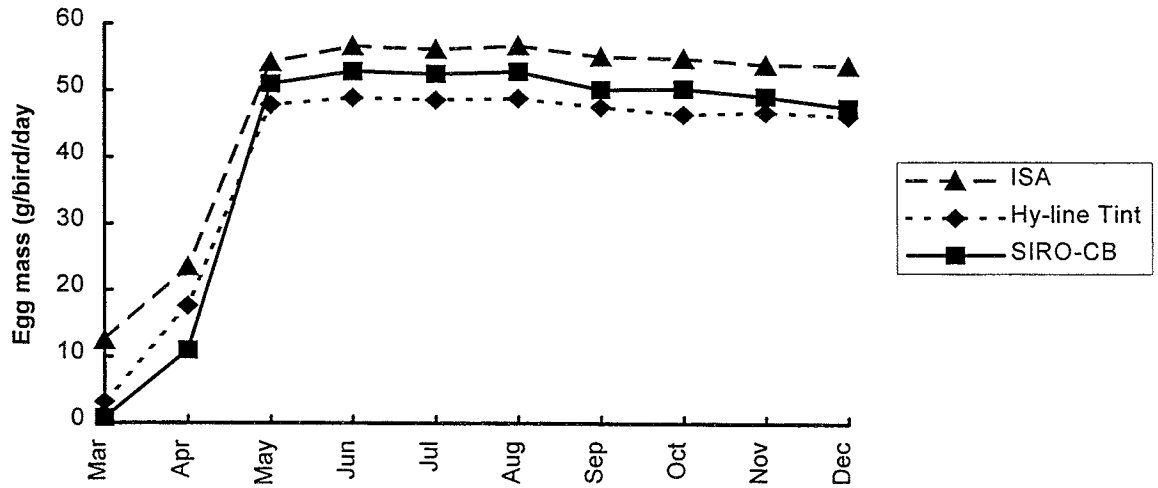


Figure 3. Egg-mass production (g/bird per day) of hens from 3 strains over a 40-week period of lay.

When the mortality was taken into account, hen-housed egg production was highest for the SIRO-CB and ISABrown hens (which did not differ significantly), and was lower ($P < 0.05$) in the Hy-line hens.

Feed conversion ratio (g feed/g egg mass) from the time of peak lay to the end of the trial was higher ($P < 0.001$) for the SIRO-CB hens than for the other two strains which did not differ significantly (Figure 4). However, feed conversion efficiency was reduced in all the strains which were housed in a shed with minimal insulation under cold winter conditions on the northern tablelands of NSW.

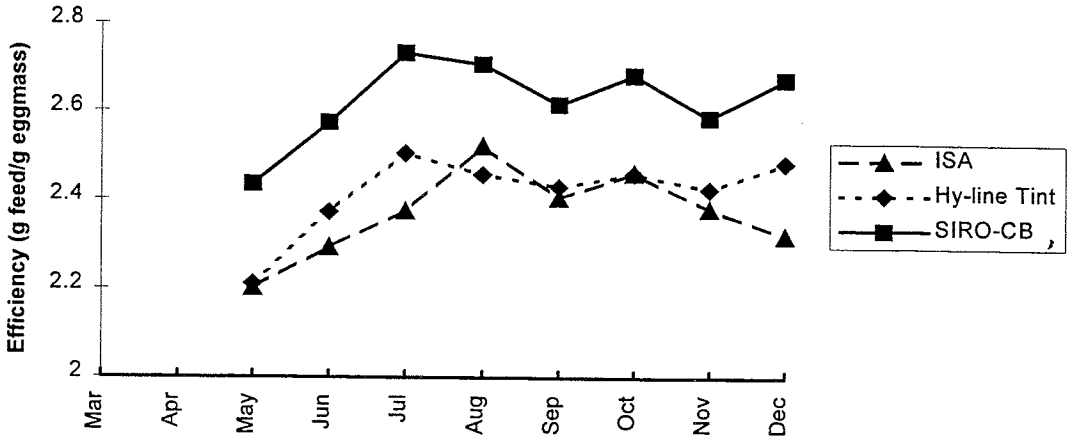


Figure 4. Feed Conversion Ratio of hens from 3 strains over a 40-week period of lay.

However, another important consideration was the overall efficiency of egg mass production from the commencement of lay to the end of the trial. Total feed intakes, total egg mass output and the overall feed efficiencies for the entire 10-month period of the study are given in Table 2. The inherently better feed conversion (Figure 5) and the earlier onset of lay, of the ISABrown hens made them the most efficient of the 3 strains.

Table 2. Production parameters for 10-month period of lay.

| Strain | Feed intake kg | Egg mass kg | Feed conversion g feed/g egg |
|---|---|---|---|
| ISA | 34.76 | 13.41 | 2.59 |
| Hy-line Tint | 30.90 | 11.26 | 2.74 |
| SIRO-CB | 35.75 | 11.73 | 3.05 |

Thus, the imported ISABrown hens had some advantages over the two local strains. Their egg mass output and feed conversion were clearly superior although this advantage was offset by their high mortality and a significant number of oversize eggs. Their high mortality also tended to nullify the advantage of their 2-3 week earlier onset of lay. Moreover, the ISABrown hens proved rather more sensitive to general stresses such as disease and day-to-day disruption which appeared to reduce egg quality, a pattern which has been evident also in a subsequent comparative layer trial at 'Laureldale'. Thus, to capture the potential benefits of this strain in terms of profitability, it is crucial to provide good management aimed at minimising nutritional inadequacies and other management stresses and reducing mortality.

The high mortality in the ISABrown and Hy-line Tint birds greatly reduced hen-housed production and the less-efficient SIRO-CB strain, with its very low mortality, was still commercially competitive.

Poultry farmers from developing nations have raised the query that smaller, higher-producing birds may be inappropriate under their conditions where husbandry practices may be less than optimal and feed formulations inconsistent. The smaller body size of some newer strains is also less desirable where the carcass value of the spent hen is an important consideration for lifetime profitability of birds.

# DUCKWEED AS AN ALTERNATIVE TO SOYABEAN MEAL IN DIETS FOR HIGH-PRODUCING LAYERS

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Summary

Laying hens were given diets in which duckweed replaced soyabean meal at concentrations of 0, 65, 95 and 128 g/kg of the diet. The diets were formulated to standard layer diet specifications but without artificial pigments. Egg size, hen-day production, egg mass output, shell characteristics and feed conversion ratio did not differ between diets. Furthermore, a volunteer tasting panel did not detect any significant difference in the eating quality of the eggs from the hens on any of the diets but strongly preferred the yolk colour of eggs from hens on diets containing duckweed. This study indicates that duckweed has the potential to replace other plant protein sources such as soybean meal in the diets of laying hens without affecting egg production or egg quality.

## I. INTRODUCTION

Duckweed (family *Lemnaceae*) is a small aquatic plant that grows on the surface of lakes and ponds in all parts of the world. There are four genera *Lemma, Spirodela, Wolffia* and *Wolffiella* and about 40 known species. As well as providing an effective means of removing unwanted nutrients from eutrophic waters, duckweed is a potential source of nutritious feed for domestic animals. Under favourable conditions it can double its biomass in less than two days and produce in excess of 30 tonnes of protein-rich dry matter (DM) per hectare annually.

Metabolizable energy (ME) content of duckweed can vary between 5 and 8.4 MJ/kg DM depending on the growing conditions and the type and physiological state of the animal to which it is fed (Haustein *et al.*, 1992). On a DM basis, duckweed usually has a crude protein (CP) content of 300 to 450 g/kg and a lipid content of 40-60 g/kg when grown in nutrient-enriched waters. This makes it comparable to other plant protein sources such as cottonseed meal and soybean meal. Duckweed has higher concentrations of the two essential amino acids, lysine and methionine, than other plant protein sources (Skillicorn *et al.*, 1993).

Duckweed also contains a high level of calcium (Ca) (10-25 g/kg DM; Haustein *et al.*, 1992) and relatively large amounts of xanthophylls and carotene which, when present in layer diets, will give rich yellow/orange yolks in the eggs (Skillicorn *et al.*, 1993). Nutrients such as aluminium, calcium, iron, magnesium, potassium and sodium will be removed from the water in sufficient quantities to eliminate pollution (Zirschy and Reed, 1988). Skillicorn *et al.* (1993) claim that once dried to 10% moisture duckweed can be stored for up to five years in cool dry conditions.

Despite its potential as a protein-rich feed there appear to have been few evaluations of duckweed in diets for laying hens, and none in Australia. The aim of this experiment was to determine the effects on egg production and the shell and eating qualities of eggs when duckweed is substituted for soybean meal in diets for Australian layers.

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## II. MATERIALS AND METHODS

Duckweed (predominantly *Spirodella punctata)* was collected from the Sewage Treatment Works at Scone in January, 1995 and transported to Armidale where it was spread out to dry in a glass-house (daily maximum 40-65 $^{o}$C). The duckweed was placed on tarpaulins on the ground to a depth of 10-30 mm and turned twice daily to facilitate even drying to less than 10% moisture.

Four, pelleted, iso-nitrogenous diets were formulated to commercial layer diet standards (except that neither synthetic yolk pigment nor ground Ca were included in the formulations) with duckweed in place of soyabean meal (Table 1). Calcium was provided *ad libitum* as oyster chips to enable the birds to balance the Ca:P ratio in their diets.

Table 1. The dietary formulations (g/kg).

| Duckweed (g/kg) | 0 | 65 | 95 | 128 |
|---|---|---|---|---|
| Wheat | 527 | 535 | 527 | 522 |
| Barley | 226 | 229 | 226 | 224 |
| Wheat bran | 58.3 | 24.8 | 42.4 | 30.9 |
| Soybean meal | 149 | 108 | 74.2 | 54.3 |
| Duckweed | 0 | 64.6 | 95.3 | 128 |
| Meat meal | 13.8 | 21.5 | 23.3 | 27.7 |
| Vegetable oil | 10.6 | 8.6 | 5.3 | 6.4 |
| Phosphorus | 8.5 | 2.2 | 0 | 0 |
| DL-methionine | 1.1 | 1.1 | 1.1 | 1.1 |
| Lysine monochloride | 1.1 | 0 | 1.1 | 1.1 |
| Sodium chloride | 3.2 | 3.2 | 3.2 | 3.2 |
| Mineral/Vitamin mix | 2.1 | 2.2 | 2.1 | 3.2 |

Forty-eight hens (SIRO-CB), approximately 18 months of age, were held singly in multi-level battery cages in an insulated shed. Artificial lighting was used to maintain day length at 17 h per day. Twelve birds were randomly allocated to each dietary treatment within 12 blocks each containing one bird on each treatment. Each pen had two feed containers, one containing the pelleted feed, the other oyster chips. The feed and Ca were provided *ad libitum*. The hens were fed a commercial layer diet for one week and then changed to their experimental diets. Feed consumed was then measured on a weekly basis for three weeks. Water was available at all times from a trough at the rear of the cage.

Eggs were collected each day late in the afternoon. Special collections of one egg from each hen were made on 4 occasions, i.e. one immediately prior to the experiment, and then three at 7 d intervals, to be used for egg-quality testing by a panel of 12 volunteers. Egg shell colour was estimated using a reflectometer, and resistance to deformation and breaking strength by quasi-static compression. The yolk colour was determined using a Roche Colour Fan. Data were analysed using either a one-way or two-way ANOVA.

## III. RESULTS and DISCUSSION

The CP content of the duckweed as determined by an automated procedure (Leco Corporation, Michigan, USA) was 324 g/kg. The protein content of the diets was also determined by the Dumas procedure and the mineral content by inductively coupled plasma spectrometry. The dietary concentrations of other ingredients were estimated from tabulated analyses (Table 2).

When duckweed replaced soyabean meal in the diets no significant ($P>0.05$) differences between the treatments were found for either total feed intake or oyster shell consumption or in measures of layer performance (Table 3).

The feed conversion ratios (FCR) were typical of those obtained from commercial layers of this age. Egg shell pigmentation, deformation resistance and shell weight did not differ ($P>0.05$) between diets. Overall, the shell breaking strengths were relatively low (22 compared with more typical values of 40 Newtons), presumably because the birds were at a late stage of lay.

Table 2. Concentrations of ME and nutrients in each of the four diets.

| Duckweed (g/kg) | 0 | 65 | 95 | 128 |
|---|---|---|---|---|
| ME (MJ/kg) | 116 | 115 | 116 | 116 |
| Crude protein (g/kg)* | 186 | 175 | 177 | 176 |
| Crude fibre g/kg | 39.0 | 38.9 | 42.3 | 42.6 |
| Total phosphorus g/kg* | 7.1 | 6.6 | 6.7 | 7.5 |
| Methionine (g/kg) | 3.0 | 2.9 | 2.9 | 2.8 |
| Met+Cys (g/kg) | 5.9 | 5.5 | 5.4 | 5.3 |
| Lysine (g/kg) | 7.0 | 6.6 | 7.2 | 7.0 |
| Tryptophan (g/kg) | 2.1 | 1.9 | 1.7 | 1.6 |
| Isoleucine (g/kg) | 6.0 | 5.9 | 5.8 | 5.7 |
| Threonine (g/kg) | 5.2 | 5.1 | 5.0 | 5.0 |
| Linoleic acid (g/kg) | 13.8 | 12.4 | 11.4 | 11.8 |
| Sodium (g/kg)* | 3.48 | 2.49 | 2.10 | 2.42 |
| Sulphur (g/kg)* | 4.33 | 2.30 | 2.42 | 2.62 |
| Potassium (g/kg)* | 15.9 | 8.65 | 9.59 | 10.7 |
| Calcium (g/kg)* | 9.63 | 4.85 | 5.26 | 6.82 |
| Magnesium (g/kg)* | 3.67 | 1.99 | 2.15 | 2.28 |
| Aluminium (g/kg)* | 0.21 | 0.28 | 0.37 | 0.40 |
| Manganese (μg/g)* | 2800 | 1540 | 1950 | 2060 |
| Iron (μg/g)* | 3350 | 3610 | 3720 | 4370 |
| Zinc (μg/g)* | 2360 | 1190 | 1220 | 1360 |
| Copper (μg/g)* | 405 | 224 | 353 | 269 |
| Boron (g/g) | 430 | 688 | 934 | 1295 |

* Determined by analysis (other values calculated using tabulated data).

Table 3. Feed and calcium grit intakes (g/kg), hen-day production (%), egg mass output (g/d) and feed conversion ratio (FCR) for hens on each of the four diets.

| Duckweed (g/kg) | 0 | 65 | 95 | 128 | Mean |
|---|---|---|---|---|---|
| Feed intake (g/d) | 140 | 136 | 146 | 137 | 140 |
| Crude protein intake (g/d) | 24.9 | 22.8 | 24.7 | 23.0 | 23.8 |
| Oyster-shell grit intake (g/d) | 6.0 | 5.7 | 6.4 | 6.3 | 6.1 |
| Calcium intake in pellets(g/d) | 1.29 | 0.631 | 0.736 | 0.893 | 0.888 |
| Calcium intake, total (g/d) | 3.46 | 2.69 | 3.00 | 3.15 | 3.08 |
| Phosphorus intake, total (g/d) | 0.94 | 0.85 | 0.94 | 0.99 | 0.93 |
| Hen-day production (%) | 79.8 | 74.2 | 82.9 | 78.6 | 78.9 |
| Egg-mass output (g/d) | 50.6 | 45.7 | 51.8 | 49.1 | 49.3 |
| FCR (g feed:g egg) | 2.77 | 2.98 | 2.82 | 2.79 | 2.84 |

Odour and taste scores recorded by the taste panel did not differ ($P > 0.05$) between the four treatments. The tasters 'preferred' the darker yolk colour of eggs from hens on the diets containing duckweed to those on the diet based on soyabean. However, tasters were unable to distinguish between the yolk colour of eggs from the duckweed diets and the yolks of locally produced commercial eggs. Interestingly, increases in pigmentation with increasing duckweed in the diet were not linear and further research is needed to determine the minimum levels of duckweed required to give a satisfactory yolk colour. Already in the USA, the use of synthetic colorants in feedstuffs has been restricted, and some of the common synthetic colorants have been banned (Papa *et al.*, 1985). In Australia, certified organic free-range egg producers can use only natural pigments. This will tend to increase the demand for, and value of, natural sources of pigments such as duckweed.

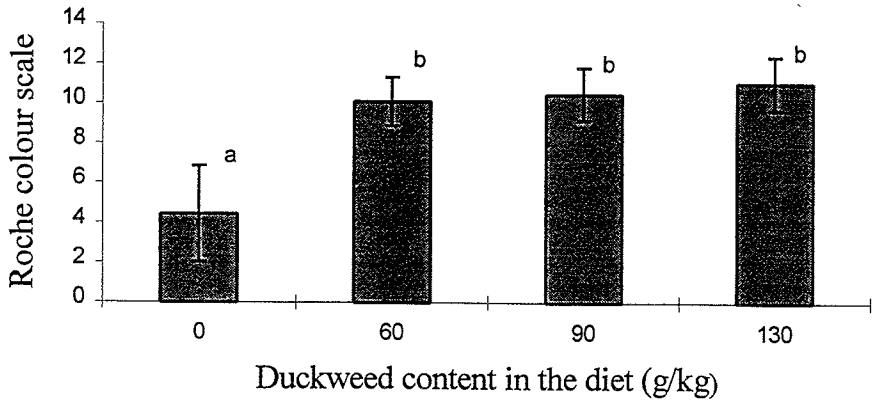


Figure 1. Variation in yolk colour of eggs from hens fed the four diets (Means ±SE).

$^{a-c}$ Bars with different annotations are significantly different at $P < 0.05$.

No decreases in acceptability or food consumption were found when the hens were changed from the commercial layer pellets to the diets containing duckweed. Hens given diets containing up to 128 g/kg of duckweed in place of soyabean meal had similar intakes of feed and granular Ca, and similar levels of egg production and efficiency of feed conversion, to hens on the diet with no duckweed. Moreover, a panel of volunteers did not detect any differences in the odour or taste of hard-boiled eggs from any of the treatments. Overseas, Haustein *et al.* (1990) found that egg production, egg weight and egg quality from hens on diets which included duckweed were equal to, or better than, those given a control diet and a taste panel was unable to differentiate between eggs from birds given diets containing different levels of duckweed.

Duckweed is an excellent source of high-quality protein, but is valuable also for its pigments, lipid, vitamins and minerals.

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# AN EXPERIMENTAL MODEL OF OSTEOPOROSIS IN THE LAYING HEN

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Summary

An experimental model has been established to monitor the progressive onset of osteopenia/osteoporosis in laying hens housed in a controlled environment. A qualitative rib abnormality index and a quantitative femur calcium determination have been used to assess skeletal density. Hens with severely deformed ribs and sternum are significantly lighter in body weight and have significantly lower femur calcium contents. This model will be used to define peak bone mass and to examine the balance of early growth and production, as important determinants of osteopenia/osteoporosis severity.

## I. INTRODUCTION

The development of an age dependent osteopenia or osteoporosis in laying hens is believed to be a normal physiological phenomenon associated with the altering hormonal status of the fowl. The progressive onset of osteoporosis with age is probably analogous to the processes occurring in many other species, including humans, and is characterised by a deficiency in the quantity of fully mineralised structural bone. The development of the osteoporosis in the laying fowl may differ, however, from other species, because of the large demands on bone calcium reserves created by sustained egg production.

The commercial laying hen has been selected for many generations for both high levels of egg production in the first eighteen months of life, and for feed conversion. This selection pressure has produced concomitant reductions in both mature body size and skeletal size. The pool of skeletal calcium available for egg shell formation is, therefore, also likely to have declined. In other words, the output of calcium has increased substantially, and the input of calcium for egg shell formation from both feed and skeletal reserves has declined. These genetic developments imply that the physiological processes which regulate absorption of calcium, and the partitioning of calcium to the skeleton and the shell gland, have increased in efficiency.

The contribution that calcium turnover makes to the development of osteoporosis in the laying fowl is generally accepted, but a precise understanding of the impact that rate of production has on accelerating osteoporosis in the laying hen remains to be described. Comparisons between male and female fowl indicate that females are subject to significant osteoporosis by 45 weeks of age and that osteoporosis is likely to be accelerated in females producing eggs (Wilson *et al.*, 1992; Wilson and Duff, 1991).

The sternum and ribs are frequently deviated in birds with severe osteopenia or osteoporosis and the long bones and vertebrae can be fractured (Riddell, 1989). A reduction in structural bone mass (cortical and trabecular bone) is believed to be responsible for the deformation of the rib cage and the enhanced bone fragility (Wilson *et al.*, 1992).

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The third component of bone, the medullary bone pool, serves as a source of labile calcium for shell formation and provides little mechanical support. The accretion of the medullary bone during follicular maturation in the hen is believed, however, to provide an important means of preventing resorption of the structural cortical and trabecular bone (Thorp *et al.*, 1993; Thorp, 1994). There appears to be limited formation of both trabecular and cortical bone during lay (Rowland and Foutz, 1990; Wilson *et al.*,1992; Thorp *et al.*, 1993), and it seems probable that the peak bone mass will occur at the onset of follicular activity (Thorp, 1994).

A clinical syndrome termed cage layer fatigue has also been recognised in laying fowl. This is characterised by paralysis, bone under mineralisation and elevated flock mortality (Riddell, 1989). The clinical syndrome is believed to be accentuated by precocious onset of egg production and by feeding diets marginally adequate in phosphorus or calcium. The disease is more common in summer-reared pullets which are producing at high levels with superior feed conversion (Grumbles, 1959). This suggests that lighter body weight birds with low feed intakes and high rates of production are more susceptible to cage layer fatigue.

The pathological symptoms of cage layer fatigue are similar to osteoporosis, and it is clear that the skeletal mineral reserves are significantly depleted in both conditions. Cage layer fatigue is normally diagnosed in younger laying flocks at or near peak egg production, whilst osteoporosis is normally described in aged hens. Given the similarity of the two conditions it is reasonable to hypothesise that there may be sub-clinical forms of cage layer fatigue largely unrecognised, and that this disease syndrome may be the precursor to the development of osteoporosis in older hens.

Clearly, the balance of growth rate, skeletal development and egg production are critical elements in the determination of the peak bone mass which, in turn, will determine the susceptibility of commercial laying hens to the development of the age-dependent osteoporosis. In an attempt to more systematically study this relationship a laboratory model was established in a controlled environment shed using commercially prepared layer feeds and brown egg layer pullets. The model was established to study the progressive onset of osteoporosis and to develop rapid methods for defining bone porosity and skeletal calcium reserves.

## II. MATERIALS AND METHODS

### (a) Qualitative Assessment of Osteoporosis

A flock of 240 brown egg layers (Australorp x New Hampshire) were housed at 18 weeks of age in a controlled environment shed and fed a commercially prepared layer ration (crude protein 165 g/kg, metabolizable energy 11.5 MJ/kg, methionine 3.4 g/kg, total lysine 8.1 g/kg, calcium 37.5 g/k g and phosphorus 8 g/kg ). The flock were housed in 4 bird cages with five replicates of 48 birds. The body weight and egg production were monitored continuously between 20 and 72 weeks of age. At 40 and 72 weeks of age, 20 and 50 birds, respectively, were randomly subsampled and assessed for deformation of the costochondral junction in the rib cage ( Grumbles, 1959). The incidence of rib abnormalities was calculated as a proportion of the sample and a severity index score developed to define the severity of the skeletal deformity. The score gave a ranking of 0 for normal ribs through to 5 for a grossly deformed costochondral junction with deformation of the rib cage.

(b) Assessment of Femur Calcium Contents

Seventy seven femurs were collected from 70-75 week old hens from a number of flocks of the same strain (Australorp x New Hampshire) and the total calcium content of the femur was related to bird body weight and the severity of the rib abnormality index (0-5).

## III. RESULTS

By 72 weeks of age the flock had produced 275 eggs on a mean hen-housed basis. The flock achieved a peak egg production of 94% at about 30 weeks of age, but persistency of production deteriorated substantially after 40 weeks of age. The flock growth rate slowed substantially between 24 and 30 weeks of age and the body weight of the flock was below accepted breed standards by approximately 10-15% at 72 weeks of age (Parkinson and Almond, 1994).

Skeletal assessments made at 40 weeks of age indicated that the flock had an incidence of rib abnormalities of 16-20% with a severity index of between 1 and 2. A similar assessment undertaken at 72 weeks of age indicated that the incidence had increased to 70 %. The severity of the rib abnormalities had increased markedly with 10% of the hens having a severity index between 3 and 4 and 60 % of the hens an index between 1 and 2.

The analysis of the femur calcium content (Y) from 77 hens of the same strain illustrated a significant correlation ($P < 0.05$ ) with body weight (X). The relationship was described by the equation $Y=-0.133 + (0.625*X)$ $R=0.498$ (Figure1).

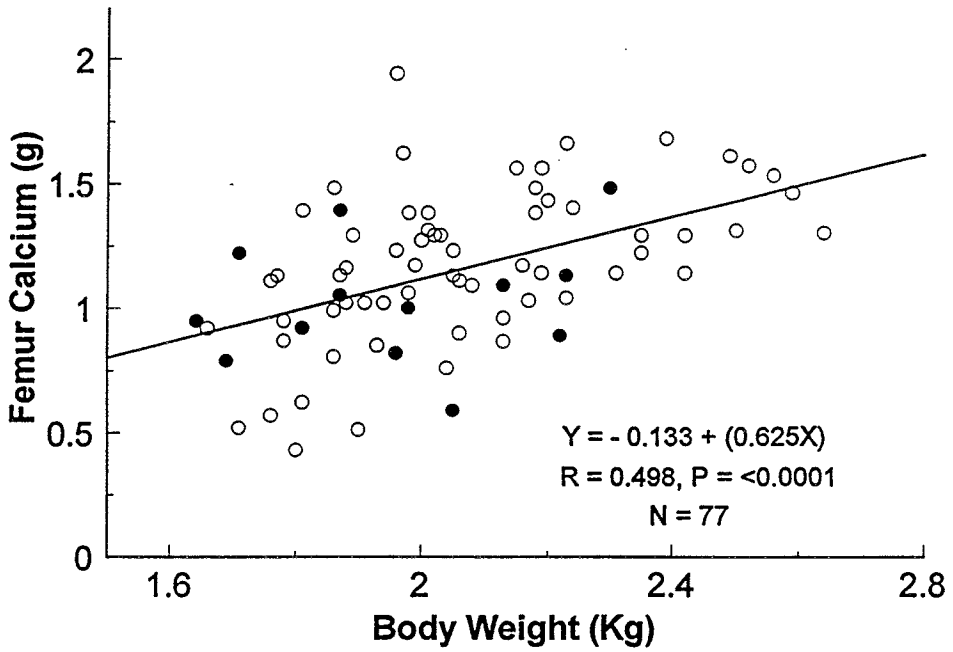


Figure 1. Relationship between body weight (kg) and femur calcium content (g) for a brown egg laying strain. (•) birds severely affected with rib abnormalities (Score 4-5). All birds were between 70 and 75 weeks of age.

The average body weight of the birds without rib abnormalities was 2.10 ± 0.06 kg whilst the birds with severe rib abnormalities had a body weight of 1.94 ± 0.04 kg ($P < 0.05$) (Figure 1). The femur calcium content was also significantly ($P < 0.05$) lower in the birds with severe rib abnormalities when compared to birds with either mild or no abnormalities. The birds with rib abnormality scores between 4 and 5 had a femur calcium content of 0.97 ± 0.05 g while the birds without rib abnormalities had a femur calcium content of 1.32 ± 0.07 g. The birds without rib abnormalities had a femur calcium content of 6.3 g/kg body weight while the birds with severe rib abnormalities had a lower concentration of 5.0 g/kg body weight. The femur calcium contents recorded in the smaller birds in this study were similar to those recorded previously in 75 week old White Leghorn hens ( Buss and Guyer, 1984).

## IV. DISCUSSION

The model demonstrated the relatively early onset of osteoporosis in the hens at 40 weeks of age. The slow growth and reduced body size in early egg production may play some role in influencing the onset of clinical osteoporosis, particularly since peak bone mass is likely to be achieved by 20 to 26 weeks of age (Thorp *et al.,* 1993; Thorp, 1994). The marked increase in severity of the osteoporosis by 72 weeks of age must be influenced by the age-dependent osteopenia, but a low peak bone mass achieved in early egg production will inevitably accelerate the severity of the disease in aged hens. The rib abnormality incidence and severity score provide an easy and rapid method for monitoring osteoporosis.

The analysis of a large subsample of laying hens for body weight, osteoporosis severity score and femur calcium, indicated that smaller birds are more likely to have severe osteoporosis, and that hens with severe osteoporosis (rib deformation score 4-5) have approximately 27% less femur calcium than hens without clinical osteoporosis (rib deformation score 0). Smaller hens with less skeletal calcium may be more susceptible to the depletion of skeletal calcium during sustained egg production and may be more susceptible to the development of osteoporosis. Alternatively, the hens which developed severe osteoporosis may experience body weight loss as a consequence of the severity of the clinical disease. Irrespective of the exact explanation the relationship between rib abnormality score and femur calcium indicates that calcium depletion is important in the development of osteoporosis in the laying hen.

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# THE EFFECT OF INCLUSION OF FOUR DIFFERENT GRAIN LEGUMES IN LAYER DIETS ON BIRD PERFORMANCE

R.A. PEREZ-MALDONADO*, P.F. MANNION*, R.J. JOHNSON**
and D.J. FARRELL*

Grain legumes are being included as protein sources in layer diets with increasing frequency but often at low levels since some contain antinutritional factors. This paper reports the first 12 weeks of a layer experiment in which four different grain legumes seeds were included at 250 g/kg in least-cost formulated isonitrogenous and isoenergetic diets. Each diet was fed to pairs of Siro-CB hens in 50 cages commencing at 25 weeks of age.

Mean values (± SEM) for production parameters between 25 and 37 weeks of age and the variety and crude protein content of the legume seeds.

| Diet | Variety | Crude protein (g/kg DM) | Hen-day production (%) | Egg weight (g) | Egg mass (g/d) | Feed intake (g/d) |
|---|---|---|---|---|---|---|
| Chick peas | Amethyst | 179 | 91.07$^{ab}$ (0.978) | 52.07$^{a}$ (0.459) | 47.735$^{a}$ (0.619) | 116.8$^{a}$ (1.06) |
| Field peas | Alma | 217 | 92.05$^{a}$ (0.659) | 52.19$^{a}$ (0.454) | 47.928$^{a}$ (0.466) | 118.3$^{a}$ (1.06) |
| Sweet lupins | Gungurru | 328 | 91.78$^{a}$ (0.711) | 51.83$^{a}$ (0.452) | 47.87$^{a}$ (0.484) | 122.7$^{b}$ (0.99) |
| Faba beans | Fiord | 245 | 89.27$^{b}$ (0.746) | 49.83$^{b}$ (0.433) | 44.889$^{b}$ (0.455) | 116.5$^{a}$ (0.99) |
| LSD (P=0.05) | | | 2.182 | 1.196 | 1.4213 | 2.954 |

* Values without a common superscript are significantly different at $P<0.05$.

The apparent metabolizable energies of the grain legumes (MJ/kg DM) were 10.61, 8.61, 10.57 and 11.08 for chick peas, sweet lupins, faba beans and field peas respectively. With the exception of the value for sweet lupins which is low the others agree with values in feed composition tables.

Overall mortality was 1.8%; on the chick pea diets it was 3%. However, spare birds fed the experimental diets were used to replace those that died. Mean hen-day production was satisfactory. Food intake was highest ($P<0.05$) on the sweet lupin-based diet. Faba beans failed to support the levels of production seen with the other grain legumes. Egg weight and egg mass were reduced ($P<0.05$) while hen-day egg production was lowest and significantly ($P<0.05$) less than on the sweet lupin diet. Faba beans are often used in dietary formulations as an interchangeable protein source with field peas but can often give depressed performance, particularly with broilers. There are several reports in the literature of antinutritional factors in faba beans.. The three other grain legumes supported excellent production. Measurement of excreta moisture gave similar values for the four diets.

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# STUDIES ON THE STABILITY OF ß-GLUCANASE AND XYLANASE ACTIVITIES OF *Trichoderma reesei* DURING FEED PELLETING

J. T. PIIRONEN

Summary

A series of pelleting studies was carried out under practical conditions to investigate the sensitivity to steam pelleting of ß-glucanase and xylanase activities from *Trichoderma reesei.* The enzyme preparations were formulated using a spray-drier or a mixer granulator. The enzyme supplemented feeds were pelleted at different temperatures and analysed for ß-glucanase and xylanase activities using dyed substrates. The results showed that ß-glucanase activity was more sensitive to steam addition than that of xylanase. By increasing the particle size, alone or especially in combination with coating, the activity recoveries were increased.

## I. INTRODUCTION

The fungus, *Trichoderma reesei,* is one of the most widely used organisms in the production of ß-glucanases and xylanases for feed applications. The enzymes are mesophilic representing a compromise between requirements to act at body temperature and to resist high temperatures during feed pelleting. However, there is a trend towards higher pelleting temperatures to eliminate salmonella contamination of feeds and this increases the need to improve the high temperature stability of enzyme preparations. The problem can be minimised by formulating dry products or by spraying feeds with liquid enzymes.

The aim of this work was to investigate the influence of different enzyme formulations on the stability of ß-glucanase and xylanase activities of *Trichoderma reesei* during feed pelleting.

## II. METHODS

A series of pelleting stability measurements was carried out under practical conditions at a feed factory in Finland.

Two commercial enzyme preparations from *Trichoderma reesei*, ECONASE Barley P as a source of ß-glucanase activity and ECONASE Wheat P as a source of ß-glucanase and endo-xylanase activities (Primalco Ltd Biotec, Finland), were formulated using a spray-drier or a high-intensity mixer granulator.

The granulation was performed using wheat flour as a filler with or without polyethylene glycol (PEG) as a coating agent. The spray-dried (instantized powder) and granulated products had a particle diameter of 45-300 and 100-900 $\mu$m, respectively. The manufactured feed was a commercial ruminant feed (Feedmix Oy, Finland) with pellet diameter of 4.5 mm. The ruminant feed was used as a starting material since it did not contain supplemental enzyme activities.

Primalco Ltd Biotec, FIN-05200 Rajamäki, Finland.

The enzyme preparations were added at a level of 600 g to each of eight batches of 1000 - 1250 kg each. Five feed samples were taken from the feed flow to measure activity levels prior to pelleting. A mean activity of these was used as an initial value.

The pelleting temperature was measured immediately after the pelleting machine by taking a sample into a thermos flask and at the same time onto a piece of paper for activity measurements. The temperature was registered as soon the value remained stable. Since enzymes are known to be particularly sensitive to moist heat the steam valve values were also recorded. Enzyme activities were measured on the feed samples using dyed substrates from Megazyme, Australia. All assays were performed in triplicate.

## III. RESULTS AND DISCUSSION

Steam addition had a marked effect on the ß-glucanase and xylanase activity recoveries. When feeds were treated with steam, instantized preparations showed lowest recoveries with a critical point at about 73°C after which a reduction in enzyme activities took place. Without steam addition the xylanase and ß-glucanase activity recoveries in the same experiment at 79°C were 90 and 67%, respectively (Figure 1).

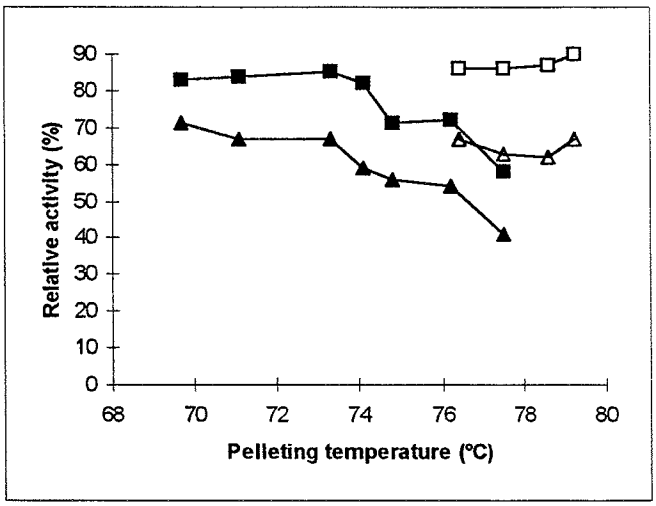


Figure 1. The influence of pelleting temperature and steam addition on ß-glucanase and xylanase activity recoveries. ß-Glucanase: ▲ with steam; △ without steam. Xylanase: ■ with steam; □ without steam.

Stability of the activities was improved when preparations were granulated or granulated and coated (Figure 2). This can be explained by a reduced surface area and the coating layer which both reduce the access of steam to the enzyme preparation. The relatively small difference in stability between granulates and coated granulates may indicate that the amount of the coating agent was too low.

Xylanase activity recovery was on average 20 percent units higher than that of ß-glucanase and the difference was not dependent on the formulation used. However, the ß-glucanase activity recovery may be underestimated since it has been reported to be tightly bound to its substrate, especially at high temperatures (Poulsen, 1994). Therefore, pelleting

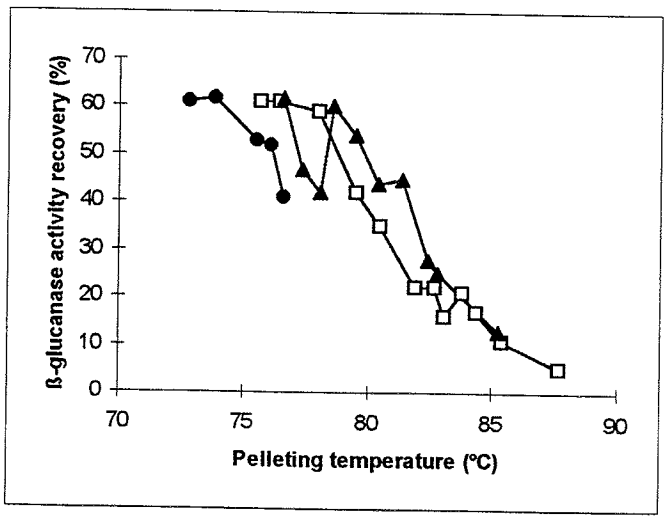


Figure 2. The effect of formulation on the ß-glucanase activity recovery. Formulations: ● instantized; □ granulated; ▲ granulated and coated.

stability studies along with performance trials are needed to demonstrate the actual stability during steam pelleting.

A drawback from the increased particle size was that it tended to increase the deviation between parallel samples which made activity analysis more difficult to carry out. Within experiments the deviation can be reduced by using higher inclusion levels and by increasing the sample size used in the assay. Unfortunately, the former is not possible in normal practice . In this experiment the enzyme inclusion levels were ten times higher than the recommended levels.

This study demonstrated that granulation, especially in combination with coating, improved the stability of enzyme activities during steam pelleting. However, with temperatures above 78°C spraying of liquid enzymes onto feed pellets is recommended so as to avoid major losses in activity and, thus, reduced animal performance.

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# RESPONSE TO SELECTION FOR BREAST MEAT YIELD IN JAPANESE QUAIL USING ULTRASOUND

B. POPOVIC and R.A.E. PYM

Breast meat commands a high price relative to other cuts of chicken, which provides an incentive to increase the proportion of breast meat in the carcass. The only feasible way of achieving this is through genetic selection given an accurate and objective measure of breast meat yield in the live bird. Such a measure has been described using data from Japanese quail (Popovic and Pym, 1995) where information on liveweight (LWT), and breast muscle depth (BD), length (BL) and width (BW) were incorporated in a multiple regression equation predicting breast meat yield. Breast muscle depth was measured using real-time ultrasound on the live bird. The present study follows on from this earlier work and describes the responses after two generations of selection for aspects of breast meat yield.

There were five lines of Japanese quail selected for: increased 42d liveweight (line LWI); increased breast meat weight (line BWI); increased breast meat proportion (line BPI); decreased breast meat proportion (BPD); or at random (line C). The lines were derived from the same base population and were each generated from matings between 12 males and 36 females, selected in subsequent generations from approximately 360 birds. Selection indices for the breast yield-selected lines were derived from prediction equations determined in a series of studies. They took the following approximate form:

Line BWI 0.04 LWT + 0.30 BL + 0.80 BD + 0.25 BW

Line BPI and BPD -0.05 LWT + 0.30 BL + 0.90 BD + 0.25 BW

Line averages (±SE) for 42d liveweight (g), breast weight (g) and breast proportion (g/kg liveweight) after two generations of selection in the five lines are shown in the Table.

| Line | 42d Liveweight (g) | Breast weight (g) | Breast proportion (g/kg) |
|---|---|---|---|
| LWI | 248 (2.3) | - | - |
| BWI | 255 (2.5) | 43.9 (0.65) | 173 (2.4) |
| BPI | 236 (2.5) | 45.0 (0.87) | 192 (2.9) |
| BPD | 228 (1.8) | 38.5 (0.48) | 170 (2.0) |
| C | 231 (2.4) | 40.8 (0.85) | 177 (3.1) |

Selection for increased breast weight, whilst undoubtedly effective in improving the selected trait, did not result in an increase in the proportion of breast meat. Selection for increased breast proportion, however, resulted not only in a considerable direct positive response in the selected trait but also in a greater increase in the weight of breast meat than in the BWI line itself. Selection for breast muscle proportion, either up or down, had no apparent effect on liveweight.

Whilst preliminary, these results suggest considerable gains can be made in breast meat yield from selection.

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# TRYPTOPHAN CONTENT OF AUSTRALIAN FEEDSTUFFS

G.RAVINDRAN and W.L.BRYDEN

Tryptophan, unlike most other amino acids, undergoes degradation during acid hydrolysis and, therefore, has to be determined separately. A multitude of methods have been published on tryptophan analysis, involving modifications in hydrolysis (broadly alkaline or enzymatic) and analytical (chromatographic, colorimetric or fluorimetric) conditions (Friedman and Cuq, 1988). A method for the analysis of the tryptophan content of feedstuffs employing alkaline hydrolysis followed by quantitation of tryptophan in the hydrolysates by ion-exchange chromatography has been developed and tested on lysozyme (Sapphire BioScience, Alexandria, NSW) and a range of Australian feedstuffs. Some preliminary results are presented in this paper.

This procedure is based on hydrolysis with sodium hydroxide in teflon containers under an atmosphere of nitrogen. Samples containing 40-50 mg protein were dispersed in 10 mL of 4.2 M sodium hydroxide containing 0.15 mM 5-methyl tryptophan as an internal standard. At this time the internal standard was also added to the standard tryptophan. Soluble starch (20 mg) was added to samples containing little or no starch as a protective agent. A drop of 2-octanol was added to prevent frothing. The medium was then sonicated, flushed with nitrogen, cooled, evacuated and purged with nitrogen. The process was repeated until all the air was removed from the samples. The samples were then hydrolysed at 120° C for 15 h, cooled, acidified to a pH of 6.5 with HCl, diluted to 50 mL with sodium citrate buffer of pH 6.5 and filtered through a 0.2 mm nylon 66 filter membrane.

Tryptophan and 5-methyl tryptophan was separated by high performance liquid chromatography on a sodium cation exchange column using a Shimadzu amino acid analysis system. Aliquots of the sample hydrolysates or standard mixtures of tryptophan and 5-methyl trypytophan were injected onto the column and eluted isocratically with sodium citrate buffer of pH 9.3 at a flow rate of 0.5 mL/min and a column temperature of 65° C. O-phthalaldehyde was used for postcolumn derivatization and fluorimetric detection of amino acids. Separation was accomplished in 20 min without any interference from other compounds in the hydrolysate.

The recovery of tryptophan from lysozyme was greater than 95% and this procedure has proved useful for the routine analysis of most feedstuffs. The tryptophan contents (g/kg air dry basis) of the following feedstuffs were determined to be: wheat, 1.22; soybean meal, 6.24; canola meal, 4.72; cottonseed meal, 5.01; sunflower meal, 3.14; lupin (*Lupinus angustifolicus*), 2.70; blood meal, 13.9; feather meal, 4.67 and casein, 10.3. These values compare well with those reported in the literature (National Research Council, 1994).

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# THE EFFECT OF AN INDUCED MOULT ON EGG SHELL QUALITY IN THREE STRAINS OF LAYING HENS

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Summary

Egg and egg shell quality were assessed in three strains of laying hen, Isa Brown, Hy-Line Tinted and SIRO-CB before an induced moult, as the birds were going out of production and following an induced moult. There were strain differences in egg and egg shell characteristics prior to the moult. All strains showed a deterioration in egg shell quality as birds were going out of production at the start of the induced moult. Egg shell quality improved following the moult, as evidenced by a significant increase in egg shell breaking strength.

## I. INTRODUCTION

The management practice of induced moulting has been shown to improve egg shell quality and egg production in an ageing flock (Karunajeewa *et al.*, 1989). A number of methods may be employed to achieve the moult (Daniel and Balnave, 1980; Abu-Serewa and Karunajeewa, 1985). The post-moult increase in egg production has been attributed to a decrease in the production of shell-less eggs (Roland and Bushong, 1978; Roland and Brake, 1982). A reduction in the incidence of shell defects such as pimpling (Roland and Bushong, 1979; Hess and Briton, 1988) and improved egg shell colour (Karunajeewa *et al.*, 1989) also have been reported as the result of an induced moult. The mechanisms by which an induced moult improves egg shell quality are not completely understood. However, it appears that best results are achieved if there is a complete cessation of lay for 4-8 weeks, a loss of approximately 50% of primary feathers and a 27-31% loss of body weight (Baker *et al.*, 1983; Brake, 1993). Recently, research has been conducted in Australia using a range of layer strains and different numbers of rest periods varying from none to three (Robinson *et al.*, 1995). These studies have found that the effects of induced pauses vary between strains and depend on the number of cycles throughout the lay period.

In the present study, egg shell quality was examined in three strains of layers prior to an induced moult, during the moult (as birds were going out of production), and at intervals after the birds resumed production.

## II. MATERIALS AND METHODS

Twenty birds of each of three strains, Isa Brown, Hy-Line Tinted and SIRO-CB were housed in individual cages in a layer shed at the University of New England's "Laureldale" farm. Moult was induced according to the commercial "Code of Welfare" and resulted in a reduction in egg production within 48 hours. All birds had gone out of lay within 8 days and did not resume lay until 2-3 weeks later. Birds were monitored to assess the effect of the moult on egg shell quality. All eggs laid by the birds were collected for one week prior to the induction of moult (Period 1), for the 8 days as the birds were going out of production (Period 2), for the first two weeks after birds came back into production (Period 3), and then after a further one month (Period 4) and two months

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(Period 5) had elapsed. Egg weight, gross egg shell defects, egg shell pigmentation (by reflectivity), egg specific gravity (by Archimedes Principle), egg length and breadth, egg shell breaking strength (by quasi-static compression), shell weight and shell thickness (using a dial comparator gauge). Shape index (breadth/length) and percentage shell (shell weight/egg weight) were calculated. Data were analysed by two factor ANOVA. Fisher's (Protected) Least Significance Difference test was used to determine differences between means. Significance was assumed at $P<0.05$.

## III. RESULTS

The results of the experiment are summarised in Table 1. Prior to the induced moult, the Isa Brown hens laid the largest eggs with the highest specific gravity, the heaviest and thickest shells, and the greatest shell weight to egg weight ratios (% shell). However, the percentage production tended to be highest in the Hy-Line tinted birds and the eggs of the SIRO-CB birds produced the shells with the greatest breaking strength. The shape index was lowest in the Hy-Line tinted birds, indicating that the eggs from this strain were narrower in relation to their length. As would be expected, the percentage reflectivity was consistently highest for the white egg shells of the Hy-Line tint birds and lowest for the dark brown shells of the Isa Brown birds.

The induced moult caused all three strains of birds to cease production and remain out of production for periods of from 16 to 19 days. Percentage production and egg weight were reduced in all strains for the eggs produced as the birds were going out of production (Period 2 of the experiment). Egg shell quality was also lower during Period 2 with lower specific gravity, shell weight, shell thickness, percentage shell and shell breaking strength. Percentage reflectivity of the brown eggs laid by the Isa Brown and SIRO-CB birds increased during this phase, indicating that these birds were producing egg shells which were lighter in colour.

Following the moult, percentage production returned to pre-moult levels in the Hy-Line birds and was increased in the Isa Brown and SIRO-CB birds. However, percentage production had declined by Period 5 (2 months after the birds had resumed production). Egg weight returned to pre-moult levels in the Isa birds but was increased for the Hy-Line and SIRO-CB birds. Following the moult, egg specific gravity, shell weight, shell thickness and percentage shell were the same as they had been prior to the moult for all three strains. However, egg shell breaking strength was significantly improved in all strains. The shape index for the shells from the Isa Brown birds increased immediately after the birds resumed production but was at pre-moult levels during Periods 4 and 5. The incidence of some superficial shell defects (pimpled, wrinkled, speckled egg shells) was higher following the moult in all strains.

## IV. DISCUSSION

The Isa Brown hens laid the biggest eggs with the heaviest and thickest shells and the greatest shell weight to egg weight ratios (% shell). Egg specific gravity was also greatest in this strain. The shells of the Isa Brown strain had the darkest colour (the lowest % reflectivity). However, the shell breaking strength of the Isa Brown birds was intermediate between the other two strains with the SIRO-CB birds having the strongest shells. All three strains laid smaller eggs with poorer shell quality as they were going out of production at the start of the induced moult.

Table 1. Effect of induced moult on egg and egg shell quality in three strains of laying hen (Mean ±SEM).

| | Strain | Period | | | | | Strain | Period | Strain* Period |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | | | |
| Egg Weight g | Isa | **67.4** ±0.4 | **65.7** ±0.5 | **68.5** ±0.3 | **67.3** ±0.4 | **67.6** ±0.8 | <.0001 | <.0001 | .0207 |
| | Hy-Line | **61.7** ±0.5 | **60.8** ±0.6 | **65.1** ±0.3 | **63.8** ±0.4 | **64.4** ±0.7 | | | |
| | SIRO-CB | **62.7** ±0.4 | **59.7** ±1.0 | **63.6** ±0.3 | **63.4** ±0.4 | **64.3** ±0.6 | | | |
| Shell Weight g | Isa | **6.18** ±0.05 | **5.19** ±0.11 | **6.23** ±0.04 | **6.10** ±0.0739 | **6.13** ±0.09 | <.0001 | <.0001 | .0462 |
| | Hy-Line | **5.38** ±0.04 | **4.55** ±0.11 | **5.72** ±0.03 | **5.70** ±0.05 | **5.57** ±0.08 | | | |
| | SIRO-CB | **5.41** ±0.05 | **4.66** ±0.09 | **5.69** ±0.04 | **5.58** ±0.06 | **5.47** ±0.10 | | | |
| Shell Thickness µm | Isa | **391.9** ±2.8 | **341.7** ±5.8 | **389.4** ±2.0 | **390.4** ±3.9 | **386.0** ±4.2 | <.0001 | <.0001 | NS |
| | Hy-Line | **360.9** ±2.6 | **313.2** ±5.8 | **362.2** ±1.9 | **369.0** ±2.5 | **359.2** ±3.6 | | | |
| | SIRO-CB | **356.4** ±2.7 | **318.6** ±5.2 | **364.5** ±1.6 | **360.6** ±2.5 | **353.9** ±4.7 | | | |
| Reflectivity % | Isa | **65.4** ±0.4 | **67.9** ±0.5 | **65.6** ±0.3 | **65.9** ±0.4 | **64.9** ±0.6 | <.0001 | <.0001 | .0041 |
| | Hy-Line | **79.0** ±0.2 | **78.9** ±0.3 | **79.0** ±0.1 | **78.3** ±0.2 | **78.5** ±0.3 | | | |
| | SIRO-CB | **72.5** ±0.2 | **73.9** ±0.3 | **72.3** ±0.2 | **72.1** ±0.2 | **71.8** ±0.4 | | | |
| Breaking Strength Newtons | Isa | **24.3** ±0.7 | **22.3** ±1.1 | **26.4** ±0.5 | **26.3** ±0.8 | **26.7** ±1.0 | .0347 | <.0001 | NS |
| | Hy-Line | **23.9** ±0.7 | **21.7** ±1.3 | **25.3** ±0.5 | **26.8** ±0.8 | **26.3** ±0.9 | | | |
| | SIRO-CB | **24.9** ±0.6 | **22.0** ±1.0 | **28.2** ±0.4 | **28.8** ±0.7 | **27.0** ±1.1 | | | |
| Specific Gravity | Isa | **1.084** ±0.001 | **1.076** ±0.001 | **1.084** ±0.001 | **1.082** ±0.001 | **1.083** ±0.002 | .0007 | <.0001 | NS |
| | Hy-Line | **1.081** ±0.001 | **1.072** ±0.001 | **1.082** ±0.000 | **1.082** ±0.001 | **1.083** ±0.001 | | | |
| | SIRO-CB | **1.080** ±0.001 | **1.072** ±0.001 | **1.084** ±0.000 | **1.081** ±0.001 | **1.082** ±0.002 | | | |
| Shape Index % | Isa | **75.1** ±0.2 | **74.3** ±0.3 | **76.2** ±0.2 | **75.0** ±0.3 | **74.6** ±0.4 | <.0001 | .0149 | <.0001 |
| | White | **73.9** ±0.3 | **73.5** ±0.3 | **74.0** ±0.2 | **74.1** ±0.2 | **74.0** ±0.4 | | | |
| | SIRO-CB | **75.7** ±0.3 | **75.7** ±0.4 | **75.3** ±0.2 | **76.0** ±0.3 | **75.0** ±0.4 | | | |
| % Shell | Isa | **9.21** ±0.06 | **7.92** ±0.16 | **9.09** ±0.05 | **9.07** ±0.10 | **9.07** ±0.12 | <.0001 | <.0001 | .0090 |
| | Hy-Line | **8.69** ±0.07 | **7.47** ±0.15 | **8.83** ±0.04 | **8.94** ±0.06 | **8.66** ±0.09 | | | |
| | SIRO-CB | **8.63** ±0.07 | **7.75** ±0.13 | **8.92** ±0.04 | **8.79** ±0.06 | **8.51** ±0.13 | | | |
| Percent Production | Isa | **82.5** ±7.6 | **41.9** ±10.2 | **86.1** ±2.4 | **82.9** ±1.5 | **75.0** ±8.7 | NS | <.0001 | NS |
| | Hy-Line | **92.5** ±5.7 | **42.8** ±11.3 | **91.8** ±2.9 | **86.4** ±4.9 | **73.3** ±4.4 | | | |
| | SIRO-CB | **80.0** ±4.8 | **44.2** ±9.4 | **85.0** ±4.8 | **77.1** ±1.8 | **78.3** ±1.7 | | | |

Egg weight increased following the moult in the Hy-Line and SIRO-CB birds but was not significantly different for the Isa strain. The greatest initial improvement in shell weight as the result of the moult was for the Hy-Line birds. All strains showed significant improvement in egg shell breaking strength, following the moult. In contrast with previously reported findings, (Roland and Bushong, 1979; Hess and Briton, 1988; Karunajeewa *et al.*, 1989), shell colour was not significantly changed by the process of moulting and the incidence of some shell defects such as pimpling increased.

An induced moult can improve some aspects of egg shell quality. However, this advantage needs to be assessed in relation to the cost of having the birds out of full production for 1-2 months.

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# SEQUENCE ANALYSIS OF THE S1 GLYCOPROTEIN OF AUSTRALIAN STRAINS OF INFECTIOUS BRONCHITIS VIRUS

S.I. SAPATS*, P.J. WRIGHT** and J. IGNJATOVIC*

Summary

Nine Australian strains of infectious bronchitis virus (IBV) have previously been divided into two genotypic groups (I and II) based upon the level of identity shown among their S1 genes. Alignment of the deduced amino acid sequences of the S1 genes of the group I strains identified two hypervariable regions between residues 52-67 and 117-146. In contrast, group II strains displayed variability throughout the entire S1 glycoprotein. Close examination of the sequences revealed that both point mutations and, possibly, genomic RNA-RNA recombination appear to have contributed to the evolution of Australian strains.

## I. INTRODUCTION

Infectious bronchitis virus causes a highly contagious disease of chickens resulting in significant economic losses to poultry industries throughout the world. The IBV genome is a single strand of positive sense RNA, 27.6 kb in length (Boursnell *et al.*, 1987). It encodes three structural proteins, the spike glycoprotein (S), the membrane glycoprotein and the phosphorylated nucleocapsid protein. The S glycoprotein consists of two subunits S1 (520 amino acids) and S2 (625 amino acids).

The S1 glycoprotein induces both neutralizing and haemagglutination inhibiting antibodies, and is the major antigen involved in protection (Cavanagh *et al.*, 1984, 1986; Ignjatovic and Galli, 1994). Sequence comparisons of the S1 glycoprotein of strains isolated in the USA, Europe and Japan have indicated that most amino acid substitutions occur within the first 365 residues of the S1 glycoprotein (Kusters *et al.*, 1989; Cavanagh *et al.*, 1992). In particular, two hypervariable regions (HVR1 and HVR2) have been identified within the S1 glycoprotein of strains belonging to the Mass serotype comprising amino acid residues 56-69 and 117-131, respectively (Niesters *et al.*, 1986). These HVRs have been shown to contain epitopes responsible for inducing neutralizing antibodies (Cavanagh *et al.*, 1988).

A major problem associated with IBV is the continuing emergence of variant strains of IBV from vaccinated flocks. Since the first isolation of an IBV in Australia in 1962 many variant strains have been isolated, many of which differ serologically and antigenically from vaccine strains (Ignjatovic and McWaters, 1991; Ignjatovic and Galli, 1995). Sequence analysis of the S1 gene of nine Australian IBV strains has previously identified two genotypically distinct groups, Vic S (vaccine virus), V5/90, N1/62, N3/62, N9/74 and N2/75 comprising group I and N1/88, Q3/88 and V18/91 comprising group II (Sapats *et al.*, In press). Group I strains were found to share only 53.8 - 61.7% identity at the amino acid level with group II strains. Such a high level of variation was unexpected and lead to the question of the mechanisms involved in the evolution of Australian IBV strains. Previous studies have indicated that point mutations and recombination play a role in the evolution of IBV strains in the USA and Europe (Cavanagh *et al.*, 1992; Wang *et al.*, 1993, 1994; Jia *et al.*, 1995).

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The purpose of this study was to examine the S1 gene of nine Australian IBV strains isolated over a period of three decades (1962 to 1991). Attempts were made to localize regions of variability within the S1 glycoprotein as well as the possible mechanisms involved in the evolution of Australian strains. The results presented show that both point mutations and recombination have played a significant role in the evolution of Australian strains. However, the exact mechanism by which group II strains have evolved remains uncertain.

## II. METHODS

### (a) IBV strains

The Vic S strain is a commercial vaccine. Strains N1/62 (synonym T) and N3/62 isolated in 1962, N9/74 and N2/75 isolated in 1974 and 1975 in the state of New South Wales, have been described previously (Wadey and Faragher, 1981). The N1/88 and Q3/88 strains were isolated in 1988 in New South Wales and Queensland, respectively (Ignjatovic and McWaters, 1991) whereas V5/90 and V18/91 were isolated by this laboratory in Victoria in 1990 and 1991, respectively.

### (b) cDNA synthesis and cloning

cDNA for Vic S, N1/88, and Q3/88 was synthesized using random primers. Sequences obtained from these clones were used to select primers for reverse transcription polymerase chain reaction (RT-PCR) of the S1 genes of Vic S, V5/90, N1/62, N3/62, N9/74, N2/75 and V18/91. All cDNA was cloned into a pUC series plasmid.

### (c) DNA sequencing and computer analysis

cDNA was sequenced using the Pharmacia T7 sequencing kit as per the manufacturer's instructions. Both strands of each cDNA clone were sequenced using the M13 universal and reverse primers as well as internal primers. A phylogenetic tree was constructed using the neighbour-joining method (Saitou and Nei, 1987).

## III. RESULTS AND DISCUSSION

### (a) Distribution of changes within the S1 glycoprotein

The deduced amino acid sequence of the S1 gene of Vic S (vaccine virus) was aligned to that of group I (N1/62, N3/62, N9/74 and N2/75) and group II strains in order to determine regions of variability. The results are shown diagrammatically in Figures 1 and 2. Among the group I strains 73% of residues were totally conserved with three variable regions identified between residues 52-146, 188-196 and 282-304 (Figure 1). Closer examination revealed two HVR's (HVR1 and HVR2) located between residues 52-67 and 117-146 in which only 31% and 40% of the amino acids were conserved, respectively. These HVRs are similar in location to the HVRs (residues 56-69 and 117-131) identified in IBV strains belonging to the Mass serotype (Niesters *et al.*, 1986). However, HVR1 of the Australian strains is shifted slightly closer to the amino-terminus of the S1 glycoprotein, while HVR2 is slightly larger. Comparison of the group II strains with Vic S (Figure 2) showed that variation was more extensive in these strains and was distributed throughout the entire S1 glycoprotein with only 50% of the mino acids being totally conserved.

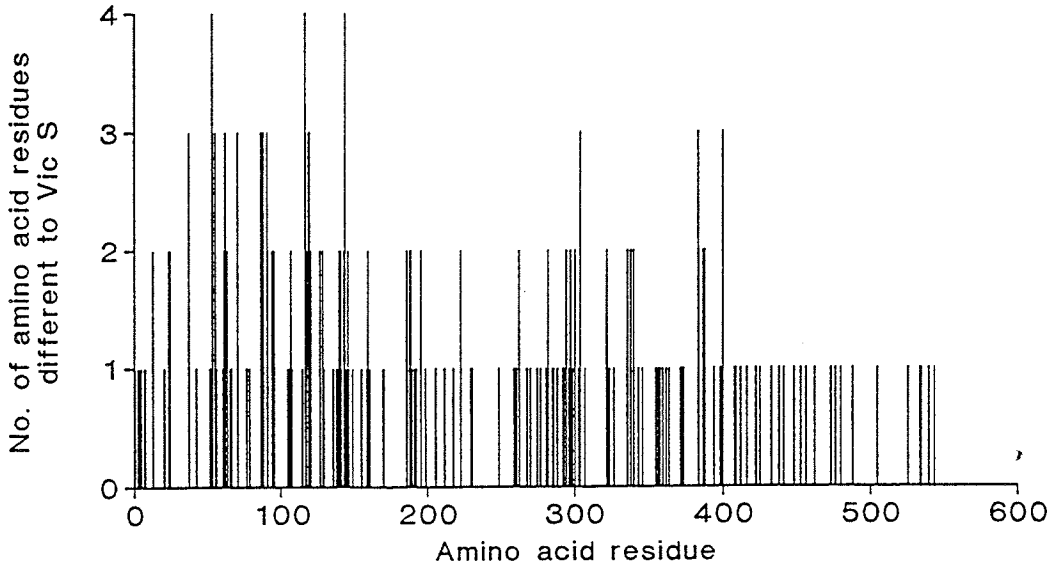


Figure 1. Diagrammatic representation of variability within the S1 glycoprotein of group I strains.

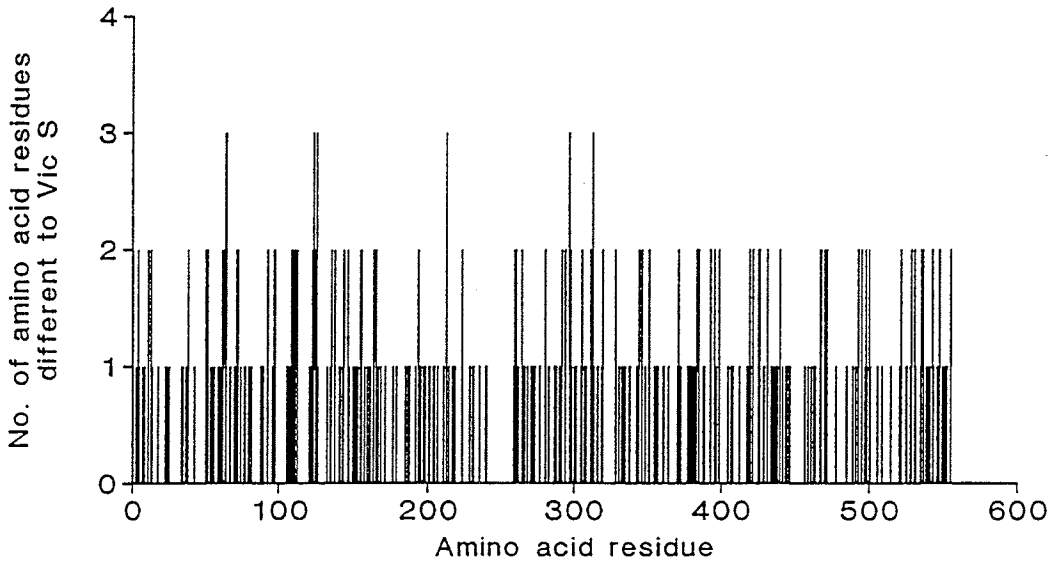


Figure 2. Diagrammatic representation of variability within the S1 glycoprotein of group II strains.

(b) Generation of N9/74 by recombination

Examination of the nucleotide sequences showed that the S1 gene of N9/74 was similar to that of N3/62 except for the region corresponding to nucleotides 438-978 where the N9/74 sequence was more similar to N1/62 (Figure 3). This suggests the possibility of recombination with two cross-overs in the vicinity of nucleotides 438 and 978. The percentage of identical nucleotides between N9/74 and N3/62 was 93%-86%-98% for nucleotides [1-437]-[438-978]-[979-1632] while the corresponding values for N9/74 and N1/62 were 86%-94%-89%. Examination of IBV nucleotide sequences has previously shown that recombination occurs between IBV strains (Kusters *et al.*, 1989, 1990; Wang *et al.*, 1993, 1994; Jia *et al.*, 1995). A number of sites within the S1 gene in the vicinity of nucleotides 20, 71, 98, 131, 1030, 1112, 1373 and 1460 have been identified as potential "hot spots" for exchanging genetic material between IBV strains (Wang *et al.*, 1993, 1994). Recombination events may occur by "jumping" of the polymerase from one template to another during the synthesis of either negative or positive strand RNA. Complex secondary structures may be involved in slowing down the polymerase thus causing the enzyme to dissociate from its template (Banner and Lai, 1991). The polymerase and nascent strand may then attach to an alternative template through base pairing of the nascent and new template strands. Wang *et al.* (1993) identified the sequence CUU(A/U)(A/U)G around every junction where a homology shift occurred in S1. This sequence is similar to the sequence CUUAAG found at the 5'end of the IBV genome and is also similar to the consensus sequence CU(G/U)AACAA required for leader primed mRNA synthesis throughout the genome (Sutou *et al.*, 1988). It was, therefore, suggested that the CUU(A/U)(A/U)G sequence may mimic these sequences and represent an alternative binding site for the IBV RNA polymerase and/or accessory factors (Wang *et al.*, 1993). On the other hand, examination of the Australian strains N1/62, N3/62 and N9/74 did not reveal any such sequences flanking the proposed cross-over sites. It has also been suggested that coronavirus recombination occurs randomly and that the detection of recombinational "hot spots" for coronaviruses results from selection pressures for certain recombinant viruses and not from restrictions on the occurrence of RNA recombination (Banner and Lai, 1991).

(c) Phylogenetic analysis

The phylogenetic relationship between Australian strains is shown in Figure 4. The Australian strains formed two distinct clusters. The six group I strains formed a distinct cluster with two sub-clusters, one containing Vic S, V5/90 and N2/75, the second N1/62, N3/62 and N9/75. The V5/90 strain was the most closely related to the vaccine virus Vic S and had undergone a number of point mutations, possibly due to immune pressure. The N2/75 strain was also related to Vic S but appeared to have accumulated a higher proportion of point mutations. The three viruses N1/62, N3/62 and N9/75 showed approximately the same degree of relatedness to one another and are the three viruses believed to have been involved in recombination. The three group II strains, N1/88, Q3/88 and V18/91 formed another cluster, well separated from all group I strains. Within this cluster it appeared that V18/91 may have originated from a strain similar to N1/88 by a process of point mutation. Hence it does not appear that N1/88, Q3/88 and V18/91 originated recently from the other Australian strains examined in this paper through point mutations or recombination. It is unknown why strains more closely related to N1/88 and Q3/88 were not isolated from chickens prior to 1988. Both strains appeared suddenly in broilers on separate geographic

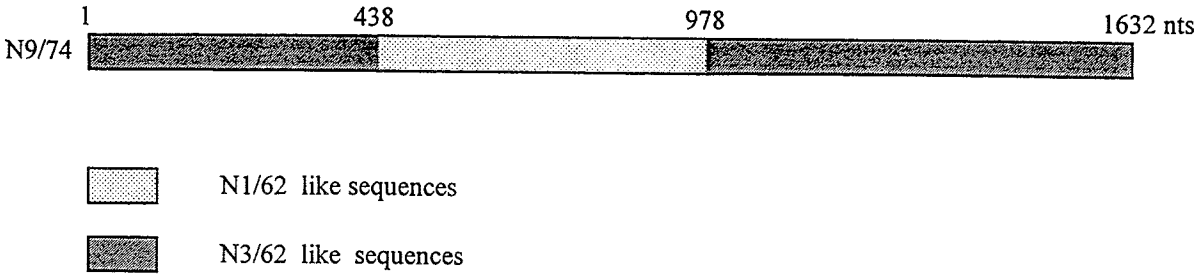


Figure 3. A schematic representation of the putative recombinant S1 gene of N9/74

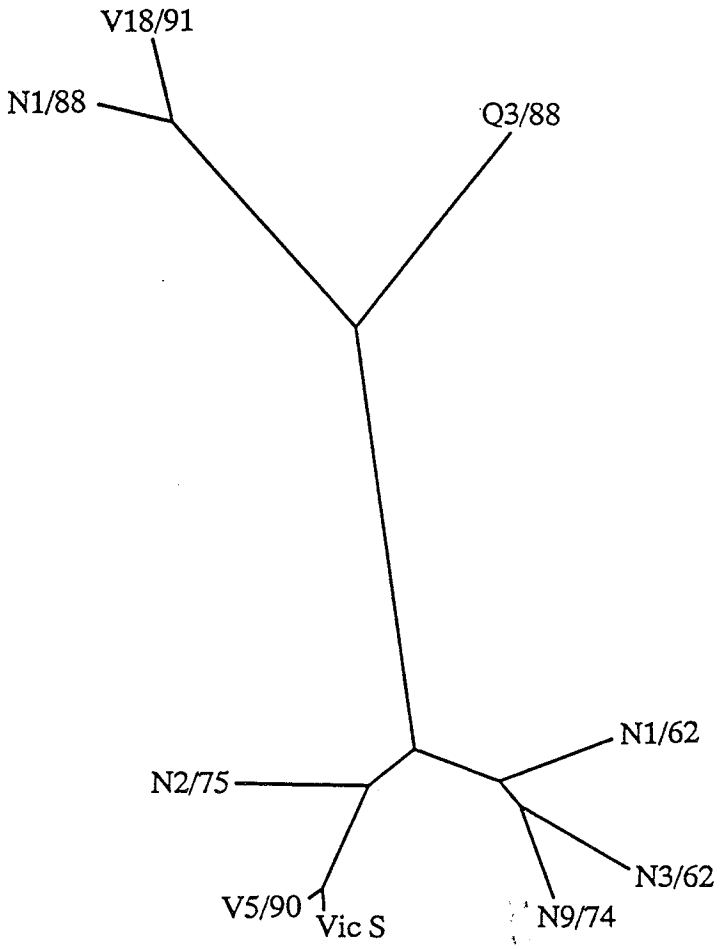


Figure 4. Phylogenetic tree showing the relationship of the S1 amino acid sequences of Australian IBV strains.

locations. It is possible that such strains existed causing only sub-clinical infections or that they originated from another avian species. It has also been suggested that the progenitors of the group II viruses may have been circulating for a prolonged period through immune layer populations without causing visible disease, but that during this time a significant number of mutations accumulated (Sapats *et al.*, In press). Thus the origin and the mechanism involved in the generation of these viruses remains uncertain.

## IV. CONCLUSIONS

Two hypervariable regions were identified within the S1 glycoprotein of group I strains between residues 52-67 and 117-146. Analysis of the S1 sequences indicated that V5/90 and N2/75 may have arisen from Vic S by point mutations, while N9/74 may have resulted from a recombination event involving N1/62 and N3/62. The strains N1/88, Q3/88 and V18/91 were unrelated to strains isolated before 1988. Sequence analysis indicated that V18/91 may have originated from N1/88 via point mutations. The exact origin of group II strains could not be determined.

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# PERFORMANCE AND WELFARE OF AUSTRALIAN LAYER GENOTYPES IN ALTERNATIVE AND MODIFIED CAGE DESIGNS - A PRELIMINARY REPORT

G.D. STEWART


## Summary

A study of the effects of stocking density and cage configuration on production and aspects of welfare in three Australian strains of laying hen indicated no production advantage, and a definite disadvantage from a 'bird welfare' point of view (increased mortality and feather pecking), where cages were modified to reduce maximum stocking density from the current 450 $cm^2$ of floor space/bird to a stocking density of 675 $cm^2$/bird. Cage partitions cannot be satisfactorily adjusted because of the placement of cage doors and waterers, and cage strength will be weakened if internal partitions are removed. This could lead to more welfare problems (increased foot pad wear) and less realizable production with the likelihood of extra egg cracking due to sagging 'roll out' trays. Increasing the stocking density from 450 $cm^2$/bird to 675 $cm^2$/bird led to increased stress and aggression which resulted in overall higher mortality.

The newly designed 'Edinburgh Modified Cage' developed by the Roslin Institute (UK) with nest boxers, scratch trays, and perches has been designed for a much smaller bird than the majority of Australian layer strains. The two heavier Australian laying strains under test performed well below their potential as measured in conventional cages. Significant concerns regarding bird welfare due to feather wear, foot pad damage and toenail damage were raised for all strains tested. Considerable cage re-design is required to make these 'modified' cages suitable for the majority of larger Australian layer strains currently available.


## I. INTRODUCTION

The 1992 (2nd Edition) Australia 'Code of Practice - Domestic Poultry' specified that all caged layers in Australia should be housed at a maximum stocking density of 600 $cm^2$/bird for 3 or more birds/cage compared with the then current 'Code' maximum stocking density of 52 $kg/m^2$ (approximately 450 $cm^2$/bird). As part of its overall responsibility in overseeing research into the effects of such proposed changes on bird welfare and productivity, the Australian Egg Industry Research and Development Council commissioned the study presented here. The aim was to investigate the effect of this stocking density change on commercial Australian layer strains, and to establish what effect 'on farm' changes to cage size might have on both production and welfare parameters.

Considerable interest is being shown in Europe in new designs of 'modified cages' which aim at providing facilities within cages (nests, scratch trays and perches) which allow birds to express a wider range of innate behaviours than is possible in current conventional cages. Appleby (1993) published research from the Roslin Institute, Edinburgh showing that the welfare of hens could be improved using the Edinburgh Modified Cage (EMC). Opponents of the conventional 'caged' housing systems in Australia have suggested that modified cages, such as the EMC, would be significantly better in welfare terms than current cage systems. The present study investigated how

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Australian layer strains would perform in the EMC under Australian conditions from both production and welfare points of view.

## II. MATERIALS AND METHODS

### (a) Modified standard cage design

Cage modifications and stacking densities are shown in Table 2. Standard single deck 'California type' back-to-back cages (30 cm wide x 45 cm deep), which under the present Code of Practice may house 3 layers of less than 2.4 kg liveweight at a stocking density of 450 $cm^2$ of floor space/bird, were modified by adjustments to the side walls to allow for the movement of birds over a greater floor area while maintaining the same stocking density. The common wall was either completely or partially (1/3) removed between adjacent cages - creating 'double' cages capable of holding 6 layers. The rationale behind removing only part of the common partition was to offer a 'hide' within the 'double' cage for the protection of birds lower in the peck order. The 1/3 partition was left at the front of the cage next to the feed trough and also maintained extra cage floor support.

In order to make a 'real time' comparison of the performance of hens at different stocking densities using current Australian laying strains under Australian conditions, these 'modified' cages were stocked with medium bodyweight layers at either 450 $cm^2$/bird in sets of 3 or 6 bird configurations, or 675 $cm^2$/bird in cage group sets of 2 or 4 bird configurations. Each cage group set was 1800 mm long and contained either 12 or 18 birds (i.e. 6 treatments). Six replicates of each of the 6 treatments (total 36 groups with a total of 540 birds) were tested, and complete production data for 52 weeks of lay were recorded including daily egg production, mortality, feed consumption, progressive body weight and egg grades. In addition to the production data, feather score and cage condition were also monitored throughout the experiment.

### (b) Alterative cages - Edinburgh Modified Cage

Eighteen modified cages built under agreement to Roslin Institute (Scotland) specifications (cage floor 95 cm x 37 cm = 878.75 $cm^2$/bird at 4 birds/cage) were trialed using 3 Australian layer strains of light, medium and heavy bodyweights for a 12 month laying period. Six replicates of each strain (total 18 groups) were tested and complete production data were recorded. Feather score and foot pad condition were also monitored for each group.

After 12 months of lay all birds were killed by neck dislocation and the incidence of broken bones was recorded. Bone strength was assessed using an Instron meter which measured the force (kg) required to break the bone when applied at right angles to, and at the approximate centre of, the longitudinal axis of the bone.

## III. RESULTS AND DISCUSSION

### (a) Modified standard cages

The overall effect of stocking density across the three cage types on production performance is shown in Table 1.

Table 1. Mean production parameters of combined high and low stocking density treatments.

| Production trait | High density (450 $cm^2$/bird) | Low density (675 $cm^2$/bird) |
|---|---|---|
| Hen-housed production (HHP) (/100 bd) | 75.0 | 77.5 |
| Hen-day production (HDP) (/100 bd) | 80.0 | 84.9 |
| Mortality (/100 b) | 11.4 | 15.3 |
| Feed consumption (g/bird/day) | 121.5 | 133.7 |
| Feed consumption (kg/dozen eggs) | 1.82 | 1.89 |
| Feed conversion (g feed/g egg) | 2.74 | 2.81 |
| Total 2nd quality gradings (/100 eggs) | 9.46 | 7.53 |
| Total cracks (/100 eggs) | 3.42 | 3.31 |
| Stained and soiled (/100 eggs) | 4.34 | 3.14 |
| Misshapen eggs (/100 eggs) | 0.81 | 0.25 |
| Weak and porous shells (/100 eggs) | 0.27 | 0.18 |

Hen-housed production (HHP) and hen-day production (HDP) were slightly improved for birds housed at the lower density of 675 $cm^2$/bird compared to birds housed at 450 $cm^2$/bird, but the return on investment/$m^2$ of house space was significantly poorer for the lower density groups. After six months of lay the net return of the high density treatments ranged from \$15 to \$20/$m^2$ of shed area greater than that of the low density treatments.

Feed consumption is affected by stocking density or, more accurately, ease of access to the available feeding space. All groups in the low density treatments consumed significantly more feed compared to the high density groups. As indicated by the poorer feed conversion and increased mortality the greater food consumption of the low density groups can lead to general welfare problems as the birds age since some of this excess feed intake is stored as body fat which can predispose the birds to disease (particularly fatty liver syndrome) and make them more susceptible to heat stress.

Treatment 1 (3 birds/single cage) had the lowest average feed consumption of any treatment at 120 g/b/d. In this cage only two birds can feed at once with the non-feeding bird required to stand back and wait its turn. In contrast, Treatment 3 (4 birds/double cage) had the highest average feed consumption of 139 g/b/d. As the dividing partition between the old single cages had been completely removed the 'usable' feeding space for this low density group was the greatest of any treatment. The more common recent design of cages with wider fronts and, consequently, greater feeding areas is considered beneficial in term of ease of access to feed and supposedly reduces aggression between cage mates.

Mortality was significantly higher for treatments at either density when birds were placed in an 'open double cage' - 29% mortality for 4 birds/double cage (675 $cm^2$/bird) and 17% mortality for 6 birds/double cage (450 $cm^2$/bird). Post mortems revealed that most deaths were due to Marek's disease but the mortality may have been exacerbated by extra stress due to vent/tail pecking in the more open double cages (see below).

By the end of the 12 months of the experiment the cages in which the full adjoining side partitions had been removed were beginning to show 'sagging' towards the centre, which in time would have led to the floor slope exceeding the recommended 8°. This would result in greater foot wear due to slippage and also more cracked eggs as eggs would tend to roll to the lowest part of the cage 'roll out'.

The results of the first assessment of feather condition made on the vent region at six months are shown in Table 2.

Table 2. Feather score (/100 birds) at six months (vent and mid-back regions).

| Vent region | | | | | | |
|---|---|---|---|---|---|---|
| Feather Score* | 3birds/cage 450cm$^2$/bird | 2birds/cage 675cm$^2$/bird | 6birds/d.cage 450cm$^2$/bird | 4birds/d.cage 675cm$^2$/bird | 6birds/d.cage + partition 450cm$^2$/bird | 4birds/d.cage + partition 675cm$^2$/bird |
| >3 | 78 | 88 | 67 | 84 | 81 | 93 |
| 3 | 16 | 12 | 27 | 3 | 9 | 2 |
| <3 | 5 | 0 | 7 | 13 | 9 | 5 |
| Mid-back region | | | | | | |
| Feather Score* | 3birds/cage 450cm$^2$/bird | 2birds/cage 675cm$^2$/bird | 6birds/d.cage 450cm$^2$/bird | 4birds/d.cage 675cm$^2$/bird | 6birds/d.cage + partition 450cm$^2$/bird | 4birds/d.cage + partition 675cm$^2$/bird |
| >3 | 90 | 100 | 87 | 77 | 88 | 93 |
| 3 | 10 | 0 | 13 | 13 | 6 | 5 |
| <3 | 0 | 0 | 0 | 10 | 6 | 2 |

* Feather score: 1 = very poor (bare skin); 5 = very good (tight even feathering).
d.cage = double cage.

The worst feather deterioration in the vent region was observed in birds in the open space 'double cages' at both high and low density. The low density 'double cage' also had the worst feather score the 'mid back' region.

Feather score for the vent region and mid-back was worst in the 'open double cages' with the low density birds showing the greatest feather damage. These results support recent UK research (Gregory, 1994) which has shown that until the space given to birds exceeds 850-900 cm$^2$/bird caged birds will engage in more pecking of the vent and tail areas of cage mates. This is because they have more room to get behind other birds as the stocking density is lowered. Beyond about 900 cm$^2$/bird the number of agressive encounters declines.

The effect of stocking density and cage configuration on body weight of the birds at caging and after 3, 6 and 12 months is shown in Table 3.

Table 3. Mean bodyweight (kg) at four periods throughout the trial.

| Mean bodyweight at: | 3birds/cage 450cm$^2$/bird | 2birds/cage 675cm$^2$/bird | 6birds/d.cage 450cm$^2$/bird | 4birds/d.cage 675cm$^2$/bird | 6birds/d.cage + partition 450cm$^2$/bird | 4birds/d.cage + partition 675cm$^2$/bird |
|---|---|---|---|---|---|---|
| Caging | 1.85 | 1.89 | 1.84 | 1.79 | 1.88 | 1.86 |
| 3 months | 2.09 | 2.15 | 2.04 | 2.04 | 2.08 | 2.09 |
| 6 months | 2.21 | 2.34 | 2.20 | 2.20 | 2.21 | 2.27 |
| 12 months | 2.26 | 2.45 | 2.36 | 2.32 | 2.30 | 2.38 |

d.cage = double cage.

Birds in the low density treatments gained more body weight than did the high density birds, commensurate with their higher food intake and poorer feed conversion (Table 1).

(b) Edinburgh Modified Cages (EMC)

The effects on the various production traits of three different layer strains housed in EMC are shown in Table 4.

Table 4. Mean production parameters for 12 months of lay for three Australian layer strains housed in Edinburgh Modified Cages.

| Production trait | Light bodyweight strain | Medium bodyweight strain | Heavy bodyweight strain |
|---|---|---|---|
| Hen-housed production (/100 bd) | 76.1 | 65.2 | 64.2 |
| Hen-day production (/100 bd) | 80.0 | 70.5 | 76.0 |
| Mortality (/100 birds) | 12.5 | 20.8 | 29.2 |
| Feed consumption (g/d) | 105.8 | 113.3 | 128.5 |
| Total 2nd quality grades (/100 eggs) | 11.6 | 12.0 | 16.5 |
| Total cracks (/100 eggs) | 3.6 | 4.6 | 3.9 |
| Stained and soiled (/100 eggs) | 5.9 | 5.0 | 9.1 |
| Misshapen eggs (/100 eggs) | 0.2 | 0.3 | 0.6 |
| Weak and porous shell (/100 eggs) | 0.07 | 0.12 | 0.30 |
| Location of lay (/100 eggs) | | | |
| Nest box | 80.4 | 69.3 | 55.4 |
| Cage front | 8.4 | 10.6 | 24.9 |
| Cage back | 0.06 | 0.12 | 1.84 |
| Sand box | 8.9 | 14.2 | 14.0 |
| Eaten | 2.2 | 5.3 | 3.9 |

The production of each strain and location of lay within the modified cages differed considerably between groups. The light bodyweight Australian strain (which more closely resembles the lighter European egg layers) showed the highest rate of production over the full laying period. The medium and heavy Australian strains performed worse than would have been expected in conventional cages and it is considered that this was due to the physical size and bodyweight of these strains and the constraining size of the EMC design. By comparison, the HHP of excess sister birds from the Edinburgh cage trial housed in conventional four bird 'flat deck' cages in the same shed and given the same feed was 82% for the light body weight strain and 79% for the heavy body weight strain respectively. There were no spare medium body weight strain birds to make a comparison of all three strains using conventional cages. These preliminary results suggest that in future trials with the EMC comparative production performance with sister birds in various types of conventional cages should be conducted to provide a statistical comparison of the performance of each strain for each cage type under similar conditions.

The results obtained from this first year of lay indicate that mortality increased with increasing body weight. The losses of 20.8% and 29.2% for the medium and heavy strains respectively are unacceptably high by normal commercial standards for these breeds. The cage dimensions as supplied by the Roslin Institute were patently not suitable for the size of the majority of current Australian laying strains.

The larger birds could not stand erect on the perches (due to lack of height above the perch to the ceiling of the cage), and did not walk on the cage floor behind the perches (they rubbed against the back of the perch and the back of the cage). Consequently, there was an unacceptable build up of manure in the cages behind the perch with both the

medium and heavy strains which required several physical clean outs during the laying period. This has both welfare and disease implications.

In this experiment 24 h access was allowed to all parts of the cage (including the nest box and scratch tray). Analysis of the location of lay showed that hens did not always choose to lay in the darkened nest box and that the larger the birds the less they chose to lay in the nest boxes. This may be an overall cage design factor for the larger Australian strains. However, there were large differences between groups of the same strain as to their preference for laying eggs in the nests. It is possible that the high intensity of natural light in Australian sheds as opposed to light-controlled sheds in Europe does not induce the birds to seek the relatively darkened nest box. One of the possible factors associated with the reduced preference for laying eggs in the nest box by the larger birds is the likely physical discomfort experienced due to the dimensions of the nest box provided, particularly the nest box height.

The effects on the condition of the toe nails and foot pads of the hens from the three strains housed in EMC are shown in Table 5.

Table 5. A comparison of the foot condition of various Australian strains housed in Edinburgh Modified Cages.

| Condition | Light bodyweight strain | Medium bodyweight strain | Heavy bodyweight strain |
|---|---|---|---|
| Birds with toe nails broken (/100 birds) | 74 | 41 | 45 |
| Birds with toe nails missing (/100 birds) | 0 | 14 | 30 |
| Birds with foot pad lesions (/100 birds) | 87 | 36 | 35 |
| Mean bodyweight (kg) | 1.67 | 2.11 | 2.39 |

The condition of the toe nails and foot pads of each strain varied considerably. The incidence of broken and missing toe nails (Table 5) was unacceptably high. Birds spent a large amount of time standing on the perch which in this design is 50 mm x 25 mm dressed pine with bevelled edges. The cage design lacks any abrasive strip which might wear down claws and, consequently, claw length often exceeded 25 mm. It is suggested that claw damage is caused by birds stepping down off the perches and the claws hitting the wire floor. Also, birds often had crossed claws when standing on the perches. From a welfare point of view there is a definite need for claw abrasives to be fitted to these cages.

Foot pad damage (Table 5) observed in all three strains was also unacceptably high. In most cases the foot pads were swollen and many had loose casts. In some cases bacterial infection was present and the condition resembled classical 'Bumblefoot'. As there were no obvious sharp areas within the cage area it is suggested that most foot pad damage must have occurred when birds stepped down from the perch onto the wire floor.

Bone strength measurements for the three strains of layer housed in EMC are presented in Tables 6 and 7. Bone strength as measured by lateral breaking force generally increased with body weight for the metatarsus, tibia and femur but decreased with increasing body size for the humerus. This decrease in humerus bone strength with body size would appear to be due to the restraining nature of the EMC design which tended to prevent wing flapping by the larger birds. This further demonstrates that the current EMC design and dimensions are not suitable for the larger Australian layer strains.

Table 6. A comparison of mean femur and humerus bone strengths at the end of lay for three Australian layer strains housed in Edinburgh Modified Cages.

| Bone | Light bodyweight strain | Medium bodyweight strain | Heavy bodyweight strain |
|---|---|---|---|
| Femur breaking strength (kg) | 28.9 | 30.2 | 37.0 |
| Humerus breaking strength (kg) | 16.2 | 15.3 | |

Table 7. A comparison of the location of bone breakages (/100 birds) at the end of lay for three Australian layer strains housed in Edinburgh Modified Cages.

| Bone | Light bodyweight strain | Medium bodyweight strain | Heavy bodyweight strain |
|---|---|---|---|
| Metatarsus | 0 | 0 | 0 |
| Tibia | 0 | 0 | 0 |
| Femur | 0 | 2.6 | 10 |
| Humerus | 0 | 33.3 | 20 |

Breakages of the humerus were substantially higher in the heavier strains in keeping with the bone strength data (Table 6), but breakages of the femur were also higher in the heavier strains suggesting possibly that, not withstanding their somewhat greater breaking strength, the legs were broken in the heavier birds as they jumped down from the perches on to the cage floor.

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# IMPROVEMENTS IN PRODUCTION OF LAYING HENS AND CHICKS GIVEN CHILLED DRINKING WATER

G.D.STEWART and J.G.DINGLE

Summary

The temperature of the drinking water made available to both brooding and laying stock can affect their water and feed consumption, and growth and production. Hens given chilled water (<25°C) produced significantly greater egg mass/bird/day during a heat wave period than did similar hens given non-chilled water. Brooding chickens (both broilers and layers) given chilled water had an increased growth rate, feed and water consumption compared with chickens given unchilled water. The results from these experiments indicate that, on the basis of the improvement in production, chilled water provided during times of high temperature has a positive effect on the welfare of commercial layer and broiler stock. This is possibly due to a reduction in body temperature and heat stress. The costs of establishing a 'cool' water system for birds in intensive management systems is thus offset by better production during a high temperature challenge.

## I. INTRODUCTION

The importance of water as one of the essential nutrients is often overlooked. However, as well as the quantity, the quality and the temperature of the water provided to poultry are assuming increasing importance as the industry expects greater productivity from each bird.

Birds do not have sweat glands and one of the major ways they dispose of excess body heat is through expired moisture from the respiratory system. Due to the location of drinking lines within the shed (often in the roof structure), the temperature of the water in the drinking lines can rise to over 40°C during 'heat wave' conditions (Stewart, 1994) and may be in excess of 30°C for long periods for several successive days during summer. Birds decrease their water consumption if water temperature rises and may cease consuming water totally when the water temperature rises above 30°C. Not only are normal metabolic processes inhibited but birds quickly become dehydrated through panting if they do not replenish body moisture. It has been found that laying hens given chilled drinking water (5°C) during periods of high environmental temperature consumed more food, and produced eggs with better shell quality than hens in the same environment receiving water at 30°C (Glatz, 1993). Broilers at a house temperature of 38°C consumed four times as much water as those at 21°C (Harris *et al.*, 1975) and for all birds the water:food intake ratio increases from approximately 2:1 at moderate temperatures of around 20°C to about 5:1 at 35°C (Balnave, 1989).

The drinking water provided to most birds (layers and rearing stock) is generally provided in a 'closed' system (i.e. long rows of nipple/cup lines or bell type drinkers). In this case the water within the drinking system will tend to heat up to the ambient temperature surrounding the drinking line at any point and the rate of water consumption is not sufficient to allow water entering the closed lines to exert more than a minimal cooling effect. The three experiments reported here used both pump recycled flowing water and an in-line mechanical 'water chiller' to cool the water.

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## II. MATERIALS AND METHODS

### (a) Chilling apparatus

A commercial water chiller (similar to that used as a public drinking fountain inside buildings) was used in conjunction with a recirculation pump to maintain water at a particular temperature by using an 'in line' thermostat which automatically switched on the chiller whenever the predetermined temperature was exceeded. The cost of the equipment was approximately $950.

### (b) Laying hens supplied with chilled water during a heat wave

Laying hens (440) were housed in 3 sets of back-to-back cages (i.e. 6 rows in total). The watering system for this shed was one line of nipple drinkers with a 'v' trough down the centre of each set of cages so that the birds in each cage had access to 2 nipples. Only the second set of back-to-back cages was fitted with the water chilling apparatus (which was set to a maximum temperature of 25° C). The sets of cages on either side of centre were provided with normal water supply at ambient temperature. During the period under test a natural heat wave occurred with temperatures within the laying shed reaching 42° C. The shed was fitted with white reflective roof paint, old foil insulation on the underside of the roof and ventilation fans on the northern side. Eggs were collected daily and weighed, and total 'egg mass' calculated daily for each of the three sets of back-to-back cages.

### (c) Brooding layer-type chickens supplied with chilled water (cage reared)

This trial was conducted on a commercial farm in a 'hot air' brooding shed containing 20,000 birds in 3 sets of back-to-back cages with a nipple/cup drinking system placed inside and towards the front of each cage. The chilling system as described for Trial 1 was installed to supply temperature-controlled water to all the chickens in both rows of the middle set of back-to-back cages. The hot air was supplied by 'direct fired' oil burners positioned at each end of the shed. Three cages were chosen at random from each chilled row and all the birds from these 6 cages and those from the 6 cages directly opposite in the non-chilled rows were weighed at 28 days of age.

### (d) Brooding broiler chickens supplied with chilled water (floor pens)

This trial was conducted using radiant heat infra-red lamps to brood four groups of 100 commercial meat chickens in floor pens from day old to 3 weeks of age. Two groups were supplied with continuously recirculating 'chilled' water (maintained at a maximum temperature of 17°C), and the remaining two groups had access only to water at the ambient temperature operating within the infra-red heated surrounds. Water and feed consumption were measured for each group and the dressed carcass (plucked empty body) weight measured for each bird at the end of the third week.

All results were subjected to statistical analysis using the SAS package.

## III. RESULTS

### (a) Laying hens

The descending order of hen-day production, egg weight and egg mass was, chilled water cages > west cages > east cages, with differences being significant ($P<0.05$) except for mean egg weight from the chilled water cages which was significantly greater than mean egg weight from the east cages only (Table 1).

Table 1. Layer production during the 8 day heatwave period.

| Cage location | Treatment | Mean HDP[1] (/100 bd) | Mean egg wt (g) | Mean egg mass /hen/day (g) |
|---|---|---|---|---|
| EAST | Non chilled | $38^{c*}$ | $57.6^{b}$ | $21.90^{c}$ |
| CENTRE | Chilled | $69^{a}$ | $59.8^{a}$ | $41.44^{a}$ |
| WEST | Non chilled | $63^{b}$ | $58.9^{ab}$ | $37.13^{b}$ |
| LSD (P=0.05) | | 3.8 | 1.5 | 1.78 |

1 HDP = Hen-day-production.
* Means without a similar superscript are significantly different at $P<0.05$.

### (b) Brooding layer-type chickens

The mean body weight of layer chicks given chilled water (<25°C) for 28 days in a hot air cage brooding shed was significantly ($P<0.05$) greater than hatch mates kept in the same shed but given unchilled water (Table 2).

Table 2. Mean body weight of layer type chickens given chilled or non-chilled water during 28 days of brooding.

| Water | Mean body weight (g) |
|---|---|
| Non-chilled | 221.6 |
| Chilled | 244.7* |
| LSD (P=0.05) | 4.5 |

* Significantly heavier at $P<0.05$.

### (c) Brooding broiler chickens

The mean dressed weight and the feed consumption of infra-red brooded chicks given chilled water for three weeks were significantly ($P<0.05$) greater than those of chicks in the same environment given unchilled water (Table 3). Approximately 6.3% more chilled water was consumed than non-chilled water and approximately 9% more feed was consumed by the birds given chilled water than by those receiving unchilled water.

## IV. DISCUSSION

### (a) Chilled water for laying hens

The laying hens in Experiment 1 were 76 weeks old and approaching the end of lay.

Table 3. Mean dressed body weight and water and feed consumption of broiler chicks given chilled or non-chilled water in an infrared brooder.

| | Chilled | Non-chilled | LSD (P = 0.05) |
|---|---|---|---|
| Mean water consumption (mL/d)[1] | 101.6 | 95.6 | NA |
| Mean food consumption (g/d) | 58.9 | 53.9 | NA |
| Mean dressed weight (g/bird)[2] | 548.7 | 519.1 | 10.1 |

[1] As only 1 water chiller was available water consumption could only be measured as a total for both groups on the chilled water regimen.
[2] Dressed weight = plucked empty body weight with feet removed.
NA = not applicable.

In this experiment, the water temperature in the chilled water row was successfully maintained at the maximum temperature of 25°C compared to the non-chilled rows where the water temperature varied up to 38°C. Due to the placement of the water reticulation system within the shed (through the roof structure) inlet water temperature was measured at up to 39°C prior to emptying into the small header tanks which supplied each row. The temperature of the water as it moved down the drinker line increased by up to 2°C by the last outlet compared with the first drinking outlet in the line, indicating that the drinking line picked up conducted, convected, and radiated heat within the shed. The significant positive response to chilled water as measured by hen-day production and egg mass per hen-day showed that the cost of providing chilled water can be offset by the extra realizable production gained. Egg production returned more quickly towards the pre-heat wave production rate in the chilled water water birds than for the non-chilled groups. The relatively poorer performance of the east set compared with the west set of cages, was thought to be caused in part by its proximity to an internal corrugated iron wall which separated a rearing section. Radiation of absorbed heat from this wall and poorer air circulation resulted. The highly significant positional effect in this study between the two non-chilled groups suggests further experimental work with more replication and randomization of treatments to unequivocally determine and quantify the benefits of chilled water.

The practical positive outcome of this work in terms of bird welfare i.e. stress alleviation, was considerable and the chilling/recirculation system as described can be easily adapted to most commercial sheds. Since the water is recirculated there is no water wastage or contamination as the recirculating water stays within the pipes. The practical level of chilling achieved will depend on the temperature of the input source, the length of the water lines within the shed and heat control within each shed (insulation, foggers, fans, etc.).

(b) Chilled water for layer-type brooding chickens (cage reared)

This experiment was undertaken in a commercial shed with 20,000 chickens so that it was not possible to measure water or feed consumption for the targeted birds. For cage

rearing systems, the application of cooled recirculated water has particular benefit in reducing the stress associated with localised 'hot spots', as birds cannot move to a cooler part of the shed.

It was noted that the humidity in this 'hot air' shed was measured at being as low as 27% relative humidity generally during the pre-dawn hours when the external shed temperature was at its lowest. It is considered that such low humidity levels within the brooding area would have a desiccating effect on the young chickens which may be partially compensated for by having access to cooler water. The addition of a humidifying system is strongly recommended in this case. Detailed analysis of the data showed a positional effect within the shed which indicated the presence of hot spots/cold spots caused by the positioning of the 'hot air' brooders and movement of air within the shed. These temperature differentials can be overcome by better shed management.

(c) Chilled water for commercial broiler chickens (floor pens)

This experiment was designed to follow the layer chicken brooding trial with the specific intention of measuring average water and food consumption as well as body weight. Broilers were used because of their fast growth rate compared to layer-type chickens. It was expected that body weight differences due to the chilled water treatment might be more pronounced. The use of 'infra red' lamps during brooding is not standard practice in the chicken meat industry, but managed properly with small numbers of birds it is still an effective brooding method.

The temperature within the brooding surrounds reached a maximum of 34°C during the early stages of brooding and consequently the non-chilled drinking water within the surrounds would have approached this temperature also. The two groups of birds with access to chilled water (maximum 17°C) consumed more water and ate more food than the non-chilled water groups. This resulted in a significant increase in body weight by 21 days.

## V. CONCLUSION

The cost of establishing a cool water system for birds in intensively managed systems is offset by increased production during periods of high temperature challenge.

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# THE REPLACEMENT OF SOYABEAN MEAL WITH PEANUT MEAL IN BROILER DIETS CONTAINING ANIMAL PROTEIN CONCENTRATE

H. SUSWANTO and G.P.D. JONES

The use of multiple protein-rich feedstuffs as dietary constituents is an important means of minimising any negative effects resulting from limitations in raw materials. Any 'supplementary effects' which improve the quality of the feed are additional benefits. Irish and Balnave (1993) indicated that retarded growth occurred in broilers fed diets containing soyabean meal as the sole dietary protein concentrate. Moreover, the combination of soyabean meal with other vegetable protein meals (sunflower meal, rapeseed meal and cottonseed meal: 75 g/kg) improved broiler growth. The present experiment examined the use of peanut meal as an alternative protein source to soyabean meal in broiler diets containing meat and bone meal.

Six replicates of eight five-day-old broiler chicks were fed one of five isoenergetic (12.36 MJ/kg) and isonitrogenous (207 g crude protein/kg) diets. These diets contained soyabean (SBM), meat and bone meal and peanut meal (PM) as protein sources. Peanut meal inclusions in the diets (replacing soyabean meal) were 0, 25, 50, 75 and 150 g/kg. The soyabean meal concentration in these diets ranged from 263 to 94 g/kg. The diets were fed from 5 to 23 days of age and the results are shown in the Table.

| Dietary protein meals | Weight gain (g) | Feed intake (g) | Feed conversion (g/g) | Dietary ME (MJ/kg) | Metabolizability (%) |
|---|---|---|---|---|---|
| SBM | 652 | 996 | 1.53 | 12.30 | 80.39 |
| SBM+PM (25 g/kg) | 637 | 975 | 1.54 | 12.43 | 81.13 |
| SBM+PM (50 g/kg) | 623 | 962 | 1.55 | 12.53 | 81.35 |
| SBM+PM (75 g/kg) | 635 | 965 | 1.52 | 12.79 | 81.92 |
| SBM+PM (150 g/kg) | 626 | 963 | 1.54 | 12.70 | 83.36 |
| LSD (P = 0.05) | 27 | 43 | 0.07 | 0.32 | 2.06 |

The replacement of soyabean meal with peanut meal in a diet containing meat and bone meal did not affect the performance (weight gain and feed conversion ratio) of the broilers. The ME values indicated a slight improvement with peanut meal inclusion. The energy metabolisability of the feeds showed that peanut meal was more digestible than soyabean meal. A significant relationship existed between metabolisability (Y) and peanut meal inclusion: $Y = 80.5 + 0.0192 X$, where X is the inclusion level (g/kg) of peanut meal in the feed ($r = 99.1\%$). Digesta viscosity and excreta moisture contents were not affected by feed treatments. Nitrogen balance was similar on all diets.

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# EUROPEAN ALTERNATIVE HOUSING SYSTEMS FOR LAYERS - HEALTH, PRODUCTION AND ENVIRONMENTAL CONSIDERATIONS

R. TAUSON

Summary

Within Europe, housing conditions for laying hens vary considerably due to national animal welfare acts and directives and also because of different structures of production. Examples are stocking densities and whether it is permissable to beak trim or even to use cages. The welfare debate on cages for laying hens has been most intensive in North Western Europe. Brittle bones, fear and lack of opportunity to express natural behaviours are arguments against cages. Unpredictability of mortality and production, unstable peck order in large flocks increasing the risk of cannibalism, parasitic disorders resulting from inferior hygiene, and dirty and difficult working environments in floor-kept flocks are proven problems. Special attention must be paid to the higher emission of ammonia and dust from litter floor systems. The alternatives comprise traditional floor keeping, multi-tiered aviaries and modified enriched cages. The latter models, for 5-10 birds with perches and nest, seem more realistic than litter floor systems for large-scale egg production.

## I. INTRODUCTION

Within Europe housing conditions for laying hens vary considerably due mainly to national animal welfare acts and directives on management procedures and also because of the structures of production. Thus, while the EU directives (from 1995) on stocking densities in cages are similar to those in Australia, i.e. 450cm$^2$ cage floor space per bird, individual member countries like Denmark and Sweden have 600cm$^2$ and Norway (not a member of the EU) 700 cm$^2$. Sweden is even facing a ban on cages in 1999 with strict requirements being placed on acceptable alternatives. The alternatives must not mean impaired animal health, increased medication, the introduction of beak trimming or impaired working conditions. The Standing Committee on Agriculture and Environment has also stated that Swedish egg production must not be out-competed by foreign cage production and also that self-sufficiency should be retained. These requirements have been very difficult to match and fulfil. Hence, 90% of the Swedish laying stock is still kept in cages less than two years before the intended last flock of pullets kept for a full production cycle can be housed. Swedish egg production is deregulated and 70% of production comes from farms with more than 10,000 hens. In Switzerland, where in contrast to most countries egg production is highly subsidised and small-scale production is dominant (60% of production originating from farms with less than 4.000 hens), conventional cages were banned in 1992.

The welfare debate on cages for laying hens (Appleby, 1993; Craig and Swanson, 1994) has been most intensive in North Western Europe. Hence, most recent studies have been performed in these countries. The criticism is not always based on facts but emotionally it is often very strong. However, the levels of brittle bones (Gregory and Wilkins, 1989), fear (Hansen, 1993), and lack of opportunity to express natural behaviours have been demonstrated and used as arguments against cages. The general unpredictability

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of mortality and production, the unstable pecking order in large flocks, inferior hygiene implying considerable risk for outbreaks of cannibalism and parasitic disorders, and a difficult working environment due to dust and floor egg collection in floor-kept flocks as compared to cages are mentioned as problems. Beak trimming, mostly in large groups of birds as a measure against cannibalism and feather pecking, is allowed and widely used in all countries except Finland, Norway and Sweden.

The alternatives to conventional cages include traditional floor keeping, multi-tiered aviaries and modified and enriched cages. This paper initially illustrates some examples of the importance of conventional cage design on health and production (Tauson, 1986) and then describes and comments on the properties of different systems, focusing on the most recent research where comparisons have been made with conventional cage systems, if possible under similar conditions. However, few such studies have been carried out. Instead, in several cases results from different farms with different conditions have been compared.

## II. PRODUCTION AND HEALTH

In the comparison and development of cage equipment it was found that stimulating manufacturers to make changes in cage design was easier when both bird welfare and the economics of egg production were improved than when both were less obvious (Tauson, 1986). This raises the much-argued question as to whether egg production is a relevant index of the total welfare of the hen. Production may well indicate very important aspects of welfare status, i.e. when the daily production records reflect the provision of high quality food and water to all birds in a flock, which is a primary component of welfare. Furthermore, birds suffering from serious disease are unable to produce at their normal capacity. On the other hand, feather condition and renewal of plumage are to some extent inversely related to production due to hormonal (oestrogen) blocking effects. Generally speaking, an almost naked hen consumes in the range of 30 per cent more feed due to heat losses than a well-feathered bird at similar production levels and hence in this sense, welfare and economy are positively correlated (Tauson and Svensson, 1980). Birds showing claw fold lesions and excessively long claws have been shown to be able to produce well (Tauson, 1986b). While these examples show that production is not always a good index of of all aspects of welfare, there is still a belief among most egg producers that production is the best overall indicator of welfare.

## III. ALTERING AND OPTIMISING DESIGN OF CONVENTIONAL CAGES

A large proportion of the findings derived from studying commercial national and international cage equipment for layers within the same building at Funbo-Lövsta Research Centre (Tauson, 1986a), came as a surprise to many manufacturers. However, through active contacts and regular presentation of the results obtained, these studies have considerably increased the awareness, involvement and responsibility of the industry.

### (a) Floor design and foot condition

Severe damage to exterior parts of foot digits (hyperkeratosis/inflammations) was found in more than 60 per cent of birds housed in cages having floors with an unnecessarily steep slope (>20%) and poor galvanising quality as compared with only 1-3 per cent damage on

lesser sloped floors (<14%) with plastic-coated wire. Today, it is difficult to find differences in foot condition between hens kept on well-galvanised or plastic floors.

Great variation among birds within the same cage suggested that there were individual differences in sensitivity or differences in behaviour in terms of the standing posture on the cage floor (Hughes and Black, 1974). The maximum floor slope allowed today in Sweden is 12 % and in the rest of the EU it is 14%. In a special study it was found that at a stocking density of 480 $cm^2$ of floor area per hen the optimal cage floor slope was about 10%, both for egg quality traits (cracks and dirties) and for foot condition. Further improvement in foot condition was not detected until the slope was 3% or lower. However, as expected, at that slope egg out-rolling efficiency was too poor and egg quality could not be retained.

(b) Feed troughs, feed wastage and neck skin condition

Due to deep (14 cm) feed troughs with sharp lips which were installed too high, a majority of birds in a well-known cage design developed severe neck skin atheroma. The manufacturer justified this trough design as a way of keeping feed wastage low when using a travelling feed hopper system. However, by using a flat chain feeder and a shallower trough only 10 cm deep flicking was prevented and feed wastage was reduced by 50 per cent while neck skin atheroma disappeared (Tauson, 1978). Hence, this defect was easily corrected and should no longer be a problem in modern cage design.

Depending on feed trough design, the ratio between feed flicked outwards, which can be checked, and inwards from the feed trough ranged from about 0.1-0.5. Feed wastage between six feeders varied between 0.6-2.7 g/hen/day. A difference in feed wastage of 2 g/bird/day in a 10,000 layer unit corresponds to a loss of about 8 metric tonnes of feed per batch.

(c) Cage design and mortality

Due to faulty cage design, especially involving cage fronts, the frequency of accidentally trapped and often killed hens in some cage designs reach more than 3.5 per cent of hens housed (Tauson, 1985). Apart from being an obvious welfare problem, such figures imply a considerable loss in production capacity, especially since most cases of trapping occur at a young age. Today, in modern designs accidents normally do not exceed 0.5 per cent, primarily due to simplifications in cage design. This also results in a saving of material and facilitates removal of hens from the cages. The latter is important since it reduces the risk of broken bones in spent laying hens (Elson, 1992).

(d) Plumage condition, cage design and stocking density

The use of solid side partitions (sheet metal or plastic) instead of wire partitions reduced feather damage due to wear and pecking between cages by 15-20 per cent (Tauson, 1984a). This damage involved mostly wing-, tail- and back-feathers. The effect of solid side partitions was similar to reducing stocking density by 25%. A further reduced deterioration in plumage was found in cages with a semi-solid rear partition letting ventilation through slats.

Also, in large-scale comparisons reduced stocking density has been shown to decrease mortality, increase production and feed consumption, implying a total better economic result (Bell, 1995). Reduction of stocking density has also been shown to reduce toe

hyperkeratosis in caged hens (Tauson and Abrahamsson, 1994a). Since a reduced stocking density improves locomotor activity it may also contribute to a stronger skeleton.

(e) Cage shape

The introduction of shallow cages instead of deep ones, implying a wider feeder space, was shown to improve production (Bell, 1972). Hughes and Black (1976) suggested that some of the advantages that they could show - higher production, less feather pecking and a lower proportion of cracked eggs - were related to the better feeding pattern and greater accessibility to the wider feed trough space in the shallow cages. Hence, relative effects registered in shallow and deep cages are likely to depend on what feed trough space is compared.

## IV. UNCONVENTIONAL DESIGN FEATURES IN CONVENTIONAL CAGES

(a) Claw abrasives

It has been found that up to 30 per cent of caged SCWL layers have broken claws after one production cycle. The excessive growth of claws due to lack of natural wear can cause trapping and bleeding at breakage as well as scratches. By introducing a claw abrasive strip at the egg guard deflector on the inside of the feed trough, the scores for claw condition improve dramatically and claws become short and blunt (Tauson, 1986b). Medium heavy hybrids have been found not to show the same severe problems of excessive growth of claws as have the SCWL hens. Recently other materials and methods have been developed by the industry in order to obtain a similar effect but eliminating the problem of the wearing down of the tape. These include the use of a mixture of paint/glue and sand or the pressing of indentations into the sheet metal of the egg guard.

(b) Perches

The introduction of a perch in conventional cages provides hens with an opportunity for an enriched behavioural repertoire as shown by Luesher *et al.* (1982), Tauson (1984b), Hughes and Appleby (1989) Braastad (1990), Duncan *et al.* (1992) and Abrahamsson and Tauson (1993). These studies show that the perch is used intensively, particularly at night. Luesher *et al.* (1982), in a pilot experiment, studied a perch which remained flush with the cage floor during the day, allowing eggs to roll over it, but which was raised during the night.

Feed consumption is reduced in perch cages (Tauson and Jansson, 1988; Braastad, 1990), probably due to better insulation of the hens' bodies at night when "clumping" on the perch (Lill, 1968) as well as by increasing the amount of resting behaviour occurring in the cage. Also, the breaking strength of parts of the skeleton increases in cages with perches by about 15% (Duncan *et al.*, 1992; Tauson and Abrahamsson, 1994a) reducing risks of bone breakage when handling/processing birds (Gregory and Wilkins, 1989). Depending on cage floor designs a slight increase in the proportion of cracked eggs is registered. Hygiene may be somewhat impaired in parts of the cage because it is more difficult to effectively trample manure through the cage floor at the rear and under the perch. Because of this there may be more dirty eggs and the perch cannot yet be given a general recommendation for all cage floor designs.

The perch, depending on the shape and the hygiene of the cage respectively, may negatively affect the conditions of the feet and keel bone (see later). However, in some studies the perch has been reported to improve foot condition (Appleby *et al.*, 1992). The use of special designs of perches for different purposes, and the recording of different kinds of foot and keel bone defects, may explain differences in research findings (Appleby *et al.*, 1992; Tauson and Abrahamsson, 1994a).

## V. NON-CAGE SYSTEMS

### (a) Traditional floor keeping and free-range

The keeping of medium heavy brown hens in traditional floor systems (7-9 birds per $m^2$ ground floor) is increasing in some European countries today. However, the market for these eggs is limited and varies. Hence, the premium for such eggs to cover extra production costs is difficult to retain when over-production of these eggs occurs. Also, the keeping of layers on free range, sometimes in order to produce so-called "ecological'eggs", has increased in countries like Denmark, France, the Netherlands and Switzerland. In Denmark it is estimated that in 1998 about 25 % of production may originate from some kind of non-cage production (1993, 12%), (Lysgaard, 1995). This is a high figure and about four times higher than in other Scandinavian countries at the moment. In Switzerland, where conventional cage keeping is banned, 47 % of the consumption of eggs comes from domestic production and 53% as imports of cage eggs (Mettler, 1995).

The practicality for free range production in the Nordic countries is limited because of the long periods of cold and wet weather. It requires large areas of land in order to fulfil the requirements placed on the area needed per bird to get a premium for the eggs but also because of the necessity to change yards regularly in order to limit hygiene problems such as parasites (Morgenstern and Lobsiger, 1993). Another critical aspect is salmonella infection through wild birds. The salmonella issue, in general, is high-lighted in Europe at the moment. There is a negative environmental effect from this form of production since a calculated 10-15 % of the manure is left in the open field which then, through rainfall, may increase pollution of neighbouring waterways (Meierhans and Menzi, 1995).

In a Danish report (Danish Poultry Council, 1994) the average mortality was 35% higher and production 8% lower in floor systems than in cages. Gunnarsson and Algers (1994) reported from an experimental study to 80 weeks of age that mortality in two flocks in a traditional 9 bird per $m^2$ floor area system with manure bins in the middle were 8.6-11.6% and in the conventional cages 5.0-6.7%. The corresponding feed conversions were 2.50-2.55 and 2.19-2.22 kg of feed per kg of eggmass and production 20.3 and 20.9 kg per hen housed, respectively. The percentages of birds with naked areas of the body were 83% and 28%, respectively. The lower production and inferior feed conversion of the floor-kept birds ratio can probably be largely explained by higher mortality and higher heat losses but also to more movement in the floor-kept birds.

### (b) Aviary systems - general aspects

Today in Europe about 4-5 multi-tiered aviary systems are used. The main differences between traditional floor keeping and aviaries are that the stocking density in the latter systems is increased by the use of wire net tiers, normally 2-3, and the manure is regularly removed by scrapers or belts under each tier, as in cages. Normally, automatic egg collection by belts in front of the laying nests are used. Thus, stocking density in

aviaries varies from 14-25 birds per $m^2$ *ground floor area*, i.e. about 8-12 birds per $m^2$ *available area*. Hence, from an energy balance point of view, most of these systems do not need the extra heating that low stocked traditional systems require. However, as pointed out by Oester (1995) pullets for these multi-tiered systems must be reared in a similar way to the laying pens, i.e. on floor with elevated perches and feed and water on elevated tiers in order for the birds to spread through the system to find feed, water and nests.

Ammonia in the air is very much reduced compared with the situation when the manure is stored inside the house. As litter is used on the bottom level the emission of nitrogen from these systems is higher than in cages (Koerkamp and Bleijenberg, 1995). However, the lower the moisture content in the manure or litter the lower the emission into the surrounding air (Koerkamp and Montsma, 1995). The levels of dust in litter-based systems are generally higher than in cages (Hauser, 1988; Tauson *et al.*, 1992; Kangro, 1993; Koerkamp and Drost, 1993). The movement of birds and the activities in the litter are important causes.

The main advantages of an increased behavioural repertoire, lower level of fear (Hansen, 1993) and increased bone strength (Abrahamsson and Tauson, 1995) in the aviaries may often be outweighed by drawbacks like outbreaks and spread of cannibalism and feather pecking (Hansen, 1993; Nørgaard-Nielsen *et al.*, 1993; Abrahamsson and Tauson, 1995). This is especially apparent in countries where beak trimming is not allowed. This measure, which is now being severely criticised in the EU and thus possibly facing a ban because of evidence of suffering to the bird (Hughes and Gentle, 1995), is almost regularly carried out in larger groups of birds. The causes of both feather pecking and cannibalism are probably multifactorial and have been occupying poultry scientists for decades. Both behaviours may appear in cages and in large groups of litter birds. However, the main and very important difference for the birds and the producer is that cannibalism does not normally spread within the conventionally-caged flock, as it frequently does in large groups. One of the most important factors believed to cause cannibalism is the difficulty birds have in creating stable pecking orders in large groups (Keeling, 1995). Hence, until now the only ways of reducing the problem are to beak trim, dim the lights or try to use hybrids where this behaviour is less prominent. However, in some countries like Sweden and Switzerland there is a directive on the requirement for natural light in new buildings. Since the light level influences the risk of outbreaks of pecking, this decision is very difficult to cope with in practice and farmers often close the windows to their barns.

When discussing the appearance of cannibalism and directives on natural light it is interesting to note that the origin of modern layer hybrids, the red jungle fowl, lives normally in small groups (5-10) (Nishida, 1993) and to a considerable extent of time in rather dim light in dense vegetation. Hence, it seems that the selection used with modern hybrids has not changed these preferences much.

### (c) Aviaries - results compared with conventional cages

With non-beak-trimmed birds, Hansen (1993) found in an experimental study of three batches of birds that production in three aviary models ("Tiered wire floor system", "Voletage" and "Marielund") was on average, 4% lower, feed consumption 4% higher, mortality 5% (units) higher and plumage condition 18% inferior to the cages. However, results varied considerably and the best aviary in this study ("Marielund"), although also showing inferior results compared with cages, had levels of production and plumage condition considerably closer to the cages than the other systems. Similar results from experimental studies were reported by Tauson *et al.* (1992) and Abrahamsson and Tauson

(1995). As reported by Engström and Schaller (1993), bumble foot syndrome as affected by the use of perches and poor hygienic conditions, and keel bone deformation resulting from the use of perches are present to a considerable extent in birds kept in aviaries but not in conventional cages. Hyperkeratosis on the digits of the feet appear in cages but not in aviaries (Tauson and Abrahamsson, 1994a). In their study it was found that aviaries may give lower proportions of cracked eggs but higher proportions of dirty eggs. It was also shown that the breaking strength of tibia bone was 30%, and the humerus bone 115 %, higher than that of birds in cages.

The proportion of eggs not laid in the nests but on litter or on wire tiers has been shown to vary considerably between systems and hybrids used. Normally, the heavier hybrids have more mislaid eggs than the lighter body weight white feathered birds. However, as with many results from the aviaries, the variation between batches may be as great as, or greater than, between the genotypes or systems used. An acceptable level of mislaid eggs is below 2% which can be achieved at optimal conditions. However, fairly frequently they may reach 4-5% or more (van Niekerk and Elhardt, 1994).

In Switzerland, where beak trimming is allowed and often practised, Meirhans' *et al.* (1992) calculated that the mortality rate in aviaries was 8.0% and in cages 5.2% and production 2.5% lower in the former system. From a large Dutch study on beak-trimmed birds it is reported that an aviary system may give production results that are similar to those in conventional cages but most often give higher feed consumption (van Niekerk and Elhardt, 1994). However, medical treatment against parasites is common (Bosch and van Niekerk, 1994). In this connection the withdrawal time of eggs for human consumption and their economic as well as health aspects should be taken into account. As shown by Morgenstern and Lobsiger (1993) and Bosch and van Niekerk (1994) fatty liver syndrome was more common in cages than in aviaries.

Blokhuis and Metz (1994) concluded that, provided improvements in working conditions and a reduction of floor egg laying and feather pecking can be obtained and the extra costs for the production is accepted, aviaries may become an applicable animal friendly husbandry system.

## VI. MAJOR ALTERATIONS TO CAGES IMPROVING THE BEHAVIOURAL REPERTOIRE OF THE HENS

### (a) The large Get-Away cage

Mainly because of unpredictable problems of cannibalism and hygiene in larger flocks of litter-kept birds, as referred to above, attention has been drawn to fully furnished cages with features intended to increase behaviours expected to be of major importance to bird welfare. These include access to a nest, a perch and litter material for scratching and sandbathing. One of the first designs of a cage to provide these furnishings was the so called Get-Away cage. This model was first described and studied by Bareham (1976) and Elson (1976) and has been studied further and the results reviewed by, among others, Wegner (1990). A great variety of models of this design as well as of stocking densities have been presented through the years by (Brantas, 1978; von Kleist, 1985; Rauch, 1994). Today, such models used in studies have a floor area of approximately $1m^3$ where normally 15-40 hens are kept (Wegner, 1990; Tauson *et* al., 1992) using time-monitored opening/closing of nests and sandbaths in order to avoid defaecation and misplacing of eggs. Unlike a conventional cage fitted with one perch this design has perches at two levels which increases bird movement and wing flapping and which has been shown to

increase skeletal strength by about 50%, which is more than in the conventional cage with a perch (Tauson and Abrahamsson, 1994a). Also tibia strength shows an increase of about 15% compared with conventional cages. The use of perches during daytime has also been shown to be considerably greater than in a conventional cage with a perch. The drawbacks of the Get-Away cage mostly concern the possibility of birds defaecating on each other (perches at two levels), the inferior egg quality and the inferior inspection possibilities, including also inferior ergonomic properties (Rauch, 1994; Abrahamsson *et al.*, 1995a). Production and mortality rates in these models have not always attained the levels in conventional cages or in cages featuring a perch (Abrahamsson *et al.*, 1995a), possibly because of the larger group size and, hence, probably greater difficulty in creating a stable pecking order. As in other systems such as litter floor systems including perches, the Get-Away cage has also been shown to cause bumble foot syndrome and keel bone deformation but not toe pad hyperkeratosis (Tauson and Abrahamsson, 1994). However, the design of the perches in this model has a significant effect on the degree of foot damage and also on keel bone deformation (Siegwart, 1990; Oester, 1994; Tauson and Abrahamsson, 1994a). Claws have been found to be more naturally worn in Get-Away cages than in conventional cages due to the scratching in the sandbath (Tauson and Abrahamsson, 1994a).

(b) Small fully-furnished cages

Small group cages (5-10 birds) including nests, perches and sandbath have been studied with increased attention during recent years. Originally these were small scale studies in Great Britain (among others Sherwin and Nicol, 1992, 1993; Appleby *et al.*, 1993; Appleby and Hughes, 1995) but later also in more applied studies in Sweden (Tauson and Abrahamsson, 1994b; Abrahamsson *et al.*, 1995a,b) and in the Netherlands (van Niekerk and Reuvekamp, 1995). The intention with a small group-modified and enriched cage is to overcome the problems of cannibalism, hygiene, working conditions and labour requirement inherent in aviary systems for large groups of birds on litter, and of the poor hygienic conditions and inspection constraints in the Get-Away cages. In a preliminary study of some prototypes Sherwin and Nicol (1992) reported that these radical modifications to cages might provide a suitable alternative housing system which satisfies both welfare and production considerations.

Appleby and Hughes (1995) reported production from 20-44 weeks of age in one specified model of a small furnished cage "The Edinburgh Modified Cage" to be similar to that in conventional cages and that the use of nests was between 95 and 100%. Studies of pre-laying behaviour have shown that in these cages this behaviour was much more settled than in conventional cages (Appleby *et al.*, 1993).

At the moment, studies are also being carried out in Germany and in Australia and further larger scale studies are presently underway in Great Britain. Recent comprehensive reports from seminars on these systems were edited by Sherwin (1994) and Kronägg (1995).

Studies of more or less commercial models in several tiers during full production cycles have been reported by Abrahamsson *et al.* (1995a,b). Hence, in order to get an indication of how far one such model has developed in relation to other systems like Get-Away cages, conventional cages and to a certain extent aviary systems, some results from recent studies are presented these models were compared using both SCWL and medium heavy brown hybrid layers (Abrahamsson *et al.*, 1995a,b; Tauson and Abrahamsson, 1995). Two kinds of furnished cages including nest, perch and sandbath were used as follows. 1) Modified and enriched small cages (MEC) with 5 light white hybrids (LSL) or 4 medium heavy

hybrids (ISA-Brown), 2) Get-Away cages (GA) with 15 birds of the same hybrids, 3) Controls were two commercial conventional cages with 4 birds (C), and 4) Conventional plastic cages with solid side- and slatted rear partitions with 3 birds (PLC), see earlier. The study comprised 1,455 non-beak trimmed cage-reared birds housed at 16 weeks of age. Records were kept from 20 to 80 weeks of age. Comparisons were possible with a parallel study with aviaries at Funbo-Lövsta using the same hybrids (hatching) with floor-reared hens from the same farm. Cage floor space per bird was 600 $cm^2$ in MEC and GA for LSL nests and sandbaths excluded, and 800 $cm^2$ with these features included i.e. the latter figure being about 35% larger than a conventional cage (C). Stocking density in PLC was 720 $cm^2$ due to the implementation in 1989 to remove one hen from existing cages at 480 $cm^2$ in order to achieve the stipulated 600 $cm^2$ per bird.

Production was higher and mortality lower in LSL hens (22.3 kg egg mass/hen-housed and 3.2% respectively) than in ISA birds (20.8 kg and 7.7%). The average for the two hybrids in MEC (22.3 kg and 2.6%) compared well with the conventional cages (21.8-21.9 kg and 4.9-5.8%) and was better than the GA (20.2 kg and 8.6%). In the aviaries, production varied between 20.8 kg and 22.5 kg (LSL) and 14.8 kg and 17.0 kg (ISA) and mortality between 3.1% and 4.9% (LSL) and between 30.0% and 45.3% (ISA). Proportions of cracked (after candling) and dirty eggs were higher and lower, respectively, in MEC (9.2% and 2.0%) compared with C (5.0% and 6.0%) and PLC (8.6% and 6.3%), both traits being lower in MEC than in GA (18.6% and 4.9%). The increased proportion of cracked eggs in fully furnished cages agrees with early results by van Niekerk and Reuvekamp (1995) and may be due to accumulation of eggs outside the nest.

Plumage condition was similar in all models except PLC, where it was better, probably due to the rear partition with slats and the lower stocking density. Hygiene of plumage was inferior in GA than in all other models and in the aviaries. Birds in the GA and in the aviaries had inferior foot condition regarding the bumble foot syndrome which is believed to be a perch design-hygiene related defect. In an introductory trial of the MEC (Tauson and Abrahamsson, 1994b) the breaking strength of the humerus was clearly affected by the furnished cage designs and was found to be considerably higher in the modified cages MEC and GA than in conventional cages. Hens in GA and MEC developed keel bone lesions but of a less severe nature than in aviaries due to the use of perches. Incidence of pecks as wounds on the skin of the comb and rear part of the body were more common in the aviaries than in the cage models. Use of Astro-turf (artificial grass) lined nests in MEC was 92% and 65% and in GA 95% and 75% in LSL and ISA, respectively. Astro-turf nest linings were used more than welded wire linings, indicating a preference for the former material. This agrees with the results of Hughes (1993) and Sherwin and Nicol (1993). The latter authors also reported that cage-reared hens housed at early age (16 w.) lay fewer floor eggs than litter-reared birds housed later. However, unlike the case in aviaries where mislaid eggs cause problems of inferior egg quality, extra work and inferior working conditions, such effects are not applicable to modified cages, at least in a system like the MEC where cage floor hygiene is similar to that in a conventional cage.

Eggs laid in the sandbath, however, may cause problems even in a cage. The average proportion of eggs laid in the sandbath in the present study was lower in MEC (0.1%) than in GA (1.7%). Age at the first opening of the sandbath in MEC (16 weeks or 26 weeks) did not affect egg location. Opening at 16 weeks compared to 26 weeks increased the frequency of birds visiting the bath from 5% to 11% of observations per day between 30 and 70 weeks. Use of perches in MEC averaged 96% during the night.

An earlier study of three different SCWL hybrids (Tauson and Abrahamsson, 1994b) revealed that genetic x environment interaction is considerable both between and within

strains. This applied to traits like production as well as health parameters like foot and plumage condition.

Thus, fully furnished cages for small groups of birds (< 10 per cage) may compete in production and mortality with conventional cages. The smaller group size and, hence, probably more stable rank order, improved hygiene resulting from perches at one level only, and easier inspection, imply benefits of MEC compared with GA cages. Results from MEC-models so far have also shown a higher predictability and achievement of normal production and mortality rates than typically found in aviaries, e.g. when using non-beak trimmed brown medium heavy hybrids. The MEC system needs further development regarding the design of the perch and possibly the egg cradle, egg collection procedure and management of the sandbath. Also, a slightly larger group size would reduce the investment cost per hen (nest and sandbath) as well as increasing the total available area per bird, although it is already 35% larger in the present model than in a conventional cage sized 600 $cm^2$. This is a very important issue since the restricted area in a cage will remain a critical point. Such studies are under way.

## VII. FUTURE OF ALTERNATIVES TO CONVENTIONAL CAGE KEEPING

The main problems with alternative litter floor systems are that behaviour (cannibalism and floor laying), animal health and egg production are more difficult to predict and production costs are higher than with cage systems. Hence, a compromise between the behavioural needs of the bird and improved environmental control will have to be found (Gerken, 1994). Apart from the degree of success in development, the possible use and acceptance of alternative housing systems for layers similar to those described above will be influenced by several factors. These include size and structure of farms, policy and veterinary legislation with regard to the egg trade between countries, interpretation of scientific results by politicians, legislation, and acceptance of higher food costs in various countries. The experience so far is that it is difficult to tell if and when modern litter floor alternatives, will approach the levels typically obtained in conventional cages with respect to bird health, working environment and general predictability. Certainly, the cost of eggs from such systems will be considerably higher, especially when the factors of labour costs and general unpredictability are included. The fact that production in aviaries, even when the problems mentioned above do not appear, is reported to be 3-5% lower than in cages (Tanaka and Hurnik, 1992; Hansen, 1993; Abrahamsson and Tauson, 1995) must also be taken into consideration.

In countries where conventional cages may have to be altered or, alternatively, be prohibited, a future alternative might be a system similar to the MEC, where the advantages of hygiene and small group sizes in cages can be combined with the increased behavioural repertoire and increased skeletal bone strength found in hens kept in loose-housing systems. Such improvements will also have associated costs, but may be less expensive than more radical alternatives, while having more predictable benefits for welfare (Duncan *et al.*, 1992).

## VIII. CONCLUSIONS

Production systems for layers vary considerably in their effects on health characters like foot, plumage and skin condition, and mortality, but also production traits like feed wastage and egg quality traits. Keeping the plumage of hens as intact as possible decreases feed requirements due to reduced heat losses. Concerns about bird welfare have led to proposals for cage modifications and to the introduction of extra features in cages, such as

claw abrasive tapes which eliminate excessive growth of claws and perches which considerably increase bone strength. For countries where conventional cages are becoming the subject of intense discussion, and especially in places where beak trimming is not allowed, it is difficult to predict if and when an accepted alternative for aviary litter birds will be ready for practical use on a scale comparable to conventional cages. Until now, furnished and modified cages for small groups of hens seem more realistic as alternatives for large-scale production than litter-kept birds in larger groups. This is due mainly to the reduced risk of spread of cannibalism and parasitic disorders within a flock and because of a more acceptable working environment. Effects on the environment as regards both dust inside the house and ammonia emission into the ventilated air in litter systems must also be considered.

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# THE EFFECT OF THE DEGREE OF FATNESS OF A RANGE OF BROILER GENOTYPES ON THE SUBSEQUENT RESPONSE TO FEEDS VARYING IN ENERGY:PROTEIN RATIO

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Summary

Responses in growth rate, feed efficiency and body fat to two feeds differing in energy:protein ratio were measured in 480 broilers of five strains following a period up to 650g (males) and 600g (females) liveweight in which different degrees of fatness were achieved by dietary means. Substantial variation in relative growth performance and feed conversion efficiency between the dietary treatment groups in the five strains suggests that considerable differences exist in the extent to which different strains deposit lipid reserves and make use of these reserves as an energy source.

## I. INTRODUCTION

The general influence of dietary energy:protein ratio on growth and body composition of broilers is well understood (Summers *et al.*, 1992). In addition, it has been shown (Gous *et al.*, 1992) that broilers respond differently to feeds differing in energy:protein ratio depending on the degree of fatness of the birds when such feeds are introduced. Obese broilers, for example, will consume small amounts of a high protein feed and exhibit very high feed conversion efficiencies which can be explained on the grounds that such birds are utilizing lipid reserves as an energy source. Lean broilers do not exhibit this improved feed efficiency presumably because of the lack of excess lipid reserves from which to draw.

The response of broilers to feeds differing in energy:protein ratio may depend not only on their state but also on the genotype. Genotypes may differ in their ability to deposit lipid and then to utilise these lipid reserves. Such information is fundamental for corroborating broiler growth models used for predicting the effects of dietary changes on growth and body composition. The present study was designed to provide such information on the response of Australian commercial and experimental broiler genotypes to variations in dietary energy:protein ratio during which the chemical composition of the birds was changed by dietary manipulation.

## II. MATERIALS AND METHODS

Four commercial broiler strains (Ingham, Steggles, Bartter and Cobb) and one experimental line (line I, selected on a feed efficiency index), were used in the study. At the request of the commercial companies, the strains have been coded in this paper. One hundred and twenty day-old chicks of each strain were obtained on the same day, sexed and reared on a crumbled high-protein starter diet (S) containing 250g crude protein (CP) and 12.5 MJ of ME/kg to five days of age when they were divided into three groups with three

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replications and given either the starter diet *ad libitum,* a crumbled commercial broiler finisher diet (F) containing 200g CP and 13.0 MJ ME/kg *ad libitum*, or the starter diet restricted to about 75% of *ad libitum* consumption. The birds were reared in strain- and sex-intermingled groups in brooder and follow-on cages until they reached 600g (females) or 650g (males), when 16 birds per strain x sex x dietary group were transferred to 480 single growing cages with individual feeders and given either the finisher or starter diet *ad libitum.* The experimental design was thus factorial with five genotypes, three initial by two final feeding treatments, two sexes and 8 individual bird replicates. Data from birds receiving the restricted starter diet during the starter phase will not be presented in this paper, reducing the comparison here to four feeding regimes.

The birds were grown in the single cages until they had doubled their weight (1200g females and 1300g males) when they were weighed and food intake measured. They were fasted overnight before being killed by neck dislocation, after which the abdominal fat pad was removed and weighed. The carcasses were then stored at -20°C for later chemical analyses. A sample of two birds at 600-650g from each of the genotype x treatment x sex sub-groups was taken at transfer to the single cages to determine the effects of the starter treatments on body composition.

## III. RESULTS AND DISCUSSION

The effect of diet on growth performance during the starter phase in the five strains is shown in Table 1.

Table 1. Growth rate (g/d) and carcass fat (g/kg liveweight) of the five strains given the starter or finisher diet during the starter phase from 5 days to 600g (females) and 650g (males).

| | | Strain | | | | | |
|---|---|---|---|---|---|---|---|
| Trait | Diet | 1 | 2 | 3 | 4 | 5 | $LSD_{0.05}$ |
| Growth | Starter | 23.5 | 23.0 | 24.0 | 22.6 | 21.9 | |
| rate | Finisher | 19.0 | 19.4 | 18.7 | 18.7 | 16.1 | 1.4 |
| Carcass | Starter | 96 | 104 | 69 | 99 | 91 | |
| fat | Finisher | 94 | 112 | 114 | 108 | 119 | 19 |

Growth performance on the finisher diet during the starter phase was depressed in all strains, but considerably more so in commercial Strain 3 and the experimental line (5) than in commercial Strains 2 and 4. Body fat at the conclusion of the starter phase was generally higher in the birds in each strain given the finisher diet, but considerable variation was evident. There was essentially no difference in strain 1 whereas there was a marked (65%) increase in carcass fat in birds from Strain 3 given the finisher diet.

The effect of the dietary regimens on growth performance during the grower and combined starter + grower phases, is shown in Table 2.

During the grower phase, all strains grew faster on the SS, FS and FF regimens than on the SF regimen, but there was considerable variation between strains in their relative performance. Birds from three strains (1, 3 and 5) in the FS group, grew at almost twice the rate of the reverse treatment birds (SF). These were the three strains which performed relatively poorly on the finisher diet during the starter phase suggesting considerable compensatory growth in these strains when provided with a high protein diet of good amino acid balance. Birds provided with the starter diet during both phases grew considerably faster than the SF group in all strains, but there was again considerable between-strain

variation. The results suggest that the protein requirements of all the strains during the grower phase were not being adequately met by the finisher diet and that this was considerably more marked in some strains (1, 4 and 5) than in others (2).

Table 2. Growth rate (g/d) of the five strains during the grower and combined starter + grower phases on the SF, SS, FS and FF dietary regimens.

| Phase | Grower (625 to 1250g) | | | | Combined (5d to 1250g) | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment Line | SF | SS | FS | FF | SF | SS | FS | FF |
| 1 | 35.9 | 54.1 | 68.8 | 49.9 | 34.3 | 40.8 | 36.6 | 32.9 |
| 2 | 42.0 | 51.8 | 68.6 | 48.9 | 35.3 | 38.4 | 35.1 | 33.1 |
| 3 | 38.2 | 56.3 | 73.0 | 50.4 | 36.8 | 43.5 | 36.3 | 33.2 |
| 4 | 35.6 | 56.9 | 64.6 | 51.1 | 33.4 | 41.4 | 35.1 | 31.4 |
| 5 | 29.2 | 49.9 | 64.5 | 41.3 | 28.9 | 37.4 | 32.1 | 27.9 |
| $LSD_{0.05}$ | | | 7.6 | | | | 5.7 | |

Combining the two phases, compared to the SF group, growth performance from 5d to about 1250g liveweight was moderately improved in the SS groups, essentially unchanged in the FS groups, and moderately depressed in the FF groups. The similar overall performance in the FS and SF groups may be explained, in part at least, by the relatively high amino acid requirements during the quite early grower phase chosen (625-1250g), than could be adequately met by the moderate protein finisher diet of possibly less than ideal amino acid composition. The improved growth rate from 650g in the FS group given the well-balanced high-protein starter diet thus more than compensated for the early growth advantage to 650g of the SF group.

The effect of the dietary regimes on feed conversion ration (FCR) during the grower phase and on abdominal fat pad expressed as a proportion of liveweight (g/kg) at about 1250g in the five genotypes, is shown in Table 3.

Relative to the SF group, all strains showed a reduction in FCR during the grower phase (i.e. an improvement in feed efficiency), in the three other groups. This was most marked in the FS group where for most strains, FCR was almost halved. There was again considerable between strain variation in the relative FCR between the treatment groups indicating differences in nutrient requirements between the strains.

Abdominal fat was lower in the SS birds than in the SF group in all strains but considerably higher in all groups given the finisher diet in the starter phase (FS and FF). In three of the strains there was more than twice as much fat in the FF groups as in the SF groups.

It would appear that the principal determinant of the much lower FCR of the FS than the SF group birds over the grower phase is the considerably higher growth rate and, hence, shorter growing period of the former group. Differences in body composition would appear to account for very little of the difference in feed efficiency since the FS group birds appear to have deposited at least as much fat over this period as the SF group. Strain differences within feeding regimes, however, show a strong negative association betwee feed efficiency and fat deposition.

Table 3. Food conversion ratio during the grower phase and abdominal fat (g/kg liveweight) at about 1250g liveweight in the five strains in the SF, SS, FS and FF dietary treatment groups.

| Trait | FCR | | | | Abdominal fat | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment<br>Strain | SF | SS | FS | FF | SF | SS | FS | FF |
| 1 | 2.47 | 1.69 | 1.42 | 2.11 | 6.8 | 5.9 | 12.6 | 15.3 |
| 2 | 2.41 | 1.77 | 1.49 | 2.08 | 9.7 | 8.8 | 13.9 | 14.3 |
| 3 | 2.35 | 1.69 | 1.38 | 2.04 | 5.2 | 4.3 | 7.7 | 11.1 |
| 4 | 2.58 | 1.65 | 1.44 | 2.09 | 8.6 | 7.6 | 12.7 | 14.3 |
| 5 | 2.89 | 1.71 | 1.41 | 2.13 | 6.3 | 5.0 | 10.8 | 14.5 |
| $LSD_{0.05}$ | | 0.27 | | | | 3.7 | | |

This study has demonstrated considerable between-strain variation in growth, feed efficiency and body composition both at the end of the starter period and following the period of fattening. This suggests that there may be substantial differences in optimum dietary amino acid concentrations and/or energy:protein ratios for the different commercial strains studied. However, each strain may be following the same rules in dealing with deficiencies and excesses, and it is only possible to determine whether this is the case by comparing the results obtained here with those predicted by means of a simulation model. This experiment was designed in such a way that when the analyses are complete, when the different genotypes used here have been accurately characterised (see Gous *et al.,* 1996) and when the results have been compared with those predicted by the Gous broiler growth model, it will be possible to determine whether there are any significant departures by broiler strains used in Australia from the rules applied in that model.

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# METHIONINE-SPARING EFFECT OF BETAINE FOR BROILERS FED A WHEAT-BASED OR A SORGHUM-BASED DIET

E. VIRTANEN and J. REMUS

Summary

Two experiments were conducted to assess the methionine-sparing effect of betaine in broiler chicks given wheat or sorghum based diets. In Experiment 1, basal wheat-soya rations were supplemented with three levels (0.5, 1.0 and 1.5 g/kg) of either DL-methionine or anhydrous betaine. The basal rations contained 4.7 g/kg of methionine from 0 to 14 days, 4.1 g/kg of methionine from 15 to 28 days and 3.9 g/kg of methionine from 29 to 42 days of age. In Experiment 2, basal sorghum-soya diets were supplemented with 1.2 g/kg (0-35 d) and 1.0 g/kg (36-49 d) of methionine or 0.6 g/kg (0-35 d) and 0.5 g/kg (36-49 d) of betaine. In one treatment, betaine (1.0/0.8 g/kg) was added to the low-methionine basal diet to replace the choline supplement on an equimolar basis.

In Experiment 1, methionine or betaine did not affect 42-day weight gain, mortality, coccidial lesion score or carcass characteristics, but significantly reduced the 42-day feed conversion ratio (FCR). The FCR responses to methionine and betaine did not differ significantly. In Experiment 2, both methionine and betaine significantly increased 49-day weight gain and decreased 49-day FCR. Only betaine decreased the amount of abdominal fat at day 49. Weight gain was similar with methionine and both levels of betaine. FCR was similar with methionine (1.2/1.0 g/kg) and the higher level (1.0/0.8 g/kg) of betaine and slightly poorer with the lower (0.6/0.5 g/kg) betaine supplement.

## I. INTRODUCTION

Pesti *et al.* (1979) demonstrated that the methionine requirement of broiler chicks fed a corn-soya diet was reduced when methyl donors were available in excess. Recently, Virtanen and Rosi (1995) reported that betaine was more efficient than methionine in promoting growth and feed efficiency in chicks when additions were made to a corn-soya basal diet 20-25 % deficient in methionine or 10-15 % deficient in total sulphur amino acids (TSAA).

Since the selection of dietary feed ingredients may affect the methionine-sparing value of betaine, experiments were conducted with wheat-soya and sorghum-soya based diets marginally deficient in methionine. The aim was primarily to compare the relative efficacies of betaine and methionine for broiler production and carcass quality.

## II. MATERIALS AND METHODS

Two experiments were conducted in practical floor-pen conditions.

Experiment 1 was carried out at the Scottish Agricultural College, Auchincruive, UK. In this experiment, 3360 one-day-old male Ross broilers were divided into 56 pens of 1.8 x 2.2 m (15.1 birds per/$m^{2)}$. The experiment was a randomized complete block design consisting of eight rooms, each containing seven pens. The birds were raised to 42 days of age using a daily photoperiod of 23.5 h and a temperature regimen descending from 31° to

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20°C during the first three weeks and, thereafter, constant 20°C until slaughter. Wheat-soybean meal-meat and bone meal based diets were used throughout the trial (for nutritional values, see Table 1). The basal diets were supplemented with 0.5, 1.0 and 1.5 g/kg of either DL-methionine or anhydrous betaine. Salinomycin was used as the coccidiostat. Each bird was allocated 0.50 kg starter crumbles and 1.00 kg grower pellets. Finisher pellets were fed to the birds until day 39 of the trial after which withdrawal pellets were given. Salinomycin was not used in the withdrawal diet.

At the hatchery the chicks were vaccinated against infectious bronchitis. Live infectious bursal disease vaccine was administered via the drinking water at 16 and 23 days.

Each pen of birds was bulk weighed on days 0, 14, 28 and 42. The uneaten feed was weighed on days 14, 28 and 42 to determine feed intake. Dead birds were weighed. On days 35, 36 and 37 one bird was randomly chosen from each pen and assessed for coccidial lesion score in four intestinal areas using a scale of 0-4 as described by Johnson and Reid (1970). On day 42, three birds near the mean body weight for each pen were selected for carcass analysis, including weights of carcass, abdominal fat pad and breast meat.

Statistical analysis of the results was made using mainly the Genstat V Mark 4.03 software package (Genstar 5 Committee, 1987).

Table 1. Calculated nutritional values of the basal diets used in Experiment 1.

| Parameter | Starter | Grower | Finisher/Withdrawal |
|---|---|---|---|
| Metabolizable energy (MJ/kg) | 12.70 | 12.95 | 13.50 |
| Crude protein (g/kg) | 230 | 212 | 196 |
| Methionine (g/kg)[1] | 4.9 | 4.3 | 3.7 |
| TSAA (g/kg) | 8.4 | 7.6 | 6.8 |
| Lysine (g/kg) | 13.5 | 12.8 | 11.0 |
| Choline (g/kg) | 1.8 | 1.6 | 1.5 |

[1]The analysed values for methionine were 4.7, 4.1 and 3.9 g/kg, respectively.

Experiment 2 was run at the Instituto Internacional de Investigación Animal, Queretaro, Mexico. In this experiment, 1280 one-day-old mixed sex Arbor Acres x Arbor Acres broilers were divided into 32 pens of 4 $m^2$ (10 birds per/$m^2$). The experiment was a randomized complete block design with eight replicates per treatment. The birds were raised to 49 days of age using a daily photoperiod of 24 h and ambient temperature. Sorghum-soybean meal-full fat soya-meat meal-corn gluten-canola meal diets were used throughout the trial (for nutritional values, see Table 2). These diets were supplemented with 1.2 g/kg (0-35d) and 1.0 g/kg (36-49d) of methionine or 0.6 g/kg (0-35 d) and 0.5 g/kg betaine (36-49 d) of betaine. In one treatment (1.0/0.8 g/kg) was added to the low-methionine basal diet to replace the choline supplement on an equimolar basis. Nicarbazin was used as the coccidiostat in the starter diet and monesin in the grower and finisher diets. 3-Nitro, Endox and Avotan were used as growth promoters, Avotan in the starter diet only. Feed was provided in the form of crumbles; starter diet from 0 to 21 days, grower diet from 22 to 35 days and finisher diet from 36 to 49 days.

At the hatchery the chicks were vaccinated against Marek's disease. Newcastle disease vaccine was given on days 6 and 42 and vaccination against infectious bronchitis was given on day 12.

Each pen of birds was bulk weighed on days 1, 21, 35 and 49. The uneated feed was weighed on days 21, 35 and 49 to determine food intake. Dead birds were weighed. On day 49, the birds were sacrificed for carcass analysis, including weights of carcass, abdominal fat pad and breast meat.

Statistical analysis of the results was made using analysis of variance.

Table 2. Calculated nutritional values of the basal diets used in Experiment 2.

| Parameter | Starter | Grower | Finisher |
|---|---|---|---|
| Metabolizable energy (MJ/kg) | 12.95 | 13.18 | 13.16 |
| Crude protein (g/kg) | 209 | 205 | 193 |
| Methionine (g/kg) | 4.1 | 3.9 | 3.4 |
| TSAA (g/kg) | 7.6 | 7.3 | 6.8 |
| Lysine (g/kg) | 12.1 | 11.8 | 9.5 |
| Choline (g/kg) | 1.7[a] | 1.7[a] | 1.5[b] |

[a]Choline-chloride-60% added at 0.9 g/kg.
[b]Choline-chloride-60% added at 0.7 g/kg.

## III. RESULTS

In Experiment 1, no significant responses in body weight and feed intake were seen, although numerically both methionine and betaine tended to increase body weight and decrease feed intake (Table 3). FCR was significantly affected in a similar way by methionine and betaine. The 42-day mortality, 35-day lesion score and the carcass characteristics were not significantly affected by the treatments.

In Experiment 2, both methionine and betaine significantly improved weight gain and feed efficiency (Table 4). Replacing the 1.2/1.0 g/kg methionine addition with 0.6/0.5 g/kg betaine produced similar growth but slightly worse feed conversion. When choline was replaced with an equimolar amount of betaine, (Treatment 4), weight gain and feed conversion were similar to the diet adequate in methionine. Both betaine treatments, but not methionine, decreased the size of the abdominal fat pad. No significant treatment effects were seen with mortality or percent breast meat.

Table 3. The effect of increasing levels of methionine or betaine upon the 42-day body weight, feed intake and mortality-adjusted feed conversion ratio of male broilers.

| | | Level (g/kg) | | | | Methionine vs betaine response |
|---|---|---|---|---|---|---|
| Parameter | Compound | 0.0 | 0.5 | 1.0 | 1.5 | |
| Body weight (kg) | Methionine | 2.21 | 2.26 | 2.31 | 2.26 | NS |
| | Betaine | | 2.22 | 2.28 | 2.26 | |
| Feed intake (kg) | Methionine | 4.95 | 4.83 | 4.90 | 4.70 | NS |
| | Betaine | | 4.93 | 4.86 | 4.86 | |
| FCR (g:g) | Methionine | 2.116[a] | 2.051[ab] | 2.036[ab] | 1.977[b] | NS |
| | Betaine | | 2.086[ab] | 2.034[b] | 2.026[b] | |

a,b,ab - Means in the same row with different superscripts are significantly different ($P<0.05$). The overall responses in body weight and feed intake were not significant.

Table 4. Effect of methionine and betaine on 49-day weight gain, feed intake, feed conversion ratio and percentage of abdominal fat in broilers fed a low-methionine basal diet with (Treatments 1-3) or without (Treatment 4) supplementary choline.

| Treatment | Weight gain (g/d) | Feed intake (g/d) | FCR (g:g) | Fat pad, (g/kg l'wt) |
|---|---|---|---|---|
| 1. Basal | 40.93[a] | 87.03[a] | 2.126[a] | 2.78[a] |
| 2. Basal + 1.2/1.0 g/kg methionine | 42.46[b] | 86.37[a] | 2.034[c] | 2.72[a] |
| 3. Basal + 0.6/0.5 g/kg betaine | 42.05[b] | 87.88[a] | 2.089[b] | 2.24[b] |
| 4. Basal - choline + 1.0/0.8 g/kg betaine | 42.03[b] | 86.95[a] | 2.049[c] | 2.21[b] |
| Significance of treatment effect | $P<0.03$ | $P<0.67$ | $P<0.02$ | $P<0.10$ |

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

## IV. DISCUSSION

In these experiments, a somewhat higher basal level of methionine was used compared to Pesti *et al.* (1979) and Virtanen and Rosi (1995). However, in relation to dietary protein the level of TSAA in the basal diets was similar at about 3.5 % of crude protein. In both experiments, methionine addition resulted in significant improvements (3-7 %) in feed efficiency, indicating that the basal diets were deficient in methionine.

The data indicate a methionine-sparing effect of betaine with wheat-soya and sorghum-soya diets similar to that seen earlier with corn-soya diets. While corn-soya diets have consistently shown betaine to be markedly more efficient than methionine in promoting growth and feed efficiency with diets 20-25 % deficient in methionine, the present results indicate less difference in the efficacy, especially with wheat-based diets. With the sorghum-based diet, replacement of 1.2/1.0 g methionine/kg with half these amounts of betaine resulted in slightly worse feed conversion but a lower body fat. Interestingly, when the supplementary choline was replaced with betaine, feed efficiency was improved to the same level as with the high-methionine diet and abdominal fat remained similar to that observed with the lower betaine supplement. This indicates a higher requirement for methyl donor activity, since betaine is reported to be a more efficient methyl donor than choline in the chick (Stekol *et al.*, 1957).

It is interesting to note that betaine had a more pronounced lipotropic effect than methionine with the sorghum-based, but not with the wheat-based, diet. The dietary dependency of the responses may be due to differences in the methionine/cystine ratio and in the availability of sulphur amino acids and methyl donors. In addition to diets, both genetic and environmental factors, such as the level of coccidiosis challenge, may also have affected the responses.

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# NUTRITIONAL EVALUATION OF ENZYME PROCESSED FEATHER PROTEIN

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The objective of the research was to produce a feather protein containing high levels of digestible amino acids using enzyme processing methods. Conventional steam-hydrolysed feathermeal may contain only low levels of digestible amino acids even if the *in vitro* pepsin digestibility is high (Latshaw, 1994).

From a common source of raw broiler feathers (approximately 30% DM) two products were manufactured using steam jacketed pressure cookers incorporating internal mixing paddles. Conventional feather meal was produced by processing feathers at 40 pounds per square inch (psi) (145°C) for 60 min, followed by drying at 100°C. Enzyme processed feather protein was produced by processing the feathers at 50°C for 60 min with an enzyme package added (Woodgate, 1994); pressure was raised to 25 psi (125°C) for 20 min followed by drying at 100°C in a cooker at atmospheric pressure.

Samples were assayed for crude protein, pepsin digestible protein (PDP) using the *in vitro* AOAC (1990) method, amino acid content and *in vivo* amino acid digestibility using the technique of McNab and Blair (1988). Digestible amino acid content was calculated for all amino acids. A summary of the results is shown in the Table.

| | Conventional feather meal | Enzyme processed feather protein |
|---|---|---|
| Protein (g/kg sample) | 850 | 844 |
| PDP (g/kg protein) | 732 | 668 |
| *In vivo* amino acid digestibility coefficient | 0.67 | 0.85 |
| Digestible amino acids (g/kg) | | |
| Lysine | 8.6 | 13.9 |
| Methionine | 3.5 | 5.4 |
| Cystine | 17.3 | 37.1 |
| Threonine | 17.6 | 27.6 |

The data presented shows that enzyme processed feathers contained higher levels of digestible amino acids than conventional feathermeal. The divergence between *in vivo* and *in vitro* data is particularly important as it indicates that routine *in vitro* analyses such as PDP can give an unrealistic evaluation of either conventional or enzyme processed feather products.

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