Proceedings of

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

1994

Volume No. 6

University of Sydney, Sydney, NSW

February 1994

AN ANNUAL SYMPOSIUM ORGANISED BY

THE POULTRY RESEARCH FOUNDATION,

UNIVERSITY OF SYDNEY

AND

THE WORLD'S POULTRY SCIENCE ASSOCIATION

(Australian Branch)

ISSN NO. 1034-6260

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

1994

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The Organisers wish to thank the Chicken Meat Research and Development Council and the Egg Industry Research and Development Council for contributing financially to the attendance of Drs. Hardy Edwards and Regina Vasilatos-Younken. Also we wish to thank Rhône-Poulenc Animal Nutrition and Alltech Inc. for joint sponsorship of Dr Colin Whitehead.

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Papers presented at this Symposium have been edited in a normal refereeing process by the Editorial Committee. Authors were responsible for the final proof reading of their respective papers.

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CONTENTS

SHORT PAPERS

EFFECT OF CAECECTOMY ON EXCRETA AMINO ACID DIGESTIBILITY IN ADULT COCKERELS

K. ANGKANAPORN and W.L. BRYDEN

The use of excreta to determine amino acid digestibility has been disputed because of the influence of microflora in the hind-gut (Raharjo and Farrell, 1984). Caecectomy has been the technique used to overcome this problem but there are few studies that have examined the influence of caecectomy on amino acid digestibility in birds fed different diets. The influence of caecectomy on amino acid digestibility is likely to depend on the diet fed and presumably reflects the quantities of undigested dietary proteins that pass into the caecum. Microbial activity in the caecum releases amino acids which are either deamminated or absorbed thus changing apparent amino acid digestibility. Amino acid uptake has been observed in the proximal caecum of chickens *in vitro* (Moreto and Planas, 1989).

In the present studies 12 caecectomised and 12 intact adult cockerels were used. In the first trial, they were fed either a normal broiler (CP 220 g/kg) or a normal layer diet (CP 170 g/kg) for 7 days and total excreta collected. In a second study the caecectomised and intact cockerels were fed a semipurified diet based on maize starch with either cottonseed (CSM) or soyabean (SBM) meals (CP 200 g/kg) as the sole source of protein. In the final trial, cockerels were starved for 48 h or fed a nitrogen-free diet. The excreta were collected, hydrolysed and analysed for amino acids.

The digestibility of a normal broiler diet was significantly reduced ($P < 0.05$) in the caecectomised as compared to intact cockerels. This was reflected in a decreased amino acid output in intact birds. In contrast, when a normal layer diet was fed the digestibilities were the same. Intact fasted cockerels excreted more amino acids ($P < 0.01$) than caecectomised birds. The quantities of amino acids excreted after feeding the protein-free diet did not differ between intact and caecectomised birds. When a semipurified diet containing cottonseed meal was fed, intact birds had a slightly enhanced apparent digestibility and excreted a significantly smaller amount of amino acids than caecectomised birds. The apparent digestibility and excreta amino acid output when a semipurified diet containing soyabean meal was fed was similar in both intact and caecectomised cockerels. From the present results, caecectomy reduces digestibility of dietary protein with a resultant increase in excreta amino acid loss. The improvement in digestibility noted in intact birds will only be of benefit to the bird if the amino acids are released and absorbed in a form that can be utilised.

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AME DETERMINATION AND ITS APPLICATION TO LUPINS

G. ANNISON*, M. CHOCT* and R.J. HUGHES**

Summary

Increasing levels of casein were added to maize and sorghum basal diets to determine whether casein AME values were affected by its level of addition. The AME value of casein was independent of level of addition (134-314 g/kg). The mean value was 20.1 MJ/kg DM. Using the same assay system the AME of lupins was determined at three levels (100, 200 and 300 g/kg) of inclusion in a basal sorghum/casein diet. The AME was calculated to be 10.26 MJ/kg DM. There was no indication of anti-nutrients effecting energy metabolisabilty being present in the lupins.

I. INTRODUCTION

The AME assay is used to determine the nutritive value of single ingredients to provide information which can be used in dietary formulation. Many rapid techniques have been developed (single dose feeding, rapid broiler assays, adult cockerel assays etc.) but if speed is not a prime consideration the classical AME assay over a period of days is the best technique. The birds eat normal amounts of feed and there is no starvation. One disadvantage is that for single ingredient assays the bird may be consuming an unbalanced diet. These considerations have led to trial diets containing vitamins, minerals and casein (a highly digestible protein) as well as the test ingredient. The AME of dietary components are assumed to be additive and thus if the AME of casein is known the AME of the test ingredient can be calculated. This procedure works well for ingredients such as cereals which are low in protein. For legumes, and particularly those devoid of starch (such as lupins), the method is unsuitable as test diets would contain high levels of protein and thus the protein:energy ratio would be far from ideal. This would lead to protein being used as energy and excretion of high levels of nitrogenous compounds which would reduce the AME value.

Lupins are used as a protein source in broiler diets. There is anecdotal evidence that even at moderate levels of inclusion (< 10%) performance suffers. Lupins contain oligosaccharides (7-8% DM) and non-starch polysaccharides (35-40% DM). There is little evidence from the literature however that these components have anti-nutritive activities although the non-starch polysaccharides of cereals are known to have detrimental effects in broiler chicken diets (Annison and Choct, 1991).

This paper describes studies investigating 1) the effect of increasing casein in cereals diets to determine whether high protein levels depress AME values provided by the assay and 2) the application of the AME assay to lupins at different levels in broiler diets to determine whether there is evidence of compounds which inhibit nutrient utilisation.

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II. MATERIALS AND METHODS

(a) Bird Husbandry, experimental diets and AME trials.

Day-old mixed-sex broilers were fed commercial rations until 28 days of age when they were transferred to individual metabolism cages. Birds were randomly assigned to treatment groups of 6-12 birds for the AME determination of trial diets. Major ingredients (cereal, casein, lupins) levels in diets were as detailed in Tables 1 and 2. The minor ingredients levels were (g/kg):- dicalcium phosphate (26); calcium carbonate (11); vitamin premix (5), sodium chloride (3.6); choline chloride (0.4). The birds were fed the cold pelleted diets for 7 days (adaptation period, 3d; collection period, 4d). The excreta voided in the collection period were saved. Gross energy of diets and excreta were determined and AME calculated.

(b) Experiment 1. Determining the AME of casein

Three AME trials were carried out to determine the AME of casein. Trial diets contained casein (0, 134, 204 and 314 g/kg) replacing either maize or sorghum. Minor ingredients (46 g/kg total) were kept constant.

(c) Experiment 2. Determining the AME of lupins.

Five trial diets, including a maize control (Diet 4A; maize 820g/kg; casein 134g/kg) were prepared with de-hulled lupin meal replacing sorghum and casein in a sorghum basal control diet (Diet 4B; sorghum, 820 g/kg; casein, 134 g/kg) at levels of 100, 200 and 300 g/kg. Minor ingredients were unchanged across diets. The AME of the diets were determined and the contribution of the lupins was calculated.

(d) AME calculations

AME values of the ingredients were assumed to be additive with the minor ingredients providing no energy. The dry matter of all ingredients and diets was determined and the actual inclusion level (ie following corrections for dry matter) were used. For clarity these corrections have not been included in the expressions below.

AME_{diet} = (energy intake - energy excreted)/ kg diet

= $(feed_{in} \times GE_f) - (ex_{out} \times GE_{ex})$/kg diet

$feed_{in}$ = feed intake, ex_{out} = excreta output

GE_f, GE_{ex} = Gross energies of feed and excreta

AME of casein in the each of the diets was calculated using the following expressions (Experiment 1).

$AME_{cas.diet} = (AME_{bas.diet} \times IL) + (AME_{casein} \times IL)$

thus

$AME_{casein} = \{AME_{cas.diet} - (AME_{bas.diet} \times IL)\}$/casein IL

cas.diet, bas.diet = casein diet, basal diet; IL = inclusion level.

The AME of the lupins (Experiment 2) was also calculated using the above expressions except the lupin levels were used rather than the casein. The sorghum/casein diet (Diet 4B) was used as the basal diet in this case.

III. RESULTS

(a) Experiment 1

The AME of the diets increased with increasing levels of casein (Table 1). The diets in the second trial had lower AME values than those in the first although in both the maize was the basal cereal. Thus the calculated AME of casein was lower in the second trial with values of 18.44-19.24 MJ/kg DM compared to 21.39-22.35 MJ/kg DM. In the third trial AME values of casein ranged from 19.01-20.14MJ/kg DM. The overall mean was 20.1 MJ/kg. DM.

Table 1 The AME of casein determined in three feeding trials in Experiment 1. Standard errors are in brackets.

Diet *(n=)*	Cereal (g/kg)	Casein (g/kg)	AME diet[1] (MJ/kg DM)	AME Casein[1] (MJ/kg DM)
Maize 1A (8)	954	0	15.05 (0.14)	-
Maize 1B (8)	820	134	15.94 (0.07)	22.35 (0.48)
Maize 1C (6)	750	204	16.32 (0.17)	21.94 (0.66)
Maize 1D (8)	640	314	16.82 (0.10)	21.39 (0.30)
Maize 2A (6)	954	0	15.19 (0.09)	-
Maize 2B (6)	820	134	15.52 (0.14)	18.44 (1.00)
Maize 2C (6)	750	204	15.81 (0.16)	19.00 (0.75)
Maize 2D (6)	640	314	16.23 (0.12)	19.24 (0.38)
Sorghum 3A (6)	954	0	14.58 (0.11)	-
Sorghum 3B (6)	820	134	15.24 (0.15)	20.14 (1.06)
Sorghum 3C (6)	750	204	15.35 (0.15)	19.01 (0.71)
Sorghum 3D (6)	640	314	15.81 (0.12)	19.43 (0.38)

AME_{casein} from the means of determinations = 20.1 MJ/kg.DM.

(b) Experiment 2

Adding lupin meal to the basal diet depressed the dietary AME ($P < 0.05$, Table 2). Although the AME of Diet 4C was significantly higher than Diets 4D and 4E the calculated AME values of the lupins were not significantly different.

Table 2 AME of lupin containing diets and controls and calculated AME value of the lupins.

Diet *n=9*	Cereal (g/kg)	Prot. Source (g/kg)	AME diet (MJ/kg DM)	AME Lupins (MJ/kg DM)
Diet 4A	820 (maize)	134 (casein)	15.49[a] (0.15)†	-
Diet 4B	820 (sorghum)	134 (casein)	15.29[a] (0.27)	-
Diet 4C	854 (sorg/cas)[1]	100 (lupin)	14.69[b] (0.11)	11.07 (1.01)
Diet 4D	754 (sorg/cas)	200 (lupin)	13.78[c] (0.22)	8.97 (1.09)
Diet 4E	654 (sorg/cas)	300 (lupin)	13.59[c] (0.24)	10.75 (0.81)

AME_{lupins} from the means of determinations = 10.26 MJ/kg DM.

[1] Sorghum:casein in ratio of 820:134 replaced by lupin meal
[a,b] Values not sharing a superscript are significantly different at $P<0.05$.
† Standard errors in brackets

Table 3 Linear model (y=ax + b) for describing AME (y, MJ/kg DM) dependence on lupin inclusion level (x; g/kg)

Parameter	Estimate	SE
intercept	15.9771	0.1936
slope	-0.00631	0.0103

IV. DISCUSSION

Experiment 1 demonstrated that increasing dietary protein considerably above the requirements of the chicken did not significantly effect the calculated casein AME value as no relationship between casein level and AME value was observed. If nitrogenous excretions had increased greatly the AME of casein would have decreased with inclusion level. The reason for the difference in the casein value obtained when maize was used (Trials 1 and 2) is unclear. It is not due to the basal AME value obtained for the maize (from which the casein values are calculated). The cause can only be due to a general increase in organic matter digestibility but there are no obvious reasons for the variation between groups of chickens held at different times as they were sourced from the same supplier and husbandry practices were uniform. The mean value for the AME of casein was 20.1 MJ/kg.DM. Values from the United States-Canadian Tables of Feed Composition and the NRC are 19.01 and 18.58 MJ/kg. DM respectively. This type of assay can be used to assess the AME of other protein sources at similar levels without concerns for the possible effects on AME of raising the protein levels above the requirements of the birds. The reproducibility of the assay can be high. Diets with the same composition being fed in different trials all being within 0.5 MJ/kg DM and several much closer (compare Diets 1A and 2A, 2B and 4A, 3B and 4B) but as can be seen by comparing the Trial 1 with Trial 2 reproducibility can on occasions be poor. This aspect

of AME methodology needs to be investigated.

The AME of lupins was assayed by replacing the sorghum and casein components of a basal diet. This approach maintained protein levels at the lowest levels of lupin (100 g/kg). The data indicate that the lupins have a lower energy value than the sorghum basal diet which is to be expected from their composition. As there is no significant difference in the calculated AME values for the lupins as inclusion levels increase there is no indication of anti-nutrients being present which effect energy metabolisability. There is a weakness, however, in this type of approach. The AME of the lupins is calculated by subtracting the AME of the diet from the AME calculated to be contributed by the sorghum:casein portion. Since this is a constant the variability in the diet AME data is transferred to the component associated only with the lupins. This results in very large variations in the calculated value for the lupin AME which is reflected in the very large standard errors shown in Table 2. An alternative approach is to regress the AME data against the lupin inclusion levels (0, 100, 200, 300 g/kg) and extrapolate the data to a level of 1000 g/kg lupins. This regression is presented in Table 3 and AME value calculated for lupins is 9.67 MJ/kg DM. This value agrees closely with values obtained by Johnson and Eason (1991). This method is probably more accurate but requires greater resources and thus is not appropriate for use with large numbers of samples. For routine assays of AME single diets with levels at 300 g/kg should give acceptible results.

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Proc. Aust. Poult. Sci. Sym. (1994) 6

WELFARE OF LAYING HENS IN ONE- AND TWO-BIRD CAGES

J.L. BARNETT

Summary

An experiment is described to determine the most suitable criteria that can be used to assess welfare in modified cages. Physiological data indicated a chronic stress response in 1-bird cages compared to 2-bird cages and these data did not correlate with changes in feather condition. The preliminary data suggest that social behaviour may be an important determinant of welfare, determined by physiological parameters, and that feather condition, while obviously affecting the cosmetic appearance of the bird, may have few implications for welfare.

I. INTRODUCTION

A number of criteria have been used to assess welfare in poultry, including lower levels of physiological stress (as evidenced by lower plasma corticosterone concentrations 'at rest' and in response to ACTH, a decreased heterophil to lymphocyte ratio and an increased immune responsiveness), reduced aggression, improved plumage, foot and claw condition and increased bone strength (Craig and Adams, 1984; Craig *et al.*, 1986; Gregory *et al.*, 1991; Tauson, 1986; Appleby, 1993). Thus, to assess welfare of poultry in modified housing systems, it is necessary to have one or more control treatments which provide a point of reference for the different welfare criteria which must firstly be defined. To achieve these objectives, a preliminary experiment was undertaken involving a number of measurements in different housing systems using two control treatments. One control treatment was hens in the top tier of a two-tier battery (Top Tier Control); this treatment was chosen to assess the magnitude of any chronic stress response since higher corticosterone concentrations (indicative of a stress response) occur in this treatment compared to the lower tier of a battery (Barnett and Hemsworth, 1989). The other control treatment was floor pens (Pen Control) in which an attempt was made to optimize the environment, in terms of space, number of nests, etc. to result in good condition of plumage, claws and feet and high bone strength, which have generally been found to be better in non-cage systems.

Thus, for welfare to be improved, hens in modified cages should have a stress response, including plasma corticosterone concentrations, that are closer to those found in the Lower Tier conventional cages than the Top Tier and Pen Controls (since the latter two treatments are known to result in elevated corticosterone concentrations). Similarly, for improved welfare, plumage, foot and claw condition and bone strength measurements need to be closer to those found in the Pen Control than the Top Tier Control treatment.

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II. MATERIALS AND METHODS

The 4 treatments were: 1. Top Tier treatment. Hens were housed 2 hens/cage in cages measuring 32 x 47 x 43 cm (w x d x h) in the top tier of a battery system in a total of 72 cages in banks of 6 cages. The 2 outer cages per bank contained non-experimental hens. Thus there were 48 experimental cages containing 96 hens and 24 non-experimental cages containing 48 birds. 2. Pen treatment. Hens were housed in 6 deep litter pens containing 10 hens/pen. The pens were 2.5 x 2.5 m, provided with nest-boxes and perches and separated by solid sides to minimize feather damage. 3 and 4. Bottom Tier treatments. Hens were housed either 1 (treatment 3) or 2 (treatment 4) hens/cage in a total of 72 cages. The arrangement of banks of cages was as in Treatment 1. There was a total of 24 experimental cages containing 2 hens/cage and 24 experimental cages with 1 hen/cage.

Tegel Tint hens (Parafield Poultry Research Centre) were housed in the treatments at 16 weeks of age. The hens were sampled at 35 and 60 weeks of age for behavioural, physiological and condition parameters. At the end of the experiment birds were killed and bones removed for assessment of shear strength. Only data for the physiological and condition measurements are presented.

(a) Measurements and statistical analysis

Blood samples were taken from the wing vein over 2 days from one hen/cage from 12 cages in Treatments 1, 3 and 4 and from 4 hens/pen from 3 pens in Treatment 2. The blood samples (about 2 ml) were collected within 45 sec of opening the pen/cage and subsequently assayed for corticosterone concentrations using a commercial diagnostic kit following extraction of the lipids (Immunodiagnostic Systems Ltd., UK), and a blood smear was prepared for subsequent determination of heterophil/lymphocyte ratio. Eight days later the same hens were given an intramuscular injection of 50 IU of ACTH (Synacthen, Ciba Geigy) and the corticosterone response was determined from a blood sample collected 45 min post-injection (Barnett and Hemsworth, 1993). Eleven days later the same hens were tested for cell-mediated immunity (Regnier and Kelly, 1981; Blecha *et al.*, 1983) by injecting 250 μg of leucoagglutinin in 0.25 ml saline into the left wattle and measuring wattle thickness prior to and 24 h post-injection with a pressure-sensitive micrometer. The increase in wattle thickness was used as a measure of the reactivity of the immune system. Three days after the first blood sample the birds were assessed for feather damage using a subjective 4 point scoring system applied to the neck, breast, back, wings and tails (Tauson *et al.*, 1984). Score 4 was for birds having very good plumage with none or few worn or deformed feathers (both birds in 2-bird cages and all birds in 3 floor pens were assessed). Score 1 was for a part of the body with heavy damage with no or only very small areas being covered with feathers. The data were analyzed by analysis of variance.

III. RESULTS AND DISCUSSION

The experiment was able to distinguish between treatments, particularly

Table 1 Effects of housing treatment (1: 2 birds/cage top tier, 2: pens, 3: 1 bird/cage lower tier and 4: 2 birds/cage lower tier) on corticosterone concentrations (nmol l^{-1}), immune response (% increase in wattle thickness in response to a mitogen), heterophil/lymphocyte (H/L) ratios and feather condition (mean of measurements at 35 and 60 weeks of age; N=12/treatment/age class for physiological measurements and N=12 and 3 for feather condition in cages and pens, respectively)

Parameter	Treatment				Age (weeks)		LSD	
	1	2	3	4	35	60	Tmt[1]	Age
Corticosterone 'at rest'[2]	1.37^{ac} (3.43)	1.63^{bc} (4.56)	1.54^{bc} (4.19)	1.20^{a} (4.11)	1.51 (3.30)	1.37	0.26	0.19
Corticosterone response to ACTH	124.3	140.4	146.6	113.4	137.6	124.8	35.1	24.8
Immune response	177.2^{b}	169.8^{b}	138.9^{a}	170.0^{b}	175.3^{y}	152.7^{x}	29.2	20.6
H/L ratio[3]	0.60	0.59	1.35	0.63	na	0.79	0.9	na
Feather condition								
Total plumage[4]	3.02^{a}	3.53^{b}	3.40^{b}	3.08^{a}	3.47^{y}	2.92^{x}	0.139	0.069
Total plumage[5]	3.02^{a}	3.30^{b}	3.40^{b}	3.07^{a}	3.46^{y}	2.89^{x}	0.138	0.068
Neck	2.70^{a}	3.55^{b}	3.33^{b}	2.88^{a}	3.27^{y}	2.76^{x}	0.329	0.163
Breast	3.10^{a}	3.43^{b}	3.63^{c}	3.21^{ab}	3.71^{y}	2.94^{x}	0.186	0.146
Back	3.42^{b}	3.18^{a}	3.50^{b}	3.35^{b}	3.82^{y}	2.99^{x}	0.161	0.126
Wings	3.02^{a}	3.52^{b}	3.50^{b}	3.08^{a}	3.42^{y}	3.03^{x}	0.180	0.090
Tail	2.88^{a}	3.97^{c}	3.04^{b}	2.85^{a}	3.12^{y}	2.89^{x}	0.234	0.116
Base of tail	3.00^{b}	2.13^{a}	3.42^{b}	3.04^{b}	3.40^{y}	2.75^{x}	0.463	0.230

[1]maximum $LSD_{(P=0.05)}$ value for pen versus cage comparisons; [2]statistics on $\log_e(x+1)$transformed data with untransformed data in parentheses; [3]data from 60 weeks of age only; [4]includes neck, breast, back, wings and tail; [5]includes neck, breast, back, wings, tail and base of tail; [abc and xy]different letters denote a significant difference at $P < 0.05$ for treatment and age effects, respectively.

between 1-bird cages, 2-bird cages and floor pens on the basis of corticosterone concentrations 'at rest' and immunological responsiveness and plumage condition (Table 1). The data suggest that corticosterone concentrations are higher in floor pens than in cages with more than 1 bird, which is in general agreement with the literature. While the higher corticosterone response to ACTH in the floor pen treatment (Treatment 2) could indicate a chronic stress response in this treatment, compared to 2-bird cages (Treatments 1 and 4) the differences are not statistically different and the immunological data also indicate no differences between floor pens and 2-bird cages. These data suggest that the increased corticosterone concentrations in floor bird pens are not indicative of a chronic stress response.

The data indicate that birds in 1-bird cages with a space allowance of 1504 cm^2/bird (Treatment 3) may be chronically stressed compared to similarly housed birds in 2-bird cages with a space allowance of 752 cm^2/bird (Treatment 4). Overall corticosterone concentrations were higher ($P < 0.05$) and the measure of cell-mediated immunity was lower ($P < 0.05$). Furthermore, ACTH response and H/L ratios were higher in 1-bird cages, although the differences were not statistically different ($P > 0.05$). These results suggest that, at the space allowances used in the present experiment, the social environment may be more important to welfare than the physical environment. This finding clearly warrants further investigation.

As expected overall feather condition was scored higher in the floor pens (Treatment 2) than the cages (Table 1). At 60 weeks of age the mean score for the neck, breast, back, wings and tail was 2.8, 3.2, 3.1 and 2.1, for treatments 1-4, respectively. However, while birds in the floor pens had full tails (mean scores were 2.8, 3.9, 3.0 and 2.7, respectively) the area around the base of the tail was in a poor condition in this treatment (mean scores were 2.8, 1.6, 3.0 and 2.7 for treatments 1-4, respectively). Feather condition was better in 1-bird than 2-bird cages. The mean values for birds from the lower tier (Treatments 3 and 4) were 3.0 and 2.7 for the neck, 3.3 and 2.8 for the breast, 3.1 and 3.0 for the back and wings and 3.0 and 2.7 for the tail, respectively.

While the physiological data indicate a chronic stress response in the 1-bird cages (Treatment 3) compared to the 2-bird cages (Treatment 4) these data do not appear to be correlated with the plumage condition data. While plumage condition and cover will affect thermoregulation and hence feed intake and production efficiency, provided that feed and appetite are not limited, feather condition may not be a good indicator of welfare. Feather condition obviously affects the cosmetic appearance of the bird but it may have few implications for welfare.

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Proc. Aust. Poult. Sci. Sym. (1994) 6

XYLANASE SOURCE, ACTIVITY AND INFLUENCE ON THE RESPONSE OF BROILERS WHEN FED WHEAT BASED DIETS

M.R. BEDFORD

Summary

Two xylanase sources were included in a wheat/triticale based diet at 4 levels of inclusion (0,1000, 3000 and 5000 U/kg feed). Weight gain, feed conversion and intestinal viscosity responded to increments in dietary xylanase in a quadratic fashion, there being an optimum at approximately 3000U for feed conversion and viscosity for both xylanases. The two enzymes were significantly different, however, in terms of gain and feed conversion but not viscosity. The data suggest that whilst viscosity reduction is important for improved performance, the source of the enzyme that can elicit such a response will also impact on the magnitude of the performance enhancement. It is suggested that excessive release of free 5-C sugars may be responsible for compromising the beneficial effects of an enzyme preparation.

I. INTRODUCTION

Viscous arabinoxylans have been identified as a contributing factor to the restriction of and variability in the utilisation of wheat by broiler chickens. (Annison and Choct, 1991; Bedford and Classen, 1992). High viscosity reduces the efficiency of digestion by reducing the rate of diffusion of solutes (Fengler and Marquardt, 1988) and changing both the quantity and species dominance of the microfloral population (Feighner et al, 1988). Quantification of the negative effects of feeding wheat and rye-based diets by Bedford and Classen (1992) indicated that as much as 60-70% of the variation in body weight and feed efficiency could be described by intestinal viscosity alone. Reduction of intestinal viscosity through use of exogenous xylanases has been demonstrated as an effective method for improving growth and feed utilisation by the broiler chick (Bedford et al 1991, Bedford and Classen, 1992).

The activity of a xylanase preparation is most often assayed using a reducing sugar method, whereas the enzyme is expected to perform at intestinal level by reduction of viscosity. It was the purpose of this trial to determine the relationship between intestinal viscosity and broiler performance when fed a wheat/triticale based diet supplemented with varying levels of one of two xylanases from different sources.

II. MATERIALS AND METHODS

Day old, Ross 1 male broiler chicks were fed on a wheat, triticale and soybean meal (48%) based diet (49.40, 15.0 and 28% respectively; supplying 12.71 MJ AME/kg and 224 g crude protein/kg) formulated to contain either 0, 1000, 3000 or 5000 units (u) of xylanase from two *Trichoderma Sp.* sources. Liveweight gain and feed intake were measured and feed efficiency (FCR, g feed/g gain) calculated at 3 weeks of age. There

Finnfeeds International Ltd, Ailesbury Court, Marlborough, Wilts. UK. SN8 1DH

were 10 replicate cages of 7 birds per replicate, all measurements being made on an entire cage basis.

At 21 days of age, 6 birds per treatment were sacrificed and their intestinal contents assayed for viscosity by the method of Bedford et al (1991).

Data were subjected to ANOVA according to the GLM procedure of SAS. The optimum concentration of each were predicted using the RSREG facility and the covariates feed intake and initial weight incorporated into the model where appropriate (weight gain, FCR and Feed intake).

III. RESULTS

The results (Table 1) indicate a benefit to xylanase inclusion on FCR and weight gain ($p=0.11$), and a trend towards improvement in viscosity ($p=0.14$). Feed intake was not influenced by xylanase inclusion level.

Differences between the two xylanase sources where detected. Xyl A significantly outperformed Xyl B with respect to weight gain ($p=0.008$) at all levels of inclusion. There was no predicted optimum for weight gain for Xyl A since the effect was for a linear increase in gain with xylanase inclusion level. Xyl B gave a predicted optimum at 2874 U with a final gain of 562 g.

FCR was optimized at approximately 3000U xylanase whether it was from Xyl B or Xyl A. The predicted optimum from Xyl A was 1.633 whereas that from Xyl B was 1.691. Since the response was quadratic, it indicates that there is to some degree a negative effect with feeding 5000U xylanase activity or higher.

Feed intake was not significantly influenced by either enzyme type or level, and as such no optimum could be predicted.

Intestinal viscosity was low, even in the control birds, which leaves little room for improvement. Nevertheless, there was a tendency for viscosity reduction due to increasing enzyme activity (no difference between the enzyme sources), with both enzymes yielding a similar predicted minimum viscosity at very similar activities.

Table 1 Effect of xylanase type and inclusion level on gain, FCR, feed intake and intestinal viscosity of broilers at 21 days of age.

Xylanase	Activity added (U/kg feed)	Gain (g)	FCR (g/feed/ g gain)	Feed Intake (g)	Viscosity (cps)
-	-	529	1.761	932	4.95
A	1000	541	1.726	935	3.42
A	3000	559	1.612	902	3.35
A	5000	566	1.673	945	3.72
B	1000	523	1.737	905	3.86
B	3000	545	1.695	925	3.82
B	5000	517	1.695	925	4.19
Pooled SD		37	0.115	63	1.05

The statistics (p-values) relating to all data are presented in the Table below.

Table 2 P-values relating independent effects to the dependents gain, FCR, feed intake and intestinal viscosity

	Gain	FCR	Feed Intake	Viscosity
Statistical effects				
Feed intake	0.0001	0.5577		
Initial weight	0.9153	0.9314	0.0611	
Enzyme type	0.0076	0.0309	0.5665	0.8564
Enzyme level	0.1060	0.0300	0.5334	0.1400
Type x level	0.2847	0.4104	0.4889	0.5144
Optima (value in brackets)				
Xylanase B Opt	2874 (562)	2695 (1.691)	NONE	2958 (3.85)
Xylanase A Opt	NONE	3370 (1.633)	NONE	3152 (3.99)

The correlation between intestinal viscosity and FCR is shown below. The equation for the relationship between FCR and viscosity is;

$$FCR = 1.467 + 0.062 \text{ x} \quad \text{(Whole model } p=0.007)$$

$$R^2 = 0.4638$$

IV. DISCUSSION

Xylanase addition to wheat based diets has previously been shown to reduce intestinal viscosity (Bedford and Classen, 1992) and as a result improve the FCR and gain of broilers at 21 days of age (Bedford and Classen, 1992;). However, the degree of response is quite variable and may depend upon the age of the bird (Petersen et al, 1993), the sample of wheat (includes varietal and climactic variations) and the quantity of enzyme used. This study indicates that when two different sources of xylanase are supplemented at similar inclusion rates into the same diet, the degree of improvement in performance was not constant. There were no significant differences between the two xylanases in their ability to reduce intestinal viscosity, which indicates that functionally they are equivalent.

However the performance of the birds did not relate well to the viscosity reduction when all data are combined. Previous work has indicated a very good relationship between viscosity and performance (Bedford and Classen, 1992, Bedford and Classen 1993), although in these studies a much wider range in intestinal viscosities was achieved. Nevertheless, this indicates that there are factors other than intestinal viscosity that mitigate the response achieved. Separating the xylanases gives a clearer picture, in that viscosity reduction for xylanase A results in a better performance enhancement than that of xylanase B. Two possibilities are suggested to explain this observation. Firstly the response may not be viscosity related. Xylanase A may have elicited a further beneficial response by increasing cell wall perforation and thereby increasing digestive enzyme access to endosperm cell contents. The fact that fat digestion is significantly enhanced by supplementation of rye (Campbell et al,

1983) and barley based diets with the relevant viscosity reducing enzymes has indicated that this may not be a significant route in high viscosity diets, although with the low viscosities encountered in this study such a mechanism cannot be ruled out.

Secondly, the main difference between the two preparations utilised in this study is that xylanase A does not release as many free sugars as xylanase B. This results in far less free xylose and arabinose, sugars which are known to be anti-nutritive in the chick (Schutte, 1990). Thus the beneficial effects of viscosity reduction may be compromised by free xylose and arabinose release to a greater extent with use of xylanase B compared to xylanase A. This hypothesis is more acceptable than the first given that excess xylanase is actually reducing performance, which may be a result of the negative effects of xylose/arabinose release over-riding the positive effects of viscosity reduction.

In summary, all xylanases are not equivalent when added to wheat based diets. They may differ in their ability to release free sugars which has an impact on the performance enhancement brought about through the reduction of intestinal viscosity. Identification of negative activities and selection of more appropriate preparations will lead to enhancements not only in the degree of response obtained through xylanase use, but also the consistency of response.

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IMPACT OF SULFUR AMINO ACIDS ON GROWTH PERFORMANCE AND CARCASS QUALITY IN BROILERS

R. Beste*, M. Pack* and J. B. Schutte**

Summary

The impact of nutritional programmes on meat yield and carcass fatness of broilers attracts increasing interest since more and more birds are further processed. Data on the influence of amino acid standards on carcass quality are still limited. Hence a feeding experiment with growing broilers was designed to study the effect of the methionine and cystine supply on growth and meat yield in male broilers (Ross).

Six graded levels of DL-methionine were supplemented to a basal diet with 7.1 g/kg methionine and cystine, 224 g/kg crude protein and 13.2 MJ ME/kg. The experimental diets were fed from 14 to 38 days of age.

The methionine and cystine level of the diet had a strong impact on weight gain, feed conversion and breast meat yield. The responses could be precisely described by exponential curve. An economic model was applied to the experimental data to enable the nutritionist to calculate optimum methionine + cystine levels for maximum profitability under given price conditions.

I. INTRODUCTION

The effects of amino acid nutrition on meat yield and carcass fatness of broilers attracts increasing interest since more and more birds are further processed. Data on the influence of individual amino acids on carcass composition are limited. Recently, several authors observed a positive effect of lysine on meat yield in broilers (Moran and Bilgili 1990; Holsheimer and Veerkamp 1992). Hickling et al. (1990) and Huyghebaert et al. (1993) reported a positive impact of increasing methionine levels on breast meat deposition in broilers.

A question still to be answered is, whether it is useful to formulate to specific amino acid standards depending on the kind of processing. That means: Should dietary amino acid specifications change, if birds are marketed as entire birds or further processed? The present study investigates the effects of increasing levels of methionine and cystine on growth, feed conversion and carcass quality in broilers. Based on the experimental results, the content of sulfur amino acids is calculated that maximizes profit for different production targets.

II. MATERIALS AND METHODS

A growth trial was performed using male Ross broilers over a period of 14 to 38 days of age. A basal diet was formulated to be deficient in methionine and cystine.

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Increasing contents of methionine and cystine were achieved by stepwise addition of DL-methionine. The ingredient composition of the basal diet is given in the Table 1. The nutrient composition (per kg) was: 3200 kcal ME, 227 g protein, 7 g methionine + cysteine, 3.4 g methionine, 13.2 g lysine.

Table 1 Composition of the basal diet (g/kg)

Maize	436
Soybean meal(48)	280
Soybeans, full-fat	95
Peas	50
Meat and bone meal	20
Feather meal, hydrol.	7
Fat blend	60
Molasses	20
Minerals, vitamins	31
Lysine·HCl	0.9

DL-methionine was added at levels of 0, 0.3, 0.7, 1.2, 1.8 and 2.4 g/kg to the basal diet, resulting in 6 dietary treatments. Each diet was fed to 4 replicates of 50 broilers. The birds were kept in floor pens. For carcass quality studies, a representative sample of 60 birds per treatment was analyzed for carcass composition with main emphasis on percentage of breast meat and abdominal fat.

III. RESULTS

Weight gain and feed conversion data are given in Figure 1, whereas breast meat yield and abdominal fat content are detailed in Figure 2.

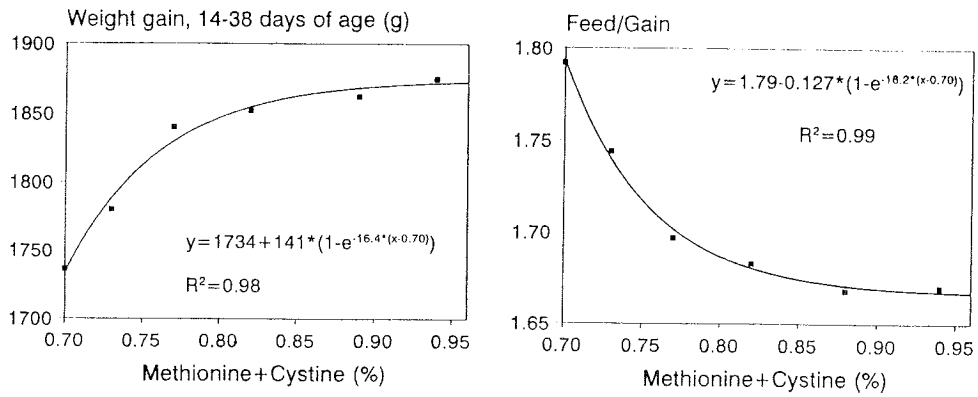

Figure 1. Weight gain and feed conversion as influenced by dietary methionine and cystine (14 to 38 days of age)

Increasing levels of methionine and cystine had a strong impact on each of the tested parameters. Breast meat percentage was highest with the highest dietary level of methionine and cystine (9.4 g/kg). The response to increasing additions of DL-methionine could be precisely described by exponential curves as indicated in the graphs.

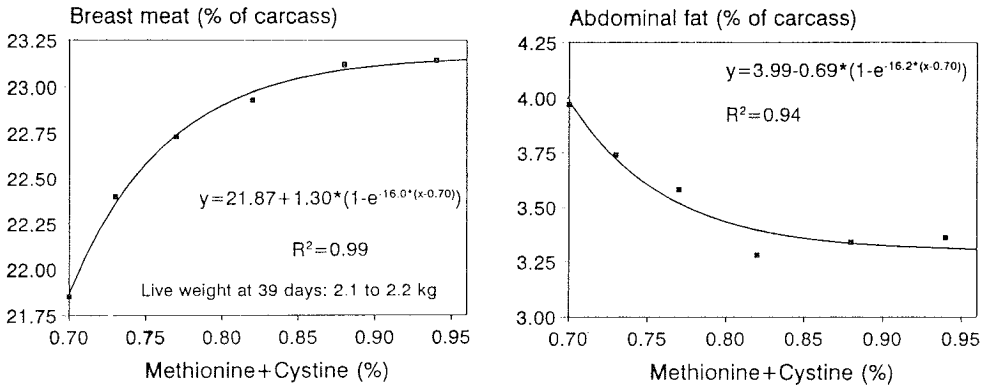

Figure 2. Breast meat and abdominal fat content as influenced by dietary methionine and cystine (39 days of age)

(a) Economic optimum

In order to calculate the level of methionine and cystine, which results in maximum profit from broiler production, the dose-response functions from Figures 1 and 2 were evaluated. In practical feed formulation for poultry, the target content of methionine and cystine is usually adjusted by supplemented DL-methionine. Hence, the optimum level of methionine and cystine can be calculated from a stepwise comparison of additional cost for added DL-methionine and of additional income from improved bird performance. In the present study, this comparison was done per step of 0.1 g/kg added DL-methionine. Additional cost and additional income from the respective last added unit of 0.1 g/kg DL-methionine were calculated. As long as the additional income is higher than the additional cost, it is still an advantage to increase DL-methionine. Profit is maximized at that level of DL-methionine, where the additional cost meets the additional income from the last added unit of 0.1 g/kg DL-methionine.

Two cases were considered:

1. Production of whole broilers, income from improved carcass composition neglected
2. Production of broilers for further processing

In case 1, only the income from savings of feed cost by improved feed conversion was considered. In case 2, the income from increased breast meat

percentage was calculated additionally. Prices were set at 330 US $/t of broiler feed and 3.60 US $/kg of DL-methionine, which corresponds to typical West European prices. For increased breast meat yield, a bonus of 3.00 US $ per additional kg of breast meat was calculated. All calculations refer to a production of 1 ton of broiler live weight.

Figure 3 gives the economic optimum for case 1 (only feed conversion evaluated) and case 2 (both feed conversion and breast meat percentage evaluated).

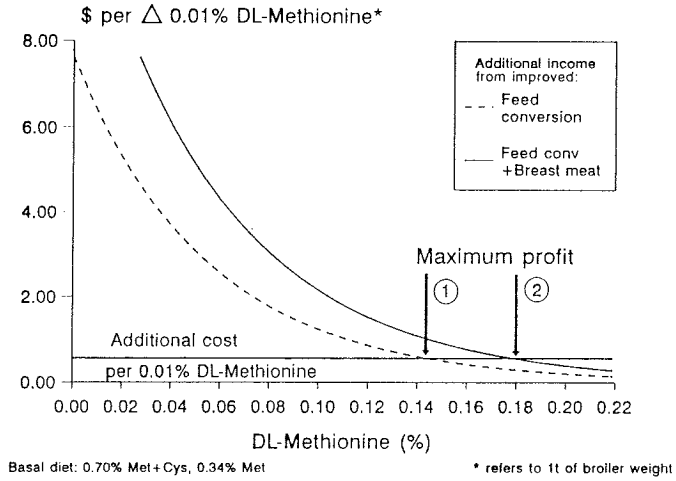

Figure 3. Economic optimum of DL-methionine addition, calculated from stepwise comparison of additional income and additional cost per 0.01 % DL-methionine
Case 1. only income from improved feed conversion calculated (- - -)
Case 2. income from feed conversion and increased breast meat percentage calculated (----)

Under the given price conditions, profit was maximized at 1.4 g/kg added DL-methionine, if only feed conversion was considered, or at 1.8 g/kg DL-methionine, if the increase in breast meat percentage was evaluated additionally. This refers to a basal diet containing 7.0 g/kg methionine+cystine and 3.4 g/kg methionine.

III. CONCLUSIONS

Depending whether broilers are sold as whole birds or further processed, the economic optimum of dietary methionine and cystine in a broiler grower diet differs considerably. If broilers are to be further processed, profit is maximized at higher methionine levels as compared to a production of whole birds. In broilers grown to 2.2 kg, about 8.4 g/kg methionine and cystine were most economical to optimize feed

conversion, whereas 8.8 g/kg methionine and cystine (7.0 g/kg from basal diet plus 1.8 g/kg from added DL-methionine) turned out to be most profitable for broilers used for further processing.

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Proc. Aust. Poult. Sci. Sym. (1994) 6

OSTRICH AND EMU EGGSHELL ULTRASTRUCTURE

C.E. BRACKPOOL and J.R. ROBERTS

The ostrich and emu are large flightless birds. Altough farming of these birds is not a new concept, over the last few years commercial production in Australia has expanded dramatically. As the industry grows then so too will the pressure on the birds to increase production. Already the hens are laying three times as many eggs per year as they would normally lay in the wild. This study has been conducted as a preliminary investigation into the ultrastructure of commercial ostrich and emu eggshells. Fundamentally ostrich and emu eggshells are similar to those of the domestic hen, in that they have pores, an inner and an outer shell membrane and mammillary and palisade layers.

	Hen	Emu	Ostrich
Body weight (kg)	1.8	40	120
Egg weight (g)	60	600	1350
Shell thickness (μm)	330	1050	2020
No. Eggs/year	>250	~45	~100

Emu eggshells are three fold and ostrich eggshells are six fold thicker than those of the domestic hen. Unlike the hen, ostriches and emus have branched pores, with ostrich pores being considerably more complex than those of the emu.

Ultrastructurally the palisade layer of the hen eggshell is further subdivided into three morphologically distinct layers, defined by vesicular density; the palisade, compact palisade and vertical crystalline layers. In the ostrich eggshell these layers are not readily distinguishable. Conversely, the Emu eggshell palisade layers are easily identified and are characteristic in that a reticulated layer replaces the vertical crystal layer. This reticulated layer comprises irregularly-shaped crystals and the spaces between the crystals interconnect to form a labyrinth of chambers (Board, 1982).

The emu eggshell also has a characteristic outer covering of dome shaped deposits. Under high power magnification the composition of these domes appears to be similar to the calcite deposits of the compact palisade layer. The mammillary layer of ostrich and emu eggshells is also distinctly different from that of the hens. Instead of a smooth cone surface, the cones have a rough crystalline surface. The rough crystalline surface is more dramatic in the emu eggshells and for the angular shaped cones the surface has a striated appearance.

The mammillary and palisade layers and, for the emu, the outer covering are quite distinct from those of the hen eggshell. It is interesting that ultrastructural abnormalities similar to the hen eggshell also exist for the ostrich and emu eggshells.

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COMPARISON OF EGGSHELL ULTRASTRUCTURE FROM EGGS LAID BY HENS EITHER EXPOSED TO HEAT STRESS OR EXPOSED TO THE MYCOTOXIN, CYCLOPIAZONIC ACID.

C.E. BRACKPOOL*, J.R. ROBERTS*, R.J. HUGHES**, W.L. BRYDEN*** and S. SUKSUPATH***

Summary

The deleterious effect of high temperatures or exposure to the mycotoxin, Cyclopiazonic Acid (CPA), on eggshell quality and eggshell ultrastructure was examined. Two independent studies were conducted. The temperature study was carried out at the South Australian Research and Development Institute, where birds were housed either in a hot (constant 30°C) or mild (constant 20°C) environment. The mycotoxin study was carried out at the University of Sydney, where birds were dosed with either 0 or 2.5 mg/kg BW of cyclopiazonic acid (CPA). Hot environmental temperatures or dosing with CPA adversely affects overall eggshell quality. Conversely, eggshell ultrastructure was improved for birds housed in hot environments or exposed to CPA.

I. INTRODUCTION

Eggshell quality may be compromised by many nutritional, environmental and management factors. This study examined the effect of two factors on eggshell ultrastructure: housing temperature and the consumption of mycotoxin.

The deleterious effect of high temperatures or mycotoxin are of particular importance to the Australian Poultry Industry. The ideal temperature for egg production in laying hens is 20-25oC (Cobb, 1991), although birds are able to adapt to variations in temperature. Egg production and eggshell quality are initally affected at 27-29oC (Say, 1987). Cyclopiazonic acid, CPA, is a mycotoxin produced by some fungal species of the genera Aspergillus and Penicillum. Such fungal species are known to grow on poor quality, immature or incorrectly stored grains (North and Bell, 1990; Chang-Yen and Bidasee, 1992). CPA primarily has a entero-nephrotic effect on birds (Suksupath et.al., 1989). Other effects are interference with the recovery from other diseases present in the flock (North and Bell, 1990) and a deterioration of eggshell quality (Suksupath et.al., 1989).

II. MATERIALS AND METHODS

Individually caged commercial White Leghorn x Australorp hens (aged 42 weeks) were kept at either a hot (constant 30°C) or a mild (constant 20°C)

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environmental temperature. Eggs laid between 0800 and 1200 hours in week 4 of theexperiment were collected. A total of 38 eggs from the mild temperature and 37 from the hot temperture was examined.

Individually caged commercial crossbred laying hens were dosed with CPA daily at either 0 or 2.5 mg/kg BW for seven days. Eggs were collected daily during the dosing period. A total of 81 control eggs and 68 CPA treated eggs was examined.

Traditional eggshell quality measurements were recorded for each egg. Egg weight, shell weight and shell thickness were measured and percentage of shell present, surface area of the shell, weight of shell per unit of surface area and shell density were calculated (Curtis et. al., 1985). The results were analysed by ANOVA.

A small equatorial piece of eggshell was prepared according to the method of Reid (1983) and viewed under a JOEL JSM35 Scanning Electron Microscope. The mammillary region of each eggshell was examined for ultrastructural characteristics as described by Solomon(1991) and scored for the degree of incidence. For each shell the ultrastructural observations were applied to the scoring system described by Nascimento (1988) to give a total structural score for each egg shell. The lower the total structural score, the better the ultrastructure of the eggshell. The ultrastructural results were analysed by Kruskal Wallis nonparametric test and the appropriate post hoc test was applied to determine significance.

III. RESULTS

Shell weight, shell thickness and the weight of the shell per unit of surface area in the eggs of birds housed in the hot environmental temperature were significantly less ($p<0.05$) than ineggs laid by birds in the mild environmental temperature (Table 1). Egg weight, percentage of shell present, surface area of the shell and shell density were not significantly different between the two housing temperatures (Table 1).

Egg weight, shell weight, shell thickness, percent shell present, surface area of shell and weight of shell per unit of surface area were significantly less ($p<0.05$) in the eggs laid by birds dosed with CPA than in the control birds (Table 1). Shell density was not significantly affected by dosing the birds with CPA (Table 1).

Ultrastructurally, the incidence of early fusion was significantly increased for eggshells of birds either housed in hot environmental conditions or dosed with CPA (Table 2). Hot housing conditions significantly reduced the incidence of late fusion and cubic formations on the mammillary layer cones (Table 2) whereas CPA significantly reduced the incidence of type B bodies in the mammillary layer (Table 2). The total score of heat stressed and CPA dosed bird's eggshells was significantly reduced (Table 2). No statistically significant differences were observed for the other ultrastructural characteristics (Table 2).

IV. DISCUSSION

Traditional egg quality parameters showed that birds housed in hot

environmental temperatures or dosed with the mycotoxin, CPA, laid inferior quality eggshells. The mammillary layer of the eggshells of heat stressed birds exhibited significantly more early fusion and less late fusion. Ultrastructurally, fusion refers to

Table 1 A comparison of the effects of temperature and mycotoxin on traditional eggshell quality parameters.

Eggshell Quality Measurement	Mild Housing Temperature	Hot Housing Temperature	Control	CPA
Egg Weight (g)	58.29	56.78	60.96**	58.61**
	(5.21)	(5.74)	(0.64)	(0.50)
Shell Weight (g)	5.36*	4.98*	5.01**	4.46**
	(0.67)	(0.68)	(0.08)	(0.06)
Shell Thickness (μm)	365.3*	344.2*	349.7**	324.6**
	(32.83)	(38.82)	(3.72)	(3.67)
% Shell Present	9.21	8.78	8.20**	7.62**
	(1.00)	(1.01)	(0.10)	(0.10)
Surface Area of Shell (cm2)	70.01	68.71	72.25**	70.30**
	(4.40)	(4.84)	(0.53)	(0.42)
Shell Weight / Unit Surface Area (mg/cm2)	76.51*	72.40*	69.11**	63.42**
	(8.05)	(8.24)	(0.87)	(0.83)
Shell Density	2.09	2.10	2.01	1.99
	(0.09)	(0.07)	(0.05)	(0.03)

Values across a row with the same superscripts are significantly different from each other. $P < 0.05$

the point where the crystal front becomes confluent. This defines the line between the mammillary and palisade layers. Bain (1990) defines eggshell strength in terms of those properties which influence shell stiffness and those properties that influence its resistance to crack growth. Stiffness characteristics are best explained in terms of the effective thickness of the shell. The contribution of early fusion to improving shell strength is two fold - by increasing the effective thickness of the eggshell (Parsons 1982) and by reducing the interstitial area between the mammillary formations. Reducing the mammillary interstitial space renders the shell less susceptible to crack along these natural fracture lines (Van Toledo et al 1982).

Eggshells of heat stressed birds also exhibited a lower incidence of cubic out-growths on the cones. These angular type cones have not previously been described as defects and may indicate a non-typical shell gland environment.

A significant reduction in the incidence of type B bodies was observed for eggshells of birds dosed with CPA. Type B bodies are small rounded bodies of calcite present between the cones of the mammillary layer that do not contribute to shell thickness.

The total score of heat stressed and CPA dosed bird's eggshells was significantly smaller. A lower score indicates an ultrastructurally superior eggshell.

A common effect of hot environmental temperatures and exposure to CPA is the reduced feed intake of hens (Say, 1987; Sukspath et.al.,1989). It is therefore suggested that during periods when eggshell calcification is restricted, the hen will calcify an eggshell as efficiently as possible in an attempt to maintain shell strength.

Table 2 A comparison of the effects of temperature and mycotoxin on ultrastructure of the eggshell mammillary layer.

Ultrastructural Characteristic (mean rank)	Mild Housing Temperature	Hot Housing Temperature	Control	CPA
Mammillary cap size	38.38	37.61	75.72	76.32
Alignment	36.40	39.65	76.71	75.18
Changed membrane	41.21	34.70	79.14	72.36
Confluence	37.50	38.51	71.80	80.86
Cuffing	34.11	42.00	72.04	80.58
Early fusion	32.99*	43.15*	66.79**	86.66**
Late fusion	43.80*	32.04*	80.94	70.29
Depression	37.50	38.51	74.27	78.00
Erosion	41.03	34.89	77.85	73.86
Hole	38.00	38.00	76.00	76.00
Type A bodies	40.54	35.39	77.61	74.14
Type B bodies	40.49	35.45	85.01**	65.57**
Aragonite	40.13	35.81	78.41	73.21
Cubics	40.43	35.50	76.36	75.58
Cubics on cones	45.67*	30.12*	77.12	74.70
Cap quality	38.36	37.64	79.62	71.81
Total score	45.08*	30.73*	84.19**	66.53**

Values across a row with the same superscripts are significantly different from each other. $P < 0.05$

V. ACKNOWLEDGMENTS

This study was supported by a grant from the Egg Industry Research and Development Council. C.E. Brackpool is also supported by fellowships from the Egg Industry and Chicken Meat Research and Development Councils.

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FEED ENZYME SUPPLEMENT IMPROVES THE APPARENT METABOLISABLE ENERGY OF LUPINS FOR BROILER CHICKENS

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The breeding of low alkaloid or "sweet" varieties of lupins and other varietal improvements such as seed shatter resistance and soft seededness (Gladstones, 1972) have considerably increased the value of this legume as a potential feed ingredient. Lupins have the potential of being an important protein source in poultry diets. However, their use has been limited by reports of depressed performance at even low dietary inclusion levels (<6%). Currently, there is an interest in defining suitable feed enzyme preparations capable of improving the nutritional value of lupins. In the current apparent metabolisable energy (AME) bioassay (Mollah *et al.*, 1983), individually caged 6-week-old broilers were used to evaluate two feed enzyme preparations. The main sources of enzyme activity in the Enzyme 1 preparation ('Lupinase' - Finnfeeds International Ltd., U.K.) were β-galactanase and minor amounts of α-galactosidase. The Enzyme 2 preparation was predominantly α-galactosidase.

Two sources of lupins were used, the first being classified as "lupin meal" and the second being cracked white lupins. These were included at a concentration of 250 g/kg in a maize-based diet. The two basal diets were fed without enzyme, with Enzyme 1 added at 1 g/kg diet or with Enzyme 2 added at 0.5 g/kg diet. The results of the bioassay are shown in the Table (mean values $\pm$ SE).

Diet		Lupin AME (MJ/kg DM)		
Lupin meal	(n=4)	8.66	$\pm$	0.405
Lupin meal + Enzyme 1	(n=5)	9.59	$\pm$	0.299
Lupin meal + Enzyme 2	(n=6)	8.79	$\pm$	0.547
Cracked lupins	(n=6)	7.98	$\pm$	0.430
Cracked lupins + Enzyme 1	(n=5)	9.84	$\pm$	0.346
Cracked lupins + Enzyme 2	(n=5)	7.13	$\pm$	0.288

Enzyme 1 gave improvements in AME of 10.7% (lupin meal) and 24.6% (cracked lupins). The latter response was significant ($P<0.01$). Enzyme 2 did not significantly affect AME but the differences in response of the cracked lupins to the two enzymes was significant ($P<0.001$).

These data indicate that the energy contained in lupins is not well utilised by broilers and that feed enzyme preparations containing β-galactanase can significantly improve the nutritional value of lupins for broiler chickens.

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THE USE OF ENZYMES IN LOW-ME WHEAT BROILER DIETS: EFFECTS ON BIRD PERFORMANCE AND GUT VISCOSITY

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Summary

The effect of a non-starch polysaccharide-degrading enzyme product (Avizyme, TX) on the performance of 4 week old broiler chickens fed wheats with high and low-AME values was studied. Supplementation with the enzyme increased the AME (MJ/kg dry matter) of a low-ME wheat from 12.02 to 14.94 and of a normal wheat from 14.52 to 14.83. This coincided with a significant fall in the digesta viscosity and marked increases in the apparent starch and protein digestibility in the small intestine. The performance of the birds fed the low-ME wheat with the enzyme was as good as those fed the maize control diet.

I. INTRODUCTION

Wheat is often used as the main energy source for poultry, but the metabolisable energy values are highly variable. This has been documented in many countries in the last three decades. In Australia Connor *et al.* (1976) first noted that the AME values obtained for wheats was 7-25% lower than that of sorghum when they were included above 50% in poultry rations. Payne (1976) postulated that some wheats may contain a "slightly toxic inhibitor". Subsequently, two major surveys were conducted in Australia to examine this phenomenon. A total of 60 wheats were assayed for AME and a number of other measurements (Mollah *et al.* 1983; Rogel *et al.* 1987). The AME values ranged from 10.35 to 15.9 MJ/kg dry matter. Increased starch excretion accompanied by poor bird performance was evident when low-AME wheats were fed to broilers.

There is now considerable evidence that the AME of wheat is affected by the soluble non-starch polysaccharides (NSP) which elicit anti-nutritive activities in poultry diets (Annison 1991; Choct 1993). The occurrence of low-AME wheats (AME <13 MJ/kg dry matter) appears to depend on the environmental and climatic conditions during maturity (Choct *et al.* unpublished data). Extensive studies are being conducted to elucidate this mechanism of action of the phenomenon and to establish means of overcoming it. One of these is the use of appropriate enzymes. Pentosanase and ß-glucanase are often used to alleviate the anti-nutritive effects of soluble arabinoxylans (pentosans) in rye and the mixed-linked ß-glucans in barley (Classen and Campbell 1990). Marked improvement of the nutritive value of wheat by enzyme supplementation has also been reported (Annison 1992). This paper presents data on the effect of enzyme supplementation on the low-AME wheat broiler diets.

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II. MATERIALS AND METHODS

(a) AME trial and digesta collection

Day-old mixed sex broiler chicks were obtained from a local hatchery and raised on commercial starter and finisher diets to 27 days. A 7-d classical ME trial was conducted using individual ME cages with a 3-d adaptation period and a 4-d collection period. Five diets (1: Maize control; 2: Low-ME wheat; 3: Low-ME wheat plus enzyme; 4: Normal wheat; 5: Normal wheat plus enzyme) were prepared with the basal composition of 80% grain, 13.4% casein and 4.6% minor ingredients (vitamins and minerals). Celite (2%) was added as a digesta marker. Each diet was pelleted and fed to 8 birds. At the end of the AME trial the birds were killed by intravenous injection of Nembutal. The small intestinal content from the beginning of the jejunum to 1 cm above the ileo-caecal junction was collected and kept on ice until viscosity was determined.

(b) Viscosity measurement

Approximately 2 g of fresh digesta were immediately centrifuged (12,000 *g*;10 min at 20°C). Viscosity was determined on a 0.5 mL supernatant using a Brookfield viscometer at 25°C. After the determination of viscosity, the supernatant was re-combined with the solid and freeze-dried for nutrient analyses.

(c) Determination of gross energy, acid-insoluble ash, starch and protein.

Gross energy of the diets and excreta samples was determined using a Parr isoperibol calorimeter. Acid-insoluble ash was measured by ashing the samples at 480°C and washing with 4M HCl. Starch was analysed using the Boehringer glucose kit after hydrolysis with amylase and amyloglucosidase. Protein was determined using a Kjel Foss apparatus. Data were analysed using analysis of variance.

III. RESULTS

Enzyme supplementation improved the nutritive values of both normal- and low-ME wheats. The improvement was large for the low-wheat but only marginal when good quality wheat was used (Table 1). This coincided with a significant fall in the digesta viscosity and marked increases in starch and protein digestibilities in the small intestine (Table 2). The AME values were predominantly dependent on the digestibility of the starch in the small intestine as shown by a highly significant relationship between the two parameters (AME vs. Small intestinal starch digestibility: $r^2=0.68$). The feed efficiency and growth rate of the birds fed the low-ME wheat diet were very poor but feed intakes did not differ among treatments. The performance of the birds on the low-ME wheat diet with the enzyme equalled that of the birds on the maize control diet.

Table 1 Effects of NSP-degrading enzymes (Avizyme TX) on the weight gain (WG; g/bird/week), feed intake (FI; g/bird/week) and feed conversion ratio (FCR; feed: gain) of broilers fed low- and normal-ME wheat diets. Apparent metabolisable energy (AME; MJ/kg dry matter) values are also shown (n=8; means ± SE).

Diet	WG	FI	FCR	AME
Maize control	438 ± 19^{a}	852 ± 23^{a}	1.955 ± 0.059^{a}	16.65 ± 0.07^{a}
Low-ME wheat	306 ± 46^{b}	771 ± 77^{a}	2.691 ± 0.183^{b}	12.02 ± 0.25^{b}
Low-ME wheat + Enzyme	397 ± 31^{ab}	786 ± 48^{a}	2.006 ± 0.078^{a}	14.94 ± 0.18^{c}
Normal wheat	383 ± 30^{ab}	779 ± 54^{a}	2.050 ± 0.063^{a}	14.52 ± 0.24^{c}
Normal wheat + Enzyme	431 ± 27^{a}	835 ± 48^{a}	1.951 ± 0.088^{a}	14.83 ± 0.22^{c}

^{abc} Unlike superscripts within a column are significantly different at $P < 0.05$.

Table 2 Effects of NSP-degrading enzymes (Avizyme TX) on the digesta viscosity (viscosity; cP), starch (SDC) and protein (PDC) digestibility coefficients in the small intestine of broilers fed low- and normal-ME wheat diets (n=8; means ± SE).

Diet	Viscosity	SDC	PDC
Maize control	3.16 ± 0.28^{a}	0.900 ± 0.012^{a}	0.769 ± 0.011^{a}
Low-ME wheat	20.28 ± 5.52^{b}	0.584 ± 0.024^{b}	0.689 ± 0.023^{b}
Low-ME wheat + Enzyme	10.36 ± 1.26^{a}	0.861 ± 0.031^{a}	0.745 ± 0.030^{ab}
Normal wheat	9.65 ± 1.30^{a}	0.815 ± 0.046^{a}	0.769 ± 0.017^{a}
Normal wheat + Enzyme	5.70 ± 0.34^{a}	0.888 ± 0.025^{a}	0.779 ± 0.018^{a}

^{ab} Unlike superscripts within a column are significantly different at $P < 0.05$.

IV. DISCUSSION

The wheats used in the current experiment had the lowest and highest ME values in our 1991/1992 survey (Choct *et al.* unpublished data) and large quantities of these two wheats were stored in rodent-proof containers for future use. The AME of the low-ME wheat increased considerably from 9.5 to 12.02 MJ/kg DM which may have been due to storage and/or assay conditions, although it still remained low in AME and broilers fed this wheat had poor growth and feed conversion. There was no change in the nutritive value of the normal wheat during storage. The gradual improvement of the nutritive value of the low-ME wheat indicates that the endogenous enzymes in the grain may slowly degrade the anti-nutritive compounds.

Supplementation of low-ME wheat with a commercial glycanase enzyme (Avizyme TX) largely eliminated its adverse effects in broiler chickens. This was manifested by increased bird performance and feed efficiency. The weight gain, FCR and AME were improved by 15%, 34% and 24% respectively for the low-ME wheat and by 4%, 5% and 2% respectively for the normal wheat. These improvements were due largely to

increased starch digestion in the small intestine. Protein digestibility in the small intestine was also significantly lower in the birds fed the low-ME wheat diet albeit the difference was less pronounced compared to that of starch. The increase in the small intestinal protein digestibility by the enzyme was not statistically significant. Protein digestibility is often influenced by the presence of endogenous and microbial proteins which in turn are affected by dietary factors such as increased level of NSP and the status of the animal. The current experiment, however, was not designed to examine these effects.

The level of soluble NSP in wheat appears to vary according to the environmental and climatic conditions (Choct *et al.* unpublished data) and is closely related to the AME in wheat (Annison 1991). The NSP are not digested by the chicken and they cause viscous digesta which impairs nutrient digestion and absorption (Choct and Annison 1992a,b). The current experiment showed that the digesta viscosity was much higher in birds fed the low-ME wheat diet than those fed the normal wheat diet and it fell significantly when supplemented with a glycanase enzyme. The fall in the digesta viscosity was, however, not proportional to the improvement in performance by the enzyme, suggesting that significant nutrient indigestibility may occur only above a certain threshold viscosity level. Nevertheless, benefits could still be obtained by further decreasing the digesta viscosity as seen with the normal wheat diet.

The enzyme used in this experiment has activities predominantly against arabinoxylans and ß-glucans. This confirms the hypothesis that NSP are a causative factor in the low-ME wheat phenomenon. Further studies are needed to investigate the exact mechanism of action and to recommend strategies to overcome this problem in a most cost effective way.

V. ACKNOWLEDGEMENTS

Thanks are extended to Mr. Mick Taylor and Mrs. Chris Adley of the Parafield Poultry Research Centre for their assistance in the animal trial and Ms. Leanne McGrath for her help in starch analysis. This work was part of a project funded by the Australian Chicken Meat Research and Development Council.

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VALIDATION OF A BEHAVIOURAL MEASURE OF FEAR IN POULTRY

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A fearful animal reacts to a threatening stimulus in a manner that is most likely to ensure its survival (Murphy, 1978). The responses open to an animal are to escape or avoid the threatening stimulus. Thus, avoidance of humans should reflect an animal's fear of humans. Since it is generally accepted that exposure to fear provoking stimuli result in a range of physiological responses in the animal, one of the most consistent being elevated plasma corticosteroid concentrations (Mason, 1968; Selye, 1976), correlations between these physiological and behavioural responses would provide evidence that avoidance behaviours are useful measures of an animal's fear of humans.

Two treatments, each comprising 40 day-old broiler chicks, were designed to provide differing levels of fear of humans. Treatment one birds received regular handling (20 minutes per day) while treatment two birds received no human contact over a six week period. At the end of the treatment period, the withdrawal response of each bird to an approaching experimenter was examined in a standard test. Immediately following this test each bird was held by the experimenter for 3, 6, 9, 12, or 15 minutes, before decapitation and subsequent collection of trunk blood. The plasma was subsequently analysed for corticosterone concentrations.

Table 1. Effect of previous handling on changes in mean plasma corticosterone concentration (nmol l^{-1}) in held birds and the proportion of birds withdrawing from an approaching human.

Variables	Handled					Non-handled				
Time held (min)	3	6	9	12	15	3	6	9	12	15
Corticosterone	8.2	5.8	6.6	5.4*	8.3	4.0	8.5	10.2	12.6	9.8
Birds withdrawing	0.46*					0.77				

* indicates a significant difference ($P<0.05$) from the corresponding treatment

Birds in the non-handled treatment showed a greater corticosterone response to handling, which was statistically significant at 12 minutes of handling ($P<0.05$; SD=4.07 and 11.31 for treatments one and two respectively), and a greater withdrawal response to the approaching experimenter than those in the handled treatment (Table 1). These data support the proposition that withdrawal responses of broilers from humans is a useful measure of the birds fear of humans.

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Proc. Aust. Poult. Sci. Sym. (1994) 6

INFECTIOUS BRONCHITIS - A PERSONAL VIEW

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Summary

Infectious bronchitis (IB) has been a major interest of mine since October 1962 and remains the most widely used poultry vaccine in Australia. This disease has not been discussed in a public forum for a long time and it is perhaps time this old problem was reviewed again. The international IB scene has been recently reviewed (McMartin 1993) and the Australian scene by Faragher (1993). I will attempt to review the more practical aspects and problems of this intriguing disease.

I. VACCINES AVAILABLE

The first Australian vaccines became available in 1966 and were derived from the Vic S Virus which was isolated in my laboratory from specimens received from a Mr L.P. Stevenson of Pearcedale in Victoria in July 1963. We had shown that this virus was mild in terms of its effect on the kidneys, killing comparatively few birds from nephritis, and protecting against the more severe viruses. This mild virus was given to various vaccine and commercial poultry firms to develop as a vaccine.

Another commercially available vaccine is the cold attenuated A3 virus, which was developed from the virus which was isolated at the Laureldale Poultry Unit of the University of New England in 1962. This virus, which was extensively tested and evaluated, was released in the late 1970s when specimens were handed to various vaccine firms and other bodies interested at the time.

To meet newer registration criteria, this virus had to undergo further refinements. Of the two vaccine firms then functioning in Australia, one (Philips Duphar) used limiting dilutions, while the second firm (Websters) had the virus plaque purified in cell culture by workers at the National Biological Standards Laboratory. Philips Duphar no longer functions in Australia and thus the only A3 vaccine virus available is the Webster A3.

Earlier work in my laboratory (Chubb & Ma 1974) had demonstrated that as few as five cell culture passages will render Australian IB virus non infective. The three cell culture passages the Websters A3 virus has undergone has markedly reduced its immunogenicity, both in terms of its protection of the kidneys (Cumming, unpublished) and the respiratory tract (Tannock, unpublished). There are thus only two vaccines registered and generally commercially available for the control of IB in Australia, the Vic S and A3. However, there are a number of other IB vaccines in use by integrated poultry companies (eg, I virus).

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II. COMPARISONS OF VACCINES

There are several reports comparing the various IB vaccines available in Australia. The early work (up until about 1973) concentrated on nephritis control and was largely from the University of New England (Ratanasethakul & Cumming 1981; Ratanasethakul & Cumming 1983a, 1983b, 1983c; Cumming 1985). Thereafter, more attention was paid to the respiratory tract with work from the University of Newcastle (Arvidson et al, 1990; Arvidson et al. 1991) and the National Standards Laboratory (Endo-Munoz & Faragher 1989), as well as the University of New England (Klieve & Cumming 1988a, 1988b, 1990).

Viewed overall, the evidence shows that the Vic S virus is extremely mild in its effect on chickens but produces an inferior resistance. The Vic S causes both respiratory and kidney complications but a fairly solid immunity. The I virus is the severest in terms of nephritis and is no more effect than the Vic S vaccine virus. The original A3 virus is very mild and yet produces the best resistance of both respiratory tract and kidneys against a variety of viruses. The Websters A3 virus produces an inferior resistance.

III. PRESENT VACCINE USE

Discussions with various poultry veterinarians around Australia reveal a rather confusing picture with quite different vaccine usages in both broilers and layers.

a) Broiler chickens

It appears that practically every organisation vaccinates at day old, and most chickens receive only one vaccination. Some apply a second vaccine (usually different from the first) by coarse spray at 10 days of age. One organisation is trialing some experimental vaccines in certain specific areas. Thus the N strain vaccine is used in New South Wales and the Q strain in some parts of Queensland.

Many veterinarians claim they have effectively controlled the disease with their vaccine procedures while others state they have continuing problems. When pressed to support these claims, no-one ever has any really hard evidence to back them up.

b) Layer chickens

Again it appears that day-old vaccination of egg type stock is extremely widely practised. This is usually followed up with a second vaccination at about 8-16 weeks of age. Again, various combinations of the vaccines mentioned above are utilised. The majority of pullets receive at least one Vic S vaccination some time in their lives.

There is quite a divergence of opinion on how egg type stock should be managed in the laying phase as far as IB is concerned. Several organisations claim that they only require vaccinations during the growing phase and confidently state they do not have any problems due to IB in their layers, which in many cases have been forcemoulted at least once and some up to three times. Thus these birds are often over 110 weeks old and apparently still not bothered by IB, although receiving

their last vaccination at 16 weeks of age.

On the other hand some of the bigger integrators, particularly in Sydney, categorically state that unless they re-vaccinate their layers at approximately eight week intervals via the drinking water during lay, that drops in egg production due to IB frequently occur. When pressed for scientific evidence to support these two very different vaccination strategies, the organisations concerned have little evidence (virus isolation, rise in serological titres) to back their opinions. Nor does anyone apparently have any scientific evidence that vaccination succeeds via the drinking water in commercial laying sheds.

With the introduction of overseas laying strains into Australia, it is obvious that day-old vaccination of egg-type stock is frowned upon in the northern hemisphere. In all cases, the overseas recommendations are that their laying pullets be vaccinated no earlier than 10 days of age. Further, the overseas organisations often recommend the additional use of inactivated vaccines in laying pullets just before the onset of production. Such vaccines are not yet available in Australia.

IV. EXTENSION PAMPHLETS

In general there is a lack of information available to poultry farmers on IB and its control from the various State Departments of Agriculture. At present there is nothing available in New South Wales or Victoria, and while a pamphlet is available from the Queensland Department of Primary Industry, it lacks a considerable amount of available information and contains several errors. Neither the Vic S nor A3 vaccines are registered for coarse spray administration at day old, yet coarse spray at day old is the most common method of application of IB in Australia, and this is stated in the Websters Poultry Biological Products Handbook. All this, despite the resolutions passed at the Poultry Review Meeting in Gosford in 1979, that updated and informative national information pamphlets be produced on important diseases.

V. PRESENT IB RESEARCH

Following the review on IB funded by the broiler and egg industries in Sydney in 1985, research was concentrated largely in the biotechnological areas of IB. Great advances were anticipated from this new approach (Ignjatovic & Bagust 1985) and around the world the biotechnologists have promised the production of highly efficacious and safe vaccines. Unfortunately, the problems have been more complex than anticipated and eight years later the results, in terms of new vaccines, are very disappointing. On the positive side, some very sophisticated and useful serological tests are now available, we understand more about the virus and its various components (Ignjatovic & McWaters, 1991; Ignjatovic, et at. 1991), and no doubt, new vaccines will eventually appear. However, it appears that it will be some years before these are available.

Very recently a project, centred on Victoria, has been supported by the egg industry to look specifically at IB problems in laying flocks.

VI. RESEARCH COMMITTEES

The research committees advising on poultry research were reconstituted in 1986 and differ quite markedly from the previous committees. The Chicken Meat Research and Development Committee essentially represents the integrators left in the industry, while the Egg Industry Research and Development Committee (EIRDC) was not very strong in the scientific areas and positively deficient in the disease areas. Neither committee had much strength in the area of independent scientific expertise. While these aspects have been improved with some recent appointments, the CMRDC is overwhelmingly influenced by the industry veterinarians. Until the present financial year, IB research was jointly funded by the two committees and it is therefore little wonder that the problem of aberrant viruses in broiler flocks has been heavily subsidised by Egg Industry research funds. At the same time, little or no money has been spent on identifying and working on the IB problems of the egg industry.

Little work has been done in Australia on the effect of IB virus on layers in production, on either internal or external egg quality parameters. It is generally accepted that depigmentation of brown shelled eggs may follow infection of partially immune birds in Europe. In fact, French research workers utilise depigmentation of brown eggs as a measure of susceptibility in long term immunity studies (Chubb, pers. comm.). This aspect has not been researched at all in Australia and this is surprising when one considers the huge increase in production of brown shelled table eggs in Australia over the past eight years.

In discussions with members of the EIRDC over the past four years, it is obvious that the industry committees do not solicit expert opinion on projects, even though the committees may be deficient in expertise on particular projects.

VII. NEGLECT OF AUSTRALIAN RESEARCH FINDINGS

The application of IB research to the field problems in Australia has been continually dogged by active opposition ever since 1962. From October 1962 to mid 1964, most Australian veterinary authorities refused to admit "uraemia", as the disease was then called, was caused by an IB virus. The electrolyte replacer formulations to reduce losses from IB nephritis, recommended in 1969 (Cumming & Heath 1969) were significantly altered by the industry to reduce costs, resulting in useless mixtures being widely sold (Cumming, 1981).

While Chubb (1973) produced compelling evidence that vaccination be delayed to 10 days of age for best results, the industry insists on vaccinating all birds at day old. Chubb's finding was supported by the British workers Darbyshire and Peters (1984). This topic was argued again in 1987 (Cumming 1987), but to no avail. Australian veterinarians all appear dogmatically wedded to day-old vaccination. It is interesting that Canada has few IB problems and their broilers are mostly vaccinated at between 7 to 10 days of age (Zellen 1988).

Chubb & Ma (1974) reported that cell culture very rapidly and dramatically alters the immunogenicity of IB viruses, yet the only available A3 vaccine virus was passaged three times in cell culture.

We at the University of New England were asked to produce a new vaccine by the research committees in 1983, and this was done with the Tasmanian isolate 2032 (Klieve & Cumming 1988a, 1988b, 1990). Yet this virus has never been trialled in the field, although the project was largely funded by the industry, both egg and broiler.

Registration of virus vaccines for poultry is now an expensive and exacting procedure in Australia. Vaccine firms are reluctant to expend the money on such registrations unless they have a reasonable chance of recouping their costs by subsequent vaccine sales. The poultry industry needs to carefully consider this situation if further producers' financial contributions to research and the efforts of researchers are not to be wasted.

In his final statement at the industry-funded review on IB held in Sydney (1988), Dr Jeff Fairbrother pleaded "a totally avirulent, efficacious and inexpensive IB vaccine is a high research priority". While the original A3 vaccine, as well as the Tasmanian derived 2032 may not exactly meet all these requirements, the industry would be a lot better off if it correctly applied the present knowledge and the best available vaccine viruses.

VIII. CONCLUSION

IB appears to be reasonable well controlled in Australia now according to industry sources, but there is not a great deal of scientific fact to back this opinion. In particular little is known of the importance of the disease in egg producing flocks. Further, the return on previous research efforts and present research would be significantly improved if past scientific findings and past experiences were applied.

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HORMONAL REGULATION OF BODY COMPOSITION IN CHICKENS

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Summary

The metabolic-hormone interrelation acting as an interface is studied in lean and fat broiler lines obtained by different selection strategies. Growth rate-selected and feed efficiency selected broiler lines clearly differ in growth hormone (GH) pulsatility. Direct selection for high or low abdominal fat while maintaining similar body weight, did not result in a different GH pulsatility between these lines. GH receptor numbers showed a strong negative relation with plasma GH concentrations. This was not parallelled by identical differences in IGF-I between the respective lines.

The lean and fat lines which did not show pronounced differences in the somatotrophic axis are however markedly different for thyroid hormone levels and/or metabolism, and vice versa.

Also differences in lipolytic activity *in vitro* between lean and fat lines seemed to be differential according to the selection parameters used. These results suggest that the same outcome, e.g. the high respectively low fat content in broilers selected in a different way may be achieved, at least partly, by different endocrine mechanisms illustrating and offering further sources of genetic variability.

I. INTRODUCTION

Selection for rapid growth in broilers in order to reach a market weight at earlier age resulted in fatter birds. According to the theory of proportional growth, selection shifted the growth speed curves for early and late maturing tissues towards an earlier age, but did more so for the late maturing tissues (fat) than for earlier growing tissues. Although the broiler is reaching its 2 kg live weight earlier, it comes closer to its maximal fat growth speed resulting also in a fatter bird. This gives us a very deterministic and mechanistic explanation of changes in broiler composition with selection for fast growth but it does not give any physiological cause for the observed facts.

Therefore, in several institutes lean and fat lines were selected in order to study more closely the linkage between rapid growth and fatness and also the strong negative relationship that exists in poultry between rapid growth and reproductive efficiency.

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The phenomena of growth and adiposity are complex processes which depend upon genotype as well as exogenous factors and for which the metabolic-hormone interrelation acts as an interface. Several hormones interact in the regulation of intermediary metabolism, with adipose tissue acting as a dynamic buffer helping to preserve a steady state for the central nutrient pool. To understand this regulatory role of hormones, it is necessary to take into account their absolute levels, tissue sensitivity for the particular hormonal signal as well as turnover rates.

Different selection strategies to breed leaner poultry have been used, and all have been successful in their primary aim but the various lines differ endocrinologically. Although the picture is yet far from complete the role of several of these endocrine factors in broiler obesity may be emphasised.

From the comparison of the different genetic models it can already be stated that, however, important such models for research into the physiological basis of broiler fatness may be, a single genetic model cannot unravel all aspects of physiological control of fatness.

Numerous studies have documented the integral role of hormones in nutrient partitioning and hence in the quality of body accretion. Effects of these hormones on intermediary metabolism are summarised in table 1.

Table 1 Effects of several hormones on intermediary metabolism

PROCESS	STIMULATING	INHIBITING
AA and glucose uptake	T_3, insulin, GH	NA, A
blood glucose	glucagon	insulin
gluconeogenesis	B, glucagon	insulin
glycogenolysis	APP, NA, A, B, glucagon	insulin
glycogenesis	insulin	glucagon
protein synthesis	GH, IGF-I, T_3, insulin	B
lipogenesis	PRL, insulin, APP, B, E	glucagon, NA, A, T
LPL activity	insulin, PRL	glucagon
VLDL synthesis	insulin, T_3	glucagon
lipolysis	glucagon, NA, A, T_3, T_4, GH	SRIF, APP, PE1

A: adrenalin; AA: amino acids; APP: avian pancreatic polypeptide; B: corticosterone; E: oestrogens; GH: growth hormone; IGF-I: insulin-like growth factor-I; LPL: lipoprotein lipase; NA: noradrenalin; PE1: prostaglandine E1; PRL: prolactin; SRIF: somatostatin; T: testosterone; T_3 triiodothyronine; T_4: thyroxine; VLDL: very low density lipoproteins

Two major axes can be identified: the somatotrophic (GH, IGF's) and the thyrotrophic axis. In addition the pancreatic hormones (insulin, glucagon, Avian Pancreatic Poylypeptide and somatostatin) are also important in the regulation of carbohydrate, protein and lipid metabolism.

II. SOMATOTROPHIC AXIS

(a) GH pulsatility

The pituitary hormone, growth hormone (GH) is a potent stimulator of growth in mammals. In fowl, hypophysectomy has also been found to reduce body and skeletal growth while GH replacement therapy restores growth.

In contrast with mammals, in juvenile meat-type chickens, there is no consistent evidence that exogenous GH administration may improve growth rate and feed efficiency although the way of administration or the age of the chickens may interact with the effect of GH on growth performance. In general, GH is known to stimulate the uptake of glucose and amino acids from the blood circulation by various tissues, to stimulate lipolysis and to promote protein deposition. Whether these effects result from a direct action of GH, or are mediated by IGF's or other hormones, is not always clear.

GH is known to be secreted in a pulsatile way and the influences of age (Vasilatos-Younken and Zarkower, 1987), sex (Johnson, 1988) or dietary protein content (Buyse et al., 1992a) on the temporal secretory GH pattern in chickens have been assessed.

Moreover, broiler selection strategy has also markedly changed the pulsatile presence of GH in the plasma. Johnson et al. (1986) as well as Decuypere et al. (1991) observed a more pronounced episodic GH pattern in slower-growing, for feed efficiency-selected broiler chickens compared to faster-growing, for growth rate-selected chickens. Also, the for feed-efficiency selected chickens resulting from the Danish selection experiment had a more pronounced pulsatile GH release compared with their for body weight-selected counterparts (Buyse et al., submitted).

Since the broiler chickens selected for feed-efficiency are also more efficient convertors of dietary protein into body protein, it is hypothesised that the episodic nature of GH secretion is related to protein conversion efficiency. Also the Scottish broiler lines selected for low plasma VLDL levels are more efficient in retaining dietary protein and show a more pronounced GH secretory pattern compared to chickens selected for high VLDL levels.

In contrast, differences in the pulsatile presence of GH in the plasma between the French broiler chickens selected for high or low abdominal fat content were very weak and not statistically discernable (Buyse et al., 1994, in press). For the Israeli chickens which are also divergently selected for high or low abdominal fat content, there is indirect evidence that there are no differences in the episodic nature of GH secretion between lean and fat birds. It seems therefore that the absence or the presence of any effect on the episodic nature of GH secretion coincides with a direct or indirect selection strategy for leanness, respectively (Table 2).

Notwithstanding their similar episodic GH release, the French LF broilers are also more efficient in retaining dietary protein compared to their HF counterparts. This does not mean that the above mentioned hypothesis linking the episodic nature of GH secretion with protein conversion efficiency is incorrect but that it is only valid *ceteris paribus*. For the French broiler lines, it has been proposed that a glucose-insulin imbalance is the primary cause for the higher fat deposition of the HF chickens (Saadoun et al., 1988).

The Dutch broiler chickens selected for feed efficiency showed a significantly higher GH response upon a TRH challenge compared to the GL chickens, which may be causative for their more pronounced episodic GH pattern (Herremans et al., 1991).

(b) Hepatic GH receptors

Broiler lines selected for growth rate, feed efficiency, and high or low abdominal fat content in different European countries were compared in one experiment conducted at COVP-DLO Spelderholt (The Netherlands). In total, 9 broiler lines from four countries were included in the experiment (Table 2).

For each line 36 males and females were raised and several zootechnical and biochemical parameters were recorded (Leenstra et al., 1992). There was a clear age-dependent pattern in GH-concentrations and in specific GH-binding.

Plasma GH levels were higher in 4 week than in 7 week old chickens while the opposite was true for hepatic GH receptor activity. Significant line and sex differences in GH concentration and the specific GH binding were found in 7 week old broilers selected for growth and feed conversion. Growth lines (both Dutch and Danish) had lower plasma GH levels and higher GH binding in comparison with lean lines selected for feed conversion. Females exhibit higher specific GH binding and lower GH concentrations in both Dutch and Danish lines. There is evidently a strong negative relation between plasma GH level and hepatic GH receptor numbers. This suggests a hepatic GH down regulation as a consequence of a continuous exposure to high circulating GH levels.

No significant sex or line differences in GH concentration or GH receptor activity were found in lines selected directly for high respectively low abdominal fat content (Table 2).

These results suggest that the same biological effect, e.g. the high respectively low abdominal fat content in broilers selected in a different way may be achieved by different endocrine regulatory mechanisms illustrating and offering further sources of genetic variability.

(c) IGF's

IGF-I is a GH-dependent peptide, which is mainly synthesised in the liver and was thought to be a true regulator of somatic growth in mammals as well as in avian species. Recent evidence however also suggests that IGF-I (and -II) may also act as an autocrine/paracrine factor. However, the physiological role of IGF-I in growth regulation is not so unambiguously established in the domestic fowl. Indeed, comparative studies often yield conflicting results as far as the relationship between circulating plasma IGF-I, plasma GH levels and growth is concerned (reviewed by Decuypere et al., 1993).

Huybrechts et al. (1992) did not observe any effect of continuous administration of rhIGF-I on growth rate or feed efficiency of broiler chickens. However, abdominal fat content, but not breast or thigh fat, was significantly reduced after 2 weeks of rhIGF-I administration.

Leenstra et al. (1991) did not find consistent differences in circulating IGF-I levels

between FC and GL chickens. However, in a subsequent study (Decuypere et al., 1993), it was observed that GL chickens had higher plasma IGF-I levels at 2, 3 and 4 weeks of age compared to those of their lean counterparts.

No consistent differences in plasma IGF-I levels were observed between the lean and fat broilers resulting from the other selection experiments (Table 2).

Data on plasma IGF-II at present are scarce. A consistent and significant higher plasma IGF-II level was found in the GL-line during the 6 wk growing period (Decuypere et al., 1993). Both lines exhibited similar age-related changes in plasma IGF-II levels with a maximum at the age of 3 weeks.

III. THYROTROPHIC AXIS

(a) Plasma T_3 and T_4

There is abundant evidence that thyroid hormones (triiodothyronine, T_3 and thyroxine T_4) are very important for normal post-hatch growth in birds (see Decuypere and Buyse, 1988). Plasma T_3 levels are positively correlated with growth rate and post-hatch growth is greatly inhibited by inducing a hypothyroid status but is restored by thyroid hormone therapy. However, as for the somatotrophic hormones, mild thyroid hormone administration to euthyroid birds hardly influences body weight gain. In contrast, inducing a hyperthyroid status in chickens decreased growth rate and feed efficiency to a great extent (e.g. Decuypere et al., 1987). Thyroid hormones also affect body composition. A hyperthyroid status in chickens is associated with a decreased fat content whereas in hypothyroid chickens, fat deposition is greatly enhanced (Decuypere et al., 1987, Cogburn, 1991). T_3 and T_4 have both been found to increase basal lipolytic activity and the glucagon-induced lipolysis in cultured broiler adipocytes (Harden and Oscar, 1991).

Differences in circulating T_3 levels were only consistently significantly different between lean and fat broilers resulting from the French and from the Israeli selection experiments.

French LF chickens are characterised by higher circulating plasma T_3 and lower plasma T_4 levels compared to their HF counterparts. In contrast, Israeli LF chickens have lower plasma T_3 and T_4 levels compared to the HF counterparts.

(b) Nuclear T_3 receptors and deiodinase activity

The Dutch lean line showed a tendency to have lower T_3 as well as T_4 levels which is paralleled by an increased type III deiodination activity (responsible for T_4 and T_3 degradation) and decreased type I deiodination activity (T_4 degradation and T_3 formation) in the lean line.

The increased T_3 and decreased T_4 in French LF-line can be explained by a higher type I deiodination activity indicating a higher T_4 to T_3 conversion. No differences in deiodination activity were found between the Israeli LF and HF in spite of marked differences in T_3 and T_4 levels indicating the existence of a difference in thyroid functioning itself (lower in the lean line) rather than in its peripheral metabolism.

At the age of 4 weeks no differences were observed between the Dutch FC and

GL line in hepatic nuclear T3 receptor capacity or occupancy (both expressed as fmol T3/μg DNA). At an identical age the French LF showed a non-significantly higher capacity [6.77 $\pm$ 2.27 (SE) for the LF vs. 2.36 $\pm$ 0.91 (SE) for the HF line]. The occupancy of liver T_3 receptors was respectively 6.50 $\pm$ 2.26 and 0.33 $\pm$ 1.07 fmol T_3/μg DNA for the LF and HF line and statistically different at the $P < 0.05$ level. From the results it can be concluded that the French, but not the Dutch, lean and fat lines showed marked differences in thyroid hormone T_3 receptor levels while the reverse picture is true for GH and GH receptor levels.

IV. LIPOLYSIS *IN VITRO*

Possible differences in lipolytic activity *in vitro* have only been investigated between lean and fat broilers resulting from the Dutch and from the French selection experiments.

Basal lipolytic activity of abdominal adipose tissue explants from FC chickens averaged higher compared to that of GL chickens (Buyse et al., 1992b). Glucagon, the most potent stimulator of lipolysis in birds, stimulated lipolytic activity of adipose tissue from FC but not from GL chickens. These observations suggest that FC chickens are able to mobilise their fat stores to a greater extent than GL chickens which contributes to the leaner carcass composition of FC chickens. In contrast, in the French broiler lines, similar sensitivity of isolated subcutaneous adipocytes from LF and HF chickens to glucagon, were observed (Leclercq et al., 1988). Again, direct as well as indirect selection are successful in obtaining a leaner broiler but the underlying mechanisms by which this is achieved, depend on the selection strategy employed.

Growth hormone is also known to stimulate fat breakdown in avian species. Incubation of abdominal adipose explants from GL chickens but not from FC chickens with GH, resulted in increased lipolytic activity. The desensitisation of adipocytes from FC chickens to the lipolytic action of GH probably results from their continuous exposure to high circulating GH levels in the plasma as is the case for the reduced specific GH binding to hepatic GH receptors of FC compared to GL chickens (Vanderpooten et al., 1993). In vivo, French LF and HF chickens also exhibit parallel changes in free fatty acid levels during the disposal of an oral glucose load (Saadoun et al., 1988).

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Table 2 Summary of the endocrine survey in selected lean and fat broiler lines

selection experiment	GH pulsatility	Hepatic GH binding	plasma IGF-I	plasma T3	plasma T4	deiodinase (4 weeks)	
						5 D (III)	5' D (I)
Dutch	FC > GL	FC < GL	FC = GL	FC ≤ GL	FC ≤ GL	FC > GL	FC < GL
French	LF = HF	LF = HF	LF = HF	LF > HF	LF < HF	=	LF ≥ HF
Danish	FC > GL	FC < GL	FC = GL	=	=	=	=
Israeli	ND	LF = HF	LF = HF	LF < HF	LF < HF	=	=
Scottish	lVLDL > hVLDL	ND	lVLDL = hVLDL	lVLDL > hVLDL	=	ND	ND

FC: selected for feed efficiency
GL: selected for growth rate
LF: selected for low abdominal fat content
HF: selected for high abdominal fat content
lVLDL: selected for low levels of very low density lipoproteins
hVLDL: selected for high levels of very low density lipoproteins
ND: not determined

Proc. Aust. Poult. Sci. Sym. (1994) 6

FACTORS AFFECTING BONE DEVELOPMENT IN BROILERS

H.M. EDWARDS, JR.

Summary

These studies have shown that specific action can be taken by producers involving genetics, environment, and nutrition that will dramatically influence the development of tibial dyschondroplasia and the resulting leg abnormalities in broilers. Careful attention to diet composition, particularly calcium, phosphorus, and chloride can result in decreased incidence of the disease. Use of fasting or lighting programs to promote fasting can be used to significantly reduce leg abnormalities in broilers. The use of vitamin D_3 derivatives will require additional research and development work before they are generally used in the poultry industry but their day should come. When these procedures are put into use by the industry they should contribute to an improved welfare of the individual chicken as well as the production of an economical and wholesome food product. Several factors such as breed and strain of bird, age, and dietary factors such as calcium, phosphorus and aluminum levels influence phytate phosphorus utilization by poultry. Supplementation of the diet with several vitamin D_3 derivatives including 1,25-dihydroxycholecalciferol resulted in 80-90% utilization of the phytate phosphorus in corn-soybean meal diets by broilers. These findings open up many avenues of research to facilitate phytate phosphorus utilization.

I. INTRODUCTION

Leg abnormalities have been a problem since poultry were first reared in barnyard flocks and continues to be a problem in large commercial production facilities of today. Recently they have received even more attention as the producer becomes more concerned with the welfare of his birds. In addition to the economic loss from mortality and loss at processing, leg abnormalities cause crippling and other ailments that cause discomfort to the bird.

In 1981, a research program was initiated at the University of Georgia with the objective of determining the cause of leg abnormalities in poultry. Since one of the primary causes of leg abnormalities in poultry is the development of tibial dyschondroplasia in the young chicken and the etiology of this condition was unknown, efforts were focused on this disease. Tibial dyschondroplasia is characterized by an abnormal mass of cartilage in the proximal end of the tibiotarsus. The abnormal cartilage is persisting prehypertrophic cartilage that is not calcified and has not been invaded by vessels from the metaphysis. Prior to this time the only nutrient known to be directly involved with the development of tibial dyschondroplasia was chloride. In fact, research workers had reported that increased levels of individual nutrients such as calcium, phosphorus, magnesium, vitamin D_3, and others had no effect on the occurrence of tibial dyschondroplasia.

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II. DIETARY CALCIUM AND PHOSPHORUS

The first major finding from this project was that broilers fed practical type corn soybean meal diets low in calcium and high in phosphorus developed very high incidences of tibial dyschondroplasia (Edwards and Veltmann, 1983). Either raising the calcium level of the diet or lowering the dietary phosphorus dramatically reduced the development of tibial dyschondroplasia (Table 1).

Table 1 Effect of dietary calcium and phosphorus levels on the incidence of the tibial dyschondroplasia in broilers.

Dietary Calcium	Incidence of tibial dyschondroplasia - %				
	Dietary phosphorus - %				
	.53	.61	.81	1.01	1.09
%			%		
1.67			8		
1.50		4		14	
1.10	0		13		32
0.70		17		39	
0.53			26		

Edwards and Veltmann, 1983

These observations have now been confirmed in laboratories throughout the world. Magnesium supplementation of the low calcium, high phosphorus diet that caused tibial dyschondroplasia is effective in preventing development of the disease, but magnesium is not as effective as calcium (Edwards, 1984). Studies on the effect of levels of other essential elements in the diet on the development of tibial dyschondroplasia confirmed the previous work indicating that high dietary chloride levels would increase the incidence of the disease (Edwards, 1984; Ballard and Edwards, 1988; Edwards, 1988 and Edwards, 1989a). These studies reaffirmed the importance of genetic selection in influencing the development of tibial dyschondroplasia. Differences in the incidence of tibial dyschondroplasia was shown to exist between various strains of commercial broilers.

III. SOYBEAN MEALS

The source of soybean meal was demonstrated to be a determining factor in the development of tibial dyschondroplasia (Edwards, 1985 and Edwards, 1985a). Soybean meals from one plant consistently produced a high incidence of tibial dyschondroplasia (34-69%); whereas soybean meals from a different plant consistently produced low incidences (14-28%). This same relationship was found with soybean meals from these two plants produced in different years (Table 2).

Table 2 Effect of soybean meal on the incidence of tibial dyschondroplasia in broilers (Edwards, 1985).

	Tibial dyschondroplasia	
Soybean samples†	Incidence	#Large cartilage plugs
	%	
Exper. 1		
A1	60	18/38
B1	14	3/38
Exper. 2		
A1	45	17/59
B1	14	0/39
Exper. 3		
A1	34	12/58
B1	18	4/59
Exper. 4		
A6	69	21/58
B2	28	8/60

† The samples A6 and B2 used in experiment 4 came from the same plants as A1 and B1, respectively; but over a year later.

IV. FASTING

Fasting of broilers results in a dramatic decrease in the development of tibial dyschondroplasia (Edwards and Sorensen, 1987). Birds that were fasted for 8 or 10 hours per day had reduced incidence of tibial dyschondroplasia as compared to birds fed for 24 hours ad libitum (5-11% vs. 59-68%). Results are shown in Table 3.

Table 3 Effect of short fasts on the development of tibial dyschondroplasia in chickens (Edwards and Sorensen, 1987).

	Hours fasted each day							
	Experiment 1		Experiment 2		Experiment 3			
	0	10	0	8	0	2	4	8
Tibial dyschondroplasia incidence %	59	11	68	5	70	54	61	17

	Days fasted (8 hours fasts)							
	Experiment 4				Experiment 5 (frequency)			
	0	4	8	16	0	1d4	2d4,8	4d4,8,12,16
Tibial dyschondroplasia incidence %	55	24	20	18	30	25	12	9

When birds were fasted for only 2 or 4 hours per day there was no effect on the development of tibial dyschondroplasia compared to ad libitum fed controls. However, birds fasted for 8 hours per day every 4th day had a reduced incidence of tibial dyschondroplasia. This knowledge is utilized to prevent the development of tibial dyschondroplasia in commercial production by limiting feed intake or by lights.

V. TRACE ELEMENTS AND ZEOLITE

Studies indicated that a trace element mixture containing aluminum, boron, bromine, chromium, fluorine, lithium, molybdenum, nickel, silicon, strontium, tin, and vanadium would slightly decrease the incidence of tibial dyschondroplasia (Edwards, 1987). This has led to extensive studies on the trace elements silicon and boron (Elliot and Edwards, 1991, 1992). However, most of the results indicate that these two elements are not closely involved in the development of tibial dyschondroplasia. Addition of synthetic zeolite to the low calcium, high phosphorus diets used to produce tibial dyschondroplasia in broilers significantly reduced the incidence of the disease (Table 4). The zeolite effect appeared at least to be partially the result of aluminum from zeolite binding phosphate thus causing a more favorable calcium to phosphorus ratio (Ballard and Edwards, 1988 and Edwards, 1988).

Table 4 Effects of natural and synthetic zeolites on phytate phosphorus retention by broilers (Elliot and Edwards, 1991).

Zeolite	Dietary calcium	Phytate phosphorus retention	
		Week 1	Week 2
	%	%	%
None	.65	28	13
Synthetic	.65	-17	-15
Natural	.65	41	28
None	1.00	26	17
Synthetic	1.00	5	7
Natural	1.00	22	19

While studying aluminum and silicon interrelationships as trace elements, it became apparent that aluminum and iron levels of the diet may influence the development of tibial dyschondroplasia probably by way of their interaction with phosphates (Elliot and Edwards, 1991a,b). This led to the observation that dietary ingredients and diets for broilers in the United States often contain levels of aluminum and iron that will cause major interference in phosphate absorption (Table 5).

It was also found that high levels of aluminum interfered with even low level utilization of phytate phosphorus in broilers.

Table 5 Effects of dietary aluminum on phytate phosphorus utilization by broilers (Elliot and Edwards, 1991 a,b).

Dietary level of aluminum (mg/kg)	Phytate phosphorus retention (%)	
	7 day	14 day
0	24	19.1
1,000	19.0	12.5
2,000	9.9	3.7
4,000	10.9	-7.0

VI. THIURAM AND DISULFIRAM

The drug thiuram has been known to cause leg abnormalities in broilers for forty years and in the mid-nineteen eighties, Brazilian researchers showed that it specifically caused tibial dyschondroplasia. This stimulated extensive studies in the laboratories at Georgia on thiuram and disulfiram (antabuse - the alcohol deterrent drug) (Edwards, 1987 and Edwards, 1989). It was thought that knowledge about the action of these drugs might be transferred to help solve the basic problem as to exactly why a chicken develops tibial dyschondroplasia. While a number of interesting studies resulted from the use of these drugs, no leads developed as to exactly why the chicken develops tibial dyschondroplasia. In fact, information eventually was collected that indicated that the way in which tibial dyschondroplasia develops as a result of feeding these drugs may be unique (Edwards, 1989).

VII. VITAMIN D_3 DERIVATIVES

Research workers in France have put forth the hypothesis and evidence that tibial dyschondroplasia resulted from a decreased synthesis of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) in the kidney as a result of a blood acidosis produced by dietary conditions. However, birds were not dosed or fed D_3 derivatives and observations on the development of tibial dyschondroplasia were not made to test their hypothesis. Early studies at Georgia tested 24,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_3$ for their ability to prevent tibial dyschondroplasia (Edwards, 1984). Results are shown in Table 6.

Table 6 Effect of daily oral administration of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) or 24,25-dihydroxycholecalciferol (24,25-$(OH)_2D_3$) on tibial dyschondroplasia in broilers (Edwards, 1984).

Experiment and treatment	Incidence of tibial dyschondroplasia (%)
Experiment 7	
Control	22
+1,25-$(OH)_2D_3$	19
Experiment 8	
Control	45
+1,25-$(OH)_2D_3$	31
+24,25-$(OH)_2D_3$	39
Experiment 9	
Control	34
+1,25-$(OH)_2D_3$	39
+24,25-$(OH)_2D_3$	43

The effects of these two compounds were negative. We now know they were negative due to the method of dosing and that an inadequate amount of the D_3 derivatives

were given to the chickens. Several years later, an extensive series of studies were conducted testing vitamin D_3 derivatives (Edwards, 1989, 1990). Results are shown in Table 7.

Table 7 The effect of cholecalciferol derivatives on incidence of tibial dyschondroplasia in broilers (Edwards, 1990).

	Tibial dyschondroplasia			
	Experiment 1		Experiment 2	
Vitamin D compound	Incidence (%)	#3Score (%)	Incidence (%)	#3Score (%)
None	54	35	63	18
D_3	44	35	82	22
1,25-$(OH)_2D_3$	18	8	56	9
24R,25-$(OH)_2D_3$	53	46	78	29
1,24,25-$(OH)_2D_3$	23	14	57	10
1α-OHD_3	29	15	42	5
1,25-$(OH)_2$-26-27$[^2H]_6$	16	7	42	0
1,25-$(OH)_2$-24R-FD_3	41	16	60	5

Those that had a hydroxy group in the 1 position on the vitamin D_3 molecule were found to dramatically prevent the development of tibial dyschondroplasia (Edwards, 1990). In the studies, feeding higher levels of cholecalciferol (D_3), 25-OHD_3, or 24,25-$(OH)_2D_3$ had no effect on the development of tibial dyschondroplasia.

VIII. ULTRAVIOLET LIGHT

The influence of ultraviolet light on the quantitative requirement for vitamin D_3 and the development of leg abnormalities is under investigation (Edwards et al., 1992, 1993). Results are shown in Table 8.

Table 8 Some data indicating that dietary vitamin D_3 may not be able to completely substitute for ultra violet light effects in the broiler chicken (Edwards et al., 1992).

Battery pen fluorescent lights	Dietary content		16 day body weight	Bone ash	Incidence of tibial dyschondroplasia
	Calcium	Cholecalciferol			
			g	%	%
Experiment 1					
+	0.95	0	425	39.6	0
+	0.95	200	435	39.7	6
+	0.95	2000	420	39.5	15
-	0.95	0	344	25.9	100
-	0.95	200	416	32.5	90
-	0.95	2000	423	39.2	53
Experiment 2					
+	0.95	0	412	39.3	54
+	0.95	200	413	39.7	20
+	0.95	2000	423	40.0	25
-	0.95	0	349	26.5	100
-	0.95	200	423	35.2	85
-	0.95	2000	419	40.0	40

The basal diet used in all of these studies contained adequate levels of vitamin D_3 and the birds were in brooders equipped with fluorescent lights that have been shown to contribute considerably to the D_3 requirement of the broiler chickens. Thus, this effect of 1,25-$(OH)_2D_3$ cannot be obtained from greater D_3 supplementation. These studies indicated that approximately 5 μg of 1,25-$(OH)_2D_3$ per kilogram of feed was needed to prevent tibial dyschondroplasia from developing. The effect of 1,25-$(OH)_2D_3$ supplementation of turkey diets on the prevention of leg abnormalities is not as clear as the work with broilers though the work to date is encouraging and further work is needed (Sanders and Edwards, 1991 and Sanders et al., 1992).

VIV. 1,25-DIHYDROXYCHOLECALCIFEROL METABOLISM

The discovery that feeding 1,25-$(OH)_2D_3$ to young broiler chickens will prevent tibial dyschondroplasia can be explained by either decreased synthesis of 1,25-$(OH)_2D_3$ in the animal or by some type of defect in quality or quantity of receptors influencing cartilage development and differentiation either directly or indirectly. The latest studies indicate that plasma levels of 1,25-$(OH)_2D_3$ are not involved in determining whether or not a chicken develops tibial dyschondroplasia; thus the inference is that synthesis of

$1,25\text{-}(OH)_2D_3$ is not the primary defect in $1,25\text{-}(OH)_2D_3$ metabolism causing the development of the disease and a defect in receptor site would be a more likely defect.

The results of these studies on tibial dyschondroplasia (Table 9) have pointed out large changes in calcium and phosphorus metabolism that result from $1,25\text{-}(OH)_2D_3$ supplementation of broiler chicken diets (Edwards, 1992 and Edwards et al., 1992). These are the focus of intense investigation at the present time.

Table 9 Effect of dietary calcium level on the response by broilers to $1,25\text{-}(OH)_2D_3$ supplementation (Edwards et al., 1992).

Dietary treatment		16-day body weight	Bone ash	Tibial dyschondroplasia incidence
Calcium	$1,25\text{-}(OH)_2D_3$			
%	μg/kg	g		
.45	0	370	31.1	85
.55	0	398	35.1	80
.65	0	410	38.7	30
.75	0	430	39.7	40
.85	0	413	40.2	58
.95	0	406	40.9	32
.45	10	356	36.8	36
.55	10	399	39.0	43
.65	10	401	40.6	45
.75	10	381	40.7	36
.85	10	373	41.3	0
.95	10	273	39.6	5

X. PHYTATE PHOSPHORUS UTILIZATION BY POULTRY

Poultry nutritionists have had a continual interest in the utilization of phytate phosphorus. During the last two decades this interest has intensified as phosphorus costs have increased with oil or fuel costs and the problems associated with phosphorus

accumulation in the environment has been better understood.

In order to gain more knowledge on how various experimental variables (genetic, environmental, nutrition, etc.) may influence phytate phosphorus utilization, it was decided in 1980 to make measurements on phytate phosphorus utilization in all of the experiments conducted on preventing leg abnormalities where there was the possibility that valuable information might be obtained. As a result of this decision, it was discovered that Single Comb White Leghorn chicks utilized almost twice as much phytate phosphorus (56%) from corn-soybean meal diets as broiler chicks (37%) (Edwards, 1983). This study also showed that both strain of chicks utilized much more phytate phosphorus when the diets were low in calcium and phosphorus. The extent of this effect of dietary calcium and phosphorus levels on phytate phosphorus utilization in broilers is readily apparent from a surface analysis (Edwards and Veltmann 1983) where dietary calcium levels ranged from 0.63 to 1.67% and dietary phosphorus levels from 0.53 to 1.07% and the phytate phosphorus utilization ranged from 11 to 39%. The poorest utilization occurring in chicks fed high calcium levels and the best utilization occurring when chicks received diets with low calcium and phosphorus levels. The effects of dietary calcium and phosphorus levels on the utilization of phytate phosphorus in young turkeys has also been studied (Sanders et al., 1992).

Table 10 Effect of 1,25-$(OH)_2D_3$ supplementation on the utilization of phytate phosphorus by broilers (Edwards, 1993).

Addition to diet			Weight	Bone	Phytate phosphorus
Phosphorus	Phytase	1,25-$(OH)_2D_3$	9 day	ash	retention
%	units/kg	μg/kg	g	%	%
0	0	0	145	24.2	31.3
0.2	0	0	170	34.8	42.3
0	75	0	154	23.7	41.6
0	0	5	172	29.8	68.4
0.2	75	0	181	35.4	44.4
0.2	0	5	186	35.9	62.5
0	75	5	167	30.1	79.4
0.2	75	5	178	35.8	67.7

Studies indicated that the addition of synthetic zeolite to the diet caused a reduction in phytate phosphorus utilization (Edwards, 1988; Elliot and Edwards, 1991b and Edwards et al., 1992). Since this effect of zeolite was thought to be caused by solubilization of the zeolite in the gastrointestinal tract, the effect of aluminum was studied. Aluminum would be released when zeolite solubilized and has previously been shown to decrease phosphate utilization in animals. Aluminum additions to chicken diets caused a definite decrease in the use of phytate phosphorus (Elliot and Edwards, 1991a). This was true at dietary aluminum levels that are in the range of dietary aluminum in commercially used practical broiler diets.

Recent studies indicate that the addition of several vitamin D_3 derivatives to broiler diets dramatically increases the utilization of phytate phosphorus (Edwards, 1992, 1993), as shown in Table 10. The development of a HPLC procedure for detection of inositol phosphates in the droppings of chickens should facilitate our understanding of phytate utilization (Sooncharernying and Edwards, 1993).

This discovery that dietary supplementation with 1,25-$(OH)_2D_3$ increases phytate phosphorus utilization is important not only from the point of view that direct application in the poultry industry would result in reducing the cost of broiler production and decrease phosphate excretion, but it indicates that the animal can have physiological changes that allow it to utilize the phytate phosphorus in the diet. This opens up a whole new area of research to look at other manipulations both genetically and physiologically that will stimulate phytate phosphorus utilization of all classes of poultry as well as other monogastric animals.

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TASTE PANEL AND STORAGE MEASUREMENTS OF COMMERCIAL EGGS AND THOSE ENRICHED WITH OMEGA-3 POLYUNSATURATED FATTY ACIDS

D.J. FARRELL and E. THOMSON

The fortification of the hen's egg with the long chain polyunsaturated fatty acids (n-3 PUFA) has been reported by Farrell (1993). Sensory evaluation of these eggs and their shelf life are important criteria. Here we report a sensory evaluation of two commercial eggs (one without (A) and one with (C) egg yolk pigment in the diet). Two enriched eggs (B and D) were also evaluated by 78 untrained individuals who were asked to score the eggs (poached or boiled only) in 5 categories by placing the egg identification letter on a scale of 1-10, eg. flavour - strong to weak. The results are given in Table 1.

Table 1 Sensory evaluation of commercial (A & C) and enriched eggs (B & D)

Egg type	Flavour (1=strong)	Taste (1=like a lot)	Colour (1=like a lot)	Texture (1=tough)	Overall (1=like a lot)
A	6.6	5.1	8.1	6.4	5.9
B	4.6	3.6	2.3	6.7	3.6
C	4.4	3.4	3.1	7.4	3.5
D	4.5	3.8	2.6	6.8	4.1
Pooled SD	2.55	2.76	2.49	3.02	2.74

Only egg A was inferior ($P<0.05$) in all categories, except texture, to the other three egg types which did not differ in any category. Haugh units were used to measure shelf life of 6 eggs from hens on either a commercial (1) or n-3 PUFA enriched (2) diet and held at 5° or 25°C for up to 30 days. The results are given in Table 2.

Table 2 Haugh units of commercial (1) and enriched (2) eggs at 5 and 25°C at 1, 15 and 30 d.

Egg type	Temperature (°C)	Days 1	15	30	Overall
1	5	87.0	79.8	72.3	LSD (P=0.05)
	25	87.0	46.5	41.3	= 5.08
2	5	92.7	82.2	79.5	
	25	92.7	43.2	42.0	

Storage life of both eggs types after day 1 were similar ($P>0.05$) at any given time within the same temperature. Eggs stored at 5°C had higher Haugh units at 15 and 30 days then eggs stored at 25°C. We conclude that sensory characteristics and storage life of n-3 PUFA enriched eggs are similar to commercial eggs.

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HAEMORRHAGIC DAMAGE TO THE INTESTINE OF CHICKENS INGESTING T-2 TOXIN MAY RESULT FROM A PRIMARY LESION IN ENDOTHELIAL

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Summary

T-2 toxin causes haemorrhagic lesions in the gastro-intestinal tract of chickens. Epithelial cells isolated from the intestine appeared refractory to normally expected doses of the toxin. Conversely endothelial cells are especially sensitive suggesting a mechanism for generation of the type of lesion encountered *in vivo*.

I. INTRODUCTION

The mould *Fusarium* which is commonly associated with cereal grains produces a range of toxic trichothecene compounds under certain conditions of which T-2 toxin is an example (Figure 1.). Indeed T-2 toxin is the most toxic member of the trichothecene family of compounds. The trigger for the production of these mycotoxins by *Fusarium* is unknown.

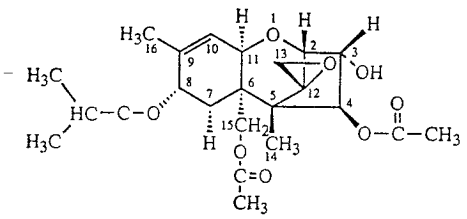

Figure 1. Structure of T-2 toxin

The physiological targets of T-2 toxin are primarily the skin, the gastrointestinal tract, the haemopoietic system and the immune system. The common feature among these systems is rapid cellular proliferation and thus toxicity of the mycotoxin is readily attributed to its high potency as a protein synthesis inhibitor. (Feinberg and McLaughlin 1989).

Chickens fed diets containing more than 0.4 ppm of T-2 toxin are affected according to the concentration of the toxic agent and duration of exposure. (Kabena *et al* 1989). A highly visible effect is ulcerative lesions of the oral cavity, which are rapidly subjected to secondary infection because of lowered immunological competence. Necrotic lesions are also found in the crop, proventriculus, the small intestine and the large intestine.

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We investigated the possibility of using isolated cells from the intestinal epithelium of chickens as an *in vitro* assay for the presence of T-2 toxin in feeds. The isolated cells were refractory to toxic affects at physiological dose levels. This paper reports that T-2 toxin-induced lesions may result from primary damage to cells of the vascular system.

II. METHODS

Intestinal epithelial cells (enterocytes) were isolated from the proximal 15 cm of the small intestine. The organ was removed rapidly from chickens killed by cervical dislocation. The pancreas was removed and the lumanal contents flushed out with a warm (37°C) buffer mixture. The intestine was everted and threaded onto a glass rod. The rod was immersed in buffer and connected to a machine which caused it to vibrate at 60 cycles per second with an amplitude of about 1 mm. The enterocytes were shed from the intestine as sheets of cells by this process. The procedure was devised by Harrison and Webster (1969), and was modified it by digesting the sheets of enterocytes with an enzyme mixture to produce a suspension of isolated single cells. After removal of the enzyme mixture by mild sedimentation the cells were resuspended in Krebs-Henseleit buffer at a density of about 5 x 10^6 cells per ml. Human endothelial cells were isolated from umbilical cords and cultured according to the method of Jaffe et al. (1973.

III. RESULTS AND DISCUSSION

Scanning electron microscopy of T-2 treated enterocyte suspension revealed that at 160μM toxin concentration many cells were subject to membrane damage. Such cells did not take up trypan blue and thus were not seen as dead cells in our assay system. Also the membrane damage seen in the electron micrograph was not apparent at toxin concentrations likely to be encountered by dietary exposure.

Figure 2 shows the effect of T-2 toxin on the viability of isolated chicken enterocytes as measured by absorption of vital dye.

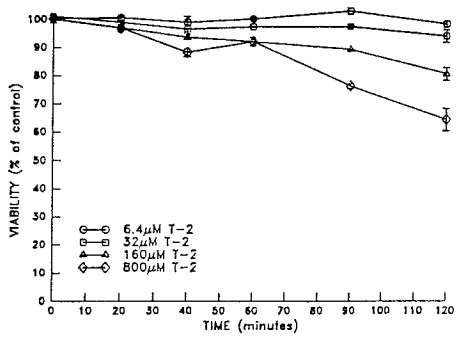

Figure 2. Decreasing viability of enterocyte preparations following exposure to T-2 toxin at the level indicated. Viability was measured as the percentage of cells in a microscopic field which did not stain with the dry trypan blue

Clearly the effect of the toxin in this system was minimal since ingestion of a 10 ppm of T-2 in the diet for 2 weeks with total retention of active toxin would produce an approximately 36 μM concentration. Interestingly the cells were more affected if they were subjected to paraoxon treatment which has the effect of inactivating a cellular esterase involved in detoxication of the mycotoxin.

Thus isolated enterocytes were not an effective *in vitro* system for the detection of T-2 toxin. However it was puzzling that haemorrhagic lesions involving these and similar epithelial cells were a major manifestation of T-2 intoxication. One possible explanation was that the site of action was on the vascular system itself with the resulting damage manifesting itself as haemorrhage and inadequate blood supply to the epithelial tissue and cell death as a consequence.

To test this possibility cultures of endothelial cells grown from the cell-layer lining the blood vessels were tested for the effects of exposure to T-2 toxin. The cells used in this experiment were primary cultures of human endothelial cells isolated from umbilical cords. The choice of human cells, rather than chicken endothelial cells was dictated by what was available.

When T-2 toxin was added to the culture medium bathing endothelial cells the cells developed gross morphological changes in which they appeared as flattened cell envelopes, or ghosts. Small spherical blebs also formed which appeared to be floating in the medium separated from the cells. Destruction of the cell monolayer was very extensive. The toxic effects did not appear before 2-3 hours after the addition of toxin and were apparent at concentrations as low as 50nM after 18h exposure. Thus the toxicity of T-2 toxin in this system is more than 3000 times greater than that seen with the chicken enterocyte preparation.

This remarkably toxic effect is substantiated by examination of biochemical changes in the endothelial cells.

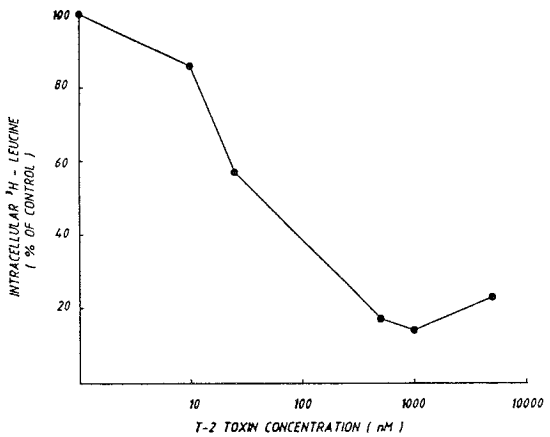

Figure 3. Incorporation of ^{3}H-leucine into protein of human endothelial cells during 30 min. exposure to the label and the effect of T-2 toxin concentration on the incorporation.

Figure 3 illustrates that uptake of ^{3}H-leucine, which presumably reflects the extent of protein synthesis in the cells is inhibited even at 10 nM T-2 toxin. The inhibition of protein

synthesis is reflected in a similar degree of DNA synthesis. There is also evidence that the cytoskeleton of the endothelial cell is damaged by T-2 toxin, and some genotoxicity is indicated by the fact that the plating efficiency of the cells is diminished by 50% after exposure to 25 nM T-2 toxin.

It is thus clear that human endothelial cells are extremely sensitive to T-2 toxin. If this sensitivity could also be demonstrated in chicken endothelial cells it would identify this tissue as the primary site of attack by T-2 toxin and account for some major aspects of the gross pathology observed.

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IMMUNITY DEVELOPMENT PROGRAMS FOR COCCIDIOSIS IN BROILER BREEDER BIRDS

P.J. GROVES and N.A. COOPER*

Coccidiosis is one of the most important diseases affecting poultry production throughout the world. Whereas this disease is normally controlled by "in feed" medication in broilers, the aim in broiler breeder birds is to develop a strong immunity to the infection. In recent years there have been attempts to give young broiler breeder birds a controlled exposure to the important coccidial species prior to the commencement of egg laying. The aim of such programs is to ensure that the birds are immune to the important coccidial species before the point of lay, so that outbreaks of coccidiosis do not occur during the production phase.

In an attempt to develop a more defined coccidial exposure program, 1 day-old Cobb 500 broiler chickens were allocated randomly to each of 9 deep litter pens at 150 per pen. The birds were vaccinated for Marek's Disease and Infectious Bronchitis and were fed a non-medicated ration for the first four weeks of life. At 7 days of age whole wheat was added to the ration at a ratio of 10% of daily intake. At 10 days of age, birds were given a measured dose of sporulated *E. tenella, E. acervulina E. maxima and E. necatrix* oocysts. The oocysts were obtained from natural field outbreaks which did show a response to medication with either amprolium or sulphaquinoxaline.

Following coccidial challenge three "in water" coccidiosis treatment regimes were compared in the trial. Each treatment was replicated three times. No untreated controls were used in the trial. All medications were given in the drinking water.

(a) Amprolium was administered on days 9-11, amprolium plus sulphaquinoxaline on days 12-14, and amprolium again on days 15-16 and 19-21 post-coccidial exposure.

(b) Toltrazuril (Baycox) was administered for 48 hours on days 9 and 10 post-coccidial exposure and again on days 14 and 15.

(c) Amprolium was administered on days 9-10, toltrazuril on days 11-13, no medication on days 14-17, sulphaquinoxaline on days 18-20 and amprolium on days 24-25 post-exposure.

Random fresh faecal samples were collected from each pen on days 7, 10, 15 and 40 and examined for oocyst presence by saturated salt flotation and microscopic examination. Lesion scores were assessed from one bird in each pen at 13 days post-coccidial exposure.

Examination of lesion scores and faecal samples showed that the birds had been exposed to the coccidial organisms. The trial also showed that the infection was equally well controlled by all medication regimes. The convenience of a 4 day toltrazuril medication program was established. In the future, coccidial challenge studies may be included to determine if any of the treatment regimes either promote or interfere with the development of active immunity to the various coccidial species.

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Proc. Aust. Poult. Sci. Sym. (1994) 6

THE INFLUENCE OF AMBIENT TEMPERATURE DURING GROWTH AND LAY ON THE PERFORMANCE OF COMMERCIAL LAYING HENS

C.C. KYARISIIMA and D. BALNAVE

In Australia, growing and laying pullets are often exposed to extremes of temperature which can influence laying performance. In particular, hot temperatures exert detrimental effects on egg production. Most of the research studies conducted to assess the effects of temperature on layer performance have applied the temperature treatments only during lay and little information exists concerning possible interactions between ambient temperatures experienced during growth and lay. The present study was designed to investigate the effect of life-time cool and hot temperatures, and their interactions in growth and lay, on the performance of laying chickens.

Three hundred day-old egg-type pullets (Tegel Superbrown) were brooded normally, and then reared at cool (10-20^{0}C) or hot (25 - 35^{0}C) temperatures until 18 weeks of age. At this age 192 pullets were transferred to laying cages. Half of the birds were kept at the same temperatures as during growth while the remainder were changed to the alternative temperature regimen. Performance during lay to 50 weeks of age is shown in the Table.

Temperature (^{0}C)		Body weight (g)		Feed intake	Egg prod^n	Egg wt
Growth	Lay	Initial	Final	(g/d)	(%)	(g)
COOL	COOL	1512^a	2475^a	145^a	86^a	57.6^a
COOL	HOT	1516^a	2341^{ab}	116^c	81^b	54.4^b
HOT	COOL	1502^b	2338^b	135^b	82^{ab}	56.9^a
HOT	HOT	1501^b	2138^c	106^d	75^c	52.4^c
SEM		2.5	46.7	2.1	1.5	0.44

Within a column mean values with the same superscripts are not significantly different ($P > 0.05$), as determined by Duncan's Multiple Range Test.

The poorest performance was observed with birds kept at the hot temperatures throughout life. Birds reared at the cool, and changed to the hot, temperatures during lay showed significantly better production than those kept at the hot temperatures throughout life. The hens kept at cool temperatures throughout life were heaviest, ate most food and had the best performance.

These results indicate that if hens are to be exposed to high temperatures during lay, their laying performance can be significantly improved by rearing them at cool temperatures. This presumably reflects the heavier body weight and greater food consumption of these hens.

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THE EFFECT OF A MICROBIAL PHYTASE IN RICE BRAN BASED DIETS FED TO GROWER-FINISHER DUCKS

E.A. MARTIN and D.J. FARRELL

Summary

An experiment was undertaken with ducks between 19 and 40 days of age on diets with 0, 300 and 600 g of rice bran per kg with (+) and without (-) a microbial phytase. Growth rate and feed conversion ratio were improved on all diets with microbial phytase as were overall dry matter, phosphorus and nitrogen digestibility and metabolizable energy but not feed intake. The effect of phytase on tibia ash was small. There was a decline in tibia ash with increasing rice bran and total phosphorus in the diet.

I. INTRODUCTION

We have shown in previous experiments with ducklings a response to a microbial phytase in diets with inadequate available phosphorus (Farrell *et al.*, 1993). Increases in the metabolizable energy of the feed and in nitrogen retention were also observed in chicks in addition to improvements in growth rate and feed conversion ratio (FCR) when a microbial phytase was used (Farrell *et al.*, 1993). In the present experiment, a microbial phytase was included in a duck grower-finisher diet containing high levels of rice bran.

II. MATERIALS AND METHODS

Ninety ducks aged 19 days were housed in 18 wire mesh cages (95 cm x 120 cm x 40 cm) in an air conditioned room . There were six dietary treatments with 0, 300 and 600 g rice bran/kg with (+) or without (-) a microbial phytase (Natuphos, Gist-brocades, The Netherlands) included at 1000 U/kg feed. One U is the amount of enzyme that liberates inorganic phosphate from 1.5 mM-sodium phytate at pH 5.5 and 37 °C at the rate of 1 mol/min. Dicalcium phosphate was added to all diets to provide 1 g of inorganic phosphorus (Pi) per kg. The ingredient and chemical composition of the diets are given in Table 1.

Feed intake was determined weekly and ducks were weighed at the start and finish of the experiment. The experiment was terminated when ducks were aged 40 days.

Digestibility measurements were made using acid insoluble ash (AIA) as an inert marker. Celite (1%) was added to all diets. Excreta were collected between day 33 and 40 of the experiment. Recovery of AIA from feed and excreta was made using a modified procedure of Van Keulen and Young (1977). Chemical analysis followed the methods of AOAC (1980). An analysis of variance was used to determine treatment efffects. Differences between means were subjected to the Least Significance test (LSD).

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Table 1 Ingredient (g/kg) and chemical composition of three diets with 0, 300 and 600 g rice bran per kg with and without a microbial phytase.

	Rice Bran		
	0	300	600
Sorghum	653.1	346.8	40.3
Rice bran	0	300.0	600.0
Vegetable oil	15.9	31.9	47.9
Soybean meal	185.8	176.3	167.0
Lupin meal	20.0	20.0	20.0
Pea (cv. Dunn)	40.0	40.0	40.0
Sunflower meal	50.0	50.0	50.0
Limestone	10.7	10.5	10.3
Dicalcium phosphate	5.0	5.0	5.0
DL-methionine	1.6	1.5	1.5
L-lysine-HCI	0.1	0	0
Salt	3.0	3.0	3.0
Vitamin-mineral premix	5.0	5.0	5.0
Celite	10.0	10.0	10.0
Calculated analysis ('as is' basis)			
ME, MJ/kg	13.0	13.0	13.0
Crude protein, g/kg	198.0	198.0	198.0
Meth + cys, g/kg	8.2	8.4	8.7
Lysine, g/kg	8.0	8.8	9.7
Calcium, g/kg	6.0	6.0	6.0
Phosphorus, total, g/kg	4.9	8.5	11.9
phytin, g/kg	2.4	6.1	9.7
non-phytin, g/kg	2.1	2.5	3.6

III. RESULTS

No ducks died during the experiment. Body weight gain, feed intake and feed conversion ratio (FCR) are given in Table 2. Daily gain and FCR were significantly improved ($P < 0.05$) in all three diets as a result of phytase addition. Dry matter (DM), nitrogen (N) and phosphorus (P) digestibilities and metabolizable energy (ME) were improved in all three diets with added phytase (Table 3); these were statistically significant ($P < 0.05$) except for N digestibility. Increase in P digestibility due to the enzyme in the diet with 600 g rice bran/kg was not statistically significant.

Table 2 Growth rate, feed intake and feed conversion ratio of ducks grown from 19-40 days on diets with (+) and without (-) a microbial phytase.

Rice bran (g/kg)	Phytase	Gain (g/bird/d)	Feed (g/bird/d)	FCR (g/g)
0	-	78.3	211.8	2.70
0	+	85.5	211.3	2.47
300	-	74.4	193.3	2.60
300	+	77.8	194.1	2.49
600	-	67.3	178.0	2.64
600	+	71.8	177.8	2.48
LSD (0.05)		2.71	5.96	0.11

Table 3 The apparent digestibility (%) of dietary dry matter (DM), nitrogen (N), phosphorus (P) and metabolizable energy (ME, MJ/kg) of diets with (+) and without (-) a microbial phytase in growing ducks (33-40 days).

Rice bran	DM		N		P		ME	
(g/kg)	-	+	-	+	-	+	-	+
0	72.2	76.6	43.8	50.0	29.0	39.3	12.8	13.4
300	66.7	68.3	48.7	52.5	17.8	27.0	13.2	13.4
600	59.2	62.2	47.2	51.8	17.6	23.4	13.5	14.0
LSD (0.05)	1.56		7.84		7.06		0.15	

Shown in Table 4 is the change in tibia ash expressed as g and as % of dry, fat extracted bone due to the addition of dietary phytase.

Table 4 Tibia ash of ducks grown on diets containing rice bran with (+) and without (-) a microbial phytase.

Rice bran (g/kg)	Phytase	Tibia ash (g)	Tibia ash (%)
0	-	2.82	52.01
0	+	2.84	53.43
300	-	2.39	51.57
300	+	2.61	52.23
600	-	2.10	50.35
600	+	2.27	50.52

Tibia ash (g and %) declined with increasing inclusion of rice bran in the diet. Any positive effect of the phytase was only marginal.

The decline in tibia ash (g, Y) was related to rice bran inclusion (%, X) by the following equation:

$$Y = 2.83 - 0.01X, \quad r^2 = 0.99, n = 9 \qquad (1)$$

and to total P (%, X) by the following equation:

$$Y = 3.29 - 0.09X, \quad r^2 = 0.99, n = 9 \qquad (2)$$

For these equations, data were combined for diets with (+) and without (-) phytase since the two equations (not shown here) were not significantly different.

IV. DISCUSSION

In contrast to our previous work with ducklings but not chicks (Farrell et al., 1993), the present study has shown not only improvements in growth rate and FCR (Table 2) with phytase addition but also improvements in apparent digestibility of some chemical components in the diet. This suggests that in addition to P, other components are in bound form and are released when phytase is added to the diet. In contrast, recent similar work with pigs failed to demonstrate any improvement in the apparent digestibility of dietary dry matter or protein with a feed phytase (Ketaren *et al.*, 1993). But another study has demonstrated an increase in N digestibility on diets adequate in P with a feed phytase (Barnett *et al.*, 1993).

Even though some diets (Table 1) contained adequate levels of total P and non-phytin P there was still a small decrease in tibia ash with increasing level of rice bran in the diet (equation 1). Addition of phytase was generally not effective in overcoming this decline but no leg problems were observed on any treatment. Tibia ash values were also higher than those reported for ducklings in a previous study (Farrell *et al.*, 1993)

The practical implications of this study are, the potential for a reduction in total dietary P to meet the P requirements of ducks, and the reduction in P voided in excreta with the use of phytase.

The unanswered question is whether the observed improvement in apparent dry matter digestibility and ME values could be maintained with addition of microbial phytase to rice bran-based diets if the diets were not deficient in Pi.

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ASSESSMENT OF FEEDING VALUE OF WHEATS FOR BROILERS IN RELATION TO PHYSICAL AND CHEMICAL CONSTITUENTS AND ENZYME SUPPLEMENTATION

K.J. McCRACKEN*, G. QUINTIN* AND M.R. BEDFORD**

Variations in the nutritive value of feed wheats for broiler production continue to be a subject of major importance to the industry. In Expt 1 three wheats were used with or without enzyme supplementation (Avizyme TX, Finnfeeds International) and in Expt 2 six different wheats were used. Wheat was incorporated at 667 g/kg with a concentrate balancer, cold-pelleted and offered to individually-caged birds *ad libitum* from 6 to 27 d. AME was determined from 13-20 day and also, in Expt 2, from 6-8 and 9-12 d (periods 1 and 2). Expt 1 was done in two replicates each using 11 birds per treatment for performance data and 5 for AME determination. Expt 2 employed 7 birds per treatment.

In Expt 1 there were significant effects of wheat ($P<0.001$) and enzyme ($P<0.05$) on weight gain and on gain:feed (Table 1).

Table 1 Effect of wheat source and enzyme supplementation on diet AME content, calculated wheat AME content (AME2) n, 10; df 52 and bird performance n, 22; df 102.

Bushel weight	69		67		57		SEM		
Enzyme	+	-	+	-	+	-	Wheat	Enz	W x E
AME (MJ/kg DM)	14.7	14.8	14.9	15.0	14.7	14.8	0.08	0.06	0.11
AME2 (MJ/kg DM)	14.9	14.9	15.2	15.3	14.9	15.0	0.11	0.09	0.16
Gain (g/d)	47.6	46.2	50.3	48.8	46.4	44.1	0.67	0.54	0.94
Gain:feed	0.73	0.72	0.76	0.76	0.73	0.70	0.005	0.004	0.007

In Expt 2 the diet AME content was affected by wheat source in all three periods ($P<0.001$, $P<0.001$, $P<0.01$) and the mean value increased from 13.7 to 14.7 MJ/kg with increasing age. The calculated wheat AME contents were significantly affected by wheat source (Table 2) the mean values for periods 1,2,3 being 13.3, 14.4, 14.8 MJ/kg DM.

Table 2 Effect of wheat source and bird age (6-8, 9-12, 13-20 d) on calculated wheat AME content (AME1, AME2, AME3, MJ/kg DM) n, 7; df 33.

Bushel weight	81	77	76	69	64	61	P=	SEM
AME1	13.9	14.2	13.9	13.2	12.4	12.2	<0.001	0.19
AME2	14.8	15.0	14.8	14.3	13.8	13.7	<0.001	0.16
AME3	15.2	15.5	15.0	14.4	14.5	14.1	<0.001	0.23

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Proc. Aust. Poult. Sci. Sym. (1994) 6

ASPECTS OF INSULIN-LIKE GROWTH FACTORS IN POULTRY GROWTH PHYSIOLOGY

JOHN P. MCMURTRY

Summary

Before biotechnology can be applied to improve production efficiency of poultry, a detailed knowledge of the genetic control of growth and the physiology and biochemistry of the regulatory factors must be clearly understood. This is best illustrated by the failure of simple and direct approaches, such as the injection of chicken ST, to have significant commercially relevant effects. Similarly, limited studies to date have failed to demonstrate that the administration of exogenous insulin-like growth factors (IGFs) will influence poultry growth and metabolism in a significant manner. Any affect that the IGFs may have on growth can not be entirely attributed to increased circulating or tissue IGF-I concentrations. Changes in insulin-like growth factor binding protein (IGFBP) physiology must also be considered as well as species specificity. It may be for example, that for exogenous IGF to be effective in positively influencing body growth and carcass composition in domesticated birds, co-administration of chicken IGFs and IGFBPs may be required. With the cloning and expression of the IGFs and the production of mass quantities of these hormones, the question of biological effects and usefulness of enhanced IGF status in poultry becomes critical.

I. INTRODUCTION

The hormonal regulation of growth and metabolism is dynamic and complex. This review has as its focus selected aspects on the role of hormones in regulating cell growth and metabolism in poultry with emphasis on the insulin-like growth factors (IGF-I and -II) and their binding proteins (IGFBPs). It is well-established that the synthesis and release of the IGFs are under the influence of somatotropin or growth hormone (Figure 1). The indirect effects of growth hormone are mediated via IGF and include increased amino acid uptake and protein synthesis, cell differentiation and proliferation (Etherton, 1993). This topic has been recently reviewed (Vasilatos-Younken and Scanes, 1991) and at this symposium (R. Vasilatos-Younken). I will focus on some recent advances in our understanding of the IGFs in poultry, and how this may have an impact on metabolic events and potential use of these hormones to enhance poultry production.

An understanding of avian growth factor biology is of importance if one considers the current problems in the poultry industry: 1) excess fatness in broiler stock, and 2) increased incidence of skeletal defects. The poultry meat industry is the most dynamic in animal agriculture. Increased poultry consumption will place significant demands on the poultry industry to increase yields of a product with proportionally greater muscle and lower fat content. These demands will not be achieved by genetic selection or

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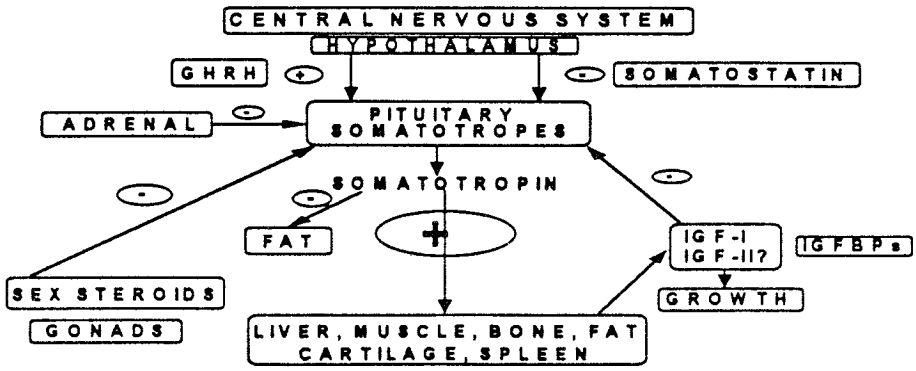

Figure 1. Somatotropin-Insulin-Like Growth Factor Axis

greater production alone. During the last decade a major portion of the market has changed from whole bird to "cut-up" products for further processing. This shift has placed more emphasis on rapid growth and a marketing of meat-type birds at heavier body weights. A combination of genetic and nongenetic factors have contributed to dramatic increases in broiler growth and body weights (Nir et al., 1989). Concomitant with these responses have been changes in feed consumption and metabolic patterns which result in excessive adiposity in broilers which is energetically costly and reduces reproductive efficiencies and lean carcass yield (Soller and Eitan, 1984). The increasing amount of excessive fat in the broiler carcass is widely recognized as one of the industry's major problems. In the future industry demands will focus on improving meat yield and meat:bone ratio of its birds for the portioning market, and at the same time lowering carcass fat. Feed costs represent 60% of turkey production costs. Therefore, any improvement in feed efficiency or time to market weight (enhanced growth rate) will result in a significant savings in feed costs alone. A better understanding of avian growth biology will provide significant information on how the growth factors affect poultry growth, thereby opening new strategies for augmenting lean tissue accretion in meat-type broiler chickens, and improving feed efficiency and decreasing the time to market weight in turkeys.

I. INSULIN-LIKE GROWTH FACTORS (IGF-I and -II)

Insulin-like growth factor-I is a 70 amino acid polypeptide hormone whose primary and tertiary structures are strikingly homologous to proinsulin. The primary structure of IGF-I is highly conserved among species. The structure and function of porcine and bovine IGF are identical (Etherton, 1993). Mature chicken IGF-I differs from mammalian by eight amino acids (Ballard et al., 1990). Whether these compositional differences are significant to biological function or metabolism remain to be elucidated.

Insulin-like growth factor-II (IGF-II) is structurally (60%) and biologically related to IGF-I. Insulin like growth factor-II contains 67 amino acids (Zapf and Froesch, 1986). Like IGF-I, chicken IGF-II contains eight amino acid differences from human or bovine IGF-II (Kallincos et al., 1990). Little is known about the function of IGF-II. This lack of information has been in part due to the absence of a reliable radioimmunoassay for IGF-II and sufficient quantities of the peptide to conduct whole animal studies. It has been hypothesized that IGF-II is crucial to embryonic and fetal development (Daughaday and Rotwein, 1989). Until sufficient quantities of the peptide are available and specific assays developed for quantifying the peptide, the physiological significance of IGF-II in development and growth remains elusive.

II. INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS (IGFBPS)

An important and unique feature of IGF physiology is the association of IGF peptides with multiple binding proteins (IGFBPs). These IGFBPs prolong the half-life of IGFs in circulating blood, *in vitro* decrease the availability of IGFs for receptor interaction, modify the biological effects of the IGFs, and are produced by a variety of tissues. Six distinct, but structurally related classes of IGFBPs have been isolated and characterized (Ballard et al., 1991). IGFBP-3 is growth hormone-dependent and carries the majority of IGF-I and IGF-II in the circulation. Recent evidence has established the presence of multimeric forms of IGFBPs in embryonic and adult chick sera (Armstrong et al. 1989; Francis et al. 1990; Schoen et al. 1992) and embryonic and adult turkey sera (McMurtry et al. 1992). Whether avian IGFBPs are similar in structure and function to that in mammals is unresolved. At this time it is difficult to assess the role of the IGFBPs on IGF action in birds.

III. REGULATION OF IGF SYNTHESIS and SECRETION

Somatotropin (ST) is the primary hormone that regulates serum IGF-I levels in most species. For many years it was believed that the liver was the primary site of IGF synthesis (Daughaday and Rotwein, 1989). More recently it has been demonstrated that the IGFs are synthesized in most tissues, and that changes in tissue levels precede any changes in circulating levels (D'Ercole, 1984; McMurtry and Brocht, 1992). This has given rise to the concept that the IGFs are produced in numerous tissues and that they act in an autocrine or paracrine manner (Daughaday and Rotwein, 1989). There is a growing body of evidence which supports the hypothesis that locally produced IGFs play an important role in regulating growth.

Unlike mammals, the functional significance of the insulin-like growth factors in birds remains an enigma. Domestic birds are species in which one might expect to find IGF-I to be very closely involved in growth and metabolism because of the very rapid growth rates in these species. Insulin-like growth factor-I (IGF-I) immunoactivity has been detected in the chicken embryo and avian yolk (De Pablo et al. 1990) and in avian liver (Haselbacher et al. 1980) and cartilage cell culture media (Burch et al. 1986) by heterologous radioimmunoassays (RIAs). Similarly, IGF-I has been detected in chicken and turkey blood using both heterologous RIAs and radioreceptor assays (Wilson and Hintz, 1982; Daughaday et al. 1985; Huybrechts et al. 1985; Huybrechts et al. 1987;

Burch et al. 1986; McGuinness and Cogburn, 1990;). In these studies, a heterologous antibody to human IGF-I has been used and the results expressed in reference to a heterologous human standard or arbitrary units of IGF-I based on an undefined pool of chicken plasma or serum. Therefore, because of the absence of a purified chicken standard and homologous antibody, the concentrations of IGF-I reported vary greatly among these studies. Moreover, the significance of the reported differences in concentrations of IGF-I is difficult to assess. To overcome this problems our laboratory has recently developed an homologous radioimmunoassay for chicken IGF-I. Preliminary findings indicate that both circulating and tissue IGF-I levels may be greater than previously reported (Fig. 2).

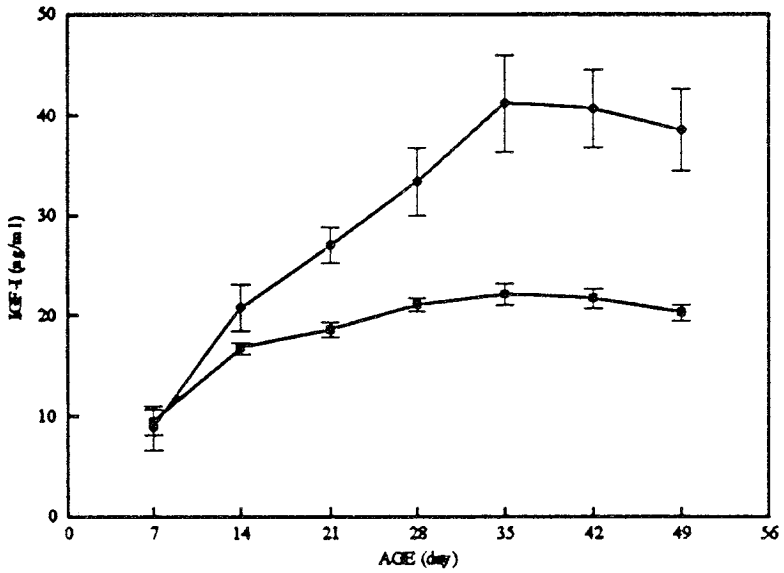

Figure 2. Comparison of plasma chicken IGF-I concentrations in male chickens at different ages as determined by homologous (−●−) and heterologous (−■−) immunoassays for chicken IGF-1 following acid/ethanol extraction. Values are means ± SEM for 8 birds at each age.

Somatotropin injected into the pig elicits a dose-response increase in circulating IGF-1 levels (Etherton, 1993). The relationship between ST and IGF-1 in birds appears to be more complex than in mammals. In the turkey embryo, IGF-1 production begins prior to ST secretion (McMurtry et al., 1990). IGF-1 gene expression precedes ST expression in the chick embryo (Kikuchi et al. 1991). In growing birds the concentration of IGF-1 in blood is dependent in part on ST status, although there is evidence for a discordant ST:IGF axis in birds. Paradoxically, in the growing chick IGF-1 increases as ST secretion declines (Johnson et al., 1990). Contrariwise, blood levels of IGF-1 are to some extent dependent on ST as hypophysectomy leads to a decrease in IGF-1 (Huybrechts et al., 1985). Administration of chicken ST to hypox chicks (Lazarus and

Scanes, 1988) or intact chicks (Vasilatos-Younken et al. 1988) increases circulating IGF-1. The manner in which ST is administered is crucial, as only when ST is given in a pulsatile manner do IGF-1 increase. Continuous infusion of ST or daily injections do not elicit an increase in IGF-1 (Vasilatos-Younken and Scanes, 1991). We have recently compared the effect of continuous versus pulsatile growth hormone administration on tissue IGF-1 protein and mRNA levels in the meat-type chicken (McMurtyry et al. unpublished observations). Changes in IGF-1 mRNA did not reflect changes in IGF-1 protein levels. The results of this study indicates that there is a differential tissue response to growth hormone in local IGF-1 production. Tissue IGF-1 messenger ribonucleic acid expression during embryonic development and post-hatch growth has also been reported (Kikuchi et al., 1991; Burnside and Cogburn, 1992). A general statement can be made regarding the secretion of IGF-1 into the circulation of chickens. Plasma concentrations vary with the physiological age and state of the bird. During late embryonic development, IGF-1 levels start to rise gradually. Circulating IGF-1 is low during the first 2 - 3 weeks of age, when as a percentage of body weight, the most rapid gains in body weight occur. Levels increase with age, plateauing at 4-6 weeks of age, and eventually decline to concentrations observed immediately post-hatching, at 3-5 months of age (Johnson et al. 1990; McMurtry et al. 1990; McMurtry and Brocht, 1992).

As compared to mammals there is not a clear relationship between plasma IGFs and growth rate in birds. For example, plasma concentrations of IGF-1 are not different between lines and are not related to line differences in chickens selected for growth rate (Goddard et al. 1988). In general, dwarf chicks are reported to have lower IGF-1 blood levels (Vasilatos-Younken and Scanes, 1991). A negative genetic correlation between IGF-1 and growth rate in chicks has been reported (Pym et al. 1991). Conversely, McGuinness and Cogburn (1990) have reported a high correlation between circulating IGF-1 and growth rate of broiler cockerels. IGF-1 levels are reduced 30 to 40% in hypophysectomized chicks (Huybrechts et al. 1985) and turkey poults (McGuinness and Cogburn, 1990). Growth rates were significantly lowered in both species. It is likely that the true secretion rate of IGF is greater than that which enters the circulation, as IGF involved in autocrine or paracrine regulation may not be observed in the vascular pool.

Nutrition is an important regulator of IGF-1 production in mammals (Phillips and Vassilopoulou-Sellin, 1979). In chickens, both caloric restriction and protein undernourishment lower circulating IGF-1, paradoxically at a time when growth hormone is elevated (Rosebrough et al., 1989; McMurtry and Johnson, 1989).

IV. BIOLOGICAL EFFECTS OF IGF-I and -II

Insulin-like growth factor-I elicits two types of biological effects *in vivo*, an insulin-like effect and a growth promoting effect in mammals (Daughaday and Rotwein, 1989). The insulin-like effects of IGF-I and II have not been reported for the domestic fowl. The observed effects of IGF on avian tissues are mainly confined to studies conducted on embryonic tissues, and have utilized human IGFs rather than the chicken growth factors. In the chick embryo IGF-I and IGF-II are mitogenic for chick osteoblasts (Slootweg et al., 1988), fibroblasts (Hasselbacher et al., 1980), heart mesenchymal cells

(Balk et al., 1984), and chondrocytes derived from growing birds (Rosselot et al., 1992) and myoblasts (Schmid et al., 1983). IGF-I increases the uptake of glucose and amino acids in chick fibroblasts (Cynober et al., 1985), and increases plasma protein synthesis and glycogen deposition in chick hepatocytes (Parkes et al., 1986). In avian fibroblasts, human IGF-I inhibits collagen synthesis and enhances collagenase activity (Granot et al., 1991), and stimulates cartilage growth (Kemp et al., 1984). IGF-I may also be an important mediator in neural development as IGF-I receptors are present in the chick brain (DePablo et al., 1990) and sclera (Waldbillig et al., 1990), and regulate lentropin, a protein controlling lens fiber formation in the chick.

There is indirect evidence that IGF-I may alter lipid metabolism in birds. Human IGF-I stimulated hepatic lipogenesis *in vitro* (Cupo and Cartwright, 1989). Other indirect evidence that IGF-I influences fat metabolism are the reports in which carcass fat content have been reduced by the administration of pulsatile growth hormone to chickens (Vasilatos-Younken et al., 1988; Rosebrough et al., 1991). In both of these studies, blood IGF-I levels were increased. This suggests that IGF-I may have an important influence on lipid metabolism in the growing chicken.

IGF-I in concert with calciotropic hormones is the most important regulator of long bone formation, both as a mediator of growth hormone and as an autocrine/paracrine affect (Daughaday and Rotwein, 1989). It has been demonstrated that both growth hormone and IGF-I injected directly into bone in rats stimulated longitudinal bone growth (Russell and Spencer, 1985). IGF-II has been shown to stimulate bone cell proliferation and type I collagen synthesis, with IGF-II being the most prevalent growth factor found in human bone (Mohan and Baylin, 1991. The significance of these peptides to *in vivo* bone growth in birds has not been assessed. It is interesting to speculate that the growth hormone-IGF relationship is critical to bone maturation in growing birds, as chicks diagnosed as tibia dyschondroplastic, have reduced episodic patterns of growth hormone secretion (Vasilatos-Younken and Leach, 1986).

There are two reports on the effect of exogenous human IGF-I administration on growth and carcass composition in meat-type chickens (McGuinness and Cogburn, 1991; Huybrechts et al., 1992). Both studies failed to demonstrate any effect of IGF-I on growth. In the first study a human analog (N-Met) form of IGF-I was injected i.m. daily. It has been previously shown that i.m. injections of IGF-I are the least effective route of administration. Huybrechts et al., (1992) infused human IGF-I into broiler chicks and observed a decrease in the size of the abdominal fat pad, albeit no change in total carcass lipid or growth rate. The authors suggest that IGF-I may function as a repartitioning agent in birds. The same authors also failed to demonstrate any affect on carcass composition, although feed efficiency tended to be improved.

One of the more dramatic properties of IGF-I is its effects on the gastrointestinal tract, (Read et al., 1991). Gut size is known to be a limiting factor to enhancing growth performance in domestic animals (National Research Council, 1987). The IGFs may offer promise as a strategy for improving gastrointestinal efficiency in domestic animals.

Chicken IGF-I and -II have been isolated, characterized, and sequenced (Kallincos et al., 1990; Ballard et al., 1990). Recently, methods for the recombinant DNA production of biologically active chicken IGF-I and IGF-II has been reported (Upton et al., 1991; Upton et al., 1992). With the cloning and expression of the IGFs and the

production of mass quantities of these hormones, the questions surrounding the biological effects and usefulness of enhanced IGF status in poultry may be answered.

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MUCOSAL IMMUNE RESPONSES IN THE CHICKEN FOLLOWING VACCINATION

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Many microbial pathogens that result in intestinal disease and reduced productivity in the chicken enter the body via mucosal organs. These could be better controlled if improved vaccination procedures directed at stimulating a local immune response at the mucosal surface were available (Husband, 1993). This study was designed to evaluate formulations for intraperitoneal (ip) delivery which have proved effective in other species as an alternative to oral immunisation for stimulating mucosal IgA antibody responses to non-replicating antigens in the chicken.

Male broiler chickens were vaccinated at 19 days of age with tetanus toxoid as a model antigen using the following procedures; oral PBS daily for 14 days, ip with Freund's incomplete adjuvant (FIA) with or without oral boost 14 days later, subcutaneously (sc) in FIA with or without an oral boost, ip in Auspharm adjuvant with or without an oral boost, and ip in Auspharm adjuvant + Quil A with or without an oral boost. The immune response was measured by the detection of antibody by ELISA, modified from Nicholas and Cullen, (1991), in serum, bile and intestinal washings. The distribution and isotype of antibody containing cells was determined by double fluorochrome immunofluorescence, adapted from Bienenstock *et al.* (1973), in the jejunum and spleen of treated birds.

Whereas repeated oral delivery only elicited a small total and IgA-specific anti-tetanus toxin antibody containing cell (ATCC) response in the intestine, ip priming promoted an enhanced IgA response especially following oral boosting. The Auspharm formulation was as effective as FIA especially when given with Quil A.

Although serum IgG titres were highest in the birds vaccinated sc with FIA, serum IgA titres were highest in birds vaccinated ip with FIA plus an oral boost and Auspharm + Quil A produced a serum IgA antibody response almost equivalent to that observed with FIA ip.

This study suggests alternative methods to oral vaccination for establishing improved IgA responses using protein or subunit antigens and indicates that the characteristic unresponsiveness to oral immunisation can be overcome by appropriate systemic priming. Although FIA is not suitable for practical use, Auspharm adjuvant is well tolerated by either ip or sc injection causing no adverse effects or carcase damage. Apart from the theoretical implications of this work, this could be of practical benefit in broiler breeders where injectable vaccines are a feasible approach, particularly for vaccination against those mucosal diseases for which replicating antigens are unavailable.

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PRENATAL ORAL VACCINATION ESTABLISHES EARLY DEVELOPMENT INTESTINAL IMMUNITY IN CHICKENS

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Contamination of chicken carcasses with *Campylobacter jejuni* is perceived as a potential public health risk and broiler chickens are considered as a major food borne source of Campylobacter infection which causes human gastroenteritis in developed countries (Evans, 1992). In order to reduce carcass contamination, it is necessary to control infection at an early age in broiler chickens. Control by vaccination depends on establishing effective intestinal immunity within the first few weeks of life. As reported by Prowse et al (1993) in ovo vaccination 3 days before hatching can improve disease morbidity and growth performance significantly. We have therefore investigated the potential for prenatal oral immunisation to establish early mucosal immunity against *C. jejuni* using protective antigen delivered in ovo into the amniotic fluid. First vaccination was given at Day 16 of incubation period by injecting purified *C. jejuni* flagellin protein into the amniotic cavity and the response observed at Day 5 post hatching. At Day 7 post hatching, some chicks received a second vaccination orally and the response observed at Day 14.

Whereas low titres of specific anti-flagellin antibody were detected in sera of unimmunised control birds (presumably reflecting passively acquired maternal antibody), high titres were observed in immunised birds, particularly associated with IgG and IgA isotypes. In contrast, the serum antibody titre of chicks receiving two immunisations were lower than in chicks receiving one immunisation. Enumeration of immunoglobulin-containing cells in the spleen and intestine by fluorescent histology revealed a dramatic increase, particularly in IgA and IgM-containing cells, in immunised birds. The immunoglobulin-containing cells in the tissues of chicks receiving a postnatal oral booster vaccination were higher than those immunised only prenatally.

These data indicate that in ovo oral immunisation stimulates the development of an early immune response and the high IgA component of this response indicates that mucosal immunity can be elicited by prenatal oral exposure. The increase in total immunoglobulin-containing cells number also suggests that prenatal oral antigen exposure may cause precocious development of the local immune system involving both antigen-specific and non-antigen-specific effector mechanisms in chickens as has been previously reported in other species (Husband, 1980).

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THE EFFECT OF EXOGENOUS INSULIN ON GROWTH AND BODY COMPOSITION OF LEAN AND FAT LINE CHICKENS

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Lines of chickens selected divergently for body fatness have been widely used in studies of metabolic and hormonal aspects of body composition in an endeavour to understand associated mechanisms. Although undoubtedly important, the precise role of insulin in lipid metabolism in chickens is not well established (Simon, 1988).

In the present study nine male birds from each of two lines selected for six generations for high (line F) or low (line L) abdominal fatness were used in the study. Following rearing to 42 d of age, 14 d mini osmotic pumps containing about 0.2 ml of either 2.5 ng/ml (0.03 ng/d) or 2.5 μg/ml (30 ng/d) insulin or 0.9% saline were implanted in each bird under the loose skin behind the neck, with three birds/line given each treatment. Following implantation the birds were weighed and placed in single cages and birds and feed weighed and blood samples taken after 14 d and body composition determined on the birds at 56 d of age. Average daily gain (ADG, g/d) and body fat (g/kg) and plasma glucose (mg/100 ml) and insulin (ng/ml) in the three treatment groups in each line are shown in the table.

	ADG		fat		glucose		insulin	
Line	F	L	F	L	F	L	F	L
Control	37.2	34.2	164	105	266	277	0.13	0.10
I_1 (0.03 ng/d)	37.2	39.6	157	115	266	295	0.13	0.12
I_2 (30 ng/d)	39.6	38.0	127	123	282	290	0.18	0.12
$LSD_{0.05}$	7.7	7.7	34	34	22	22	0.06	0.06

Insulin administration decreased body fat in line F but had the opposite effect in line L. In both lines there was a slight increase in plasma glucose with the higher level of insulin infusion. Endogenous plasma levels of glucose and insulin in line F were lower and higher respectively than in line L.

The differential response in body lipid to exogenous insulin administration suggests major differences in hormonal regulation of body composition in the two lines. Chronic exogenous insulin infusion was lipolytic in the fat line but mildly lipogenic in the lean line, and did not result in hypoglycaemia in either line. The increased incorporation of glucose into lipid in the liver in the fat line would thus not appear to be facilitated by a high secretion rate of insulin, despite the slightly higher endogenous plasma levels of insulin in this line.

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ULTRASTRUCTURE OF THE EGGSHELLS OF LAYING HENS: EFFECT OF STRAIN, SALINE DRINKING WATER AND THE STAGE OF SHELL FORMATION

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Eggshell ultrastructure was assessed in two strains of laying hen (Strains A and B) and birds were assigned to experimental groups based on the drinking solution and the quality of egg shells. There were four groups of Strain A birds: saline drinking solution and good quality egg shells (SG), saline drinking solution and poor quality egg shells (SB), deionised water and good quality egg shells (DG) and deionised water and poor quality egg shells (DB). For the Strain B birds, only the first three groups were represented.

Time of lay was assessed by use of a time lapse video recorder. Eggs were expelled from the birds at two hour intervals from 14-24 hours postoviposition. Each egg was subjected to a standard series of eggshell quality measurements. Shells were prepared for ultrastructural assessment by the process of plasma ashing followed by coating with gold-palladium and were viewed under the scanning electron microscope. Ultrastructural abnormalities in the mammillary region of the shells were assessed as described by Solomon (1991) and were scored for incidence. A total structural score was also calculated according to Nascimento (1992).

For Strain A, the stage of eggshell formation had no significant effect on the incidence of ultrastructural features. In addition, there were no significant differences between groups except for the incidence of "cuffing" which was lowest in the birds receiving deionised water and laying poor quality eggshells. For Strain B, the total structural score was lowest at 18-20 hours for the birds receiving a saline drinking solution and laying poor quality eggshells. There were no consistent effects of saline drinking water on eggshell ultrastructure.

One conspicuous feature of the eggshells in this study was the high incidence of gross membrane defects. Membrane whorls and other major membrane abnormalities were seen in 11% of all shells examined (a higher incidence than is usually seen) and occurred in all groups of both strains. This may be due to the age of the hens (90 weeks).

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AGRO-INDUSTRIAL BY-PRODUCTS AND NON-CONVENTIONAL FEED INGREDIENTS IN POULTRY RATIONS IN INDIA

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Summary

Research work in India on utilization of agro industrial by-products and non-conventional feeds to replace costly ingredients like corn, groundnut cake and fish meal in poultry rations has indicated that many such products can be successfully used to prepare practical poultry rations for starter - grower chicks, layers and broilers. Many such ingredients can be used in combination to formulate least cost rations. Further research is necessary to examine these products for nutritional factors limiting their utilization.

Laboratory work has also shown that it is possible to use the non-conventional energy supplements to formulate non-cereal poultry diets so that the cereal grains required for India's largely vegetarian human population can be spared. Commercialization of this aspect of the work is indicated.

I. INTRODUCTION

India has about one sixth of the world's human population, which is mostly vegetarian and mainly depends on cereal grains, as the source of energy. During the last three decades, the poultry industry has grown substantially. Today, India is the world's fifth largest egg producer. Traditionally, corn (50 to 60%) has been used in poultry rations as the main source of energy, while groundnut (peanut) oil cake (20 to 30%) and fish-meal (5 to 10%) have been used as protein sources. In view of the need to conserve available feed resources and to minimise the competition for food between man and animals, considerable attention has been given to research on the utilization of cheaper agro-industrial by-products and waste materials and non-conventional poultry feeds, with a view to replacing traditional feed ingredients. Proximate compositions of some of the major useful ingredients are given in Tables 1 and 2. These ingredients can be broadly grouped as (a) Energy supplements and (b) Protein supplements.

II. ENERGY SUPPLEMENTS

(a) Rice polish and deoiled rice polish

Rice polish is a by-product of the rice milling industry and is a rich source of energy, due to its oil content. It was shown that it could be used upto 50% in chick starter rations (Rao et al., 1966; Malik and Ichhaponani 1968). Rawany (1970),

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showed that development of rancidity in rice polish and its resulting adverse effect on chick growth can be prevented by 0.02% supplementation with the antioxidant "Ethoxyquin". The work on deoiled rice polish has shown that it can be used upto 35% in broiler rations (Nagpal et al., 1968) and layer rations (Rao et al., 1966). It appears that low utilization of deoiled rice polish is due to its low in-vivo protein digestibility (Bakshi 1971).

Table 1 Proximate composition of important agro-industrial by-products and some traditional items of poultry feed

Name of the ingredient	On dry matter basis (%)						
	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total Ash	Calcium	Available phosphorus
Maize yellow	8.90	4.26	2.09	82.74	2.01	0.31	0.16
Sorghum	10.20	5.00	3.74	78.98	2.08	0.18	0.15
Salseed meal	10.40	1.22	1.10	84.33	2.95	0.35	0.06
Tapioca chips	2.80	0.90	2.99	90.26	3.05	0.02	0.06
Rice polish	12.00	12.80	10.96	51.06	13.18	0.27	0.62
Deoiled rice polish	14.50	1.53	14.10	53.99	15.88	0.40	0.56
Groundnut cake	41.70	7.10	3.59	41.44	6.17	0.30	0.24
Deoiled groundnut cake	45.00	0.74	6.25	41.42	6.59	0.34	0.49
Sesame cake	38.00	9.22	3.20	39.57	10.01	2.51	0.51
Safflower cake	39.90	9.97	6.46	34.84	8.83	0.44	0.15
Sunflower cake	34.50	7.18	14.50	37.03	6.79	0.75	0.49
Soyabean meal	44.90	1.63	7.45	37.57	8.45	0.64	0.29
Cottonseed cake	39.00	4.50	10.80	39.47	6.23	0.39	0.47
Fish meal	55.10	2.94	1.59	25.80	14.57	2.32	2.08
Sterlized meat meal	59.50	10.58	1.50	18.74	9.68	1.58	2.18
Sterilized liver meal	63.50	14.40	1.50	12.75	7.85	0.68	1.42
Silkworm pupae meal	67.50	2.85	4.20	16.22	9.23	0.35	0.69
Blood meal	73.40	-	0.70	-	6.00	0.32	0.31
Liver meal	65.40	15.80	1.30	11.90	5.60	?	?
Hatchery by-product meal	30.00	29.80	-	-	25.70	?	?
Poultry manure	25.70	2.50	15.20	31.20	25.40	3.86	1.42
Maize gluten feed	26.90	4.80	5.10	59.60	3.60	0.15	0.28
Maize gluten meal	49.90	4.20	2.00	41.40	2.90	0.22	0.35
Molasses	2.80	-	-	86.60	10.90	1.51	0.66
Niger cake	36.00	8.90	18.60	28.30	8.20	0.62	0.96
Wheat (damaged)	9.50	2.30	2.30	81.90	4.00	0.08	0.41

(b) Salseed cake

Salseed is available abundantly in the forest areas. After extraction of oil, the salseed cake can be used as livestock feed. Experiments have shown that it can be used upto 5% in chick rations and 10% in layer rations (Panda 1970). Presence of large quantities of tannic acid was found to be a major inhibiting factor in the cake. Washing of the product in cold water overnight was found to remove about 56% of

the tannic acid and improve its utilization for poultry (Baruah et al., 1978). This work has not been developed on an industrial (commercial) scale.

Table 2 Metabolizable energy (ME) and amino acid content for agro-industrial by-products and some traditional items

Name of feed ingredients	M E Kcal/kg	Lysine (%)	Methi-onine (%)	Cystine + Meth. (%)	Argi-nine (%)	Trypto-phan (%)
Maize yellow	3309	0.17	0.15	0.23	0.44	0.09
Sorghum	2645	0.35	0.18	0.43	0.69	0.12
Salseed meal	3096	0.60	0.38	0.70	0.72	0.41
Tapioca chips	3000	0.58	0.06	0.12	0.07	0.01
Rice polish	2937	0.43	0.23	0.32	0.42	0.09
Deoiled Rice polish	2235	0.54	0.43	0.86	0.80	0.21
Groundnut cake	2596	1.63	0.58	1.20	5.66	0.48
Deoiled groundnut cake	2328	1.56	0.63	1.07	5.77	0.52
Sesame cake	2414	1.11	1.20	1.72	4.09	0.67
Safflower cake	2594	1.36	0.63	1.28	5.47	0.48
Sunflower cake	2230	1.81	1.45	2.17	3.80	0.55
Soyabean meal	2694	2.77	0.82	1.43	2.67	0.61
Cottonseed cake	1820	1.61	0.62	1.43	4.05	0.62
Fish meal	1834	5.72	1.95	2.73	2.44	0.45
Sterilized meat meal	2319	4.23	0.89	1.56	4.12	0.34
Sterilized liver meal	3000	4.58	1.24	2.11	3.91	0.57
Silkworm pupae meal	3000	3.85	2.97	5.84	3.99	0.72
Blood meal	1420	4.79	0.66	?	2.49	3.06
Liver meal	3650	3.86	0.98	?	2.52	0.50
Maize gluten feed	3315	0.59	0.45	?	0.69	0.20
Pencillin mycelium residue	?	1.24	0.46	?	1.38	0.37

(c) Lesser millets

As compared to corn, sorghum grain is cheaper. The white variety is found to successfully replace 50% of corn in chick and layer rations. Lesser millets like kodon and sawan are grown in large quantities in arid and low rainfall areas in the northern parts, while ragi, kumbu and cholam are grown in southern India. They have a lower energy content than corn. They also have tannic acid as a limiting factor for poultry. It has been shown that about a 10 to 14% saving in feed cost can be achieved by replacing 24% of corn with kodon and sawan (Michael and Gupta 1967). Presence of husk (crude fiber) has been found to be a major limiting factor in kodon. De-husking of kodon and its washing by water and acid treatment has been found to increase its nutritional value. Supplementation of kodon with the enzyme takadiastase at the rate of 1.1 g per kg. of diet has been found to improve its

nutritional value for chicks (Rodey 1972).

(d) Damaged grains

Large quantities of insect infested (damaged) grains are available in warehouses every year. The portions considered unsuitable as human food are sold for livestock feeding. They have been found to replace about 30% of corn in chick starter rations. Presence of uric acid and mold infection (aspergillus) have been found to be the major factors inhibiting their large scale utilization by poultry.

(e) Tapioca-meal (Cassava, Manihot)

The tapioca-meal prepared from parts of tubers which are not suitable as human feed can be used upto 50% in poultry rations. Presence of prussic acid and the powdery texture of the meal are major limitations. Pelleting of tapioca-meal has been found to increase its utilization.

(f) Cane molasses

Utilization of this by-product of the sugar industry has been limited due to its liquid consistency. Work has shown that liquid molasses can be used only upto 10% in poultry feeds. Higher levels cause problems of wet droppings (wet litter). At 2 to 3%, it has been used as a good binding material for pelleting poultry feed.

III. PROTEIN SUPPLEMENTS

(a) Oil cakes

Earlier experiments showed that groundnut (peanut) cake could be used upto 50%, replacing even some portion of the corn in chick and layer rations, affecting economy of feeding (Sathe 1961, Rao et al., 1966). Many experiments have shown that it is possible to efficiently replace a traditional oil cake like groundnut cake with a mixture of other oil cakes, such as soyabean, sunflower, safflower, sesame and cotton seed (degossypolised) cakes. Complimentary effects of sesame cake and groundnut cake for growing chicks have been reported by Panda (1966). Coffee cake, a by-product of the coffee industry, has been used only upto 3 to 4% in poultry rations, the major limiting factor being the presence of tannins and caffeines. Niger (black sesame) cake has about 32 to 35% crude protein and is grown widely in central India. Experiments have shown that it can replace upto 50% of groundnut cake, on a protein equivalent basis, in chick starter diets. Addition of upto 0.2% methionine in diets greatly enhance its growth promoting ability for chicks (Kolhekar and Sathe 1974). One of its major limiting factors appears to be the presence of free unsaturated fatty acids which can cause rancidity in the oil cake if it is not properly stored, especially during humid monsoon weather. Sadagopan et al., (1981) have shown that expeller processed mustard oil cake and solvent processed cake could be used at 14% and 28% respectively in chick rations. Perhaps some of the deleterious

factors are removed during solvent extraction.

(b) Meat meal and blood meal

Sathe and Bose (1962) and Panda (1966) showed that about 50% of fish meal could be replaced by good quality meat meal in chick starter and grower rations. Variations in nutritional quality of meat meals were largely found to be determined by their content of calcium and available essential amino acids (Sathe et al., 1964). A useful quick laboratory test involving a micro-biological assay using Tetrahymena pyriformis was developed by Sathe (1965). Sun dried or steam dried blood meal, upto 3 to 5%, was found to efficiently replace fish meal in grower rations (Desai et al., 1961; Sathe and Bose 1962; Panda 1966).

(c) Liver meal

After preparation of "liver extract" as a human medicine, the residual liver tissue is dried and used as liver meal. Results have indicated that it could be used upto 10%, replacing fish meal, in chick rations (Desai et al., 1961; Sathe and Bose 1962; Venkatachalam et al., 1965).

(d) Silk worm pupae meal

After reeling of silk, the silk worm cocoon is dried and used as a meal. The meal can be used upto 10% in grower and layer rations (Bora and Sharma 1965). It is rich in protein and some of the essential amino acids (Panda 1970). Now it is deoiled, which helps to prevent rancidity.

(e) Guar meal

After extraction of guar gum from the seed, the residual product is used as guar meal. The guar seed could be used only upto 6.5% in chick rations (Sathe 1961). Lyton et al., (1966) found that it could be used upto 20% if it was toasted or supplemented with 0.1% hemi-cellulase enzyme.

(f) Hatchery by-product meal

Results have shown that the incubator (hatchery) waste can be steam dried and used upto 33% in grower rations, replacing fish meal and groundnut cake (Verma et al., 1975). However, industrial application of these findings has not yet been taken up.

(g) Dried cow manure and poultry manure

Properly sun dried or steam dried cow manure and poultry manure have been found to be useful, replacing part of corn and oil cakes. However, its utilization for poultry is limited to 15% (Sadagopan et al., 1977). The ME content was 786 kcal/kg

(Sinha et al., 1977). There was a marked improvement in egg quality when it was fed to layers (Dinanath Prasad and Sadagopan 1979). Recent studies under a USAID project (Anon 1992) have shown that fresh poultry manure can be ensiled and used as a good source of feed for ruminants.

IV. FORMULATION OF NON-CEREAL POULTRY DIETS

In view of the large demand for cereal grains as human food in India and with a view to minimizing competition between humans and poultry for cereal grains, several investigations have been made to formulate practical type non-cereal poultry rations. Ichhaponani and Malik (1972) found that in the case of chicks and layers it was possible to prepare such rations primarily using rice polish, and replacing corn completely (Table 3). Similar results have been obtained for growing chick rations in which corn could be completely replaced by combinations of ingredients such as rice polish, molasses and groundnut cake (Table 4). Large scale experiments for commercialization of these concepts are necessary.

Table 3 Some promising Non Cereal rations

Ingredient	Percent of feed					
	C-1	C-2	G-1	G-2	L-1	L-2
Rice polish	31	61	51	61	30	55
Wheat bran	10	-	10	10	10	10
Maize grit	20	-	10	-	-	-
Maize gluten feed	15	10	-	-	20	-
Maize gluten meal	-	-	5	5	-	10
Groundnut cake	10	15	10	10	10	15
Fish meal	10	10	10	10	-	5
Meat/Silk worm pupae meal	-	-	-	-	5	-
Molasses	-	-	-	-	15	-
Pencillin mycelium	-	-	-	-	5	-
Limestone	1.5	1.5	1.5	1.5	3	3

Note: (1) C, G and L are chick, grower and layer rations, respectively.
(2) All diets contained 1% steamed bone meal, 0.5% common salt and 1.0% vitamin-mineral mixture.

V. FORMULATION OF LEAST COST RATIONS

In many of the experiments reported earlier, effects of replacing only 1 or 2 conventional ingredients with 1 or 2 agro-industrial by-products and non-conventional feeds were studied. In more recent work, Rao (1993) has studied the possibility of preparing least cost rations incorporating a large number of agro-industrial and non-conventional by-products for chick starter, layer and broiler rations. About 35 feed

ingredients and 55 constraints (limitations of nutrient levels, levels in the feed, cost of ingredients etc.) were used and the rations were tested using birds. A reference control diet was used. The results showed that it was possible to prepare least cost poultry diets containing many agro-industrial/non-conventional ingredients which are as efficient as control rations. In the case of chick grower and broiler rations, there were savings in feed cost ranging from 4 to 14.4% in different test rations. In the case of layers, all test rations gave higher returns over feed cost (Table 5). These results have indicated a great potential to commercially exploit the research on these feed ingredients.

Table 4 Composition of some promising non-cereal rations for chicks

	(Percent of feed)			
	1	2	3	4
Wheat bran	15	6	-	-
G.N. cake	39.5	30	23	15
Fish meal	5	3	6	6
Meat meal	5	-	-	-
Tapioca meal	25.5	-	-	-
Toasted guarmeal with 1% cellulase	-	-	20	20
Rice polishing	-	20	40	40
Blood meal	-	4	-	-
Liver meal	-	3	2	-
Brewer's yeast	-	2	-	-
Cow manure (dried)	-	13	-	-
Dried green	-	5	-	-
Molasses	-	20	8	16
Dried alfalfa	5	-	-	-
Limestone	3	-	-	-
Bone meal	1	-	1	1
Common salt	0.5	-	-	-
Shark liver oil	0.5	1	-	1

Note: Ration 1 : Sathe and Bose (1962)
Ration 2 : Moore (1962)
Rations 3 and 4 : Lyton et al. (1966)

VI. CONCLUSION

The laboratory work shows that a large number of industrial by-products and non-conventional feeds can be used to formulate cost effective poultry rations. The nutritional factors limiting their utilization need to be studied.

The work also shows that it is possible to prepare non-cereal poultry rations. Commercialization of this concept is required.

Table 5 Composition of least cost poultry rations using agro-industrial by-products

Ingredients	Percent Composition of Diet								
	CHICKS			LAYERS			BROILER		
	C1	C2	C3	L1	L2	L3	B1	B2	B3
Maize	35.0	32.4	32.3	43.0	38.4	37.6	50.0	42.0	43.0
Sorghum	-	10.0	-	-	20.0	-	-	10.0	-
Salseed meal	-	5.0	-	-	7.0	7.0	-	5.0	-
Tapioca chips	-	-	10.0	-	-	12.0	-	8.8	9.0
Rice polishings	10.0	-	-	16.8	-	-	19.8	-	16.4
Deoiled rice bran	20.5	16.0	18.1	9.5	-	7.6	-	-	-
Groundnut cake	17.4	10.0	-	9.1	9.7	-	9.7	8.0	-
Safflower cake	-	-	2.8	-	-	4.0	-	-	4.0
Sunflower cake	-	10.0	10.0	-	3.0	4.0	-	5.0	5.0
Soyabean meal	7.0	-	8.0	8.0	-	4.4	8.0	-	-
Maize gluten meal	-	5.3	10.0	-	9.0	10.0	-	6.2	7.2
Fish meal	5.0	3.0	3.0	4.0	1.0	2.0	5.0	3.3	4.2
Meat meal (sterlized)	2.5	-	-	2.0	-	-	3.0	-	2.5
Liver meal (sterlized)	-	2.6	-	-	2.0	-	-	3.0	-
Silkworm pupae meal	-	3.0	3.0	-	1.4	3.0	-	3.0	4.0
Feed cost (Rs./100 kg)	227.1	203.6	209.2	216.6	195.9	202.0	270.5	243.9	242.4

Note: (1) C1, L1 and B1 are control rations
(2) Rs.32/- (approx.) = US Dollar 1.00

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MEASUREMENT OF FEEDING VALUE OF FEED WHEATS WITH OR WITHOUT ENZYME SUPPLEMENTATION FOR BROILERS

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In the present study, five different feed wheats were substituted (70%) into a basal diet and then (1) unsupplemented, (2) supplemented with a cellulase enzyme or (3) supplemented with cellulase and a lipase enzyme. The 15 diets (5 wheat x 3 enzyme) were fed ad lib to 3 pens (20 birds/pen) of male broilers from 0 to 42 d. Excreta (24 h collection) from each cage was collected at 7, 21 & 35 d; ileal digesta was collected at 21 & 35 d. Diets, excreta and ileal digesta were analyzed for moisture, marker (celite, for acid insoluble ash), gross energy, nitrogen and crude fat. Apparent metabolizable energy (AME) and retention of fat and nitrogen (N) were determined for each cage of birds at 7, 21 and 35 d of age.

Enzyme supplementation produced an overall increase ($P<0.01$) in AME of wheat diets by 5.7 and 4.5% based on respective excreta and ileal measurement, and enzyme supplementation increased N (3.9%, $P<0.01$), but not fat, retention. Significant variation in AME and N retention between wheat samples was found. The bird response to enzyme supplementation varied between wheats for: AME (excreta) -1.0 to 12.3%; AME (ileal) 0.2 to 12.2%; N retention 1.4 to 5.5%; and fat retention -6.1 to 13.5%. Differences in ileal and excreta values were partially due to inclusion of 7 d excreta samples in the analysis. The lack of response in AME of Wheat 4 was unexpected since a significant decrease in digesta viscosity was observed with enzyme supplementation, suggesting that other factors besides level of non-starch polysaccharides may be involved.

Table. AME of diets based on excreta and ileal digesta, and excreta N and Fat retention for diets without enzyme control and the response (+E, % change from control) to enzyme supplementation.

	AME(excreta)		AME(ileal)		NITROGEN		CRUDE FAT	
	Control MJ/kg	+E %	Control MJ/kg	+E %	Control %	+E %	Control %	+E %
WHEAT1	12.38^{bc}	4.7	12.92^{ab}	5.6	82.6^{ab}	5.0	66.2^{a}	-3.9
WHEAT2	12.42^{b}	3.2	12.51^{b}	0.4	83.2^{a}	3.1	64.5^{a}	-0.3
WHEAT3	12.22^{b}	9.3	12.59^{b}	4.1	81.5^{b}	5.3	58.6^{a}	6.1
WHEAT4	11.46^{c}	-1.0	12.38^{b}	0.2	79.1^{c}	1.4	61.0^{a}	-6.1
WHEAT5	12.76^{a}	12.3	13.43^{a}	12.2	83.7^{a}	5.5	51.0^{a}	13.5
Mean	11.59	5.7	12.22	4.5	78.9	3.9	60.3	1.4
Std Error	0.64		0.26		2.9		16.4	

abc Different superscripts indicate significant ($P<0.01$) differences between mean values.

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DESCRIPTION OF A CHICKEN MEAT PRODUCTION MODEL

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Summary

In this paper a general description of meat chicken growth and production is discussed. A description of the equations involved in terms of energy intake, available energy, energy partition, protein and fat accretion and the prediction of growth is provided. Results from validation experiments are discussed together with some implications for further research.

I. INTRODUCTION

The prediction of an animal's response to nutrients is of obvious economic importance to those who feed animals. It has been argued (Fisher, 1983) that the failure to incorporate the theoretical principles of prediction into the scientific development of poultry nutrition has both limited progress and led to poorly designed, repetitive experimental work. Since prediction of response is quantitative, this leads to consideration of simulation and modelling and their role in nutrition and animal production.

It is accepted that the response to nutrients and the requirements for nutrients depend on many factors. The animal, the feed and the environment are just some of those that have to be included as explicit variables in the prediction equation. In practice the type of animal (genotype), major dietary variables, environmental temperature and economic conditions are probably the minimum requirements for a crude working model. The potential of such models can be extended to used to devise feeds which optimise the economic performance of given genotype in given environmental conditions. By combining the quantitative information from genetics, nutrition and the environment, biological models can be constructed which can make estimates of expected performance from feeding given diets to given genotypes in given environmental conditions.

In this paper the general description of growth provided by Emmans (1981) and the equations provided by Whittemore and Fawcett (1976) and Isariyodom *et al.*, (1988) will be discussed. These equations have been moulded into a model which is described in terms of energy intake, available energy within the system, energy partition, energy associated with protein and fat accretion and weight gain estimation.

II. FOUNDATION EQUATIONS

The idea underlying Emman's model is: an animal has a potential rate of normal growth at a given time and it seeks to eat an amount of a given feed which will allow this to be achieved. Its degree of success depends on the feed offered and the environment in which it is kept. Daily weight gain (WG) is determined from

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retained protein (PR) and fat (FR).

$$WG = (aPR + bFR) \quad (1)$$

The retention of protein and fat depends on energy and protein intake. (See Sections 3 and 4).

(a) Metabolizable energy (ME) intake

ME intake (MEI, kJ/day) historically has been expressed as a function of body weight (W, g) and ambient temperature (T, °C) (Davis *et al* 1973). Because modern meat chickens are much faster growing the coefficient needs to be increased and Isariyodom *et al.*,(1988), suggested a value of 1165. The equation thus obtained is:

$$MEI = C_2((1165-4.73T)((W/1000)^{0.75}))+C_1 \quad (2)$$

where C_1 represents a correction factor of MEI; ie. (60 - 9.12W) when body weight is less than 500g, or zero when body weight is equal to or more than 500g, and C_2, a multiplicative factor for photoperiod adjustment.

The desired quantity of feed intake is determined as MEI divided by the ME value of the diet.

$$DFI = MEI/ME \quad (3)$$

(b) Energy partition

Total available ME is partitioned largely into three forms, ie maintenance, protein accretion and lipid deposition.

Maintenance energy (MAE) is calculated by using the equation :

$$MAE = C_4 . C_5 . 675 (W/1000)^{0.75} \quad (4)$$

where C_4 and C_5 are an arbitrary correction factor and a multiplicative factor due to the change in ambient temperature, respectively. The latter was a function of age and temperature, and is taken from Barott and Pringle (1946). The term $675 (W/1000)^{0.75}$ is for MAE requirement taken from Siregar and Farrell (1980).

(c) Available ME within the system

Total available ME within the system is from both protein-free and protein fractions.

Available ME from protein-free fraction (EPF) in a diet is calculated by taking the difference between total digestible energy intake (DEI) and energy from digestible crude protein intake (DCPI).

$$EPF = (DEI - DCPI) \quad (5)$$

Digestibility of dietary protein and essential amino acid index (EAAI) is assumed to be 80% and 100, respectively. Digestible crude protein intake (DCPI) and biological value (BV) of dietary protein is then calculated according to Whittemore and Fawcett (1976) as:

$$DCPI = (DCP * DFI) \quad (6)$$

$$BV = (1.09 * EAAI - 11.7)/100 \quad (7)$$

From 5 and 6 available protein (AP) is determined as

$$AP = DCPI * BV \quad (8)$$

Protein retained is estimated from the following equation:

$$PR = AP \,.\, Z \,.\, C_3 - 1.26\ (W/1000)^{0.75} \quad (9)$$

where PR, AP, Z and C_3 represent daily protein retention (g/day), available protein, gross efficiency of utilisation of dietary crude protein as a function of body weight, and an inhibition constant of protein necessary for obligatory loss (Scott *et al* 1982).

The ME yield from protein is the difference between gross energy value of protein (taken as 23.6 kJ/g) and the sum of energy associated with urinary nitrogenous compounds and for their synthesis. Protein from urinary loss (PL) is determined as :

$$PL = DCPI - PR \quad (10)$$

The net yield of returnable ME from protein deamination to the system (EPL) is calculated as 9.1 kJ/g (Whittemore and Fawcett, 1976), which is considerably lower compared with the ME values for various protein sources. The estimate would be true only for the protein fraction not retained and eventually deaminated to lead to excretion.

Thus, the total ME derived from protein (Qp) is calculated as :

$$Qp = EPL + (23.6* PR) \quad (11)$$

Absolute protein synthesis needs to be determined before protein retention can be calculated. Muramatsu and Okumura (1985) and Muramatsu *et al* (1987), have used fractional synthesis rate (FSR, %/day) of whole body protein in chickens to determine the rate at which protein is synthesised. They described FSR as:

$$FSR = 0.9\ (36.0 - 0.3857\ A) \quad (A \leq 30 \text{ days}) \quad (12)$$

or $$FSR = 0.9\ (24.93 - 0.0167\ A) \quad (A > 30 \text{ days}) \quad (13)$$

where A corresponds to the age of birds (days). From this the absolute protein synthesis rate (PS, g/day) is calculated as:

$$PS = (PM \,.\, FSR)/100 \quad (14)$$

where PM is the protein mass (g). The value for PS is then used to calculate the energy cost for protein deposition which is the sum of the energy for PR and PS. The energy cost for protein retained is, then, used to calculate the available energy for lipid deposition as described below.

(d) Energy associated with protein and fat retention

The energy cost attributed to protein retention should not relate only to the synthesis of accreted protein but to total synthesis of protein. Thus

$$EPR = (7.3 * PS + 23.6 * PR) \quad (15)$$

where EPR is the energy cost of protein accretion, PS is the total protein synthesis (both new synthesis and re-synthesis) and PR is protein retained from new synthesis with energy content of protein being 23.6 kJ/g.

Estimates for daily rate of protein accretion indicates the maximum PR is often not reached before 500 g liveweight (Isariyodom, 1988). Therefore limitations for a maximum PR is imposed as 2.5, 5.0, 7.5 and 10.0 g/d for body weight of birds up to 100, 200, 500 and above 500g respectively.

The total ME derived from protein (TEP) is

$$TEP = 9.1(DCPI - PR) + (23.6 * PR) \quad (16)$$

Fat retention is calculated according to equations provided by Whittemore and Fawcett (1976) which was modified by Muramatsu (1988).

$$FR = (TEP - MAE + 0.365 * PM - EPR)/51.0. \quad (17)$$

(e) Predicted daily weight gain

As stated in equation 1 daily weight gain (WG, g) is determined from retained protein and fat. The equation is rewritten by Isariyodom *et al.*,(1988) as follows:

$$WG = 1.082\ (4.4*PR + 1.1*FR) \quad (18)$$

where PR and FR stand for protein retained (g/day) and lipid retained (g/day) respectively.

III. VALIDATION OF MODEL

Three sets of data derived from a nutritional experiment (Redlands), an Industry standard and observed performance from a commercial farm were simulated. The relationships between observed growth and predicted growth in chickens 1 to 7 weeks of age are shown in the Table 1 and illustrated in Figure 1.

Table 1 Regression equations of observed liveweights on simulated liveweight in meat chickens.

Data set	Slope ± SE	Intercept ± SE	r^2
Redlands Expt.	1.10±0.02	47.2±28.2	0.99
Industry Standard	1.08±0.03	48.6±41.2	0.99
Farm	0.99±0.02	23.5±28.2	0.99

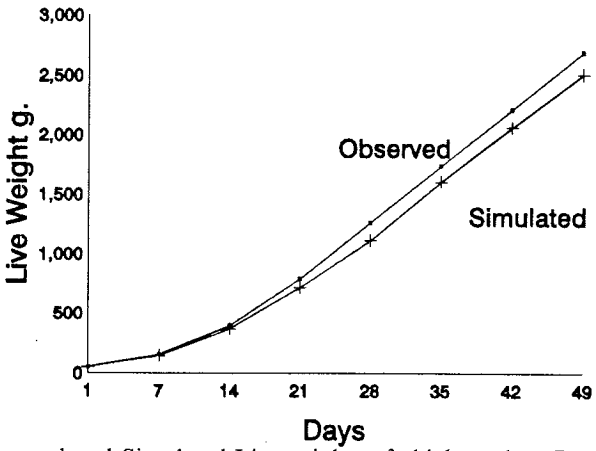

Figure 1 Observed and Simulated Liveweights of chickens 1 to 7 weeks old (Pooled)

The high correlation coefficients for each set of data indicates excellent agreement between observed values and those predicted by the model.

IV. CONCLUSION

The model appears to simulate growth adequately but further validation and testing is warranted. Further research on efficiency of protein utilisation for a wide variety of strains will assist in refining the model. Also there is a need to incorporate economic aspects to the model so that management decisions can be made using the model. With simulation models it is possible to take into account all the interacting factors and gain a better understanding of the processes involved in meat chicken growth and feed utilisation.

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LAYERS LEARNING TO CHOICE FEED

SRI SULANDARI, J.V. NOLAN and R.B. CUMMING

Animals in the wild have the ability to select feeds from those available so that they can grow and reproduce. In modern production units, birds do not have the opportunity to learn from parents or other experienced birds, and therefore have to learn from their personal experience of the metabolic consequences of eating particular feeds. In this 11-week study, 18 commercial layers (New Hampshire X White Leghorn) were reared to 21 weeks of age on a commercial crumble diet, then placed in individual cages until 24 weeks of age, then abruptly changed to choice feeding in their 25th week. The objective was to determine whether birds of this age could learn quickly to choose a well-balanced diet without affecting their subsequent production.

The choice-fed birds were offered ingredients with the following composition (per kg): whole wheat (116 g protein; 13 MJ ME), protein-rich pelleted concentrate (333 g protein, 10 MJ ME, 31 g Ca with added minerals and vitamins), and oyster shell chips (each ingredient in a separate bin in front of each bird). The experiment was run for 11 weeks in a room maintained at 20°C with lights on between 04.00 and 20.00 hours. A further 6 birds were fed a normal layer diet formulated from the above ingredients. They consumed 122 g/d of this feed and had an egg production of 93%.

During the first week of choice-feeding, the 18 choice-fed birds consumed 120 $\pm$ 5.6 g/d of wheat, protein concentrate and oyster shell but had quite variable intakes of these three components. In this week, ten hens appeared to balance their diets reasonably well, consuming daily (mean $\pm$ SE) 1.24 $\pm$ 0.058 MJ of ME, 19.9 $\pm$ 1.14 g protein and 4.1 $\pm$ 0.31 g Ca, whereas eight did not. The latter obtained sufficient ME, i.e. 1.08 $\pm$ 0.068 MJ, but ingested excessive amounts of the protein concentrate (25.3 $\pm$ 2.89 g protein) and insufficient Ca (2.1 $\pm$ 0.53 g Ca). From week 3 to week 6, the eight hens that were eating inappropriately had their dietary ingredients placed in a single feed bin with the shell chips on top. This appeared to cue the birds to select all three feed ingredients and to solve most of the problems of inappropriate feed selection. From week 7 to week 11, these birds were again offered the three feeds in separate containers, and six of these hens then selected the feeds appropriately. However, even after 11 weeks two birds were ingesting slightly more protein concentrate than wheat. One bird that did not ever consume protein concentrate or Ca chips dropped rapidly in production and was removed from the trial after three weeks. Most of the birds continued to lay well even though some took time to learn to choice feed (mean egg production/bird over 11 weeks was 90.8%, range 81-95%).

In summary, when birds with no experience of choice feeding were placed in individual cages and offered whole wheat, protein concentrate and oyster chips, they produced well. Some were apparently slow to develop appropriate feeding behaviour but it was possible to train them by offering mixtures of the three feeds in a single container. We conclude that, ideally, layers should be trained to choice feed in the growing phase thus avoiding the need to re-train them later.

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CHOICE FEEDING COMMERCIAL BROILER BREEDERS

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This study was conducted to compare the production of broiler breeder females when offered (a) restricted amounts of "standard" breeder crumbs, or (b) restricted amounts of similar but calcium (Ca) insufficient diet and free-choice Ca.

Commercially reared breeders were randomly allocated to 2 treatment groups, each with 4 replicates. Each replicate group containing 127 females and 12 males, was allocated to a pen in one of 2 experimental laying sheds, so that each shed housed two of the replicate groups for each treatment. Until 21 weeks of age, one treatment group was offered a "standard" pullet developer crumb (11.09 MJ ME/kg ME, 150 g crude protein/kg, 11 to 12 g Ca/kg) and the other treatment group was offered whole grain sorghum/protein concentrate mash (to "standard" pullet developer specifications) plus free access to washed, graded shell in the feed trough.

The experimental diets for females, offered from 21 to 60 weeks of age, were: conventional - "standard" breeder crumb (60 g/kg limestone) containing (per kg) 11.5 MJ ME, 150 g crude protein, 28-30 g Ca,; choice - whole grain sorghum/protein concentrate mash (no limestone) plus free access to washed, graded shell. Again, the choice diet was to the commercial "standard" specifications. Males were barrel fed on commercial breeder crumbs. All birds were hand-fed but all other management practices and production recordings were according to commercial procedure. Egg fertility, hatching egg weights and hen bodyweights were recorded weekly. Hatching egg specific gravity (SG) was recorded at 27, 28 and 31 weeks of age and weekly thereafter. The data were analysed weekly using AOV based on a two factor factorial design, the first factor being shed, the second being diet.

Production results to 60 weeks of age are shown in the table.

Table The effect of feeding either a conventional diet or offering choice feeding on broiler breeders from 24 to 60 weeks of age.

Feeding Method	Eggs/Hen Housed Total	Hatch	Bodyweight (g)	Kg feed/bird 24 - 60 weeks
Conv'l	143.0	126.0	4022	37.92
Choice	142.0	126.6	3948	36.65

Peak hen-week production of the choice-fed birds (76.6%) was reached at 30 weeks of age, one week later than that of the conventionally fed birds (79.3%); production of the former was lower ($P<0.05$) until the birds were 30 weeks of age. However, to 60 weeks of age, total egg production and total eggs hatched did not differ between treatments. SG was greater ($P<0.05$) in the choice-fed birds during weeks 38, 41, 48 and 49 and tended to be higher during other weeks. Fertility, hatchability and liveweight did not differ ($P<0.05$) between treatments.

C/- Southern Cross Concrete Ltd., Sydney

PRODUCTION AND CHARACTERIZATION OF RECOMBINANT CHICKEN INSULIN-LIKE GROWTH FACTORS

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Summary

Little is known about the functions of insulin-like growth factors (IGFs) in avian growth and development, this primarily being due to the low amounts of pure peptide available for testing. In order to overcome this we have developed gene-fusion strategies and downstream processing procedures to produce recombinant chicken IGFs from *Escherichia coli* (*E. coli*). We report here the production and characterization of recombinant chicken IGF-I and IGF-II, as well as an analogue which exhibits reduced binding affinity for IGF receptors and carrier proteins.

I. INTRODUCTION

Insulin-like growth factors (IGFs) are mitogenic proteins with structural homology to insulin. They elicit both insulin-like metabolic effects as well as growth promoting effects. The biological actions of the IGFs are generally considered to be mediated via interactions with the type-1 IGF receptor. These interactions are in turn modulated by specific IGF carrier proteins, IGFBPs (review, Walton *et al.* 1990). We have previously reported the amino acid sequences of the first non-mammalian IGFs characterized, namely chicken IGF-I (cIGF-I) (Ballard *et al.* 1990) and chicken IGF-II (cIGF-II) (Kallincos *et al.* 1990). Protein sequencing indicated that cIGF-I has eight amino acid differences compared with human IGF-I (hIGF-I). The sequence deduced from a cDNA for cIGF-I by Kajimoto & Rotwein (1989) confirms the protein sequence. Chicken IGF-II on the otherhand has 12 amino acids which differ from human IGF-II (hIGF-II). The isolation of a partial antisense transcript to the cIGF-II gene confirms most of the changes we identified (Taylor *et al.* 1991). Although we have evaluated the serum-derived cIGFs in a limited range of *in vitro* test systems, complete assessment of the *in vitro* and *in vivo* biological effects was not possible as insufficient quantities of the growth factors were available for testing. In order to overcome this we have developed gene-fusion and downstream processing strategies to produce recombinant IGFs from *E. coli*.

II. PRODUCTION OF RECOMBINANT CHICKEN IGFS

Recombinant chicken IGFs were produced in *E. coli* using a gene-fusion system similar to that we have developed for producing human IGFs. Synthetic genes with nucleotides optimized for protein synthesis in *E. coli* were generated for the required cIGFs. The synthetic genes were then subcloned into an *E. coli* bacterial expression vector which, when induced, expresses the cIGFs linked to a leader peptide. Codons for

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specific amino acids were engineered to be immediately upstream of the cIGFs to facilitate cleavage of the cIGFs from the expressed fusion protein with either hydoxylamine or a genetically engineered serine protease, subtilisin. The cIGF fusion proteins deposited in bacterial inclusion bodies were dissolved under reducing conditions, desalted, subjected to anion exchange chromatography to remove bacterial proteinases, refolded and partially purified by high performance liquid chromatography. The fusion proteins were then cleaved with hydroxylamine in the case of cIGF-I or with subtilisin for cIGF-II. The peptides were subsequently purified to homogeneity via three additional chromatographic steps (Upton *et al.* 1992).

III. *IN VITRO* CHARACTERIZATION OF RECOMBINANT CHICKEN IGF-I AND IGF-II

We have previously reported that serum-derived cIGF-I had about half the biological potency of hIGF-I (Dawe *et al.* 1988). We have now ascertained that cIGF-I is in fact equipotent with hIGF-I in biological assays measuring either the ability of the peptides to stimulate protein synthesis (Fig. 1a) or inhibit protein degradation in rat myoblasts (results not shown). The biological actions of IGFs are generally considered to be mediated by the type-1 IGF receptor, thus not suprisingly, recombinant cIGF-I and hIGF-I exhibited similar affinities for this receptor in rat myoblasts (Fig. 1b). Moreover, hIGF-I and cIGF-I were equally potent in both biological and receptor-binding assays performed in chick embryo fibroblasts (Fig. 2). Both peptides were also equivalent in their affinities for the IGFBPs present in rat or chicken serum (results not shown).

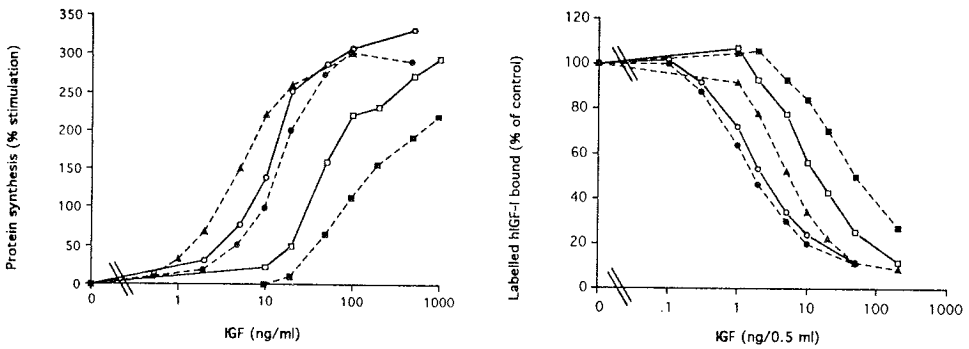

Figure 1 Effects of IGFs on a) protein synthesis and b) competition for binding of [^{125}-I]-labelled human IGF-1 to type-1 IGF receptors in rat myoblasts. The proteins tested were chicken IGF-1 (●), human IGF-1 (○), chicken IGF-11 (■), human IGF-11 (□) and the chicken IGF-1 analogue (▲).

In vitro analysis of recombinant cIGF-II, on the otherhand, has revealed differences between cIGF-II and its human counterpart. For example, recombinant cIGF-II is 3.5-fold less potent in stimulating protein synthesis in rat myoblasts (Fig. 1a) apparently due to a decreased affinity for the type-1 IGF receptor (Fig. 1b). The human

and chicken peptides are equipotent, however, in studies assessing binding to the type-2 IGF receptor and to IGFBPs (results not shown). Moreover, recombinant cIGF-II and hIGF-II are equipotent in both biological (Fig. 2a) and receptor-binding (Fig. 2b) studies in chick embryo fibroblasts, suggesting that there may be a difference between mammalian and avian type-1 IGF receptors.

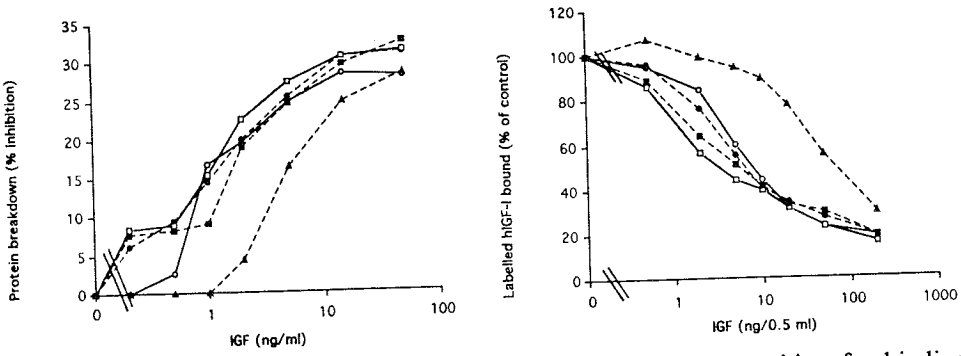

Figure 2 Effects of IGFs on a) protein breakdown and b) competition for binding of [^{125}-I]-labelled human IGF-1 to type-1 IGF receptors in chick embryo fibroblasts. The proteins tested were chicken IGF-1 (●), human IGF-1 (○), chicken IGF-11 (■), human IGF-11 (□) and the chicken IGF-1 analogue (▲).

IV. ANALOGUE OF CHICKEN IGF-I THAT BINDS POORLY TO IGF BINDING PROTEINS

A recombinant analogue of cIGF-I with an eighteen amino acid N-terminal extension as well as a substitution of Arg for Glu at position 3 in cIGF-I has also been produced. This particular analogue was engineered because *in vitro* and *in vivo* studies with the human equivalent of this protein have shown that it is a more potent form of IGF-I. The increased potency is not associated with an increase in affinity for the type-1 IGF receptor. Instead, the increased potency is a result of decreased affinity for IGFBPs. The recombinant chicken homologue we produced also has decreased affinity for IGF receptors (Fig. 1b. and 2b) and for IGFBPs (results not shown), while exhibiting enhanced biological potency in rat myoblasts (Fig. 1a). The analogue, however, was less effective than cIGF-I at inhibiting protein breakdown in chick embryo fibroblasts (Fig. 2a). The difference in biological potency between the rat and avian cell lines is caused by the difference in the concentrations of IGFBPs produced by the cells. Thus, rat myoblasts produce abundant IGFBPs whereas chick embryo fibroblasts produce little, if any IGFBPs (Ross *et al.* 1989).

V. DISCUSSION

The production of the recombinant cIGF-I and cIGF-II, as well as the cIGF-I fusion-protein analogue, will permit not only a wide range of *in vitro* studies, but also

experiments involving exogenous administration of chicken IGFs. Furthermore, the recombinant peptides will allow the development of homologous radioimmunoassays for measuring absolute levels of IGFs in chicken serum. This in turn will be useful in establishing whether serum IGF levels can be used as indicators or predictors of potential differences in growth and nutrient partitioning in birds. Considerable research effort is still required to determine if IGFs play any roles unique to avian growth, or indeed whether cIGFs, or analogues of cIGFs, can be used to improve growth performance of birds. However, if IGFs are in fact involved in as many varied and important functions as is found in mammals, the benefits to the poultry industry could be substantial.

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RESPONSES OF LAYING HENS TO WHEAT-BASED DIETS CONTAINING VARIOUS PHOSPHORUS CONCENTRATIONS

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Estimates of the available phosphorus requirements of laying hens vary from 2.5 to 4.8 g/kg. The current study was designed to examine the responses of an Australian White Leghorn x New Hampshire cross to dietary available phosphorus concentrations between 1.2 and 5.1 g/kg.

Six replicates of six hens (22 weeks of age) were allocated to each of four experimental diets. Diet 1 consisted of a basal wheat-based diet in which the phosphorus was only supplied from plant ingredients. Three additional diets were formulated in which the dietary phosphorus was increased through the use of dicalcium phosphate. The four concentrations of available phosphorus (1.2, 2.5, 3.8 and 5.1 g/kg) were calculated using the bioavailability data derived by Cromwell and Coffey (1993) using growing chicks. They corresponded to 3.0, 4.1, 5.3 and 6.6 g determined total phosphorus/kg. The diets were fed for a total of 28 weeks in a commercial layer shed. Feed intake, egg production, egg weight, the incidence of egg shell defects and egg shell quality were monitored. Results are in the Table.

Dietary Available-P (g/kg)	Food Intake (g/h/d)	Egg Production (%)	Egg Weight (g)	Shell breaking Strength (g)	Shell Thickness (μm)
1.2	109.0	87.4	61.2	2275	373
2.5	108.8	88.1	60.5	2189	366
3.8	107.6	86.8	61.2	2241	366
5.1	109.0	87.5	61.1	2223	359
LSD (5%)	6.4	3.9	2.4	240	17

Dietary phosphorus concentration had no significant effect on feed intake, production or egg shell quality. The results indicate that the phosphorus requirement of hens fed these wheat-based diets was met by the diet containing 1.2 g available phosphorus/kg. Assuming each egg contains 0.12 g phosphorus, calculations show that 80% of the available phosphorus intake was deposited in the egg. Similar requirements (1.6 g/kg) for laying hens fed wheat-containing diets have been suggested by Vogt (1992). Presumably, the presence of high intrinsic phytase activity in wheat (Sauveur, 1984) contributes in a significant way to this response.

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Proc. Aust. Poult. Sci. Sym. (1994) 6

ENDOCRINE REGULATION OF POST-HATCH GROWTH AND METABOLISM IN CHICKENS

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Summary

It has been well documented that the single most important endocrine factor regulating postnatal somatic growth in mammals is somatotropin [a.k.a. growth hormone (GH)]. In such species, GH clearly has effects [many mediated by insulin-like growth factor (IGF)-I] on lean (muscle) and adipose tissue accretion, as well as skeletal growth. Despite clear evidence in mammals, significant, "true growth" effects of this hormone in the chicken have not been similarly demonstrated. Specifically, skeletal muscle deposition, the most economically relevant target tissue for enhancement in meat animals, has not been consistently or dramatically increased in studies where the species-specific hormone (ie., either recombinant or pituitary-derived chicken GH) has been used. Alternatively, GH does appear to have important metabolic effects in the chicken, which ultimately impact on the efficiency of gain and on net adipose tissue deposition. This review will emphasize the demonstrated metabolic effects of the hormone, and explore why a positive effect on skeletal muscle accretion has not been realized.

I. INTRODUCTION

The process of growth is unquestionably complex and under the coordinated control of a multitude of factors. The homeostatic and homeorhetic mechanisms governing nutrient partitioning, cell proliferation, differentiation and hypertrophy, and degradative processes are implemented by a variety of hormones and growth factors, which are subject to environmental influences, nutritional constraints and genetic limitations. It is beyond the scope of this paper to provide a comprehensive review of all such factors (see Scanes et al., 1984; Leung, 1986; Scanes, 1987), however, several of those playing a major role in the post-hatch growth process will be discussed briefly. These major players include the IGFs (which are expertly reviewed in the accompanying paper by J. P. McMurtry), thyroid hormones (primarily the active form, T_3), insulin and glucocorticoids. A variety of peptide growth factors including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGF-beta) also have critical roles in growth and developmental processes (see Dayton and Hathaway, 1991).

Insulin is clearly an anabolic hormone, with pleiotropic effects ranging from stimulation of cellular transport and utilization of substrates to cell proliferation. Insulin enhanced cellular uptake of thymidine into DNA by neonatal chicken muscle satellite cells in culture (Duclos et al. (1991), an effect that is likely mediated via the IGF-I receptor. Similarly, insulin stimulated proliferation of chicken heart mesenchymal cells in primary culture, an effect which was synergistic with EGF, but required high

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concentrations of insulin (Balk et al., 1982). Anabolic processes are enhanced and degradative processes inhibited by insulin, which increased amino acid transport and glucose oxidation in thymocytes (Simon, 1979), and inhibited protein degradation in isolated muscle preparations (Klasing and Jarrell, 1985) from growing chickens. A dominant role for insulin in lipid metabolism is supported by both direct and indirect evidence. Insulin is required to maintain high rates of lipogenesis in cultured chicken hepatocytes (Tarlow et al., 1977), and inhibits stimulated lipolysis in adipose tissue in vitro (Campbell and Scanes, 1988). In vivo, insulin administration enhances substrate incorporation into hepatic lipids (Kompiang and Gibson, 1976) and lipid deposition into large vessels, with elevation of serum lipids (Stout et al., 1973). Hypothyroid states which result in increased adiposity are proposed to be mediated via alterations in the insulin:glucagon ratio (involving simultaneous increases in circulating insulin and decreases in glucagon), as were observed in PTU-induced hypothyroid chicks (Raheja et al., 1980). In vivo studies also suggest a role for insulin in stimulating feed intake and, consequently, body weight gain (Shanawany et al., 1979; Sonoda, 1983).

Corticosterone is also a key metabolic hormone, particularly with regard to fat deposition. The hormone increases hepatic lipogenesis in vitro (Kafri et al., 1988), and markedly inhances fat deposition in vivo, with high levels of the glucocorticoid resulting in reduced overall growth rate, feed efficiency, and skeletal muscle mass (Gross et al., 1980; Davison et al., 1983). The fattening effect has been demonstrated to involve decreased T_3 and IGF-I status (Buyse et al., 1987), which may also be involved in the growth depressive effects of the hormone.

No hormone is more centrally involved in directing the rate of metabolism than triiodothyronine (T_3), the active form of thyroid hormone in the chicken. The importance of thyroid hormones in thermoregulation in birds is well established (Nobukumi and Nishiyama, 1975; Lam and Harvey, 1990), with rapid peripheral conversion of T_4 to T_3 proposed as a "first response" mechanism to cold exposure, prior to pituitary thyrotropin release (Rudas and Pethes, 1984). The involvement of T_3 in post-hatch growth as well is supported by the demonstration that early thyroidectomy dramatically retards growth of neonatal chickens, with restoration of growth achieved by T_3 replacement therapy (see King and May, 1984, for review). Similarly, broiler chickens rendered hypothyroid by methimazole treatment exhibit depressed growth in conjunction with reduced IGF-I levels (despite high GH) (Decuypere et al., 1987). The role of T_3 in growth is also implicated by the endocrinological profile of the sex-linked dwarf (SLD) chicken, which is characterized by elevated circulating GH, decreased IGF-1 (associated with a reduced GH receptor status in target tissues, leading to GH resistance), and decreased T_3 (Huybrechts, 1985; Leung et al., 1987; Kuhn et al., 1989;). The T_3 deficiency is likely related to the GH resistant state, given the known action of GH in stimulating peripheral conversion of thyroxine (T_4) to T_3 in the chicken (Kuhn et al., 1987). Replacement therapy with T_3 increases somatic and skeletal growth in the SLD (Marsh et al., 1984), again underlining the requisite need for some basal or threshold level of T_3 for normal growth to be expressed. Alternatively, in normal T_3 states, thyroid hormone supplementation to the point of T_3 excess depresses growth (Bowen et al., 1987; Decuypere et al., 1987), noteably in the face of normal IGF-I levels (Decuypere et al., 1987). Thus, although, adipose tissue accretion is reduced as a result of an increased metabolic rate (and, possibly, reduced insulin:glucagon ratio - see above)

in hyperthyroid states, at too high a level the potential for compromising overall somatic growth, including that of lean tissues, must be recognized (May, 1980; Suthama et al., 1989; Wang et al., 1989). The results of the above studies further suggest a role for T_3 in maintenance of IGF-I production, which may represent at least part of the mechanism for the growth retardation of T_3-deficient states. Whereas GH receptor status was not assessed in the above studies, given the profile of the SLD, and the relative GH responses in the study by Decuypere et al. (1987), regulation of the GH receptor by T_3 as a means of mediating IGF-I should be a logical focus of future investigations.

II. GROWTH HORMONE

(a) Early Versus Late Post-Hatch Growth

If the collective body of studies in which exogenous chicken GH (cGH) was administered to early post-hatch (one day through six weeks of age) chickens is reviewed, there is little indication of a substantial or consistent improvement in one or more growth performance parameters (including rate, efficiency or composition of gain) (Table 1). It is generally concluded that growth of pituitary-intact, normal broiler chickens is not improved by exogenous cGH enhancement during the early post-hatch growth period. Several factors are believed to mitigate this consensus, including the status of endogenous GH secretion over this period, which is maximal relative to later ages and characterized by high amplitude pulses (Vasilatos-Younken and Zarkower, 1987; Johnson et al., 1987). This high level of endogenous GH secretion is in conjunction with maximal growth velocity (relative to later ages) during this period, suggesting that target tissue responsiveness may already be at threshold levels. The inability to measure substantial hepatic GH binding in young broilers, prior to sexual maturity (Leung et al., 1987; Vanderpooten et al., 1991), may in fact reflect rapid turnover of a finite pool of membrane receptors in response to high circulating concentrations of GH during this period. This is supported by the observation that removal of GH by means of hypophysectomy in young chicks increased apparent hepatic GH binding, which was subsequently reduced to control levels by GH replacement (Vanderpooten et al., 1991b).

In contrast to earlier ages, during the late post-hatch period (8 to 12 weeks of age), endogenous GH secretion is low to non-measureable, and growth rate has slowed dramatically (Vasilatos-Younken and Zarkower, 1987). Based on studies in red meat animals where the repartitioning effects of GH have been demonstrated (Chung et al., 1985; Campbell et al., 1988;), it would be expected that GH administration would be most effective when nutrient partitioning is shifting towards proportionately greater adipose tissue deposition, and efficiency of gain is markedly declining. This point is approximately 6 weeks of age in modern, commercial broiler chickens (Leeson and Summers, 1980). It has also been suggested that it is energetically more favorable to redirect nonlipid precursors away from the site of lipogenesis (ie, liver, in the chicken) to other tissues (eg, muscle), than to expend ATP for the processes leading up to and including lipogenesis, and then catabolize storage lipid (adipose tissue) to subsequently supply fuel for (extrahepatic) tissues (Etherton, 1989). This predicts that GH, as a repartitioning agent, would be expected to reduce adipose and enhance lean tissue

deposition at least partly by inhibiting lipogenesis, which has been convincingly demonstrated in swine (see Etherton, 1989 for review).

Table 1 Growth-related responses of normal, pituitary-intact chickens to recombinant (r-) or pituitary-derived (p-) chicken growth hormone (cGH) during the early post-hatch period (hatching through six weeks of age).

Age at administration	Hormone/ dosage (ug/kg BW/d)	Response	Reference
1 to 15 days	p-cGH 250 to 90	BW gain from 17 to 30 d after termination of cGH in males	Scanes et al., 1986
21 to 25 days	p-cGH 60	BW gain to 3 d post-treatment	Scanes et al., 1984
4 to 6 weeks	p-cGH 5 to 2.5	BW gain at 7 d but not at 14 d of treatment	Leung et al., 1986
1 to 28 days	p-cGH 100	No growth improvement	Bowen et al., 1987
3 to 5 or 6 weeks	p-cGH 100 or 200 composition	No effect on BW gain, feed efficiency, or carcass	McGuinness and Cogburn, 1988; Cogburn et al., 1989a
3 to 5 weeks	p-cGH 100 or 200	No effect on BW gain; Increased carcass fat	Cogburn et al., 1989b
	r-cGH 200	No increase in carcass fat	
2 to 24 days	r-cGH 1500	Increased carcass fat in females at end of treatment; Decreased fat in males at 24 d post-treatment	Burke et al., 1987
2 to 5 weeks	p-cGH 20	No effect on growth performance	Cravener et al., 1989

Given the above, it is not surprising that cGH administration to meat-type chickens during the late post-hatch period has been effective in improving measures of growth

performance. Growth rate and breast muscle mass were increased in cockerels administered cGH from 12 to 15 weeks of age, and breast muscle and dressed carcass yield were improved when cGH was given from 8 to 11 weeks of age (Scanes et al., 1990). Growth rate, feed efficiency and carcass composition were improved in pullets administered cGH from 8 to 11 weeks of age (Vasilatos-Younken, et al., 1988), however, the effects on carcass composition were expressed as a marked reduction in carcass fat content (31%), with no significant alteration in skeletal muscle deposition. In the latter study, positive reponses to GH were obtained only when the hormone was administered intravenously in a pulsatile pattern to mimic the endogenous pattern characteristic of young, rapidly growing broilers. When the identical dose of p-cGH was administered as a continuous infusion, resulting in chronic exposure of tissues to a markedly elevated baseline level of GH, growth performance parameters were either negatively affected (eg, feed efficiency) or unaffected (eg, carcass fat content), suggestive of inhibition of GH action (Vasilatos-Younken, et al., 1988).

(b) Pattern of Exposure to GH

The differential response to GH pattern was further explored in pullets administered the same dosage of p-cGH, beginning at the same age, as in our previous study (Vasilatos-Younken et al., 1988), in either a pulsatile or continuous pattern of infusion (Rosebrough et al., 1991). Pulsatile cGH reduced in vitro hepatic lipogenic rates (determined at the end of in vivo treatment delivery) over 80% compared to control levels, whereas continuous cGH had no effect. Gross measures of lipid deposition were consistent with in vitro responses, with mass of the abdominal fat pad reduced 32% by pulsatile cGH, but not altered by continuous delivery of the hormone (Rosebrough et al., 1991). These dramatic differences in lipogenic response to GH pattern of delivery suggest that regulatory mechanisms exist in the bird to prevent "over-stimulation" by GH.

Recently, further insights into this phenomenon were provided by the construction of a hybrid receptor containing the extracellular binding domain of the human GH receptor (GH-R) linked to the intracellular domains of the murine granulocyte colony-stimulating factor (the latter to provide a cellular signalling assay) (Cunningham et al., 1991; Fuh et al., 1992;). It was demonstrated that at low GH concentrations, the hormone is bound sequentially at two sites to the extracellular binding domains of two individual receptors, resulting in formation of a GH-$[GH\text{-}R]_2$ complex. This dimerization of the receptor is believed to be essential for signal transduction. At high GH concentrations, "excess" GH molecules are bound exclusively at one site, preventing requisite dimerization and, consequently, signal transduction, with loss of biological action (Cunningham et al., 1991; Fuh et al., 1992). Thus, continual exposure to high GH concentrations may lead to loss of GH action due to blocking of the pathway of signal transduction. Although this mechanism remains to be demonstrated in the chicken, it's existence would be consistent with the "anti-GH" effects reported (eg, increased carcass fat deposition; Cogburn et al., 1989b) with chronic GH exposure.

(c) GH Metabolic Effects

When cGH is administered to late post-hatch chickens in an appropriate pattern of exposure to maintain resposiveness to the hormone, dramatic reductions in lipid deposition occur, as reflected by reductions in mass of the abdominal fat pad, total carcass fat content, and hepatic lipogenic rates (Vasilatos-Younken et al. 1988; Rosebrough et al., 1991). In reviewing physiological responses presented in the above cited studies, a picture emerges which describes the metabolic hormone profile when GH is effective in reducing net fat deposition. This picture is one of elevated circulating T_3 concentrations, with no significant increase in the insulin:glucagon ratio or in corticosterone levels. Alternatively, states of chronic or excessive GH enhancement are characterized by elevated insulin and corticosterone, lower T_3 levels (than with pulsatile or an effective GH pattern), and no reduction in net fat deposition. Thus, it appears that when GH exposure pattern is appropriate to allow for tissue resposiveness to the hormone, the overall metabolic response includes increases in some (T_3) and decreases in other (insulin, corticosterone) hormones, which may then mediate the observed metabolic effects of GH. Further evidence that the reduction in hepatic lipogenesis by GH is indirect is the recent report that GH was not directly effective in altering lipogenic rates in either freshly isolated or cultured hepatocytes (Griffin and Windsor, 1993).

An additional GH effect that is integral to its ability to reduce net adipose tissue accretion is reduction of voluntary feed intake. This effect has largely gone unrecognized due to the paucity of studies where appropriate conditions as described above existed for expression of responsiveness to GH. In all studies with red meat animals where GH is effective as a repartitioning agent, improvements in efficiency of gain partly reflect reductions (10-15%) in feed intake (Etherton et al., 1989). Sufficient studies on cGH administration to broiler chickens are beginning to accumulate to allow for the observation that broilers may be even more sensitive in this regard, to the point where growth of desirable tissues (eg, muscle) is compromised if excessive dosages of the hormone, or nutritionally-limited basal diets are employed. Voluntary feed intake was reduced by 10.7% during the first week of pulsatile cGH infusion when positive growth responses were obtained in a previous study (Vasilatos-Younken et al., 1988). Similarly, in a recent study where endogenous pulsatile GH secretion was enhanced 6 to 7-fold by means of human pancreatic GH-releasing factor (hp-GRF) administration, feed intake was reduced 8%, and BW gain was increased 20% in comparison to controls over a four-day treatment period (Vasilatos-Younken et al., 1992). However, a higher dose of hp-GRF that enhanced plasma GH even further (10-fold) reduced feed intake by 33%, resulting in a 130% reduction in net weight gain over the same short treatment period (Vasilatos-Younken et al., 1992). Clearly, lean tissue accretion can not be supported when nutrient intake is reduced too severely, regardless of the degree of improvement in efficiency of protein synthetic processes. In an unpublished study (Johnson, McMurtry and Vasilatos-Younken, 1989), feed intake was reduced so severely in broilers fed a low energy diet (11 MJ/kg) and administered cGH in a pulsatile pattern, that 60% of birds exhibited total anorexia within several days after initiation of cGH treatment. This effect was immediately reversible by termination of cGH infusion, and not observed in control birds fed the same diet but infused with vehicle. In contrast, birds maintained on a 14 MJ/kg diet and administered the same dosage of cGH consumed

7% less feed, with a 13% improvement in feed efficiency, compared to controls on the same diet. These data suggest an intake suppressive effect of GH beyond the level of feed intake reduction accounted for by an improvement in the efficiency of lean tissue synthesis and synergistic with marginal energy density diets. The pharmacological (dose-response) relationship for the suppressive effect of GH on feed intake, and its interaction with dietary nutrient density must be further explored.

III. CONCLUSIONS

Our understanding of the growth process in poultry is still relatively naive. As genetic selection programs continue to improve productivity, growth-related disorders such as skeletal abnormalities, ascites, increased mortality and, in breeder stocks, decreased reproductive efficiency, underline the need to expand our basic knowledge of the regulation of growth processes and interactions among metabolic hormones. The delineation of the metabolic effects of GH and its apparent suppression of voluntary feed intake are important contributions to this understanding, but only a fraction of what must surely be a much larger picture yet to be unveiled.

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RESPONSE OF PLASMA CREATINE KINASE TO DIETARY VITAMIN E IN HEAT STRESS IN BROILERS

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Heat stress may lead to tissue damage by a variety of mechanisms including changes in the acid-base balance, impaired immunological reactivity and hormonal responses. Production of active oxygen free radicals results in lipid peroxidation and damage to organelle and cell membranes. In normal animals there is sufficient endogenous antioxidant capacity to remove active oxygen but this mechanism may be inadequate in stressed animals. Major cellular changes in heat stress give rise to an efflux of creatine kinase (CK) from cells into plasma. Since cell membrane stability is influenced by vitamin E, an experiment was carried out to investigate responses of CK and other stress- related characteristics to dietary vitamin E.

The experiment involved rearing 60 broilers of a commercial strain (Ross) on practical starter and grower diets containing either 0 or 300 mg supplemental vitamin E/kg diet under normal conditions to 8 weeks of age and then exposing the birds to an acute heat stress. The heat stress conditions were chosen to resemble the acute stress that might be encountered when birds are being transported to a processing factory. This involved confining the birds in crates in an environmentally-controlled room at 35°C for 2 hours. Blood samples were taken prior to (t_0) and after the period of stress (t_1).

Both groups of birds attained a mean weight of 2.8 kg. Pre-stress body temperatures were very similar; post stress temperatures were elevated by 3°C in both groups but there was no beneficial effect of vitamin E supplementation. Mean pre-stress CK activities were the same in both groups (495 IU/l) but were higher in the control group post-stress. When the CK changes for each bird were analysed, there was a significant ($P<0.05$) effect of vitamin E, those birds supplemented with vitamin E showing a smaller mean increase (237 IU/l) than the control birds (389 IU/l). This difference was also apparent in the CK ratio (t_1/t_0) where the vitamin E supplemented birds exhibited a significantly lower ($P<0.05$) CK ratio (1.48) than the controls (1.97). There were differences in the lactate dehydrogenase activities pre- and post-stress but no effects of vitamin E. Haematological characteristics showed a basophilia in the post-stress samples consistent with acute heat stress. Thirteen of the vitamin E supplemented birds and nine of the control birds exhibited a leucopenia post-stress. There were no significant differences in the heterophil/lymphocyte ratio pre- or post-stress.

The results showed that feeding a high level of vitamin E to broilers could alleviate one of the characteristic metabolic abnormalities associated with heat stress. CK is released from muscle cells in stressed birds in response to an intracellular influx of calcium, and the results suggest that vitamin E must interact with this mechanism, either by inhibiting the calcium influx or the CK efflux. Effects of vitamin E on muscle cell membrane integrity during heat stress could have an important implication for meat quality in birds being transported to a processing factory.

We acknowledge support from the Ministry of Agriculture, Fisheries and Food and Rhône-Poulenc Animal Nutrition for this work.

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Proc. Aust. Poult. Sci. Sym. (1994) 6

NUTRITIONAL AND CELLULAR FACTORS AFFECTING TIBIAL DYSCHONDROPLASIA IN BROILERS

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Summary

The results of a series of experiments have shown that tibial dyschondroplasia (TD) can be prevented by supplementing diets with 1,25-dihydroxyvitamin D and that this can lead to an improvement in broiler leg health. A slowing of the rate of differentiation of growth plate chondrocytes leads to TD, and 1,25-dihydroxyvitamin D has been shown to speed up this process. However a comparison with another analog of vitamin D has suggested the effect on differentiation may be indirect. 1,24,25-trihydroxy- but not the 24,25-dihydroxy-derivative has also been shown to reduce TD.

There is an interaction between dietary 1,25-dihydroxyvitamin D and Ca concentrations. Higher levels of both can lead to hypercalcaemia and growth depression. Ascorbic acid appears to have an additive or synergistic effect in minimising TD when fed in conjunction with 1,25-dihydroxyvitamin D. A possible strategy for minimising TD might involve feeding a relatively low dose of 1,25-dihydroxyvitamin D (2 μg/kg) in conjunction with ascorbic acid.

I. INTRODUCTION

Angular and rotational deformities of leg bones are widespread in young birds, especially in broilers but also in turkeys. Dyschondroplasia is a widely observed defect in the growth plates of leg bones. It occurs most noticeably in the growth plate of the proximal tibia, the fastest growing bone in the young broiler, and is frequently referred to as tibial dyschondroplasia (TD). Resulting from a failure of growth plate chondrocytes to differentiate fully, it leads to a build up of a mass of prehypertrophic chondrocytes. The lesions develop between the ages of 2 and 5 weeks and can regress thereafter.

The importance of TD in causing bone deformity has been assessed by carrying out sequential radiography of the proximal tibias of broilers growing up to 16 weeks of age. At this age the tibias were dissected and the angulation of the proximal end (tibial plateau angle, TP°) was measured (Lynch *et al.*, 1992). The severities and durations of the TD lesions, assessed by radiography, were found to be highly correlated with the TP°. Birds with abnormally large TP° were obviously lame. These observations confirm that TD can lead to distortion of bone growth and that this can contribute to lameness in broilers.

It has been reported (Edwards, 1989;1990) that dietary supplementation with 1,25-dihydroxyvitamin D can reduce the incidence of TD. More recently, Rennie *et al.* (1993) have shown, using histological techniques to accurately diagnose TD, that 1,25-dihydroxyvitamin D can completely prevent TD. The present paper summarises a series of nutritional experiments that have been carried out to investigate the role of 1,25-dihydroxyvitamin D in preventing TD and its interactions with other dietary factors,

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particularly calcium and ascorbic acid. Results of cellular studies on the mechanism bywhich 1,25-dihydroxyvitamin D might prevent TD are also reported.

II. MATERIALS AND METHODS

(a) Nutritional Studies

Six experiments are described. In Experiments 1,2,4, 5 and 6, groups of 20 male broiler chicks were reared up to 3 weeks of age in tier brooders on different dietary treatments. Birds were killed when they were 3 weeks of age and tibial growth plates were examined visually for the presence of lesions. Samples of growth plates were then fixed in formalin and the presence or absence of TD was confirmed histologically.

In Experiment 3, groups of 150 broilers were reared in litter floor pens and fed either a control diet or this diet supplemented with 5 μg 1,25-dihydroxyvitamin D / kg up to 3 weeks of age. All birds were then given the same grower diet, without vitamin D metabolite supplementation, up to 8 weeks of age when leg health of all birds was assessed visually.

(b) Cellular studies

Alkaline phosphatase (ALP) is produced in differentiating chondrocytes and its concentration can be taken as a marker of the extent of differentiation. Sections of growth plates from normal (i.e. non TD) birds fed diets with or without supplementation with 1,25-dihydroxyvitamin D were reacted for ALP. The amounts of ALP in different zones of the growth plate were measured by densitometry. Birds were also injected with bromodeoxyuridine (BrdUrd) as a marker of cellular proliferation. Birds were killed after 21 h and cells containing BrdUrd were detected in tissue sections by immunofluorescence. method. This enabled calculation of rate of proliferation (labelling index) and rate of chondrocyte movement down the growth plate.

The measurements on ALP were repeated, this time with dietary treatments involving a control diet alone or with regular oral dosing with 1,25-dihydroxyvitamin D or 1,25-dihydroxy-22-ene-24-hydroxymethylvitamin D, a potent inhibitor of proliferation and stimulator of differentiation of some cells. Its effects on chondrocytes are unknown.

III. RESULTS AND DISCUSSION

(a) Nutritional studies

The results of experiments 1 and 2 are shown in Table 1. They show that, when a diet of relatively low Ca content (7.5 g/kg) was used to maximise the incidence of TD, dietary supplementation with 1,25-dihydroxyvitamin D decreased the incidence of TD in a dose dependent manner. A dietary level of 5 μg/kg gave a zero incidence at 3 weeks of age without significant effect on growth. Body weight was not significantly ($P < 0.05$) affected. In contrast, supplementation with extra vitamin D itself did not decrease TD. More details of these experiments are given in Rennie *et al.* (1993).

In Experiment 3, it was found that at 8 weeks of age the leg health of birds fed

the starter diet containing 1,25-dihydroxyvitamin D was much superior. The proportion of birds with apparently normal legs increased from 19 to 34% whilst the proportion of birds with severely deformed legs dropped from 5 to 1% of the flock. These results thus demonstrated that prevention of TD by 1,25-dihydroxyvitamin D has the potential to improve leg health in broilers. However a slight but significant growth depression was noted in the treated group. Since the diets in this experiment contained higher contents of Ca (12 g/kg) than in the earlier study, an experiment was carried out to investigate possible interactions between dietary Ca and 1,25-dihydroxyvitamin D.

Table 1 Effects of different levels of vitamin D_3 and 1,25-dihydroxyvitamin D_3 in diets of imbalanced Ca/P (7.5 g Ca/kg; 7.5 g P/kg) on the growth and incidences of TD and rickets as assessed histologically at 3 weeks of age in groups of 20 broilers (Expts 1 and 2).

	Diet content		Weight	%TD	%rickets
	D_3(μg/kg)	1,25-D_3(μg/kg)			
Expt 1	25	0	535	21	14
	75	0	533	46	0
Expt 2	25	0	532	36	11
	25	2.5	549	19	0
	25	5	516	0	0
	25	10	522	0	0

The factorial treatments and results of Experiment 4 are summarised in Table 2. Interactions between dietary Ca in the range of 7.5 to 12.5 g/kg and 1,25-dihydroxyvitamin D occurred for growth and incidence of TD. The incidence of TD was much higher with the diet of lowest Ca content but dietary supplementation with 3.5 μg 1,25-dihydroxyvitamin D/kg reduced TD to low incidences (0-5%) at all dietary Ca levels. At these low incidences, the severities of TD lesions are invariable mild and unlikely to lead to clinical lesions. Growth depression occurred with diets of higher Ca and 1,25-dihydroxyvitamin D content and this was found to be related to elevated plasma ionic Ca concentrations (hypercalcaemia). The lowest level of 1,25-dihydroxyvitamin D found not to cause growth depression at any Ca level was 2 μg/kg, but this did not prevent TD completely.

The experiment on ascorbic acid was carried out after findings in earlier studies that dietary supplementation with ascorbic acid seemed to reduce the incidence of TD, though not prevent it completely. Previous reports have suggested that ascorbic acid can stimulate vitamin D hydroxylation (Weiser *et al.*, 1988). Experiment 5 therefore investigated the possibility that addition of ascorbic acid to a diet containing 2 μg 1,25-dihydroxyvitamin D/kg might exert an additional effect on TD. The results, shown in Table 3, indicate that supplementing a diet of 7.5 g Ca/kg with 2 μg 1,25-dihydroxyvitamin D only decreased the incidence of TD from 32 to 12.5%, but that the further addition of 250 mg ascorbic acid/kg to this diet eliminated TD.

Table 2 Effects of different dietary levels of calcium and 1,25-dihydroxyvitamin D_3 on bodyweight, incidence of TD and plasma ionic Ca at 3 weeks of age (Expt 4).

Diet content		TD(%)	Weight(g)	Ionised Ca (mmol/l)
Ca(g/kg)	1,25-D_3(μg/kg)			
7.5	0	50	741[a]†	1.50[a]
	2	15	748[a]	
	3.5	5	731[a]	
	5	0	738[a]	1.56[b]
10	0	10	739[a]	
	2	20	733[a]	
	3.5	5	712[a]	
	5	5	699[b]	
12.5	0	15	710[a]	1.55[b]
	2	15	730[a]	
	3.5	0	677[b]	
	5	0	617[b]	1.89[c]

† Means within columns which are not the same differ significantly ($P<0.05$).

Table 3 Effect of supplementing low and high calcium diets with 2 μg 1,25-dihydroxyvitamin D alone or combined with 250 mg ascorbic acid (AA)/kg on incidence of TD, bodyweight and plasma ionised Ca at 3 weeks of age (Expt 5).

Diet content			TD(%)	Weight(g)	Ionised Ca (mmol/l)
Ca(g/kg)	1,25-D_3(μg/kg)	AA(mg/kg)			
7.5	0	0	32	576[a]†	1.42[a]
7.5	2	0	12.5	605[a]	1.45[ac]
7.5	2	250	0	612[a]	1.46[ac]
12.5	0	0	16	586[a]	1.53[b]
12.5	2	0	0	613[a]	1.53[b]
125	2	250	5	576[a]	1.50[bc]

† Means within columns which are not the same differ significantly ($P<0.05$).

The sixth experiment involved supplementing a diet containing 7.5 g Ca/kg with ascorbic acid or different metabolites of vitamin D. The results are given in Table 4 are provisional because they are based on visual rather than histological diagnosis of TD. This perhaps explains the very high apparent incidence (90%) of TD in the control diet,

because some lesions that visually resemble TD are found subsequently not to contain the cellular changes characteristic of TD. Supplementation with 24,25-dihydroxyvitamin D had little effect on apparent TD incidence but supplementation with 1,25-dihydroxyvitamin D (2 μg/kg) or 1,24,25 -trihydroxyvitamin D (5 μg/kg) decreased the incidence. Adding either 250 or 1000 mg ascorbic acid/kg did not affect TD but combining the higher level of ascorbic acid with the 1,25-dihydroxyvitamin D supplement reduced TD to its lowest incidence.

Table 4 Effect of supplementing a diet containing 7.5 g calcium/kg with derivatives of vitamin D and/or ascorbic acid on the incidence of TD at 3 weeks of age (Expt 6).

Diet supplement	TD (%) (assessed visually)
Control	90
1,25-dihydroxyvitamin D (2 μg/kg)	35
24,25-dyhydroxyvitamin D (5 μg/kg)	71
1,24,25-trihydroxyvitamin D (5 μg/kg)	20
Ascorbic acid (250 mg/kg)	68
Ascorbic acid (1000 mg/kg)	75
1,25-dyhydroxyvitamin D (2 μg/kg) plus ascorbic acid (1000 mg/kg)	11

Chondrocytes cultured in the presence of ascorbic acid have been found to synthesise large amounts of 24,25- but only little 1,25-dihydroxyvitamin D. In feeding experiments, 1,25-dihydroxyvitamin D has been shown to elevate plasma levels of 24,25-dihydroxyvitamin D, an effect that was enhanced by adding ascorbic acid to the diet also (Berry *et al.*, 1993). These findings raise the possibility that 24,25-dihydroxyvitamin D might have a role in TD, but the lack of response to feeding this metabolite suggests that this is not so. However, the response to 1,24,25-trihydroxyvitamin D implies that 1-hydroxylation is an important feature associated with the involvement of vitamin D metabolites in TD.

The observation in Experiment 5 that ascorbic acid could enhance the effectiveness of low doses of 1,25-dihydroxyvitamin D was reproduced in Experiment 6. These observations suggest that a dietary combination of 1,25-dihydroxyvitamin D and ascorbic acid may be an effective way of preventing TD without risking growth depressions from the hypercalcaemia induced by combinations of higher dietary levels of Ca and 1,25-dihydroxyvitamin D.

(b) Cellular studies

Measurements of BrdUrd incorporation into chondrocytes showed that rate of proliferation of chondrocytes was unaffected by dietary 1,25-dihydroxyvitamin D. However labelled chondrocytes moved 12% faster down the growth plate with this treatment. This conclusion was supported by measurements of alkaline phosphatase in different maturation zones of the tibial growth plate. ALP activities were low and similar in proliferating chondrocytes in control and 1,25-dihydroxyvitamin D-treated birds and

reached similar high activities in fully differentiated chondrocytes in both groups. However activities were up to 100% higher in transitional chondrocytes in the treated birds. This result indicates that chondrocyte differentiation proceeded faster in birds given 1,25-dihydroxyvitamin D.

It has previously been suggested by Thorp *et al.* (1993) that TD is caused by a slowing of the rate of differentiation. This is on the basis of observations on lines of broilers selected for and against TD using an X-ray imaging device (Lixiscope) that the high TD line showed both focal accumulations and broad bands of transitional (but not dyschondroplastic) chondrocytes. A possible interpretation of the present results is that dietary supplementation with 1,25-dihydroxyvitamin D prevents TD by directly preventing the slowing down of differentiation that leads to TD. 1,25-dihydroxyvitamin D is known to control cell regulatory factors such as TGF-β and *c-myc* proto-oncogene and both of these factors have been shown to be decreased in TD chondrocytes (Loveridge *et al.*, 1993).

The results of the second cellular experiment, however, cast doubt on whether 1,25-dihydroxyvitamin D acts directly at the chondrocyte level in preventing TD. This experiment showed that, far from preventing TD, 1,25-dihydroxy-22-ene-25-hydroxymethylvitamin D increased the incidence and delayed chondrocyte differentiation as assessed by the distance of maximal ALP activity from the top of the growth plate. These distances were: control, 360 μm; $1,25(OH)_2D$, 300 μm; analog, 480 μm. This observation leaves open the possibility that vitamin D metabolites act at some site other than the growth plate by some other, perhaps calcaemic, mechanism that gives an indirect stimulation of differentiation.

It is of interest to note that ascorbic acid stimulates chondrocyte differentiation *in vitro*. It has been thought that it exerts this effect through stimulation of collagen synthesis and subsequent cell-matrix interactions. However it is not known at present whether the apparent interaction between 1,25-dihydroxyvitamin D and ascorbic acid involves these compounds acting *via* the same or complementary mechanisms.

ACKNOWLEDGEMENTS

We thank the Ministry of Agriculture, Fisheries and Food, Commission of the European Communities and F. Hoffmann-La Roche & Co for support for this research.

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