Proceedings of

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

1992

Volume No. 4

University of Sydney, Sydney, NSW

February 1992

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

1992

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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 Dr. R.F. Wideman at this year's Symposium.

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Current proceedings represent Volume No. 4. Previous proceedings of the Australian Poultry Science Symposium include; 1989 (Volume No. 1), 1990 (Volume No. 2) and 1991 (Volume No. 3).

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EFFECT OF WHEAT PENTOSANS ON ENDOGENOUS AMINO ACID SECRETION IN BROILER CHICKENS

K. ANGKANAPORN*, M. CHOCT*, G. ANNISON**, W.L. BRYDEN* and E.F.ANNISON*

Adding highly purified wheat pentosans (arabinoxylans) to broiler diets depressed nutrient utilization resulting in poor growth and feed efficiency in the birds (Choct and Annison 1990). Elevated levels of dietary fibre have also been shown to increase endogenous protein losses in chickens (Siriwan et al. 1990). To further characterize the anti-nutritive effect of wheat pentosans, the effect of this fibre on the ileal digestibility and endogenous amino acid secretion was examined using homoarginine as a marker (Siriwan et al. 1987).

Twenty-four 5 wk old broiler chickens were allocated into 3 groups and fed purified diets containing 200 g/kg casein as the sole protein source and wheat pentosans (0 g/kg, 15 g/kg or 35 g/kg). Wheat pentosans (720 g/kg arabinoxylans) were isolated as described by Choct and Annison (1990). The birds were fed the experimental diets ad libitum for 4 days. On the fifth day, the chickens were precision-fed with the same diets except that casein was replaced by "guanidinated" casein (Siriwan et al. 1987). Ileal contents were collected 3 h after precision feeding and analyzed for amino acid and acid insoluble ash (a digestibility marker). Endogenous amino acid secretions were calculated from the ratio of homoarginine and amino acids in experimental diets and ileal contents.

Pentosans (g/kg diet)	% Apparent Digestibility	% True Digestibility	Endogenous Lysine (g/100g CP intake)
0	76.97 ± 3.01^a	96.51 ± 0.03^a	0.47 ± 0.05^a
15	59.07 ± 4.75^b	94.76 ± 0.09^a	1.04 ± 0.04^b
30	60.01 ± 4.40^b	88.32 ± 0.18^b	0.88 ± 0.04^b

Addition of wheat pentosans at 15 and 35 g/kg significantly reduced the apparent ileal digestibility of amino acids ($P < 0.05$). Endogenous secretion of amino acids was enhanced by the presence of pentosans and at a level of 35 g/kg, the true digestibility of amino acid was depressed ($P < 0.01$). The findings indicate that the anti-nutritive effect of wheat pentosans in depressing apparent protein digestibility is the result of an increase in endogenous amino acid secretions. At higher dietary levels of pentosans, there is also a decrease in protein digestion. Both effects represent a loss to the birds which will adversely affect performance.

CHOCT, M. and ANNISON, G. (1990). Br.Poult.Sci. 31:811.

SIRIWAN, P., BRYDEN, W.L. and ANNISON, E.F. (1987). Proc.Nutr.Soc.Aust. 12:120.

SIRIWAN, P., BRYDEN, W.L., and ANNISON, E.F. (1990). Proc.Nutr.Soc.Aust. 15:112.

* Department of Animal Science, The University of Sydney, Camden, NSW, 2570

** CSIRO Division of Human Nutrition, Adelaide, SA 5158.

THE STRUCTURE OF WHEAT ARABINOXYLANS

G. ANNISON*, M. CHOCT**, and N.W. CHEETHAM**

One experimental approach used to establish that wheat arabinoxylans (pentosans) have an anti-nutritive activity when fed to broilers has been to isolate pentosans and examine their effects when added to experimental diets (Choct and Annison 1990). It is important to demonstrate that the isolation procedures do not cause structural changes in the pentosans which may affect their activity. Water-soluble (WEPBP) and alkali-soluble (AEPBP) wheat pentosans were isolated by a large-scale procedure from a milling by-product and by a small scale, gentle laboratory procedure (WEPW and AEPW) directly from wheat. The preparations were analysed by ^{1}H-nuclear magnetic spectometry (NMR). The chemical shifts of the anomeric resonances (and integrals) of wheat arabinoxylans are shown in the Table.

Assignments	Large-Scale Isolation		Laboratory Isolation	
	WEPBP	AEPBP	WEPW	AEPW
α-ara-3X	5.32 (0.24)	5.31 (0.24)	5.32 (0.16)	5.31 (0.23)
α-ara-3(A2)X[a]	5.22 (0.25)	5.21 (0.24)	5.21 (0.14)	5.21 (0.17)
α-ara-2(A3)X	5.14 (0.29)	5.14 (0.28)	5.14 (0.17)	5.14 (0.28)
α-glc?			5.22-5.27 (0.47)	
β-xyl	4.35-4.45 (1)	4.35-4.45 (1)	4.35-4.45 (1)	4.35-4.45 (1)

[a] α-arabinose linked to O3 of xylose also substituted with arabinose at O2.

From the ^{1}H-NMR data it is clear that the chemical structure of the four pentosan preparations are similar. The poorly resolved extra peak in the WEPW spectrum is probably glucose from residual starch. It can be concluded that it is possible to isolate wheat pentosans in large amounts with little change to the structure. Anti-nutritive activity associated with such preparations will probably also be exhibited by the pentosans in wheat-based diets. There appears to be little difference in the structure of the pentosans extracted by water and by alkali. These preparations exhibit similar anti-nutritive properties when added to broiler diets (Choct and Annison 1992).

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* CSIRO Division of Human Nutrition, O'Halloran Hill, SA 5158,Australia
** Dept. of Animal Science, Sydney University, Camden, NSW 2570,Australia
*** School of Chemistry, University of New South Wales, Kensington, NSW 2033, Australia.

CALCIUM AND CARBONATE SUPPLY POTENTIAL IN THE SHELL GLAND OF HENS LAYING EGGS WITH EITHER GOOD OR POOR SHELL QUALITY

D. BALNAVE and N. USAYRAN EL-KHATIB

Egg shell quality varies markedly in any population of hens. While many hens lay eggs with good quality shells others produce eggs with thin or weak shells. The present study was carried out to determine whether this variation in shell quality is associated with changes in the concentration of calcium-binding protein (CaBP) and the activities of the calcium ATPase (Ca ATPase) and carbonic anhydrase (CA) enzymes in the shell gland mucosa.

Forty eight 40-week-old laying hens from the same hatch were kept in individual cages in each of two temperature-controlled rooms at 25°C. Light was provided for 16 h daily, between 03.30 and 19.30 h in one room (normal light) and between 15.30 and 07.30 h in the other (reverse light). After 4 weeks the six hens producing eggs with either the best or the poorest shell strength in each room were killed between 09.00 and 12.00 h for shell gland assays. This time period corresponded to the time of active shell formation in hens kept in the reverse lighting regimen.

Shell breaking strength (SBS) and shell gland assays

	SDS (g)	CaBP (% bound)	Ca ATPase (μmol Pi/mgP/5 min)	CA (Units/mgP)
Light				
Normal	2206	71.2	0.44	0.75
Reverse	2011	66.2	0.48	0.57
Shell Quality				
Good	2500	68.4	0.49	0.68
Poor	1717	69.0	0.43	0.63
SEM		1.91	0.041	0.073

Factorial analysis of variance showed no significant treatment or interaction effects. The present data for CaBP and CA agree with other reports. However, Watanabe et. al. (1989) reported differences in shell gland ATPase activity between two lines of White Leghorn hens divergently selected for shell strength. The fact that all these markers are reduced significantly in hens rested from lay (Balnave et al. 1991) suggests that their physiological significance is related more to whether or not hens are producing eggs rather than to the absolute requirements for calcium and carbonate.

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Department of Animal Science, University of Sydney, Camden, NSW, 2570

LONG-TERM RESPONSES IN EGG SHELL DEFECTS RESULTING FROM THE SHORT-TERM USE OF SALINE DRINKING WATER IN EARLY LAY

D. BALNAVE and D. ZHANG

Although drinking water containing up to 2 g NaCl/l significantly reduces egg shell quality and significantly increases the incidence of egg shell defects in laying hens of all ages, the replacement of saline water with town water has little effect on the incidence of egg shell defects (Balnave, 1991) except when hens receive the saline water for a limited time in early lay (Yoselewitz and Balnave, 1989). This latter response was investigated further in the present studies.

Two studies were conducted using White Leghorn X New Hampshire hens. In Experiment 1 the treated hens received town water supplemented with 2 g NaCl/l between 23 and 28 weeks of age and in Experiment 2 between 26 and 30 weeks of age. Prior to, and subsequent to, this period the hens received town water. Controls were given town water throughout life. All hens received a proprietary layer mash (11.00 MJ of ME and 160 g CP/kg) throughout lay. Two replicates of 45 hens per treatment were used in Experiment 1. In Experiment 2 six replicates of 90 hens received the saline drinking water and 5 replicates of 84 hens were used as controls. In this experiment the incidence of shell defects in the 58 hens producing eggs with defective shells during the NaCl treatment was subsequently determined separately to those of the controls and the remainder of the NaCl-treated hens.

The incidence of shell defects was similar for controls (2.5%) and treated (2.8%) birds during the final week of the NaCl treatment in Experiment 1. In Experiment 2 a significant ($P<0.001$) increase in shell defects (6.3 vs 1.4%) was observed in birds on the NaCl treatment at this time but values were similar (1.7%) one week after the withdrawal of the saline water. In Experiment 1 an increase ($0.05<P<0.1$) in shell defects (10.4 vs 6.2%) was observed between 56 and 72 weeks of age in the NaCl-treated hens, with values of 13.6 and 7.2% respectively during week 72. During the similar period (55-71 weeks of age) in Experiment 2 shell defects were significantly ($P<0.05$) increased (10.5 vs 7.9%) in hens which had previously received the saline water for 5 weeks at the start of lay. However, the incidence of shell defects from the 58 hens which produced eggs with shell defects during the period of saline water treatment at the start of lay, and which subsequently overcame this problem after the withdrawal of the saline water, showed the highest incidence of shell defects at the end of lay. Shell defects in these hens increased from 7.3 to 20.9% between 55 and 71 weeks of age compared with increases from 7.1 to 12.5% in the remaining saline water-treated hens and 6.9 to 8.6% in the controls.

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Department of Animal Science, University of Sydney, Camden, NSW 2570

ASCORBIC ACID CONCENTRATIONS IN THE BLOOD AND SHELL GLAND OF LAYING HENS GIVEN TOWN OR SALINE DRINKING WATER AND ASCORBIC ACID SUPPLEMENTS IN THE DRINKING WATER OR DIET

D. BALNAVE* , D. ZHANG* and L. VOLKER**

Recent studies have shown that simultaneous supplementation with ascorbic acid alleviates the decline in egg shell quality that occurs when laying hens receive saline drinking water (Balnave et al., 1991). However, the mechanism by which ascorbic acid exerts this effect is unknown.

Two replicates of 50, 60-week-old laying hens (White Leghorn X New Hampshire) were allocated for 6 weeks to each of 5 treatments (see Table). Town water and a proprietary layer mash (11.00 MJ of ME and 160 CP/kg) were provided ad libitum with the supplements added as shown in the Table. Five hens from each shell quality group (see Table) were then selected for the ascorbic acid measurements. Samples were stabilised using 5 per cent metaphosphoric acid prior to the determination of ascorbic acid (Vuilleumier and Keck, 1989).

Ascorbic acid (AA) concentrations (SD) in tissues

Treatment and Supplement	Shell Type	Plasma (μmol/l)	Shell Gland mucosa (μmol/g)	Shell Gland Fluid (μmol/l)
1) None	Good	59.4(4.91)$^{a+}$	0.13(0.016)^{ab}	2.9(1.94)^{a}
2) NaCl(2g/l)	Poor	47.7(14.9)^{a}	0.13(0.039)^{ab}	11.2(5.46)^{ab}
	Good	58.9(7.87)^{a}	0.11(0.030)^{a}	10.9(3.05)^{ab}
3) AA(1g/l)	Good	143.7(29.6)^{c}	0.21(0.037)^{c}	21.3(6.06)^{b}
4) Tr2+AA(1g/l)	Good	100.9(10.5)^{b}	0.16(0.043)^{bc}	18.2(12.2)^{b}
5) Tr2+AA(2g/kg)	Good	124.7(12.8)^{c}	0.19(0.056)^{c}	17.7(7.11)^{b}

Values within a column with similar superscripts are not significantly different ($P>0.05$).

Shell defects were significantly higher (18.5%) on Treatment 2 compared with all the remaining treatments (range 7.2 - 10.1%). The data from Treatments 1 and 2 indicate that this effect was not related to differences in the ascorbic acid levels in the blood or shell gland. Ascorbic acid supplementation of the drinking water (Treatments 3 and 4) or diet (Treatment 5) increased its concentration in all three tissues. Maximum concentrations were obtained with ascorbic acid supplementation of the town water.

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* Department of Animal Science, University of Sydney, Camden, NSW 2570
** F. Hoffmann-La Roche AG, CH-400Z Basle, Switzerland

THE MORPHOLOGICAL DIFFERENCES IN EGG SHELL ULTRASTRUCTURE OF HENS RECEIVING EITHER DEIONISED OR SALINE DRINKING WATER

C.E. BRACKPOOL and J.R. ROBERTS

Electrolytes in drinking water have been shown to have a deleterious effect on egg shell quality (Balnave and Scott 1986). Shell strength is the main factor which determines shell quality. The most direct means for measurement of shell strength is shell thickness which is due to the palisade layer. The quality of the palisade layer depends largely on the shell's initial building blocks, the cone layer.

Abnormalities of the cone layer that may affect the quality and thickness of the palisade layer are:

(1) irregular cone shapes;
(2) lower density of cones where the cones are multinucleated, and larger and more widely spaced, resulting in a more porous egg shell;
(3) fragmentation of the cones;
(4) the cones are not firmly attached to the outer membrane;
(5) a proliferation of round calcified bodies lying upon the outer membrane;
(6) the plane of fracture is through the cones and not between the columns (Tullet 1987).

We examined the ultrastructure of eggs laid by Hyline Red hens which were drinking either de-ionised water or saline water (2g NaCl per litre of water) and laying predominantly either good ("good layer") or poor ("bad layer") quality egg shells. The results are shown below:

Average Number of Ultrastructural Defects per Egg Shell

	Good Layer Eggs:Good	Bad Layer Eggs:Good	Bad Layer Eggs:Broken on Handling	Bad Layer Eggs:Broken
Con Layer of Egg Shell				
De-ionised	2.6	3.5	3.0	4.4
Saline	3.0	3.1	3.8	4.7
Palisade Layer of Egg Shell				
De-ionised	3.1	3.7	3.6	4.8
Saline	3.4	3.9	4.8	6.0

This preliminary investigation indicates that eggs classified as "good" have intrinsic alterations to the cone and palisade layers, although not to the same extent as eggs that are classified as "broken". Also, eggs laid by saline-treated birds had more intrinsic alterations than eggs laid by hens receiving de-ionised water.

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SOME OCCUPATIONAL HEALTH ASPECTS OF WORK IN BROILER SHEDS

A.M. BROWN

Summary

Surveys of the chicken meat industry have shown that farmers have high prevalences of chronic bronchitis and asthma. The development of these conditions is related to age smoking atopy and exposure to the chicken shed environment. There is evidence that the surveyed populations represent survivors and the true extent of the problem may be greater. Most farmers do not use respiratory protective equipment regularly. The dust levels to which farmers are exposed in the sheds may exceed recommended standards.

I. INTRODUCTION

Traditionally agricultural occupations have been regarded as healthy at least compared to factory work. This is despite Ramazzini, 'the father of occupational medicine', having described respiratory disease in grain handlers in his famous book of 1713 (Ramazzini 1713). In recent years there has come the general realisation that rural occupational are not free from occupational hazards.

Over the years, broilers growers have been concerned about the possibility of respiratory disease from their work in chicken sheds. This concern was fuelled by anecdotal reports of prominent growers being warned off their sheds for health reasons.

There is some theoretical basis for such concern. The environment in a chicken shed contains a host of contaminants, such as feed dust, litter dust, feather and skin dust, micro-organisms, ammonia and other chemicals. Various infectious and non-infectious respiratory diseases might be expected from exposure to such an environment. Outbreaks of psittacosis have been reported in Australian poultry flocks (Barr et al 1986) but chickens are an uncommon source of human disease (Yung and Grayson 1988). Histoplasmosis is rare in humans and no clear link with chickens has been established in Australia (King 1982). Contact with bird proteins is responsible for extrinsic allergic alveolitis. It has been reported from exposure to hen feathers (Korn Florman and Gribetz 1968) but other birds, especially pigeons (Reid Sosman and Babee 1965) are more important. Asthma has been reported in Israeli chicken farmers from sensitivity to the Norther Fowl mite (Lutsky Teichtahl an Bar-Sela 1984). This paper outlines some epidemiological surveys of broiler farmers and some occupational hygiene measurements in sheds.

University of Newcastle, Faculty of Med. Disc. Envir. & Occup. Health, 86 Platt Street, Waratah, NSW, 2298

II. EPIDEMIOLOGY

(a) Methods

In Victoria, a survey was undertaken of all the broiler growers in the State (Brown 1990). Each farm on the mailing list of the Chicken meat Group of the Victorian Farmers Federation was contacted and given an appointment for an interview at which a standard respiratory symptom questionnaire (Ferris 1978) was administered and respiratory function testing performed. A total of 278 adult males and 94 adult females completed the interviews. The conditions of interest were all respiratory symptoms, chronic bronchitis (chronic cough and sputum) and asthma (defined as a positive response to the questions "Have you ever had asthma? Was it confirmed by a doctor?")

A similar survey was carried out in the Hunter Valley of NSW. Each farm contracted to a single processing plant was contacted and sent a general questionnaire about the farm and an individual health questionnaire for each person who worked in the sheds. A total of 173 men and 107 women from 123 farms responded.

(b) Results

From the Victorian survey, 74.5% of adults (77% of males and 68% of females) reported at least one respiratory symptom, while in the Hunter 66.3% of men and 54.7% of women reported symptoms. The prevalences of chronic bronchitis, asthma and eye irritation are given in Table 1.

Table 1. Prevalences of specific symptoms in Broiler Farmers

		Chronic Bronchitis	Asthma	Eye Irritation
Males	Victoria	12.6%	9.7%	71.6%
	Hunter	16.2%	11.0%	58.4%
Females	Victoria	8.5%	13.8%	66.0%
	Hunter	10.3%	12.1%	46.7%

There are many things that influence the prevalence of respiratory symptoms, notably age, atopy (allergic tendency) and smoking. In this situation the other influence of interest is exposure to the chicken shed environment. A cumulative exposure variable was created from the reported hours per day spent in the sheds at different times in the batch multiplied by the number of batches per year and the number of years working in the industry. These variables, age, atopy, smoking and shed exposure, were considered using the statistical technique of logistic regression to calculate Prevalence Odds Ratios (PORs) for each factor adjusting for all the others. PORs represent the odds that a person with the disease has been exposed to the factor being considered. A POR of one indicates no effect or no risk from the exposure and values above one represent increased risk. The PORs from the Victorian study are given in Table 2.

Table 2. Prevalence Odds Ratios controlling for other variables. 95% Confidence intervals in brackets

Variable		Chronic Bronchitis	Asthma	Eye Irritation
Age (years)	≤30	1	1	1
	30-40	1.43 (0.22-9.49)	0.98 (0.17-5.60)	0.51 (0.14-1.80)
	40-50	2.10 (0.36-12.29)	1.24 (0.23-6.83)2.02	2.24 (0.54-9.24)
	50-60	2.23 (0.35-14.09)	(0.30-13.57) 0.13	1.58 (0.36-6.96)
	>60	0.77 (0.04-7.07)	(0.01-2.25)	0.76 (0.15-3.84)
Smoking	non-smoker	1	1	1
	ex-smoker	0.73 (0.21-2.54)	2.73 (0.68-10.97)	3.33 (1.12-9.91)
	current smoker	10.95 (3.03-39.49)	6.02 (1.23-29.51)	5.47 (1.44-20.69)
Family history of atopy	absent	1	1	1
	present	3.86 (1.12-13.26)	31.12 (7.37-131.531)	7.35 (1.61-33.46
Exposure (shed years)	≤0.25	1	1	1
	0.25-0.75	3.85 (0.99-14.91)	0.64 (0.18-2.35)	2.25 (0.85-5.91)
	>0.75	9.18 (2.30-36.68)	1.01 (0.25-4.19)	3.75 (1.29-10.88)

This shows that age has little influence on symptoms but atopy and smoking are important. Cumulative exposure to the chicken shed environment is also important.

The findings from the Hunter survey were similar.

The prevalences of chronic bronchitis, asthma and eye irritation are high, but this situation appears not to be unique to Australia. Morris Lenhart and Service (1991) surveyed 59 male chicken catchers in and found 32.2% had chronic cough, 39.0% had chronic sputum and 27.1% had chronic wheeze (adjusted for smoking).

In any survey it is likely that the most severely affected individuals will have left and so the true prevalence of illness is under estimated. To assess this survivor population in the Hunter, efforts were made to trace the 55 farms that had left the industry in the previous five years. Of these, 49 were found and 47 responded to a questionnaire 30% of enterprises reported health was the main reason for leaving the industry and a further 12.5% reported that health contributed to the decision to leave.

One would hope that the use of respiratory protective equipment (RPE) might protect people from developing symptoms. Few people regularly used RPE 19% always used it and 45% never did. When the use of RPE was added to the logistic regression model it appeared that there was no protective effect. The most likely explanation for this is that farmers tended to use a mask only after they started to get symptoms. There is some evidence that RPE could be useful, only 11% of regular mask users had chronic bronchitis while 18% of the never-users did.

III. OCCUPATIONAL HYGIENE

(a) Methods

Occupational hygiene measurements have been made in a small number of sheds in the Hunter Region. Sampling was done while the farmer was performing normal work first thing in the morning, usually while collecting dead birds. Dust samples were collected using seven hole samplers for inspirable dust and cyclones for respirable dust. Static samples were taken at a height of approximately 1.5 m from the floor. Personal samples were collected using sampling equipment worn by the farmer and sampling the breathing zone. Ammonia was measured using either a portable MIRAN infra-red analyser or Dråger tubes, temperature and humidity were measured using a sling hygrometer and air movement was measured using a direct reading hot wire anemometer.

(b) Results

The sampling procedure is time consuming. Results reported here are for two farms where samples were collected weekly for a whole batch. This only a small number of situations and individual results. They may not be typical of the industry as a whole, but are the only data available.

For inspirable dust the geometric mean was 13.8 mg/m^3 with a range of 5.9 to 41.0 mg/m^3 of and respirable dust the geometric mean was 1.7 mg/m^3 with a range of 0.7 to 13.6 mg/m^3. The ratio of these is not constant and this suggests that the type of dust varies. Figure 1 shows the inspirable and respirable dust levels by age of the birds. This suggests the dust levels increase with the age of birds.

There is no specific workplace standard set for organic dust. WorkSafe Australia (the National Occupational Health and Safety Commission) has issued standards for many other materials and include a standard for "dusts not otherwise classified". This is a time weighted average (TWA) of 10 mg/m^3 measured as inspirable dust and it is often recommended that respirable dust not exceed a TWA of 5mg/m^3. In this study dust, samples were taken over a period 60 to 90 minutes during the busiest part of the farmer's day, so average exposures over a whole day would be lower. However levels of up to 40 mg/m^3 are very high over any period and the standard provides that if the full day TWA is not exceeded, never should the level exceed three times the standard.

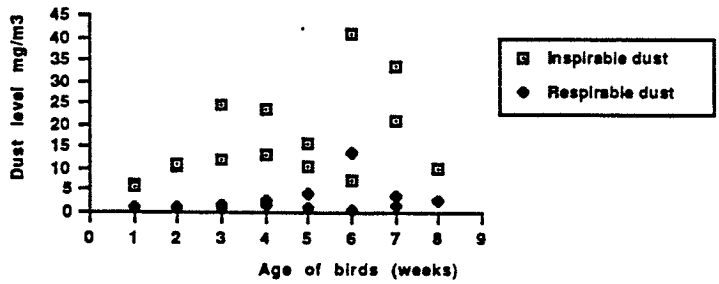
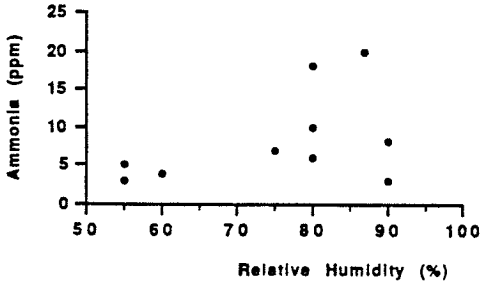


Figure 1. Inspirable and respirable dust levels dust (TWA, personal samples) by age of birds

Ammonia levels varied considerably. The variation was not directly with the age of the birds and ammonia levels appeared to be heavily influenced by variables such as humidity and general shed ventilation. (Figures 2 and 3)



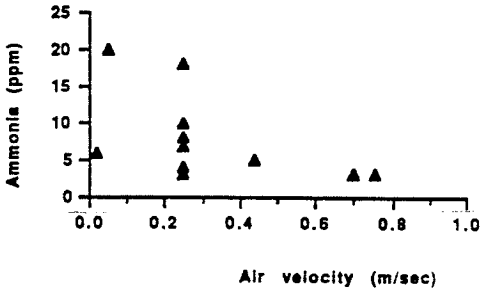


Figure 2. Ammonia by Humidity Figure 3. Ammonia and Air velocity

Under some circumstances it is possible for ammonia levels to be quite high. There are anecdotal reports that at times some farmers have had ammonia levels over 50 ppm. The Workplace Standard for ammonia is 25 ppm TWA with a short term exposure limit(STEL) of 35 ppm (NOHSC 1990). Usually chicken sheds have levels below this but continual exposure to ammonia as well as dust adds an extra load to respiratory tract defences.

Ventilation would seem to be the way to control both dust and ammonia but this may have costs in terms of temperature control or energy use. Farmers need to use RPE to control their exposure. The commonly used dust masks provide little

or no protection from ammonia and in fact simple masks appropriate to ammonia exposure are not readily available.

IV. CONCLUSION

Broiler growers suffer a various respiratory conditions and the risk of developing them increases with age smoking atopy and exposure to chicken sheds. The dust and ammonia levels in sheds can be high and farmers should try to reduce their exposures as much as possible by shed ventilation and using appropriate respiratory protective equipment.

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ANTI-NUTRITIVE EFFECT OF PENTOSANS: ROLE OF VISCOSITY

M. CHOCT* and G. ANNISON**

It has been demonstrated that the pentosans in wheat exhibit an anti-nutritive activity which is manifested by a general inhibition of nutrient digestion accompanied by poor bird performance (Choct and Annison 1990). The mechanism of this effect, however, is not established. NSP increase intestinal viscosity, which inhibits nutrient absorption and, hence depresses the growth of the chickens (White et al. 1981).

Wheat pentosans were isolated and AME trials were conducted as described by Choct and Annison (1990). Some of the pentosans were depolymerized using a β-xylanase. Wheat pentosans, commercial pentoses and depolymerized wheat pentosans were all added at 30 g/kg to a sorghum-based control diet. The results for the AME, weight gain (WG), feed conversion ratio (FCR) and feed intake (FI) are presented in the Table.

Effects of pentosans and depolymerized pentosans on the performance of broiler chickens (Mean $\pm$SE; n=8)

Diet	AME (MJ/kg DM)	WG (g/wk)	FCR (g:g)	FI (g/wk)
Control	$16.1 \pm .06^{a1}$	430 ± 17.1^{a}	1.59 ± 0.029^{a}	681 ± 22.5^{a}
Pentosan	14.5 ± 0.46^{b}	325 ± 19.8^{b}	1.96 ± 0.114^{b}	622 ± 24.9^{a}
Depolpen[2]	15.7 ± 0.12^{a}	404 ± 20.5^{a}	1.65 ± 0.040^{b}	661 ± 22.1^{a}
Pentoses	16.2 ± 0.13^{a}	394 ± 31.0^{a}	1.69 ± 0.059^{a}	658 ± 38.6^{a}

[1]Unlike superscripts within a column differ at $P<0.05$.
[2]Depolymerized pentosans.

The wheat pentosans significantly ($P<0.01$) depressed AME, weight gain and feed efficiency. The depolymerization decreased the viscosity (relative to water) of the wheat pentosans 4-fold, and this treatment largely eliminated their anti-nutritive effect.

The results from the current experiment suggest that the anti-nutritive effect of pentosans in poultry is due predominantly to the ability of these polysaccharides to increase the viscosity of the intestinal contents, which in turn reduces nutrient assimilation.

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* Department of Animal Science, The University of Sydney, Camden, NSW, 2570, Australia.
** CSIRO, Division of Human Nutrition, Glenthorne Laboratory, Majors Road, O'Halloran Hill, SA, 5158, Australia.

MECHANISMS OF BIOLOGICAL CONTROL OF COCCIDIOSIS IN CHICKENS

R.B. CUMMING

Summary

This paper focuses on ways of reducing the incidence of coccidiosis in poultry by dietary manipulation and presentation, rather than by chemotherapy. Use of high vs. low fibre diets, ingestion of grit and free choice feeding showed good promise. The beneficial effects of these strategies appear to be related to gizzard development. The importance of gizzard size and function are discussed. The beneficial effect of establishing an adult gut flora in young chickens on coccidiosis is discussed as well.

I. INTRODUCTION

In the modern broiler industry, coccidiosis continues to be a major health and production problem. As discussed by Reid et al (1984) in Hofstad et al (1984), there are seven possible methods of coccidiosis control and these are, in ascending order of usefulness: genetically resistant birds, nutrition, quarantine and depopulation, sanitation, reducing exposure, immunity and chemotherapy.

Nowadays, the major reliance is on chemotherapy to control the disease, as well as improved standards of hygiene on broiler farms: all-in, all-out farming, new litter for each batch and impervious floors.

The modern anti-coccidial drugs are remarkably proficient when compared with those used forty years ago, but the disease continues to cost the broiler industry dearly. While there have been many changes in breed and husbandry over the same forty years, one of the major alternations has been the drive for high energy, high protein diets. By definition these diets have become lower and lower in fibre and many commercial broiler rations now contain under 2% fibre. Over the years I had observed that coccidiosis appeared to be less severe in birds on high fibre diets and in 1985 I commenced a small investigation into this topic.

A further observation has been that chickens reared in a backyard situation and brooded under hens seldom if ever succumb to coccidiosis. These birds are reared in what are badly contaminated areas when compared to a modern broiler shed. Chickens hatched in incubators and not brooded under hens in these backyard situations appear to suffer from many diseases, including coccidiosis.

We therefore investigated the role of adult bird intestinal flora in coccidial infections.

Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, NSW Australia

II. DIETARY FACTORS AND FEEDING SYSTEMS

(a) High Fibre vs High Energy Low Fibre Diets

In these trials, the basic methods have been to feed crossbred cockerels on their respective diets for 3 to 4 weeks. The diets were composed of normal Australian poultry feed ingredients and mixed in our laboratory. The chickens were challenged by individual intubation with a field strain of coccidiosis. The challenge material, which was largely Eimeria tenella, was titrated in the same age birds to establish a dose that would cause about 10% mortality. On the 6-8th day post challenge all excreta were collected, weighed and an aliquot taken. The aliquots were later pooled and oocyst counts made.

The first trial produced an exciting result, with the high fibre fed birds suffering a lower mortality and producing far fewer oocysts than the chickens fed the high energy, high protein (and low fibre) diet (Cumming 1986).

(b) High Fibre vs High Energy Low Fibre Diets vs Free Choice Feeding

The experiment was then repeated with the addition of a group of chickens on free choice feeding i.e. chickens fed a high (42%) protein concentrate and whole wheat. Again the chickens on the conventional high energy, high protein diet experienced the greatest mortality and oocyst output, with the birds on the high fibre diet being lower in both categories. The choice fed birds had the lowest mortality and oocyst output, despite their fibre intake being even below that of the chickens on the high protein, high energy diet (Cumming 1987).

(c) High Energy Low Fibre Diets vs Free Choice Feeding - Natural Challenge

A third trial was then conducted, with chickens in floor pens and only comparing the high energy, high protein diet, and free choice feeding. The litter was seeded with infected litter from the University's Laureldale Poultry Unit thus providing a "natural" challenge. Again the choice fed birds had a lower mortality and oocyst output than those fed the conventional high energy, high protein diet (Cumming 1989).

(d) Individual Gizzard Size and Oocyst Output

Male broiler chickens were reared to 6 weeks of age, on either a conventional high energy, high protein diet or free choice feeding. They were then individually housed and individually challenged with sporulated oocysts, using a dose that would cause no mortality. Oocyst counts were made from each individual bird. Ten days post infection, the birds were starved overnight, weighed and killed. The proventriculus and gizzards were then removed from each bird and the empty weight recorded. The weight of the gizzard was then expressed as a percentage of the body weight.

The results (Table 1) show the difference between the birds fed the complete diet and free choice fed, with those from the free choice fed birds being significantly heavier and producing significantly fewer oocsyts. More importantly, within each group the birds with the largest percentage of gizzard tended to have the lowest oocyst output.

Table 1. Relationship between gizzard size and oocyst output of individual broiler cockerels fed a complete diet or free choice fed

Diet		Complete		Free Choice	
Relative		% Gizzard* Relative size	oocyst output	% Gizzard size	oocyst output
Bird	1	1.09	180,000	2.28	< 10,000
	2	1.21	170,000	2.20	< 10,000
	3	1.55	150,000	1.74	10,000
	4	1.87	140,000	2.21	< 10,000
	5	1.65	150,000	1.86	20,000
	6	1.77	140,000	2.01	< 10,000
	7	1.17	170,000	2.07	< 10,000
	8	1.70	150,000	1.96	10,000
	9	1.61	150,000	1.82	10,000
	10	2.01	100,000	2.53	< 10,000

*Expressed as % of total body weight

(e) Effect of Insoluble Grit

Crossbred cockerels were reared on a high energy, high protein diet or free choice fed. From ten days of age half of each group were offered small (2-4mm diameter) pieces of granite grit, which was scattered over their feed. Previous work had demonstrated that all chickens will ingest insoluble granite grit when offered in this manner. When four weeks of age, all groups were individually challenged by intubation of sporulated oocysts.

Table 2. The effect of insoluble grit on the oocyst output of crossbred cockerels fed a complete diet or free choice fed

Diet	Relative oocyst output
Complete	150
Complete + grit	130
Choice fed	63
Choice fed + grit	29

The results (Table 2) show that the ingestion of insoluble grit reduced the impact of the coccidiosis challenge, in terms of oocyst output. In the choice fed birds, the feeding of insoluble grit was additive to that of the diet.

(f) Adult Intestinal Flora and Coccidiosis

In these trials crossbred cockerels were fed on a conventional chicken starter crumble diet free from coccidiostat throughout. The intestinal flora was obtained from a six year old backyard male over a three hour period. The faeces produced were diluted 1 in 3 with distilled water and passed through a muslin filter and then a suitable bacterial filter to withhold any protozoan organisms.

Twenty-five three week old cockerels were then drenched individually with 1 ml of this solution. The drenched birds were housed separately from 25 controls. All birds were challenged by individual intubation one week later and oocyst counts made as previously described.

Table 3. The effect of drenching chickens with the intestinal flora from an old bird one week before coccidiosis challenge

Treatment	Mortality*	Relative oocyst output
Control	2	17,700
Drenched	Nil	3,500

*25 chickens per treatment

As shown in Table 3 two controls died, while all drenched birds survived. The oocyst counts from the drneched birds were markedly lower than those of the controls.

III. DISCUSSION

(a) The Importance of Gizzard Size and Function

The common factor observed in birds fed high fibre diets and fed free choice on whole grain is the gizzard size - these diets lead to a "normal" active organ. Chickens fed on modern high energy, high protein (and low fibre) diets develop a small atrophied gizzard (Riddell 1976). In fact, the atrophied gizzard is a common observation of broilers all over the world, when they are fed on conventional modern diets. The physiological alterations of the atrophied gizzard are fairly substantial but generally disregarded by poultry nutritionists. The two major differences are that the atrophied gizzard does not function as a grinding organ and the pH does not fall to the "normal" low level (Hill 1971).

The small atrophied gizzard acts more like a conventional portion of intestinal tract in chickens fed on finely ground food i.e. modern pelleted rations. The feed material, plus any ingested oocysts, are not exposed to any significant grinding by the gizzard, the pH is higher and the period of exposure to low pH is reduced, as feed material passes rapidly through the atrophied gizzard "and appears in the

duodenal content as a suspension of relatively unchanged particles" (Hill 1971).

The release of the sporozoites from the oocyst is enhanced by the action of trypsin, which enzymes are generally far more effective at low pH - below pH4. Thus in birds with atrophied gizzards the action of these enzymes would probably be less marked, compared to their action in a normal gizzard.

Actual release of the sporozoites appears to take place in the anterior portion of the small intestine, although there is little published data on the exact locality.

The apparent correlation of gizzard size and oocyst production (Table 1) suggests that oocyst or sporozoite destruction may be related more to the mechanical action of the gizzard. This is based on the assumption that a large muscular organ can exert greater grinding pressure than a small gizzard. The reduction in oocyst production in both conventionally fed and choice fed chickens given insoluble grit (Table 2) supports the idea that mechanical destruction of the oocysts/sporozoites in the gizzard is probably occurring.

Taking all the above into consideration, we propose that an active, normal gizzard is part of the fowl's natural resistance against coccidiosis. Exactly what happens in the gizzard is still unclear and requires further investigation to elucidate the actual procedures.

The application of this hypothesis to the modern broiler industry is difficult. The modern high energy, high protein diets are naturally low in fibre. The high fibre diets used in these experiments (>6%) would be quite uneconomic but further work may identify whether a lower level of fibre is sufficient to allow a more normal gizzard to develop. An interesting observation from a very successful broiler nutritionist was that a small proportion of oats in broiler rations frequently improved performance. Was this improvement due to the high fibre content of the oats which improved coccidiosis control?

The Australian broiler industry is very resistant to applying free choice feeding commercially and is not funding any research in this area. This is unfortunate as the feeding of whole wheat to broilers is now widespread in Scandinavian and some other countries. This is generally done by feeding a 24% protein broiler starter ration from day old to slaughter and adding increasing amounts of whole wheat from about 8 days of age, reaching a maximum of about 40% whole wheat by three weeks of age. Despite the fact that only normal levels of coccidiostats are added to the 24% broiler starter ration, these growers have not reported any coccidiosis problems.

Similarly in Scotland (W. Michie - personal communication, 1989) coccidiosis has not been a problem in broilers choice fed in the same way as the Scandinavians.

Generally, poultry processors prohibit the feeding of insoluble grit to broilers, as the grit damages the blades on the gizzard cutters. However, if the use of grit can significantly improve coccidiosis problems in the field, this objection may have to be overruled. After all, what is the financial return on gizzards compared to a reduction in coccidiosis problems? Further, the ingenious poultry industry could readily overcome the processing problem if grit was shown to be of real value in the field.

The dynamics of coccidiosis challenge are better understood now, with the rapid build up in oocyst numbers that commences at about 21 days of age (McDougald 1988). Thus any benefits from an active gizzard need to be implemented early in the chicken's life, to enable the normal active gizzard to

develop and act on the ingested oocysts.

This biological control measure of the chicken needs to be kept in perspective. We have seen in a number of experiments that the resistance of a "normal" active gizzard can be overwhelmed by a massive coccidiosis challenge. Thus the role of the gizzard should be regarded as an addition to the procedures we employ to control coccidiosis in the modern intensive industry. An active gizzard may well extend the useful life of several existing coccidial drugs.

Finally, the role of the normal gizzard in digestion should be further elucidated. The gizzard of the modern broiler is in a sad state of disrepair and does not function normally. Other disease problems, such as Escherichia coli infections and necrotic enteritis may be diet induced by our modern feeding practices.

(b) The Role of the Intestinal Flora

The results of the one trial reported here are in complete agreement with more extensive trials carried out in our laboratory a decade ago (Wolfenden and Cumming - unpublished). In those trials a lower mortality and lower oocyst output was recorded in three experiments.

An explanation for these results is not readily available, but the area does warrant further investigation. Modern broiler production practices generally deny the young chicken the benefit of quickly developingt a "normal" gut flora from the broody hen. As in salmonella infections, this may be of importance in overcoing coccidiosis, one of the diseases that has tended to increase rather than decrease in importance over the years in the modern broiler industry.

IV. ACKNOWLEDGEMENTS

I am grateful for the support for this work by the University of New England Poultry Research Sundry Donors Fund and ACIAR. My thanks also to Jenny Schaefer, Amanda Choice, and in particular, Wendy Ball.

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EGG QUALITY AND COOKING RESPONSES INFLUENCED BY STORAGE

B.M. DAVIS and H.P. STEPHENSON

Summary

Storage has a profound influence on the maintenance of egg quality and the eggs' cooking response. During the course of egg quality surveys in north Queensland storage conditions on farms, at grading floors and in shops were noted. Controlled experiments using these storage methods and others, which the researchers thought to be superior, were tested. It was demonstrated that when eggs were held at ambient temperature, Haugh units (freshness measurement) declined within 7 days to unacceptable levels for retailing. The quality of eggs held in air-conditioning, which operates at 22-24°C, also declined rapidly. The majority of eggs in Australia are sold in air-conditioned shops. Professional chefs showed that cooking responses were related to freshness.

I. INTRODUCTION

In 1981 motel managers in the Cairns region of north Queensland complained to suppliers about the quality of eggs. Managers claimed some eggs had broken yolks and many eggs did not fry or poach satisfactorily. Poor cooking responses resulted in many eggs being dumped which meant dissatisfaction and a loss of income. As a result of complaints, the North Queensland Poultry Farmers Association approached the Queensland Department of Primary Industries and asked for assistance to resolve the problem.

II. MATERIALS AND METHODS

(a) Market Surveys

Market surveys were conducted at Cairns and Townsville in 1981, 1985/1986 and 1989. Four dozen eggs were purchased from retail outlets at these centres and quality measurements made. In 1981 and 1985/1986 eggs had been stored at ambient temperature, in air-conditioning or under refrigeration. In 1989 oiled eggs and unoiled eggs were purchased from similar storage conditions.

(b) Farm and Grading Floor

Farm and grading floor surveys were conducted over the same period. Again four dozen eggs were used as a representative sample size. Most eggs on farms were held in cool rooms which operated at 13°C. All eggs were held in cool rooms at the grading floor.

Oonoonba Veterinary Laboratory, Queensland Department of Primary Industries, Townsville, QLD 4810.

(c) Controlled Storage Trials

Controlled storage trials were conducted in 1986, 1987, 1989 and 1990 and emulated storage conditions observed in shops and those thought to be superior. They included ambient conditions, oiled plus ambient conditions, refrigeration, oiled plus refrigeration. · Oiling on the day of lay was compared with oiling 3 and 7 days after lay.

III. RESULTS

Eggs oiled on the day of lay and held in refrigeration gave the best result (see Fig 1). Unoiled eggs held at room temperature gave the poorest response to the Haugh unit test. Unoiled eggs stored under refrigeration and oiled eggs stored in ambient conditions gave intermediate responses. In a previous trial, eggs held in air-conditioning gave a response similar to unoiled ambient eggs in the 1989 trial.

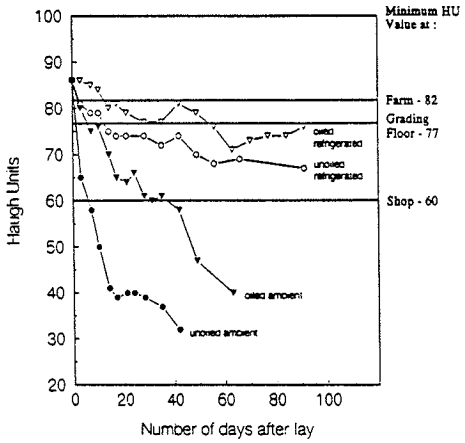

Figure 1. Results for four storage methods (1989 Trial)

IV. DISCUSSION

Prior to the commercial oiling of eggs in north Queensland freshness fail rates for eggs in shops at Cairns ranged from 25 to 58 percent and from 34 to 66 percent at Townsville. After the application of oil, the fail rate at both Cairns and Townsville was reduced to 13 percent (Davis and Stephenson, 1989). Other workers, notably Pugh et al (1977, 1978) and Choice (1981), showed high fail rates for egg freshness in retail outlets in other regions of Australia.

The single biggest factor influencing the maintenance of egg quality is environmental temperature. Eggs should not be stored at temperatures exceeding 13°C at any time between production and consumption. The 1989 survey results showed that only 20 percent of retail eggs are held in refrigeration at Cairns and 7 percent at Townsville. By cooling in summer or

heating in winter, temperatures in retail outlets are set for human comfort not for the maintenance of egg quality. Figure 2 shows maximum temperatures throughout the year in Darwin and Hobart. Maximum temperatures in the other capital cities lie between these extremes.

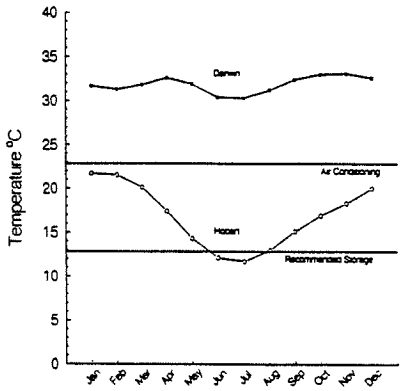

Figure 2. Mean daily maximum temperatures for Darwin and Hobart

Egg quality has a profound influence on cooking responses. During 1990 and 1991 TAFE Tourism and Hospitality students throughout Queensland, Defence Force personnel and private sector chefs were instructed on the attributes of egg quality. Eggs had been stored under the conditions depicted in Figure 1 and students were asked to cook with these and rate cooking responses. On the day of each lecture freshly laid eggs were collected and used as controls.

Students found a strong relationship between storage and cooking responses. It was particularly strong for poached and fried eggs. Figure 3 shows the trend for fried eggs. This information was compiled from 20 groups located at different centres between Cairns and Brisbane.

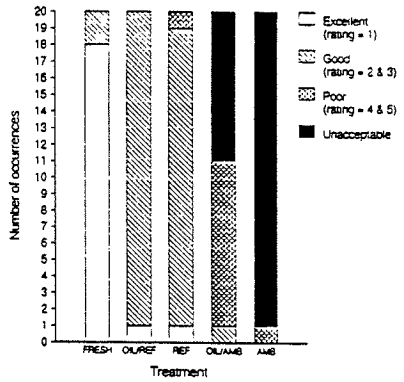

Figure 3. Fried Eggs - cooking responses to different storage conditions.

With other products, extra eggs in the ambient treatment had to be used due to difficulties in separation. There was little indication that fresh eggs always gave better results and the randomness of the results suggested that the cook's ability was more important. It was observed, however, that with mayonnaise, fresher eggs needed less oil to thicken. For demonstrations, when batches of mayonnaise were prepared by the same person using an electric beater, the volume of oil and the length of time needed to bring each batch to the same consistency were recorded. Results are tabulated in Table 1.

Table 1. The effect of storage conditions of eggs on oil quantities (mls) and preparation time (mins) to produce mayonnaise.

	Eggs	Farm Fresh	Oiled/ Refrig.	Refrigerated	Oiled/ Ambient	Ambient
1	Mls of oil	400	500	750	800	1000
	Mins	10	7	10	10	15
2	Mls of oil	200	450	500	700	1000
	Mins	1	1	1.5	2	3
3	Mls of oil	350	375	400	450	500
	Mins	3	3.5	4	5	7
4	Mls of oil	320	350	500	450	500
	Mins	6	8	9	10	17
5	Mls of oil	180	240	275	355	375
	Mins	1	1	1.5	1.75	2

The recommendation of egg oiling clearly translates into superior cooking responses ensuring that consumers do receive value for money in the form of a fresh product.

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EFFECT OF FEED ENZYMES ON EGG PRODUCTION AND CHOLESTEROL OUTPUT OF LAYERS FED WHEAT, TRITICALE OR RYE

J.G. DINGLE and T.M. SHAFEY

Plasma and egg cholesterol have been found to be reduced by the polysaccharide components of certain cereals in the diet (Qureshi et al 1980, McDonald and Shafey 1989). This trial fed high levels (750 g/kg) of wheat, triticale or rye as the sole cereal in a layer diet with or without a commercial polysaccharide enzyme mixture (200 ppm Roxazyme® Roche) containing cellulase, β-glucanase, xylanase, pectinase and amylase, to examine the interaction between polysaccharides and cholesterol in the gut. The results of the seven week trial are shown in the Table.

Egg production and cholesterol responses of layers fed sole grain diets and polysaccharide enzymes.

Treatment	Rate of Lay (egg/hen/day)	Egg Mass (g/day)	Egg Yolk Cholesterol Content (mg/egg)	Egg Cholesterol Output (mg/day)
Grain				
Wheat	0.87^{a}	47.4^{a}	295.0^{a}	254.7^{a}
Triticale	0.82^{b}	44.2^{b}	288.1^{a}	234.2^{b}
Rye	0.82^{b}	44.7^{b}	250.6^{b}	203.1^{c}
LSD (P<.05)	0.03	1.9	14.4	9.4
Enzyme				
Nil	0.81^{y}	44.3^{y}	275.2	222.2^{y}
Supplement	0.86^{x}	46.6^{x}	280	237.3^{x}
LSD (P<.05)	0.02	1.5	NSD	7.7

LSD = Least significant difference. NSD = No significant difference.
Within columns, treatment means with different superscripts are significantly different.

Both triticale and rye produced a significant decrease in rate of lay and egg mass, compared with the wheat diet. The polysaccharide enzyme mixture significantly increased the rate of lay and egg mass of birds fed each of the cereals. The enzyme mixture did not significantly increase the cholesterol content of eggs but did significantly increase the egg cholesterol output per day. This indicates that polysaccharide breakdown increased cholesterol uptake from the gut and supports the hypothesis that polysaccharide inhibition of bile salt cholesterol reabsorption occurs in the small intestine.

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The University of Queensland, Gatton College, Lawes, Queensland, 4343.

EFFECTS OF LINSEED OR LINOLA™ MEAL INCLUSION IN LAYER DIETS

D.J. FARRELL* and A.G. GREEN**

We have reported previously results of experiments with poultry offered diets with linseed or Linola™ meal (Farrell and Green 1991). Here we provide further results of a layer experiment in which a total of 100 individually caged layers of a commercial strain (AZTEC 300) were offered least-cost formulated diets (17% CP and 11.3 MJME/kg DM) that contained 50 or 100 g of linseed or Linola meal per kg feed. A commercial diet (17% CP) was used as a control. Birds were given the diets at 20 weeks of age and data are reported here from 26 to 54 weeks (see Table).

Responses of laying hens to the five diets.

Inclusion (g/kg)	Linseed (50)	Linola (50)	Linseed (100)	Linola (100)	Control	LSD (P,0.05)
Feed (g/d)	115	113	118	120	121	3.5
Egg prod. (%)	88.2	86.1	83.5	82.9	89.9	2.46
Egg weight (g)	60.3	60.7	60.7	60.8	61.5	0.88
Egg mass (g/d)	53.2	52.1	50.6	50.4	55.1	1.16
Feed effic. (g egg/g feed)	2.2	2.2	2.3	2.4	2.2	0.08
Bodyweight (kg)	1737	1722	1737	1684	1818	9.41

Mean egg production on all diets during the 7 months was high. Only the diet with 50 g linseed meal per kg gave the same egg production as did the commercial diet. However egg mass was always less ($P<0.05$) on the four experimental diets due to the greater number and larger eggs produced on the commercial diet compared to the experimental diets.

For diets with 100 g of the linseed and Linola meal per kg, egg production was reduced substantially compared to 50 g inclusions although egg weight was similar. Overall there was a tendency for diets with Linola meal to give poorer egg production than diets with linseed meal but these differences were not significant ($P>0.05$). Egg shell quality, measured by specific gravity, did not differ due to treatment.

These results support previous data which suggested that linseed and Linola meal tended to depress performance when included at 50g/kg feed or higher in laying diets (Farrell and Green 1991).

We thank Wendy Ball, Norma Ducker, Evan Thomson, Mick Burke and Kitty Woods for technical assistance and John Spragg (Fielders Agricultural Products) for formulating the diets.

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*Department of Biochemistry, Microbiology and Nutrition, University of New England Armidale, N.S.W. 2351
**CSIRO Division of Plant Industries, Canberra, ACT 2601
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THE NUTRITIVE VALUE OF BORAGE MEAL IN LAYER DIETS

D.J. FARRELL

Borage meal is the residue of the seeds of the borage plant (*Borago officianalis*) following mechanical extraction of the oil which is rich in gamma-linolenic acid (all cis 18:3 n6). This fatty acid is claimed to have therapeutic value in humans. The meal contains, on an 'as is' basis, about 270g crude protein, 125-150g oil, 10g lysine, 5.3g methionine and 8.2g threonine per kg. The ash content is 120g and the calcium and phosphorus contents are 16 and 8g/kg respectively.

Thirty nine individually-caged 24-week old hens of a commercial strain (WL x NH) were divided into three groups and given least-cost formulated diets (164g CP and 11.5 MJ ME/kg DM) based on wheat, soybean meal and meat and bone meal with either 100 or 200g borage meal per kg. A commercial layer diet (165g CP/kg) was used for comparison. Data were computed every 4 weeks to 48 weeks of age.

Production data from hens on the three diets

	Borage Meal Diets		Commercial Diet	LSD (P = 0.05)
	(100 g/kg)	(200 g/kg)		
Feed intake (g)	118	125	121	4.8
Egg production (%)	89.4	88.6	89.6	2.95
Egg weight (g)	56.4	57.2	57.7	1.99
Egg mass (g/d)	50.4	50.6	51.5	2.08
Feed efficiency (g/d)	2.39	2.49	2.41	0.148

There was no effect of treatment on any parameter (Table) except for a reduced feed intake on the diet with 100g borage meal/kg. Specific gravity of eggs was measured eight times and showed no effect of treatment on shell quality.

Production was very high indicating that borage meal can be included in layer diets at up to 200g/kg diet (or perhaps higher) without any adverse effect on production. However due to a limited supply of borage meal the experiment ran for only 6 months. After this time differences between treatments may have become apparent.

Fatty acid analysis of egg lipid showed a small but significant increase in gamma-linolenic acid in eggs (n = 5) from hens on a diet with 150g borage meal/kg compared with hens (n = 3) on a vegetarian diet (0.7 ± 0.1 vs 0.1 ± 00%).

I thank Wendy Ball, Norma Ducker, Evan Thomson, Mick Burke and Kitty Woods for technical help.

Department of Biochemistry, Microbiology and Nutrition, University of New England Armidale, N.S.W. 2351

THE IMPROVEMENT IN PHOSPHORUS AVAILABILITY WHEN PHYTASE IS ADDED TO BROILER DIETS

D.J. FARRELL, J.J. du PREEZ, M. BONGARTS, M. BETTS, A. SUDAMAN, E. THOMSON and W. BALL

Summary

Five diets containing increasing amounts of phosphorus (P) from soybean meal or from CaHPO4 were fed, with or without phytase, to 3 replicate groups each of 10 chicks from 1 to 18 d of age. Balance measurements and carcass retention of P and nitrogen (N) and other parameters were measured. Feed intake, growth rate and feed conversion ratio (FCR) responded to increased dietary P and to the addition of phytase except for the diet with the highest concentration of inorganic P. Mean utilization of P increased with phytase from 57 to 67%, and retention of P from 47 to 55%. Metabolizable energy (MEn), N retention and utilization also responded to phytase addition. Ash and P in tibia bone responded to dietary P and to phytase.

I. INTRODUCTION

Much of the phosphorus (P) in feedingstuffs of plant origin is in the form of phytic acid and therefore poorly available especially to young chickens. Phytic acid may also reduce the bioavailability of other dietary minerals and perhaps of other dietary components (Maga 1982). The improved technology in the production of enzymes for use in pig and poultry diets has been reviewed (Jacques 1990). We report here the results of an experiment with growing chickens in which a phytase, Natuphos (Gist-brocades), was added to the diet at 750 international feed units (FTU)/kg feed.

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

Diet No.	1	2	3	4	5
Sorghum	450	450	450	450	450
Soybean meal	300	400	500	300	300
Starch	155	95		155	155
$CaCO_3$	9	11	12	10	11
$CaHPO_4$	1	1	1	4	7
Sunflower oil	11	10	25	11	11
Lysine	3			3	3
Methionine	4	3	4	3	3
Cellulose	58	21		45	51
Premix	5	5	5	5	5
NaCl	4	4	4	4	4
Protein (g/kg)	188	230	273	188	118
ME (MJ/kg)	11.6	11.6	11.6	11.6	11.6
Phosphorus(g/kg)	3.6 (4.1)[a]	4.3 (4.9)	4.9 (5.3)	4.3 (5.1)	4.9 (5.7)

[a]Determined values in brackets

Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale N.S.W. 2351

II. MATERIALS AND METHODS

Three hundred, one-day old male broiler chicks of a commercial strain were allocated to 10 dietary treatments each replicated thrice. The chicks, in wire mesh cages, were in a room initially at 33°C and gradually reduced to 25°C at 18 of age and given feed and water ad libitum. There were five diets without (-) phytase and five diets with added phytase (+) (Table 1).

Feed intake and corresponding excreta output were measured at d 3-8, 8-13 and 13-18 d of age. Birds were weighed at the end of each collection period. At 18 d of age birds were starved for 6 h then weighed and killed. The tiba was removed from each leg of half of the chickens in each group, and the remainder were frozen for subsequent carcass analysis For P and nitrogen (N), measurements were made on feed and excreta to obtain balance, and on the wet carcass and dry, fat-free carcass and tibia to obtain P retention. Chemical analyses followed the methods of the AOAC (1980). Phosphorus was determined in feed, excreta, bone and carcass tissue using an autoanalyser technique (Williams and Twine 1967).

Table 2. Effect of diets with (+) and without (-) phytase on biological performance and feed utilisation or retention.

Diet	Enzyme	Gain (g/d)	Feed intake (g/d)	FCR	DM dig (%)	ME_n (MJ/kg)	Nitrogen	
							Util (%)	ret (%)
1	-	17.2	26.8	1.56	78.8	13.03	57.8	57.8
	+	19.6	30.2	1.54	79.6	13.00	58.7	59.5
2	-	18.2	27.1	1.49	75.1	12.46	51.5	52.0
	+	23.1	31.1	1.35	77.7	13.00	56.8	56.7
3	-	22.8	30.6	1.35	69.7	12.42	49.2	50.0
	+	24.4	31.8	1.31	72.6	13.00	51.2	50.4
4	-	18.3	28.1	1.54	79.5	13.06	61.4	58.2
	+	20.6	30.6	1.48	80.2	13.25	61.2	60.4
5	-	19.7	30.4	1.54	79.1	13.03	59.9	61.9
	+	19.7	30.6	1.54	80.4	13.21	59.9	60.5
SEM		0.48	0.052	0.019	0.32	0.024	0.75	0.36
Mean	-	19.2	28.6	1.49	76.5	12.83	55.9	56.0
	+	21.5[c]	30.9[c]	1.44[c]	78.1[c]	13.10[c]	57.6[b]	57.5[a]

[a]$0.10 < P < 0.05$; [b]$P < 0.05$; [c]$P < 0.01$

Table 3. Measurements of utilisation or retention of phosphorus (P) in five diets with (+) and without (-) phytase

Diet	Enzyme	P balance		P retention		Tibia P		Tibia ash	
		(mg/d)	Util (%)	(%)[a]	(%)[b]	(mg)	(%)	(g)	(%)
1	-	70.6	55.7	47.5	46.1	44.6	6.5	0.29	42.3
	+	96.4	67.4	53.0	52.0	68.2	8.1	0.41	48.0
2	-	85.5	54.7	42.3	40.2	56.1	7.1	0.34	43.1
	+	125.2	70.7	59.0	61.7	81.4	7.8	0.50	48.4
3	-	106.7	55.6	56.3	56.6	77.1	7.5	0.57	45.7
	+	123.9	62.7	57.5	54.4	95.0	8.0	0.59	49.0
4	-	99.6	60.1	42.6	44.8	66.7	7.8	0.41	47.4
	+	125.5	70.4	53.1	54.0	74.2	8.0	0.46	50.0
5	-	114.3	57.1	46.4	46.0	69.5	7.8	0.44	49.1
	+	130.3	63.8	47.5	52.2	7.0	7.9	0.49	49.9
SEM		3.08	1.24	1.73	2.37	8.21	0.55	0.044	0.02
Mean	-	95.1	56.6	47.0	46.7	62.8	7.3	0.39	45.5
	+	120.3[c]	67.0[d]	54.0[c]	79.4[d]	79.4[d]	8.0[c]	0.49[c]	49.1[c]

[c]$P<0.01$, [d]$P<0.05$
[a]Measured in the fat-free dry carcass and [b]wet carcass

III. RESULTS AND DISCUSSION

The determined P contents of the diets were higher than calculated (Table 1). This may explain the small differences in biological response by chickens on diets 4 and 5 and a lack of response when phytase was added to diet 5 (Table 2). The improvement in feed intake, weight gain and FCR on diets 1-3 without added phytase was due to the increased contribution of P provided by the increase in soybean content of these diets. Diet 1 was calculated to meet the amino acid and other nutrient requirements of the chick. Phytase had a significant and substantial positive effect on all parameters measured (Tables 2 and 3). Not only was the P content of the fat-free, dry tibia increased with phytase addition but P balance and P retention were also improved. The overall effect of phytase was to increase apparent P retention (measured in the wet and fat-free, dry carcass) by 18 and 15%, and P balance by 18%. However balance gave consistently higher values (%) than retention. For nitrogen, the two methods gave similar results. It should be noted that balance measurements were made over three periods each of 5 days, from 3 days of age and values were combined; while retention was determined over 18 d. No account was taken of endogenous P. Differences in dietary crude protein and intake tend to make interpretation of these results difficult. Balance measurements gave a similar value of about 55% for P in the soybean meal/sorghum diets 1-3 without added phytase. Since the increase in soybean meal from 30 to 50% of the diet did not change this value, it appears that sorghum and soybean meal have a similar P availability.

It is apparent that the effects of phytase extend beyond those of P utilization. Phytase significantly improved dry matter (DM) digestibility, metabolizable energy of the diet, and to a lesser extent N utilization. The latter may have reflected the increased feed intake observed when phytase was included in the diets.

Simmons et al. (1990) have observed improvements in biological performance in pigs and poultry on a grain/soybean meal diet with the addition of phytase. The maximum response by broilers was observed when 750 FTU of phytase/kg diet was fed. P balance, measured only once over 3 d, gave initial values of 45-52% depending on dietary P and Ca levels, and increasing to a maximum of 64% with phytase addition.

The mean balance values, with and without added enzyme, are in agreement with these availability values. Interestingly, retentions of P (mg/bird per d) gave very similar values on diets 2-5 when the enzyme was added (Table 3).

It was hoped that the slope-ratio assay (Major and Batterham 1981) could be used here to determine the availability of P in the soyabean meal/sorghum diet using one of a number of the parameters determined in Tables 2 and 3. The higher than expected P levels, particularly in diet 5, precluded a significant increased response of most parameters to this diet. The overall response was therefore not linear, a pre-requisite for a valid slope-ratio assay. Ketaren et. al (1991) have demonstrated in similar P studies with pigs that the slope-ratio assay is insensitive to the majority of parameters and frequently gives spurious values for P availability.

From the measurements made here, it appears that P and ash measured in the dry, fat-free tibia and P balance data are the most sensitive parameters for determining P utilization in a feed.

We thank Gist-brocades (The Netherlands) for donating the phytase.

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SURVEY OF CHICKEN PRODUCTION SYSTEMS IN WEST TIMOR

A.M. FUAH, R.A.E. PYM and W.A. PATTIE

Approximately 74% of chickens found in West Timor are of an indigenous breed known as kampung chickens. The breed is popular with most farmers in villages since they require simple low cost management and are highly adapted to scavenging in the village environment. They are typically raised close to the household where their main sources of feed is that scavenged from the environment and kitchen waste. Although egg production is low (60-70 eggs/annum), and chick mortality high (approximately 25% to 8 weeks of age), the bird makes a significant contribution to poultry meat and egg consumption in the country.

A three month survey was conducted between October and December 1989 in which one hundred and twenty farmers from three villages which represented three agricultural systems (rice field and mixed garden areas, RMG; mixed garden and grazing areas, GG; and dry field, grazing and forest areas, DGF) were chosen for interview, their animals observed and measurements made on the birds.

The results showed that the average numbers of birds kept by each family in the RMG, GG and DGF systems were 19 + 3; 27 + 6.8 and 11 + 2.8 (SE) respectively. The distribution of chickens thus appears to be associated with the agricultural system practiced. Performance traits were, however, relatively similar in the three production systems. Approximately 94% of the farmers raised their chickens extensively during both the dry and wet seasons. Animals scavenged around the owners' house and relied on the occasional availability of kitchen waste.

Age of females at commencement of lay was 32 weeks, and the average number of eggs per clutch cycle produced to 36 months of age was 11.2. Of these, 90% were set under the hen whilst the rest were available for sale and home consumption. Hatchability averaged 84% and mortality of chicks between 1 and 6 weeks of age was 24%, caused mostly by infectious disease and accident. Newcastle Disease occasionally causes mortality up to 75% in chicken flocks in this region. Average weights of the birds at 4 and at 26 weeks were 287 g and 1540 g for males and 215 g and 1250 g for females respectively. The average contribution of the chickens to annual farm income in the three agricultural systems was Rp 128.000, equal to US $ 68, which was received from an average of 40 birds sold. Annual farm income ranged from US$130 to $195.

The contribution of the kampung chicken in providing food for human needs and cash earnings for farmers in villages of West Timor is already significant, but there is a considerable potential for improvement. Better management and effective but inexpensive methods are required to reduce mortality and increase the number of chickens and eggs available for sale and consumption.

Department of Farm Animal Medicine and Production, University of Queensland, St Lucia 4067, Qld, Australia

PRODUCTION RESPONSES OF HENS FED COMMON VETCH

P.C. GLATZ, R.J. HUGHES and R.C. WOOLFORD

Home-mixers have shown interest in vetch (*Vicia sativa*) as a protein source in laying diets. Castanon and Perez-Lanzac (1990) showed that inclusion of 15% common vetch (unstated cultivar) in a layer diet resulted in a decrease in performance. Some cultivars of common vetch fed to pigs give a reduction in performance whilst other cultivars Languedoc and Blanchefleur have no effect (Bull and Mayfield, 1988). We considered that there may be potential in feeding Blanchefleur to hens.

In one experiment three diets 0, 100 and 200 g/kg vetch (cultivar Blanchefleur) with similar nutrient content (12.6 MJ AME, 180 g crude protein and 38g fat/kg) were fed in a coarse mash to a commercial laying strain from 26-33 weeks. Birds were fed the control diet from 18-25 weeks and returned to this diet from 34-41 weeks. From 42-49 weeks in a second experiment, the same birds were fed 0, 50 and 100 g/kg vetch (11.6 MJ AME, 196 g crude protein and 30g fat/kg) in a very finely ground mash. Production responses of layers fed common vetch in two experiments is shown in the table below.

Treat (vetch) (g/kg)	Food intake (g/b/d)	Rate of lay (%)	Body wt change (g)	Egg weight (g)	Egg income ($)
0[1]	119.0[a]	88.8[a]	92[a]	55.5[a]	5.50[a]
100	115.2[a]	82.8[a]	- 19[b]	54.1[b]	5.11[a]
200	102.1[b]	63.6[b]	-169[c]	53.7[b]	3.71[b]
0[2]	111.6[a]	82.3[a]	- 35[a]	59.4[a]	5.29[a]
50	110.4[a]	81.6[a]	- 78[b]	58.8[a]	5.28[a]
100	106.0[b]	81.4[a]	- 93[b]	58.7[a]	5.22[a]

Means within a row within experiments with different superscripts are significantly different ($P<0.05$). [1] Experiment 1 (26-33 weeks of age); [2] Experiment 2 (42-49 weeks of age).

From 26-33 weeks, hens on 100 and 200 g/kg vetch had a greater change in body weight and lower egg weight than controls. Birds on 200 g/kg vetch showed a drastic reduction in all production variables relative to controls (Table). In the second trial, birds on 100 g/kg vetch in a very fine mash showed a reduction in food intake and change in body weight relative to controls. Clearly vetch inclusion of 100 g/kg or more in a layer diet causes production problems in hens. While 50 g/kg vetch did not cause a reduction in performance of hens 42-49 weeks of age, we need to confirm these findings with hens fed over a normal laying cycle.

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Parafield Poultry Research Centre
230 Salisbury Highway, Parafield Gardens, South Australia, 5107

DIETARY MINERAL SUPPLEMENTATION AND BROILER PERFORMANCE AT HIGH TEMPERATURES

I. GORMAN, D. BALNAVE and Y. MOLLAH

Summary

A variety of mineral supplements were added to broiler finisher diets to test the relevance of two popular mineral balance equations as predictors of performance at high temperatures. No correlation was observed between performance and either of these equations. Supplementation with sodium bicarbonate gave the best results, with further indications that metabolisable anions may play a significant role.

I. INTRODUCTION

Many attempts have been made to define a simple mathematical relationship between broiler performance and dietary mineral content as an aid in formulating rations. Two equations commonly tested are the dietary electrolyte balance equation [EB, where EB = (Na + K - Cl) mEq/kg], and the cation-anion balance equation [(cat-an), where (cat-an) = (Na + K + Ca + Mg) - (Cl + P + S) mEq/kg]. However, experimental support for these equations has been inconsistent.

The following studies aimed to test the usefulness of the above equations at high ambient temperatures. While some limitations of these equations have been illustrated by specific ion effects independent of the equations (Nesheim et al. 1964; Melliere and Forbes 1966; Johnson and Karunajeewa 1985; Hulan et al. 1987), the range of minerals tested at high temperatures is relatively small. Furthermore, while many mineral requirements of poultry have been established at moderate temperatures, the effect of high temperatures on these requirements has not been well defined. The following studies examined whether growth performance was similar at given dietary EB or (cat-an) levels when these levels were obtained using different mineral supplements. Specific ion effects were also examined in some cases. Mineral retentions were measured to test whether retained levels are more suitable for use in balance equations than dietary levels.

II. MATERIALS AND METHODS

(a) Bird Management and Sample Collection

Day-old male broiler chickens were obtained from a commercial hatchery and brooded to three weeks of age with free access to commercial starter crumbles and town-water. Birds were then allocated on the basis of liveweight into groups of six and transferred into grower cages within controlled temperature rooms where they were then randomly allocated to treatments and given free access to the treatment diets and town-water. Feed intakes and weight gains were measured from three to six weeks of age. Excreta samples were collected over a 24 hour

Department of Animal Science, University of Sydney, Werombi Road, Camden, N.S.W. 2570.

period during the final week of eachtrial and dried for 48 hours at 80°C.

(b) Experimental Diets

Basal mash diets were formulated to approximate a typical commercial finisher ration, with the inclusion of celite as an inert dietary marker (measured as acid insoluble ash) and either rice hulls or solka floc as an indigestible filler to enable the amounts of salts added to the diet to be varied without affecting the level of any other nutrient.

(c) Chemical Analyses

Samples of the basal diets and the excreta were analysed for minerals other than chloride by atomic emission spectrophotometry. Chloride was measured by potentiometric titration with silver nitrate. All samples were analysed for the dietary marker by dry-ashing, followed by boiling in 4N HCl and weighing back the insoluble ash.

III. RESULTS

In Experiment 1 male broiler chicks were housed at a constant 30°C and given free access to one of twelve experimental diets. The particular salt supplements and their levels of inclusion were chosen to enable simultaneous comparisons between EB, (cat-an) and specific ion effects.

Table 1. Performance of 3-6 week old broilers in Experiment 1.

Diet	Dietary ion (g/kg)	EB (cat-an) (mEq/kg)	Gain (g)	F.C.R. (g/g)
Control	-	186 485	1247 bcd	2.03 bcd
$CaCO_3$	Ca 15.24	186 787	1129 a	2.10 d
$MgCO_3$	Mg 5.55	186 786	1210 b	2.04 cd
K_2CO_3	K 18.29	487 786	1219 bc	2.04 bcd
Na_2SO_4	Na 3.98			
	S 1.08	286 484	1234 bcd	2.00 abc
Na_2CO_3	Na 8.62	488 787	1259 bcde	2.01 abc
NH_4Cl	Cl 5.95	85 384	1260 bcde	1.99 abc
NaH_2PO_4	Na 3.98			
	P 3.09	286 405	1264 bcde	1.99 abc
NaCl	Na 3.98			
	Cl 5.95	183 483	1269 cde	1.99 abc
KCl	K 10.49			
	Cl 5.95	186 485	1277 cde	1.97 abc
$(NH_4)_2SO_4$	S 1.11	186 381	1282 de	1.96 ab
$NaHCO_3$	Na 8.61	487 787	1317 e	1.93 a
SEM			20.5	0.03

Within a column values with the same suffix are not significantly different ($P > 0.05$).

The data in Table 1 show that there was no significant relationship between broiler performance and the dietary levels of either of the two balance equations. A significant improvement in performance with the $NaHCO_3$ supplement was observed. Other studies in this department have also shown the beneficial effects of $NaHCO_3$ supplementation at high temperatures (Fixter et al. 1987; Balnave and Oliva 1991). Also interesting to note was the poor performance of the carbonates. This, along with the noticeable difference in the responses to Na_2CO_3 and $NaHCO_3$ at the same dietary sodium concentration, indicates that metabolisable anions may need to be considered when predicting performance.

The mineral retentions on each diet are shown in Table 2 except for the retentions of calcium and chloride which were not significantly affected by diet.

Table 2. Mineral retentions of broilers in Experiment 1.

Diet	Na	K	Mg	P	S
		(mg retained/g weight gain)			
Control	0.69 a1	2.07 ab	0.49 a	4.07 abc	1.99 ab
$CaCO_3$	0.66 a	1.93 ab	0.40 a	3.34 a	2.13 abc
$MgCO_3$	0.64 a	3.07 bc	3.94 b	4.39 bc	2.31 c
K_2CO_3	0.72 a	8.61 d	0.59 a	4.22 abc	2.16 bc
Na_2SO_4	2.41 b	2.74 bc	0.52 a	4.35 abc	2.93 d
Na_2CO_3	3.11 c	0.66 ab	0.38 a	4.07 abc	1.96 ab
NH_4Cl	0.68 a	2.56 abc	0.43 a	4.36 abc	2.00 ab
NaH_2PO_4	3.08 c	3.03 bc	0.76 a	7.38 d	2.10 abc
NaCl	1.98 b	2.41 abc	0.49 a	4.76 c	2.13 abc
KCl	0.67 a	4.61 c	0.43 a	4.50 c	2.07abc
$(NH_4)_2SO_4$	0.86 a	3.15 bc	0.52 a	4.50 c	2.93 d
$NaHCO_3$	2.17 b	0.05 a	0.30 a	3.47 ab	1.86 a
SEM	0.17	0.89	0.17	0.36	0.10

1 See footnote Table 1.

As shown in Table 2, the $NaHCO_3$ and Na_2CO_3 supplements consistently resulted in low retentions of minerals other than sodium where they ranked among the other high sodium diets. The low retentions associated with $NaHCO_3$ occurred despite these birds having the best growth and feed conversion. Re-evaluation of the two balance equations using retained levels also failed to show any correlation with performance.

Experiment 2 was designed to test the effects of different metabolisable anions. The bicarbonate, carbonate and acetate salts of sodium and potassium were tested on birds subjected to constant ambient temperatures of either 30°C or 33°C. These temperatures kept the birds just below or just above their panting threshhold. The basal diet had a calculated EB of 175 mEq/kg and a (cat-an) of 365 mEq/kg. All other diets had an EB of 385 mEq/kg and a (cat-an) of 575 mEq/kg.

Results from Experiment 2 are presented in Table 3. Raising the temperature by 3°C caused significant reductions ($P<0.05$) in growth and feed efficiency. There was no significant effect of diet on weight gain or feed conversion. However, at 33°C the growth rate was noticeably better with the sodium salts, while the effects of the potassium salts were inconsistent.

In this experiment the lack of significant production responses to the mineral supplements were mirrored by few significant effects on mineral retentions. The only significant ($P<0.05$) diet effects were the high sodium retentions of birds on the sodium-supplemented diets and the high potassium retentions of birds on the potassium-supplemented diets. The absence of the significant diet effects that had been observed in Experiment 1 may be related to a lower measured level of sodium in the control diet of Experiment 2 (0.64 g/kg versus 1.09 g/kg). The measured levels of all other minerals were similar in the control diets in both experiments.

Table 3. Responses of broilers in Experiment 2.

Diet	Temp (°C)	Gain (g)	F.C.R. (g/g)	Na (mg retained/g	K weight gain)
Control	30	1276	1.98	0.07	2.83
	33	918	2.14	0.60	2.76
$NaHCO_3$	30	1281	1.99	4.41	2.75
	33	958	2.13	6.07	2.37
Na_2CO_3	30	1258	1.99	4.42	2.36
	33	952	2.13	6.78	2.64
CH_3COONa	30	1248	2.04	6.44	0.23
	33	954	2.13	7.57	2.75
$KHCO_3$	30	1273	2.00	-1.35	9.94
	33	910	2.14	0.48	7.76
K_2CO_3	30	1273	2.03	0.00	8.71
	33	959	2.11	0.51	9.07
CH_3COOK	30	1250	2.08	-0.01	6.85
	33	887	2.24	0.51	8.30
DIET SEM		23.4	0.03	0.22	0.76
TEMP SEM		12.5	0.02	0.12	0.41

Temperature exerted a significant effect on the retentions of all minerals other than potassium. At 33°C higher retentions were observed for sodium and chloride and lower retentions for magnesium, phosphorous and sulfur.

IV. CONCLUSIONS

The trials reported in this paper failed to provide any support for the use of EB or (cat-an) equations as predictors of broiler performance at high temperatures. Since any performance responses observed were more likely due to the specific effects of particular salts the conclusions of Fixter et al. (1987) that broilers at high temperatures require a higher EB than broilers at moderate temperatures may merely reflect the higher EB associated with $NaHCO_3$ supplementation.

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BEHAVIOUR AND PRODUCTIVITY OF LAYING HENS AND BROILER CHICKENS

P.H. HEMSWORTH and J.L. BARNETT

Summary

Two studies examined the between-farm relationships between the behavioural responses of birds to humans and the productivity of laying hens and broiler chickens. A number of the behavioural variables were moderately to highly correlated with food conversion of broiler chickens and egg production of laying hens. The existence of these negative relationships between level of fear of humans and productivity of commercial poultry indicates the potential to improve productivity by minimizing the birds' fear of humans.

I. INTRODUCTION

Intense or prolonged fear is generally considered as an undesirable emotional state which may have adverse consequences on the animal's productivity and welfare (Craig and Adams 1984; Jones 1989; Mills and Faure 1990). A number of experiments have been conducted to examine the consequences of a high level of fear of humans on the productivity of intensively-managed farm animals. Research on pigs has shown that high levels of fear of humans can cause a chronic stress response or a series of acute stress responses which may severely depress the productivity of the animal (Hemsworth and Barnett 1987; Hemsworth et al. 1989). Recent research on experimental laying hens also indicates that high levels of fear of humans may adversely effect the productivity of intensively-managed farm animals (Hemsworth and Barnett 1989). Furthermore, although some studies with poultry have indicated either no effects or negative effects of handling on growth performance in poultry (McPherson et al. 1961; Buckland et al. 1974; Reichmann et al. 1978; Freeman and Manning 1979), several studies have shown positive effects (Thompson 1976; Gross and Siegel 1979, 1980; Jones and Hughes 1981; Collins and Siegel 1987). These latter studies provide some support for the contention that regular handling of a positive nature, by reducing fear of humans, may improve growth performance of poultry.

This paper describes two studies in which the relationships between fear of humans and the productivity of laying hens and broiler chickens were investigated.

II. MATERIALS AND METHODS

Poultry at 16 layer sheds (from 14 commercial layer farms) and 22 commercial broiler farms were studied. The level of fear of humans by birds in each shed was quantified on the basis of the behavioural responses of a sample of birds to an experimenter in a series of tests. Two main tests were conducted on laying hens aged 40 to 54 weeks. The ST (Shute) and AH (Approaching Human) tests involved measuring the withdrawal responses of birds to an approaching experimenter. In each shed the ST test was conducted on 40 birds

Victorian Institute of Animal Science, Department of Agriculture, 475 Mickleham Road, Attwood, Victoria, 3049.

which were individually placed on a table at the end of a narrow long arena. In contrast, the AH test was conducted in the bird's home cage and approximately 90 birds were tested in each shed. Two main tests were conducted on 5-week old broiler chickens. In addition to conducting the ST test on 40 birds in each shed, a MT (Movement) test was conducted within each shed to measure the withdrawal responses of birds to an experimenter moving through the shed.

Records on egg production of laying hens from 26 to 54 weeks of age and growth rate and food conversion of broiler chickens to 7 weeks of age were collected. A correlation analysis was used to examine the associations between behaviour and productivity.

Table 1. Proportion of variance accounted for ([adjusted]R^2) and level of significance (from the analysis of variance) from the stepwise multiple regression analyses with production as the dependent variable and bird (laying hen) behaviour as the independent variable.

Dependent variable	Independent variable	R^2	P <
Peak hen day production	M_{ST} E_{AHT}	0.53	0.01
Number of weeks within 2 percentage points of peak production	FM_{AHT} M_{ST} F_{AHT}	0.63	0.005
Number of weeks within 5 percentage points of peak production	$PROPOF_{AHT}$	0.30	0.05
Hen day production from 26 to 54 weeks of age	M_{ST}	0.28	0.05

$_{ST}$ and $_{AHT}$ indicate the Shute Test and Approaching Human Test, respectively. M - the proportion of birds that moved more than 42 cm from the approaching human; E - the number of times birds adopted an erect posture; F and FM - the number of times birds were in the front 10 or 20 cm of the cage, respectively; PROPOF - the proportion of birds that oriented forwards.

III. RESULTS

Significant associations were found between level of fear of humans and the productivity of both laying hens and broiler chickens. In the laying hen a number of behaviour variables were moderately to highly correlated with production variables. For example, the proportion of laying hens that withdrew from an

approaching experimenter in the ST test was significantly correlated with peak hen day production ($r = -0.66$, $P < 0.01$). Furthermore, the behavioural responses to humans accounted for between 23 and 63% of the variation in a number of production variables (Table 1), including peak hen day production (53% of variation) and the number of weeks within 2 and 5 percentage points of peak hen day production (63 and 30% of variation, respectively).

In the broiler chicken the average number of birds that remained within 75 cm of the experimenter in the MT test and the number of birds that remained forward and oriented forward when an experimenter approached in the ST test were significantly correlated with the feed to gain ratio ($r = -0.57$ and -0.44, respectively, $P < 0.01$). The former behavioural variable accounted for 29% of the variation in feed conversion at the farms (Table 2).

Table 2. Proportion of variance accounted for ([adjusted]R^2) and level of significance (from the analysis of variance) from the stepwise multiple regression analysis with food conversion as the dependent variable and bird (broiler) behaviour as the independent variable.

Dependent variable	Independent variable	R^2	$P <$
Food conversion	Birds remaining	0.29	0.01

Birds remaining - mean number of birds remaining within 75 cm of a moving experimenter.

IV. DISCUSSION

The results of these studies at commercial farms demonstrate significant associations between level of fear of humans and the productivity of poultry. These negative relationships between fear of humans and productivity agree with the findings for other farm species and support the proposal that high levels of fear of humans may limit the productivity of intensively-managed farm animals.

In the laying hen experiment it could be argued that factors associated with shed type, i.e. full environmental control versus open-fronted, could have a major effect on birds' responses in the tests. However, the correlation matrices for behaviour and production variables for the 2 shed types (7 with full environmental control and 9 open-fronted) showed similar relationships to the overall data although the coefficients tended to be higher for the controlled environment sheds. For example the correlation coefficients for the variable 'the proportion of birds that moved more than 42 cm from the approaching experimenter' in the Shute Test (M_{ST}) and 'peak hen day production' were -0.89 ($P < 0.05$) and -0.57 ($P > 0.05$) for the environmentally controlled and open-fronted sheds, respectively. Similarly, the coefficients between the variables 'the proportion of birds that oriented forwards' in the Approaching Human Test ($PROPOF_{AHT}$) and the 'number of weeks within 2 percentage points of peak hen day production' were 0.91 and 0.70, respectively ($P < 0.05$).

Future research is required to examine whether this fear-productivity relationship in poultry is a "cause and effect" relationship. The existence of a

"cause and effect" relationship in commercial farms provides the opportunity to improve productivity by reducing the bird's fear of humans.

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SELF-SELECTION OF CALCIUM IMPROVES SHELL QUALITY

R.J. HUGHES

Summary

Different ways of enabling each bird in the flock to fine-tune her intake of calcium according to her physiological needs were studied under housing, management and feeding conditions commonly used in Australia.

Giving pullets access to marble chips in the late rearing phase was neither detrimental to sexually maturing pullets, nor beneficial to shell quality later in life.

Hens near peak production were able to satisfy their appetite for calcium by self-selecting marble chips. They responded by producing larger eggs with better shells, but at a slightly lower rate of lay. Hens nearing the end of their laying life also responded by producing larger eggs with better shells, but without any effect on rate of lay. Inclusion of marble chips in the latter stages of the laying cycle improved the bone strength of hens. Excessive dietary phosphorus negated these benefits.

I. INTRODUCTION

Each year in Australia producers suffer losses of about $10-20 million from damage to egg shells incurred on the farm and on the way to market. The value of eggs is further reduced by damage in the market place. The prevalence of defective shells is thought to be a significant factor in reluctance of shoppers to purchase eggs. Similar incidences of egg loss due to poor calcification of the shell occur in other countries.

The ability of the hen's egg to withstand shell damage is largely dependent on the amount of Ca secreted by the shell gland during the 18 or so hours it takes to form the shell. A 60g egg contains about 2g of Ca in the shell. Because Ca is not stored in the shell gland, it must be drawn from the blood supply at a rate in excess of 100 mg/hour to meet this need. Ca in the blood stream is replenished by increased absorption from the gut and increased reabsorption of bone.

Heat stress is a significant factor in the high incidence of shell damage on farms and in the marketing system. Poor shell quality during heat stress is not merely the result of reduced feed intake. Basic changes occur in the rates of uptake and utilisation of various nutrients essential for egg shell formation. That is, the feeding of diets of high nutrient density can ease, but not fix, the problem. Ideally, producers should aim at minimising heat stress on laying stock. However, the high capital cost of modifying or replacing poultry sheds can be prohibitive for many producers. Other ways of coping with heat stress should be considered.

This paper examines different ways of enabling each bird in the flock to fine-tune her intake of Ca according to her physiological needs.

Parafield Poultry Research Centre, Parafield Gardens, SA 5107.

II. SELF-SELECTION OF MARBLE CHIPS DURING REARING

Immature female birds are similar to other animals in their Ca requirement. However, with the approach of sexual maturation, there is a dramatic increase in Ca uptake in association with the development of medullary bone, a specialised tissue which forms in the medulla of long bones, e.g., femur and tibia (Hurwitz 1987).

There is no point in providing additional Ca before bone tissue has been primed by ovarian steroids which signals the onset of sexual maturity. Also, there are risks in providing excessive dietary Ca to pullets, e.g., retarded development, and urolithiasis (Wideman and Cowen 1987).

Proper development of medullary bone reserves of Ca in the 2-3 weeks prior to sexual maturity is an important factor in the regulation of Ca balance in laying hens. Classen and Scott (1982) showed that pullets given access to shellgrit during rearing (5-18 weeks of age) produced better shells in the period 25-31 weeks of age. The purpose of this experiment was to verify these findings and to see whether benefits extended into the laying cycle. Each pullet was able to self-select marble chips according to her increased appetite for Ca as she matured sexually.

(a) Methods

About 1100 commercial pullets were reared on litter from hatch to 18 weeks of age in a controlled environment shed. Marble chips (1%) were included in the rearing feed from 15 or 17 weeks of age, or not at all. The marble chips were 2.5 - 4mm in size and boosted dietary Ca to 13.5g/kg.

At 18 weeks of age, 840 pullets were transferred to single bird cages in a layer shed equipped with ventilation fans and an evaporative cooling system. All pullets received a layer diet containing 11.7 MJ AME, 177g protein and 36.5g Ca/kg from 18 weeks of age.

Blood samples were taken from 36 pullets (twelve per treatment) at 18 weeks of age. Testosterone concentrations in plasma were measured. The pullets were killed by cervical dislocation, then weighed. Ovaries, femur and tibia were removed and weighed. Tibia were assayed for ash, Ca and P content. Cortical thickness of tibia was determined by radiography. Tibia, kidney and parathyroid glands were examined histologically.

When hens were 28, 38, 46, 50, 60 and 77 weeks of age, all eggs laid between 0800 and 1000h from Monday to Thursday were kept for shell measurements. Occasionally, this period was extended from 0730 to 1030h.

(b) Results

Inclusion of marble chips in rearing diets had no effects on femur (7.9g), tibia (10.2g) or body (1.33kg) weights, or ash (64%), Ca (38.6%), P (17.7%) or cortical thickness of tibia. Histological examination of parathyroid glands, tibia and kidneys revealed no differences due to rearing diets, nor signs of urolithiasis or other disorders.

Testosterone concentrations in plasma from 22 pullets averaged 0.19ng/ml. Concentrations were below the limit of detection (0.07ng/ml) in the other 14 pullets . There were no correlations between testosterone and ovary or bone weights, or with bone ash, Ca or P contents.

At 28 weeks of age, egg weight (52g), shell weight (5.12g) and thickness (375μm) were unaffected by rearing diets.

At 38 weeks of age, after hens were subjected to dietary treatments involving marble chips and high dietary P for 8 weeks (see Section III), inclusion of marble chips in the rearing feed from 15 weeks of age had deleterious effects on egg weight (57.8g), shell weight (5.38g) and shell thickness (371μm) compared with control hens (58.6g, 5.52g and 376μm, respectively). Similarly, inclusion of marble chips in rearing feed from 17 weeks of age significantly affected shell weight (5.44g) and tended to affect egg weight (58.1g) and shell thickness (372μm).

At 46 weeks of age), after receiving layer treatments involving sodium zeolite and high dietary P for 4 weeks, shell thickness was significantly lower for hens given marble chips during rearing from 17 weeks of age (362μm) than controls (366μm). Hens given marble chips from 15 weeks of age were not affected (367μm).

At 50 weeks of age, there was no difference in shell thickness (357μm). In contrast, at 60 weeks of age, hens given marble chips during rearing from 17 weeks of age had significantly poorer shells (347μm) than controls (353μm), whereas hens given marble chips from 15 weeks of age did not differ (349μm).

Shell quality at 66 and 77 weeks of age was not affected. Egg weight, shell weight and shell thickness averaged 63.2g, 5.32g and 346μm at 66 weeks, and 62.9g, 5.34g and 345μm at 77 weeks, respectively.

(c) Discussion

The lack of effects on pullets of 1% marble chips in rearing feed from 15 or 17 weeks of age indicates that higher than usual intakes of Ca were excreted without difficulty, and did not result in higher than normal storage of Ca in bones.

There was no evidence of increased secretion of shell material by the uterus. Any improvements in shell thickness attributable to marble chips during rearing were associated with a reduction in egg size. That is, thicker shells were the result of smaller egg masses being encased in the same amounts of shell material, and not due to greater availability of Ca.

III. SELF-SELECTION OF MARBLE CHIPS DURING LAYING

Modern breeds of poultry are able to self-select some of their nutritional requirements such as Ca (Hughes 1984). This inherent ability is not expressed if food is offered as pellets or finely-milled mash (Mongin and Sauveur 1974).

A continuous supply of Ca from ingested food is a critical factor in the hen's ability to produce eggs with adequate shell quality. The hen will draw on her bone reserves of Ca to meet any shortfall in the supply from the gut (Etches 1987). In general, the more a hen relies on her bone reserves then the more

likely it is that the subsequent shell will be weak (Farmer et al. 1986), and that she will suffer leg weakness later in the laying cycle (Keshavarz 1987).

The purpose of this study was to enable each hen to self-select marble chips (2.5-4mm in size) according to her increased appetite for Ca at the beginning of the shell calcification period.

(a) Methods

A total of 840 hens received four experimental diets comprising two levels of each of total dietary P (0.6 and 1%) and marble chips (0 and 5%). Marble chips replaced ground marble to provide about half of the total dietary Ca (3.8%). The diets were fed for 8-week periods from 30 and 69 weeks of age. Shells were measured when hens were 38 and 77 weeks old.

At 77 weeks, 36 hens were weighed then killed by cervical dislocation. Tibia were weighed and analysed for ash, Ca and P content.

(b) Results

Young hens

Egg weight (58.6g) was significantly higher, and shell weight (5.48g) and shell thickness (375μm) tended to be higher in hens given marble chips compared with control hens (57.9g, 5.42g and 372μm, respectively). High dietary P depressed shell thickness (372 vs 375μm).

Specific gravity, and shell proportion, density and thickness were affected by an interaction between marble chips and dietary P. Marble chips were beneficial only when dietary P was not excessive.

Hens given access to marble chips laid at a significantly lower rate (85%) than control hens (87%), but did not differ in feed intake (109 g/bird.day), over the 8-week period. The drop in production was most evident in the first 4 weeks.

Old hens

Hens given access to marble chips laid larger eggs (63.7 vs 62.2g) with more shell (5.43 vs 5.26g) than control hens. Shell density was higher (72.8 vs 71.8 mg/cm^2) and shells tended to be thicker (346 vs 343μm) for hens given marble chips. These differences were not reflected in shell proportion (8.5%) or specific gravity (1.080).

Rate of lay (68%) and feed intake (105 g/bird.day) were unaffected by marble chips or dietary P measured over the 8-week period.

Tibia Ca was increased by marble chips (41.3 vs 39.7%) if dietary P was low (0.6%), but decreased (39.3 vs 40.7%) if dietary P was high (1%). Body weight (2.1kg), tibia weight (12g), tibia ash (68%) and tibia P (17%) were unaffected by marble chips or dietary P.

(c) Discussion

Inclusion of marble chips in the laying diet to provide half of the dietary Ca resulted in larger eggs with more shell, and consequently better shell quality from young hens providing dietary P was not excessively high, and from old hens irrespective of dietary P.

The beneficial effect of marble chips on egg size and quality was offset by a small drop in production in young hens soon after the hens were given this experimental diet. Thereafter, production picked up.

Strength of leg bones of old hens was probably affected by marble chips in the feed. The Ca content of tibia was increased in hens given access to marble chips in a low P diet (0.6%), whereas tibia Ca decreased in hens given marble chips in a high P diet (1%).

IV. ACKNOWLEDGEMENTS

I am grateful to the Egg Industry Research and Development Council for financial support for this work.

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PROTEIN SOLUBILITY AS A MEASURE OF SOYABEAN MEAL PROCESSING

G.G. IRISH and D. BALNAVE

Recently attention was drawn to a series of studies in which poor growth was observed when broilers were fed diets in which soyabean meal was the sole dietary protein concentrate. The fact that growth was improved by replacing about one quarter of the soyabean meal with either sunflower meal or mixed plant protein meals suggests that in some instances the feeding quality of the soyabean meals available in Australia may be questionable (Irish and Balnave, 1991).

Dale et al. (1987) recently published a procedure by which the protein quality of soyabean meal is estimated from the percentage protein solubility in KOH solution (2 g/kG, pH 12.5). Protein solubility identifies both overprocessed (<75%) and under-processed (>85%) soyabean meals.

A number of samples of soyabean meals were obtained from eastern Australia in 1991 and, in addition to three imported meals from the USA (Samples 8-10), were analysed to evaluate processing efficiency. The results are shown in the Table.

Chemical evaluation of soyabean meals.

Samples	Crude Protein (g/kg)	Urease Activity (%)	Protein Solubility (%)	FDNB-Reactive Lysine (%)
1	373	6.9	82.8	97.8
2	404	12.8	79.4	97.8
3	453	3.4	72.1	97.4
4	462	1.7	74.5	97.8
5	463	0.5	76.1	97.9
6	466	1.7	78.5	97.6
7	480	2.6	80.9	98.1
8	480	1.1	81.6	97.9
9	492	0.7	81.5	98.0
10	492	7.7	82.9	98.0

The urease activity was low in all cases (0-25 represents relatively inactive urease activity). However, the data indicate that two of the seven Australian samples showed evidence of over-processing. This was not identified by the available lysine data although a significant ($P<0.05$) correlation was observed between protein solubility and available lysine ($r^2=0.46$). Protein solubility is a simple procedure which identifies both under- and over-processing of soyabean meals and should be considered as a routine screening procedure for protein quality.

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Department of Animal Science, University of Sydney, Camden, NSW, 2570

IMMUNE RESPONSES OF BROILER CHICKENS TO WHEAT PROTEINS

R.J. JOHNSON*†, J. SKERRITT**, G. ANNISON***‡ and P.J. EASON*

Malabsorption syndromes can be elicited in young piglets and in calves due to ingestion of dietary antigens (Porter and Barratt, 1987). Malabsorption due to immunological disturbance of gut function may lead to diarrhoea and marked depression in feed intake. Certain wheat proteins may cause immunological reactions in humans. There is no information on broiler chickens but certain wheats are poorly digested by broilers (Mollah et al, 1983). The present study was carried out to determine if broilers could develop immunological responses to wheat proteins which could lead to malabsorption.

Groups of eight broiler chickens were fed a wheat-free diet (sorghum 800 g/kg, casein 130 g/kg) from day-old to 14 d of age. Groups were then either maintained on sorghum/casein or wheat/casein diets using four different wheat samples for a period of 7 d. This was followed by a 14-day wheat-free diet again then another 7-day period of feeding the test diets. Blood samples were collected from individual birds at 15, 22, 36 and 43 d of age, and plasma was assayed for IgY by indirect ELISA. Three wheat protein antigens were studied, namely total wheat flour proteins, wheat flour salt-soluble proteins, and gliadin proteins. Apparent metabolisable energy (AME) was determined in the two 7-day periods when the wheat-based diets were fed (14-21 d and 35-42 d of age).

There was evidence of significant levels of antibodies, with especially high responses in some birds. Antibody responses to the wheat gliadin fraction was greatest, as has been noted in human wheat intolerance (Skerritt, 1988). These antibody levels increased with age in the chickens and following the second exposure to the wheat-based diets. Mean (±SD) AME (MJ/kg DM) of the four wheats determined twice from 14-21 d and from 35-42 d of age were: Wheat A 13.00 (±1.82), 13.41 (±1.33); Wheat B 13.59 (±1.79), 15.01 (±0.75); Wheat C 12.79 (±1.68), 13.41 (±1.70 and Wheat D 13.66 (±0.83), 14.18 (±1.08) respectively.

There was no clear-cut relationship between AME and antibody levels for the wheats but this may have been due to the small numbers of birds used. However this study is the first to show that broiler chickens develop immunological reactions to certain dietary components. Further studies are required to determine if immune - related malabsorption may occur in broiler chickens.

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*Victorian Department of Agriculture Animal Research Institute, Werribee, 3030.
**CSIRO Division of Plant Industry, North Ryde, NSW, 2113.
***Department of Animal Science, University of Sydney, Camden, NSW, 2570.
†Present Address: Rhone Poulenc Animal Nutrition Pty Ltd, West Footscray, 3012.
‡Present Address: CSIRO Division of Human Nutrition, O'Halloran Hill, SA, 5158.

THE EFFECT OF STRAIN AND SEX ON GROWTH AND PHYSIOLOGICAL RESPONSES TO EARLY GROWTH RETARDATION IN BROILER CHICKENS

R.J. JOHNSON*†, J.P. McMURTRY** and P.J. EASON*

Summary

Broiler chickens from two commercial strains were subjected to feed restriction (R) from 6 to 12 d of age. Ad libitum feed intake was allowed thereafter and growth and hormone physiology were measured compared to controls (C). Body weights were lower ($P<0.05$) for R(2.171 kg) than C(2.260 kg) birds. Feed conversion ratio (FCR) was lower for R(1.932) than C(1.975) birds. Plasma GH was increased ($P<0.05$) during compensatory growth (21 to 49 d of age) in the R birds, associated with marked changes in GH secretory patterns. Plasma IGF-1 levels were depressed by feed restriction but levels increased rapidly during compensatory growth.

I. INTRODUCTION

Broiler chickens subjected to a short period of feed restriction at an early age subsequently grow at faster than normal rates when allowed ad libitum feed intake (Plavnik and Hurwitz, 1985). Improvements in feed efficiency and a reduction in abdominal fat content have been observed although age-related body weight may be reduced (Plavnik and Hurwitz, 1985; 1988).

Reasons for the marked compensatory growth observed in broilers, as in other animals, may be related to efficiency of energy utilization, maintenance energy requirements, food intake capacity and/or alterations in composition of body gain (Farrell and Williams, 1989). Homeostatic mechanisms relating to endocrine regulation of growth may be altered during compensatory growth but there is no information on this for broiler chickens. The present study was carried out to determine the effect of early feed restriction on subsequent growth hormone (GH) and insulin-like growth factor-1 (IGF-1) levels in two commercial strains of broiler chickens.

II. MATERIALS AND METHODS

Two hundred male and 200 female broiler chickens from Strain A and 150 male and 150 female broiler chickens from Strain B were wing banded and allocated at random to cages in a conventional brooder unit. Temperature during the first 7 days was 35°C and was gradually reduced to 25°C by 21 d of age. At 21 d of age the birds were transfered to grow-out wire cages.

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*Victorian Institute of Animal Science, Department of Agriculture, Werribee, Victoria, 3030

**USDA, Agricultural Research Service, Beltsville Agricultural Research Centre, Animal Science Institute, Beltsville, MD 20705.

†Present Address: Rhone Poulenc Animal Nutrition Pty Ltd, 19-23 Paramount Road, West Footscray, Victoria, 3012

At 6 days of age birds from each strain and sex were weighed individually and allocated to one of the two treatments, either (1) ad libitum - fed controls (C) or (2) restricted-fed from 6 to 12 d of age (R). The restricted-fed birds received approximately 60% of ad libitum feed intake over the 6-12 d of age period as described by Plavnik and Hurwitz (1988). At 13 d of age the restricted birds were weighed and returned to ad libitum feeding.

Standard broiler starter (0-21 d) and finisher (21-49 d) diets were fed. Lighting was 23L:1D throughout the trial. Feed intake, body weight and mortality were measured at 7 day intervals. Blood samples were taken from a wing vein of representative birds (8 birds/sex and strain) at 6, 12, 21 and at 7 day intervals thereafter. Blood was collected into heparinized (1×10^6 IU sodium heparin/l) syringes and plasma recovered by centrifiguration. Samples were collected commencing at the same hour of the day (0800 h) at each age.

Serial blood samples (0.5 ml) were collected from a jugular vein cannula (Johnson, 1988) at 10 min intervals from six male and six female birds from each treatment for 5 and 7 h at 29 and 43 days of age respectively. Plasma samples were assayed for growth hormone (GH) and insulin-like growth factor-1 (IGF-I) by radioimmunoassay as described by Johnson et al. (1990).

III. RESULTS

(a) Body Weight and Feed Efficiency

Body weights are given in Table 1. There was no evidence of strain or sex interactions on the effect of the restriction treatment on body weight at 49 d of age. Mean body weights (kg) for both strains were lower ($P<0.05$) for R (2.171) than C (2.260) and body weight gain (g/d) from day-old to 49 d of age was lower ($P<0.05$), for R (43.4) than C (45.3) treatments. However, from the end of restriction at 12 d of age body weight gain was greater ($P<0.05$) for R (54.4) than C (52.5) treatments. The overall feed conversion ratio (FCR, g feed/g body weight gain) from day-old to 49 d of age was lower ($P<0.05$) for Strain A (1.819) than for Strain B (2.136) and for R (1.932) than C (1.975) treatments. Again, there was no evidence of strain or sex interactions with treatment.

(b) Plasma GH Concentration

Plasma GH was lower ($P<0.05$) in both male and female R birds at 12 d of age (at the end of feed restriction). However, from 21 to 42 d of age, plasma GH was higher ($P<0.05$) for both male and female R birds than C birds. This pattern was largely the same for both strains, as shown in Figure 1 for Strain A birds.

Temporal GH secretory patterns were markedly altered by feed restriction from 6 to 12 d of age. This was seen at 29 d of age in that R females had greater ($P<0.05$) GH peak amplitude and frequency (no. peaks/h) than C females. Also, at 43 d of age, in male R birds, GH pulse amplitude (ng/ml) and frequency were greater ($P<0.05$) than in C birds.

(c) Plasma IGF-1 Concentration

Plasma IGF-1 was depressed ($P<0.001$) in both male and female R birds at

12 d of age, similar to plasma GH. At 12 d of age, plasma IGF-1 had increased such that levels were not significantly different from C birds thereafter, as shown in Figure 2 for Strain A birds.

Table 1. Effect of feed restriction from 6 to 12 days of age on body weight of male and female broilers of two commercial strains.

	Males				
	Strain A		Strain B		
Age (d)	Control	Restricted	Control	Restricted	SEM+
6	123	127	125	123	3
12	315	159	224	155	5
21	718	573	739	580	16
28	1159	987	1195	1032	29
35	1587	1419	1642	1467	48
42	2033	1873	1966	1909	60
49	2424	2302	2379	2305	64
	Females				
6	128	126	125	125	
12	313	159	315	153	
21	680	545	699	553	
28	1064	907	1114	948	
35	1433	1295	1494	1354	
42	1779	1660	1833	1709	
49	2100	2022	2137	2054	

+ SEM is the standard error of a mean (df=33) determined in a factorial analysis of variance at each age (2 strains x 2 treatments x 2 sexes).

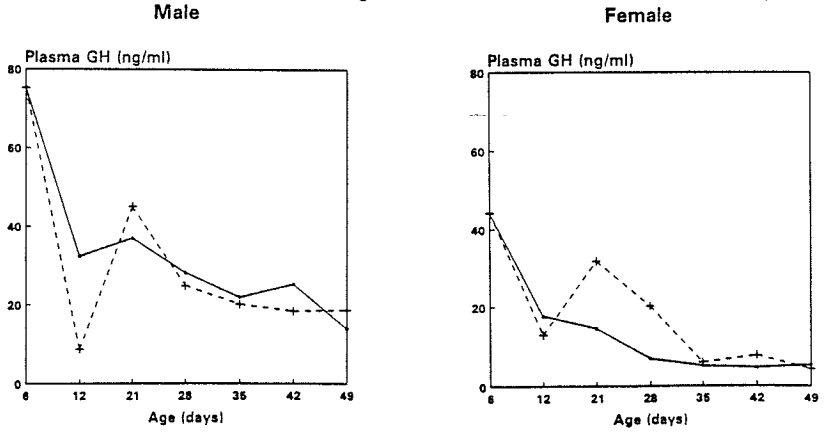

Figure 1. Plasma GH concentrations (ng/ml) in broiler chickens (Strain A) either allowed ad libitum feed intake (C) (-) or restricted from 6 to 12 d of age (R) (---).

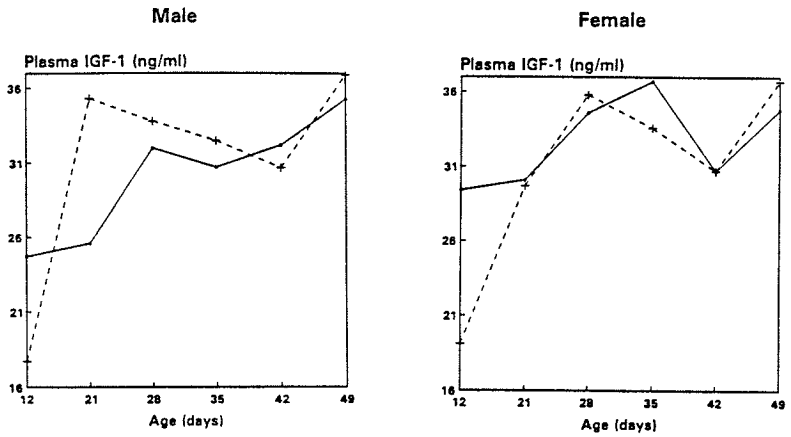

Figure 2. Plasma IGF-1 concentration (ng/ml) in broiler chickens (Strain A) either allowed ad libitum feed intake (C) (-) or restricted from 6 to 12 d of age (R) (---).

IV. DISCUSSION

The present study confirms that broiler chickens subjected to a short period of feed restriction subsequently undergo compensatory growth with an overall improvement in feed efficiency (Plavnik and Hurwitz, 1985; 1988). There were clear effects on the endocrine regulatory processes caused by feed restriction associated with the observed compensatory growth. Plasma GH concentration was elevated during this period, with significant alterations in GH pulse parameters such as amplitude and frequency. Plasma IGF-1 concentration also increased markedly after cessation of feed restriction. The results support the notion that GH is the principal endocrine regulator of growth through modulation of IGF-1 production. Plasma IGF-1 concentrations have been shown to be positively correlated with rates of protein accretion (Tomas et al., 1991). As amino acid turnover is the greatest energy cost of protein deposition (Buttery and Annison, 1973) the present results indicate a possible hormonal basis for the observed increases in efficiency associated with early feed restriction in broiler chickens.

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THE GROWTH OF BROILERS IN A SUPEROXYGENATED ATMOSPHERE

G.P.D. JONES and D.J. FARRELL

The domestic fowl is particularly susceptible to ascites (Scheele et al. 1991) due to low hypoxia and to the inability of the lung to adequately oxygenate the blood (Sykes, 1960). The growth rate of the broiler is positively related to the incidence of ascites (Julian et al, 1987).

Two strains of broilers, selected for feed conversion (Line F) or unselected (Line C) were grown in environmental cabinets modified to house chickens. Temperature remained high (29-32°C) throughout and CO_2 levels ranged from 0.35 to 0.80%. Four groups of 15 broilers of each strain were grown for 4 weeks under continuous lighting under a normal atmosphere (21% oxygen) in one cabinet or in the second, further modified cabinet into which oxygen was continuously bled so that the atmosphere in the cabinet was maintained at 23% oxygen.

	Line F		Line C		LSD
	21%	23%	21%	23%	(P=0.05)
Bodyweight (g) @ 14 d	452	461	424	428	21
@ 21 d	759	802	707	701	29
@ 28 d	1089	1187	1030	1067	80
Feed conversion ratio	1.63	1.43	1.71	1.65	0.15
Abdominal fat pad (g)	5.3	6.3	11.2	11.9	2.6
(% BW)	0.50	0.54	1.08	1.08	0.21
Arterial pressure index	25.4	23.3	23.3	21.5	1.8

Bodyweight of the two lines was not different under normal oxygen levels, however, under a 23% oxygen environment, Line F birds were heavier after 14 d than Line C birds grown under either oxygen level as well as Line F birds grown under 21% oxygen (see Table). The increase in growth was concurrent with an improvement in FCR. There was no effect on body fat, as indicated by the abdominal fat pad (AFP). The arterial pressure index (API), a measure of susceptibility to ascites, was decreased by increasing atmospheric oxygen. Line F, selected for FCR, had a greater API, regardless of oxygen level.

The results indicate that selection for FCR has increased the potential for the incidence of ascites to be seen in Australian broiler flocks and point to the need for careful management practices. This techniques has no apparent commercial application, however, to ensure that the occurrence of the ascites syndrome is minimised, adequate shed ventilation is essential.

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Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, NSW, 2351

SUBSTANCE P-IMMUNOREACTIVE NERVE FIBRES IN THE CHICKEN BEAK

C.A. LUNAM* and P.C. GLATZ**

Physiological and behavioural data suggest that following beak trimming birds suffer both acute and chronic pain (Breward and Gentle 1985, Glatz 1987, Gentle et al 1990). Evidence demonstrating the presence of nociceptive fibres has been provided by electrophysiological recordings of nerves supplying the beak (Breward 1983). In mammals nociceptive nerves classically contain the peptide, substance P. This peptide is considered to be important in the transmission of nociceptive signals. To date, neuropeptides have not been identified in the avian beak. It was the intention of this study to determine whether nerves innervating the chicken beak contain substance P.

At hatch, a commercial laying strain was killed by cervical dislocation and the whole of the upper and lower beak removed and processed for single labelling immunofluorescence as described previously (Lunam 1989). The distribution of substance P-immunoreactive nerves was determined from photographic montages prepared from transverse sections taken through each beak at intervals of 500 microns.

Both the upper and lower beaks were innervated by a dense meshwork of intensely fluorescent fibres. This meshwork was observed over the entire extent of each beak. Perivascular nerves showed reactivity for substance P. Free nerve endings, immunoreactive for substance P, were present in the dermis and occasionally nerves penetrated into the basal layers of the epidermis.

These results demonstrate that the chicken beak is densely innervated by sensory nerves containing substance P. The presence of free nerve endings immunoreactive for substance P provides direct anatomical evidence for nociceptive fibres in the avian beak.

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* Department of Anatomy and Histology, Flinders University, South Australia, 5042.
** Parafield Poultry Research Centre, Parafield Gardens, South Australia, 5107.

A COMPLETE MORTALITY SURVEY OF A COMMERCIAL BROILER FLOCK

G.M. MALANYAON and B.I. REMINGTON

Mortality of day old to processing commercial broiler continues to plague the poultry industry, indicating a varying percentage depending on the incidence and occurence of diseases. At present, limited surveys have been carried out to investigate the causes of mortality in Australia (Jackson et al 1972) and other countries (Hemsley 1968; Brigden and Riddell 1975). Results of their completed studies showed a mortality range of three to five percent. The present study was conducted to identify the major causes of mortality and to find out the reasons for varying mortality rate. Necropsy procedures and diagnosis of dead and culled birds were done based on gross pathological changes. The result of the study is shown in the Table.

Causes of mortality and culling among 10,700 broiler chickens

Causes of Mortality	No. of Death	Mortality (%)	Proportion of total death (%)
Acute Death Syndrome	193	1.803	47.54
Yolk Sac Infections	89	0.831	21.92
Starvation Syndrome	56	0.523	13.79
Runts/culls	33	0.308	8.13
Non-specific cause	11	0.102	2.71
Infectious Bronchitis	7	0.065	1.72
Others	17	0.155	4.21
Totals	406	3.787	100.00

A total of 406 birds (3.787%) died or were culled. Heavy losses were attributed to Acute Death Syndrome (1.809%) with total death proportion of 47.54%. The 1.809% incidence of ADS found in this survey is within the range of 1.13 to 2.46%. (Riddell and Orr 1980; Steele and Edgar 1982). Incidence of males dying from ADS is 93% and usually the heaviest among the flock are found lying on their backs.

The results show Acute Death Syndrome had the highest mortality rate than other diseases. These losses due to Acute Death Syndrome are vital to the broiler industry as death occurs throughout the production period.

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Department of Farm Animal Medicine and Production, University of Queensland, Australia

DEVELOPMENT OF AN ELISA FOR THE ALTERNARIA TOXIN TENUAZONIC ACID

I. McCAULEY and A.D. HUNTINGTON

Alternaria is a widely distributed genus of fungi that produces over 30 mycotoxins, which is found as both a pre- and post-harvest contaminant of grains, fruit and vegetables. Alternaria has been found in many samples of Australian grains which have been shown to produce toxicity in animals. The most important Alternaria toxin on a weight basis, tenuazonic acid is also a good indicator of a toxic species, being present in over 80% of toxic isolates. Quantitation of TA by HPLC requires that biological material be purified by a number of organic extractions, stringent control of assay conditions and use of specific equipment to achieve reasonable accuracy and precision. Studies of the significance of Alternaria contamination have been hampered by the cumbersome assays needed to identify toxic isolates and to measure specific toxins. For this reason we have attempted to develop an ELISA to tenuazonic acid.

The major restriction to the development of an ELISA to TA is the difficulty in preparing an immunogenic conjugate as there is no suitable group on the toxin to couple it to a carrier. We took the approach of synthesising homologous tetramic acids, replacing the isoleucine found in tenuazonic acid with amino acids which had side chains suited for linkage to proteins. Using modifications of the method of Lacey (1954) the glutamic acid homologue (Glu-TA) was synthesised which then provided a carboxyl group suitable for coupling to proteins. Glu-TA was coupled to ovalbumin using water-soluble carbodiimide and an antisera was successfully developed. In addition, the N-hydroxysuccinimide derivative of glu-TA was prepared and coupled to peroxidase.

A competitive antigen-capture ELISA has been designed using Protein A to selectively bind anti-Glu-TA to the wells. A fixed amount of peroxidase-labeled Glu-TA is then added with the sample. The antisera shows 100% cross-reactivity with tenuazonic acid, its copper salt and the tyrosine homologue and no significant reactivity with either glutamic acid or isoleucine. The assay has a detection limit of 0.25 pmol which is equivalent to 0.05 μg/mL in an extract of contaminated grain. Recovery of TA added to extracts in the range of 0.5 to 15 μg/mL was 99.1 $\pm$ 11% and between and within assay coefficients of variation are 14% and 6% respectively. This is the first report of an ELISA for an Alternaria toxin.

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Victorian Institute of Animal Science, Attwood, Victoria

MATHEMATICAL MODELS OF EGG PRODUCTION. CAN THEY BE USED FOR PREDICTION?

M.W. McDONALD

Summary

A modified compartmental model of egg production has been fitted to hen day production from 15 entries into the Victorian Random Sample Test and from two commercial flocks. Some consistency was demonstrated between some parameters of the model when the same strains of layers were compared in the RST data. The commercial data showed that the model overestimated production at the end of the cycle when extrapolating from part records.

I. INTRODUCTION

Mathematical modelling of the egg cycle, initially based on Wood's curve (Wood, 1967), an algebraic model for describing the dairy lactation curve, was first reported by McNally (1972). This model described the production curve very accurately. The constants, however, lacked any obvious biological meaning.

Adams and Bell (1980) examined the averaged production of 232 commercial flocks and showed that a modified Wood's equation proposed by McNally predicted an increase in egg production after about 70 weeks which was not evident in their data. They proposed a model composed of two sections - a growth curve describing the effect of maturation on production and a linear ageing effect. Their equation contained five constants, again with little meaning biologically.

A compartmental model, originally developed for studying insects, was applied to poultry by Gavora et al (1982). They defined its four constants in biological terms. McMillan et al (1986) considered this compartmental model to be superior to the Wood's model; Cason and Britton (1988) found that the Adams-Bell model produced a better fit than the compartmental model in 19 commercial flocks. Coefficients of determination of .97 to .99 were common with both Adams-Bell and compartmental models, and .9 to .95 with the Wood's model.

Recently, Yang, Wu and McMillan (1989) proposed a modified compartmental model, adding the variation in age of sexual maturity to the model. This model gave higher coefficients of determination than either the Wood's curve or the compartmental model and predicted age and rate of peak production with greater accuracy than either of the other models, when fitted to their data.

The present investigation uses the modified compartmental model to examine the consistency of the coefficients of the model as effected by such factors as genetic differences and time differences. It also examines the effect of length of data collection on the reliability of extrapolations of the fitted curve in predicting future production.

P.O. Box 2003, Toowoomba Qld. 4350.

II. MATERIALS AND METHODS

The modified compartmental model describes egg production by:

$$Y(t) = ae^{-bt} /(1 + e^{-c(t-d)})$$

where Y(t) is production during week t,
a is a scaling factor,
b is the rate of decrease in laying ability,
c is a reciprocal indicator of the variation in sexual maturity
and d is the mean age of sexual maturity;
e is the exponential constant.

Data on hen-day production from the 28th, 30th and 31st Victorian Random Sample Tests and from two commercial flocks were analysed. Data from the 29th RST were not used as diets differing in protein level were fed to some strains. The parameters of the modified compartmental model were estimated by an iterative process, the criterion for goodness of fit being the minimization of the residual mean squares between observed and predicted production at each age. This was preferred to the maximization of the correlation between observed and predicted production as the correlation coefficient frequently exceeded 0.98 and hence was very insensitive to small changes in the values of the parameters being fitted.

The Random Sample data were for flocks of 144 birds to 72 weeks of age; data were presented as summaries of 28 day periods. Data on the Victorian Department's files were incomplete for the 28th RST. The two commercial flocks were 1700 birds to 78 weeks of age and 10 000 birds to 70 weeks; data were summarised for seven day periods. Both commercial flocks were SiroCTs.

III. RESULTS AND DISCUSSION

Fifteen sets of Random Sample Test data were analysed, involving three tests and seven strains. In all cases the correlation coefficient exceeded 0.99, the lowest being 0.9916 and the highest 0.9984. Six strains were represented in two tests and one in three tests.

Strains showed consistency in the estimated values for the parameters. While the scaling factor, *a* in the equation, ranged from 100.0 to 127.43, the largest difference between estimates for the same strain was 11.11. The parameter, *b*, measuring the decline in production with age also showed consistency. The range was 0.0052 to 0.0117 but the largest difference between estimates for the same strain was 0.0025. The parameter *(c)* measuring variation in sex maturity was less consistent, ranging from 0.51 to 1.45; the largest difference in the estimate for the same strain in different years was between 0.84 and 1.45. The parameter *(d)* measuring average age of sex maturity also showed considerable variation, ranging from 20.42 to 25.03 weeks; strains were consistent, particularly the earlier maturing strains for which estimates differed by as little as 0.05 weeks.

Estimation of the standard deviation of the parameters was difficult because the effect on the residual mean square of altering the estimate of a parameter was not always symmetric. Thus for one flock for which an optimum value for $c = 0.84$ was estimated, the lower estimate of *c* which increased the residual standard

deviation by one half its minimal value was found to be 0.549 but the upper estimate which produced the same effect was in excess of 2.0. The parameters b and d had a less asymmetric effect on error.

Table 1 summarizes estimates of the parameters for some of the more significant commercial strains of layers.

Examining table 1, the fit for the 30th RST was better than for the 28th. This resulted from the absence of any data in the 28th between weeks 21 and 28, a critical period for estimating parameters c and d.

The parameters of the production curves for the two commercial flocks are presented in table 2 ($r = 0.9793$ and 0.9814 for the smaller and the larger flocks, respectively). To test the reliability of the estimates for extrapolation to later ages, the curves were refitted to data from sexual maturity to 55, 60, 65 and 70 weeks of age. These are also summarized in table 2.

Table 1. Parameters of the Modified Compartmental Model.

Strain	RST	Parameters				RMS†	r‡
		a	b	c	d		
Hyline WxNH	28	103.73	0.0056	0.84	24.26	3.5528	0.9973
	30	103.21	0.519	1.45	23.04	1.5521	0.9984
SiroCT	28	123.03	0.0083	0.73	24.9	7.0029	0.9951
	30	121.90	0.0075	1.01	22.98	1.7891	0.9985
SiroCB	28	126.27	0.0088	0.63	24.82	13.487	0.9917
	30	116.32	0.0066	0.99	23.03	1.7483	0.9987
Hichick	30	110.70	0.0076	0.65	20.42	2.0945	0.9964
	31	100.00	0.0071	0.72	20.48	0.5735	0.9981

†RMS = Residual Mean Square
‡ = Correlation coefficient

Table 2. Production Curve for two commercial flocks

Flock	Period	a	b	c	d	RMS†	r‡
Small	55 wks	103.4	0.0049	0.656	24.58	8.00	0.9918
	60 wks	103.0	0.0048	0.652	24.58	7.77	0.9907
	65 wks	106.0	0.0056	0.623	24.56	11.53	0.9846
	70 wks	108.4	0.0061	0.610	24.58	13.93	0.9798
	78 wks	107.5	0.0060	0.631	24.64	12.64	0.9793
Large	55 wks	96.4	0.0051	0.595	24.45	12.72	0.9895
	60 wks	102.4	0.0066	0.568	24.50	11.93	0.9886
	65 wks	104.8	0.0073	0.562	24.50	12.65	0.9863
	70 wks	111.7	0.0088	0.523	24.62	15.66	0.9814

Using the estimated parameters, the theoretical production curves were fitted and the predicted production to the end of observation compared with the

observed production. For the large flock, observed production in the 70th week was 61.9%; this compared with estimates of 60.3, 62.9, 64.5 and 67.4% using parameters estimated from the full 50 weeks of records, or the first 45, 40 or 35 weeks. Similarly for the small flock the observed production for week 78 was 67.2% compared with estimated production of 67.1 using parameters calculated to week 78 and 66.9, 68.3, 70.6 and 70.2 using parameters calculated to weeks 70, 65, 60 and 55 respectively.

These results indicate that the modified compartmental equation overestimates production towards the latter end of the production cycle if the parameters of the curve are estimated only from the earlier part of the cycle. The reason for this is probably that *b*, the rate of decline in production, does not stay constant but increases with age.

IV. ACKNOWLEDGEMENTS

The project was funded by the EIRDC. Data were supplied by the Victorian Department of Agriculture, Gatton College and Loveday's Attinga Hatchery, Beerburrum Queensland.

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GRAIN LEGUMES FOR BROILER PRODUCTION

H. M. MILLER and J.H.G.HOLMES

I. INTRODUCTION

Grain legumes are a valuable source of feed for all classes of livestock, either as whole seed grown especially as animal feed, as the oil-extracted bi-product of vegetable oil production or as culls from grain legumes used for human food. They are a rich source of protein and energy but also may contain one or more Anti-Nutritional Factors (ANF) such as trypsin and chymotrypsin inhibitors (TI and CTI) , lectins (haemagglutinins), tannins, saponins, alkaloids, goitrogens and phytic acid (Laralde and Martinez, 1989). For human usage, most of these ANF are removed by decortication, soaking or cooking, while when fed to ruminants, several of the protein ANF's are destroyed by fermentation in the rumen (Holmes et al., 1991). When grain legumes are used as feed for poultry or pigs, ANF's may present problems of reduced appetite, reduced FCR's or overt intoxication (Batterham and Egan, 1986).

The problem is made complex by (i) the enormous number of grain legume species and varieties, with about 10,000 varieties recorded for Phaseolus vulgaris alone; (ii) the large number of toxins, with lectins and tannins being large classes of compounds with a wide range of activity; (iii) the interacting nature of the toxins, several of which affect digestion or absorptive capacity of the intestine and (iv) the induceable nature of many ANF's which are produced in a variable amounts as the result of attack by some plant pathogens. Consequently there is not sufficient knowledge available of the ANF content or efficacy of individual varieties, nor are laboratory assays sufficiently well developed to permit prediction of the feeding value of specific batches of seed.

Australian production of mungbeans, pigeon peas, chickpeas and field peas has increased enormously in the last decade, with, for example, a twenty-fold increase in chickpeas in only four years (1984-1988). Although most of these are produced for human consumption, a stockfeed market would provide a secondary market for reject grain and for the excess production which is likely to occur occasionally in a rapidly expanding industry, while providing the livestock industries with alternative sources of protein and energy-rich feeds.

This paper reports the results of three feed evaluation trials with the objectives of (i) determining the maximum safe levels of inclusion in broiler diets and (ii) estimating the levels of some ANF's in the grain and correlating these levels with reductions in chick performance. The fourth feeding trial was an attempt to detoxify grain legumes with enzymes and other feed treatments.

School of Agriculture and Forestry, Univesity of Melbourne, Parkville, Victoria, 3052.

II. MATERIALS AND METHODS

(a) Feeds

The grain legumes studied in feeding experiments were:- Experiment 1, Mung Beans (cv. Satin) and Kabuli Chick Peas (cv. Opal);
Experiment 2, Pigeon Peas (cv. Quantum) and Desi Chick Peas (cv. Tyson);
Experiment 3, Field Peas, (cv. Dun and Wirrega).

Apparent ME values were determined for each grain by total collection of faeces for four days using four replicates of eight three-week old male broiler chicks (Table 1). These data were used to formulate rations with the maximum possible inclusion of grain legume and containing, on an air-dry basis, 12 MJ ME/kg, 11 g/kg lysine, about 200 g/kg crude protein and satisfying the ARC (1975) nutrient requirements in all other respects. The diets were pelleted without steam. Levels of inclusion were :

Experiment 1: Both Mung Beans and Kabuli Chick Peas at 0, 100, 200, 300, 400 and 505 g/kg.

Experiment 2, Desi Chick Peas at 0, 50, 100, 150 and 200 g/kg, and Pigeon Peas at 0, 40, 80, 120 and 160 g/kg.

Experiment 3, both varieties of Field Peas at 0, 100, 200, 300 and 400 g/kg. A commercial broiler diet was used as a second control.

Table 1. Nutrients and ANFs in grain legumes fed to broilers

	ME MJ/k g/DM	CP g/kg	Ether Extract g/kg	TI mg/g	CTI mg/g	Lectin HU/g	Tannin mg/g
Mung Beans	11.4	261	9	3.4	0.2	51.2	9.4
Kabuli Chick Peas	13.8	257	53	3.2	4.3	6.4	6.4
Pigeon Peas	5.5	218	13	1.9	1.8	51.2	4.0
Desi Chick Peas	8.1	172	45	1.6	3.1	12.8	8.5
Field Peas (Dun)	11.4	-	-	-	-	-	-
Field Peas (Wirrega)	12.4	-	-	-	-	-	-
Desi Chick Peas (exp.4)	-	-	-	2.5	4.3	-	-

In Experiment 4, Desi Chick Peas of known high ANF concentration (Table 1) were fed only at 300 kg in eight treatments: 1, Mash, no additives; 2, pelleted, no additives; 3- 8 , mash +: 3, 20% extra methionine; 4, bacterial protease; 5, lipase; 6, fungal protease; 7, cellulase and 8, β glucanase (Alltech, Inc., USA). Each commercially produced enzyme mixture was fed at the recommended level of 0.1% of the diet.

Concentrations of TI and CTI were measured by the methods of Kakade (1969) and lectins by the method of Jaffe (1969).

(b) Birds

Day-old broiler chicks purchased from commercial producers were housed in brooder stacks, three tiers high with four cages per tier and fed commercial starter diet for four days, after which extreme fast and slow growers were culled. Males were used in Experiments 1 and 2 but mixed sexes in Experiments 3 and 4. Feed and water were available ad libitum throughout the experiments. From day 5 to 28, each diet was fed to four replicates of eight birds, randomised across brooder stacks and tiers.

(c) Measurements

Individual chicks were weighed on Days 0, 4 and 28. Replicate groups were weighed each week, feed consumption was recorded daily and weight gain, feed intake and FCR were calculated. Total faecal collection from Day 19 to 23 permitted measurement of ME of all diets fed. On Day 28, in each replicate the two birds which weighed closest to the replicate mean were identified, fasted overnight and slaughtered in a commercial abattoir. Each was dissected and the weights were recorded of the plucked bird, carcass, pancreas, liver and intestine (duodenum to cloaca, not emptied).

III. RESULTS

Experiment 1: ME values of the rations (Table 2) indicate similar values for ME of each grain legume in properly balanced rations to those in the simple two-ingredient mixtures used in ME trials (Table 1). However, ME of the experimental diets declined slightly with increasing inclusion of either bean. Protease inhibitors in the diets (Table 2) are less than expected from the amounts in the ingredients. This indicates that there has been some destruction of ANF's during the compounding and pelleting of the diets.

Intake generally declined with increasing inclusion of each grain although at 100 g/kg and 200 g/kg inclusion of mung beans, intake increased slightly (Table 3). FCR increased with increasing amounts of each legume, the effect being greater with chickpeas. Carcass weight decreased with increasing inclusion of each grain ($P<0.02$) while the weight of the pancreas and its proportion of LW increased. The liver weight remained constant, increasing as a proportion of LW ($P<0.01$). Intestine weight increased ($P<0.002$) with both legumes and as a proportion of LW the increase was significantly greater for Chick Peas at high levels. The deleterious effects of both grains were apparent at the lowest levels fed.

Experiment 2: Intake was higher with Pigeon Pea diets than with Desi Chick Pea diets ($P<0.001$) and declined at higher levels of inclusion (Table 4). FCR became poorer as intake increased with each grain so that LW gain did not differ between diets. ME's for all experimental diets ranged between 13.43 and 13.70 MJ/kg DM with no trends. The weights of pancreas, liver and intestine did not differ between

Table 2. ME and protease inhibitors (actual and predicted from composition of ingredients) of broiler diets containing grain legumes, Experiment 1.

Dietary Treatment (g/kg)	DM (g/kg)	ME MJ/kgDM	TI		CTI	
			Actual	Expected	Actual	Expected
Control	892	14.08	0.4	-	0.00	
Mungbeans						
100	879	13.92	.39	.34	.01	.02
200	879	13.55	.57	.67	.05	.05
300	888	13.60	.68	1.00	.08	.07
400	882	13.40	1.05	1.33	.14	.10
500	891	13.34	1.24	1.68	.20	.12
Chickpeas (Kabuli)						
100	893	13.75	.27	.33	.74	.43
200	888	13.83	.46	.65	1.10	.86
300	887	13.20	.65	.98	1.14	1.29
400	888	13.10	.86	1.30	1.26	1.72
500	883	12.89	1.05	1.63	1.32	2.15

Table 3. Liveweight gains,feed conversion ratio and pancreas weights of male broiler chicks grown on diets containing increasing levels of mung beans or kabuli-chickpeas from 5 to 28 days of age.

Dietary Treatment (g/kg)	Liveweight gain (g/bird/d)	Feed Intake (g/day)	Feed Conversion Ratio	Pancreas Weight (g)
Control	41.1^{a}	70.4^{ab}	1.56^{a}	2.28^{a}
Chickpeas (Kabuli)				
100	43.6^{a}	70.6^{ab}	1.62^{a}	2.68^{a}
200	41.3^{a}	68.6^{a}	1.66^{b}	3.05^{b}
300	41.9^{a}	71.6^{a}	1.71^{b}	3.05^{b}
400	39.0^{b}	68.6^{a}	1.76^{b}	3.24^{b}
500	37.4^{b}	68.1^{a}	1.82^{b}	3.21^{b}
Mung Beans				
100	44.0^{a}	74.4^{b}	1.69^{b}	2.50^{a}
200	45.4^{a}	76.3^{b}	1.68^{b}	2.86^{a}
300	41.3^{a}	71.0^{ab}	1.72^{b}	2.66^{a}
400	41.6^{a}	70.7^{ab}	1.70^{b}	2.95^{a}
500	37.8^{b}	66.9^{a}	1.77^{b}	2.65^{b}
SED	1.607	1.82	0.030	0.170
Significance:				
Bean	NS	**	***	***
Level	***	**	***	***

grains or levels of inclusion. Although chicks grew at least as well on the diets containing Desi Chick Peas and Pigeon Peas as on the control diet, these grains could only be fed at low levels of inclusion due to low ME values. When these values are accepted, the grain diets are as nutritionally valuable as the control.

Experiment 3: ME of the diets declined from 15.47 MJ/kg DM with no Field Peas to 14.83 MJ/kg DM for diets with each variety included at 400 g/kg of the diet ($P<0.01$). These values were considerably above the calculated values for all diets.

The intake of the experimental diet without grain legume was low and LWG was poor (Table 5) but intake, LWG and FCR improved with increasing inclusion of Field Peas, with no difference between varieties. At 400 g/kg inclusion, intake, LWG and FCR were the same as with the commercial diet.

The weight of the liver did not differ between legumes or levels but was proportionately larger in the slower growing chicks receiving low levels of legumes . The weight of the pancreas was less in the birds receiving low levels of legumes but at high levels was slightly below those from the commercial diet (Table 5). The weight of the intestine increased with inclusion of grain legume but this was not associated with production of wet faeces. The reverse occurred, with an increase in faecal DM with increasing inclusion of each variety of Field Peas ($P<0.001$).

Experiment 4: Seven diets were fed as dry meal to avoid the destruction of ANF's and enzymes by the heat produced by pelleting. Pelleting produced the most significant effect; this may have been due to greater intake for physical reasons or breakdown of ANF's (Table 6). The addition of extra methionine had little effect, indicating that the ANF's were not acting by TI and CTI creating a secondary S-amino acid deficiency. The enzymes did not increase performance to the same extent as pelleting. The most effective enzymes were those which are not part of the normal array of digestive enzymes in monogastric animals, i.e. the cellulase and β-glucanase, which augmented the normal digestive processes.

IV. DISCUSSION

The poor performance of Mung Beans and Kabuli Chick Peas conflicts with our results with quail which received up to 450 g/kg Mung Beans and with the data of Johnson and Eason (1990) who fed 200 g/kg Kabuli Chick Peas to broilers without any adverse effect on performance in either case. In the present experiment, the concentration of TI in both grains and of CTI in Kabuli Chick Peas was unusually high. Multiple regression analysis of these data was carried out on performance indices using as predictors TI, CTI, tannins and ME; this revealed significant correlations between ANF's and performance (Table 7). Tannins and TI were so closely correlated that their effects cannot be separated. TI/tannin and CTI were associated with poor growth, as might be expected. However pancreas weight appeared to be unrelated to TI but related to CTI and ME of the diet, liver weight was related to CTI and ME while gut weight was negatively related to ME value. These empirical relationships cannot be easily explained without further physiological studies.

Table 4. Liveweight gains, feed conversion ratios and pancreas weights of male broiler chicks grown on diets containing increasing levels of pigeon peas or desi-chickpeas from 5-28 days of age.

Dietary Treatment (g/kg)	Liveweight gain (g/bird/d)	Feed Intake (g/day)	Feed Conversion Ratio	Pancreas Weight (g)
Control	47.3	71.9[ab]	1.57[a]	2.25
Pigeonpeas				
40	48.2	74.5[a]	1.55[a]	2.71
80	48.3	72.8[ab]	1.50[b]	2.60
120	48.4	75.1[a]	1.55[a]	2.65
160	48.0	72.5[ab]	1.51[ab]	2.44
Chickpeas (Desi)				
50	46.5	71.5[ab]	1.54[a]	2.39
100	48.1	73.5[a]	1.53[ab]	2.58
150	46.7	69.1[b]	1.49[b]	2.59
200	47.1	69.4[b]	1.47[b]	2.80
SED	1.078	1.587	0.028	0.176
Significance:				
Bean	NS	***	NS	NS
Level	NS	**	*	NS

Table 5. Performance and organ weights of broiler chicks fed diets including two varieties of field peas.

Dietary Treatment (g/kg)	FI g/d	LWG g/d	FCR	Carcass Weight	Pancreas g	Liver g	Gut g
Basal	54.6	35.0	1.56	604	1.97	23.5	33.6
Dun							
100	57.8	38.4	1.51	680	2.20	22.5	37.6
200	63.4	41.2	1.54	748	2.46	24.5	38.9
300	67.0	42.9	1.56	756	2.15	23.8	30.8
400	68.4	45.7	1.49	784	2.86	22.3	38.6
Wirrega							
100	61.0	40.0	1.53	710	2.35	21.6	34.2
200	64.1	42.5	1.52	728	2.56	21.6	36.2
300	66.3	43.7	1.52	747	2.32	23.4	38.1
400	65.3	45.0	1.45	766	2.45	23.1	39.7
Commercial	67.0	44.2	1.52	753	2.67	22.4	35.3

Table 6. Performance of chicks fed a diet containing 30% Desi Chickpea treated in an attempt to reduce ANFs.

Diet	Feed Intake g/d	Wt Gain g/d	FCR	Fasted Liveweight
Control Diet Mash	58.4^{ab}	33.7^{d}	1.73^{c}	856^{b}
Pelletted Diet	61.9^{a}	41.0^{a}	1.51^{a}	982^{a}
Mash + 20% Extra Methionine	57.1^{b}	33.9^{d}	1.69^{c}	851^{b}
Mash + Bacterial Protease	55.8^{b}	35.8^{bc}	1.56^{ab}	877^{b}
Mash + Lipase	58.8^{ab}	34.4^{cd}	1.71^{c}	863^{b}
Mash + Fungal Protease	59.3^{ab}	35.2^{cd}	1.68^{c}	862^{b}
Mash + Cellulase	59.5^{ab}	36.1^{bc}	1.65^{bc}	886^{b}
Mash + β glucanase	61.6^{a}	37.3^{b}	1.65^{bc}	906^{b}

In Experiment 2, both legumes supported similar growth rates as the control diet, with slightly enhanced feed efficiencies; the levels of CTI in Chick Peas and of lectins in Pigeon Peas were apparently inadequate to affect the birds. The unexplained low ME values found in a conventional ME trial and confirmed in the feeding trial with complete rations preclude their widespread use in the raw form.

Both varieties of Field Peas, unprocessed, supported rapid growth and FCR below 1.50 at 400 g/kg inclusion in our diets, at least equal to a commercial broiler grower diet. The increase in pancreatic weight was only up to that found with the commercial diet and the enlarged intestine was not associated with reduced performance or moist faeces. Nevertheless, the occurence of these two effects, although at non-deleterious levels, indicates the need for caution even with Field Peas.

The lack of response to exogenous proteases and lipase shows that the lack of endogenous enzymes was not the primary limiting factor in poor performance. Alternatively, they were not added in sufficient amount to counteract the anti-enzymes such as TI, CTI and tannin which would act synergistically to reduce digestion.

Table 7. Multiple correlations of carcass and organ weights with ME and ANF's

Dependent Variable	Prediction and significance
Live Weight	TI/Tannin, $P<0.001$; CTI. $P<0.05$
Carcass	TI/Tannin, $P<0.001$; CTI, $P<0.02$
Pancreas	CTI, $P<0.01$; ME, $P<0.01$
Liver	CTI, $P<0.007$; ME, $P<0.04$
Intestine	ME, $P<0.04$

The discrepancy between our results with Mung Beans and other reports may be due to variation in ANF's as a result of insect attack during growth. ANF's are part of the legume plant's defence against pathogens; at least some ANF's are induceable and concentrations will vary according to the severity of attack. While there is scope for plant geneticists to reduce the capacity to produce ANF's, this is at the expense of disease resistance and will render such a modified grain legume less attractive from the agronomic aspect.

A broad classification of grain legume species is possible on the basis of likely ANF levels but it is not possible to catalogue species and varieties precisely as to their levels of ANF's, even if assay procedures can predict accurately the nutritional and anti-nutritional characteristics of a given batch of grain. Each harvested batch requires separate assessment. These experiments indicate the resources required to achieve this level of precision in measuring the nutritional value of grain legumes. The problem of variation of ANF's between batches of grain legumes for poultry feed may be avoided by treatment of all batches, e.g. by steam-pelleting, or by biological treatment. Assaying each batch for safety requires a greater understanding of the action of ANF's than now available and the availability of assays which measure the deleterious effect of ANF's on the target animal rather than the presence of classes of compounds of widely varying toxicity. This is not possible now and may never be practical except for extremely large batches.

V. ACKNOWLEDGEMENT

These experiments were supported by the Chicken Meat Research Council, Grain Legumes Research Council and the Australian International Development Assistance Bureau.

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FACTORS INFLUENCING OOCYST OUTPUT FROM CHICKENS INFECTED WITH *EIMERIA SP.*

W.I. MUIR and W.L. BRYDEN

Infection of growing chickens with *Eimeria sp* results in a reduction in growth rate, fall in feed intake and impaired feed conversion efficiency. It has been shown that different coccidiosis (Long, 1968) and that oocyst output is reduced in chickens fed diets containing higher levels (63 g/kg) of dietary fibre (Cumming, 1986). As the first step in a programme examining the effect of manipulating the gut environment on the severity of coccidiosis we have examined the resistance of local chicken strains, different sexes and the influence of dietary fibre.

Day-old male chickens of two broiler strains and one layer strain along with females of one broiler strain were purchased from commercial hatcheries for three experiments. Unless otherwise stated chickens were housed in cages, fed a normal starter diet, challenged at 3 weeks of age with a mixed culture (*E. acervulina* and *E. tenella*) of coccidia (2.0×10^4 oocysts) and lesion scored 7 days after challenge. Birds were weighed, excreta collected and oocyst output measured.

A comparison of th three strains in Experiment 1 demonstrated that the layer strain was more susceptible to coccidiosis as there was a significant ($P<0.05$) strain X infection interaction and this strain had a higher oocyst output than the other strains. In the second experiment females had a greater oocyst output than males of the same strain. In both of these experiments and in the final experiment a significant drop ($P<0.05$) in body weight was observed in infected chickens compared to uninfected controls. In Experiment 3 graded levels of dietary fibre (0, 30, 60, 90 g/kg) were added to the control diet and fed to chickens from day-old. Increasing the level of dietary fibre halved oocyst output and resulted in an increase ($P<0.05$) in relative gizzard weight. Despite the difference in oocyst output there was no difference in either duodenal or caecal lesion scores in chickens fed diets containing different levels of fibre.

It is suggested that care should be taken when selecting strain or sex of chicken and formulating diets for studies with coccidia.

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Department of Animal Science, The University of Sydney, Camden, NSW, 2570.

ENZYME REGULATION OF UTERINE FUNCTION AND SHELL QUALITY OF COMMERCIAL LAYING HENS

A.M. OSMAN*, R.J. HUGHES* AND H.A. MORRIS**

Summary

Intra-cellular concentration of Ca^{++} and various enzymes associated with shell formulation were measured in laying hens producing thick and thin shells on diets adequate or low in Ca, in hot and mild conditions. Ca^{++}-ATPase with high specific activity (17μmol Pi/mg protein/h) was detected in uterine mucosa. Specific activity was inhibited by Mg^{++} at concentrations higher than 6mM, but was stimulated by HCO_3^- and by a crude extract of uterine protein, but not by purified calmodulin (Sigma).

Specific activities of enzymes involved in Ca^{++} and HCO_3^- transport in the uterus were significantly affected ($P<0.05$) by temperature. At 30°C, carbonic anhydrase was lower in hens producing thin shells compared with thick shell layers (44 vs 54 U/mg protein), but there was no difference between thick and thin shell producers at 20°C (54 U/mg protein). Increased Ca^{++}-ATPase activity at 30°C approached significance (16.6 vs 14.2μmol Pi/mg protein/h). Stimulation of Ca^{++}-ATPase by uterine protein was greater at 30°C than 20°C (22.4 vs 18.6μmol Pi/mg protein/h), irrespective of shell thickness.

None of the these effects were significant when activities were calculated over the entire uterine mucosa. Hence, total activities of these enzymes were not the underlying reasons for shell thinning in the hot and mild conditions used in this study.

I. INTRODUCTION

Shell strength is dependent on the amounts of calcium (Ca^{++}) and bicarbonate (HCO_3^-) ions secreted by the uterus in the 18-20h needed to form the shell. For example, an adequate shell on a 60g egg will contain at least 2.2g Ca and 3.2g carbonate.

Carbonate for the shell is formed from HCO_3^- ions actively and passively secreted into the luminal fluid surrounding the developing egg. HCO_3^- ions are formed by hydration of carbon dioxide produced by uterine mucosal cells, and not simply drawn from the blood supply as carbon dioxide or HCO_3^-. The enzyme carbonic anhydrase is involved in synthesis and secretion of HCO_3^- by uterine cells. Inhibition of carbonic anhydrase by acetazolamide results in egg shell thinning (Lundholm 1990a).

Nys and de Laage (1984) found that active transport of Ca^{++} across uterine tissue required an active form of Mg^{++}-HCO_3^--ATPase, which in turn required carbonic anhydrase. Hence, carbonic anhydrase is involved in secretion of both Ca^{++} and HCO_3^- ions in the shell gland. Active secretion of both ions uses ATP as the energy source. Castaldo and Maurice (1990) reported that

* Department of Agriculture, Adelaide, SA 5000.
** Institute of Medical and Veterinary Science, Adelaide, SA 5000.

activity of ATPases in uteri of hens producing strong shells was higher than that of hens producing weak shells. On the other hand, Grunder (1983) found that uterine ATPase required Mg^{++} for maximum activity, was inhibited by Ca^{++}, but was not related to shell quality. Similarly, Heald et al. (1968) found nocorrelation between shell strength and carbonic anhydrase activity.

We examined the activities of carbonic anhydrase and Ca^{++}- and Mg^{++}-ATPase in uteri from commercial hens forming thick or thin shells in hot and mild conditions to determine whether any of these enzymes had rate limiting effects on shell formation.

II. METHODS

(a) Birds, Housing and Management

A total of 192 commercial hens were housed in single bird cages in two controlled temperature rooms. Food and water were freely available. Each kg of food provided 11.5MJ AME, 174g protein, 37.6g Ca and 9g total P. Incandescent lights were turned on at 0400h and off at 2000h, daily.

Commencing at 43 weeks of age, room temperatures were set to constant 20 or 30°C. Three weeks later, all birds were categorised according to shell thickness of eggs laid between 0700 and 1600h in a 4-day period.

In the following week in a 4-day period, 20 hens from each of the top and bottom quartiles of shell thickness in each room were killed. Half of the number in each room were taken soon after oviposition between 0900 and 1200h, while the remaining hens were taken 20h after laying an egg, i.e., during the rapid stage of shell formation of the following egg.

(b) Enzyme Assays

The uterus was removed immediately after slaughter, cleared of extraneous fat and connective tissue, then frozen. Mucosa was scraped from the thawed uterus then homogenised in 5mM Tris-PIPES buffer pH 7.4 at 4°C.

Protein and carbonic anhydrase were measured by the methods of Lowry et al. (1951) and Maren (1960), repectively.

Ca^{++}- and Mg^{++}-ATPases were measured in a volume of 0.5ml containing 50mM Tris-PIPES buffer pH 7.4, 2mM Na azide, 2mM Na_2ATP and homogenate containing 2-3mg of protein. The control, in addition contained 1mM EDTA. Different combinations of $CaCl_2$ or $MgCl_2$ in varying concentrations, with and without 10mM $NaHCO_3$ or crude calmodulin extract (Lundholm 1990b), were added to the incubation medium. The reaction was started by addition of Na_2ATP and stopped with 1ml mixture of molybdate/Tween 80 for direct measurement of inorganic P (Daly and Ertingshausen 1972).

III. RESULTS

(a) Presence of Ca^{++}- and Mg^{++}-ATPases in Uterine Mucosa

The separate and combined effects of adding $CaCl_2$ and $MgCl_2$ to the incubation medium are shown in Table 1. Three distinctly different patterns of ATPase activity are evident. Addition of $CaCl_2$ alone clearly stimulated ATPase activity in a manner which resembles an enzyme substrate saturation effect. $MgCl_2$ produced an even greater effect. When both ions were included, the pattern of activity was qualitatively and quantitatively different from those exhibited by either cation used alone. Activity was at an intermediate level up to 6mM, but fell below that of Ca^{++} when concentrations exceeded 6mM. This suggested that $MgCl_2$ activated Ca^{++}-ATPase at concentrations below 6mM and inhibited it above that concentration, whereas $CaCl_2$ inhibited Mg^{++}-ATPase at all concentrations. An alternative explanation is that both enzymes were inhibited by the other cation, the overall activity reflecting the residual activities of both enzymes. A similar study of duodenal mucosa revealed no antagonistic effect, the level of activity in the presence of both cations exceeding the levels reached by either cation used alone.

Table 1. Effects of concentrations of Ca^{++} and Mg^{++} on specific activity of ATPase (μmol Pi/mg protein/h) from uterine mucosa.

Ion	Concentration (in mM)								
	0	1	2	4	5	6	8	10	12
Ca^{++}	2.0	8.2	12.1	12.4	12.6	12.7	13.3	12.5	13.2
Mg^{++}	2.0	18.1	20.8	20.3	20.8	20.3	20.9	19.9	20.7
Both	2.0	12.5	15.1	14.8	14.6	13.8	12.6	11.0	10.2

(b) Effects of Environmental Temperature, Time of Sampling and Shell Thickness on Uterine Enzyme Activity

Specific activities of Ca^{++}- and Mg^{++}-ATPase and carbonic anhydrase in uterine mucosa from hens producing thick or thin shells in hot (30°C) and mild (20°C) conditions are summarised in Table 2.

Specific activities were generally affected by temperature. At 30°C, carbonic anhydrase was significantly lower ($P<0.05$) in hens producing thin shells compared with thick shell layers (44 vs 54 U/mg protein), but there was no difference between thick and thin shell producers at 20°C (54 U/mg protein). Increased Ca^{++}-ATPase activity at 30°C approached significance (16.6 vs 14.2μmol Pi/mg protein/h). Stimulation of Ca^{++}-ATPase by uterine protein described as calmodulin by Lundholm (1990b) was greater at 30°C than 20°C (22.4 vs 18.6μmol Pi/mg protein/h), irrespective of shell thickness.

IV. DISCUSSION

We measured Ca^{++}-ATPase with high specific activity in uterine mucosa (Table 2), much higher than was reported for purified plasma membrane from uterine mucosa (4.8 to 9μmol Pi/mg protein/h) and other tissues (Coty and McConkey 1982). Antagonism between Ca^{++} and Mg^{++} (Table 1) might explain lower ATPase activity measured by Coty and McConkey (1982), who used 5mM $MgCl_2$ in the incubation medium, and reduction in shell strength often associated with excessive dietary intake of Mg.

Table 2. Specific activities of Ca^{++}- and Mg^{++}-ATPase (μmol Pi/mg protein/h) and carbonic anhydrase (units/mg protein) in uterine mucosa from hens producing thick or thin shells in hot (30°C) and mild (20°C) conditions. (Figures in parenthesis are standard deviations of means of five hens).

Temp. (°C)	Time (h)	Ca^{++}-ATPase (5mM Ca^{++})		Mg^{++}-ATPase (5mM Mg^{++})		Carbonic anhydrase	
		Thick	Thin	Thick	Thin	Thick	Thin
30	0	13.4	14.8	22.4	24.9	52.3	42.9
		(2.9)	(1.8)	(3.1)	(4.8)	(13.7)	(7.3)
30	20	17.0	14.0	24.9	20.9	56.6	44.3
		(1.1)	(3.1)	(2.5)	(3.5)	(6.1)	(9.3)
20	0	12.7	14.0	18.6	23.2	56.7	54.0
		(1.1)	(3.6)	(1.2)	(7.4)	(10.9)	(6.4)
20	20	14.1	12.8	19.9	19.3	51.2	55.6
		(0.9)	(1.9)	(1.5)	(5.1)	(9.3)	(8.2)

In our study, Ca^{++}-ATPase activity was stimulated by HCO_3^- and also by a crude extract of calmodulin prepared as described by Ludholm (1990b), but not by calmodulin purified from bovine brain (Sigma). The stimulation of Ca^{++}-ATPase by a crude protein extract is of particular interest. It indicates that uterine Ca^{++}-ATPase has potential for considerably greater activity than previously considered, which may be important during periods of sub-optimal intake of dietary Ca.

We expected to see large changes in activities of these enzymes during active shell formation (20h post-oviposition) compared with activity just after oviposition when the gland is at rest. However, in general, activities were not significantly higher during calcification, presumably because these were measured under identical conditions which might differ from those in the intracellular environment.

It is clear from our observations that, while specific activities of some enzymes were affected by environmental temperature and differed between thick and thin shell producers, total activity of these enzymes calculated over the entire uterine mucosa did not differ significantly. This does not in any way detract from the importance of the role of these enzymes in the transport of Ca^{++} and HCO_3^- ions for shell calcification. For example, changes in

concentrations of ionised Ca^{++} in the vicinity of the enzyme would alter its activity, leading to differences in amounts of Ca^{++} transported.

These results also highlight the fact that activities of enzymes involved in transport of Ca^{++} and HCO_3^- across uterine mucosa can be decreased after oviposition and increased upon arrival of another egg. The mechanism behind this regulation is not yet known, but we can say from our findings that changes in intra-cellular concentration of ionised Ca^{++} may regulate the activity of Ca^{++}-ATPase.

Further studies are underway on factors which regulate the intra-cellular concentration of Ca^{++} in uteri of commercial hens producing thick and thin shells on diets adequate or low in Ca, in hot and mild conditions.

V. ACKNOWLEDGEMENTS

We thank the Egg Industry Research and Development Council for financial support, and Prof.B.P. Setchell, Chairman, Department of Animal Sciences, University of Adelaide, for use of laboratory facilities.

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RICKETS AND TIBIAL DYSCHONDROPLASIA IN AUSTRALIAN BROILER CHICKENS

G. PARKINSON, S. VAIANO and J. AZUOLAS

Summary

Ten separate broiler flocks across the three major strains in Victoria have been studied to define the incidence and prevalence of osteochondrodystrophy (OCD). Longitudinal samples from day old to processing were taken by randomly collecting 30 birds from each flock. The birds were euthanised, then the tibial ash content and the incidence of OCD determined. This study has demonstrated: 1) very rapid bone growth during the first week post hatch, 2) Rickets-like lesions seen at 2 weeks of age develop into tibial dyschondroplasia lesions seen at 3-4 weeks of age, and 3) the pathology patterns demonstrated two different models. The first being flocks with a high level of OCD at 2 weeks of age which progressively declined, and the second being flocks in which the incidence of lesions increased between 2-4 weeks of age then declined. These models may represent different metabolic/physiological problems and is the basis for further research in the area.

I. INTRODUCTION

Osteochondrodystrophies (OCD) such as rickets and tibial dyschondroplasia (TD) have been recognised as major underlying cause(s) of lameness and leg deformation in the broiler industry for many years (Leach and Nesheim, 1965; Hemsley, 1970; Wise, 1975; Poulos et al, 1978; Riddell, 1981 and Reece et al, 1985).

Despite the research endeavours of the last twenty-five years, a precise understanding of the full economic consequences of OCD syndromes to the industry remains to be described. The inability of researchers and the industry to accurately quantify the impact of OCD on broiler performance is perhaps, in part, due to the unpredictable nature of the condition(s), varying from acute outbreaks of flock morbidity to subclinical conditions frequently unrecognised by the industry (Hemsley, 1970 and Prasad et al, 1972).

In broad terms, culls and mortality attributable to leg weakness or deformation has been estimated at 1-2 percent in Canada, (Riddell and Springer, 1985) and the United Kingdom (Siller, 1970) and a similar incidence is believed to occur in Australia (Reece et al, 1985). Carcass condemnations at processing because of leg deformation or fracture is estimated at a further 0.6-0.7 percent (Riddell and Springer, 1985) with the total loss due to leg weakness estimated at 1.7 percent. Applying these estimates in Australia, losses would equate to approximately 2.8 million birds as culls or mortality and a further 2.0 million carcasses downgraded at processing.

Several studies in Australia have attempted to partition the role that OCD plays in the development of clinical leg weakness or deformation, Hemsley, 1970 reported that OCD lesions were associated with 0.02-0.45 percent of mortality,

Victorian Institute of Animal Science, Attwood, Victoria, 3049.

whilst Reece et al, 1985 indicated that OCD was associated with between 0.5-5.0 percent of mortality in 153 of 622 Australian broiler flocks. Many authors, both in Australia and overseas, indicate, however, that flock morbidity associated with OCD can reach levels of up to 40 percent (Prasad et al, 1971; Reece et al, 1985 and Parkinson and Scott, 1989). The incidence of broiler chicks with OCD but without lameness has been estimated at 7 percent (Hemsley, 1970) and many flocks are believed to have a subclinical form of the condition (Hemsley, 1970; Poulos et al, 1978; Burton et al, 1980 and Riddell, 1981).

A comprehensive survey of tibial dyschrondroplasia or focal osteochrondrodystrophy was undertaken in Australia by Burton et al, 1980, and these authors indicated that the incidence of TD at the processing plant varied from 14 to 35 percent depending on the strain. TD was also observed in between 14-42 percent of downgraded carcases, and leg deformation or fracture was highly correlated with the severity of the TD lesion (Burton et al, 1980). The prevalence of TD in commercial broilers and the correlation between lesion score and leg deformation, implies that the condition is likely to be a major factor contributing to the numbers of birds debilitated or with bone fractures.

Rickets, an analogous form of osteochondrodystrophy, is commonly diagnosed in 1-2 week old chicks (Riddell, 1981), has a flock morbidity of 20-100 percent (Riddell, 1981) and is characterised by soft bones, abnormal gait and leg abnormalities. Subclinical forms of the disease are common in modern broilers (Wise, 1975) and the condition is also likely to be unrecognised by the industry, unless a high incidence of flock immobility is manifested.

The relative importance of these two OCD syndromes in the aetiology of leg weakness is unclear in the literature. Given the similarity of the two forms of osteochondrodystrophy, the possibility remains that the conditions may occur concurrently, or alternatively, that a rachitic condition may predispose to the tibial dyschondroplasia (Long et al, 1984 and Riddell and Pass, 1987).

The variation in incidence of OCD described by many authors is believed to be determined primarily by the genotype of the stock (Leach and Nesheim, 1965 and Burton et al, 1980) and may be correlated with factors such as growth rate (Siller et al, 1970; Poulos et al, 1978) and relative skeletal growth. Research on dietary factors indicates however that there are several important nutritional components which interact with genotype to substantially alter the incidence of OCD (Leach and Nesheim, 1965; Riddell and Pass, 1987; Edwards and Veltman, 1983 and Edwards, 1987).

The incidence of subclinical OCD (rickets and tibial dyschondroplasia) described by Hemsley, 1970; Wise, 1975 and Riddell, 1981, implies that the full economic cost of these bone abnormalities is likely to be under rather than overestimated by industry.

The main concerns of the Australian broiler industry appear to be the refinement of nutritional or management practices to moderate clinical episodes associated with a high incidence of flock immobility. In the longer term, however, the industry should consider the association between growth rate and osteochondrodystrophy and recognise that bone metabolism problems represent a serious constraint to further economic development unless effective control strategies can be developed. The effective control of OCD in Australian commercial broilers is likely to reduce culls and mortality by 0.2-1.0 percent and reduce carcass downgrading by up to 0.3 percent.

II. METHODS

Over the last two years an extensive epidemiological study has been undertaken to define the incidence and prevalence of OCD using the three major broiler strains available in Victoria. These studies have been designed to investigate (1) the pathological nature of the bone mineralisation defects in broiler chickens, (2) define the underlying mechanisms responsible for the genetic predisposition, and (3) identify nutritional or management factors which accentuate the bone defects and the development of clinical leg weakness.

To date, ten separate broiler flocks have been studied using longitudinal sampling between day old and processing, across the three major strains (A, B, C). In each flock, thirty birds were randomly collected from commercial sheds at weekly intervals, and transported to the laboratory for post mortem.

The birds were destroyed by cervical dislocation and the left tibia was removed from each bird. The muscle and connective tissue removed and the bone then soaked in diethyl ether for 1 hour to remove fat. The bones were then washed again with diethyl ether, air dried for 2-3 hours and then oven dried for 12 hours at 50°C to determine the dry weight. The tibia was then ashed in a muffle furnace at 600°C for up to 8 hours and a bone ash measurement calculated as a proportion of the dry weight.

In birds aged 7 days or older, the right tibia was removed and examined by a longitudal section of the proximal tibial metaphysis. The cartilagenous growth plate was assessed for the presence of rickets or TD and an incidence calculated as a percentage of the 30 bird sample. For five of the ten Flocks, 100 bones were collected at processing and an incidence of OCD calculated from this sample.

III. RESULTS

In each of the strains studied to date, the initial osteochondrodystrophic lesions have been observed between 7-14 days of age (Table 1) and are characterised by a uniformly thickened cartilagenous growth plate in the proximal tibial metaphysis, similar in gross appearance to rickets (Lacey and Huffer, 1982). The growth plate thickening precedes a second phase between 21-28 days of age where the hypertrophic zone in the cartilagenous metaphysis lengthens and assumes the characteristics defined as tibial dyschondroplasia (Riddell, 1981 and Siller, 1970).

The variation in incidence observed in the strain B birds (B1-5, B6 and B7) indicates that factors other than inherent bone metabolism can substantially alter the pattern of pathology. The Flock B6 has a significantly higher incidence of lesions at 14 days of age, and the incidence persists at a higher level throughout the life of the flock (Table 1).

In the two flocks in which the proximal tibial metaphysis was examined at 7 days (B5, B7) (Table 1), cartilagenous lesions were recorded which appeared correlated with the incidence at 14 days.

The strain C birds appear to have a lower predisposition to the initial OCD lesions and the incidence appears less persistent than in the other two strains (Table 1).

All nine flocks studied are considered subclinical by the industry, despite the variations in OCD recorded at processing of between 1-30 percent.

Studies on tibial dry weights undertaken on the samples described (Table 2) indicates that tibial growth follows an exponential pattern. Dry weights increase some five fold in the first seven days, 2-3 fold in the second week, and between 1.7-2.0 fold each subsequent week. Percentage ash measurements undertaken on these bones indicates that mineral content increases some 30 percent in the first week, 8 percent in the second week and remains stable or declines as a proportion of tibial weight thereafter (Table 2).

Table 1. Incidence of Metaphyseal cartilagenous lesions (%) in three strains (A,B,C) and ten flocks between 7 days of age and processing.

Strain	A	B	B	B	B	B	B	B	C	C
Flock No	1	1	2	3	4	5	6	7	1	2
Age (days)										
7-8						6		7		
14-15	50	12	13	16	13	19	50	7	14	5
21	50	24	23				30		6	0
28	30	34	20	23	20		50		6	0
35			14							
42	15*	5*	5*				30			
49							30*	1*		

n = 30 birds.
* 100 bones collected at processing.

Table 2. Tibial dry weights (g), ash (%) and ash weights (g) in the three strains (A, B and C), between day old and 21 days (n ≈ 80 bones).

Age (days)	Tibial Dry Weight (g)	Tibial Ash %	Ash Weight (g)
1-2	0.08 ± 0.01	29.1 ± 1.7	0.024
7-8	0.41 ± 0.06	38.4 ± 1.9	0.161
14-15	1.04 ± 0.16	41.3 ± 2.3	0.429
21	2.08 ± 0.29	41.1 ± 1.1	0.855

* Single tibia used for these measurements.

IV. DISCUSSION

The studies on the tibial dry weight changes and the bone ash measurements undertaken on the broiler chicks in this study provide evidence of the rapid bone growth and maturation of the embryonic bone in the first week post hatching, and are similar to the finding of Wise, 1970. The rate of bone maturation will create a large demand for minerals such as calcium and phosphorus to be partitioned to the developing bone (Jubb and Kennedy, 1970). These minerals must be obtained either from the resorption of the yolk sac in the first few days of life or from the diet. The dietary mineral content and the efficiency of absorption are critical factors in ensuring that sufficient calcium and phosphorus is available to be partitioned to the rapidly growing bone. The profound changes

in bone ash content appear to coincide with the development of the initial OCD lesions, suggesting that the initial bone lesions may develop earlier in the chick's life than previously believed between day old and seven days.

The progressive changes in the nature of the metaphyseal cartilagenous lesions with age, is similar to the pathology patterns described by Long et al, 1984; Riddell and Pass, 1987. Long et al, 1984 produced an experimental calcium deficiency in broiler chicks and described a transition in the pathology between a rickets-like lesion at 2 weeks of age to tibial dyschondroplasia lesions in 3-4 week old chicks. Furthermore, Long et al, 1984 suggested that TD may be a manifestation of an exaggerated, though ineffective, attempt to repair endochondral ossification.

The patterns described in flocks B1-5 demonstrates a relatively consistent trend with a moderate incidence of lesions at 14 days followed by an upsurge in incidence in the grower phase and then declining between 4 weeks and processing. The upsurge in incidence of TD in 3-4 week old birds is similar to the observations of Wise and Jennings, 1972 who indicated that the incidence and severity of TD is higher at 4 weeks than 2 weeks of age. The declining incidence of metaphyseal lesions between 4 weeks and processing observed in the majority of flocks may be accounted for by the resolution of cartilagenous lesions, as described by Poulos et al, 1978 in birds as young as 48 days.

The pattern of OCD recorded in the B6 flock deviates substantially from the general pattern observed in the strain B flocks with a high incidence of lesions at 14 days which persist until processing. The transformation of the pathology pattern may provide an insight into the changes required to transform flocks from subclinical OCD to a clinical state.

The pathology patterns consolidated from these studies form two models. The first being flocks with a high initial incidence of OCD at 2 weeks which progressively declines, and the second being flocks in which the incidence of lesions increases between 2-4 weeks of age and declines thereafter. The two models proposed may represent different metabolic/physiological problems, with a different nutritional basis. It seems likely, however, that the clinical outbreaks of debilitation and leg weakness of concern to the industry, will be triggered by the development of severe early lesions between days 1-14 which persist until processing.

Assuming that the initial bone lesions are rachitic in nature, a clear understanding of the roles of vitamin D, calcium and phosphorus metabolism in the development of the bone pathology is dependent on accurately modelling the dynamic state of the bone metabolism, as the embryo is transformed to a chick in the first week to ten days.

Further studies on the aetiology of OCD lesions in the broiler chicken will focus on the development of calcium and vitamin D metabolism in the broiler chick between hatch and ten days of age and include consideration of rates of bone growth, changes in bone ash percentage, plus more detailed histopathological studies of the changes in the cartilagenous metaphysis in birds between 1-10 days of age.

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PERFORMANCE OF BROILER CHICKS SUBJECTED TO EARLY AGE FEED RESTRICTION

I. PLAVNIK and S. HURWITZ

Summary

Growth, feed efficiency, abdominal fat and breast meat were evaluated in broiler chicks which had been subjected to different feed restriction regimes at the age of 3 to 11 days. During the restriction period of 6 to 14 days, the birds received an energy allowance calculated to maintain from 0% to 75% of the normal growth. Following restriction, a high rate of weight gain up to marketing age overcame growth retardation due to restriction, except for birds which had been subjected to the most severe restriction regimes. In cases of the mild restriction regimes, body weights even exceeded those of the ad libitum - fed controls. Feed efficiency was significantly improved and abdominal fat was reduced, regardless of the severity of restriction. Compensatory growth and response of feed efficiency to feed restriction are considerably less pronounced in females than in males. The practical choice of a proper restriction regime involves economic considerations. A general optimisation computer model to aid in the selection of an economically optimal growth trajectory, effected by feed restriction regimen has been constructed.

I. INTRODUCTION

Feed efficiency is the most important economic factor in broiler operations. One of the means of improving feed efficiency is to change the convex to a more concave growth curve as illustrated in Figure 1. This change results in a reduction in the total energy "wasted" on satisfying the maintenance needs. Growth-restriction (by feed restriction) at an early age and the ensuing compensatory growth may provide the desired concave growth curve. Previous research showed that due to compensatory growth (Wilson and Osborn, 1960; Auckland and Morris, 1971) the loss in weight gain due to early feed restriction was regained when marketing age was reached (Plavnik and Hurwitz, 1985). Body weights of chicks following the restriction could even exceed those of the ad libitum-fed (Plavnik and Hurwitz, 1990), with restriction regimes less severe than those originally evaluated (Plavnik and Hurwitz, 1985). Early age feed restriction resulted in an improved feed efficiency in broiler chickens (Plavnik and Hurwitz, 1985, 1988a; McMurtry et al., 1988) as well as in growing turkeys (Plavnik and Hurwitz, 1988b).

The efficacy of compensatory growth in regaining the growth lost by feed restriction up to marketing age, depends on the severity and duration of the restriction period. Within practical limits, a long or severe restriction period results in an increased concavity of the growth curve and improved feed efficiency but body weight may not fully recover at marketing age. It was therefore the purpose

Institute of Animal Science, Agricultural Research Organisation, the Volcani Center, Bet Dagan Israel

of our studies to determine the relationships between performance on the one hand, the severity and duration of restriction, on the other, so that the economically optimal restriction regimen could be selected.

In addition to improved feed efficiency, early age feed restriction also resulted in improved carcass quality in terms of reduced fat and increased muscle yield (Auckland and Morris, 1971; Plavnik and Hurwitz, 1985, 1988a; McMurtry et al., 1988).

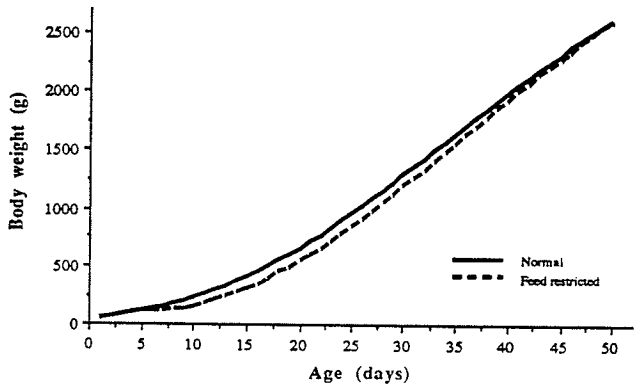

Figure 1. Increased concavity of the growth curve in response to feed restriction in male broiler chicks.

II. CALCULATION OF THE RESTRICTION ALLOWANCE

During the growth restriction period, the birds were given daily allowances of energy (EI,kcal/d), calculated to maintain body weight and support a rate of growth, varied according to treatment, using an equation modified from Hurwitz et al., (1980):

$$EI = M.W^{2/3} + D.G$$

Where W (g) is the body weight at the beginning of the restriction period, M ($Kcal/g^{2/3}$) is the coefficient of maintenance requirement, D (kcal/g) is the coefficient of growth requirements, and G (g/day) is the rate of growth. Forsum et al (1981) and Plavnik and Hurwitz (1985; 1988a) showed that the maintenance energy requirements (M) decreased considerably during feed restriction. On the basis of the results of Plavnik and Hurwitz, (1985) an approximate dependence of maintenance requirement (M) on the degree of restriction between 2.0 and 1.0 $kcal/g^{2/3}$, proportional to the degree of restriction was estimated. The value for D varied with the proportion of carcass fat gained, also in proportion to the degree of restriction.

In all experiments, the diets were designed to satisfy the recommendations of the National Research Council (1984) and were composed primarily of corn, sorghum grain and soybean oil meal.

III. DURATION OF FEED RESTRICTION

The Results of Plavnik and Hurwitz (1985) showed that the weight gain lost during periods of feed restriction lasting 2 to 4 weeks could not be regained during the period of refeeding of up to 8 weeks of age. The results of shorter periods of restriction are summarised in Table 1. In males, feed restriction for 6 days hardly influenced body weight at 8 weeks of age. The weight decreased progressively in the 10- and 14-days restriction groups. In females, 8-week body weight was reduced in all restriction treatments.

Overall feed efficiency was improved by feed restriction compared with the control in both sexes, but there were no significant differences in feed efficiency among the restricted treatments. Abdominal fat at marketing age decreased progressively in the treatment groups with the duration of the early age feed restriction.

Table 1. Effect of feed restriction on performance of male and female broiler chickens

	Control	Restriction period, days			SE
		6	10	14	
Males					
Body weight,g 1-w	114	114	114	114	
Body weight,g 8-w	2275.^{a}	2257.^{a}	2095.^{ab}	1960.^{b}	47
Feed intake,g 1-8w	4611.^{a}	4225.^{ab}	3968.^{bc}	3516.^{c}	98
Gain/feed ratio	.468^{b}	.508^{ab}	.500^{ab}	.525^{a}	.01
Abdom.fat,% of BW	2.28^{a}	1.87^{b}	1.64^{b}	1.54^{b}	.08
Females					
Body weight,g 1-w	111	111	111	111	
Body weight,g 8-w	1916.^{a}	1832.^{b}	1703.^{c}	1505.^{d}	21
Feed intake,g 1-8w	4493.^{a}	4031.^{b}	3792.^{c}	3404.^{d}	35
Gain/feed ratio	.402^{c}	.427^{a}	.420^{ab}	.410^{bc}	.005
Abdom.fat,% of BW	2.62^{a}	1.88^{b}	1.81^{b}	1.62^{b}	.16

^{a,b,c}Means within measurements with different superscripts are significantly different ($P<0.05$).

The trends apparent in the growth curves, suggests that complete compensation might have occurred had the experiments been continued to a later age. In similar studies with turkeys, which are kept for longer growing periods, full body weight compensation was noted by the age of 20 weeks, following a 2-week restriction periods (Plavnik and Hurwitz, 1988b).

IV. AGE OF FEED RESTRICTIONS

According to theory, it is advantageous to apply feed restriction at the earliest age possible. However, it appeared likely that the compensatory growth response would vary with age. A summary of the results of feed restriction of male chicks of various ages, is given in Table 2. Overall weight gain was not affected by early feed restriction, regardless of age (3, 5, or 7 days). Feed intake was significantly reduced by feed restriction, without any difference due to age at restriction. Similarly, feed efficiency was improved significantly by feed restriction, again with no age effect.

The size of the abdominal fat pad was reduced by restriction, with no differences among groups restricted at different ages.

V. SEVERITY OF RESTRICTION

A wide range of feed restriction regimens has been investigated in male and female broilers in an effort to achieve greater flexibility in the use of feed restriction technique. The experiments were started with 7-day-old male or 6-day-old female chickens and included an ad libitum fed control and 3 feed-restriction treatments, designed to support 75% (Treatment A), 50% (Treatment B), 25% (Treatment C) of the control growth. Duration of feed restriction was 7 d in males and 5 d in females.

Table 2. Effect of age of feed restriction on performance of growing male chicks.

	Control	Age at start of restriction			SE
		3 d	5 d	7 d	
Trial 1					
Body weight, 3 d,g	70	70	70	70	1
Weight gain,g/56 d	2375.[a]	2370.[a]	2406.[a]	2357.[a]	21
Feed intake,g/56 d	6442.[a]	5897.[b]	6058.[b]	5862.[b]	138
Feed efficiency,g/g	.369[b]	.402[a]	.397[a]	.403[a]	.009
Abdom.fat% of BW	1.82[a]	1.28[b]	1.16[b]	1.12[b]	.22
		7 d	9 d	11 d	
Trial 2					
Body weight, 7 d,g	140	140	140	140	1
Weight gain,g/49 d	2272.[a]	2220.[a]	2277.[a]	2238.[a]	21
Feed intake,g/49 d	5305.[a]	4909.[b]	5013.[b]	4912.[b]	102
Feed efficiency,g/g	.428[b]	.452[a]	.454[a]	.456[a]	.007
Abdom.fat% of BW	2.22[a]	1.46[b]	1.60[b]	1.38[b]	.22

[a-c]Means within variables followed by different superscripts are significantly different ($P<0.05$).

During the restricted period, daily weight gain of the restricted males slightly differed from those planned (Table 3). Compensatory growth following growth retardation resulted in minimisation of the difference in body weight between the restricted and the control birds at 56 days of age (Table 3). Body weights were reduced by the most severe restriction regimes but tended to be higher than controls in treatments A and B. Overall feed intake was lower and feed efficiency was superior in all restricted treatments than in the controls. Abdominal fat at marketing age was reduced by early age feed restriction, with inconsistent differences due to the degree of restriction.

Weight gain of the females during the restriction period was slightly lower than planned. Compensatory growth during the refeeding period resulted in a narrowing of the differences between the control and the restricted birds (Table 3). However, body weights of restriction treatments B and C remained significantly lower than those of the controls. Feed efficiency was improved by early feed restriction during the refeeding period, but there were no significant differences among treatments in overall feed efficiency.

Regardless of sex, early age feed restriction of different severity resulted in a reduction of abdominal fat at marketing age of 7-8 weeks (Table 3).

VI. EFFECT OF FEED PELLETING

Since pellets are used in modern broiler industry and since feed intake is markedly affected by diet form, it appeared of importance to compare the responses of chicks fed pellets or mash, to feed restriction. It was also theorised that if voluntary feed consumption limited the compensatory response during refeeding, the feeding of pellets would augment the response. The results (Table 4) demonstrate that weight gain was improved by feeding pellets, through increased feed intake, whereas feed efficiency remained unaffected. As expected, body weight was depressed by feed restriction at the end of the restriction period. However, at the age of 51 days, accelerated growth eliminated any gap in body weight. Overall feed efficiency was improved by early feed restriction. Abdominal fat content was reduced by feed restriction without any changes due to pellet feeding. There were no significant diet form x feed restriction interactions in any of the variables measured, indicating that stimulation of feed intake does not modify the response to feed restriction.

The lack of interaction between feed restriction and pelleting also suggests that ad libitum energy consumption is not limiting during refeeding. This conclusion is further supported by the fact that abdominal fat, which is reduced at the marketing age by early feed restriction, also does not change by feed pelleting.

Table 3. The performance of broiler female and male chicks following early age feed restriction of different severity.

	Control	Restriction treatment			SE
		A	B	C	
Males					
Body weight,g 14 d	385.^{a}	285.^{b}	246.^{c}	203.^{d}	3.1
Body weight,g 56 d	2974.^{a}	3046.^{a}	2983.^{a}	2879.^{b}	27.7
Feed intake,g 7-56 d	5787.^{a}	5747.^{a}	5690.^{a}	5452.^{b}	44.1
Feed efficiency, g/g 7-56 d	.486^{b}	.503^{a}	.497^{ab}	.499^{a}	.004
Abdominal fat, %	2.56^{a}	1.98^{b}	2.06^{b}	1.93^{bc}	
Breast meal, %	14.8^{ab}	15.2^{a}	14.4^{ab}	13.9^{bc}	
Females					
Body weight,g 11 d	247.^{a}	199.^{b}	174.^{c}	150.^{d}	3.1
Body weight,g 50 d	2025.^{a}	2044.^{a}	1945.^{b}	1956.^{b}	21.0
Feed intake,g 6-50 d	4023.^{a}	4024.^{a}	3814.^{b}	3846.^{b}	41.2
Feed efficiency, g/g 6-50 d	.472	.477	.481	.477	.003
Abdominal fat, %	3.23^{a}	2.62^{b}	2.63^{b}	2.73^{b}	.12

$^{a-c}$Means within variables followed by different superscripts are significantly different ($P<0.05$).

Table 4. Response of feed-restricted broilers to dietary form during refeeding

Diet form	Mash		Pellets		SE
Restriction	No	Yes	No	Yes	
Body weight,g,13 d	300	190	319	190	5.3
Body weight,g,51 d	2242	2233	2483	2415	35.1
Feed intake,g,7-51 d	4827	4464	5337	4986	86.2
Feed efficiency, 7-51 d	.432	.466	.434	.463	.004
Abdominal fat,% of BW	2.12	1.64	2.27	1.92	.11

VII. CONCLUSIONS

At marketing ages of less than 49 days, the practical benefit of using feed restriction regimen would depend on the cost of delaying marketing due to a reduced body weight, against the advantage of improved feed efficiency. The use of the milder restriction regimens could allow for a quicker recovery of body weight, but would minimise the advantage of feed efficiency and probably also the effect on carcass fat. The practical decision must thus involve complex considerations.

A general optimisation computer model of non-linear optimisation, to aid in the selection of an optimal growth trajectory effected by feed restriction regimen, was proposed by Talpaz and Hurwitz (1988). The model is based on biological

simulation of the response of broilers to feed restriction under different dietary and environmental conditions and contains economic calculations.

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SELECTION FOR FEED EFFICIENCY IN CHICKENS : EFFECTS OF FOOD INTAKE MEASUREMENT INTERVAL, FEATHERING RATE AND INITIAL SELECTION FOR BODY WEIGHT

R.A.E. PYM

Summary

Genetic and phenotypic parameters for growth, food intake and feed efficiency measured over different growth intervals were estimated by sib analysis in six hatches of progeny reared either in cages or on deep litter and produced from matings within a crossbred commercial broiler population. On the basis of parameters estimated for traits measured at or over the 21 to 42d or 42 to 52d intervals, selection to improve the efficiency of broiler production would appear better to be based on traits measured over the earlier rather than the latter interval.

Rapid feathering birds were more efficient to 42d of age than their slow feathering counterparts but subsequent to this, the superiority was lost. Analysis of data from the cage-reared birds indicated that it should be possible to cull about two thirds of the males and half of the females on the basis of low 21d liveweight without significant effect upon response to selection for economic efficiency.

I. INTRODUCTION

In recognition of the importance of feed efficiency as a major determinant of profitability in broiler production, most commercial breeders around the world now select at least some of their lines for improved food utilisation efficiency. Various methods have been employed and breeders have had to make decisions as to how to combine the relevant traits to optimise economic response with respect to body weight and food intake, over what interval food consumption should be measured and, whether individual food consumption should be measured on all birds or on a pre-selected group.

There is little published information available to breeders to assist them in making these decisions and, as a consequence, there appears to have been a wide range of approaches adopted. The aim of the present study was to provide information on genetic and phenotypic parameters for the relevant traits measured over different intervals, on the effect of feathering rate on feed efficiency and on the effect of pre-selection on initial body weight on predicted response to selection. The data were obtained from the base population of a selection experiment currently underway, in which lines of birds are selected on indexes incorporating body weight and food intake measured over a range of intervals.

II. MATERIALS AND METHODS

Parents of the base population were commercial four-way crossbred feather-

Department of Farm Animal Medicine and Production, The University of Queensland, St. Lucia, 4072, Qld, Australia

sexable broiler chickens. At 30 weeks of age, these birds were mated together to produce the first of six hatches of the base population. Each hatch of the base population was produced by mating 27 cockerels each with four hens with the same parental stock used, apart from mortalities, to produce the six hatches. The sex-linked genes for rapid (*k*) and slow (*K*) feathering were segregating in the base population birds with approximately half of each sex exhibiting each of the two feathering types.

In each hatch, full pedigree information was recorded on each chick at hatching and the chicks were wingbanded and vaccinated against Marek's disease. Chicks produced were subjected to different rearing regimens depending upon the line to which they were allocated. Chicks from hatches 1, 2 and 5 were brooded in cages whilst those in hatches 3, 4 and 6 were brooded on deep litter. All chicks were given a commercial broiler starter diet containing 12.5 MJ of ME and 220g CP/kg from hatching and a broiler finisher diet containing 12.8 MJ of ME and 210g CP/kg from 3 weeks.

Chicks in hatches 1, 2 and 5 were weighed and placed in single cages with individual feeders at three weeks of age and body weight and food consumption were measured individually at 42 and 52 days of age. Chicks in hatches 3, 4 and 6 were weighed at 21 and 42 days of age and then transferred from deep litter to single cages with measurement of body weight and food intake to 52 days of age. There were approximately 300 birds per hatch and all birds were scored for feathering type at 10 days of age.

Sib analysis estimates for the heritability of and genetic and phenotypic correlations between the various traits were obtained using Harvey's (1977) Mixed Model Least Squares and Maximum Likelihood Computer Program. The model used for the analyses was:

$$Y_{ijk} = u + s_i + d_{ij} + e_{ijk}.$$

Where Y_{ijk} is the trait value for the kth individual from the jth dam (d) mated to the ith sire (s) adjusted for sex and feathering type, u is the subclass mean, s_i is the random effect of the ith sire, d_{ij} is the random effect of the jth dam mated to the ith sire, and e_{ijk} is the random residual effect of the kth bird of the ijth family. Analyses were performed within hatch but estimates were pooled across hatches within rearing regimens. Heritability and genetic correlation estimates were obtained from sire components, from dam components and from the sum of sire and dam components. The latter estimates are presented here.

Selection indexes aimed at optimising economic response with respect to body weight and food intake to 6 weeks of age were calculated from the data on cage-reared birds in hatches 1, 2 and 5. The effect of pre-selection based on 21-day weight upon the capture of birds with the highest indexes was determined from the hatch 5 data.

III. RESULTS

Heritability and genetic and phenotypic correlation estimates between; 21, 42 and 52d liveweight (3WW, 6WW and 8WW respectively); 21 to 42d weight gain (WG3-6), food consumption (FC3-6) and FCR (FCR3-6) and; 42 to 52d weight gain (WG6-8), food consumption (FC6-8) and FCR (FCR6-8); calculated in the two rearing environments, are given in Table 1.

Table 1. Pooled sire plus dam component estimates of heritabilities and genetic and phenotypic correlations between the various growth, intake and feed efficiency traits determined in birds in the base population reared either in cages (c) or on deep litter (dl). Genetic correlations above diagonal, phenotypic correlations below diagonal. Standard errors in parenthesis.

Traits	Rearing	3WW	6WW	8WW	3-6WG	3-6FC	3-6FCR	6-8WG	6-8FC	6-8FCR
3WW	c	.33(.15)	.86(.10)	.71(.22)	.74(.18)	.78(.15)	.01(.37)	.04(.50)	.26(.36)	.29(.60)
	dl	.55(.17)	.95(.07)	.95(.14)				-.34(.78)	.53(.33)	.74(.25)
6WW	c	.74	.52(.15)	.86(.10)	.98(.01)	.95(.03)	-.30(.33)	-.30(.41)	.53(.24)	.32(.52)
	dl	.79	.63(.18)	.99(.07)				-.55(.87)	.43(.33)	.80(.30)
8WW	c	.55	.80	.50(.18)	.86(.10)	.86(.10)	.21(.38)	.29(.40)	.87(.10)	-.16(.61)
	dl	.54	.72	.20(.14)				-.62(.60)	.59(.29)	.99(.44)
3-6WG	c	.54	.95	.80	.52(.15)	.95(.03)	-.40(.36)	-.36(.41)	.58(.23)	.31(.53)
3-6FC	c	.66	.92	.77	.90	.53(.16)	-.07(.32)	-.21(.40)	.60(.21)	.43(.49)
3-6FCR	c	.25	-.18	-.03	-.35	.07	.33(.14)	.26(.43)	.15(.36)	.50(.66)
6-8WG	c	-.10	-.11	.60	-.10	.02	.18	.35(.20)	.79(.19)	-.90(1.39)
	dl	-.19	-.20	.51				.07(.13)	.33(.71)	.12(1.0)
6-8FC	c	.22	.48	.81	.52	.54	.04	.73	.48(.17)	-.61(.75)
	dl	.06	.14	.65				.71	.31(.16)	.70(.46)
6-8FCR	c	.24	.33	-.13	.32	.30	.03	-.76	-.10	.11(.14)
	dl	.28	.37	-.25				.05	-.25	.25(.15)

There were moderate to high estimates for the heritability of most of the traits with generally good agreement between estimates in the cage- and deep litter-reared birds. There was however, an indication of lower estimates for 8WW and WG6-8 in the deep litter-reared birds. Heritability estimates for FCR6-8 were low in both groups. Genetic correlation estimates between the different liveweight measures in both environments were generally large and positive, whilst those between 6WW or WG3-6 and FC3-6 were very large with small standard errors. Although, of moderate magnitude, correlations between 6WW or WG3-6 and FCR3-6 had relatively large standard errors. Nearly all the genetic correlations involving the three traits measured over the 42 to 52d period had large standard errors. Phenotypic correlations were generally of similar magnitude to the genetic correlations.

The effect of feathering rate on feed efficiency measured over the two intervals in birds from the two rearing regimens, is shown in Table 2.

Table 2. The effect of feathering rate and sex on feed efficiency between 21 and 42d (FCR3-6) and 42 and 52d (FCR6-8) reared either in cages or on deep litter. Standard errors in parenthesis.

Sex	Feathering	FCR 3-6	FCR 6-8	
		Cage	Cage	Deep Litter
Male	rapid	1.90(.01)	2.45(.03)	2.53(.05)
	slow	1.96(.01)	2.55(.06)	2.50(.03)
Female	rapid	1.96(.01)	2.78(.04)	2.61(.03)
	slow	2.02(.01)	2.75(.04)	2.76(.02)

In both males and females reared in cages, rapid feathering birds had significantly lower FCR over the 21 to 42d interval than their slow feathering counterparts. The only significant effect of feathering type on FCR from 42 to 52d was for females reared on deep litter, in which case the rapid feathering birds were the more efficient. Over both intervals, males were more efficient than females in converting food into body weight.

The economic optimum selection index calculated from the hatch 5 data took the form: I_1 = 6WW - 0.32 FC 3-6 - 0.80 3WW with a relative economic weighting of body weight to food of 3 to 1. A second index giving somewhat lower emphasis to food consumption with relative economic weighting for body weight to food of 4 to 1, took the form: I_2 = 6WW - 0.20 FC 3-6 - 0.80 3WW.

For the males, two thirds of the top 10% of birds ranked on index 1 were represented in the heaviest 20% at 3 weeks whilst all of the top 10% of birds ranked on the index were represented in the heaviest 40% at 3 weeks. For females, half of the top 20% of birds ranked on index 1 were represented in the heaviest 25% at 3 weeks whilst only two thirds of the top 20% of birds ranked on the index were represented in the heaviest 50% at 3 weeks. There was only a minor change in the effect of pre-selection on 3WW on the capture of birds with the highest indexes, with use of the second index.

IV. DISCUSSION

The relatively low heritability of FCR6-8 combined with the large positive genetic correlations between this trait and both 6- and 8-week weights and the low genetic correlation with WG6-8 in the deep litter-reared birds, together with the low phenotypic correlation with FCR3-6 in the cage-reared birds, suggests that the testing of food utilisation efficiency in cages subsequent to measurement of growth rate to slaughter weight on deep litter may be an inappropriate method of improving the economic efficiency of broiler production. It is not unlikely that quite different physiological components of feed efficiency are expressed in the pre- and post-slaughter weight periods.

On the basis of the parameters estimated over the 21 to 42d period, selection for improved growth and efficiency should be reasonably effective although the very high correlations between either 6WW or WG3-6 and FC3-6 together with the moderate correlations, (with their high standard errors) between these weight/gain traits and FCR3-6, suggests that there might be less scope for improvement of efficiency of broiler production in these than in earlier measured stocks (Pym 1990).

Any benefit conferred by rapid feathering on feed efficiency appears to be lost by about 6 weeks of age. The role of feather cover in insulation to heat loss is well accepted and effects on feed efficiency, particularly in sub-optimal temperature conditions have been reported (Pym 1985). The requirement to use the rapid/slow feathering allelic system for sex identification means that some parental lines require to be slow feathering. Selection for growth and efficiency in these lines could, depending upon the temperature conditions in the selection environment, utilise quite different physiological mechanisms than in rapid feathering lines.

Notwithstanding the negative weighting to initial body weight in the economic optimum indexes, an assessment of the effect of pre-selection for 21d weight on capture of the birds ranking highest on the index, showed that the large majority of the top 10% of males ranked on the index were represented in the heaviest one third of the birds at 21d. Thus, it should be possible to cull the lightest two thirds of the birds at 21d without losing a significant number of the top birds. In the case of females, assuming that the best 20% of birds ranked in the index would be required for selection, at least the heaviest half of the flock at 21d should be tested in the single cages. Moderate changes in the relative weighting to food intake in the index do not appear to greatly influence the proportion of the flock that can be culled on 21d body weight.

V. ACKNOWLEDGMENTS

This work was supported by a grant from the Australian Chicken Meat Research and Development Council. The technical assistance of Michael Cumes and Kurt van Velthuizen is gratefully acknowledged.

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NON-INFECTIOUS SKELETAL DISORDERS OF CHICKENS

C. RIDDELL

Skeletal disorders in commercial poultry are common and cause considerable economic loss for the poultry industry, but few detailed surveys have been conducted on commercial flocks to define the incidence and significance of different disorders. In a 1983 survey of 51 broiler chicken flocks killed at about 6 weeks of age in Western Canada, the average incidence of chickens with skeletal deformities was 1.72% (Riddell and Springer, 1985). This figure included 1.10% chickens culled in the field and 0.62% chickens condemned or trimmed as carcasses. In a recent informal survey of several integrated broiler chicken operations in the USA conducted by the author, the average incidence of mortality and culls due to leg weakness was estimated to be 1.17% and 2.00% for birds killed at 45-47 and 50-55 days, respectively. The lack of a standard terminology has hindered detailed definition of this problem. In this paper, the author will attempt to define the current major non-infectious skeletal disorders and summarise current understanding of their causes. The disorders will be classified into developmental, degenerative and metabolic diseases.

I. DEVELOPMENT DISORDERS

Most developmental disorders have a genetic basis and occur at a low incidence in most flocks. The incidence may be increased by environmental or nutritional factors.

(a) Spondylolisthesis

Spondylolisthesis is characterised by a clinical picture of posterior paralysis due to deformation and displacement of the sixth thoracic vertebra resulting in a pinched spinal cord. Spondylolisthesis was reviewed by Wise (1975) and Riddell (1981). The condition is restricted to broiler chickens and occurs in most broiler chicken flocks. The highest incidence the author has encountered is 2%. In the commonest form of spondylolisthesis, the body of the sixth thoracic vertebra of affected birds is titled in a longitudinal plane. The anterior end is displaced ventrally and the spinal cord is pinched due to a kyphotic angulation of the floor of the spinal canal at the posterior end. Recently, another form of spondylolisthesis was described in which step-like defects separated the bodies of the fifth, sixth and seventh thoracic vertebrae. Each vertebral body was displaced ventrally relative to the vertebral body in front, resulting in damage to the spinal cord (Duff, 1990). A marked difference in incidence of spondylolisthesis is apparent between different strains of broiler chickens and the incidence of spondylolisthesis can be increased by genetic selection.

Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N OWO

(b) Valgus and Varus Deformation of the Intertarsal Joint

Valgus and varus deformation of the intertarsal joint (VVD) is the commonest form of long bone distortion found in broiler chickens. VVD has been reviewed by Riddell (1981, 1991). The incidence of VVD in broiler chickens varies from 0.5-2.0% but occasionally it reaches 5-25% in problem flocks. The majority of affected birds are males; when the defect is unilateral, the right leg is more commonly affected. Valgus deformation is more common than varus deformation. The major defect is angulation at the distal end of the tibiotarsus but lesser angulation may also occur in the proximal tarsometatarsus. The pathogenesis and etiology of VVD is poorly defined. It is probable that multiple factors can produce an identical deformity. A deformity similar to VVD has long been recognised as a significant feature of nutritional deficiencies affecting the development of the growth plate, the classical example being manganese deficiency. The syndrome caused by manganese deficiency used to be called perosis. The term chondrodystrophy is now used instead of perosis. In most cases of VVD in modern commercial poultry, chondrodystrophy does not appear to be involved. Chondrodystrophy is discussed in more detail in this paper under metabolic disease.

In the rapidly grown modern broiler chicken the vascular morphology of the growth plate is more irregular than in the leghorn chicken (Thorp, 1988). This irregularity may predispose the broiler chicken to VVD. Some workers (Dämmrich and Rodenhoff, 1970) have noted differences in cortical bone differentiation in broiler chickens and have suggested that a delay in consolidation and formation of a tangential lamella structure, as opposed to a radial structure, may increase the susceptibility to VVD. VVD has been associated with the rupture of ligaments in turkeys (Julian, 1984). Surgical severing of the gastrocnemius tendon resulted in similar angulation to VVD (Riddell, 1975). Conformation may predispose to VVD (Randall and Mills, 1981; Riddell et al., 1983).

The incidence of VVD can be reduced by slowing the growth rate. Growth rate has been slowed by feed restriction (Riddell, 1983) and shortened photoperiods (Classen and Riddell, 1989). In both cases it was probable that the birds were more active. The higher incidence of VVD which occurs when birds are kept in cages may be explained by reduced activity (Haye and Simons, 1978). Increased exercise has been shown to increase the tangential lamellar structure of cortical bone (Rodenhoff and Dämmrich, 1973) and to produce a more regular growth plate (Thorp and Duff, 1988). Reduced photoperiods may affect circadian hormonal rhythms which may influence bone structure and development (Classen et al., 1991).

(c) Rotated Tibia

Rotated tibia (RT) is a less common form of long bone distortion found in broiler chickens. RT has been reviewed by Riddell (1991). The incidence is generally less than 0.2% but a higher incidence has been observed in some flocks. Development of RT is most common before 6 weeks of age. Birds affected with RT have difficulty in standing. The affected leg is abducted and the birds are often described as having "spraddle legs". The abnormal rotation of the tibiotarsus is commonly 90° and the tarsometatarsus is directed laterally when the tibiotarsal-tarsometatarsal joint is flexed. There is no angulation of the long bones and the

intertarsal joint is normal. The defect can be bilateral but is more commonly unilateral. Either leg may be involved and there is no evidence of a sex predisposition in broiler chickens. The above observations confirm that RT has a different pathogenesis than VVD. The lesion also occurs in guinea fowl, pheasants and leghorn chickens. This indicates that growth rate is not important in development of the disorder. Some external rotation of the tibiotarsus is normal in development of the broiler chicken (Duff and Thorp, 1985). RT appears to represent excessive external rotation. In guinea fowl, RT has been associated with early rickets (Bergmann and Pietsch, 1976).

(d) Tibial Dyschondroplasia

Dyschondroplasia is a common lesion of the growth plate in broiler chickens. The lesion is characterised by persistence of an abnormal mass of cartilage in the metaphysis below the growth plate. The lesion can occur at many different sites and the pathogenesis may vary between sites (Duff, 1989a). The proximal tibia is the most commonly affected site and has the most severe lesions. Tibial dyschondroplasia (TD) represents a well-defined syndrome in which the abnormal cartilage appears to be the primary lesion. Dyschondroplasia in the femoral head and in vertebral bodies may be secondary to necrotic and traumatic lesions in the growth plate (Duff, 1984d; Riddell, et al., 1983) The author uses the term osteochondrosis to describe disturbed endochondral ossification associated with necrotic and traumatic lesions. Osteochondrosis will be discussed later in the paper. The following discussion will concentrate on tibial dyschondroplasia which was reviewed previously by Riddell (1981, 1991). The incidence of TD in many broiler chicken flocks is 30% or greater. In most affected birds the lesion is subclinical. Severe lesions are associated with marked anterior to lateral bowing of the tibiotarsus, fractured fibulas and abnormal gaits. In some cases fractures occur in the tibiotarsus below the abnormal cartilage. If affected birds are not culled, considerable wastage can result from trimming at processing. A proportion of birds with TD also have abnormal cartilage in the proximal metatarsus and fewer birds have abnormal cartilage in the distal tibiotarsus.

In dyschondroplasia there is a failure of hypertrophy, mineralisation, vascular invasion and removal of cartilage of the growth plate. Prehypertrophic cartilage persists and chondrocytes die. The pathogenesis of the lesion is not understood. Mechanisms which have been suggested include a failure of hypertrophy which may prevent invasion of cartilage by metaphyseal vessels. This may result from thrombosis and occlusion of vessels in the epiphysis and zone of proliferation. These lesions have not been described in published reports of tibial dyschondroplasia (Duff, 1989a). A failure of mineralisation may prevent hypertrophy. A second possible mechanism is delayed development of metaphyseal vessels resulting in reduced invasion and removal of cartilage. Identical lesions to dyschondroplasia have been induced by surgical blockage of the metaphyseal blood supply. Similar blockage may follow trauma to metaphyseal trabeculae. A third possible mechanism is a failure of chondrolysis due to a lack of chondroclasts. These three different theoretical mechanisms may result in a similar lesion.

The incidence of TD is influenced by genotype, nutrition and management. It is possible to increase the incidence by genetic selection and by changing the

cation-anion ratio in the ration. Feeding high levels of phosphorus relative to calcium will significantly increase the incidence of TD but it is impossible to eliminate tibial dyschondroplasia by manipulating these minerals (Edwards and Veltmann, 1983; Riddell and Pass, 1987). High levels of some vitamin D metabolites may reduce the incidence or severity of tibial dyschondroplasia (Edwards, 1990). Severe feed restriction will reduce the incidence of TD (Riddell et al, 1983) as well as daily fasting (Edwards and Sorensen, 1987).

II. DEGENERATIVE DISORDERS

Degeneration in the skeleton is often considered to be due to trauma, particularly "wear and tear". Immature skeletons in rapidly growing meat-type birds may be susceptible to trauma.

(a) Osteochondrosis

Osteochondrosis is used by the author to refer to disturbed endochondral ossification associated with degenerative and traumatic changes in growing cartilage of the growth plate and epiphysis of domestic poultry. These degenerative lesions are often microscopic and subclinical. A brief review on osteochondrosis as defined above has been written by Riddell (1991). The incidence of osteochondrosis in broiler chickens may reach 50%. In the broiler chicken the lesions are most commonly found in the growth plates and epiphyses on either side of the synovial joints adjacent to the sixth vertebral body (Duff, 1989b) and of the proximal femur (Duff and Randall, 1987) and less commonly in the growth plate and epiphysis of the distal tibiotarsus (Duff, 1989a). No clinical significance has been attached to osteochondrosis in the thoracic vertebrae (Riddell, et al., 1983). In most cases of osteochondrosis in the femoral head described by Duff (1984b,c,d), affected birds had other skeletal deformities. However, in at least 7 birds in one study lameness could only be attributed to lesions in the femoral head (Duff, 1984a). Femoral heads were asymmetrical and misshapen with thickened or shortened femoral necks. Microscopic lesions of osteochondrosis in broiler chickens include thrombosed and occluded vessels both in the epiphysis and zone of proliferation of the growth plate, eosinophilic streaks, matrix necrosis, lakes of amorphous material and distinct tears or clefts in the cartilage. Growth plates may be thickened with no clear separation between zones of proliferation and hypertrophy. Chondrocytes may be in irregular "swarms" or multicellular clusters.

In birds with greater angular limb deformity in one limb, the more severe lesions of osteochondrosis in the femoral head occurred in the contralateral limb suggesting weight-bearing may contribute to osteochondrosis (Duff, 1984b). The lesions of osteochondrosis are commonest adjacent to joints in which the epiphyses are exposed to shear forces rather than in joints where pressure forces predominate. This suggests that shear forces may cause the lesions (Riddell et al., 1983). Osteochondrosis may predispose to epiphyseolysis of the femoral head (Duff and Randall, 1987) and to degenerative joint disease (Duff, 1985a). Osteochondrosis is probably a biomechanical disease in rapidly growing poultry in which developing cartilage is susceptible to weight bearing trauma.

(b) Epiphyseolysis of the Femoral Head

Epiphyseolysis describes separation of the epiphysis from the bone. It has been recognised in the femoral head of broiler chickens at processing when there is often associated haemorrhage in the surrounding muscles (Duff and Randall, 1987). It has also been recognised in lame, immature broiler chickens (Duff, 1984c), and mature broiler chickens (Duff and Hocking, 1986). The growth plate may be normal or thickened. Associated eosinophilic streaks or clefts and occluded vessels in the growth plate are common (Duff and Randall, 1987). The cause of the lesion appears to be trauma. Osteochondrosis may predispose to epiphyseolysis (Duff and Randall, 1987).

The terminology applied to lesions of the femoral head in poultry is confusing. This is compounded by the indiscriminate use of the term "femoral head necrosis". Epiphyseolysis must be differentiated from the irregular separation of the femoral head at necropsy due to osteomyelitirs (Mutalib et al., 1983) and from the disintegration of the femoral head and neck at necropsy due to osteoporosis (Van der Heide, Lutticken and Horzinek, 1981). In osteomyelitis yellow discoloured friable areas are generally visible in the metaphysis and , though necrosis is present, the primary change is inflammation. In osteoporosis long bones are easily broken and growth plates may be irregular. No necrosis is present. Neither of these two conditions nor the epiphyseolysis of the femoral head discussed previously should be called femoral head necrosis. The author recommends that the use of the term be discontinued.

(c) Degenerative Joint Disease

Degenerative joint disease (DJD) is common in older animals and birds. It is characterised by erosions and fibrillation in articular cartilage and in some cases by the formation of flaps of the same cartilage associated with formation of periarticular osteophytes and fibrosis of the joint capsule. In Duff (1984a) reviewed early reports of DJD in poultry. A brief review on more recent reports was written by Riddell (1991). DJD may be common in mature meat-type breeding birds (Duff and Hocking, 1986). The clinical significance of DJD is difficult to determine from the literature. DJD will cause chronic pain in turkeys (Duncan, et al., 1991) and may explain lowered fertility in male broiler breeders (Hocking and Duff, 1989). In meat-type poultry the most common site reported for DJD has been the coxofemoral joint (Duff and Hocking, 1986). In the coxofemoral joint, the antitrochanter, trochanter, femoral head and acetabulum may be affected (Duff, 1984a, 1985a,b). Fissures, tears, or erosions in the articular cartilage and attached or free flaps of cartilage, particularly in the antitrochanter along with osteophytes may be grossly visible (Duff, 1984a). DJD is common in many older animals and is considered to be due to repeat trauma, abnormal conformation or instability of joints (Doige, 1988). DJD in some instances in poultry may be secondary to osteochondrosis (Duff, 1985a).

III. METABOLIC DISORDERS

Metabolic bone disease occurs when there is failure in the production of cartilage or bone matrix, or in their mineralisation or maintenance. Most metabolic

bone diseases in poultry are caused by primary nutritional deficiencies but they may also be caused by impaired utilisation of nutrients, hormonal effects, physical factors or toxins.

(a) Rickets

Rickets is a disease of young growing poultry characterised by poorly mineralised bones and thickened and irregular growth plates. The primary defect is a failure of mineralisation of cartilage and bone due to a lack of vitamin D, calcium or phosphorus, an imbalance between the two minerals or interference with the utilisation of one of the nutrients. Rickets in poultry was reviewed by Riddell (1981). Affected birds are reluctant to move, remain sitting on their hocks when startled and may use their wings for balance. On necropsy, bones of affected birds are soft and break easily. The growth plates are thickened.

In some outbreaks of rickets the cause is a simple nutritional deficiency due to a feed mixing error. A common finding of subclinical rickets in broiler chickens characterised by uniformly thickened growth plates is most probably due to a marginal level of dietary calcium and excess dietary phosphorus (Riddell and Pass, 1987). In many cases of rickets, particularly in turkeys, it is difficult to implicate a feeding mixing error. Such cases have been described as "field rickets" implying that a deficiency in the feed may not be the cause. The possibility of infectious agents causing a malabsorption syndrome and interfering with vitamin D was proposed by workers from Holland (Kouwenhoven et al., 1978). This general concept was supported in an excellent but rarely cited study by Troup (1981) in which field rickets was reproduced by inducing a severe enteritis in experimental poults. This work has recently been confirmed (Perry et al., 1991). It is probable that the so-called brittle bone disease (Van der Heide, Lutticken and Horzinek, 1981), and the skeletal lesions associated with malabsorption syndrome (Page et al., 1982) and infectious stunting (Bracewell and Randall, 1984) have a similar pathogenesis. In most of these syndromes loss of body weight is often a more important feature of the disease than the skeletal lesions.

(b) Cage Layer Osteoporosis

Cage layer osteoporosis (fatigue) (CLO) is the most significant disease of the skeleton in mature chickens used for egg production. It is characterised by fragile bones and fractures which often result in birds becoming paralysed in their cages. Osteopenia has recently been used by Randall and Duff (1988) to describe the syndrome. CLO has been reviewed by Riddell (1981). Clinical outbreaks of CLO continue to be diagnosed (Randall and Duff, 1988) but bone breakage due to osteoporosis in laying hens at processing may be a more significant problem and has significant implications with regard to animal welfare (Gregory and Wilkins, 1989;Nørgaard-Nielsen, 1990). Clinical signs in birds with CLO are variable and include posterior paralysis or acute death with or without changes in egg production and shell quality. Paralysed birds initially are alert. They may recover if removed from their cages and given ready access to feed and water. If left in their cages they will generally die from dehydration. On necropsy, birds have fragile bones and sometimes fractures. The changes in the bones are generally most striking in the sternum and ribs. Collapse and infolding of the ribs due to

fractures at the junction of the sternal and vertebral components are very common. Recently, avulsion of the patellar ligament has been described in affected birds as a possible cause of disability (Randall and Duff, 1988).

In the opinion of the author, clinical cage layer osteoporosis is primarily a nutritional disease (Riddell, 1981). Osteoporosis can be caused by a vitamin D, calcium or phosphorus deficiency. There have been numerous studies on the calcium and phosphorus requirements of egg-type birds and requirements reported by research workers have changed with time (Roland, 1986). The recommended requirements for calcium have increased while, surprisingly, those for phosphorus have decreased. Some current problems with osteoporosis may be due to inadequate phosphorus. Calcium particle size and time of ingestion of calcium may influence the amount of calcium available for eggshell formation. Prelay feeding of calcium may influence the retention of calcium associated with the formation of medullary bone prior to egg production. These factors may be important in preventing osteoporosis. Cage layer osteoporosis, as the name implies, has been restricted to chickens housed in cages. Chickens kept in cages have weaker bones than chickens kept in more extensive systems. In one study chickens kept in cages had a higher incidence of recently broken bones at processing but chickens kept in more extensive housing systems had more old breaks (Gregory et al., 1990). In more extensive housing systems, increased bone strength has corresponded to increased movement (Knowles and Broom, 1990:Nøgaard-Nielsen, 1990). Providing hens in cages with perches has been shown to increase bone strength (Hughes and Appleby, 1989).

(c) Chondrodystrophy

Chrondrodystrophy is a generalised disorder of the growth plates of long bones such that growth is impaired, while mineralisation and appositional growth remain normal (Wise, 1975). It occurs in young growing poultry and is caused by vitamin or trace mineral deficiencies. Little chondrodystrophy has been recognised in commercial broiler chickens for many years. Chondrodystrophy as defined above results in shortened long bones, enlargement of hock joints and often secondary valgus or varus deformation. In severe cases, the gastrocnemius tendon may become displaced from the intercondyloid groove. Characteristic microscopic lesions occur in the growth plates. In the zone of proliferation, there is a lack of columnar arrangement and an absolute lack of chondrocytes, particularly distal from penetrating blood vessels from the epiphysis. Though chondrodystrophy is not considered to be important in commercial poultry today, the possibility of marginal nutritional deficiencies causing minor chondrodystrophy and resultant leg deformities should not be ruled out without careful investigation. Interactions among trace minerals and vitamins or with other feed ingredients may result in chondrodystrophy (Sauveur, 1984).

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THE ROLE OF THE KIDNEYS IN THE REGULATION OF WATER AND ELECTROLYTE LEVELS IN CHICKENS

J.R. ROBERTS

Summary

The kidneys of birds carry out a range of vitally important functions: excretion of waste products, regulation of water levels in the body, regulation of the levels of electrolytes and, in conjunction with the respiratory system, acid-base balance. Birds respond to conditions of osmotic stress by releasing hormones such as arginine vasotocin, prolactin and angiotensin II. The role of these hormones, as well as others such as aldosterone and atrial natriuretic factor, in the regulation of water electrolyte levels in the body is discussed. Imbalances of body water and electrolytes can ocur if the levels of water and/or minerals ingested are too high or too low, or if there is a disease of the kidneys. Some examples of these disorders are described.

I. INTRODUCTION

The kidneys of all avian species carry out a range of essential functions. Not only do the kidneys excrete the nitrogenous end products of protein metabolism but they also regulate the levels of water and electrolytes (such as sodium, chloride, potassium, calcium, phosphate and magnesium) and contribute to the acid-base balance of the body.

The regulation of the water levels in the body is under the control of the avian antidiuretic hormone, arginine vasotocin (AVT) which comes from the hypothalamus of the brain via the posterior pituitary gland. The levels of electrolytes are controlled by a variety of complex mechanisms.

Some disorders of kidney function are of interest to the poultry industry. These include the phenomenon of diuresis, urolithiasis, the kidney damage associated with diseases such as infectious bronchitis and excessive losses of electrolytes in urine.

II. STRUCTURE AND FUNCTION OF THE AVIAN KIDNEY

(a) Structure

The kidneys of avian species are recessed in the bony synsacral region. Each kidney consists of three lobes - cranial, middle and caudal - with the ureter running centrally along the ventral surface. Each lobe is further subdivided into lobules. The kidneys consist of cortical and medullary regions although the line of division between the two regions is indistinct (Skadhauge 1981; Sturkie 1986; Dantzler 1989). The arterial blood supply comes from three pairs of arteries, the anterior which arise directly from the aorta and the middle and posterior arteries which arise from the external iliac artery. The venous supply to and from the kidney is much more complicated than that which is found in mammals owing to

Department of Physiology, University of New England, Armidale, N.S.W. 2351

the presence of the renal portal system (which is found also in amphibians and reptiles). This portal system brings blood from the legs and tail of the bird to the kidneys. The presence of the renal portal valve enables blood to be shunted either through the kidney or through vessels which bypass the kidney. Thus the avian kidney is perfused by a mixture of arterial and venous blood. The significance of this process is unknown but there have been suggestions that it is associated with the production of uric acid as the main end product of nitrogen metabolism.

Within the avian kidney, there are two major types of functional units or nephrons. These are the reptilian-type nephrons which are located near the surface of the kidney in the cortical lobules and the mammalian-type nephrons which occur in the deeper regions of the kidney and have loops of Henle which extend into the medullary cones. The relative proportions of the two nephron types vary among the different species but most nephrons are of the reptilian-type.

(b) Function

There have been only a few studies of the avian kidney at the level of the individual nephron. These include my own studies (Roberts and Dantzler 1990; in press) as well as those of Laverty and Dantzler (1982, 1983), Nishimura et al. (1986) and Miwa and Nishimura (1986). Most studies have involved the whole kidneys or regions of the kidneys (Wideman and Gregg 1988). In general, it is assumed that the processes which occur in the individual nephrons of the avian kidney are similar to those which have been described for mammals.

The blood which passes to the kidneys is filtered at the glomeruli of the individual nephrons. The glomerulus is a tuft of capillaries which is in close contact with the cup-shaped Bowman's capsule of the nephron. Blood flows into the glomerulus through the afferent arteriole and leaves via the efferent arteriole. Both of these arterioles are able to constrict and dilate and thereby change the blood pressure in the glomerular capillaries. The rate of filtration at the glomerulus is influenced by a number of factors including the circulating levels of the avian antidiuretic hormone, arginine vasotocin (AVT).

The filtrate passes into the proximal tubule of the nephron where considerable reabsorption of filtered substances occurs. Water, sodium (Na), chloride (Cl), potassium (K), calcium (Ca), phosphate (P), bicarbonate, glucose and amino acids are reabsorbed here and there is evidence for net secretion of Ca and P (Laverty and Dantzler 1982; Roberts and Dantzler 1990). Substances such as hydrogen ions, uric acid, other organic acids and organic bases are also secreted into the proximal tubule. The tubular fluid then passes into the loop of Henle which is present only in the mammalian-type nephrons and which is responsible for the building-up of concentration gradients within the kidney tissues. It is these concentration gradients which enable the kidney to conserve water. Of the vertebrates, only birds and mammals are able to produce a urine which is more concentrated than plasma.

Some reabsorption of calcium occurs in the thick ascending limb of the loop of Henle in mammals so it is likely that a similar process occurs in birds. From the loop of Henle, the tubular fluid proceeds to the distal tubule where more Na is reabsorbed, K may be either reabsorbed or secreted, and hydrogen ions are secreted. Finally, the tubular fluid passes through the collecting ducts where, under appropriate conditions, water may be reabsorbed back into the kidney tissue

and from there into the general circulation. The role of the kidney in acid-base balance lies in the ability of the kidney tubules to vary the amount of hydrogen ions secreted, and bicarbonate reabsorbed, according to the acid-base status of the body.

The urinary end-product of nitrogen metabolism is uric acid. Uric acid forms a precipitate in the final stages of urine formation and this precipitate is able to incorporate other ions such as Na and K. Precipitated material does not contribute to the overall osmolality of the urine. Few birds are able to produce a fluid urine which is more than two and a half times the osmolality of plasma. The excretion of uric acid as a suspension or a precipitate, with the "trapping" of ions within the precipitate, enables the avian kidney to conserve more water than the maximum urine : plasma ratio for osmolality would indicate.

Urine passes down the ureters and enters the cloaca. From there it may move into the rectum (colon) and caeca where water and salts may be reabsorbed. It is still not known under what physiological conditions such retrograde movement of urinary products occurs. The ability of the rectum and coprodaeum to reabsorb ions and water is well-established (see Goldstein 1989). Hormones such as aldosterone and possibly prolactin, are involved in the regulation of this transport. The role of the caeca in avian osmoregulation has received considerable attention in recent years. Water and electrolytes can be reabsorbed in the caeca and this may be important in birds which are dehydrated and on a low salt diet (Rice and Skadhauge 1982). Overall, postrenal modification of urine may be important in some physiological conditions.

III. REGULATION OF WATER AND ELECTROLYTE LEVELS

In this paper, I will deal primarily with the regulation of water and electrolytes such as Na, Cl and K (Dr. Wideman will be discussing Ca and P). When avian species are subjected to osmotic stress, several hormones are released in the body. These include the avian antidiuretic hormone, arginine vasotocin (AVT), prolactin and angiotensin II (Scanes et al. 1976; Arad and Skadhauge 1984; Gray and Erasmus 1988). Arginine vasotocin is synthesised in the hypothalamus and released from the posterior pituitary gland in response to appropriate stimuli. The most potent stimulus for the release of AVT is thought to be an increase in plasma osmolality (caused by either a loss of water from the body or an increase in the level of an electrolyte such as Na). The effect of changes in circulating fluid volume has been less certain because haemorrhage led to an increase in plasma AVT in some species (Simon-Oppermann et al. 1984; Gray and Erasmus 1989; Bottje et al. 1989) but not in others (Stallone and Braun 1986).

Recent studies using the feral chicken (Roberts 1991a,b) have shown that the sensitivity of release of AVT (i.e. the amount of AVT released per unit increase in plasma osmolality) was greater in birds which were dehydrated than in birds which received a hypertonic salt infusion. This indicates that the expansion of extracellular fluid volume which accompanies a hypertonic salt infusion tends to suppress the release of AVT which is stimulated by the rise in plasma osmolality. In contrast, in the dehydrated birds the rise in plasma osmolality was accompanied by a reduction in the circulating fluid volume which would further stimulate the release of AVT. Therefore, it is concluded that the primary stimulus for the release of AVT is an increase in plasma osmolality with the volume of the extracellular fluid

exerting a modulating influence.

Arginine vasotocin appears to act on the kidney in two basic ways. One site of action is the vasculature, specifically the afferent arterioles of the reptilian-type nephrons, where vasoconstriction occurs in the presence of AVT. This results in a decrease in the single nephron glomerular filtration rate (SNGFR) or even a cessation of filtering (Braun and Dantzler 1972, 1974). Secondly, AVT appears to act on the distal region of the nephron in a manner which is similar to the action of arginine vasopressin (the mammalian antidiuretic hormone) on the mammalian distal nephron. The permeability to water of the distal tubule and collecting duct increases in the presence of AVT. This allows water to move by osmosis from the tubular fluid into the medullary interstitium as the result of the concentration gradient which has been built up by the loops of Henle of the mammalian-type nephrons.

The effect of AVT on individual nephrons may lead to a reduction in whole animal glomerular filtration rate (GFR) as was found in the dehydrated feral chickens (Roberts 1991a). A reduction in GFR and greater reabsorption of water from the distal nephron would contribute significantly to water conservation. However, when AVT levels increase in response to the infusion of a hypertonic saline solution, the results reported in the literature are conflicting. Most authors have reported a decrease in GFR (e.g. Braun and Dantzler 1972) or no change (Gerstberger et al. 1985). However, in the study of Roberts (1991b) and that of Laverty and Wideman (1989), GFR increased during an infusion of hypertonic saline. Therefore, the relationship between plasma AVT levels and GFR appears to be complex and is probably influenced by factors such as the extent of expansion of the extracellular fluid volume.

The hormones prolactin and angiotensin II also increase when birds are subjected to osmotic stress. Angiotensin II is known to stimulate drinking (Snapir et al. 1976) and also to stimulate the release of AVT in some species (Goto et al. 1986). The effects of prolactin on avian renal function is uncertain. The elevation of plasma prolactin levels in domestic fowl following water deprivation or salt loading suggests that this hormone is involved in electrolyte and water balance. When kidney function was assessed by micropuncture and clearance methodology in starlings which were in moult (a time when endogenous levels of prolactin are elevated), these birds showed greater reabsorption of Na and water and reduced secretion of P in the proximal tubules of the reptilian-type nephrons (Roberts and Dantzler 1990). In another study, prolactin was infused into starlings and kidney function was assessed. Prolactin resulted in elevated levels of plasma urate, a higher urine flow rate, higher fractional excretion of Na and Cl and lower fractional excretion of Mg and K. At the level of the proximal tubule of the reptilian-type nephrons, there was a slight reduction in the net reabsorption of Na and Cl (Roberts and Dantzler in press).

In another study the effect of exogenous prolactin on whole animal renal function in feral chickens was investigated. High (pharmacological) doses of prolactin tended to reduce the fractional excretion of Na and Cl in birds which were infused with isotonic NaCl solution. Chronic administration of prolactin (twice daily injections for 7 days prior to renal function experiments) had no effect on plasma and renal function parameters. It is likely that the effects of prolactin on renal function in birds (and mammals) are modulated by a range of other conditions including electrolyte status, degree of volume expansion and whether prolactin

administration is chronic or acute.

Aldosterone, from the adrenal cortex, is an important regulator of the sodium levels of the body. The release of aldosterone is activated via the renin-angiotensin system which involves a specialized region of the kidney, the juxtaglomerular apparatus. Aldosterone enhances the reabsorption of sodium (and the secretion of K) in the distal tubule and also has effects on the uptake of sodium from the rectum and caeca.

The role of the atrial natriuretic hormone (ANF) in avian salt and water balance has received some attention. Synthetic chicken ANF had diuretic and natriuretic effect and antagonized the renin-angiotensin-aldosterone system in ducks (Gray et al. 1991).

IV. DISORDERS OF WATER AND ELECTROLYTE LEVELS

(a) Ingestion of Electrolytes

Avian species are susceptible to a range of imbalances of water and electrolyte levels. Some of these are related to kidney diseases, which have been reviewed in detail by Siller (1981). An increased intake of ions such as sodium and chloride, which may occur if birds are drinking underground water containing mineral salts, may affect the rate of excretion of ions such as calcium. The decrease in egg shell quality which occurs in birds ingesting saline drinking water (Balnave and Scott 1986; Balnave and Yoselewitz 1987) may be due in part to an increase in the amounts of calcium which are excreted in the urine (Roberts and Balnave 1990; Keshavarz and Austic, 1990). A similar effect has been observed in mammalian species and is thought to be due to reduced calcium reabsorption in the kidneys (caused by expansion of the extracellular fluid volume and changes in the flow through the kidney tubules). Changes in the intestinal calcium absorption and vitamin D synthesis may also be involved (Breslau et al. 1982). This phenomenon is of interest in humans also, as excess dietary salt is thought to be a risk factor for osteoporosis (McParland et al. 1989). It is also possible that the ingestion of excess chloride ions affects the acid-base balance of the birds and thereby affects the functioning of the shell gland.

(b) Infectious Bronchitis

Diseases such as infectious bronchitis virus (IBV) affect water and electrolyte balance in chickens. Some strains of IBV, including the Australian strains, cause kidney damage (Cumming 1963) which is often referred to as IB nephritis. Heath (1970) found elevated plasma levels of uric acid in Australian birds suffering from IB nephritis. Uric acid is removed from the body primarily by secretion into the renal tubules. The tubular damage caused by IBV affects the capacity for tubular secretion of uric acid. Heath also reported abnormally low plasma levels of sodium and potassium (presumably the result of impaired tubular reabsorption of these ions) and suggested "electrolyte replacment" treatment of infected chickens. Condron and Marshall (1985) did not measure plasma electrolyte levels but found that birds infected with the same strain of IBV were in negative sodium and potassium balance. The histopathology of two Australian strains has been described by Siller and Cumming (1974) and ultrastructural

changes by Condron and Marshall (1986).

Experiments were conducted in this laboratory in which renal function was assessed in male broilers following acute challenge with Australian T-strain IBV (Afanador and Roberts, in preparation). Three groups of birds were used: control birds which were unvaccinated and unchallenged, birds which were vaccinated at 2 days of age with one dose of Webster Vic-S strain IBV intraocularly and challenged at 15 days of age with Australian T-strain IBV, and a third group which was not vaccinated and challenged at 15 days of age with T-strain IBV. Renal function experiments were conducted from 9 to 14 days post-challenge. Standard renal clearance methodology was used on anaesthetised birds. At the end of the experiments, alcian blue was infused to stain the functional glomeruli. Histological specimens were collected from birds which were not used for the renal function experiments.

Body weights were highest in the control (C) birds and lowest in the birds which were unvaccinated and challenged (UC). The vaccinated challenged (VC) birds were of intermediate weight. Kidney weights were not significantly different between groups due, in part, to hypertrophy of affected kidneys. Of the blood and plasma parameters measured, haematocrit was higher in C than in either UC or VC and plasma uric acid concentrations were significantly higher in the unvaccinated challenged group. However, the plasma osmolality and levels of sodium, chloride, potassium, ionised calcium, total calcium and phosphate were not significantly different between groups. Therefore, these findings are different from those of Heath (1970) although it should be emphasised that none of the birds was "very sick". In any case, such animals would have been unlikely to survive the experiments.

Urine flow rate and free water clearance were higher in the experimental groups than in the control group, with the degree of diuresis being greatest in the UC group. Glomerular filtration rate was higher in the VC than the C and UC birds. The significance of this is not clear although vaccination may have had some effects on the glomerulus. In humans, a common cause of glomerular disease is thought to be the interaction of immune complexes with the glomerular basement membrane.

The fractional excretion of sodium was much higher in the UC group than in the other two groups although the fractional excretion of chloride was not significantly different between groups. The fractional excretion of potassium was lowest in the C group and highest in the UC birds, with the VC group intermediate. The fractional excretion of calcium was elevated in the UC birds whereas phosphate fractional excretion was similar for all groups. Plasma clearances of para-aminohippuric acid and uric acid were not significantly different between groups. Therefore, renal losses of sodium and calcium were associated with IB nephritis.

The number of functional glomeruli (per animal, per kg body weight or per g of kidney) was highest for the control group and lowest for the UC group, with the VC birds intermediate. In addition, there were some differences in the profiles for glomerular diameter in the three groups. Histological examination of kidneys revealed pathological changes similar to those described by Siller and Cumming (1974) although, again, none of the birds was "very sick" and therefore the extreme damage found by these authors was not observed.

(c) Other Renal Diseases

In the above experiments the Australian T-strain of IBV was associated with a diuresis, as has been described for North American strains (Wideman and Stanick 1989). However, the Australian strains of IBV which cause IB nephritis do not seem to be associated with urolithiasis as is the case in the United States (Glahn et al. 1989). Urolithiasis is a degenerative kidney lesion which is known to affect caged layer flocks in the U.K. and the U.S.A. but which appears to be relatively uncommon in Australia. Suspected causes include excess calcium fed during the pullet growing phase, IBV, water deprivation, and metabolic alkalosis induced by elevated sodium plus potassium to chloride ratios. The effects of excess calcium may be accelerated if the diet contains low available phosphorus (Wideman et al. 1985).

In urolithiasis, kidney stones (uroliths) composed of monosodium urate and/or calcium urate occur in the ureters and collecting ducts of the kidneys. Portions of the kidney may atrophy and the remaining kidney tissue undergoes hypertrophy. "Visceral gout" where uric acid is deposited in the tissues, may be present. Hens in full production die suddenly without external symptoms. In most cases during an outbreak of urolithiasis death is due to blockage of the urinary tract and resultant renal failure. Acidification of the layer ration by the addition of ammonium chloride reduces the incidence of urolithiasis (Glahn et al. 1988).

V. CONCLUSION

The kidneys play a major role in regulating the water and solute composition of the internal environment of birds. Siller (1981) states that "although kidney diseases of poultry are responsible for considerable mortality and production losses, they are among the most neglected areas in avian pathology". Physiological research tends to concentrate on mammalian species because of the direct relevance to humans. Therefore, mammalian kidney function is better understood than is avian renal function. More basic research needs to be conducted on avian species in order to clarify the basic mechanisms involved in kidney function and fluid and electrolyte balance. In addition, more information is required about the effects of disease on overall water and electrolyte balance.

VI. ACKNOWLEDGEMENTS

Some of the results presented are from studies supported by the Egg Industry Research and Development Council and the Australian Research Council.

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THE EFFECT OF SALINE DRINKING WATER ON SHELL GLAND FUNCTION IN TWO STRAINS OF LAYING HENS: ACID-BASE AND ELECTROLYTE STATUS

J.R. ROBERTS*, V.D. REED* and D. BALNAVE**

Summary

Shell gland function was assessed in two strains of Australian layers. Birds were drinking either deionised water or a saline solution (2g NaCl per litre of water) and laying either good or poor quality egg shells. The ingestion of saline solutions and the laying of poor quality egg shells was associated with higher concentrations of calcium and chloride in the shell gland fluid, lower blood pH and higher blood concentrations of phosphate in one strain. The only effect found in the other strain was the tendency for reduced blood pH in birds laying poor quality egg shells.

I. INTRODUCTION

The presence of electrolytes in drinking water has been shown to cause a deterioration in egg shell quality in laying hens (Balnave and Scott 1986; Balnave and Yoselewitz 1987). Previous studies indicated that ingestion of saline drinking solution was associated with an increased calcium excretion in birds which were laying poor quality egg shells (Roberts and Balnave 1990) although the availability of ionised calcium in the blood did not appear to be a limiting factor (Roberts et al. 1991). Saline drinking water has been found to affect the composition of shell gland fluid (Balnave et al. 1989) and the activity of carbonic anhydrase in the shell gland mucosa (Yoselewitz and Balnave 1989). The present study investigated shell gland function in two strains of laying hens which were receiving either deionised water or 2g NaCl per litre of water and laying either good or poor quality egg shells. Shell gland function was examined at two hourly intervals from 14 to 24 hours post-oviposition.

II. METHODS

(a) Experimental Groups

For each of the two strains of hens (Strains A and B) birds were assigned to experimental groups based on the drinking solution and the quality of egg shells which were being produced. There were four groups of Strain A birds based on the type of drinking solution received and the quality of egg shells laid: drinking saline solution and laying good quality egg shells (SG), saline 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 as there were insufficient birds laying poor quality egg shells while drinking deionised water to constitute an experimental group.

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* Department of Physiology, University of New England, Armidale N.S.W. 2351
** Department of Animal Science University of Sydney, Camden, N.S.W. 2570.

(b) Monitoring of Egg Shell Quality

Eggs were collected daily and the time of lay was assessed by use of a time lapse video recorder. Each egg was subjected to a standard series of egg shell quality measurements. Eggs were identified as either good or poor quality with the poor quality eggs being categorised as either soft-shelled, broken, cracked or broken on handling. Egg weight, shell weight and shell thickness were measured and shell weight : egg weight ratio, shell weight : surface area ratio and shell density calculated from the equations of Curtis et al. (1985).

(c) Collection of Blood and Shell Gland Fluid

Blood and shell gland fluid were collected at two hour intervals from 14 to 24 hours post-oviposition. Blood samples were collected from either the brachial or jugular vein into a 2 ml heparinized syringe, all bubbles were expelled and the syringe sealed with an air-tight cap. The blood samples were then stored in an ice/water mixture until initial analysis on the blood gas analyzer. Eggs were expelled manually by applying firm but constant pressure to the anterior end of the egg so that the egg shifted caudally and finally exited via the everted vagina. The shell gland fluid which "gushes" out after the egg was expelled was collected in a vial, taken up immediately into a 2 ml syringe and treated in the same way as the blood samples.

(d) Analyses

Within one hour of collection, whole blood and shell gland fluid were analysed for pH, partial pressure of carbon dioxide (pCO_2) and bicarbonate (HCO_3) on a Corning 168 pH/Blood Gas Analyser at 37°C. The values obtained were corrected to a temperature of 41°C. All remaining blood was then centrifuged and the plasma removed for the determination of osmolality (Wescor 5100B Vapour Pressure Osmometer), chloride (Cl) (Radiometer CMT10 Chloridometer), sodium (Na) and potassium (K) (AVL 984 Electrolyte Analyser), and total calcium (Ca) and phosphate (P) (Cobas Bio Spectrophotometric Autoanalyser).

(e) Eggshell Water Vapour Conductance

Egg shell water vapour conductance was determined on all expelled eggs. Eggs were weighed and placed in a desiccator containing dry silica gel crystals and maintained at 20°C in an incubator. Eggs were weighed daily and mass water loss determined after the method of Ar et al. (1974).

(f) Carbonic Anhydrase Activity

Carbonic anhydrase (CA) activity was measured by the simplified micromethod of Maren (1960) on shell gland tissue taken from hens at 16-18 hours post-oviposition. CA activity was assessed colorimetrically from the rate of hydration of CO_2.

III. RESULTS

(a) Egg Shell Quality

The groups were defined by the percentage of good quality egg shells produced. Poor egg shell quality was associated with lower egg shell thickness and shell weight : egg weight ratios. Percentage production was not significantly different between groups for the Strain B. However, in the Strain A birds, the lowest production was found for the birds receiving saline solution and laying poor quality egg shells.

(b) Blood and Plasma Parameters

There were no consistent patterns in changes in blood pH over the laying cycle although there was a tendency for blood pH to be lowest in the birds drinking saline water and laying poor quality egg shells (Table 1). Blood HCO_3 levels tended to be lowest towards the end of shell deposition. Plasma total calcium showed some tendency to decrease from 14 to 24 hours post-oviposition in both strains. For Strain A plasma phosphate was higher in the birds drinking saline water. However, this trend was not consistent in Strain B (Table 2). The plasma osmolality and the concentrations of Na, Cl and K remained relatively constant over the lay cycle. Exceptions were the elevation of Na at 20-22 hours post-oviposition in the Strain B birds laying poor quality egg shells while drinking saline water, a higher concentration of K at 20-22 hours in Strain B birds drinking saline water and laying good egg shells and a tendency for chloride levels to be highest towards the end of the lay cycle in both strains of birds on deionised water and laying good egg shells.

Table 1. Blood pH

	Strain A				Strain B		
Time hrs	SG	SB	DG	DB	SG	SB	DG
14-16	7.323	7.310	7.346	7.350	7.374	7.325	7.349
	(0.016)	(0.028)	(0.023)	(0.030)	(0.015)	(0.013)	(0.021)
16-18	7.347^{ab}	7.335^{ac}	7.383^{b}	7.298^{c}	7.352	7.323	*7.346
	(0.012)	(0.017)	(0.007)	(0.030)	(0.013)	(0.016)	(0.014)
18-20	7.370	7.294	7.351	7.325	7.349	7.331	7.342
	(0.033)	(0.013)	(0.006)	(0.021)	(0.010)	(0.012)	(0.016)
20-22	7.315	7.282	7.333	7.304	7.343	7.320	7.337
	(0.011)	(0.027)	(0.006)	(0.010)	(0.015)	(0.018)	(0.011)
22-24	7.310	7.313	7.303	7.265	7.313	7.294	7.308
	(0.015)	(0.011)	(0.024)	(0.024)	(0.014)	(0.021)	(0.016)

Note: Values across a row with different superscripts are significantly different from one another.

* Significant differences between strains.

Table 2. Plasma Phosphate mM

Time	Strain A		Strain B	
	SG + SB	DG + DB	SG + SB	DG
14-16	1.16 ± 0.17	0.64 ± 0.14*	0.96 ± 0.14	0.80 ± 0.15
16-18	0.92 ± 0.17	0.63 ± 0.11	0.85 ± 0.13	1.07 ± 0.16
18-20	1.06 ± 0.14	0.75 ± 0.14	0.68 ± 0.14	0.92 ± 0.12
20-22	0.87 ± 0.13	0.65 ± 0.14	0.90 ± 0.12	0.80 ± 0.08
22-24	1.05 ± 0.16	0.82 ± 0.14	0.82 ± 0.18	0.66 ± 0.15

(See Table 1).

(c) Shell Gland Fluid Parameters

Shell gland fluid (SGF) pH was lowest during the period 16-20 hours post-oviposition and tended to be highest towards the end of the lay cycle when bicarbonate concentrations also tended to be highest. The partial pressures of carbon dioxide were very high in SGF especially during the most active period of shell formation (18-20 hours post-oviposition). A significant inverse relationship existed between Na and K concentrations in SGF, with Na decreasing and K increasing during the 14 to 24 hour post-oviposition period. The osmolality of SGF tended to increase as egg shell formation proceeded whereas total Ca and Cl levels remained relatively stable. The Ca and Cl concentrations in SGF were highest for Strain A birds receiving saline drinking water and laying poor quality egg shells (Tables 3 and 4).

Table 3. Shell gland fluid chloride mM

Time hrs	Strain A				Strain B		
	SG	SB	DG	DB	SG	SB	DG
14-16	64.4	63.9	61.5	68.3	62.6	68.7	63.1
	(4.3)	(4.9)	(3.5)	(3.7)	(4.4)	(9.9)	(7.6)
16-18	58.8	70.4	57.7	56.5	59.2	65.0	57.5
	(4.3)	(10.3)	(4.0)	(5.2)	(5.2)	(2.7)	(6.2)
18-20	57.7	61.7	58.9	58.0	63.4	57.0	57.2
	(2.8)	(3.4)	(3.9)	(6.2)	(6.3)	(1.5)	(3.9)
20-22	56.8	73.8	56.9	58.9	62.3	59.4	58.7
	(2.8)	(10.6)	(2.5)	(5.2)	(4.5)	(4.9)	(4.2)
22-24	61.2^{ab}	78.1^{ac}	60.9^{d}	53.6^{bc}	59.8	63.4*	51.6
	(2.3)	(8.2)	(4.4)	(4.4)	(4.8)	(2.9)	(3.9)

(See Table 1).

(d) Eggshell Water Vapour Conductance

Mass water loss from expelled eggs decreased up to 20 hours post-oviposition and then remained relatively stable. Values were still very high at 16-18 hours post-oviposition in birds receiving deionised water and laying poor quality egg shells.

Table 4. Shell gland fluid calcium mM

Time Hrs	Strain A SG	SB	DG	DB	Strain B SG	SB	DG
14-16	2.11	2.98	2.53	2.53	2.02	5.27	3.11
	(0.41)	(0.56)	(0.42)	(0.69)	(0.43)	(1.42)	(1.14)
16-18	1.59	3.81	1.83	2.11	2.18	2.06	2.42
	(0.27)	(1.33)	(0.35)	(0.58)	(0.56)	(0.25)	(0.58)
18-20	2.22	2.36	2.23	1.94	2.15	2.10	2.19
	(0.35)	(0.48)	(0.50)	(0.38)	(0.38)	(0.22)	(0.34)
20-22	2.27	2.74	2.86	1.45	2.39	2.65	2.72
	(0.09)	(0.91)	(0.24)	(0.38)	(0.40)	(0.56)	(0.55)
22-24	2.60	3.71	2.06	1.90	3.67	2.59	2.56)
	(0.60)	(1.64)	(0.24)	(0.52)	(0.29)	(0.35)	(0.62)

(See Table 1).

(e) Carbonic Anhydrase Acitivity

No significant differences were found between groups in the carbonic anhydrase activity of the shell gland mucosa.

IV. DISCUSSION

In general, blood parameters were maintained relatively constant throughout the period of egg shell formation. The tendency for blood HCO_3 levels to decrease over the laying cycle suggests that at least some bicarbonate is withdrawn from the blood by the shell gland tissue. The lower pH of the blood of birds drinking saline water and laying poor quality egg shells may result from an increased extraction of blood bicarbonate by these birds (and may be the result of a reduced generation of HCO_3 within the shell gland tissue). The higher blood phosphate levels in birds drinking saline water could be due either to an increased mobilization of bone or a reduction in the renal excretion of phosphate. Higher levels of blood phosphate may have led to small amounts of phosphate in shell gland fluid (below those which could be detected by our assay) and consequent interference in the co-precipitation of calcium carbonate. The regulation of blood Na concentrations was not affected by saline drinking water in either strain. However, in Strain A, blood Cl concentration at 14-16 hours post-oviposition was significantly higher when salt was present in the drinking water.

The high partial pressures of CO_2 created in the SGF during egg shell formation indicate that a large gradient for CO_2 can be sustained across the shell gland mucosa. During the process of egg shell formation, Na is removed from the SGF at the same time as K and HCO_3 move into the fluid. The relatively constant concentrations of Cl suggest that this ion is not moving in parallel with the Na ions. Chloride concentrations were higher in the shell gland fluid obtained from the Strain A birds which were receiving saline water and laying poor quality egg shells. In these birds, Cl ions may have been complexing with Ca, thus interfering with calcium carbonate co-precipitation.

The high mass water loss at 16-18 hours post-oviposition of Strain A birds drinking deionised water and laying poor quality egg shells indicates that these egg shells were more porous than those of the other groups. This may have been the result of a slower rate of formation of the egg shell.

In the birds studied, carbonic anhydrase (CA) activity was not significantly different between groups. This suggests that CA activity was not limiting, at least in this study, although another study found that CA activity was lower in birds receiving saline drinking water (Yoselewitz and Balnave 1989). The finding that blood pH was lower in birds drinking saline water and laying poor quality egg shells could be explained by a reduction in the CA activity within the shell gland mucosa which, in turn, resulted in an increased extraction of HCO_3 from the blood.

V. ACKNOWLEDGEMENTS

This study was supported by a grant from the Egg Industry Research and Development Council.

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PRELIMINARY MODEL FOR HAUGH UNIT SCORE OF FRESH AND STORED EGGS

D. ROBINSON and K.M. BARRAM

The maintenance of high standards of internal quality is becoming increasingly important in the marketing of shell eggs. A detailed model relating the factors which influence internal quality would enable producers and marketing agents to determine the most economic flock and product management strategies to maintain freshness and optimise the quality of eggs reaching the consumer. The most widely used measure of internal quality is the Haugh Unit score (H), a measure of albumen condition. Of the many factors which influence H, the most important are age of the bird which laid the egg and the conditions under which the egg is subsequently stored.

The object of the current project is to develop and validate a model for predicting H for fresh and stored eggs using Australian data and taking into account the interactive effects of strain and age of the birds, flock management techniques, period and temperature of storage of the egg and the application of mineral oil to the egg shell. Initially a model was constructed on the basis of both overseas and Australian information. A large trial (in progress) was then set up to address various deficiencies in the published information and to provide data relevant to local conditions. The constants in the equations below are derived mainly from the data collected in this trial.

The model has the basic form H = L- D, where L is the value of H at time of lay and D is a component representing albumen deterioration during subsequent storage. L is essentially a function of flock age but also contains a genetic factor which interacts with flock age. D is a function of L, storage time and storage temperature, and also includes an oiling factor which may itself interact with storage temperature. The final model will also include flock management components, a means of evaluating the effects of different successive storage conditions and an estimator of the variance of H, which is important for calculating the proportion of eggs complying with a given minimum standard.

By fresh laid H value is related to flock age (w, weeks) by $L = 109 - 0.628w + 0.00255w^2$. While many strains of bird appear to have similar H profiles, some are different particularly in respect of the rate of decline of H with bird age. The H loss during storage of untreated eggs depends mainly on the storage time (d, days) and the mean ambient temperature (t, ^{o}C). Results obtained to date indicate that the generally assumed square root depreciation of H with time underestimates the curvature of the decline over the first four weeks, which is more closely represented by $D = 0.0076\ Ltd^{0.28}$. For eggs that have been treated by spraying with mineral oil, the decline in H is reduced by about 40%. The data do not as yet support previous evidence which suggests that oiling is relatively more effective at higher storage temperatures. There are indications that the variation in H within a flock increases as the flock ages.

Redlands Poultry Research Centre, Queensland Department of Primary Industries, Alexandra Hills, Queensland, 4161

PROSPECTS FOR THE CONTROL OF THE CONTAMINATION OF POULTRY CARCASSES WITH SALMONELLA DURING PROCESSING

K. SANDERSON and T.A. McMEEKIN

Summary

The strategies proposed for decontamination of poultry carcasses during processing are reviewed. Treatments applied during scalding and chilling have limited effectivness due to rapid inactivation of chemicals and quality defects. The importance of poultry skin structure and water absorption during processing in relation to decontamination is stressed.

I. INTRODUCTION

Salmonella food poisoning continues to be a major problem in developed and developing countries alike, with the disease being responsible for about 3000 deaths per year in the United States (Todd, 1989). Epidemiological studies have consistently linked consumption of poultry products to salmonellosis (Humphrey et al., 1988; D'Aoust, 1989) and incidence surveys have shown that on average 25-50% of poultry carcasses are contaminated with Salmonella (D'Aoust, 1989; Izat et al., 1991; Jones et al., 1991). Although the proportion of Salmonella positive live birds arriving at a processing plant varies considerably, it is generally recognised that more birds (carcasses) are Salmonella positive after processing (D'Aoust, 1989; Lillard, 1990), which demonstrates the importance of cross-contamination during processing in contamination of poultry.

The aim of this paper is to examine the effectiveness of decontamination strategies in relation to the stages of processing where contamination may occur.

II. PROCESSING OPERATIONS

(a) Scalding

Birds are scalded by immersion in tanks of hot water or less frequently by spraying with hot water (Parry, 1989). Two scalding regimes are used. Soft scalded birds are immersed in water at 50-51.5°C for three and a half minutes, while hard scalded birds are immersed in water at 56-60°C for two minutes. Hard scalding removes the outer cuticle of the skin (Thomas and McMeekin, 1980) while it is retained during soft scalding (Thomas, McMeekin and McCall, 1987). Retention of cuticle on carcasses that are to be water (immersion) chilled is not necessary, however, it is essential on carcasses that are to be air chilled as otherwise chilling results in severe discolouration and drying of the skin (Parry, 1989). Hard scalding facilitates removal of feathers by the pluckers to a greater degree than soft scalding.

Birds entering the scalder carry large numbers of viable microorganisms on their skin or in soil and faecal material on the feathers and many of these

Department of Agricultural Science, University of Tasmania. GPO Box 252C, Hobart, Tasmania, Australia, 7001

organisms will become suspended in the scald water (Mead, 1989). As scalding is a continuous operation these microorganisms may be transferred to subsequent birds entering the scald water. In addition to this surface contamination, it has been demonstrated that scald water can contaminate the trachea, oesophagus, lungs, crop, gizzard and air sacs resulting in low-level contamination of the edible offal (Lillard, 1973).

Carcasses leaving the scalder are essentially free of some microorganisms such as psychrotrophic Pseudomonas spp., indicating that they are destroyed during scalding (Thomas and McMeekin, 1980; Mead, 1989). Other microorganisms such as Salmonella are more resistant to the temperature effect of scald water, particularly since the pH of scald water is maintained close to pH 6.0, by dissociation of ureate present in faeces on the feathers, which is near the optimum pH for heat resistance of salmonellas (Mead, 1989). Although this means that Salmonella may persist within the scald water resulting in cross contamination of carcasses, the importance of this in terms of overall contamination is unclear with some investigations finding no increase in the number of Salmonella positive carcasses after scalding (Lillard, 1990) and others reporting increased contamination (Mead, 1989).

Since thermal inactivation of Salmonella is prevented by the scald water pH, attempts have been made to reduce carcass Salmonella levels by raising the pH of the scald water. Adjustment of scald water (to pH 9.0 or above) by the addition of NaOH has been shown to dramatically reduce the total number of viable organisms in the scald water but it has no effect on the proportion of Salmonella positive carcasses (Izat et al., 1989; Mead, 1989). Treatment of scald water with other chemicals such as organic acids (Lillard, 1987) or chlorine (Izat et al., 1989) has similarly reduced the number of viable organisms within the scald water but has not reduced the number of organisms on the carcass surface (reviewed by Mead, 1989). Izat et al. (1989) reported that addition of 0.5% hydrogen peroxide to scald water resulted in significant reductions in the incidence of Salmonella positive carcasses but caused undesirable changes to carcass appearance.

Failure of sanitising treatments to effect the carcass appear to be due in part to the presence of organic material in the scald water which combines with and inactivates antimicrobial compounds and inactivation of compounds through reaction with the the carcass surface (Mead et al., 1975; Mead, 1989). The high buffering capacity, high organic content and high temperature of scald water, mean that relatively high concentrations of chemicals have to be added to be effective. It follows that such treatments are usually not cost effective, in addition the volatile nature of the chemicals eg organic acids, chlorine coupled with the high temperature of the scalders poses a health hazard for workers.

Spray scalding and simultaneous scalding and plucking do appear to provide benefits in carcass hygiene but the high water usage involved and occurrence of quality defects has inhibited commercial adoption (Parry, 1989). Dickens (1990) described and experimental spray-scalder that produced results similar to conventional immersion scalding, with an estimated water usage of 6L per carcass. In contrast American regulations require that a mimimum of 1L of scald water leaves the scalder for each carcass entering (Parry, 1989).

III. PLUCKING

Feathers are removed mechanically by banks of rubber "fingers" mounted on rotating discs and are flushed away by water sprayed continuously on the fingers and carcasses (Parry, 1989). Additional banks of pluckers are required when processing soft scalded carcasses because the soft scalding process does not loosen the feathers to the same extent as hard scalding (Parry, 1989). After plucking the head and feet are removed and the carcass rehung automatically on fresh shackles (Gregory, 1989). Carcasses usually pass through a spray washer before evisceration.

It has been demonstrated by introduction of a marker strain onto a carcass before plucking and examination of subsequently plucked carcasses that plucking is a major site of cross contamination (Mead et al., 1975). In addition to transfer of microorganisms from the surface of one carcass to others by the fingers of the plucker certain organisms may colonise the plucker and be distributed on all carcasses entering the plucker (Parry, 1989). For example colonisation of the plucker with a strain of Staphylococcus aureus resulted in counts of Staphylococcus aureus on the skin increasing almost one thousand fold during plucking (Dodd et al., 1988).

Earlier reports in the literature agreed that plucking not only lead to an overall increase in contamination but to an increase in the number of Salmonella positive carcasses (Mead, 1989), however, a recent survey found no increase during plucking in the incidence of Salmonella positive carcasses and a slight decrease in the total aerobic count (Lillard, 1990). While this might suggest that improvements in processing have occurred it should be noted that the data came from a single plant and should therefore be interpreted with caution.

As noted earlier simultaneous scalding and plucking provides hygiene benefits but has not been widely adopted. Lillard et al. (1987) reported that spraying carcasses with 1% acetic acid during plucking provided no significant reduction in bacterial numbers. This would be expected since the problems experienced during scalding would also affect any treatment applied during plucking.

IV. EVISCERATION

Evisceration of broilers is a fully automated process, however hand evisceration is still used for turkeys as available machinery cannot accommodate the variability in carcass size (Parry, 1989). During evisceration a circular cut is made around the vent and enlarged to permit removal of the viscera (still attached to the vent) without damage. Edible viscera are separated and pooled for washing and later packaging. Following removal of the neck in the neck cracker the carcass is spray washed inside and out to remove bacteria that may have been released during evisceration (Parry, 1989).

Although evisceration would seem to be the most likely place for contamination of the carcass with faecal material and hence with enterobacteriaceae, several studies have found no increase in the levels of enterobacteriaceae following evisceration (Parry, 1989; Lillard, 1990). Using marker organisms it has been shown that cross contamination may occur during evisceration (Mead et al., 1975), however, it is not clear whether this is significant

as spray washing after evisceration removes a significant number of bacteria from the carcass (Parry, 1989).

V. CHILLING

Due to the speed of processing there is little loss of heat from the carcasses during processing and carcasses reaching the chiller often have a temperature over 30°C (Mead, 1989). Chilling is therefore required to prevent the growth of spoilage or food poisoning organisms. Continuous in-line immersion chillers are the most widely used form of chilling, although air-chilling is used in Europe (Veerkamp, 1989). The washing effect of immersion chilling removes bacteria from the carcasses and thus may result in cross contamination. Efficiently run counter flow immersion chillers appear to reduce significantly carcass coliform and total viable counts (Bailey et al., 1987; Mead, 1989), however, the effect on the proportion of Salmonella positive carcasses is less clear (Bailey et al., 1987). Some surveys have found a decrease in the incidence of Salmonella positive carcasses during immersion chilling (Green et al., 1982; Bailey et al., 1987), while others have found an increase in the incidence (Campbell et al., 1983; Lillard, 1990). Indeed in the latter survey, the only significant increase in the incidence of Salmonella positive carcasses during processing, occurred on carcasses sampled after immersion chilling, indicating that chilling was the procedure most responsible for contamination (Lillard, 1990).

Some control of cross contamination during chilling is effected by the use of counter flow chillers. Addition of hyperchlorite to the chiller water, a procedure which is commonly used in several countries (Veerkamp, 1989), also appears to be an effective means of preventing cross contamination (Mead, 1989). Although chlorination prevents cross contamination it does not have a sanitising effect on the carcass, as chlorine is rapidly inactivated at the carcass surface (Mead, 1989). Izat et al. (1989) reported that addition of 0.5% acetic acid, 0.5% hydrogen peroxide or hyperchlorination of chiller water significantly reduced the incidence of Salmonella, however, all treatments resulted in adverse quality effects of the carcasses. Similarly, addition of slow release chlorine dioxide to chiller water has been reported to eliminate Salmonella from turkey carcasses, however, the treatment resulted in discolouration of the carcass and is not approved by health authorities (Villarreal et al., 1990).

One potential problem associated with treatments applied during immersion chilling is absorption of water by the carcass. Water absorbed during chilling may account for up to 7% of the carcass weight thus there may be a residue problem. Chlorination of chiller water for example has been shown to result in formation of mutagens (Schade et al., 1990).

VI. POST CHILLING

Following chilling the carcasses are graded by weight and packed on trays covered with plastic film or in bags (Parry, 1989). Further control of bacterial contamination, other than refrigeration to prevent microbial development, is not usually applied. The only commercially applicable procedure available for the sanitisation of processed poultry is the use of ionising radiation. Exposure of carcasses to a dose of 3kGy eliminates Salmonella and extends shelf life (Mossel,

1987). Although food irradiation has been approved by the United Nations (Mossel, 1987) and other health authorities (Elias, 1987) it has not been widely accepted due, in part, to consumer resistance (Elias, 1987; .Mossel, 1987).

Spraying beef and lamb carcasses with organic acids has proved beneficial in reducing bacterial levels (Smulders, 1987). Similarly dipping or spraying poultry carcasses with lactic acid dramatically reduced the incidence of Salmonella positive carcasses, however, effective treatments also had a deleterious effect on carcass appearance and acceptability (Izat et al., 1989). Slavik et al. (1991) reported, in a preliminary communication, that electrical stimulation reduced levels of bacteria on chicken legs. They concluded, however, that the technique was not currently applicable due to problems with muscle discolouration and the technological aspects of applying the procedure commercially (Slavik et al., 1991).

It has been reported that application of sodium diacetate powder to carcasses can be used to control contamination with Salmonella (Moye and Chambers, 1991). While this procedure clearly extends the shelf life of processed poultry, the modest decrease in the incidence of Salmonella positive carcasses demonstrated (from 33% for the control to 24% for the treated samples) seems inadequate to provide any effective control of Salmonella contamination.

A direct and simple procedure for improving the bacteriological quality of carcasses was proposed by McMeekin et al. (1984). They suggested that removal of the neck flap would extend both shelf life, since the neck flap is the most heavily contaminated part of the carcass and the region where spoilage odours are first detected, and reduce contamination with Salmonella since the neck flap constitutes the largest expanse of subcutis exposed on the carcass and several studies have shown that Salmonella adhere in high numbers to this tissue (Thomas and McMeekin, 1981; Lillard, 1988; Sanderson et al., 1991). Commercial acceptance of this procedure is however, unlikely since neck flap removal results in a reduction in yield of about 3% (McMeekin et al., 1984).

VII. CONCLUSIONS

With some notable exceptions (McMeekin et al., 1984; Slavik et al., 1991; Moye and Chambers, 1991) proposed poultry decontamination strategies have been empirically derived from procedures developed for other food products. In the main these strategies have proved ineffective either because they had no effect on Salmonella incidence or had deleterious effects on product quality. One factor contributing to the failure of these techniques is the ineffectiveness of permissible antibacterial agents in the highly complex organic milieu of scald and chiller water. A further factor, that has been largely overlooked by investigators, is the effect of skin structure on decontamination.

In an electron microscopy study of the contamination of poultry skin during commercial processing, Thomas and McMeekin (1980) noted that microbial contaminants were present in a fluid film on the skin surface and within deep skin channels and proposed that bacteria located within deep skin channels would be less accessible to physical removal than bacteria in more superficial parts of the film. While the microtopography of the outer surface of poultry skin changes as a result of water absorption (Thomas and McMeekin, 1982), very little penetration of water occurs through the skin surface (Jones and Grey, 1989). Most of the absorbed water enters into the carcass through tears in the skin and cut surfaces

and is retained by connective tissue rather than being absorbed by the skin or muscle (reviewed by Jones and Grey, 1989). Fascia (also called aureolar or loose connective tissue) underlies the dermis in poultry (Lucas and Stettenheim, 1972) and consists of a loose meshwork of collagen and elastin fibres interspersed in the viscous semifluid matrix (Davies and Davies, 1962). Absorption of water by chicken muscle fascia causes the tissue to expand forming a dense network of fibres (Thomas and McMeekin, 1981). Movement of water into the tissue may therefore facilitate transport of bacteria within this fibre meshwork where they could become entrapped. In addition to physcial entrappment several studies have demonstrated that Salmonella specifically adhere to fascia (Thomas and McMeekin, 1981; Campbell et al., 1987; Lillard, 1988; Benedict et al., 1990; Sanderson et al., 1991).

Cells attached to or physically entrapped within the fibre network or within crevices in the skin are unaffected by rinsing (McMeekin et al., 1984; Lillard, 1988; Benedict et al., 1990). Hence these bacteria represent the recalcitrant population. Effective treatment is limited by inability to exchange contaminated water with clean water or with water containing antimicrobial agents and the ineffectiveness of permissible antibacterial agents within the highly complex organic milieu of the tissue.

It follows that reduction in the incidence of Salmonella-positive carcasses and an improvement in general bacteriological quality requires the development of processing procedures to minimise water uptake by connective tissue. In the case of value added, specialty products, such as fillets where connective tissue is completely exposed, meticulous attention should be given to contact surfaces to prevent transfer to the product.

Attention should also be drawn to the potentially dangerous practice of inferring from experiments designed primarily to evaluate shelf-life extension, that a given procedure will significantly reduce the proportion of Salmonella positive carcasses. While there are many reports indicating significant increases in shelf-life as a consequence of chemical additives, no acceptable process exists for reducing the proportion of Salmonella positive carcasses. Proper evaluation of the effect on the incidence of Salmonella can only be made following a direct search for the pathogen on a large number of treated and control carcasses.

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FACTORS AFFECTING EGG FATTY ACID AND CHOLESTEROL CONTENT

T.M. SHAFEY and J.G. DINGLE

Summary

The effects of type of cereal grain (wheat, triticale, rye or oat fractions), soy oil, strain and age of the laying hen on yolk fatty acid and cholesterol concentrations were studied. The addition of soy oil to a wheat or triticale based diet has been shown to give eggs containing the healthy combination of low cholesterol and a high unsaturated to saturated fatty acid ratio. Several of the oat fractions decreased yolk cholesterol. Genetic differences in yolk cholesterol that occurred between strains of laying hens were mainly due to differences in responses in egg traits and not to responses in daily cholesterol output. Smaller eggs contained a significantly lower cholesterol content than larger eggs. Lower egg cholesterol can be achieved by choosing laying strains that are highly productive at a young age.

I. INTRODUCTION

Current health recommendations have encouraged individuals to reduce the consumption of total lipid, saturated fatty acids and cholesterol (CHL) and to increase the proportion of monounsaturated and polyunsaturated fatty acids in their diets (Walsh et al., 1975). Reducing CHL and increasing the unsaturated to saturated fatty acid ratio of eggs would increase their perceived health status. CHL and fatty acid compositions of egg yolk are influenced by diet, genetic background and age of the hen (Marion et. al., 1966; Hargis, 1988; McDonald and Shafey, 1989; Stadelman and Pratt, 1989).

II. EXPERIMENTS

The experiments described in this report were carried out on three different strains of laying hens. Birds were housed in a flat deck cages. Birds were fed the experimental diets for 11-12 weeks. Only a brief description of each experiment is supplied.

(a) The Effects of Dietary Cereal Grain and Fat

1. Comparison between wheat, triticale, rye and soy oil.

McDonald and Shafey (1989) reported that inclusion of triticale in laying hen diets tended to reduce yolk CHL concentration. Other dietary factors have been shown to affect yolk CHL and fatty acid content. Large amounts of soy oil in the diet of laying hens have affected the fatty acid composition of egg yolk (Sim et. al., 1973) but not yolk CHL (Weiss et al., 1967). The effects of wheat, triticale, rye and soy oil on yolk lipids were studied (Table 1). Pullets fed wheat or triticale based diets had lower yolk CHL concentration than pullets fed rye based

University of Queensland, Gatton College, Lawes, Queensland, 4343.

diets, however there were no significant differences between grains in yolk CHL content. There was no significant difference between the three cereals in yolk concentration of palmitic, stearic and oleic acids. Pullets fed triticale based diets had higher yolk linoleic acid concentration and lower yolk oleic:linoleic ratio than pullets fed rye or wheat based diets. The addition of 2% soy oil to the diet increased yolk linoleic concentration and yolk unsaturated to saturated fatty acid ratio but reduced yolk oleic:linoleic ratio. It was concluded that the addition of soy oil to wheat or triticale based diet gave eggs containing the healthy combination of low CHL and a high unsaturated to saturated fatty acid ratio.

Table 1. Effects of type of grain on CHL and fatty acid concentrations of egg yolks.

Type of grain	CHL Concentration (mg/g yolk)	CHL Content (mg/yolk)	Linoleic acid % 18:2%[1]	Uns/sat ratio[2]	18:1 18:2 ratio[3]
Wheat	11.5b	181.8	12.50ab	1.49b	3.03a
Triticale	11.7b	186.1	14.65a	1.58a	2.53b
Rye	12.8a	197.1	11.79b	1.47b	3.09a
LSD[4]	0.9	19.1	2.58	0.08	0.31
Oil					
Control diet	12.1	188.0	11.39	1.48	3.32
Added oil	12.0	188.4	14.57**	1.55*	2.48**
SE[5]	0.2	4.7	0.74	0.02	0.09

1 % of total methyl esters of yolk fat.
2 The proportion of unsaturated [oleic (18:1) and linoleic (18:2)] to saturated [palmitic (16:0) and stearic (18:0)] fatty acid in yolk fat.
3 The proportion of monounsaturated (oleic) to polyunsaturated (linoleic) fatty acid in yolk fat.
4 Least significant differences ($P<0.05$).
5 Standard error of the means.
ab Means within columns followed by different superscripts are significantly different ($P<0.05$).
* ($P<0.05$). ** ($P<0.01$).

2. The effect of various components of oats

Dietary oats in layer diets have been shown to reduce yolk CHL concentration (McDonald and Shafey, 1990, Moreng et al., 1990). Turk and Barnett (1971) reported that the addition of 15% oat hulls to wheat based diets reduced yolk CHL. The effects of feeding various fractions of oats (whole oats, dehulled oats, whole oats plus soy oil, dehulled oats plus oat hulls and wheat plus oat hulls) in a wheat based diet were investigated (Table 2). There was no significant difference between pullets fed the different oat fractions in daily CHL output or yolk concentration of oleic acid. Pullets fed whole oat grain had lower

yolk CHL concentration than those fed the wheat diet and the addition of soy oil further lowered yolk CHL and increased yolk linoleic acid concentration. Pullets fed the dehulled oats diet significantly increased their yolk oleic to linoleic acid ratio when compared with those fed the whole oats. The addition of oat hulls to a wheat based diet did not significantly affect any of the observations measured.

It was concluded that whole oats did not significantly alter daily yolk CHL output but did significantly decrease yolk CHL concentration. The oat fraction causing the lower CHL concentration was not identified as the inclusion of dehulled oats and oat hulls, separately or together, did not produce yolk CHL concentrations as low as did whole oats.

(b) The Effects of Age and Strain

The relationship between egg yolk CHL and age of the hen has been reported by Bair et. al. (1978) who found a decrease in yolk CHL as age of the hen increased. In contrast, Menge et al. (1974) found that yolk CHL increased with age of the hens. The relationships between egg yolk CHL and egg production, egg weight, yolk weight and age of the hen in three strains of Australian commercial layers during their laying cycle are shown in Tables 2 and 3).

Table 2. The effects of strain of laying hens on egg weight, yolk weight, rate of lay and egg CHL content.

S^t	Egg weight (g)	Yolk weight (g)	Rate of lay[1]	CHL concentration (mg/g yolk)	CHL content (mg/yolk)	CHL concentration (mg/g egg)	CHL output (mg/day)
S1	54.2b	16.2b	.89a	11.8b	190.8b	3.52	169.6
S2	58.2a	17.5a	.83b	11.9b	208.1a	3.58	172.3
S3	58.2a	16.8ab	.84b	12.6a	211.9a	3.63	177.2
LSD	1.0	1.1	.02	0.4	15.3	0.28	13.9

S^t Strain of birds. [1] egg/hen/day. ab See Table 1.

Genotypes differed in egg weight, yolk weight, rate of lay, yolk CHL concentration and yolk CHL content. Differences between strains were not significant when CHL was calculated as the concentration of the egg (mg cholesterol/g egg). Age of the hen was significantly positively correlated with egg weight, yolk weight, yolk CHL concentration, yolk CHL content and daily yolk CHL output. Age of the hen was significantly negatively correlated with rate of lay. Smaller eggs contained a significantly lower CHL content than larger eggs. Yolk CHL content at 30 weeks of age was approximately 19% lower than that produced at 56 weeks of age. The percentage increase in egg yolk CHL content of large eggs was approximately equivalent to the percentage increase in their egg and yolk weights. Significant genotype differences occured between correlated egg traits. It was concluded that differences in yolk CHL that occurred between strains of laying hens were mainly due to differences in responses in egg traits and not to responses in daily CHL output. Lower egg CHL can be achieved by choosing laying strains that are highly productive at a young age.

Table 3. The effects of age of laying hens on egg weight, yolk weight and egg yolk CHL and daily CHL output.

Age (week)	Egg weight (g)	Yolk weight (g)	CHL concentration (mg/g yolk)	CHL content (mg/yolk)	CHL concentration (mg/g egg)	CHL output (mg/day)
30	53.7b	16.2bc	11.3e	186.2d	3.48bc	161.9c
34	54.6b	16.1c	12.2bc	196.4cd	3.59abc	173.3abc
39	59.0a	17.1abc	11.5de	196.8cd	3.34c	171.3abc
47	57.6a	17.3abc	11.6cde	201.2bcd	3.49bc	164.4bc
49	58.2a	17.8abc	12.7ab	226.2ab	3.88ab	186.2ab
52	58.5a	18.0a	13.0a	232.8a	3.98a	191.9a
56	58.4a	17.9ab	12.4ab	221.8abc	3.80abc	182.2abc
LSD[4]	1.7	1.8	0.7	26.7	0.49	24.3

abc, [4] See Table 1.

III. CONCLUSIONS

Low cholesterol eggs may be produced by adopting a combination of nutrition, genetic and management means. Altering egg yolk fatty acids can be achieved by dietary means. We are now looking at the mechanisms by which lipids are transported to the yolks. Analysis of lipoprotein classes of hen blood may help us to understand these mechanisms.

IV. ACKNOWLEDGEMENT

Dr. M.W. McDonald is thanked for his valuable advice. The technical assistance of F. Gorbacz, P. Kalinowski, R. Englebright and A. Goodwin is gratefully acknowledged. This study was funded by the Egg Industry Research and Development Council.

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WHAT IS THE NEW SELECTION LIMIT TO RATE OF LAY?

B.L. SHELDON and B.H. YOO

Summary

Recent results of long-term selection experiments aimed at breaking through the old selection limit of one egg per 24 h are summarised. Selection for short interval between eggs within clutches in continuous light has resulted in a mean interval in one line of 21 h in continuous light and 23.5 h in the normal 24 h light-dark cycle. This and associated results in another line and in ahemeral cycles confirm that the old selection limit has been surmounted by this method. The foreseeable new selection limit is estimated to be 18 h in continuous light or in an 18 h ahemeral cycle, and below 22.75 h in the normal 24 h cycle, utilising only the pathway of reducing the time the egg spends in the oviduct. If the alternative pathway of earlier ovulation relative to time of oviposition is also utilised the ultimate limit is expected to be up to 5 h shorter in the normal 24 h light-dark cycle.

I. INTRODUCTION

It has been generally accepted by poultry geneticists for over 30 years that a limit to selection for rate of lay under natural cycles of light and dark would eventually be reached at one egg per 24 hours. This would occur because the endogenous circadian rhythm controlling the open period for ovulation-inducing LH release is entrained to the 24 h light-dark cycle. Therefore detection and selection of hens capable of ovulating at intervals below 24 hours is expected to be virtually impossible under these natural lighting conditions.

The reality of this "old" selection limit has been confirmed in recent years by long-term layer selection lines, research or commercial, selected under these natural conditions (see Yoo et al. 1986; Fairfull and Gowe 1990). The mean interval between eggs within clutches at peak of lay in these populations approaches closer and closer to 24 h with continued selection but does not reach it. The distribution of intervals is strongly skewed and leptokurtic, i.e. most intervals are in fact 24 h, with very few or virtually none below 24 h and the remainder in a narrow range above 24 h.

Despite the expectation and reality of this selection limit, remarkably few research groups and apparently no commercial breeding companies during the past 30 years have investigated how to overcome the problem. The few groups involved have all recognised the primary need to remove the controlling influence of the 24 h light-dark cycle, and so have studied the effect of selection for egg production or rate of lay under different lighting regimes, mainly ahemeral light-dark cycles of less than 24 h or continuous light (Abplanalp 1966, 1968; Foster 1985; Marks et al. 1968; Morris 1961; Naito et al. 1989). The most extensive and sustained of these research programs has been that of the CSIRO group, who have used continuous light as the selection environment. We have also used a

CSIRO Division of Animal Production, Poultry Genetics Unit,
P.O. Box 184, North Ryde, NSW 2113.

different primary selection criterion, namely reduced interval between eggs within clutches, measured at peak of lay. The earlier results of these experiments summarised by Sheldon et al. (1984) indicated that the "old" selection limit of one ovulation per 24 h light-dark cycle was starting to be overcome by this selection regime.

The purpose of the present report is to review the recent results of this program which confirm that the previous selection limit can be overcome by this approach. They also allow informed speculation on what the new selection limit might be under such selection regimes.

II. REVIEW OF RECENT PROGRESS IN CSIRO EXPERIMENTS

(a) Responses to Selection

The several selection lines described in Sheldon et al. (1984) have been continued under the same husbandry and selection regimes since then, i.e. for a further 8 generations of selection. The two main long-term selection lines, Australorp (AS) and Synthetic (SS), have continued to respond in this period. Their mean intervals between eggs within clutches in continuous light (CL) are now approximately 21 h in AS at the 29th generation and 22.2 h in SS at the 22nd generation. This represents a further 1.2 h in AS over the 8 generations but only 0.5 h in SS over 7 generations, indicating that the rate of response in SS has slowed. There is also some indication of a slowdown in the last three generations of AS. These further responses in mean interval have been accompanied by the expected increases in rate of lay in the test period of 4 weeks in CL and in the proportion of birds laying more than 28 eggs in 28 days in CL. In AS occasional birds now lay 35 eggs in 28 days in CL.

However, it is the performance of these lines in the normal environment (NL) of 15.25 h light:8.75 h dark which indicates the success of the method in breaking through the barrier of one egg per 24 h in that environment. The results presented in Sheldon et al. (1984) showed that the mean interval in NL starts to move slowly below 24 h only when the response in CL reaches and moves below 23 h. The relatively few experimental results on this question in recent generations (e.g. Yoo and Sheldon 1988) indicate that the mean interval in NL is reduced by about 0.25 h below 24 h for one hour of reduction below 23 h in CL. Our best estimates of current levels in NL are about 23.5 h in AS and 23.75 h in SS. The more important feature of this trend is that the flock distribution of intervals in NL, which became highly skewed and concentrated at 24-25 h as the mean approached 24 h (Yoo et al. 1986), starts to return to a normal distribution again as the mean reaches and starts to decline below 24 h. This trend has continued so that over half the hens in AS, and probably also in SS, now have mean intervals below 24 h. These correlated responses in NL are reflected in higher rates of lay in NL in both the pure lines and in crossbreds derived from them. In the absence of corrective selection for egg size and shell quality, however, these two traits have continued to deteriorate as in previous generations.

The two selection sublines ASS and SSS were derived from AS and SS at generations 18 (1980) and 15 (1983) respectively to investigate whether egg size and shell quality could be increased to previous levels or improved further, while retaining the response in mean interval. This was at that time about 23 h in CL in

both AS and SS. In 10 and 7 generations of selection respectively the mean interval in CL has been held at or just below 23 h, while egg weight has been increased by 3 to 5 grams, incidence of blood spots reduced and shell quality and brown shell colour improved. The performance of their cross bred progeny, especially in egg size, has correspondingly improved. The ASS line is the dam line for the SIRO-CT and SIRO-CB crossbred layers marketed over this period by Australian Poultry Ltd and its predecessors.

Three other selection lines had been started in 1984 from an industry White Leghorn Strain Cross (LN) base population which was much closer to the "old" selection plateau of 24 h between eggs than the base populations for AS and SS. It also had satisfactory levels of egg size, age at first egg, shell quality and body weight. The basic aims of this selection experiment were to compare selection for short interval in CL (line LN2) with selection for short interval in NL (line LN3) and with conventional selection for rate of lay in NL (line LN1). None of the important comparisons involved in this experiment had so far been investigated by anyone. While the earlier experiments with lines AS, ASS, SS and SSS had used an independent culling level approach to selection for short interval and against low egg number and aberrant patterns of lay, this new experiment used the restricted selection index of Yamada et al. (1975). The selection indices were constructed so as to hold the already satisfactory levels of egg weight and shell quality constant in each line while increasing full year rate of lay through reducing interval between eggs in CL (LN2) or NL (LN3) or selecting for part-period rate of lay in NL (LN1). Theoretically LN2 and LN3 should be 50-60% more efficient at improving full year rate of lay than LN1.

The results of the first four generations of selection in these lines appeared to follow prediction reasonably closely, but the unselected control line was not included in the comparison in those generations. When the control line was included in the fifth generation the agreement with prediction for rate of lay was not good: the response in rate of lay was significant ($P<0.05$) only in LN1, but egg weight and shell quality did not change significantly in all three lines. A repeat of this full comparison in the sixth generation confirmed little or lack of response in rate of lay in all three lines; the reason for the disagreement is still being investigated. Because the effect of CL in all base populations not previously selected in CL is to increase the mean interval between eggs by about one hour, the six generations of selection so far have been too few to allow confirmation of the prediction that line LN2 selection will allow the 24 h barrier to be surmounted while selection in LN1 or LN3 will not. However the responses so far in mean interval between eggs have been as expected from this prediction. Line LN2 selected in CL already has the lowest mean interval in both CL and NL.

(b) Physiological Basis of Selection Response In Short Interval

A key question since the beginning of these selection experiments has been whether the reduction in interval between eggs is due to earlier ovulation relative to time of oviposition or to a reduction in time spent by the egg in the oviduct. When Sheldon and Bobr (unpublished data) investigated a sample of the AS line at generation 16 it appeared that the first of these pathways had been involved, because some 30% of hens autopsied immediately after egg lay had already ovulated. At that time the established dogma was that the domestic fowl ovulates

only after egg lay (Melek et al. 1973). However, when they compared line AS with its control line in the next generation they found no difference between the lines. Both had 30% early ovulators. The selection response in AS of some 3.5 h reduction in mean interval in CL was therefore due to a reduction of 3.5 h in the time spent in the oviduct, mainly in the shell gland. Yoo and Sheldon (unpublished) have recently shown that the same basis still applies for the reduction in AS of over 5 h in mean interval in CL shown at generation 28. Preliminary observations on line SS by Sheldon and Bobr (unpublished) indicated a similar basis for the selection responses in that line. The reasons for the lack of utilisation of the first pathway in the response pattern to selection for short interval in continuous light has yet to be elucidated especially as Naito et al. (1990) suggested some reduction in time of ovulation after lay in a White Leghorn line selected for rate of lay under an ahemeral 23 h light-dark cycle.

(c) Selection for Earlier Ovulation Relative to Time of Oviposition

The finding (Sheldon and Bobr, unpublished) that both the AS selection line and its control line had a high incidence of ovulation before lay led to their further investigation of two questions. Firstly, how widespread was the tendency to early ovulation in different breeds and strains? Observations by them on a large number of such different populations at CSIRO and at the Poultry Research Centre, Roslin, Scotland, have since shown that virtually all populations have some incidence of ovulation before lay, but varying between 1% and 30%. The details will be published elsewhere. Secondly, was there a sufficient genetic component to the early ovulation phenomenon in the AS line and its control line to allow selection directly on this pathway? We therefore initiated from the AS control line a line selected for earlier ovulation relative to time of lay. After five generations the incidence of pullets ovulating before lay had doubled so that the mean time of ovulation was now 5-10 min before lay rather than 10 min after lay as in the control. By generation 8, however, no further response had occurred. In addition, no other differences between the selection line and its control could be detected except for a reduced mean interval between eggs of about 20 min at generation 5. The reality of the apparent selection plateau is being investigated.

(d) Effect of Ahemeral Light Cycles on Lines AS and SS

Because the correlated response in mean interval in NL is small compared with the primary response in CL, and continuous light is not a feasible husbandry procedure for commercial poultry flocks, we have investigated ahemeral cycles less than 24 h as an alternative management procedure for such material. The light cycles used were 23 h (15.25 L:7.75 D) or 22 h (15.25 L:6.75 D) at generations 21, 23 and 24 of AS and generation 18 of SS. The results (Yoo et al. 1987; Yoo and Sheldon 1988) show that an ahemeral cycle which approximates the mean interval of these lines in CL, i.e. their free-running endogenous rhythm, allows full entrainment of this rhythm to the light-dark cycle and maximisation of rate of lay. However, egg size is depressed in the favourable ahemeral cycle as much as it is in CL and much more than in a 24 h cycle. This is probably due mainly to a reduction in yolk size associated with the shorter ovulation cycle.

III. DISCUSSION

The recent results of the long-term selection lines AS and SS have now confirmed beyond any reasonable doubt that the physiological barrier of 1 egg per 24 h can be broken by this selection method. Therefore the old selection limit is no longer an insuperable problem. So what is the new selection limit? Can we expect an indefinite continuation of the response in these lines? While we alluded to some slowing of the rate of response in recent generations, we should also point out that the rate of response has not been uniform throughout the 29 and 22 generations of their history. It has gone through faster and slower phases. Therefore it is possible that the recent trend is a temporary one. A better indication of how much further interval between eggs can be reduced is given by the extreme individuals in the present populations. In line AS it is not uncommon now for pullets to lay 33 to 35 eggs in the 28 days of the CL test period at regular intervals of about 18 hours. Therefore we can reasonably expect that the line mean can be reduced to this level of 18 h between eggs in CL. In view of the time scale involved we can settle on 18 h as a realistic interim target.

At the current correlated rate of reduction in interval in NL, i.e. approx. 0.25 h below 24 h for each hour of reduction below 23 h in CL, the mean interval in NL will be about 22.75 h in the AS line by the time it reaches 18.0 in CL. There is some possibility that the rate of correlated response in NL might increase as the influence of the 24 h light-dark cycle diminishes further. In this case the mean interval in NL would be considerably below 22.75 h but how far is impossible to predict. The important point is that this approach allows the possibility of future progress in rate of lay in a normal environment of 24 h light-dark cycle at least at the rate that has occurred in the past 20 years of conventional selection. If the problem of egg size in the appropriate ahemeral cycle can be solved, presumably by genetic means, the selection limit in ahemeral cycles will approximate the limit in CL. There is also at least a possibility that direct selection for short interval in NL might now be more efficient than selection in CL in terms of response in NL, for lines AS and SS where the distribution and mean in NL are now sufficiently below 24h.

The discussion so far has dealt with the improvement possible as a result of selection operating only on the second of the two alternative pathways, i.e. by reducing the time the developing egg spends in the oviduct. This has been based on the assumption that the response in AS will continue to be due to utilisation of that pathway alone. The possibility of also utilising the first pathway, i.e. by selecting for earlier ovulation relative to time of oviposition, is supported only by minimal experimental evidence at this stage. The evidence comes from (i) our findings that abundant variation exists within and between populations of hens for tendency to ovulate before lay, (ii) our demonstration that some response to direct selection for earlier ovulation can be obtained, and (iii) the indication from Naito et al. (1990) that selection for rate of lay in a 23 h ahemeral cycle resulted in a reduction in mean interval which was due partly to both pathways. If the pathway of earlier ovulation proves to be exploitable, then theoretically it would be possible to produce a line ovulating up to 4.5 to 5 h before lay without running into the complication of having two eggs in the shell gland at the one time. The potential continuing improvement in rate of lay from this source for the foreseeable future would extend the new selection limit well beyond that possible from the second

pathway alone, as already discussed for CL selected material in a 24 h light-dark cycle.

It is clear that an intense and sustained research effort over a long period will still be needed to provide the scientific basis for efficient exploitation of the above areas of investigation in poultry breeding. Apart from the main directions discussed above the usual array of significant questions requiring further investigation has arisen from the existing research program. These include an unusual reduction in hatchability in sub-line ASS (Yoo and Wientjes 1991), a complex relationship between leucosis status, interval between eggs and genotype at the major histocompatibility complex (Yoo and Sheldon 1991a,b), the possible hormonal bases underlying the selection responses, and the possibility of identifying, isolating and cloning major genes involved in the selection responses. If the latter could be accomplished then the rate of application of the new technology could be accelerated by molecular transfer of the relevant gene(s) into current industry breeding lines developed by conventional methods.

As indicated in the Introduction only a few laboratories throughout the world have been involved in investigating how to break through the old barrier of one egg per 24 hours. Therefore it is by no means clear that the intense research effort still required to investigate adequately the "new" selection limit can be generated and sustained in the near future. The global trend continues in reduction of government and industry support for poultry production research generally and long-term poultry genetic studies in particular. Unless this trend is reversed the realisation of the full potential of the area of poultry science discussed in this paper may well be deferred indefinitely.

IV. ACKNOWLEDGEMENT

The work reported here has been supported in part by the Egg Industry Research and Development Council. We also thank the technical staff of the CSIRO Poultry Genetics Unit for their technical support in all of this work.

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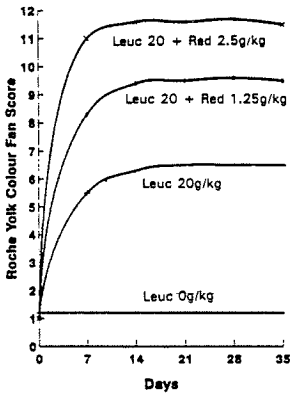
EFFECTIVE YOLK PIGMENTATION USING *LEUCAENA*

D.N. SINGH and J.S. KOPINSKI

Leucaena leucocephala is a legume shrub which grows well in the Queensland climate. It is primarily used as a protein source for grazing ruminants. *Leucaena* is also a rich source of xanthophylls and consequently has been used as a 'natural' yolk pigmenter. Tapin et al. (1981) found that 20-25g *Leucaena*/kg of diet achieved yolk colour of 7 on the Roche Yolk Color Fan (RYCF). The present study was undertaken to determine the levels of *Leucaena* and red pigment (Bio-red®) required to achieve an acceptable RYCF score (9-12) for Queensland consumers.

A total of 240 birds in each of two factorial experiments had their egg yolk colour responses examined when fed diets containing *Leucaena* with and without Bio-red®. Birds were given a nutritionally adequate basal diet consisting of wheat, sorghum, soybean and meatmeal. This diet was designed to deplete the pigment reserves of the laying hens within 3 weeks. In the first experiment, groups of 40 individually caged birds were fed the basal diet supplemented with one of six levels of *Leucaena* (0-25g/kg). In the subsequent experiment following depletion, groups of 40 birds each were fed either 20 or 25g *Leucaena*/kg of diet with Bio-red® (at 0.6, 1.25 or 2.5g/kg). Yolk pigmentation was assessed independently by two observers by weekly RYCF scoring.



The results indicate that effective yolk pigmentation (RYCF score of 9-12) using *Leucaena* can only be achieved with the further addition of a red pigment source. In this study the desired RYCF score was achieved using *Leucaena* at 20-25g/kg of diet with Bio-red® at 1.25-2.5g/kg of diet. Further studies are continuing on alternative 'natural' red pigment sources which can be used in combination with *Leucaena* to achieve optimum yolk pigmentation.

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URIC ACID EXCRETION FROM BIRDS DOSED WITH CYCLOPIAZONIC ACID

S. SUKSUPATH*, Y. MOLLAH*, R.J. COLE** and W.L. BRYDEN*

Cyclopiazonic acid (CPA) is a mycotoxin produced by several Penicillium and Aspergillus spp. Previous studies (Suksupath et al. 1990) indicate that birds receiving CPA had decreased protein digestibility but the digestibility of individual amino acids was not affected except for glycine. It is possible that the poor protein digestibility might reflect kidney dysfunction following CPA dosing and a concomittant increase in uric acid excretion. This study was designed to examine this possibility.

Twenty seven, six week old, broiler chickens were divided into three groups of nine on the basis of body weight and dosed for five days with either 0, 1.5 or 3.0 mg/kg body weight. All chicks were fed a semi-purified diet. Excreta were collected throughout the experiment and on day six, blood and lower ileal contents were also collected. Excreta were analysed for uric acid and amino acids and the latter analysis was also conducted on plasma and intestinal contents. The results in the table show mean + standard error.

CPA (mg/kg BW)	Excreta Uric Acid (g/kg DM)	Excreta Glycine (g/kg DM)	Plasma Glycine (umol/ml)	Ileum Glycine (g/kg DM)
0	104±9	28.0±2.0	0.73±0.05	3.2±0.22
1.5	127±6	30.2±1.4	0.76±0.03	3.1±0.51
3.0	157±17	39.4±6.4	0.62±0.06	3.6±0.51

Uric acid concentration in excreta was significantly ($P<0.05$) increased by CPA and this was reflected in a significant correlation ($r^2 = 0.87$) between excreta uric acid and glycine concentrations. Although both the glycine and total amino acid concentrations in excreta appeared to increase, the changes were not significant. Similarly there were no changes in the levels of glycine in the lower ileum or in plasma. The increase in uric acid excretion following dosing with CPA suggests that the associated increase in excreta nitrogen content ($r^2 = 0.79$) is the result of kidney dysfunction. Dorner (1982) has reported mild kidney tubular degeneration in broilers consuming CPA contaminated diets.

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* Department of Animl Science, The University of Sydney, Camden, NSW, 2570
** ARS, USDA, National Peanut Research Laboratory, Georgia, U.S.A.

DIETARY PROTEIN LEVEL AND NITROGEN EXCRETION FOR LAYERS

J.D. SUMMERS

With the ever increasing concern about environmental pollution the animal industry is beginning to look more at requirements to optimise rather than maximise product output. This is especially true for nitrogen and phosphorus, two nutrients considered environmentally unfriendly if high concentrations find their way into ground water.

Dietary protein levels ranging from 190 to 90 g/kg were fed to laying hens from 36 to 56 weeks of age and nitrogen excretion determined during the last week of the study. The nitrogen (dry weight basis) in the excreta was reduced, in a linear manner, from 65.5 to 36 g/kg for the 170 versus the 90 g/kg protein diet. Values for nitrogen excretion were 1.99 versus 0.57 g/d/d. Egg mass for the 110 g/kg protein diet was reduced by 2.7% versus the 190 g/kg protein diet. However, nitrogen excretion was reduced by 44.7%.

In a similar study 45-week old layers were fed diets containing 190 to 70 g protein/kg with methionine balanced in relation to dietary protein level. Nitrogen excretions were 63.9 versus 26 g/kg dry excreta and 2.13 versus 0.56 g/d/b; nitrogen excretion was reduced linearly. Comparing the egg mass output per day for the 170 versus the 110 g/kg protein diet showed a 9% decrease. However, a similar comparison for nitrogen excretion per day showed a 52% reduction.

Pullets fed diets containing 4.0 versus 2.0 g available phosphorus/kg (5.0 and 3.7 g total phosphorus/kg) gave similar egg production to approximately 30 weeks of age after which performance decreased with the low phosphorus diet. Phosphorus excretion between 31 and 32 weeks of age was 0.47 versus 0.28 g/b/d for the high versus the low phosphorus diets. Approximately 75% of the phosphorus intake appeared as apparent excretion.

The data presented suggest that a significant decrease in nitrogen and phosphorus excretion may be achieved with present-day laying hens, with little change in product output, with closer examination of nutrient requirement levels.

If the cost of disposing of poultry wastes become a reality, in the cost of production formula, then the emphasis would probably be stimulated to shift to optimum rather than maximum product output per bird per day.

Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario NIG 2WI, Canada.

HETEROTIC, MATERNAL AND SEX-LINKED EFFECTS ON PRODUCTION TRAITS IN LINES OF CHICKENS SELECTED FOR HIGH OR LOW FATNESS

H. SUTEDJO, R.A.E. PYM and W.A. PATTIE

Overall response to selection for leanness in chickens, can be optimised by exploiting additive genetic variation together with non-additive genetic and environmental effects upon the trait.

In the present study, two lines of chickens selected for six generations for high (line F) or low (line L) abdominal fat, using special abdominal fat calipers (Pym, 1987) were used. Twelve males per line were each mated to one female from each line to produce in one hatch, about 25 progeny within each of the four pure or crossbred classes. Unselected control line birds were also tested. The aim of the study was to estimate heterotic, maternal and sex-linked effects on several broiler performance traits. Maternal effects were estimated in the male progeny and the sex-linked effects, estimated in the female progeny, were adjusted for maternal effects.The line means for each trait are given in the table below.

Within sex means for 8-(8WW, g) week weight, 3 to 8 week food consumption (FC, g) and FCR and fat caliper measure (CM, mm) for birds in the five pure/cross line groups together with heterotic, maternal (FL-LF, i.e. L♀♀-F♀♀) and sex-linked (FL-LF, i.e. F♂♂ - L♂♂) effects expressed in the above units and, in parenthesis, as percentage changes.

	8WW		FC		FCR		CM	
Line Sex	M	F	M	F	M	F	M	F
FF	1379	1332	2580	2581	2.7	2.9	9.5	11.3
LL	1700	1402	3092	2634	2.3	2.6	3.3	4.1
FL	1730	1412	3105	2674	2.4	2.6	5.4	6.5
LF	1662	1344	2998	2537	2.5	2.7	5.8	6.1
CC	1642	1372	3007	2600	2.4	2.6	5.0	5.9
Effects:								
Heterotic	84(6)		107(4)		-.08(-3)		-1.1(-16)	
Maternal	63(4)		99(3)		-.07(-3)		-0.5(-8)	
Sex-linked	13(1)		50(2)		-.06(-2)		0.8(12)	

There were significant heterotic effects for 8WW ($P<0.05$) and caliper measure ($P<0.01$), but for sex linkage the only trait approaching significance was caliper measure ($P<0.08$). Maternal effects for all traits were not significant ($P>0.05$). Abdominal fatness thus appears to exhibit substantial negative heterosis.

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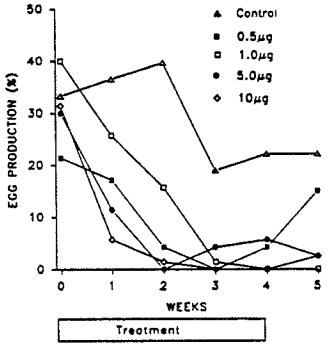
Department of Farm Animal Medicine and Production, The University of Queensland, St. Lucia 4072, Qld, Australia.

EFFECT OF A GnRH AGONIST ON EGG PRODUCTION IN BROILER BREEDERS

A.J. TILBROOK*, P.J. EASON* and R.J. JOHNSON**

In laying hens chronic treatment with an agonist of gonadotrophin releasing hormone (GnRH) desensitises the pituitary gland to endogenous GnRH with a consequent reduction in the secretion of luteinizing hormone (LH) and oestradiol and a short-term reduction in egg production (Tilbrook et al. 1992). Our aim was determine if egg production would also be reduced in broiler breeders following treatment with a GnRH agonist.



Broiler breeders were divided into 5 groups of 10. Mini-osmotic pumps were used to administer saline (control) or the GnRH agonist ({D-trp^6-pro^9 N-ethyl amide}GnRH) for 4 weeks. The doses of agonist were 0.5, 1.0, 5.0 and 10μg/kg liveweight/day. Egg production from all birds and plasma LH from 5 birds in each group were measured weekly from before treatment to one week after treatment.

Treatment with the GnRH agonist caused cessation of egg production. The 1.0, 5.0 and 10.0μg/kg/day doses of agonist were similarly effective in suppressing egg production and were more effective than the 0.5μg/kg/day dose. One week after treatment, egg production was similar to before treatment for the 0.5μg/kg/day dose but was still significantly ($P<0.05$) suppressed in the other treatment groups. The concentrations of plasma LH were also significantly ($P<0.05$) suppressed in birds treated with the GnRH agonist but these effects were not as marked as the effects on egg production. The greatest suppression of LH occurred in the birds treated with the 10μg/kg/day dose of GnRH agonist.

These data show that, similarly to laying hens, constant treatment of broiler breeders with a GnRH agonist suppresses plasma LH and egg production. An understanding of the endocrine control of egg production may form the basis to develop methods of improving egg production in broiler breeders.

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* Victorian Institute of Animal Science, Attwood Vic. 3049.
** Rhone-Poulenc Animal Nutrition, West Footscray, Vic. 3012.

ANONYMOUS CLONES AS MARKERS FOR CHICKEN GENOME MAPPING

A.A. TOYE*, C. MORAN*, F.W. NICHOLAS* and B.L. SHELDON**

Molecular biology has made it easier to study, understand and manipulate the chicken genome. Knowledge gained from such research will be applied to the production of healthier chickens that yield cheaper and better meat and eggs. Studying, understanding and manipulating the genome is made so much easier when a fine-scale genome map is available. A map of the chicken genome already exists. However, problems associated with the map include its incomplete coverage (of the genome) and a bias towards morphological markers (Van Hest et al. 1992). For a chicken genome map to be of major use in practical breeding and research, around 200 evenly spaced markers are required, with each marker existing in several variants, i.e. being polymorphic, in most populations. The only way that this can be achieved is with DNA markers. Markers are ordered on the map by observing their co-segregation during meiosis, in specially designed back-cross or inter-cross populations. Ideally, each DNA marker would correspond to a structural gene of known function. However, the limited number of genes that have been isolated in chickens coupled with the low level at which they detect polymorphisms when used as probes, justify a search for alternative approaches to map development.

One approach is to take pieces of chicken DNA that have no known function (hence anonymous) and to use them as probes for mapping. Such probes are obtained by individually screening large numbers of bacteria which are engineered to contain random pieces of chicken DNA (so-called clones). Clones that contain repetitive sequences present a complicated picture when used in mapping; it is therefore desirable to avoid them where possible. Such clones are identified through their intense hybridisation to ratio-labelled genomic DNA. Clones that do not contain repeated DNA are then tested to see if they identify polymorphisms in the chicken population intended for use in mapping. This is done by conventional restriction fragment length polymorphism (RFLP) analysis.

By screening 250 chicken DNA clones, we have produced ten probes that identify single, polymorphic marker loci. More than 20 other probes which identify single loci have also been produced, but these are not polymorphic.

We are currently searching for more clones that identify useful markers. Additionally we are using the polymerase chain reaction (PCR) to identify random amplified polymorphic DNA (RAPD) and microsatellite markers, as they tend to be much more polymorphic than RFLPs.

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* Department of Animal Science, University of Sydney,Sydney, NSW 2006
** Poultry Research Unit, CSIRO Division of Animal Production, North Ryde, NSW 2113

LINKAGE MAPPING OF THE CHICKEN GENOME

B.J. VAN HEST*, P.L. MOLLOY** and B.L. SHELDON*

A comprehensive linkage map of the chicken genome would provide information on associations between gene region and production traits of value in chicken breeding programmes. The most recently prepared map, however, comprises 50 loci contained in only six linkage groups (Bitgood and Somes, 1990). This map was derived mainly from studies of the co-inheritance of certain phenotypic traits. In the Poultry Gene Mapping Project established by the CSIRO's Division of Animal Production, and aimed at extending the current linkage map, co-inheritance of genes at the molecular level is to be studied. This approach will provide an efficient means of establishing linkages, especially between genes that product no phenotypic effect.

A resource population was established specifically for the project. Two genetically diverse chicken lines, a high rate of lay Australorp and a meat chicken male line, were initially crossed. Individuals from the F1 generation were intercrossed to establish 12 large half-sib families. The F2 generation, comprising about 1000 individuals, is being assayed for production characteristics such as egg weight and carcass composition.

Since DNA is required for the linkage analyses, blood samples from relevant individuals in all three generations are stored for DNA extraction as required.

Linkages are to be determined using restriction fragment length polymorphism (RFLP) technology (Botstein et al., 1980) and a comparative mapping approach (i.e. genes linked in other species, such as man and mouse, are to be examined for concordant linkage in the chicken). More than 50 genes have been obtained from national or international laboratories and include the chicken genes for luteinizing hormone, growth hormone, oestrogen receptor and feather keratin. Since RFLPs are restriction enzyme specific, eleven enzymes have been used routinely to screen members of the parental generation for gene/enzyme combinations that result in polymorphisms.

RFLPs have been found for 13 of 17 genes screened, including ovalbumin, avian myelocytomatosis virus oncogene, growth hormone and oestrogen receptor. Seven three-generation families, chosen according to full-sib numbers in the F2, are now being examined for segregation of these RFLPs. Pedigrees are being compiled for each family at each gene locus and compared. No linkage has been identified between the three genes, feather keratin, growth hormone and vitellogenin, so far examined.

We wish to thank the Chicken Meat and Egg Industry Councils for their funding support.

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* CSIRO Division of Animal Production, North Ryde, NSW, 2113

** CSIRO Division of Biomolecular Engineering, North Ryde, NSW, 2113

THE CONTROL OF CALCIUM AND PHOSPHORUS METABOLISM BY THE KIDNEYS

ROBERT F. WIDEMAN

Summary

The kidneys co-regulate calcium (Ca) and inorganic phosphorus (Pi) metabolism by excreting these minerals when they are present in excess and retaining them when a deficit exists. Parathyroid hormone (PTH) is secreted in response to low plasma Ca, and acts on the kidneys to decrease Ca excretion and increase Pi excretion. PTH also accelerates the renal activation of vitamin D. The active metabolite of vitamin D enhances intestinal absorption of Ca and Pi, but has uncertain effects on renal Ca and Pi transport. Several other factors contribute to the variability in renal Ca and Pi transport, including: dietary Ca/Pi ratios; acid-base balance; and, kidney damage associated with infectious bronchitis virus or mycotoxins. The influence of maturation and genetics remain to be clarified, although circumstantial evidence exists for age- and strain-related differences in renal Ca and Pi transport.

I. INTRODUCTION

Because preliminary analyses indicated that calcium (Ca) concentrations can be relatively low in the urine of laying hens, several investigators assumed that the kidneys of domestic fowl do not make a dynamic contribution to overall Ca metabolism (Etches, 1987; Hurwitz et al., 1987). This viewpoint is flawed because it fails to acknowledge the highly variable nature of urinary Ca excretion. It also fails to account for the large quantities of plasma ionized Ca (Ca^{++}) constantly undergoing glomerular filtration, and it fails to acknowledge the importance of renal co-regulation of Ca and inorganic phosphorus (Pi) metabolism (Wideman, 1987). For example, individual hens have variable diurnal rates of urinary Ca excretion, depending on: their nutritional status (G.I. tract empty or full); the presence of particulate Ca sources in the diet (oystershell or limestone "grit"); and, the stage of eggshell formation (Fussell, 1960; Wideman, 1987). A number of additional factors also can significantly influence urinary Ca and Pi excretion, including nutritional variations in Ca/Pi or cation/anion (acid/base) ratios, and variations in kidney function resulting from disease, mycotoxins, age (maturation) or genetics (Glahn et al., 1988, 1989, 1991; Hnatow and Wideman, 1985; Wideman et al., 1985, 1989; Wideman and Satnick, 1989). The objective of this paper is to summarize our current understanding of the renal influence on avian Ca metabolism, and to emphasize the fact that Ca and Pi metabolism are intimately co-regulated.

Department of Poultry Science, The Pennsylvania State University, University Park, PA 16802.

II. FILTRATION, REABSORPTION AND EXCRETION

Based on estimates that adult domestic fowl produce 2ml of glomerular ultrafiltrate per kg body weight per minute, it can be calculated from average plasma volumes that the entire plasma Ca^{++} and Pi content will be processed by the kidneys and thus potentially could be excreted every 20-25 minutes (Wideman, 1987). The simplest view is that the kidneys are designed to stabilize plasma Ca and Pi by functioning as an "overflow" system. According to this admittedly oversimplified model, the kidneys respond to increases or decreases in plasma Ca^{++} and Pi concentrations by increasing or decreasing the urinary excretion rates for these minerals. For example, assuming the glomerular filtration rate (GFR) remains constant, an increase in the concentration of plasma ionized Ca^{++} ($[Ca^{++}]p$) causes an increase in the rate at which Ca is filtered into the renal tubules (calculated as: Ca Filtration Rate = $[Ca^{++}]p$ x GFR). If tubular maximal reabsorptive rates (TmR) limit the rate at which filtered Ca^{++} and Pi can be returned to the plasma, then filtration rates exceeding the TmR will result in increased urinary excretion (calculated as: Excretion Rate = Filtration Rate - TmR). Indeed, when intravenous infusions were used to artificially elevate plasma levels of Ca^{++} and Pi, concomitant increases in urinary Ca and Pi excretion were triggered without significantly altering the GFR (Wideman and Braun, 1981; Wideman et al., 1989). Consequently, small changes in plasma Ca^{++} and Pi concentrations have a profound impact on urinary Ca and Pi excretion rates. Conversely, small changes in the tubular reabsorptive rates for Ca (TmR Ca) or Pi (TmR Pi) can have an immediate impact on plasma Ca and Pi concentrations, and on overall Ca and Pi balance.

II. ROLE OF PARATHYROID HORMONE

Parathyroid hormone (PTH) exerts primary control over the tubular reabsorption rates (TmR) for Ca and Pi (Wideman, 1987). Low plasma Ca^{++} stimulates the parathyroid glands to secrete PTH, which acts on the kidneys to reduce urinary Ca excretion while simultaneously increasing urinary Pi excretion (Wideman and Braun, 1981; Wideman, 1984). These renal responses to PTH are best understood by integrating them with the responses of other systems involved in maintaining Ca homeostasis. For example, during the 20 hr required to form an eggshell, the shell gland removes 2200mg of Ca^{++} from the plasma. This rate of Ca utilization quantitatively necessitates the complete turnover of all plasma Ca^{++} every 2 to 3 minutes. When this rate of Ca utilization cannot be sustained by intestinal absorption, plasma Ca^{++} concentrations decline and PTH is secreted. PTH acutely supports a return to normocalcemia by minimizing urinary Ca excretion (increasing the TmR for Ca), and by mobilizing bone mineral. The decline in plasma Ca^{++} further reduces urinary Ca excretion by reducing the filtered load of Ca (Wideman, 1987). PTH also stimulates the renal production of the active vitamin D metabolite 1,25-dihydroxycholecalciferol ($1,25[OH]_2D_3$), which promotes increased intestinal absorption of Ca and Pi. In addition, $1,25[OH]_2D_3$ enhances the PTH-induced mobilization of Ca and Pi from bone mineral.

PTH-induced mobilization of bone mineral adds both Pi and Ca to the plasma in a ratio of 1Pi:2.5Ca, representing an "excess" release of Pi that cannot be incorporated into whole eggs having a ratio of 1Pi:20Ca (Wideman, 1987). The potential therefore exists for Pi to accumulate in the plasma (hyperphosphatemia), resulting in a further depression of plasma Ca^{++} when the $[Ca^{++}]x[Pi]$ solubility product constant is exceeded (Wideman et al., 1980). However, hyperphosphatemic depression of plasma Ca^{++} normally is avoided because PTH acts on the kidneys to increase the rate of urinary Pi excretion (Wideman et al., 1980; Wideman, 1987).

It is important to recognize in this complex scheme that Ca and Pi metabolism are inseparably co-regulated by several endocrine systems and target tissues. Only the renal response to PTH provides an opportunity for poultry to dissociate the directional fluxes of Ca and Pi by increasing Pi excretion while reducing Ca excretion. Once excreted in the urine as a "waste" product, Pi serves an important secondary role as the major urinary buffer available to absorb acid (H^+) produced by the shell gland during the formation of carbonate for the eggshell (Hodges, 1969). This excreted Pi eventually must be replenished from nutritional sources during the non-calcifying phase of eggshell formation, otherwise skeletal re-mineralization cannot occur, and net bone demineralization will ensue.

III. INFLUENCE OF DIETARY CALCIUM/PHOSHORUS RATIOS

In poultry as in other animals, large changes in Ca or Pi intake are directly correlated with changes in their rates of urinary excretion (Wideman et al., 1985, 1989; Stanton et al., 1989). This would be expected if the kidneys are viewed in terms of the simple TmR limited overflow model presented in section I above, particularly with the inclusion of PTH as an endocrine modulator of TmR values. For example, excessive intestinal absorption of Ca elevates plasma Ca^{++}, resulting in an increase in urinary Ca excretion due both to an increased filtered load of Ca, and to a reduced rate of PTH secretion by the parathyroid glands (lower tubular reabsorption of Ca). In fact, the expected acute hypercalciuric response to decreased PTH secretion has been reproduced experimentally by surgically removing the parathyroid glands (Wideman, 1987; Clark and Wideman, 1990).

Inhibition of PTH secretion cannot solely account for the fact that the hypercalciuric response of poultry fed high dietary Ca is greatly amplified by low dietary available Pi (aP) (Wideman et al., 1985, 1989; Wideman, 1987). Aside from the indirect consequences of solubility interactions between Pi and Ca^{++} in plasma (Wideman et al., 1980), neither dietary aP nor plasma Pi concentrations are known to directly influence PTH secretion. Instead, the available evidence currently indicates that low aP, probably "sensed" as low plasma or intracellular Pi, stimulates the renal synthesis of $1,25[OH]_2D_3$. This active vitamin D metabolite tends to normalize plasma Pi by enhancing intestinal Pi absorption. Unavoidably, $1,25(OH)_2D_3$ also further increases intestinal Ca absorption in spite of the fact that "excess" dietary Ca already was available to the bird. When additional Ca flows from the intestine into the plasma, urinary Ca excretion increases due to the increased filtered load of Ca, and due to hypercalcemic suppression of PTH secretion (Wideman, 1987; Wideman et al., 1985, 1989). Both of these influences probably contribute to the capacity of low aP diets to amplify urinary Ca excretion. Possible direct effects of vitamin D metabolites on tubular Ca and Pi transport

remain unknown.

Because diets containing low aP and high Ca "flood" the bird with Ca, such diets have been used successfully to improve eggshell quality and improve bone strength in ageing hens. Evidently the high rate of intestinal Ca absorption eliminates the need for hens to utilize bone mineral during eggshell calcification, thereby reducing the requirement for dietary aP to replenish bone mineral. These diets also flood the urine with Ca, tending to predispose hens to Ca-induced kidney damage (urolithiasis) and excessive flock mortality (Glahn et al., 1988; Wideman and Lent, 1991; Wideman et al., 1985; 1989).

IV. EFFECT OF METABOLIC ACIDOSIS

The kidneys maintain acid-base balance by excreting excess acid (H^+) produced as a byproduct of metabolism. Most H^+ excretion is buffered by other metabolic wastes, such as Pi ($PO_{4=}$), sulfate ($SO_4^=$) and ammonia (Wolbach, 1955; Craan et al., 1982; Long and Skadhauge, 1983). Metabolic acidosis tends to develop in hens as a normal metabolic consequence of eggshell formation. In addition to normal metabolic processes, the acid-base status of poultry also can be influenced by dietary proportions of "fixed" cations and anions, which generally can be calculated from the intake of Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, $PO_4^=$ and $SO_4^=$ (Austic and Patience, 1988). For example, diets high in Ca and low in Pi ($PO_4^=$) have an elevated cation:anion ratio, increasing (alkalinizing) the pH of blood and urine (Wideman et al., 1989). The increase in urine pH accelerates Ca-induced kidney damage when high Ca low aP diets are fed to poultry (Wideman et al., 1989). Overall, the kidneys stabilize blood pH by adjusting the excretion rates of H^+, fixed cations and fixed anions.

When metabolic acidosis develops as a result of nutritional imbalances or renal inadequacy, several aspects of Ca and Pi metabolism can be altered (Austic and Patience, 1988; Wideman, 1987; Wideman and Buss, 1985 a, 1985b; Wideman et al., 1989). Acidosis directly inhibits carbonate formation by the shell gland, potentially resulting in thin eggshell formation. Acidosis directly inhibits vitamin D activation by the kidneys, potentially reducing intestinal Ca and Pi absorption. Acidosis increases bone mineral solubility, thereby accelerating skeletal demineralization and increasing the filtered load of Ca and Pi. Acidosis titrates Ca from anionic binding sites on plasma proteins and organic molecules, increasing the proportion of total plasma Ca occurring in the ultrafilterable ionized form (Ca^{++}), thus further increasing the filtered load of Ca. Acidosis also appears to increase urinary Ca excretion by directly inhibiting tubular Ca reabsorption. For example, urinary Ca excretion was significantly increased during Ca loading studies when pullets were fed diets supplemented with high levels of methionine. Excess methionine is metabolized to release $SO_4^=$ in the form of sulfuric acid, causing metabolic acidosis (Wideman et al., 1989). In a separate experiment using six week old pullets, acute intravenous infusions of 0.2N HCl increased urinary Ca excretion and decreased Pi excretion (Glahn et al., 1988). Obviously the acid-base status of poultry can significantly influence the renal regulation of Ca and Pi metabolism.

V. EFFECT OF KIDNEY DAMAGE

Because the kidneys regulate both mineral metabolism and acid-base balance, kidney dysfunction has the potential to profoundly influence Ca and Pi balance. For example, infectious bronchitis virus (IBV) can cause significant reductions in the number of nephrons per kidney (Niznik et al., 1985; Wideman and Cowen, 1987), and significant "hidden" kidney damage can occur under field conditions (Wideman, 1989). During the course of investigating a kidney damage problem attributed to the Arkansas strain of IBV, affected broiler breeder hens were found to exhibit symptoms of metabolic acidosis and they had significantly increased rates of urinary Ca^{++} excretion when compared with unaffected hens (Wideman and Satnick, 1989). Several studies have demonstrated that kidney damage due to IBV or mycotoxins such as citrinin, ochratoxin and aflatoxin can result in altered plasma levels or urinary excretion rates of Ca and Pi (Glahn et al, 1988; Glahn et al., 1989; Glahn et al., 1991; Hnatow and Wideman, 1985; Wideman and Satnik, 1989). Kidney damage caused by aflatoxin also can inhibit renal activation of vitamin D (Glahn et al., 1991).

VI. MATURATION AND GENETICS

At the present time, relatively little information is available concerning maturation of the renal capacity to regulate Ca and Pi metabolism. It is known that profound changes occur in the structure of domestic fowl kidneys between the time of hatch and 30 weeks of age (Wideman, 1989). Interestingly, PTH stimulates Pi excretion but has little influence on Ca excretion in chicks (Clark and Mok, 1986; Clark and Wideman, 1990). However, the normal influence of PTH on both urinary Ca^{++} and Pi excretion is fully established in pullets, cockerels and adult hens (Kissell and Wideman, 1985; Koch et al., 1984; Laverty and Wideman, 1985; Wideman and Youtz, 1985). Patterns of urinary Ca excretion also change as birds reach reproductive maturity. For example, ten week old pullets responded to high Ca low aP diets with a significant increase in urinary Ca excretion, but Ca excretion was not significantly elevated when sexually maturing 18 week old pullets were fed the same diet (Glahn et al., 1988). Presumably the older pullets conserved extra Ca during the formation of medullary bone. High Ca low aP diets apparently do not trigger the same degree of hypercalciuria in laying hens compared with immature pullets (Rao and Roland, 1990; Rao et al., 1991), presumably because much of the "excess" Ca is used by laying hens for eggshell formation.

Evidence for a genetic influence on renal function remains circumstantial at the present time. Different commercial strains of domestic fowl are known to possess significant differences in kidney structure (Wideman and Nissley, 1992). The same commercial strains also exhibit strikingly different degrees of Ca-induced kidney damage when raised on high Ca diets (Wideman and Lent, 1991). These observations suggest that different strains may have quantitative if not qualitative differences in their tubular transport mechanisms for Ca and Pi.

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MEASUREMENT OF METABOLISABLE ENERGY FOR POULTRY FEEDSTUFFS BY NIR SPECTROSCOPY

W.R.WINDHAM*, P.C.FLINN** and R.J.JOHNSON***

Summary

Near infrared reflectance (NIR) spectroscopy was used to predict apparent metabolisable energy (AME) in poultry feed ingredients. Standard errors (SE) for the prediction of AME varied from 0.38 MJ/kg dry matter (DM) for cereals to 1.15 MJ/kg DM for a combined population of cereals and animal and vegetable protein meals. These data indicate that NIR can be used to measure AME of poultry feed ingredients, particularly cereals. Further work is needed to refine the equations and to incorporate additional samples of animal and vegetable protein meals.

I. INTRODUCTION

Metabolisable energy (ME) is one of the most expensive components in a diet and has a critical bearing on animal performance. The determination of ME is a complex, time consuming and expensive procedure. In the case of poultry feeds, bioassays are used, ranging from the conventional method taking 9d to the 3d rapid method of Farrell (1978). Despite the development of rapid in vivo assays of apparent ME (AME) for broiler (Johnson 1987) and adult (Farrell 1978) poultry, the industry requires an in vitro estimate of AME for immediate assessment of feed ingredients.

Near infrared reflectance (NIR) spectroscopy is widely used for the analysis of quality components in agricultural samples. The ability of NIR to determine these components is based on the fact that each of the major chemical components in a feed sample has near infrared absorption properties which can be used to differentiate one component from the others. A derived mathematical relationship between the optical and chemical data is used to measure various properties of similar unknown samples. The major advantages of NIR include simplicity of sample preparation and speed of analysis. The present study was conducted to assess the NIR technique for measurement of in vivo AME in a range of feed ingredients for both broiler chickens and adult cockerels. A preliminary report on this work was given by Johnson et al. (1991).

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* US Dept. of Agriculture, Agricultural Research Service, Athens, Georgia 30613, USA.
Present Address: Pastoral Research Institute, Hamilton, Vic. 3300.
** Dept. of Agriculture, Pastoral Research Institute, Hamilton, Vic. 3300.
*** Rhone Poulenc Animal Nutrition, 19 Paramount Road, West Footscray, Vic. 3012.

II. MATERIALS AND METHODS

One hundred and thirteen samples of feed ingredients, including cereals (N=38), cereal by-products (N=12), vegetable protein meals (N=29), and animal protein meals (N=34) were assayed for AME using rapid broiler and adult cockerel assays (Johnson 1987). The AME values varied from 5.8 to 15.8 MJ/kg DM. Approximately 2 g of each sample was packed in NIR reflectance sample cells, and NIR spectra were obtained using an NIR Systems 6250 scanning monochromator coupled to a personal computer. Spectra consisted of 700 absorbance values, measured as log 1/R (where R = reflectance), between the wavelengths of 1100 and 2498 nm, at 2 nm intervals. NIR spectra were collected and equations developed using Infrasoft International (ISI)[1] software, which utilises the technique of partial least squares (PLS) regression. Each feed sample was ranked according to its Mahalanobis distance from the average spectrum of all 113 samples using the program CENTER (Shenk and Westerhaus 1991).

III. RESULTS

The first histogram (Fig. 1) shows seven samples as outliers due to extreme standardised H statistic values (i.e. Mahalanobis distance). Samples with values greater than 3.0 were spectrally different from the average spectrum. These included two samples each of corn gluten meal, sunflower meal and blood meal, and one sample of cottonseed meal.

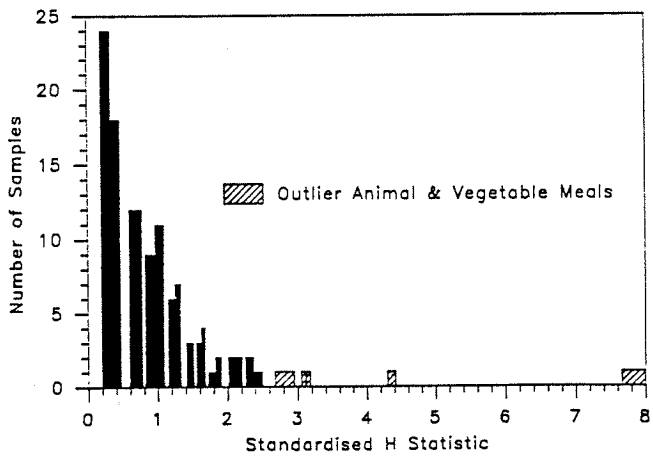

Figure 1. Histogram of sample distribution according to the Mahalanobis distance from the average spectrum of all samples (N=113).

[1]Infrasoft International, 109 Sellers Lane, Port Matilda, PA, USA

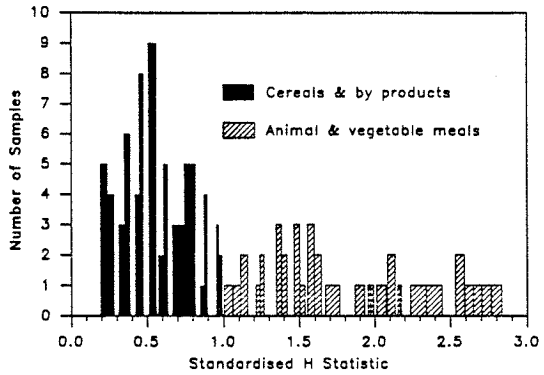

Figure 2. Histogram of sample distribution according to the Mahalanobis distance from the average spectrum (N = 106) after the removal of the seven outlier samples in Figure 1.

Samples were again ranked according to their Mahalanobis distance (Fig. 2) after elimination of the animal and vegetable meals in Fig. 1 which were outliers. Although Fig. 2 indicates no outlier samples, it is an example of a population with many extreme samples. The cereals and cereal by-products contained more samples at a given standardised H value and were separated from the animal and vegetable meals.

Clear differences were apparent between the log 1/R spectra of the seven outlier samples and the average spectra of the remaining samples (Fig. 3).

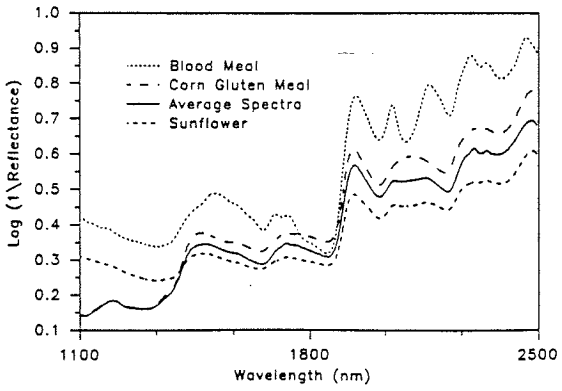

Figure 3. The log (1/R) spectra of the outlier samples (Fig. 1) and the average spectra of the remaining samples.

The calibration statistics for measurement of AME are shown in Table 1.

Table 1. Prediction of in vivo AME (MJ/kg DM) in poultry feed ingredients by NIR spectroscopy.

Population	N[A]	Broiler SECV[B]	Broiler R^{2C}	Adult SECV[B]	Adult R^{2C}
All	116	0.96	0.82	1.15	0.76
All minus outliers	106	0.67	0.89	0.56	0.92
Cereals	38	0.41	0.68	0.38	0.69

[A] Number of samples [B] Standard error of cross validation
[C] Coefficient of determination

Standard error of cross validation (SECV) in this case refers to the error measured when every fourth sample was set aside for validating the calibration derived using all other samples, such that each sample in turn was used in both calibration and prediction. The SECV values were highest when all 116 samples were present in the calibration. Elimination of the seven outlier samples (Fig. 1) reduced SECV to 0.67 and 0.56 MJ/Kg DM respectively for broilers and adult cockerels.

Calibration using cereal grains alone reduced SECV by 39% and 32% respectively. The R^2 values for cereals were low due to the limited range of AME in the cereal population. The range and standard deviation of AME values was 11.1 to 13.7 and 0.73 MJ/kg DM for broilers, and 11.8 to 14.0 and 0.68 MJ/kg DM for adult cockerels.

IV. DISCUSSION

NIR relies heavily on the collection of an appropriate population of samples for calibration. Accurate calibrations are based on samples representative of the product or feedstuff of interest. The CENTER algorithm was developed to define a population. Populations containing a number of samples extremely different from the majority (Fig. 1) will increase calibration and validation error. Although the extreme samples in Fig. 1 were part of the study they were not valid extensions of the calibration population, being the only samples of their type in the population. Including them in the calibration lowered the accuracy of cross validation.

The distribution of standardised H statistic values are also important in defining a calibration population (Fig. 2). The cereals and cereal by-product spectra were closer to the average spectra and there were more samples at a given distance than for the protein meals. As the standardised H statistic increased, there were fewer samples present at a given distance from the average spectra, and in some cases only one. This indicates that many more samples of animal and vegetable meals are needed for calibration. In fact, the majority of samples with an H value greater than 1.5 were animal meals, suggesting that this population should be calibrated separately from vegetable meals.

In conclusion, these results show that NIR has considerable potential for measuring AME of poultry feed ingredients. However, these ingredients are spectrally diverse, and many samples will be necessary in each feed category in order to obtain accurate NIR calibrations.

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PLASMA INSULIN, TRIIODOTHYRONINE (T_3) AND THYROXINE (T_4) IN LINES OF CHICKENS SELECTED FOR INCREASED OR DECREASED FATNESS

J.X. WU and R.A.E. PYM

There is limited information on the effect of insulin and thyroid hormones on protein and lipid metabolism in chickens. The present study was designed to determine the effects of dietary protein on growth performance and body composition and on plasma levels of insulin, total triiodothyronine (tT_3), total thyroxine (tT_4) and free thyroxine (fT^4) in lines of chickens selected for high or low body fatness.

Two experimental diets containing 13 MJ ME/kg with either 150 or 203 g crude protein/kg were used. The birds used in the study were sampled from the ninth generation of lines selected for six generations for increased (line F) or decreased (line L) abdominal fat. Food intake and growth rate were measured individually from 21 to 49 days of age in 48 birds per line. The birds were killed on day 50 for subsequent measure of abdominal fat and total body moisture and fat. A 2ml sample of blood was taken from the wing vein of each chicken at seven weeks of age, and radioimmunoassay techniques were used to analyse for plasma tT_3, tT_4 and fT_4. The results of the study are shown in the table.

Growth rate (GR, g/d), FCR, Abdominal fat (AF, g/kg), body moisture (%) and lipid (%) and plasma insulin (ng/100ml), tT_3 (ng/ml), tT_4 (ug/100 ml) and fT_4 (ng/100 ml) in the two lines of chickens given two isoenergetic diets varying in protein concentration (g/kg).

	GR	FCR	AF	Moist	Lipid	Insulin	tT_3	tT_4	ft_4
Line									
F	32.2	2.46b	30.5b	58.8a	17.3b	.165b	1.75a	1.41	1.67a
L	32.3	2.37a	16.2a	62.9b	13.1a	.133a	2.11b	1.62	2.01b
Diet									
150	31.2a	2.54b	26.6b	59.7a	16.3b	.145	1.93	1.44	1.82
203	33.3b	2.29a	20.2a	61.9b	14.1a	.153	1.93	1.59	1.86
LSD 0.05	1.9	0.07	2.5	0.9	1.3	.020	0.20	0.21	0.24

Plasma insulin, abdominal fat, body lipid, and FCR were significantly greater and plasma tT_3, fT_4 and body moisture were lower in birds from the fat line than in their lean line counterparts. Growth rate and carcass moisture increased, and abdominal fat, FCR and body lipid decreased with increase in dietary protein. There were significant interactions between line and diet for tT_4 and fT_4 ($P<0.05$) due to a substantial reduction in T_4 plasma levels of the fat line birds given the high protein diet but an increase in levels in the lean line birds on this diet.

Department of Farm Animal Medicine and Production, The University of Queensland, St Lucia, Qld, 4072.