Proceedings of

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

1993

Volume No. 5

University of Sydney, Sydney, NSW

February 1993

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

1993

PROGRAMME COMMITTEE

Associate Professor D. Balnave, (Chairman)
Professor E.F. Annison
Dr. W.L. Bryden
Professor D.R. Fraser
Dr. R.J. Johnson
Mr. I. Littleton
Dr. R.A.E. Pym
Dr. B.L. Sheldon

EDITORIAL COMMITTEE

Dr. R.J. Johnson, (Chairman)
Professor E.F. Annison
Associate Professor D. Balnave
Dr. W.L. Bryden
Professor D.J. Farrell
Professor D.R. Fraser
Dr. S. Prowse
Dr. R.A.E. Pym
Dr. B.L. Sheldon

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.M. Gous at this year's Symposium.

PROCEEDINGS OF THE SYMPOSIUM

The comments and views expressed in the papers are entirely the responsibility of the author or authors concerned and do not necessarily represent the views of the Poultry Research Foundation or the World's Poultry Science Association.

Every attempt has been made to edit all papers to conform with a standard in "Instructions to Authors", subject to the constraints imposed by the necessity to publish the Proceedings in time for distribution at the Symposium.

Enquiries regarding the Proceedings should be addressed to:

The Director,
Poultry Research Foundation,
Department of Animal Science,
RMC Gunn Building,
University of Sydney,
Sydney, NSW, 2006.

CONTENTS

SHORT PAPERS

NUTRITIONAL SIGNIFICANCE OF ϖ-3 FATTY ACID-ENRICHED CHICKEN MEAT

A.O. AJUYAH*, R.T. HARDIN and J.S. SIM

Research interest on the fatty acid composition of chicken meat, particularly the omega-3 fatty acids composition (Farrell and Gibson 1990; Ajuyah et al. 1992), is the result of positive correlation between dietary fat (quality and quantity), cholesterol, total calorie intake and aetiology of certain degenerative diseases. Many investigators have added fish products or oil seeds (e.g. canola and flax) to poultry diets as energy, protein and fatty acid sources (e.g. Noble et al. 1988; Phetteplace and Watkins 1989). These studies indicated the fatty acid composition of poultry meats, particularly the ϖ-3 fatty acid composition, was influenced by dietary C18:3ϖ3. Substantial amounts of ϖ-3 fatty acids in poultry meat decrease the level of saturation and enhance lipid oxidation. Products of lipid oxidation such as malonaldehyde may be toxic to humans (Fritsche and Johnson 1988). In the present study a corn soybean meal control diet, one including canola seeds, and another including flax seeds, with and without certain natural antioxidants (mixed tocopherol plus or minus canthazanthin), were fed to broiler chickens from day 7 to slaughter age (42 days).

The incorporation of flax seed into poultry diets resulted in substantial tissue enrichment of ϖ-3 fatty acids (C18:3ϖ3, C20:5ϖ3, C22:5ϖ3 and C22:6ϖ3). Total ϖ-3 fatty acids for white (W) and dark (D) meat in mg per 100 g lean meat were: 76 (W), 120 (D) for control; 138 (W), 186 (D) for canola, and 211 (W), 379 (D) for the flax fed group. Dietary antioxidant significantly reduced concentration of malonaldehyde in the freshly harvested and cooked enriched meat, particularly dark meat, and produced white and dark meat with sensory characteristics that were similar to the control group. The order of magnitude of antioxidant activity was: mixed tocopherol plus canthazanthin > mixed tocopherol > canthazanthin. The incorporation of ϖ-3 fatty acids into poultry meat by dietary management without antioxidant may affect the sensory and storage stability of the enriched chicken meat. Such effects could be minimised by the dietary addition of appropriate antioxidant.

AJUYAH, A.O., LEE, K.H., HARDIN, R.T. and SIM, J.S. (1992). J.Food Sci. 57: 338.

DYERBERG, J., BANG, H.O. and HJORNE, N. (1975). Am.J.Clin.Nutr. 28: 958.

FARRELL, D.J. and GIBSON, R.A. (1990). In Proceedings of the Inaugural Massey Pig and Poultry Symposium, Massey University, Nov. 26 - 28 pp 164 - 179.

FRITSCHE, K.L. and JOHNSON, P.V. (1988). J.Nutr. 118: 425.

KAGAWA, Y., NISHIZAWA, M., SUZUKI, M., MIYATAKE, T., HAMAMOTO, T., GOTO, K., IZUMIKAWA, H., HIRATA, H. and EBIHARA, H.J. (1982) J.Nutr. Sci.Vitaminol. 28: 441.

NOBLE, R.C., CONNOR, K., MaCARTNEY, R. and BROWN, D. (1988) Res.Vet.Sci. 44: 33.

PHETTEPLACE, H.W. and WATKINS, B.A. (1989). J.Food Comp.and Anal. 2: 104.

Department of Animal Science, University of Alberta, Edmonton, Canada, T6G 2P5.

* Current address, 17 Oxford Street, Newtown, NSW, Australia 2042

BEHAVIOURAL, PHYSIOLOGICAL AND PRODUCTION RESPONSES OF LAYING HENS TO ADDITIONAL HUMAN CONTACT

J. L. BARNETT and P.H. HEMSWORTH

Summary

Laying hens were exposed to either 'Additional' visual human contact from 19 to 36 weeks of age or 'Minimal' human contact where the visual exposure to humans was limited to that involved in routine husbandry and reduced by the use of blinds in front of the cages. The 'Additional' treatment resulted in lower levels of fear of humans, reduced corticosterone response to handling, improved immunological responsiveness and higher hen day production.

I. INTRODUCTION

An association between fear of humans and production by laying hens has been shown both experimentally (Hemsworth and Barnett, 1989) and at commercial farms (Barnett *et al.*, 1992). Physical handling of chickens (layers and broilers) affects growth rate (Buckland *et al.*, 1974; Thompson, 1976; Reichmann *et al.*, 1978; Freeman and Manning, 1979; Gross and Siegel, 1979, 1980; Jones and Hughes, 1981; Collins and Siegel, 1987) and location in a shed affects egg production (Grover *et al.*, 1972; Sefton, 1976; Sefton and Crober, 1976; Jackson and Waldroup, 1987). It is possible that the human effects are mediated via fear responses. The experiment by Barnett *et al.* (1992) showed highly significant relationships between production and behavioural responses to humans. The present experiment examined whether the amount of visual contact with humans affects the behavioural (withdrawal responses to humans), physiological (corticosterone and immunological) and production (egg production) responses of laying hens.

(a) Materials and Methods

144 laying hens were housed in 2-bird cages (0.31 x 0.47 x 0.43 m; width x depth x height) in the lower tier of a 2-tier battery. There were 4 rows of 36 cages in the shed and the 2 central rows (2 and 3) were used for the experiment.

The hens were a commercial strain (SIRO CT; White Leghorn x Australorp) purchased at 18 weeks of age. Five days after arrival, a blind, the length of the shed was placed in the central corridor in front of the cages in row 3 (Minimal treatment) to reduce visual contact with humans. There were 2 treatments:

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

1. 'Minimal'.

   Birds in row 3 received minimal human contact. Eggs were collected daily and birds were examined daily for mortalities, by looking through a gap in the blind that allowed the eggs to roll out. Feed was given twice weekly by partially raising the blind to above the level of the food troughs so that feed could be added.

2. 'Additional'.

   Birds in row 2 received a total of 15 min additional (to routine husbandry) human contact/day for 5 days/week. This contact involved one of three experimenters slowly walking up and down the aisle in front of the 36 cages of birds in a manner designed to encourage approach of the birds to humans. The experimenter randomly stopped in front of a cage and slowly extended the hand to either the front of the cage or to manipulate the feed. If the bird withdrew, the experimenter moved on.

The treatments were imposed for 17 weeks, at which time the blinds were removed and all birds then received routine husbandry, involving daily egg collections, inspections of birds and feeding and checking drinkers twice per week. Throughout the experiment routine husbandry was as above. Egg collection and feeding took approximately 10 min each. The following measurements were made on a total of 36 cages/treatment, 12 cages on each of days 2, 10 and 17 after removal of the blind.

Behavioural responses to humans were determined in a 'Shute Test' (ST) and 2 days later in an 'Approaching Human Test' (AHT); these tests have been described elsewhere (Barnett *et al.*, 1992). Basically these tests involved measuring the withdrawal responses of birds to an approaching experimenter.

The corticosterone response to handling was determined from a blood sample taken from the wing vein within 30 s of the end of the ST. The plasma was stored frozen until analysis for corticosterone concentrations using a commercial diagnostic kit (Cambridge Medical Technology Corporation, Billerica, Masachusetts, USA). Immunological responsiveness was assessed on the basis of a cell-mediated response following injection with a mitogen (Regnier and Kelley, 1981; Blecha *et al.*, 1983; Westly and Kelley, 1984). Over 2 days, starting 3 days after the AHT and using the bird from that test 250 μg of leucoagglutinin (PHH-L with a mitogenic activity at < 5 μg/ml; Sigma Chemical Company, St. Louis, USA) in 0.25 ml saline was injected into one wattle. The wattle thickness was measured prior to and 24 h post-injection with a pressure sensitive micrometer and the increase in wattle thickness was used as a measure of the reactivity of the cell-mediated immune system.

Egg production was determined between 23 and 36 weeks of age (weeks 5-17 of treatment); these data were analysed on the basis of cage averages.

The physiological and production data were analysed by analysis of variance for the effects of treatment and time (Genstat, Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The behavioural data were analysed by both analysis of variance and the chi-square test.

II. RESULTS

The behavioural responses in the ST showed no significant treatment effects ($P > 0.05$; Table 1). However, there was a trend for fewer withdrawal responses to the approaching experimenter in the 'Additional' treatment; a lower proportion of birds moved more than 42 cm away from the experimenter ($P = 0.06$).

In the AHT the number of times that birds were in the front 10 or 20 cm of the cage and oriented forwards as the experimenter approached was greater ($P < 0.01$) in the 'Additional' treatment.

Table 1. The effects of modifying the amount of human contact on laying hens (mean values for days 2, 10 and 17).

Parameter	Treatment		LSD	Chi-square
	Additional	Minimal	$P = 0.05$	$P =$
Proportion of birds that withdrew in the ST	0.41	0.64	0.230	0.06
Times in front of cage (max = 3) in the AHT	2.12^{x}	1.22^{y}	0.530	0.001
Times in front/middle of cage in the AHT	2.69^{x}	1.95^{y}	0.412	0.001
Times orient forward in the AHT	2.54^{x}	1.80^{y}	0.450	0.001
Hen day production (%)	89.4^{a}	83.1^{b}	5.68	-
Corticosterone concentration (nmol/l)[1]	2.06^{a} (8.6)	2.33^{b} (11.9)	0.243	-
Increase in wattle thickness (mm; day 1)	2.1^{a}	1.4^{b}	0.297	-
Increase in wattle thickness (mm; overall)	2.00	1.73	0.270	-

[1] $\log_e$ transformation with untransformed data in parentheses;
^{ab} and ^{xy} denote significant differences at $P < 0.05$ and $P < 0.01$, respectively.

Corticosterone concentration in response to handling was greater and hen day production was lower, respectively ($P < 0.05$) in the 'Minimal' than the 'Additional' treatment (Table 1). There was a significant treatment x period interaction in cell-mediated immunological responsiveness ($P < 0.001$; Table 1). This was due to a significant treatment effect at period 1 ($P < 0.05$) with an increased response in the 'Additional' treatment and no significant differences at periods 2 and 3. There were no effects of time on the other variables.

III. DISCUSSION

This experiment has shown that altering the amount and nature of human contact can have effects on the behavioural, physiological and production responses of laying hens. Increasing the amount and improving the nature of human contact reduced the level of fear shown by birds to humans, on the basis of their withdrawal responses to experimenters in the two behavioural tests and decreased corticosterone response to handling with consequent positive effects on production. The mechanism may be a chronic stress response on the basis of (limited) changes in cell-mediated immunological responsiveness. Increasing the amount of human contact involved changes in both the quantity and quality of the contact and further research is required to distinguish between these components. It should be emphasized that changes in quality of human contact in this experiment were likely to have involved human behaviours that encouraged approach behaviour of the birds to humans; thus experiments involving human behaviours which are fear-provoking may have produced different results. Nevertheless, the results indicate that human contact may be an important determinant of subsequent bird behaviour and production. In addition, the physiological changes suggest that human contact may also be an important determinant of welfare.

REFERENCES

BARNETT, J.L. HEMSWORTH, P.H. and NEWMAN, E.A. (1992). Br.Poult.Sci. 33:699.

BLECHA, F., POLLMANN, D.S. and NICHOLS, D.A. (1983). J.Anim.Sci. 56:396.

BUCKLAND, R.B., GOLDROSEN, A. and BERNON, D.E. (1974). Poult.Sci. 53:1256.

COLLINS, J.W. and SIEGEL, P.B. (1987). Appl.Anim.Behav.Sci. 19:183.

FREEMAN, B.M. and MANNING, A.C.C. (1979). Rev.Vet.Sci. 26:223.

GROSS, W.B. and SIEGEL, P.B. (1979). Avian Dis. 23:708.

GROSS, W.B. and SIEGEL, P.B. (1980). Avian Dis. 24:549.

GROVER, R.M., ANDERSON, D.L., DAMON, R.A. and RUGGLES, L.H. (1972). Poult.Sci. 51:565.

HEMSWORTH, P.H. and BARNETT, J.L. (1989). Br.Poult.Sci. 30:505.

JACKSON, M.E. and WALDROUP, P.W. (1987). Poult.Sci. 66:907.

JONES, R.B. and HUGHES, B.O. (1981). Br.Poult.Sci. 22:461.

REGNIER, J.A. and KELLEY, K.W. (1981). Am.J.Vet.Res. 42:294.

REICHMANN, K.G., BARRAM, K.M., BROCK, I.J. and STANDFAST, N.F. (1978). Br.Poult.Sci. 19:97.

SEFTON, A.E. (1976). Poult.Sci. 55:1922.

SEFTON, A.E. and CROBER, D.C. (1976). Can.J.Anim.Sci. 56:733.

THOMPSON, C.I. (1976). Dev.Psychobiol. 9:459.

WESTLY, H.J. and KELLEY, K.W. (1984). Proc.Soc.Exp.Biol.Med. 177:156.

ULTRASTRUCTURE OF THE EGGSHELLS OF LAYING HENS: DIFFERENCES BETWEEN STRAINS AND THE EFFECT OF HEN AGE

C.E. BRACKPOOL*, J.R. ROBERTS*, R.J. HUGHES** and S.E. SOLOMON***

Summary

Shell quality was assessed in four strains of birds at three different ages. Traditional eggshell quality measurements indicated that one strain laid superior quality eggs whereas ultrastructural examination revealed the egg shells of another strain to be structurally inferior. Using conventional egg quality measurements, as the age of the birds increased, egg weight increased and percent shell present decreased. The aging process also resulted in ultrastructural variations in the quality and arrangement of the mammillary caps and variations in the incidence of erosion and changed membrane.

I. INTRODUCTION

Significant attempts have been made by geneticists and nutritionists to improve eggshell quality. However, the number of eggs downgraded still represents a significant loss to the industry. Research conducted at Glasgow University suggests that new criteria need to be applied for the determination of shell quality. These criteria are based on the fact that unless an eggshell is structurally sound, it will fail regardless of its thickness or specific gravity. The structural quality of an eggshell, as for any structure, is dependent upon its initial building blocks. Solomon (1985) identified and described a number of ultrastructural characteristics of the egg shell mammillary layer that deviate from the norm. We examined the egg shells of four Australian commercial layer strains and investigated the effect of bird age on eggshell structure.

II. METHODS

(a) Experimental groups

Eggs were collected from four strains of Australian commercial layer hens at 33, 41 and 57 weeks of age. This study used only those eggs that were calcified overnight.

(b) Eggshell quality

Traditional eggshell quality measurements were recorded for each egg. Egg weight, shell weight and shell thickness were measured and specific gravity, percentage of shell present and shell density were calculated.

---

*Department of Physiology, University of New England, Armidale N.S.W. 2351
**Department of Primary Industries, Parafield Gardens, S.A. 5107
***Poultry Research Group, Department of Veterinary Anatomy, University of Glasgow, U.K.

(c) Preparation for ultrastructural observation

A small equatorial piece of eggshell was cut out and the inner membrane manually removed. The outer membrane was removed by ashing, using a Bio Rad RF Plasma Barrel Etcher PT 7150 after the method of Reid (1983). Each shell was then mounted, inner surface uppermost, onto an aluminium stub and coated with gold/palladium using a Sputter coating unit. The samples were viewed under a JOEL JSM35 Scanning Electron Microscope, at an accelerating voltage of 15 KV and a working distance of 15 mm.

(d) Ultrastructural examination

The mammillary region of each eggshell was examined for ultrastructural characteristics as described by Solomon(1991). Mammillary cap size was ranked as follows; 1 = similar; 2 = variable; and 3 = highly variable. Alignment, changed membrane, confluence, cuffing, depression, erosion, hole, type A bodies, type B bodies, aragonite, cubics and cubics attached to the cones were each given a rank for degree of incidence, where; 1 = none; 2 = isolated; 3 = moderate; 4 = extensive. Early fusion and late fusion where ranked as 1 = extensive; 2 = moderate; and 3 = isolated. Quality of the caps was also ranked, according to size of the cap in relation to its cone and the degree of membrane attachment and were as follows; 1 = good; 2 = good minus; 3 = poor +; 4 = poor; and 5 = poor minus. For each shell the ultrastructural observations were applied to the scoring system described by Nascimento *et al.* (1992) to give a total structural score for each egg shell. The total structural score reflects the degree of incidence and the influence of the ultrastructural variations on the functional properties of the shell (Solomon,1990). The lower the total structural score, the better the ultrastructure of the eggshell.

III. RESULTS and DISCUSSIONS

Table 1 Traditional eggshell measurements (mean ± SE) on four strains of layer hens, averaged over three different ages.

Eggshell measurements	Strain A	Strain B	Strain C	Strain D
Egg weight (g)	59.11^{a}	60.80^{b}	57.79^{a}	59.37^{ab}
	(0.651)	(0.551)	(0.598)	(0.597)
Shell weight (g)	5.235	5.618	5.22	6.135
	(0.061)	(0.055)	(0.068)	(0.801)
Shell thickness (μm)	357.7^{a}	374.7^{b}	357.5^{a}	359.1^{a}
	(2.524)	(3.087)	(3.579)	(2.839)
% Shell	8.868^{a}	9.248^{b}	9.037a	8.904^{a}
	(0.061)	(0.084)	(0.077)	(0.1)
Specific gravity	1.084	1.087	1.084	1.122
	(0.0005)	(0.001)	(0.001)	(0.041)
Shell density	73.86^{a}	77.80^{b}	74.72^{a}	74.50^{a}
	(0.519	(0.651)	(0.672)	(0.671)

Values across a row with different superscripts differ significantly $P < 0.05$

Table 2 The mean rank (Kruskal Wallis nonparametric test) of ultrastructural observations of the eggshell mammillary region in four commercial layer strains averaged over three different ages.

Ultrastructural characteristic	Strain A	Strain B	Strain C	Strain D
Mammillary cap size	138.9	142.6	115.3	140.7
Alignment	135.9	141.2	130.6	148.4
Changed membrane	137.6	139	131	148.4
Confluence	158.3	139.8	132.8	127.1
Cuffing	153	126.7	124.6	153.1
Early fusion	139.5	144.4	148.9	123
Late fusion	107.7[a]	132.5	154.6[b]	158.6[b]
Depression	145.3	131.9	135.5	143.8
Erosion	150	135.9	138.8	132.1
Hole	139	139	139	139
Type A bodies	158	131.8	141.2	126.3
Type B bodies	153.9[a]	140.7	146	116.2[b]
Aragonite	141.9	132.2	151.5	130.3
Cubics	148.4	125.4	149.5	133.1
Cubics on cones	157	125.2	147.5	127.3
Cap quality	147	139.4	137.5	132.7
Total score	171.2[a]	134[b]	134.2[b]	119.1[b]

Values across a row with different superscripts differ significantly $P<0.05$

Table 3 Traditional egg quality measurements (mean ±SE) for three ages of layer hens, using the average of four commercial layer strains.

Eggshell measurements	33 weeks	41 weeks	57 weeks
Egg weight (g)	55.87[a]	59.35[b]	62.27[c]
	(0.468)	(0.414)	(0.477)
Shell weight (g)	5.213	5.358	6.06
	(0.63)	(0.05)	(0.583)
Shell thickness (μm)	364.8	364.1	358.1
	(2.601)	(3.088)	(2.454)
% Shell	9.232[a]	9.03[b]	8.807[c]
	(0.083)	(0.066)	(0.63)
Specific gravity	1.12	1.085	1.079
	(0.033)	(0.0005)	(0.001)
Shell density	75.72	75.35	74.70
	(0.585)	(0.552)	(0.575)

Values across a row with different superscripts differ significantly $P<0.05$

Table 4 The mean rank of ultrastructural observations of the eggshell mammillary region at three different ages, using the average of four commercial layer strains.

Ultrastructural characteristic	33 weeks	41 weeks	57 weeks
Mammillary cap size	122.6	134.7	143.9
Alignment	157.4[a]	120.6[b]	139.9[a]
Changed membrane	164.8[a]	130.8[b]	123.3[c]
Confluence	151.3	137.4	129.3
Cuffing	129.7	129.1	157.1
Early fusion	137.3	140.9	138.7
Late fusion	135.8	146.4	134.8
Depression	127.1	135.9	152.9
Erosion	105[a]	149.1[b]	160.4[c]
Hole	139	139	139
Type A bodies	119.8	136.9	158.6
Type B bodies	131	132.8	152.4
Aragonite	128.3	138.4	149.4
Cubics	140.9	127.2	148.7
Cubics on cones	151.3	129.8	136.6
Cap quality	148.7[a]	117.9[b]	150.6[a]
Total score	127.9[a]	126.8[a]	161[b]

Values across a row with different superscripts differ significantly $P<0.05$

By conventional eggshell quality measurements, Strain B birds laid superior quality eggs (Table 1). However, on the basis of ultrastructural characteristics, the eggshells from birds of Strain B were not significantly better than those from Strains C and D whereas the shells from Strain A birds were ultrastructurally inferior on an overall basis and at 57 weeks. Strain D birds had ultrastructurally inferior eggshells at 33 weeks (Table 2). The significantly higher total score for Strain A was due to a lower incidence of late fusion and a higher incidence of type B bodies, and at 57 weeks a greater incidence of cubics and cubics formation on the cones. The former three abnormalities have been described as non-beneficial characteristics (Nascimento *et al.*, 1992). As the birds aged, egg weight increased in the absence of corresponding significant increases in shell weight and shell thickness. This resulted in a significant reduction in the percentage of shell present (Table 3). At the ultrastructural level, the total structural score remained constant from 33 to 41 weeks of age but was significantly higher at 57 weeks (Table 4). As the hen ages her ability to produce quality caps randomly organised in the mammillary layer initially improves and then declines. Aging also has a significant affect on the incidence of erosion, increasing both on the caps and in the cones of the mammillary layer. Interestingly, the incidence of changed membrane declines with age. The examination of aging effect revealed that while traditional egg quality measurements show similar trends within each Strain the ultrastructural variations do not. The incidence of certain ultrastructural abnormalities increase with age for Strains A and D and reduce for Strains B and C.

V. ACKNOWLEDGMENTS

This study was supported by a grant from the Egg Industry Research and Development Council and by a Junior Research Fellowship from the Egg Industry Research and Development Council and Chicken Meat Research and Development Council to C. Brackpool.

REFERENCES

NASCIMENTO, V.P., CRANSTOUN, S. and SOLOMON, S.E. (1992) Br.Poult.Sci. 33: 37-48.

REID, J., (1983) Br.Poult.Sci. 24 : 233-235.

SOLOMON, S.E.(1985).Poult.Inter 24.: 58

SOLOMON, S.E. (1990) Proc 8th Australian Poult Feed Conv 174

SOLOMON, S.E. (1991) Egg and eggshell quality Wolfe Publishing Ltd, London.

FEED MANAGEMENT FOR OPTIMUM GROWTH PATTERN OF BROILER BREEDERS

JOHN T. BRAKE

Summary

Broiler breeders have been reared with the sexes mingled for many years with good results. However, this practice now appears to produce inferior results when compared to sex-separate rearing. Broiler performance has been improved in the female parent line by faster growth rate, faster feed consumption and increased sensitivity to dietary protein while the male line has been selected for feed conversion and reduced leg problems which is associated with slower feed consumption during the early rearing period. Therefore, the early growth rate of the female needs to be slowed while the early growth rate of the male needs to be increased to regain the historical relationship. However, large males have excess breast muscle which can cause imbalance during mating. This can be corrected by slow feed increases late in the rearing period. Females require an increase in breast muscle late in the rearing period and this can be achieved by an increased rate of feed consumption or by an increase in the dietary protein to metabolizable energy ratio.

I. INTRODUCTION

Broiler breeder performance in the USA has suffered a considerable decline during the past four years. However, this has not been observed in many countries outside of the USA. A striking aspect of the international situation is the low metabolizable energy to crude protein (ME/CP) ratio of the diets when compared to U.S. diets. This is due to the low cost and high availability of metabolizable energy in the U.S. and typically high cost and low availability internationally. These observations suggested that dietary factors might play a role in this decline in broiler breeder reproduction.

(a) Female Management

Several studies which examined dietary, muscle development and frame issues have been conducted. In one experiment, breeders were grown on a broiler starter (BS) or a pullet grower (PG) diet from hatch to about 25 weeks after which a breeder diet was fed to both groups. As shown in Table 1, the BS birds had more breast muscle tissue as judged by Pectoralis major muscle at 18 and 25 weeks. The BS birds had less fat pad at 18 weeks but were equal to the PG birds at 25 weeks. The BS birds added abdominal fat during this period of rapid reproductive development. Note the difference in body composition even though the body weights were similar. There appeared to be a performance enhancing effect of the better breast muscle development during the onset of lay (Table 2).

Department of Poultry Science, Box 7608, North Carolina State University, Raleigh, NC 27695-7608 USA

Table 1. Effect of a broiler starter (BS) or pullet grower (PG) diet on broiler breeder pullet growth and development

Diet	ME/CP[1]	Body weight (g)			%Pectoralis major		% Fat pad	
		18wk	25wk	27wk	18wk	25wk	18wk	25wk
BS	134.9	1652	2211	3183	4.0	4.8	0.19	1.39
PG	182.4	1643	2243	3246	3.7	4.6	0.42	1.37

[1] Based on kcal/kg per % CP. Ref: Lilburn and Myers-Miller, 1990

Table 2. Effect of a broiler starter (BS) or pullet grower (PG) diet on early broiler breeder egg production

Diet	ME/CP[1]	Egg Production (%) 28-32 wk	32-36 wk	36-40 wk
BS	134.9	29.1	78.4	82.0
PG	182.4	17.5	72.4	80.9

[1] Based on kcal/kg per % CP. Ref: Lilburn and Myers-Miller, 1990

Research has been initiated at NCSU to investigate the effect of various diets on growth, frame, muscle development and reproduction. Slow-feathering females were given diets calculated to contain 11%, 14%, 17%, or 20% crude protein and 2926 kcal ME/kg. Equal amounts of these diets were fed for 4 weeks.

Table 3. Effect of diet on body weight where feed allocations were altered after 4 weeks of age to achieve similar body weights at 18 weeks of age

Grower diet % CP	ME/CP[1]	Prelay Diet % CP	Body weight (g) at wk of age 4	18	20	23	26	36
11	266	17	177[d]	1696	1964	2472	3125	3479
14	209	17	295[c]	1746	2028	2536	3003	3316
17	172	17	363[b]	1796	2019	2563	3012	3466
20	147	17	426[a]	1755	2109	2454	3012	3461

[a,b,c,d] Statistically significant differences ($P \leq .05$).
[1] Based on kcal/kg per % CP.

Body weights were recorded for all birds. Dietary protein appears to increase the early female growth rate irrespective of energy consumption (Table 3). Feed allocations were then adjusted so that similar body weights were achieved by 18 weeks. The 11% group suffered severe leg problems and extremely poor

feathering. There was some evidence of wrinkled feathering in the 14% group. This suggests a ME/CP ratio of 209 to be marginal. The 20% group suffered some coccidiosis due to low feed intake limiting the coccidiostat. From 18 to 25 weeks, all groups received the 17% diet followed by a 16% breeder thereafter. Daily feed allocations were identical after 18 weeks and body weights were similar (Table 3). Obviously, the cumulative consumption of energy and protein differed dramatically and breast muscle development differences were felt easily by handling the birds.

Keel lengths were measured at 18, 23, and 36 weeks of age. Juvenal primary wing feathers left to drop and evidence of "feather drop" (adult primary flight feathers lost out of sequence) were determined at 26 and 36 weeks of age (Table 4).

Table 4. Effect of grower diet on keel length and primary feather pattern

Grower Diet		Prelay Diet	Keel Length (mm)			Juvenal primary feathers remaining		Adult "Feather Drops" per bird	
% CP	ME/CP	% CP	18wk	23wk	36wk	26wk	36wk	26wk	36wk
11	266	17	119^{C}	135^{C}	137^{B}	1.6^{C}	1.13^{D}	$.60^{A}$	.04
14	209	17	124^{B}	137^{B}	137^{B}	1.8^{C}	1.54^{C}	$.10^{B}$	.03
17	172	17	130^{A}	140^{A}	142^{A}	2.0^{B}	1.74^{B}	$.06^{B}$	.09
20	147	17	127^{B}	137^{B}	137^{B}	2.4^{A}	2.12^{A}	$.06^{B}$	.07

^{A,B,C,D} Statistically significant differences ($P \leq .01$).
[1] Based on kcal/kg per % CP.

Although keel length appeared initially to be related to dietary protein, it was noted that the 20% group had shorter keels than the 17% group and possessed good breast muscle development at 18 weeks. Handling of the birds between 18 and 20 weeks revealed that many of the 20% group exhibited spreading of the pubic bones. This suggests some production of estrogens by the ovary. These hormones are known to stop bone growth and feather loss and would explain the shorter keels and more primary feathers remaining on birds in this group at 26 weeks. Primary feathers remaining at 36 weeks were directly related to grower dietary protein and suggests that cumulative dietary protein consumed is related to sexual maturity. "Feather drop" syndrome, indicative of delayed sexual maturity, was observed in the 11% group irrespective of the similar body weight for all groups from 18 to 26 weeks. Extremely poor fleshing prior to 18 weeks cannot be completely compensated for by a higher protein feed from 18 to 26 weeks. However, the marginal breast muscle development of the 14% group could be compensated for as evidenced by egg production to 48 weeks (Table 5).

The high initial mortality in the 11% hens was due to cuts caused by male mating. The male mortality was confined to one pen and was not thought to be typical. It would appear from these data that the 14% group which exhibited the most increase in breast muscle development from 18 to 26 weeks, irrespective of body weight gain and keel length, came into production faster and peaked higher. The data also suggest that well muscled birds at 18 weeks must continue to be fed

well thereafter to lay well.

Table 5. Effect of grower diet on egg production and mortality through 48 weeks of age

Grower Diet % CP	ME/CP[1]	Prelay Diet % CP	Eggs/hen	% HDP	Mortality (%) Female	Male
11	266	17	71.1[C]	39.9[B]	17.4[A]	16.7[A]
14	209	17	96.9[A]	50.2[A]	2.8[B]	8.3[A]
17	172	17	87.8[B]	45.5[A]	2.8[B]	12.5[A]
20	147	17	91.7[AB]	48.1[A]	4.3[B]	8.3[A]

[A,B,C] Statistically significant differences ($P \leq .01$).
[1] Based on kcal/kg per % CP.

What might be an explanation for these phenomena? When protein is given in excess, much of the surplus is turned into heat and body fat deposits. These body fat depots are necessary for sexual function to begin. This is termed "sex fat" in the human nutritional literature (Frisch, 1980). During the latter growing and prebreeder periods, energy is limiting and more energy produces more weight gain, not muscle (see fat pad data in Table 1 and the 11% CP group in Table 3 as examples). This latter group achieved normal body weights at 17 weeks but possessed poor breast muscle development. These birds had to complete breast muscle development before they could initiate reproduction and thus exhibited "feather drop" syndrome and poor rate of lay. Their keels continued to grow until they began to lay.

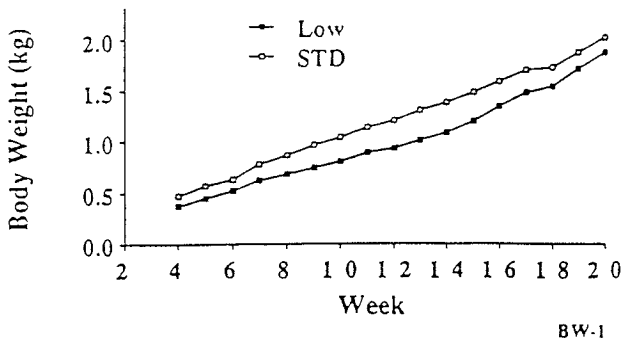


Figure 1. Standard (STD) or low (Low) body weight growing groups during the growing period.

The importance of a rapid increase in female feed allocations after 16 weeks is illustrated by another experiment where a standard (STD) and low (Low) body weight growing program was used (Figures 1 and 2). Feed amounts were increased rapidly for the Low birds late in the growing period up to 25 weeks (Figures 3 and 4). Feed allocations were the same thereafter (Figure 4). The Low

group produced 10 more eggs per hen (Figure 5). Better persistency of lay was apparent. This was probably due to increased breast muscle development observed in the low group.

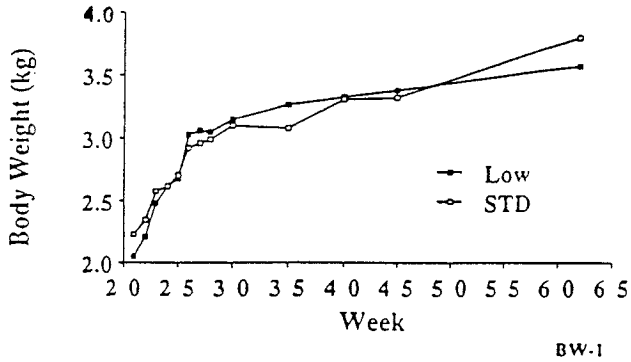


Figure 2. Standard (STD) or low body weight growing groups during laying period.

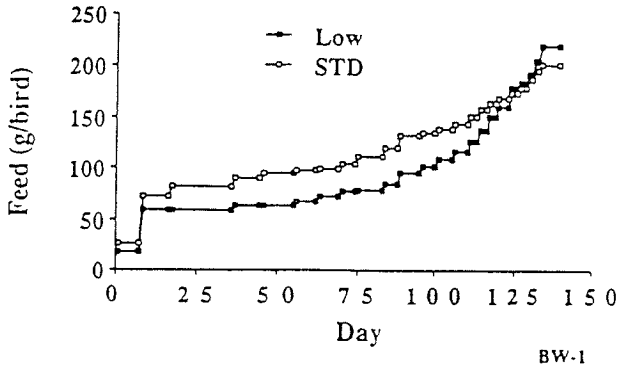


Figure 3. Standard (STD) and low feed intake programs during the growing period.

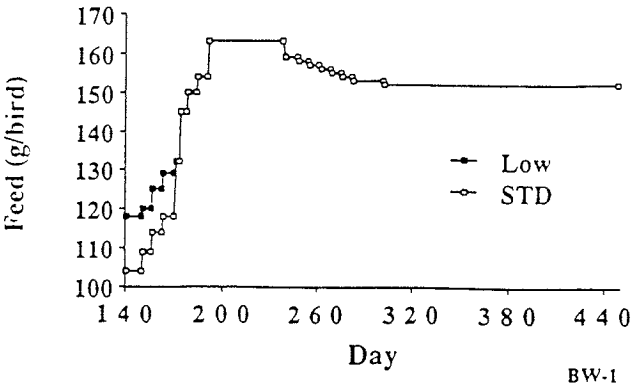


Figure 4. Feed amounts using an every-day feed program during laying period for standard (STD) or low body weight growing programs.

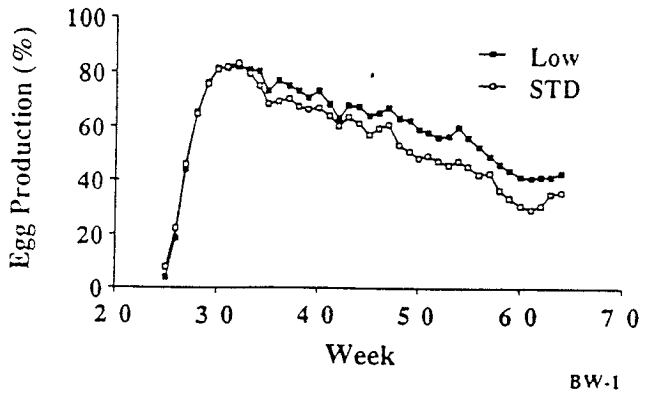


Figure 5. Egg production of standard (STD) or low growing body weight groups.

During the past 20 years we have developed the belief that control of body weight alone is the only factor of major importance when managing broiler breeders. Obviously, we need to be moving more toward a feeding and nutrition program approach rather than a body weight program approach, at least during the late growing and prebreeder periods.

(b) Male Management

Genetic selection has gradually slowed the rate of feed consumption in the male during the early phase of the rearing period. Females can consume more feed and often outgrow their male penmates. This ultimately leads to poor fertility when these underweight males fail to mature at the proper time or have lower social status.

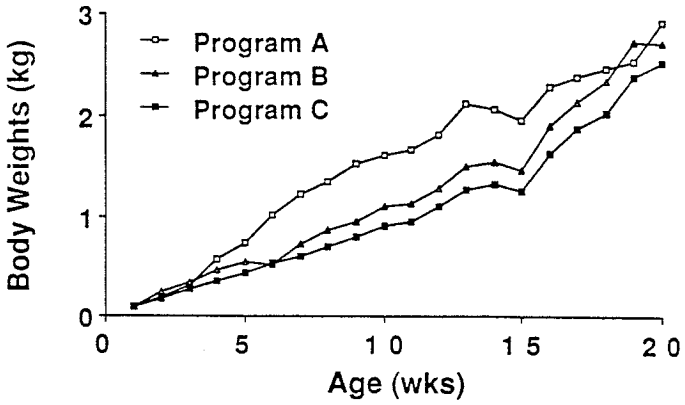


Fig. 6. Male body weights during rearing. Program A males were grown sex-separate and full-fed for first 4 weeks. Program B males were started on broiler starter for 10 days and then grown with females. Program C males were grown sex-separate but fed similar to females.

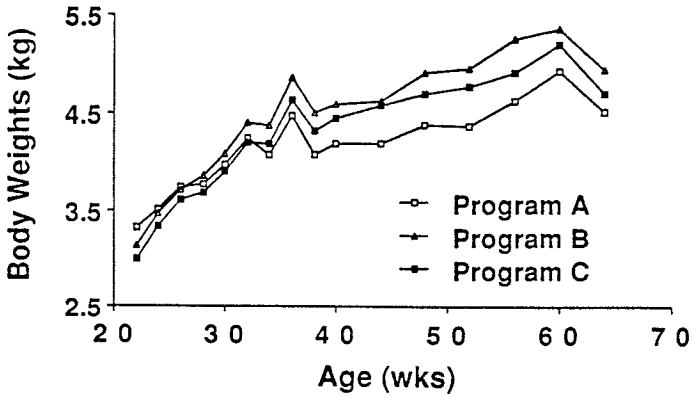


Fig. 7. Body weights during lay of males shown in Figure 6. During lay, all males and females were given similar feed allocations using FOG and MOG grills in a sex-separate feeding system.

The male body weight at 4 weeks of age is critical. Males, unlike females, appear to function best when they have a rapid early growth phase and achieve a high 4-week body weight. This produces males which possess long legs. Low male body weights at this age cannot be overcome by adequate body weight at 20 weeks. An experiment (Figure 6) was conducted where males were grown separate from females to 20 weeks of age (Program A), and were full-fed for the first four weeks followed by a declining rate of weekly feed increase late in the growing period. Program B males were fed a broiler starter ration for 10 days and then mixed with females. Program C males were grown separate from females but on a reduced feed intake similar to that of the females. Male body weights during lay for these three growing programs are shown in Figure 7. During lay, separate feeding with both female only grills (FOG) and male only grills (MOG) was employed. The Program A males became the smallest males during lay. Life-of-flock fertility was 91.3%, 81.0%, and 83.8% for Programs A, B, and C, respectively.

REFERENCES

FRISCH, R.E. (1980). Natural History 89:16-27.
LILBURN, M.S. and MYERS-MILLER, D.J. (1990). Poultry Sci. 69:1126-1132.

ROLE OF ALBUMEN QUALITY IN EMBRYONIC DEVELOPMENT AND HATCHABILITY

J. BRAKE and T. J. WALSH

Summary

Prior to establishment of the chorioallantoic blood circulation, gases (O_2, CO_2, H_2O) must passively diffuse across the eggshell and albumen to or from the blastoderm. Albumen quality determines the distance and viscosity of the medium from the blastoderm to the eggshell and the availability of readily diffusible labile water lost during early incubation in exchange for oxygen. Albumen quality is greatest when breeder flock age is young and eggs are fresh. Early embryonic mortality is typically highest at this time. The incidence of early embryonic mortality in eggs from a young breeder flock stored two days was decreased as storage temperature increased from 12.8°C to 23.8°C. Conversely, the albumen quality of old breeder flocks was poor and the incidence of early embryonic mortality in eggs stored two days increased as storage temperature increased from 12.8°C to 23.8°C.

It is suggested that albumen quality and its degradation contribute to the regulation of gas exchange during early embryonic development. This phenomenon helps explain certain aspects of flock age related patterns of embryonic mortality and length of incubation.

I. INTRODUCTION

There is conflict in the scientific literature with regard to the effects of egg holding conditions on hatchability. This is probably due to many experiments having been conducted without consideration of flock age and length of egg storage. Broiler hatching eggs are held an average of four to five days. However, it is not uncommon to set fresh eggs (one to two days) or eggs that have been held eight to 10 days. There is field evidence that setting of fresh eggs from young breeder flocks (less than two days) is associated with reduced hatchability, late hatches, and poor chick quality. Meuer and Baumann (1988) suggested that the egg albumen presents a significant barrier to gas exchange during the initial stages of incubation. Since albumen quality (Romanoff and Romanoff, 1949) is known to change with flock age and storage time, it was hypothesized that changes in albumen quality might be involved in many unexplained aspects of embryonic mortality and hatchability.

(a) Albumen Quality and Early Embryonic Mortality

Increased early embryonic mortality associated with short-term storage of eggs from young breeder flocks is postulated to be partly due to poor positioning of the blastoderm at the onset of incubation. When an egg is first laid the yolk is held in the center of the egg away from the eggshell. This is a protective

Department of Poultry Science, Box 7608, North Carolina State University, Raleigh, NC 27695-7608 USA

mechanism to prevent microbial contamination of the blastoderm. Prior to incubation, the yolk appears to "float" up as the albumen degrades so that the blastoderm comes in closer proximity to the eggshell. Extended periods of egg storage may allow the albumen to degrade too much and cause early embryonic mortality to increase.

It has been suggested that broiler hatching eggs need to be stored for two days for optimum hatchability (Hodgetts, 1988). At the generally recommended storage condition of 18.3°C, regardless of relative humidity, two days were required for the albumen index of eggs from a young flock to decline significantly (Figures 1 and 2). Although the albumen index of the old flock was initially significantly lower than that of the young flock it did not remain lower when the relative humidity was high (Figures 1 and 2). This suggests that high relative humidity helps maintain albumen quality of eggs from old flocks at a level similar to that of young flocks from 1 to 3 days of storage. Low storage temperatures (12.8°C or 18.3°C) combined with high relative humidity (75%+) can actually result in an increase in albumen quality in young flocks (Walsh, 1992). This results in increased early embryonic mortality of eggs from young flocks (Figure 3). On the other hand, higher storage temperature combined with low humidity for two days can decrease early deads in young flocks while increasing early deads in old flocks (Figure 3) (Walsh, 1992).

A relationship between albumen quality and early embryonic mortality is clearly shown in eggs from a 38 week old flock stored zero, one, two, or three days at either 12.8°C or 18.3°C and then incubated (Figure 4). The nadir in early embryo deaths occurred at about 6.75 mm albumen height regardless of temperature although this occurred one day earlier at the higher storage temperature.

(b) Albumen Quality and Length of Incubation

Young flocks generally have better albumen quality than old flocks. Albumen quality is also maintained longer in young flocks (Walsh, 1992). This may explain why eggs from old flocks can and should be set quicker than eggs from young flocks, and why old flocks do not hatch as well when stored for periods over four days.

Late hatches are also associated with fresh eggs, especially from young flocks. This may be due to a reduced rate of oxygen exchange because of the additional barrier to oxygen influx created by the viscous albumen. It is also possible that water contained within the fresh egg, which is normally lost during storage as a result of dissolution of the albumen, must be lost during incubation. This requires additional incubation time. Temperature, humidity, flock age, and storage time must be considered when attempting to optimize hatchability.

REFERENCES

HODGETTS, B. (1988). Hatch Handout 10, ADAS, Wolverhampton, U.K.

MEUER, H.J. and BAUMANN, R. (1988). Resp. Physiol. 71:331-342.

ROMANOFF, A.L. and ROMANOFF, A.J. (1949). The Avian Egg. New York, John Wiley and Sons.

WALSH, T.J. (1992). M.S. Thesis, North Carolina State University, Raleigh, NC.

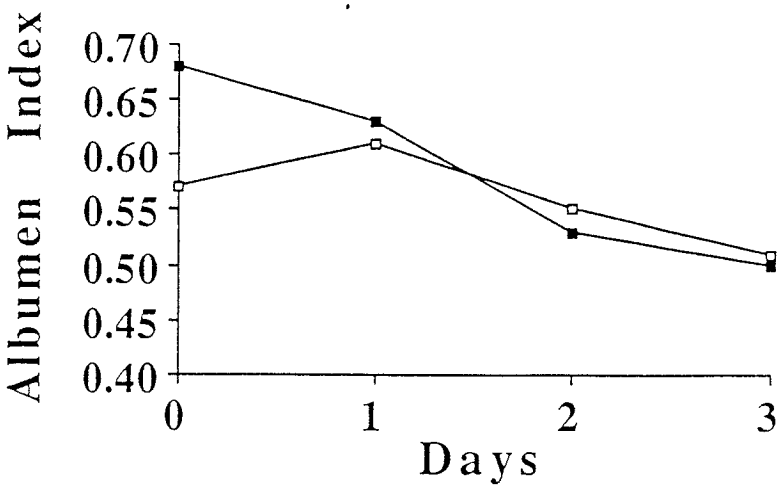


Figure 1. Rate of change of egg albumen index from a young (■) and old (□) broiler breeder flock when stored at 80% RH at 18.3°C for three days. (From Walsh, 1992).

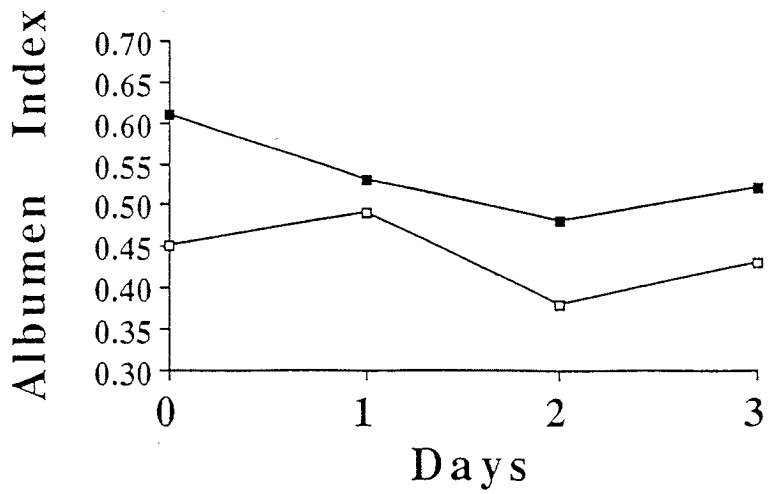


Figure 2. Rate of change of egg albumen index from a young (■) and old (□) broiler breeder flock when stored at less than 20% RH at 18.3°C for three days. (From Walsh, 1992).

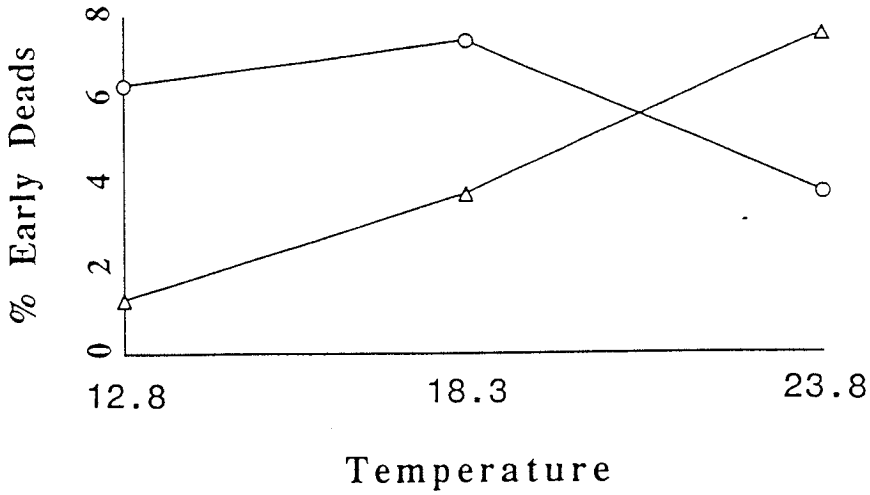


Figure 3. Early embryonic mortality of eggs from a young (○) and old (△) broiler breeder flock when eggs were stored for two days at 12.8°C and 80% RH, at 18.3°C and 75% RH or at 23.8°C and 20% RH. (From Walsh, 1992).

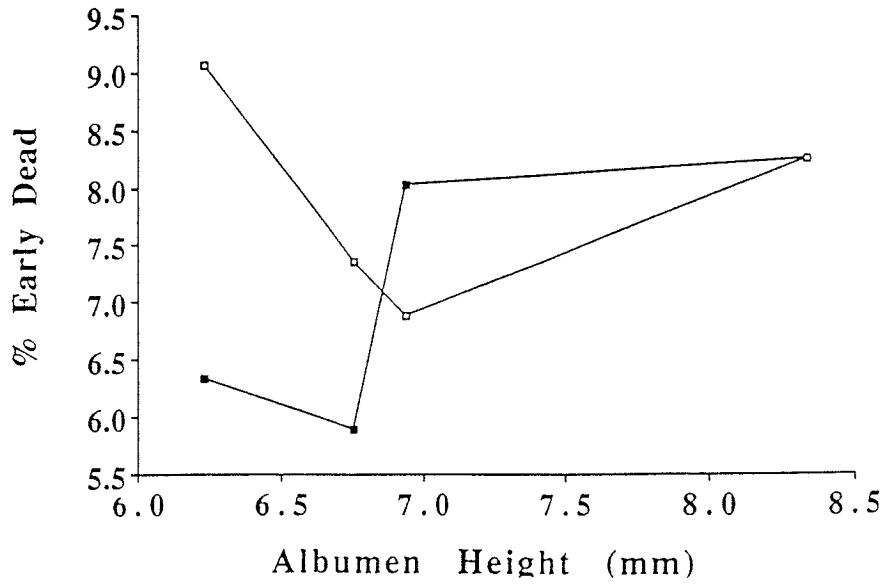


Figure 4. Relationship between albumen height and early embryonic mortality in eggs from a 38 week old flock stored for zero, one, two, or three days at either 12.8°C (■) or 23.8°C (□). (From Walsh, 1992).

EXTRACT VISCOSITY AS A PREDICTOR OF THE NUTRITIVE QUALITY OF WHEAT IN POULTRY

M. CHOCT , G. ANNISON and R.P.TRIMBLE

The nutritive value of wheat varies greatly in poultry, especially in young broilers, due to low apparent metabolisable energy (AME) contents of some wheats (Rogel et al. 1987). The low-ME wheat phenomenon has three major economic implications: (1) increased feed cost due to poor feed efficiency; (2) elevated litter problems due to watery and sticky excreta; and (3) inaccuracy in least cost formulations. Rogel et al. (1987) examined the physical structure of the starch granule, grain hardness and the activity of trypsin inhibitors in low-ME wheats and excluded them as causative factors. Recently, Annison (1991) demonstrated that the soluble non-starch polysaccharides (NSP) in wheat are closely related to the AME values. Furthermore, supplementation of NSP degrading enzymes significantly improved the nutritive value of wheat in poultry (Annison 1992; Friesen et al. 1992). These results suggest that the NSP in wheat exhibit anti-nutritive activity and low-ME wheat phenomenon is a consequence of variation in the soluble NSP contents. The randomness of the problem demands a rapid assay for the prediction of the AME of wheats.

Soluble NSP increase digesta viscosity which inhibits nutrient digestion and absorption (Choct and Annison 1992). In the current study, the possibility of using extract viscosity as a predictor for the AME of wheat has been investigated. Classical AME trials were conducted on 16 wheats (mostly grown in South Australia) using 25 or 27-day old broilers. Each trial had a maize control. For extract viscosity measurements, finely-ground wheat samples were extracted using (a) water alone; (b) acetate buffer (pH5.2) with enzymatic removal of starch, and (c) NSP isolated and re-dissolved in water. The AME values of the wheats ranged from 9.52 to 14.08 MJ/kg dry matter. The r values between the AME and the relative viscosity of extractions a, b and c were, -0.45 ($P<0.08$), -0.60 ($P<0.01$) and -0.61 ($P<0.01$), respectively.

Extract viscosity accounts for more than half of the variability of AME, but it does not hold promise as a precise predictor of wheat AME. This may imply the inadequacy of the current *in vitro* viscosity assay to predict *in vivo* conditions but also it may highlight the fact that viscosity is not the sole factor in the anti-nutritive effect of soluble NSP and that other mechanisms may play a significant role.

ANNISON, G. (1992). Anim.Feed Sci.Technol. 38:105.
ANNISON, G. (1991). J.Food Agric.Chem. 39: 1252.
CHOCT, M. and ANNISON, G. (1992). Br.Poult.Sci. 33:821.
FRIESEN, O.D., GUENTER, W., MARQUARDT, R.R., and ROTTER, B.A. (1992). Poult.Sci. 71: (in press).
ROGEL, A.M., ANNISON, E.F., BRYDEN, W.L. and BALNAVE, D. (1987). Aust.J.Agric.Res. 38:639.

CSIRO Division of Human Nutrition, Majors Road., O'Halloran Hill, S A 5158.

EFFECTS OF DIETARY NITROGEN SOURCES ON EXCRETION OF AMINO ACIDS IN CECECTOMIZED COCKERELS

T. K. CHUNG* and D. H. BAKER**

Summary

Sixteen cecectomized cockerels were used to study effects of dietary nitrogen sources on excretion of amino acids. Across all treatment groups, glycine, glutamate, aspartate, serine, leucine and threonine were excreted in largest quantity; methionine and tryptophan in lowest quantity. Lower levels of all amino acids were voided by fasted cockerels than by cockerels fed casein diets. With the exception of arginine, fasted cockerels excreted lesser quantities of all amino acids than cockerels fed amino acid diets. Fasted cockerels excreted less threonine, cystine and glycine than cockerels fed a protein-free diet. Isoleucine and valine were among the indispensable amino acids that were excreted in greater quantity by cockerels fed casein than by those fed amino acid diets.

I. INTRODUCTION

Amino acids (AA) voided by fasted birds or birds fed protein-free (PF) diets are referred to as endogenous AA losses in chickens. Casein has been shown to be almost completely digested by chicks (Parsons et al., 1982). The digestibility of crystalline AA is estimated to be near 100% in chicks (Izquierdo et al., 1988). However, it is unknown if the absolute quantities of AA excreted by fasted cockerels and cockerels fed PF, AA and casein diets were of equal magnitude.

II. METHODS

A cornstarch-sucrose basal diet (Table 1) was used in this experiment. The PF control diet was devoid of nitrogen or protein. The AA and casein diets were constructed by adding a mixture of crystalline AA and casein, respectively, to the basal diet. Sodium bicarbonate was added to both AA and casein diets so as to buffer the acidity of HCl forms of lysine and histidine and the acidic nature of casein.

Sixteen adult, cecectomized Single Comb White Leghorn cockerels averaging 2350 g were used in a completely randomized design. Details of cecectomy have been described previously by Parsons et al. (1983). Following a 24-hour fast, four cockerels per diet were force-fed (via crop intubation in a single dose) 30 g of the experimental diet (Table 1). Four fasted cockerels were served as negative controls. Birds were kept in individual cages with raised wire floors in a laboratory room with an illumination period of 16 hour. Water was available *ad libitum* during the excreta collection period. A 48-hour collection period was carried out and complete intestinal clearance of undigested AA was assumed (Parsons et al., 1982). Collected excreta were freeze dried, weighed and ground. Representative samples of excreta were subjected to 6N HCl acid hydrolysis for 24 hour. Acid

*ADM Bioproducts Division, Archer Daniels Midland Co., Singapore 0923.
**University of Illinois, Urbana, Illinois 61801, USA.

hydrolysates were subsequently analyzed for AA contents by ion-exchange chromatography. The content of sulfur AA was quantified in acid hydrolysates of performic acid preoxidized samples. Tryptophan analysis was accomplished after alkaline (lithium hydroxide) hydrolysis.

Table 1. Composition of experimental diets

Ingredient	Protein-free (%)	Amino acid (%)	Casein (%)
Cornstarch	65.8655	49.8455	48.1555
Amino acid mixture[1]	-	15.02	-
Casein[2]	-	-	16.71
Sucrose	20.00	20.00	20.00
Corn oil	5.00	5.00	5.00
Solka floc	3.00	3.00	3.00
Mineral mixture	5.37	5.37	5.37
Sodium bicarbonate	-	1.00	1.00
Vitamin mixture	0.20	0.20	0.20
Choline chloride	0.20	0.20	0.20
DL-α-tocopheryl acetate	0.002	0.002	0.002
Ethoxyquin	0.0125	0.0125	0.0125

1 Amino acid mixture: 0.44% L-arginine; 0.38% L-histidine.HCl.H20; 1.33% L-lysine.HCl; 0.41% L-tyrosine; 0.45% L-phenylalanine; 0.14% L-tryptophan; 0.32% DL-methionine; 0.32% L-cystine; 0.59% L-threonine; 0.90% Leucine 0.59% L-isoleucine; 0.62% L-valine; 0.30% L-proline; 0.90% glycine; and 7.33% glutamate.

2 Casein at 16.71% of the diet provided: 0.63% arginine; 0.48% histidine; 1.37% lysine; 0.93% tyrosine; 0.86% phenylalanine; 0.22% tryptophan; 0.47% methionine; 0.06% cystine; 0.70% threonine; 1.63% leucine; 0.84% isoleucine; 1.14% valine; 1.91% proline; 0.28% glycine; 3.8% glutamate; 1.18% aspartate; 0.95% serine; and 0.50% alanine.

Analysis of variance for completely randomized designs was used to analyze experimental data. Differences among treatments were established using the lest significant difference pairwise multiple-comparison procedure.

III. RESULTS

Similar quantities of total, indispensable and dispensable AA nitrogen were excreted by fasted cockerels and cockerels fed PF, AA and casein diets. Regardless of treatment groups, large quantities of glycine, glutamate, aspartate, serine, leucine and threonine were voided in cockerel excreta. Methionine and tryptophan were voided in lowest quantity. Fasted cockerels excreted lower levels of all AA than cockerels fed the casein diets, and fasted cockerels voided lesser quantities of all AA than cockerels fed the AA diet, with the exception of arginine. Among the indispensable AA, isoleucine and valine were excreted in greater quantity by cockerels fed casein than by those fed amino acid diets. Cockerels fed

the PF diet excreted more threonine, cystine and glycine than fasted cockerels.

Table 2. Quantities of nitrogen and amino acids excreted during the 48-hour period

Item	Fasted	Protein-free diet	Amino acid diet	Casein diet	SEM
			(mg/48 hour)		
Nitrogen					
Total	944	862	1003	1010	151.9
Indispensable amino acid	35.6	45.4	55.2	64.8	13.16
Dispensable amino acid	42.2	58.2	68.9	89.8	19.84
Indispensable amino acid					
Lysine	25.0a	30.8ab	38.7bc	47.3c	6.62
Tryptophan	5.5a	9.5a	11.3b	11.5b	1.64
Threonine	32.3a	48.5b	53.3b	60.3b	7.67
Methionine	6.0a	7.0ab	11.0bc	15.0c	2.45
Histidine	20.8a	24.8ab	25.7bc	30.5c	2.78
Leucine	46.5a	66.8ab	84.3b	82.3b	11.32
Isoleucine	16.3a	21.8a	32.9b	51.8c	5.34
Valine	25.5a	33.9a	44.3b	59.3c	6.20
Phenylalanine	18.0a	23.3ab	29.0bc	30.5c	4.37
Arginine	27.3a	30.5a	36.7ab	41.8b	5.76
Dispensable amino acid					
Cystine	18.5a	33.5b	32.0b	33.0b	7.31
Tyrosine	23.5a	30.8ab	32.3b	38.0b	5.07
Glutamate	64.8a	82.3a	144.7b	183.5b	28.17
Aspartate	46.3a	63.0ab	67.0b	90.0c	7.84
Alanine	25.0a	32.8ab	36.0b	54.0c	4.92
Serine	35.8a	46.3ab	54.0b	102.0c	7.32
Glycine	74.3a	109.5b	121.3bc	140.0c	12.51
Proline	37.8a	46.8ab	52.3b	64.8c	7.35

1 Means of four cecectomized cockerels for the fasted control and for the protein-free, amino acid and casein diets.

abc Means within a row having different superscripts differ ($P < 0.05$).

IV. DISCUSSION

Excreta harvested from fasted cockerels and those fed PF diets contained roughly the same quantities of AA, except threonine, cystine and glycine were higher in cockerels given the PF diet. Differences in excreted quantities of these AA may have resulted from increased mucus production in cockerels fed the PF

diet compared with those that were fasted. A PF diet has been associated with greater fecal outputs of several AA than is the case with fasting cockerels (Muztar and Slinger, 1980). Also, Parsons et al. (1983) indicated that AA excretion was greater in cockerels fed a PF diet than in fasted cockerels. In contrast, Chae and Han (1984) found that AA outputs of fasted cockerels were greater than those of cockerels fed a PF diet, while Yamazaki (1983) concluded that AA excretion of fasted cockerels was similar to that cockerels fed PF diets. In our study, excretion of most AA in cockerels fed casein was higher than that in cockerels fed AA diet, although the latter excreted more valine, isoleucine and glutamate than cockerels fed the PF diet. In rats, the contents of AA found in digesta upon feeding PF or AA diets were similar, but AA excretion was somewhat lower when AA were fed than when casein was fed (Darragh et al., 1988).

V. CONCLUSIONS

Different sources of dietary nitrogen induced different rates of amino acid excretion, with protein-bound amino acids producing greater responses than free synthetic amino acids. That fasted cockerels excreted less threonine than protein-free-diet fed cockerels suggests that using fasting cockerels to estimate endogenous threonine excretion may underestimate the true digestibility of threonine in highly digestible nitrogen sources such as casein.

REFERENCES

CHAE, B. J. and HAN, I. K. (1984) Korean J. Anim. Sci. 26:372

DARRAGH, A. J., MOUGHAN, P. J. and SMITH, W. C. (1988) J. Sci. Food Agric. 44:227.

IZQUIERDO, O. A., PARSONS, C. M. and BAKER, D. H. (1988) J. Anim. Sci. 66:2590.

MUZTAR, A. J. and SLINGER, S. J. (1980) Nutr. Rep. Int. 22:901

PARSONS, C. M., POTTER, L. M. and BROWN, R. D., JR. (1982) Poult. Sci. 61:939.

PARSONS, C. M., POTTER, L. M. and BROWN, R. D., JR. (1983) Poult. Sci. 62:483.

YAMAZAKI, M. (1983) Jap. J. Zootech. Sci. 54:729.

ROLE OF SINGLE ACTIVITY XYLANASE ENZYME COMPONENTS IN IMPROVING FEED PERFORMANCE IN WHEAT BASED POULTRY DIETS

W.D. COWAN*, O.B. JOERGENSEN**, P.B. RASMUSSEN** and P.E. WAGNER**

The negative effect of wheat arabinoxylans on the performance of broilers fed wheat based diets is well documented. The antinutritional effect has been associated with an increased gut viscosity that depresses nutrient absorption and influences the gut micro flora.

Arabinoxylanases alleviate this problem by hydrolysis of the non-starch polysaccharide (NSP) *in situ*, reducing gut viscosity and improving nutrient absorption. Concern however, has been raised that liberation of large amounts of arabinose and xylose, sugars which are poorly digested could depress growth and performance. A pure endoxylanase, free of xylosidase activity could therefore provide a better solution than the mixed enzyme preparations in use today.

In this study an endo-xylanase from *Humicola insolens* with a pH optimum of 6.0-7.0 was cloned into an *Aspergillus* expression system. This strain normally produces a complex mix of enzymes including cellulase, β-Glucanase, endo-xylanase and xylosidase and the product from this fermentation has been shown to be very effective in feeding trials on wheat based feeds.

The pure and mixed endo-xylanases were then compared in an *in vitro* viscosity assay and in a feeding trial using broiler chickens receiving a wheat based diet supplemented with one of the two enzyme preparations.

The substrate for the *in vitro* assay was the isolated NSP fraction from wheat. After hydrolysis and chemical analysis this was found to contain 60% xylose, 37% arabinose and 3% glucose residues. The molecular weight distribution was 99% $>DP_{12}$ and 1% $<DP_{12}$.

When dosed at equal levels of xylanase activity in a viscosity assay, both at pH 5.5 and 6.5 the viscosity reducing effect was effectively equal for both preparations. However, in feeding trials the performance of the pure endo-xylanase was slightly inferior to that of the mixed enzyme complex. As the NSP substrate also contains significant amounts of arabinose it is speculated that arabinose activity may also be an important part of the enzyme complex or that other endo/exo-xylanases may play a synergistic or supplementary role and that viscosity reduction is not the only desired effect. This hypothesis is now being evaluated using a combination of the *in vitro* and *in vivo* test systems described above.

* Novo Nordisk BioIndustries UK, Farnham, England
** Novo Nordisk A/S, Copenhagen, Denmark

CHANGES IN NUTRIENT ABSORPTION DURING ACUTE HEAT STRESS

J.J. DIBNER and C.A. ATWELL

Dr. Jadie

Summary

In vitro experiments were conducted to further elucidate changes in nutrient absorption during heat stress. Passage of nutrients across the wall of intact segments of small intestine from either control (TN) or heat stressed (HS) birds was measured. Normal histological appearance was maintained for the entire 45 min organ culture. Nutrients tested in this system include DL-methionine, 2-hydroxy-4-(methylthio)butanoic acid, L-leucine and D-glucose. Transfer of methionine and leucine decreased, while transfer of 2-hydroxy-4-(methylthio)butanoic acid and glucose was significantly increased using intestine from heat stressed birds. Epithelial uptake experiments indicated that nutrient transport systems are altered in heat stress and that absorption of nutrients may be a performance factor during heat stress.

I. INTRODUCTION

The observation that physiological and pathological changes can affect transport of nutrients is not new. Intestinal digestive enzyme and transport systems undergo dramatic ontogenetic changes following birth or hatching (Shehata et al., 1981; Moreto et al., 1991; Nitsan et al. 1991; Sell et al. 1991). Adaptive regulation of adult transport systems also follows fasting (Kotler *et al.*, 1980), changes in diet (Kotler et al., 1980; Ferraris and Diamond, 1989) and onset of disease (Hopfer, 1975).

The role of increased temperature or heat stress on intestinal absorption has not been widely studied, although Wallis and Balnave (1984) reported decreased digestibility of amino acids and Mitchell et al. (1989) reported increased transport of hexoses from the jejunum of heat stressed broilers.

II. EXPERIMENTS

The experiments described in this paper were carried out using Cobb x Cobb cockerels 4-6 weeks old. Acute heat stress (HS, 1-3 days) was achieved using a constant temperature of 33 C. Birds from the same hatch were maintained at a temperature of 22 C and were used as thermoneutral (TN) controls. Chicks were housed in flat deck cages and were euthanized using carbon dioxide inhalation. Birds were fed a corn soy broiler starter ration supplemented with equal amounts of 2-hydroxy-4-(methylthio)butanoic acid (HMB, Alimet®) and DL-methionine (DLM). Intestine, 10 cm anterior and posterior to the yolk stalk, was rinsed and used for nutrient transfer or epithelial uptake experiments. Differences between TN and HS in nmol/gm transferred or taken into the cells were considered significant at a probability value of < 0.05.

Novus International, Inc., 530 Maryville Centre Drive, St. Louis, MO 63141 USA
® Registered Trademark, Novus International, Inc.

(a) The Effects of Acute Heat Stress on *In Vitro* Nutrient Transfer

In these experiments, the effect of heat stress on absorption was studied using an in vitro model and methods described previously (Dibner et al., 1992). Intestine segments were rinsed and filled with a buffered salt solution containing the nutrient being studied. Samples of outside buffer were taken every 15 min and analyzed by scintillation counting or chromatography. Initial experiments using HMB and DLM revealed that transfer of HMB from the lumen to the outside buffer was consistently increased using intestine from HS birds while transfer of DLM was decreased (Dibner et al., 1992). L-Leucine was examined using this system to determine whether transfer of amino acids other than DLM was affected. Figure 1 shows the HS effect on L-leucine transfer. Showing similarity with DLM, radiolabeled leucine transport was reduced using intestine from HS broilers. In contrast, the transfer of D-glucose was significantly increased by HS (Figure 1).

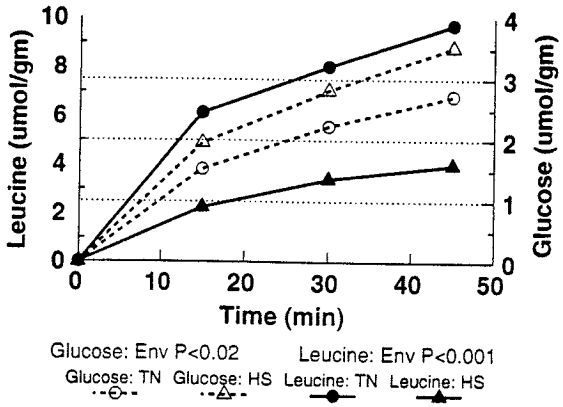


Figure 1

Changes in the rate of transfer of amino acids, hydroxy acids and sugars as have been observed in these studies may be due to macroscopic, microscopic or molecular changes in the intestinal tract. Examples of macroscopic changes include thinning of the intestinal wall or shortening of the intestinal villi, both of which could increase the absorption of HMB which occurs mainly by diffusion (Knight and Dibner, 1984). Examples of microscopic factors which could influence rate of absorption include changes in the proportion of absorptive cells in the villus epithelium and examples of molecular changes include increases or decreases in nutrient transport molecules or the energy available to drive them. Such changes would be likely to affect rate of absorption of amino acids and sugars which involve not only diffusion, but carrier specific mechanisms, either energy dependent or energy independent mechanisms or both (Lerner and Messier, 1980; Stevens et al., 1982).

The actual cellular processes involved in this transfer assay include uptake at the apical cell membrane, extrusion at the basal cell membrane and diffusion through the mucosal and submucosal connective tissue before material will appear in the outside buffer. In order to clarify the mechanism responsible for changes in nutrient transfer, epithelial uptake experiments were performed. With the uptake assay, only the transport systems of the apical membrane are detected.

(b) The Effects of Acute Heat Stress on Nutrient Uptake by Intestinal Epithelial Cells

Figure 2 shows the results of uptake studies for D-glucose. The rate of glucose diffusion was not affected by HS, but the carrier specific transport system at the apical cell membrane was significantly increased in HS intestine, resulting in a significant increase in total D-glucose uptake.

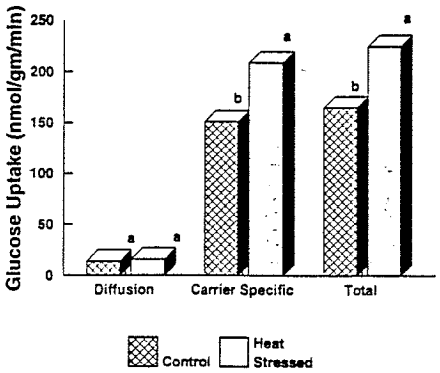


Figure 2

For L-leucine, (Figure 3) results were similar to those observed for methionine. A significant decrease in carrier specific transport resulted in an overall decrease in total amino acid uptake.

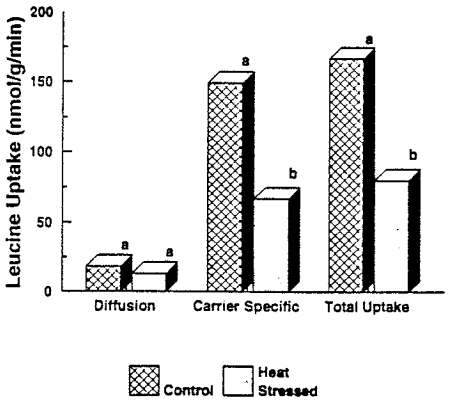


Figure 3

The results reported here for methionine, leucine and glucose are consistent with previously published changes in blood parameters in heat stressed poultry. For example, Donkoh (1989) and Ostrowski-Meissner (1981) both report that circulating glucose is increased in heat stressed birds. In addition, Ostrowski-Meissner (1981) observed reduced plasma essential amino acid levels in heat stressed cockerels. Finally, it has been demonstrated in numerous feeding studies that the growth depression associated with heat stress cannot be overcome by increasing nutrient density (Adams and Rogler, 1968; Cowan and Michie, 1968;

Kubena *et al.*, 1972). These findings would be predicted if heat stress had a primary effect on nutrient transport.

III. CONCLUSIONS

In summary, passage of amino acids, hydroxy acids and sugars across intact segments of small intestine from either control (TN) or heat stressed (HS) birds was measured. For DLM or leucine placed in the intestinal lumen, appearance in the outside buffer was reduced using tissue from HS birds in comparison to tissue from TN birds while the opposite was seen with HMB and glucose, i.e., appearance in the outside buffer was greater when intestine from HS birds was used. Further study of epithelial cell uptake in TN and HS intestine revealed that differences in transfer are associated with changes in carrier specific transport systems. These results indicate that absorption of nutrients may be a performance factor during acute episodes of heat stress.

REFERENCES

ADAMS, R.L., and ROGLER, J.G. (1968). Poult. Sci. 47:579.

COWAN, P.J., and MICHIE, W. (1968). Br. Poult. Sci. 19:601-605.

DIBNER, J.J., ATWELL, C.A. and IVEY, F.J. (1992). Poult. Sci. 71:(In press).

DONKOH, A., (1989). Int. J. Biometeorol. 33:259-265.

FERRARIS, R. P., and DIAMOND, J.M. (1989). Annu. Rev. Physiol. 51:125-141.

HOPFER, U., (1975). Proc. Nat. Acad. Sci. USA 72:2027-2031.

KNIGHT, C.D. and DIBNER, J.J. (1984). J. Nutr. 114:2179-2186.

KOTLER, D.P., LEVINE, G.M. and SHIAU, Y.F. (1980). Am. J. Phsiol. 238:G219 G227.

KUBENA, L.F., LOTT, B.D., DEATON, J.W., REECE, F.N. and DAY, J.D. (1972). Poult. Sci. 51:517-522.

LERNER, J. and MESSIER, D.L. (1980). Comp. Biochem. Physiol. 65A:363-365.

MITCHELL, M.A., PALMER, A.J., and BROWN, V. (1989). Br. Poult. Sci. 30:972. (Abs.)

MORETO, M., AMAT, C., PUCHAL, A., BUDDINGTON, R.K. and PLANAS, J.M. (1991). NITSAN, Z., BEN-AVRAHAM, G., Zoref, Z.and Nir, I. (1991). Br. Poultry Sci. 32:515-523.

OSTROWSKI-MEISSNER, H. T., (1981). Comp. Biochem. Physiol. 70A; 1-9.

SELL. J. L., ANGEL, C.R., PIQUER, F.J., MALLARINO, E.G. and AL-BATSHAN, H.A. (1991). Poult. Sci. 70:1200-1205.

SHEHATA, A.T., LERNER, J. and MILLER, D.S. (1981). Am. J. Physiol. 240:G102-G108.

STEVENS, B.R., ROSS, H.J. and WRIGHT, E.M. (1982). J. Membrane. Biol. 66:213-225.

WALLIS, I.R. and BALNAVE, D., (1984). Br. Poultry Sci. 25:401-407.

PRODUCTION RESPONSES OF HENS FED BLANCHEFLEUR VETCH

P.C. GLATZ and R. J. HUGHES

In Australia, grain legumes are an important source of protein for poultry. Some grain legumes such as vetch contain γ-L-glutamyl-L-β-cyanoalanine which is neurotoxic (Arscott and Harper, 1963). Inclusion of 10% Blanchefleur vetch resulted in an immediate drop in feed intake and rate of lay despite selective avoidance by hens (Glatz et al., 1992).

In this study, we included Blanchefleur vetch at 0, 50 and 100 g/kg in fine mash or cold-pelleted diets to prevent selective feeding. Each diet was fed continuously to 36 hens from 26-66 weeks of age. Each kg of feed provided 11.4 MJ AME, 178 g protein, 36.5 g calcium and 7.4 g total phosphorus.

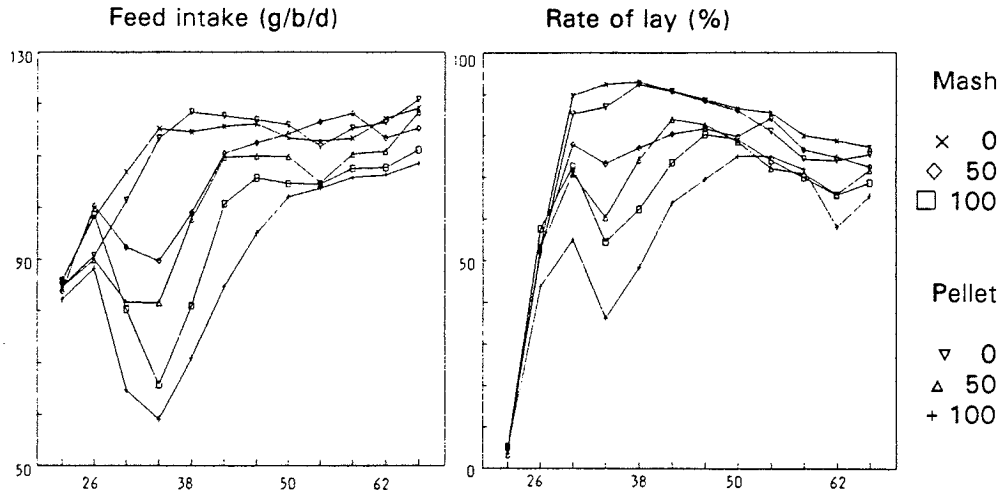


Figure 1. Feed intake and rate of lay from 18-66 weeks of age.

Inclusion of vetch at 50 and 100 g/kg resulted in immediate declines in feed intake and rate of lay (Figure 1). However, it was apparent that some hens were largely unaffected and that most of the others adapted to the dietary vetch as indicated by the slow return to normal egg production.

Possible mechanisms for the recovery include detoxification by gut microflora, conversion to asparagine by mucosal enzymes or degradation with storage. Further studies are planned to examine these possibilities and to find practical methods of removal or inactivation of toxins.

ARSCOTT, G.H. and HARPER, J.A. (1963). J. Nutr. 80: 251.
GLATZ, P.C., HUGHES, R.J. and WOOLFORD, R.C. (1992). Proc. Aust. Poult. Sci. Symp., p.142 ed. R Johnson.

Parafield Poultry Research Centre, SA Department of Primary Industries, 230 Salisbury Highway, Parafield Gardens, South Australia, 5107

THE USE OF SIMULATION MODELS IN ESTIMATING THE NUTRITIONAL REQUIREMENTS OF BROILERS

R M GOUS

Summary

Genotypes used in the broiler industry have changed significantly in the past 40 years, and continue to change at the same rate today. Different selection criteria are used by the major breeding companies, leading to widely different genotypes being available to the broiler industry. Yet nutritionists have largely ignored these changes when formulating feeds and feeding strategies for broilers. The method presently used to formulate feeds for broilers relies on tables of nutrient requirements for different phases in the life of the broiler. These tables do not reflect the requirements of broilers capable of growing at different rates, or of broilers having different genetically-determined degrees of fatness.

The only defensible way for nutritionists to improve the efficiency of feeding broilers is by the use of simulation modelling. This technique integrates information about the bird, the feed and the environment, which can then be used to make accurate predictions of food intake and growth rate for any given bird, in any given state, and in any given environment. With such a model it is possible to determine the most economical method of feeding broilers under a wide range of economic conditions.

In this paper the philosophy behind the use of simulation models is given; concepts, such as potential growth rate, that are essential in understanding how models work, are discussed; and examples are given of how models can be used to explain apparent anomalies in nutrition research and to determine the most economical method of feeding broilers.

I. INTRODUCTION

Amino acid requirements of broilers are invariably estimated from the results of experiments in which broilers of a given age have been offered diets containing graded supplements of the amino acid under study. The concentration of amino acid in the diet producing the maximum growth response was regarded as being the requirement for that amino acid. Although this method is fraught with potential errors (Gous and Morris, 1985) it is still the preferred method by some nutritionists and committees. The resultant requirement is expressed as a fixed concentration in the diet, ideal for constructing tables of requirements and for least-cost formulation of feeds.

It is impossible to apply an accurate cost/benefit analysis to such numbers. With a fixed requirement, there is no way of determining the effect on growth, food intake or carcass composition of either increasing or reducing the concentration of an amino acid in the diet. It is also not possible to suggest how, or even whether, these requirements should change with the genotypes available to the broiler industry. In fact, little, if any, notice has been taken of the changes

Department of Animal Science and Poultry Science, University of Natal, Pietermaritzburg, South Africa.

that have been made to broiler genotypes, even though these have been extremely impressive. It is assumed by most nutritionists that faster-growing broilers would exhibit a higher food intake, which would compensate for the lower-than-required amino acid content of the diet. But Morris and Njuru (1990) have demonstrated convincingly that this does not happen.

Fisher, Morris and Jennings (1973) showed that there was an advantage in seeing the requirements of animals as variable, dependent on the marginal cost of the amino acid and the marginal returns of the product. Prediction equations could then be used to determine the optimum amino acid intake for a given set of economic conditions. In spite of a general acceptance of the theory that requirements for amino acids should not be seen as being fixed, the tendency remains to do so.

The major reason for this apparent reluctance to move from the outdated and inaccurate system of seeing requirements as fixed, to the more dynamic theory, is that there are so many factors that have to be integrated before the optimum economic feeding schedule can be determined. This is especially true of feeding programmes for growing animals. Factors to be considered include the potential of the genotype; differences between individuals at a time and within individuals over time; the effect of different nutrient concentrations and energy-to-protein ratios, on food intake, carcass composition and protein gains; the effects of high or low environmental temperatures on all of the above; and the constraints placed on the animal by the environment and by the feed, which prevent the animal from consuming the necessary amount of a feed to grow at its potential.

It is only through the development of a plausible theory, and the advent of computers, that it has been possible to integrate all of these factors into a workable form. Simulation models are capable of rendering assistance to nutritionists, geneticists and producers wishing to make the broiler production enterprise more efficient. In fact, there is no defensible way to estimate the nutrient requirements of growing animals other than by the use of models.

(a) Theory of Growth and Food Intake

1. Potential growth rate

Predicting the performance of animals is a general problem in animal production. The solution to this problem depends, in part, in being able to describe the animals adequately (Emmans and Fisher, 1986). There is no consensus nor any general discussion in the literature on methods of defining genotypes that would allow similarities and differences between animals to be compared. However, with the advent of simulation models for describing the growth and food intake of animals, an adequate description of the genotype becomes essential.

The concept of a *potential growth rate* is often misunderstood by poultry scientists and producers. It is not the maximum growth rate that can be achieved by a flock of broilers of a given genotype under commercial husbandry conditions, but the maximum growth rate that the genotype can possibly achieve when given perfect nutritional and husbandry conditions. The carcass composition of a bird grown under such conditions would reflect the genotypic composition of the bird, unaltered by external factors - this being particularly important in respect of the lipid content of the bird, which is significantly influenced by deviations from a

perfect feed, feeding strategy and environment.

The approach used by Emmans (1989) to describe and evaluate different genotypes begins with a definition of potential protein growth, and the live weight of the animal is built up from this, using the allometric relationships that exist between protein, water, ash and lipid, i.e. a 'bottom-up approach. He has shown that a few, simple, assumptions can lead to a description of an animal that is sufficient for predicting its performance in non-limiting conditions and for calculating what these are. It seems sensible to be able to predict performance in non-limiting conditions before the more difficult question is tackled, namely, that of defining growth in limiting conditions.

Values for the genetic parameters which define an animal can be measured by rearing animals in environmental conditions which are as near to ideal as possible. Under these conditions, growth curves are obtained which represent the genetic potential for a particular genotype. The growth curves obtained in this way allow comparisons to be made between breeds and strains.

2. Predicting nutrient requirements

For a model of growth to be successful it must be able to calculate the nutritional and environmental requirements of the bird that are needed for potential growth, and it must be able to predict the consequences of deviations from these optimum conditions. A growing animal needs to be supplied with nutrients in order to meet the requirements for maintenance of the body and for the growth of all other components of the body, including feathers. The resources needed to meet these requirements can be determined from a knowledge of the growth rate and composition of the various components of the body. The resources available for supplying these requirements, which are present in various feedingstuffs, need to be described in the same terms as are used to describe the nutrient requirements.

Emmans (1984) has shown that the metabolizable energy (ME) scale is not a sufficiently accurate means of describing the energy content of a feedstuff. The ME scale is unable to differentiate between the efficiency of utilization of the energy emanating from the three digestible components of protein, lipid and carbohydrate, nor does it take account of the effect of indigestible organic matter on the energy available to the animal from the diet. The energy scale proposed by Emmans (1984) to take account of these deficiencies in the ME system is known as the effective energy (EE) scale and is the preferred energy system to be used in simulation modelling.

The requirement for protein depends on the amino acid composition of that protein and the rate at which it is being produced. The sum of each amino acid required for the maintenance and the growth of feather and of body protein constitutes the daily requirement for each of the amino acids. The retention of lipid, water and ash have no protein requirement. The scale on which the amino acids required by the animal are measured, and on which the amino acids in the feed are described must be the same. The conventional wisdom is to express this in terms of digestibility. The marginal efficiency with which the first limiting amino acid is used for protein retention above maintenance is not necessarily constant, but can be modified by the supply of other amino acids and by the supply of energy.

3. Predicting voluntary food intake

The implication from the above discussion is that an animal has requirements for certain resources that it needs in order to maintain its current state and to grow according to its growth plan. Because the animal is motivated to grow at this potential rate, the acquisition of food as a means of obtaining the required resources becomes a priority. Appetite can be seen to be dependent on the nutrient requirement of the animal and the content of those nutrients in the food (Emmans and Fisher, 1986).

In order to predict how much food the animal will consume when given *ad libitum* access to a food, it is necessary first to be able to predict the rate of intake on a balanced food in a thermally neutral environment. This rate of intake is termed the desired food intake (Emmans and Fisher, 1986) and is that which will allow the potential growth rate to be attained. Because the animal is assumed to eat to satisfy its requirement for the first limiting feed resource, food intake would be expected to deviate from the desired intake when the food is imbalanced in some way or if the animal were placed in an unfavourable environment. In the case of a marginal deficiency of an essential nutrient the animal may be capable of consuming sufficient of that imbalanced food to grow to its potential, but as the deficiency was made more severe, protein growth would fall below the potential. The inability of the animal to eat sufficient of such an imbalanced food is due to the constraints of feed bulk, and the inability to lose to the environment the additional heat that would be produced if more food were consumed. This theory of food intake is explained more comprehensively by Emmans and Fisher (1986).

The most important consequence to the commercial broiler producer of providing broilers with a feed marginally deficient in an amino acid is that the bird will overconsume energy in an attempt to obtain sufficient of the limiting resource, and this energy will be deposited as lipid. It has been shown that broilers exhibit statistically significantly higher feed conversion efficiencies and lower lipid contents when higher concentrations of amino acids than are conventionally used in the broiler industry are included in the feed (Gous, Emmans, Broadbent and Fisher, 1990).

As the potential growth rate of broilers is increased by genetic selection, so the daily amino acid and energy requirements of the bird are increased, but these do not increase in the same proportion. The amino acid requirements increase proportionately faster than does the energy requirement, thus a higher amino acid to energy ratio is required in faster growing strains of broiler. This was well illustrated by the results of an experiment by Morris and Njuru (1990) in which diets of increasing protein content were fed to broilers and laying-type cockerels. The maximum gain in weight and in protein content in the cockerels was achieved on diets with considerably lower protein contents than was needed to maximise the gain in the broilers.

4. Dealing with the environment

The rate of intake of a given food by a given type of bird in a given state will depend on the temperature of the environment in which it is kept. Emmans and Fisher (1986) have suggested that the heat loss of the bird varies in some way with the temperature of the environment, and as the ability of the bird to store

heat is effectively zero, its rate of heat loss must equal its rate of heat production. The environment clearly places an upper limit on the amount of heat that a bird can lose to the environment and this has important consequences in the design of feeds for fast growing broilers. As the rate of growth of broilers has been improved, so the environmental temperature at which they would be comfortable has been reduced, although this constraint on the potential growth rate of modern fast-growing broilers has largely been ignored.

Heat production will increase as food intake increases providing that, at all intakes, the environment is thermally neutral. It follows that the temperature of the environment that is thermally neutral will decrease as the rate of food intake increases. Food intake is predicted to increase as the feed content of a limiting nutrient is decreased, so the growth rate, which defines the requirement, and the composition of the feed, which determines the desired food intake, interact to define the temperature that will be thermally neutral for the bird at that time. Feeds with a low nutrient to energy ratio will cause the thermoneutral temperature to decrease. The calculations involved in determining the consequences of these interactions are complex and therefore benefit from well-constructed simulation models.

(b) Estimating Amino Acid Requirements of Broilers

In order to determine the optimum dietary intakes (and hence concentrations) of amino acids at different stages of the growing period, the response of broilers in protein gain or liveweight gain to a range of intakes of each of the important amino acid must be known. Such responses can be obtained from well-conducted growth trials, or by simulation. The most important feature of the response surface, from the point of view of economic decision-making, is the shape of the curve around the point where the requirement of the average individual in the flock is met.

The shapes of the response curves relating amino acid input to output are similar for broilers and for laying hens, but are produced in different ways, which alter the way in which such responses are interpreted and used. The characteristic shape of the response curve in laying hens is brought about by differences in the potential egg output of the hens in the population (Fisher *et al.*, 1973). Responses of broiler chickens to amino acids are of a similar shape to those in laying hens, but the curvature of the response is not only the result of differences in potential growth rate, but, in addition, to changes in food intake, maintenance requirements and body growth and composition that take place in each broiler over time. The effect of time on these components is not considered in any growth assay, which is a serious drawback when the results of empirical experiments are used to determine nutrient requirements.

Some dietary treatments, which are inadequate at the start of a growth assay become adequate as the broiler grows (Gous, 1986). The curvature in response to such dietary treatments will differ depending on the length of the assay period. Some dietary treatments will, of course, be inadequate throughout the assay period, while others will always be adequate, but it is the intermediate diets that create an additional source of curvature of the response curve. In order to interpret the responses to amino acids in growing animals, it is essential to separate out these confounding effects of time on the shape of the response curve.

Without simulation models, this is impossible to do.

The most promising method of interpreting response curves in growing animals is to reduce the curve to that of an individual at a time, and then to integrate such curves for all of the individuals in a population at that time. Separate population response curves can then be constructed for each day of the growing period and feeding strategies can be optimized in this way. The method has been outlined by both Emmans and Fisher (1986) and Gous (1986). The difference in response between the average individual in a population and the population mean is illustrated in Figure 1, in which the response of male broiler chickens to a range of diets differing in lysine concentration was simulated, over the period seven to 21d of age. The response to lysine of the average individual in the population is clearly not the same as the average response of the population, as has been demonstrated by Fisher *et al.* (1973) with the Reading Model, when this was applied to laying hens.

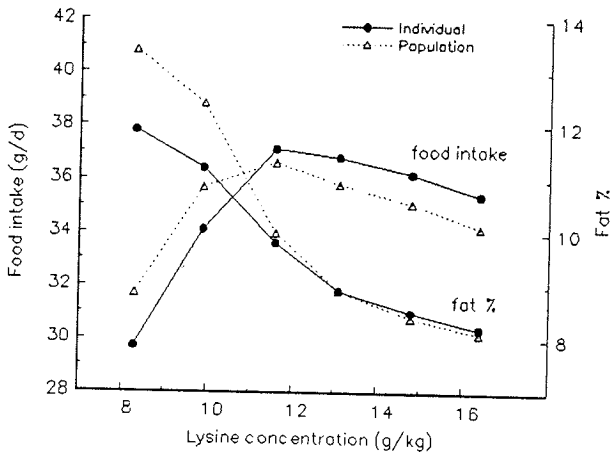


Figure 1. The simulated response in food intake and body fat content to dietary lysine during the period seven to 21d of age. The responses are for the average individual in a population, and for the average of a population.

A simulated cumulative distribution of the requirements for lysine of a population of 1000 broilers at 28d (Figure 2) illustrates that if this flock were fed according to the average individual within the population, i.e. 11.0 g lysine/kg of diet, only half of the individuals would be able to meet their requirements without having to resort to overconsumption of the food. This would constitute virtually all of the females and none of the males. The requirement for the average individual would therefore have to be increased to approximately 12.0 g lysine /kg feed (i.e by ten percent), in order to satisfy the requirements of approximately 0.90 of the population. The requirements for males is considerably higher than for females, indicating the difficulty of providing a single feed that would ensure maximum profitability when the sexes are mixed. If an 'average' feed were offered

to a flock of mixed sexes, such a feed would be either marginally or severely deficient for the males. Broilers have the ability to deal successfully with a marginal deficiency of an amino acid only as long as they are kept in an environment that will allow them to lose the excess heat that is produced as a result of their overconsumption of food. In a hot environment, therefore, the effect of a marginal deficiency of a nutrient would be more severe than in a cool environment.

The above discussion on the estimation of the amino acid requirements of broilers indicates that it is not possible to determine accurately such requirements without considering the potential rate of growth and the state of the broilers, as well as the temperature of the environment in which the birds are to be housed. Estimating the optimum feeding programme for broilers during the growing period is made more difficult because of the interaction between the bird, its feed and its environment.

(c) Optimising the Feeding Programme for Broilers

The optimum feeding programme for broilers is that which results in the highest profit for the enterprise. Determining the optimum number of feeds, the optimum concentrations of nutrients relative to energy in each of those feeds, and the optimum length of time that each feed should be fed, is therefore an economic decision.

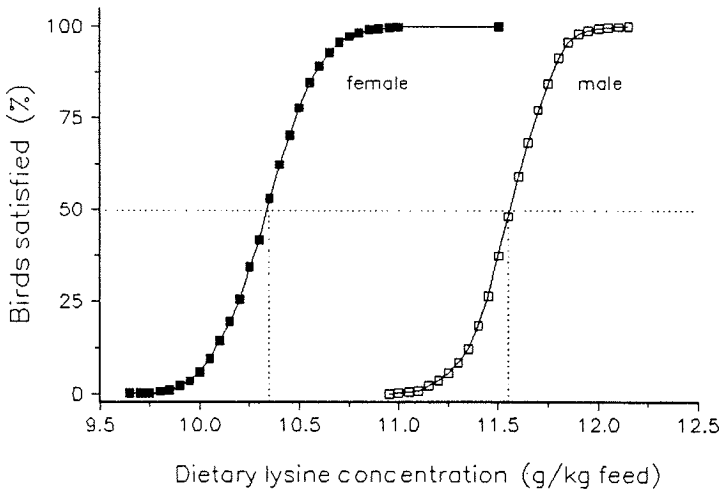


Figure 2. Simulated cumulative distribution of the lysine requirements of male and female broilers of 28d of age.

Because of the many interacting factors that influence the food intake and growth rate of broilers, it is naive to imagine that any one, or even any series, of experiments could begin to address the question of defining the optimum feeding programme for broilers.

Consider some of the procedures that would be necessary in optimising a feeding programme: It would be necessary to determine the potential growth rate and potential degree of fatness of the birds to be fed; the distribution of potential growth rates (greater when mixed sexes are used); the environmental conditions that could be provided, and the cost of altering the prevailing conditions; the costs of a range of feeds differing in nutrient density at each given energy-to-protein ratio; the cost of mixing and then transporting these feeds to the production site (which would place an upper limit on the number of feeds that could be considered in any production cycle). Consider then that the birds can adjust their intake of a given food to an extent, this being limited by the environmental conditions; that the effect of feeding a relatively low quality food initially can be compensated for at a later stage if the conditions are such that the bird can either consume more food later, or draw on lipid reserves, thereby exhibiting an improved feed conversion efficiency. Consider, too, that the amount of lipid in the gain is of importance to some producers, but not to others; and that the length of the production cycle can be altered considerably by the use of different feeding programmes. It becomes clear that only with the use of an accurate simulation model could such an optimisation being contemplated, and only if many iterations of the model are used.

In the absence of an accurate theory of food intake and growth it is possible to provide the birds with a feed that is close to their requirements on each day of the growing period. Choice feeding is one such method, although it is difficult to implement practically in a broiler unit because of the need to have the two feeds on offer within close proximity to one another. An alternative method, more suited to broiler production, is to have available on site two feeds differing in protein content. These two feeds could be blended together in varying proportions, to provide the broilers with the most appropriate protein (amino acid) concentration on each day of the growing period.

However, even with this system, in order to make the correct decision about the optimum blend of the two feeds, it is necessary to know the nutrient requirements of broilers, not at an age (which might be inappropriate, given any set-back in growth) but at a weight and state. By continually monitoring the environmental conditions in the broiler house, as well as the food intake and the weight of the broilers it is possible to estimate the state of the broilers at any one time, and hence the optimum protein concentration that should be fed during the next day (or even hour) of the growing period. This method would be even more successful if the sexes could be reared separately. The differences in nutrient requirements of male and female broilers are considerable, and may be worth exploiting if it were possible to sex broilers inexpensively, and arrange the production cycle accordingly.

Where it is possible to manipulate the environmental temperature in a broiler house, there would be a trade-off between the quality (and hence cost) of the feed and the cost of reducing the house temperature. This may also be worth exploiting. Evaluating such a decision, like so many others, would not be possible without access to a simulation model.

III. CONCLUSION

The correct interpretation of the results of response experiments of broiler growth is made difficult because of the many interacting factors influencing the growth and food intake of these birds. Not all broilers are the same, nor are the environments in which they are housed. Also, they are not all in the same state at the beginning of a response trial. It is only with the use of simulation models that it is possible to gain a better understanding of the processes involved in broiler growth and to be able to take all these interacting factors into account when optimising a feeding programme for broilers. There is no defensible way of estimating nutrient requirements of broilers other than by the use of simulation models.

REFERENCES

EMMANS, G.C. (1984). Animal Production 38: 538.

EMMANS, G.C. (1989). in : Recent Advances in Turkey Science. British Poultry Science Symposium 21: 135 - 166.

EMMANS, G.C. and FISHER, C. (1986). in: Nutrient Requirements of Poultry and Nutritional Research. British Poultry Science Symposium 19: 9 - 39.

FISHER, C, MORRIS, T.R. and JENNINGS, R.C. (1973). British Poultry Science 14: 469 - 484.

GOUS, R.M. (1986). in : Nutrient Requirements of Poultry and Nutritional Research, British Poultry Science Symposium 19: 41 - 57.

GOUS, R.M., EMMANS, G.C., BROADBENT, L.A. and FISHER, C. (1990). British Poultry Science 31: 495 - 505.

GOUS, R.M. and MORRIS, T.R. (1985). British Poultry Science 26: 147 - 161.

MORRIS, T.R. and NJURU, D.M. (1990). British Poultry Science 31: 803 - 809.

MARBLE AS A CALCIUM SUPPLEMENT FOR LAYING HENS

R.J. HUGHES

Hughes (1992) reported that young hens given the opportunity to self-select marble chips produced larger eggs with better shells at a lower rate of lay. Old hens also responded by laying larger eggs with better shells but without loss of production. Marble chips providing about half of the calcium in the diet, were included for only short periods (eight weeks).

In this study, marble chips (2.5 - 4 mm) were given continuously from 18 - 70 weeks of age to determine the effects on shell quality and laying performance. Marble chips replaced one third, two thirds or all of the crushed marble (7.5%) in the control diet. The diets were given to 960 hens housed in single-bird cages in a controlled environment shed. Each kg of feed provided 11.7 MJ AME, 178 g protein, 37 g calcium and 7.1 g total phosphorus.

Inclusion of marble chips in the diet had no effect on rate of lay or feed intake at any time during the experiment, which suggests that loss of production observed previously (Hughes, 1992) could have resulted from reduced feed intake rather than a change in calcium metabolism.

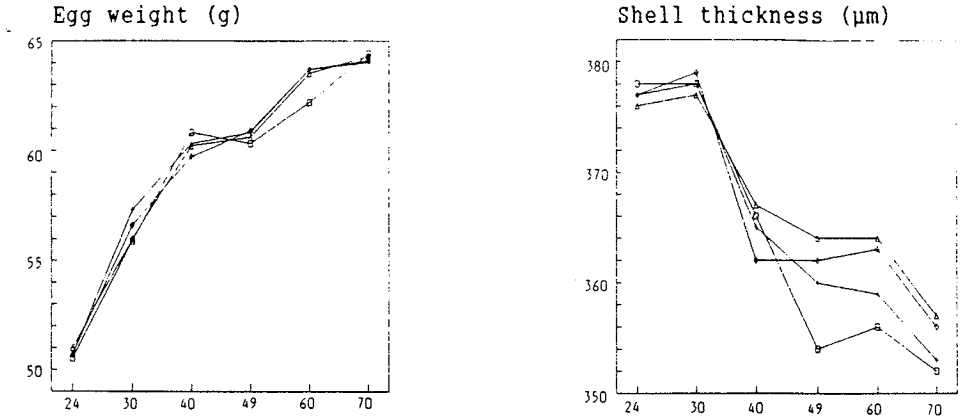


Figure Egg weight (g) and shell thickness (μm) at 24, 30, 40, 49, 60 and 70 weeks of age. No chips (□), ⅓ chips (+), ⅔ (◇), all chips (△).

Hens nearing peak production in warm weather laid eggs that were 1.4 g heavier when marble chips replaced one third of the crushed marble supplement (see figure). In contrast to earlier results, there was no improvement in shell thickness, but neither was there loss of production.

Near the end of lay, during cool weather, marble chips boosted egg weight by 1.3 - 1.5 g (see figure). Decline in shell thickness with age was slowed by the use of marble chips. The best result came from the diet in which marble chips provided all of the supplementary calcium.

HUGHES, R.J. (1992). Proc. Aust. Poult. Sci. Symp., p.120 ed. R Johnson.

Parafield Poultry Research Centre, SA Department of Primary Industries, 230 Salisbury Highway, Parafield Gardens, SA 5107.

COMPARATIVE CHANGES IN COMPOSITION OF THE UTERUS AND BLOOD PLASMA FROM HEAT STRESSED HENS DURING SHELL FORMATION

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

Summary

Hens 43 weeks of age were kept at 22 or 28°C for one week then at 20 or 30°C for a further three weeks. Hens categorised as thick (TK) or thin (TN) shell producers were weighed and bled then killed within 1 h after oviposition or 20 h later during shell formation.

TK hens did not differ in egg weight but secreted more shell material than TN hens. There were no relationships between shell quality and body or uterus weights. Increased uterine moisture during shell formation was evident in TN hens at 20 and 30°C, whereas there was no change in TK hens.

A lowering of protein in uterine mucosa could account for shell thinning associated with heat stress, but not for natural differences between TK and TN hens. Similarities between TK and TN hens in the constancy of mass and concentration of protein during shell formation in both hot and mild conditions suggest that *de novo* synthesis by uterine mucosa is not essential for formation of thick shells.

Lower concentrations of total Ca and inorganic P in plasma from TK and TN hens indicate that bone resorption was diminished during heat stress. However, increases in plasma Ca and P during shell formation in TK hens but not in TN hens suggest that reduced mobilisation of medullary bone could be the cause of natural shell thinning in mild conditions.

I. INTRODUCTION

Cyclic changes in tissue structure and composition coincide with the passage of the developing egg along the oviduct (Solomon, 1983). Increases in lipid, collagen, water and electrolytes (Cipera, 1980a; Mongin, 1978) are thought to be necessary for the uterus to accommodate the egg.

Cipera and Grunder (1977) concluded that consistent differences in body weight between hens genetically selected for production of eggs with high or low specific gravity could point to underlying metabolic differences. Later, Cipera (1980b) reported that while there were no consistent differences in uterine dry matter, better shell quality was associated with lower oviducal tissue weight. In contrast, Castaldo and Maurice (1988) observed no difference in oviduct weight among thick and thin-shell producers from the same genetic line.

Mongin (1978) observed that water content of the uterus increased by 42% during calcification, but only by 16% in the presence of acetazolamide, an agent known to cause egg shell thinning (Lundholm, 1990). Thus, egg shell thinning could be the result of reduced water uptake by the uterus.

This study examined whether (a) changes in uterine weight and moisture during shell calcification were associated with natural variation in shell thinning

* Department of Primary Industries, Parafield Gardens, SA 5107.
** Institute of Medical and Veterinary Science, Adelaide, SA 5000.

amongst hens from the same genetic line, (b) changes in blood plasma reflected underlying differences in bone resorption, and (c) these changes differed during heat stress.

II. METHODS

Commercial White Leghorn X Australorp hens were housed in individual cages in two controlled temperature rooms. Birds were given 16 h light per day and free access to food and water. Each kg of food provided 11.5 MJ AME, 174 g protein, 37.6 g Ca and 9 g total P. Room temperature was maintained at 25 ± 1°C prior to the 4-week experimental period. At 43 weeks of age, temperatures were set at 22 and 28°C for one week in the rooms designated mild and hot, respectively, then at 20 and 30°C for three weeks.

In week 3 of the experiment (hens 46 weeks of age), ovipositions were observed and recorded hourly from 0700 to 1600 for four consecutive days. Egg weight, shell weight and equatorial shell thickness were measured. Average shell thickness was calculated for each hen which laid two or more eggs. Hens in the top and bottom quartiles in each room were categorised as thick (TK) and thin (TN) shell producers, respectively.

In week 4 (hens 47 weeks of age), 20 TK and 20 TN hens from each room were killed. Hens were taken soon after oviposition between 0900 and 1200, or 20 h later, i.e., during calcification of the next egg which was measured as described above. Immediately prior to slaughter, each hen was weighed then blood sampled. Blood plasma was analysed for total calcium and inorganic phosphate. Uteri were analysed for dry matter and moisture content or for protein concentration of the mucosa.

III. RESULTS

Fully formed eggs from TK hens did not differ in egg weight, but had heavier shells and, hence, thicker shells than eggs from TN hens in both hot (constant 30°C) and mild (constant 20°C) environments (table 1). The same pattern of results was evident for partially calcified eggs taken from the uterus (Table 1). Egg weight, shell weight and thickness of fully and partially formed eggs were unaffected by room temperature.

Body weight (1.87 kg) was unaffected by room temperature, nor were there any differences between TK and TN hens at either temperature.

Uterus weight increased by about 3 g in the period 0 to 20 h after oviposition in TK and TN layers at 30°C (Table 2). Analysis of covariance indicated that at least 2 to 2.5 g of the increase was independent of differences in body weight. There were no significant increases in uterus weight at 20°C. Uterus weight was unaffected by room temperature, nor were there any differences between TK and TN hens at either temperature.

TN hens kept at 20°C showed a significant increase in uterine moisture content in the period 0 to 20 h after oviposition (Table 2). At 30°C, the increase in uterine moisture content in the period 0 to 20 h was nearly significant ($P = 0.053$) for TN hens. TK hens showed no change in uterine moisture content.

Table 1. Characteristics of eggs laid normally or taken from the uterus at 20 h after the previous egg laid by thick (TK) and thin (TN) shell layers kept at 20 or 30°C.

	30°C		20°C	
	TK	TN	TK	TN
Laid normally	$n=19$	$n=18$	$n=19$	$n=19$
Egg weight (g)	57.4	56.1	59.4	57.2
Shell weight (g)	5.46*	4.47	5.85*	4.86
Shell thickness (μm)	377*	310	391*	340
Taken from the uterus	$n=10$	$n=10$	$n=10$	$n=10$
Egg weight (g)	56.1	53.9	57.8	54.8
Shell weight (g)	4.43*	3.73	4.81*	4.13
Shell thickness (μm)	316*	270	334*	301

* Significantly different ($P<0.05$) from TN hens at the same temperature.

Table 2. Uterus weight and moisture content for thick (TK) and thin (TN) shell layers kept at constant 20 or 30°C and killed immediately after oviposition or 20 h later during shell formation.

	30°C				20°C			
	TK		TN		TK		TN	
	0 h	20 h	0 h	20 h	0 h	20 h	0 h	20 h
Uterus weight (g)	$n=9$ 12.7*	$n=10$ 15.3	$n=8$ 12.0*	$n=10$ 15.6	$n=9$ 13.7	$n=10$ 14.5	$n=9$ 13.7	$n=10$ 14.3
Uterus moisture (%)	$n=3$ 84.4†	$n=5$ 84.6	$n=3$ 82.1	$n=5$ 83.9	n=4 82.3	$n=5$ 82.8†	$n=4$ 82.8*	$n=5$ 84.8

* Significant difference ($P<0.05$) between 0 and 20 h within the same shell thickness category and temperature.

† Significant difference ($P<0.05$) between thick and thin-shell layers within the same sampling time and temperature.

Hens kept at 30°C had a significantly lower concentration of protein in uterine mucosa than hens kept at 20°C (103 vs. 125 mg/g), but did not differ in mucosa weight (1.52 vs. 1.65 g). Protein concentration and mucosa weight did not differ between TK and TN hens at either temperature and were unaffected by time after oviposition.

Hens kept at 30°C had a significantly lower concentration of total calcium in blood plasma than hens kept at 20°C (6.51 vs. 6.75 mmol/L) and tended ($P<0.1$) to have a lower concentration of inorganic phosphate (2.23 vs. 2.38 mmol/L). TK hens kept at 20°C showed significant increases in total calcium (from 6.3 to 7.1 mmol/L) and inorganic phosphate (from 2.0 to 2.7 mmol/L) during shell formation.

IV. DISCUSSION

Hens categorised as thick or thin-shell layers differed in shell weight and thickness, but not egg weight, in both hot and mild conditions. The lack of statistical effect of raised temperature on egg and shell characteristics was unexpected. Perhaps the temperature differential was too small or the period of exposure too short to result in smaller eggs with thinner shells.

It was likely that the rapid stage of shell formation was still in progress 20 h after the previous oviposition because shell weight of eggs taken from the uterus ranged from 80 to 85% of the shell weight of fully formed eggs (Table 1). Differences between TK and TN hens in shell weight at 20 h post-oviposition indicate that there was a more rapid rate of accretion of $CaCO_3$ in the shell for TK hens, irrespective of temperature.

We observed no inverse relationship between shell quality and body or uterus weight, in contrast to the findings of Cipera (1980b). Castaldo and Maurice (1988) also reported no relation between uterus weight and shell surface density in hens from the same genetic line. This might indicate that lines of hens selected for high or low specific gravity, such as those used by Cipera and others, differ in other ways to naturally good and poor shell producers within a line not divergently selected for shell quality.

Our results are in marked contrast to those of Mongin (1978) who concluded that egg shell thinning could be the result of reduced water uptake by uterine mucosa. We believe that failure to limit uterine water uptake during shell formation is linked with reduced secretion of shell material by TN hens.

Concentration of protein in uterine mucosa was the only variable measured in this study which was unconditionally affected by room temperature. Shell thinning due to elevated temperature might arise from reduced concentration or amount of protein in uterine mucosa, and that had this study continued longer, differences in shell weight and thickness would have become evident. Furthermore, because TK and TN hens did not differ in amount or concentration of protein in uterine mucosa at either temperature, we suspect that a separate mechanism for shell thinning not associated with exposure to raised temperature was responsible. Also, similarities between TK and TN hens in the constancy of mass and concentration of protein during shell formation suggest that *de novo* synthesis by uterine mucosa is not a prerequisite for formation of thick shells in either hot or mild conditions.

Lower concentrations of total Ca and inorganic P in plasma from hens kept at 30°C suggest that bone resorption was diminished at the higher temperature, although this was not reflected in differences in shell weight or thickness (Table 1). On the other hand, significant increases in plasma Ca and P during shell formation in TK hens at 20°C but not in TN hens imply that reduced ability to mobilise medullary bone could be an underlying cause of shell thinning in mild conditions.

V. ACKNOWLEDGMENTS

We thank the Egg Industry Research and Development Council for financial support.

REFERENCES

CASTALDO, D.J. and MAURICE, D.V. (1988). Poult. Sci. 67: 427.
CIPERA, J.D. (1980*a*). Poult. Sci. 59: 635.
CIPERA, J.D. (1980*b*). Poult. Sci. 59: 2797.
CIPERA, J.D. and GRUNDER, A.A. (1977). Poult. Sci. 56: 1324.
LUNDHOLM, C.E. (1990). Comp. Biochem. Physiol. 95C: 85.
MONGIN, P. (1978). Br. Poult. Sci. 19: 501.
SOLOMON, S.E. (1983). In: Physiology and Biochemistry of the Domestic Fowl Vol 4 (Editor, Freeman, B.M.) pp 379-419 Academic Press, London.

STABILITY AND MODE OF ACTION OF POULTRY FEED ENZYMES IN DIETS BASED ON WHEAT AND BARLEY

J. INBORR, M.R. BEDFORD and H. GRAHAM

Summary

An experiment was carried out to investigate the pelleting stability of a commercial poultry feed enzyme and the mechanisms involved in the enzyme induced performance response in broiler chickens. The enzyme product was found to maintain its activity through conditioning at 95°C for 15 minutes followed by pelleting. Reduction in the viscosity of the digesta explained the response to enzyme addition.

I. INTRODUCTION

Wheat and barley both contain chemical components that interfere with digestion and nutrient absorption when fed to poultry. These components have been identified as the soluble non-starch polysaccharides (NSP). In wheat the main component of the soluble NSP fraction are the arabinoxylans (pentosans) (Annison, 1991; Fengler and Marquardt, 1988) and in barley the mixed-linked β-glucans (White et al., 1983; Classen et al., 1985). These soluble fibres possess gel-forming properties and exert their anti-nutritional effects by increasing digesta viscosity in birds (Bedford et al., 1991). Supplementation of wheat and barley based feeds with fibre-degrading enzymes has resulted in marked improvements in the performance of broiler chickens (Pettersson et al., 1990; Choct and Annison, 1992; Edney et al., 1989). Bedford and Classen (1992) demonstrated that reduction in digesta viscosity due to pentosanase supplementation of wheat and rye based feeds was responsible for the performance improvement of broiler chickens.

With the advent of commercially available feed enzymes - a consequence of numerous publications showing efficacy and a true demand within the feed industry -process (heat) stability has become a major concern, in particular, where high pelleting temperatures are employed.

II. EXPERIMENTS

The first objective of the experiment described in this paper was to investigate the effect of steam pelleting on enzyme activity by either *in-vitro* (enzyme activity analysis) or *in-vivo* (bird performance, digesta viscosity) methods. The second objective was to correlate performance with chick digesta viscosity to try and explain the mechanism involved in the enzyme response.

(a) Feed enzyme survivability through steam pelleting.

A barley/wheat-based feed supplemented with (A) or without (C) a

Finnfeeds International Ltd., Market House, Ailesbury Court, High Street, Marlborough, Wiltshire SN8 1AA, United Kingdom.

commercial feed enzyme product (Avizyme SX®) was pelleted at three conditioning temperatures (targets: 75, 85 and 95°C) after conditioning for either 30 seconds (C1-3 and A1-3) or 15 minutes (C4-6 and A4-6). Pellet diameter was 3.5 mm and die thickness 65 mm.

1. *In-vitro* assessment.

After cooling, β-glucanase activity of the feeds was measured by a viscosimetric method (modified from Bathgate, 1979) to determine the survivability of the enzyme.

The measured conditioning temperatures were slightly below the targets, whereas pelleting tended to increase the temperature of the feeds (Table 1). The control mash feed contained detectable amounts of endogenous β-glucanase, which was largely inactivated at pelleting. Enzyme recovery in the supplemented feeds decreased with increasing conditioning temperatures and time.

Table 1. Measured conditioning and pellet exit temperatures (°C), and β-glucanase activities in mash and pelleted feed samples.

Treatment	Conditioner		Pellet exit	β-glucanase,
	Target	Measured	Measured	IRVU/kg
C (mash)				4
C1	75	74	78	2
C2	85	84	84	1
C3	95	90	94	1
C4	75	70	82	2
C5	85	77	83	2
C6	95	89	92	1
A (mash)				26
A1	75	74	81	16
A2	85	83	82	16
A3	95	91	82	3
A4	75	71	86	11
A5	85	83	88	8
A6	95	91	88	4

2. *In-vivo* assessment.

The pelleted feeds were fed to one-day-old broiler chickens (Ross 1) for 19 days with six replicates of five birds per treatment. Live weights and feed consumption was measured on days 1 and 19. On day 19, four birds of each treatment group was randomly selected and digesta viscosity of the proximal half of the small intestine determined as described by Bedford and Classen (1992).

Enzyme supplementation significantly ($P<0.0001$) improved the performance of the birds up to 19 days of age at all treatment conditions employed, whereas increased temperature reduced ($P<0.0001$) performance

linearly (Table 2). The greatest responses to the enzymes were obtained with the long term conditioning treatments, which would suggest that prolonged time of exposure to high temperatures is not an important factor with regard to enzyme survivability in pelleting. There were no significant enzyme x temperature x time interactions.

Table 2. Average live weight gain (g), feed intake (g), feed conversion ratios and gut viscosities (cPs) at day 19 of birds fed the experimental diets.

Treatment	Live weight gain	Feed intake	Feed:Gain	Gut viscosity
C1	544	891	1.64	26.8
C2	556	889	1.60	20.7
C3	483	844	1.75	30.6
C4	487	809	1.66	35.7
C5	539	884	1.64	12.8
C6	495	857	1.73	27.7
A1	562	854	1.52	4.6
A2	561	842	1.50	6.1
A3	510	867	1.68	8.7
A4	580	865	1.49	6.3
A5	540	810	1.50	11.1
A6	513	831	1.62	26.7
Pooled sd	29.6	133.1	0.10	

(b) Mode of action of supplementary feed enzymes.

Plotting gut viscosity values against bird live weight gains and feed conversion efficiency (FCE) of each treatment group revealed a high correlation between these parameters (Figures 1 and 2).

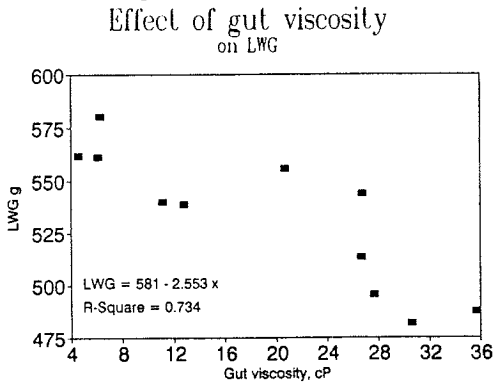


Figure 1. Effect of digesta viscosity on live weight gain to 19 days of age.

The slope of the relationship between gut viscosity and live weight gain was -2.553 (R^2=0.734) and between gut viscosity and FCE -0.00269 (R^2=0.653). These values are close to the ones reported by Bedford and Classen (1992), using wheat and rye and would suggest that digesta viscosity is also the main factor determining the performance of broiler chickens fed barley-based diets.

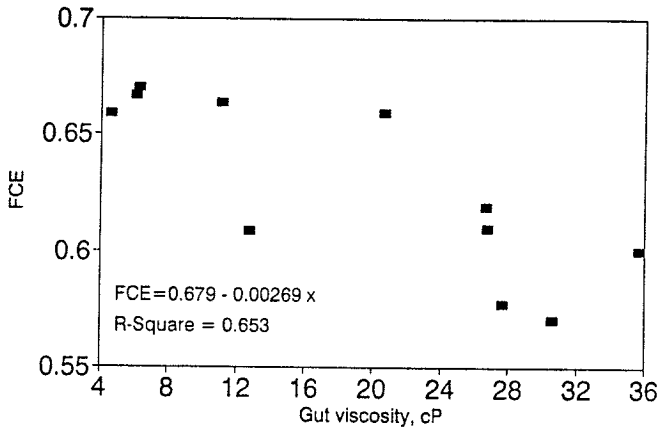


Figure 2. Effect of digesta viscosity on FCE up to 19 days of age.

III. CONCLUSIONS

The results form this experiment would suggest that the commercial feed enzyme product employed maintained its activity in conditioning temperatures up to 95°C followed by pelleting as judged by the bird performance and digesta viscosity response. Enzyme activity analysis in the pelleted feeds failed to show this. Digesta viscosity in the small intestine proved to be highly correlated with performance and could thus be used to assess the effect and relative activity of supplementary enzymes in barley/wheat-based diets for broiler chickens.

REFERENCES

ANNISON, G. (1991). J. Agric. Food Chem. 39:1252
CHOCT, M and ANNISON, G. (1992). Br. J. Nutr. 67:123
BATHGATE, G.N. (1979). J. Inst. Brew. 85:92
BEDFORD, M.R., CLASSEN, H.L. and CAMPBELL, G.L. (1991). Poult. Sci. 70:1571
BERFORD, M.R. and CLASSEN, H.L. (1992). J. Nutr. 122:560
CLASSEN, H.L., CAMPBELL, G.L., ROSSNAGEL, B.G., BHATTY, R and REICHERT, R.D. (1985). Can. J. Anim. Sci. 65:725
EDNEY, M.J., CAMPBELL, G.L. and CLASSEN, H.L. (1989). Anim. Feed Sci. Technol. 25:193
FENGLER, A.I and MARQUARDT, R.R. (1988). Cereal Chem. 65:298
PETTERSSON, D., GRAHAM, H. and ÅMAN, P. (1990). Anim. Prod. 51:201.
WHITE, W.B., BIRD, H.R., SUNDE, M.L., MARLETT, J.A., PRENTICE, N.A. and BURGER, W.C. (1983). Poult. Sci. 62:853

SOYABEAN MEAL AS THE SOLE PROTEIN CONCENTRATE IN BROILER DIETS AND THE EFFECT OF ADDITIONAL PLANT PROTEIN CONCENTRATES

G.G. IRISH and D. BALNAVE

We have recently reported reduced growth in broilers fed diets devoid of animal proteins and containing soyabean meal as the sole dietary protein concentrate (Irish and Balnave, 1991). Growth was improved in these studies by replacing part of the soyabean meal with either sunflower meal or mixed plant proteins. The object of the present study was to determine whether this response was specific to sunflower meal or whether substitution with other vegetable protein concentrates would produce the same beneficial response.

Six replicates of eight one-day-old male broiler chicks were assigned to each of four diets. These were isoenergetic (12.34 MJ/kg) and isonitrogenous (193 g crude protein/kg). They consisted of diets based on vegetable protein concentrates. Diet 1 contained soyabean meal (263 g/kg) as the sole protein concentrate. In Diets 2, 3 and 4 part of the soyabean meal was replaced with 75 g/kg of sunflower meal, rapeseed meal or cottonseed meal, respectively. The soyabean meal concentration in these diets ranged from 205 to 210 g/kg. The diets were fed from 1-21 days of age and the results are shown in the Table.

Dietary Protein meals	Weight gain (g)	Feed intake (g)	Feed conversion (g:g)
—			
Soyabean	343	657	1.92
Soya-Sunflower	407	684	1.69
Soya-Rapeseed	386	713	1.85
Soya-Cottonseed	455	754	1.66
LSD ($P<0.05$)	32	70	0.18

Broilers fed the diet containing soyabean meal as the sole protein concentrate showed significantly poorer growth than broilers fed any of the alternative diets. The feed conversion ratio was also significantly poorer than that of broilers fed the soyabean-sunflower and soyabean-cottonseed combinations. Maximum improvement in performance was observed with the soyabean-cottonseed combination, with the response being mediated through an improvement in feed intake as well as feed utilisation. The relative performance of the broilers on the different diets was not related to differences in ileal apparent amino acid digestibilities.

IRISH, G.G. and BALNAVE, D. (1991). "Rec.Adv.Anim.Nutr.Aust. 1991". p. 26A, (ed. D.J. Farrell, Uni. of New England, Armidale).

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

ASCITES, RESTRICTED FEEDING AND THE PROVENTRICULUS

G.P.D. JONES and R.B. CUMMING

There is some research that suggests that the use of restricted feeding, whilst reducing feed conversion ratio and body fat in broilers, may also lower mortality caused by the ascites syndrome. The decrease in mortality has not been explained. Studies have indicated that distension of the proventriculus may increase pressure on the heart leading to acute death syndrome or ascites. The work presented here examined the effect of restricted feeding on proventriculus size and the incidence of ascites in broilers. Commercial, unsexed broilers were raised in brooders until 6 d of age whereupon they were divided into groups of six. Four replicate groups were fed either *ad libitum* or feed restricted (Jones and Farrell, 1992) for 4 d followed by *ad libitum* feeding. Control birds were grown to 35 d. Restricted fed birds were grown to equivalent bodyweight. The birds were slaughtered and dissected. Growth performance and organ weights are shown in the table.

	Ad libitum	Restricted	LSD† (P=0.05)
Bodyweight (g)	1177	1208	168
FCR	1.97	1.75	0.15
Arterial Pressure Index	30.2	27.4	4.8
Heart (g)	6.9	7.0	0.96
Liver (g)	31.5	39.4	6.8
Gizzard (g)	22.0	20.3	3.6
Proventriculus (g)	6.8	7.2	1.5

† Least significant difference

Bodyweight loss due to feed restriction was recovered in 3 d. FCR was improved as found previously (Jones and Farrell, 1992). The arterial pressure index (API), a measure of pulmonary arterial pressure and an indicator of the onset of ascites, was non-significantly decreased by restricted feeding. Heart, gizzard and proventriculus size were unaffected by restricted feeding. Liver size was markedly increased in agreement with previous research (Gous, 1977). There was a significant relationship between API and proventriculus weight in the restricted fed birds (API = 19.6 + 0.93 Proventriculus wt (g); R^2 = 0.53, P = 0.001). No relationship was established in the fully fed broilers. The data presented here, have failed to fully establish the effects of restricted feeding on the onset of ascites caused by proventricular distension. However, if distension is rapid this will not be detected in an experiment such as that reported in this paper.

GOUS, R. (1977). Br. Poult. Sci. 18: 511.
JONES, G.P.D. and FARRELL, D.J. (1992). Br. Poult. Sci. 33:579.

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

SENSORY NERVES WITHIN THE BEAK OF THE DOMESTIC FOWL: THEIR CONTRIBUTION TO BEHAVIOUR AFTER BEAK TRIMMING

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

Summary

The aim of this work was to determine if the extent of sensory innervation of the beak at the time of trimming affects the subsequent behaviour of the adult hen. Using histochemistry techniques the distribution of nerve fibers and numbers of specialised sensory receptors were found to be similar in beaks of newly hatched chicks and in beaks of 10-day-old chickens. Significant differences however, were observed in the behaviour of adult hens that were beak trimmed at hatch compared to those trimmed at 10 days of age. We suggest that at the time of hatch the sensory receptors are not yet completely functional. Consequently, the removal of a large proportion of receptors during trimming of newly hatched chickens limits the establishment of behavioural patterns.

I. INTRODUCTION

The avian beak is a complex sensory organ that undertakes a wide variety of tasks pivotal to the normal physiological and social behaviour of fowls. The beak is not only essential for eating and drinking, but serves to grasp and discriminate between food particles. The beak is also used for grooming, nesting and for aggressive and defensive activities. With such a diverse range of tasks it is not surprising that a variety of specialised receptors have been described in the beaks of various species of bird (Gottschaldt and Lausmann, 1974; Berkhoudt, 1980; Gentle and Breward, 1986).

Electrophysiological studies indicate that the beaks of adult birds are innervated by different types of sensory nerves; some of which function as pain and temperature receptors (Gentle, 1989). Removal of these receptors and severing of the nerves during beak trimming is therefore likely to affect significantly the behaviour of the birds.

The fear response does not develop fully in birds until 2-3 days of age (Jones, 1987). Our previous work has shown that chickens trimmed on day of hatch consume less food during the laying period than chickens trimmed at 10 days (Glatz, 1990). One explanation for this response is that the sensory nerves and receptors within the beak are not fully functional until several days after hatch. Thus beak trimming at hatch, before the sensory innervation of the beak is completed, would be expected to induce less trauma and behavioural changes than later trimming.

This study was designed to investigate whether behaviour of adult hens is affected by the extent of sensory innervation within the beak at the time of trimming.

---

* Department of Anatomy and Histology, Flinders University, Bedford Park, South Australia 5042.

** Parafield Poultry Research Centre, Parafield Gardens, South Australia, 5107.

II. METHODS

(a) Birds and Beak Trimming

Birds used in this study were a commercial laying strain (White Leghorn x Australorp). Beaks were trimmed either on the day of hatch (day 0) or at 10 days of age. Beak trimming involved removal of half the upper beak and one third of the lower beak in a single cutting action with a heated blade which both cut and cauterised.

(b) Visualisation of Sensory Nerves and Receptors in the Chicken Beak at Hatch Compared to 10 Days of Age

Immediately after killing by cervical dislocation the upper and lower beaks were removed from 3 chickens at hatch and 3 chickens at 10 days of age.

Tissue sections of the beaks were prepared for identification of sensory nerves at the light microscope level using the indirect immunohistochemistry procedure of Coons and Kaplin (1950). Neuron specific enolase, predominantly a nerve specific protein, was used as a marker for the sensory nerves. Free nerve endings that labelled for the peptide substance P were considered to be nociceptive nerves specialised for transmission of pain and temperature. We have previously reported the presence of substance P-labelled nerves in the beak of newly hatched chickens (Lunam and Glatz, 1992).

Sensory receptors were visualised using either Nomarski interference contrast microscopy or Verhoff and Van Geison stain.

(c) Behaviour of Layers Beak Trimmed at Hatch Compared to Trimming at 10 Days of Age

Adult hens, at least 30 weeks of age, were selected at random from caged birds housed under commercial conditions, that had been trimmed at hatch (day 0) or 10 days of age.

Within each cage the hens were marked with coloured dyes on their backs. Time lapse video recordings (1/8th speed) were made over a 4 h period.

Data on behaviour were obtained from watching video records and manually keying observations into a hand held micro-computer. The activites recorded were time and bouts of pecking at food, drinking, preening, sitting and number of pecks made at the cage and other birds. Two separate bouts of behaviour were recorded if the activities were separated by a pause of at least 5 sec duration.

III. RESULTS

Both the upper and lower beaks of all chickens were innervated by many nerve fibers. These displayed bright fluorescence for neuron specific enolase. No differences could be observed in either the distribution or density of the nerves between the beaks at hatch and those of 10-day-old birds. Many nerves were present in the distal regions of the beak and were often observed less than 1mm from the beak tip.

Free nerve endings labelled for substance P. Although these nerve fibers formed only a small percentage of the total number of nerves present in the beak (< 5%), they were distributed throughout the entire upper and lower beak. No differences were observed in the number and distribution of these nerves in the beaks at hatch compared to 10 days of age.

Three types of sensory receptors were identified. Herbst corpuscles were concentrated on the dorsal surface whereas Grandry and Merkel corpuscles were found predominantly on the ventral surface. In both the upper and lower beaks of all chickens examined, the majority of these receptors were located within 2-3mm of the beak tip.

No differences were observed in the feeding behaviour of adult hens beak trimmed at hatch compared to those trimmed at 10 days of age (Table 1).

Birds trimmed at 10 days of age pecked less at the environment but engaged in more bouts of hen pecking than birds trimmed at hatch (Table 2).

Table 1. Effects of age of trim on feeding, drinking and sitting behaviours of adult hens recorded over a 4 hour interval

Age of trim	n†	Feeding time (sec)	Food bouts	Drinking time (sec)	Drink bouts	Sitting time (sec)	Sitting bouts
Day 0	16	3760	54.6	790	21.9	2572	10.1^{a}‡
Day 10	16	3891	54.5	666	19.8	2292	12.5^{b}
LSD		598	5.9	153	4.2	646	2.2

† n Is number of birds

‡ ab Means within columns are significantly different ($P < 0.05$)

Table 2. Effects of age of trim on preening and pecking behaviours of adult hens recorded over a 4 hour interval

Age of trim	n†	Preening time (sec)	Preen bouts	Hen pecks	Hen peck bouts	Cage pecks	Cage peck bouts
Day 0	16	1403	31.8^{a}‡	57	19.1^{a}	76^{a}	18.7^{a}
Day 10	16	1533	40.4^{b}	75	23.9^{b}	34^{b}	12.3^{b}
LSD		195	4.9	23	4.0	20	4.3

† n Is number of birds

‡ ab Means within columns are significantly different ($P < 0.05$)

IV. DISCUSSION

This study demonstrated that the age of beak trimming, (0 days compared to 10 days after hatch) was associated with significant alterations in the behaviour of adult birds.

Day 10 trimmed birds engaged in more bouts of hen pecking than day 0 birds. This is consistent with the day 10 birds being more aggressive than birds trimmed at hatch. One explanation is that chickens trimmed at day 10 were able to establish patterns of pecking and aggressive behaviours. In contrast, chickens trimmed at hatch were not able to fully establish pecking behaviours and the initial aggressive behaviour was thwarted by reduced pecking effectiveness after trimming. Further evidence in support of this view is that chickens trimmed at hatch engaged in fewer bouts of preening than day 10 birds.

The beaks of chicks at hatch were innervated by similar numbers of nerves and receptors as the beaks of ten day old birds. Thus, differences in behaviour of adult birds is unlikely to result from differences in the extent of sensory receptors within the beak at the time of trimming. It is possible however, that the sensory nerves at the time of hatch are not yet optimally functional. This would explain why behavioural patterns, which are expected to be dependent on sensory transmission, are not established on the day of hatch. Perhaps the sensory nerves do not become fully functional until 2-3 days of age, the time of development of the fright response.

V. ACKNOWLEDGMENTS

We thank the Egg Industry Research and Development Council of Australia for financial support of this work.

REFERENCES

BERKHOUDT, H. (1980) J. Zool. 30:1.
COONS, A.H. and KAPLIN M.V. (1950) J. Exp. Med. 91:1.
GENTLE, M.J. (1989) J. Comp. Physiol. 164:763.
GENTLE, M.J. and BREWARD J. (1986) J. Anat. 145:79.
GLATZ, P.C. (1990) Aust. J. Exp. Ag. 30:349.
GOTTSCHALDT, K-M and LAUSMANN S. (1974) Cell Tiss. Res. 153:477.
JONES, R.B. (1987) In: Cognitive Aspects of Social Behaviour in the Domestic Fowl. (eds. R. Zayan and I.J.H. Duncan), Elsevier, Oxford.
LUNAM, C.A. and GLATZ, P.C. (1992) Proc. Aust. Poult. Sci. Symp. 4: p145 ed. R Johnson. University of Sydney.

THE BENEFICIAL EFFECTS OF A MICROBIAL PHYTASE IN RICE BRAN BASED DUCKLING DIETS

ERNESTO MARTIN and DAVID J. FARRELL

Microbial feed phytase was effective in releasing phosphorus (P) from an all vegetable ingredient diets based on sorghum-soybean meal fed to ducklings (Farrell and Martin 1992). Here we used the same feed phytase (Natuphos, Gist-brocades) in duckling diets 0, 200 and 400 g rice bran/kg diet. All of the P was provided by vegetable ingredients and two levels of inorganic P (Pi) were added as $CaHPO_4$. The performance of the ducklings fed these diets is shown in the Table.

Table. Performance of ducklings grown from 2-19 d diets with (+) or without (-) a feed phytase (E) with inorganic phosphorus (Pi) at 1 or 3 g/kg of the diet and with rice bran included at 0, 200 or 400 g/kg.

Rice bran (g/kg)	Pi (g/kg)	E	Gain (g/d)	Feed (g/d)	FCR (g/g)	Tibia ash (g)	Tibia ash (%)
0	1	+	52.6	88.1	1.67	0.98	43.9
0	1	-	44.2	71.7	1.62	0.71	36.1
200	1	+	53.1	93.5	1.76	1.23	47.4
200	1	-	50.2	84.7	1.69	1.01	42.5
200	3	+	53.6	90.2	1.68	1.21	46.1
200	3	-	53.5	90.1	1.68	1.15	46.5
400	1	+	51.6	91.9	1.78	1.10	45.3
400	1	-	49.0	88.7	1.81	1.01	44.4
400	3	+	50.0	87.0	1.74	1.07	45.3
400	3	-	47.3	83.4	1.76	1.02	44.7
LSD (P=0.05)			2.26	3.62	0.056	0.176	2.48

The basal diet without rice bran contained 5.75 g/kg total P and gave the best response to phytase addition. There was a consistent response on the other diets to phytase at the lower (1 g/kg) Pi inclusion rate. There was a Pi x diet interaction ($P<0.01$). The addition of 3 g Pi/kg depressed growth and feed intake on diets with 400 g rice bran/kg. However, the phytase improved performance on these diets, although tibia bone measurements showed only small non-significant differences. It is concluded that rice bran although high in phytic acid (4.5%) responds to a feed phytase which improved P availability even though total P was over 10 g/kg in diets with 400 g rice bran/kg.

FARRELL, D.J. and MARTIN, E. (1992). Proc. 9th Int. Symp. on Waterfowl. Pisa, Italy (in press).

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

ANTI-NUTRITIVE ACTIVITY OF ISOLATED WHEAT PENTOSANS MAY NOT APPLY TO WHEAT

Y. MOLLAH

Summary

The anti-nutritive activity of wheat pentosans isolated from a milling by-product has been suggested to be applicable to native wheat pentosans. But the milling by-product, when included in a wheat based diet, did not show this activity, indicating that the activity was likely caused by the extraction process. The pentosans isolated from the by-product do not represent water soluble pentosans in wheat (WSPw). The mild viscous nature and the low level of WSPw, make their effect practically negligible. The anti-nutritive activity of total pentosans of a cereal has not been established. Observations indicate that the anti-nutritive activity of wheat pentosans may not be the cause of low-ME wheat.

I. INTRODUCTION

The reports on the activity of wheat pentosans are contradictory. Isolated wheat pentosans have been reported to be pro-nutritive (McLaren *et al.* 1979) and anti-nutritive (Choct and Annison, 1990, 1992a). Total pentosans, composed of isolated wheat pentosans and native pentosans of sorghum/soyabean, has been reported to be anti-nutritive (Choct and Annison, 1990, 1992a). But the total native wheat pentosans is not related to the anti-nutritive activity (Annison, 1990, 1991). It appears that the isolation process dictates the activity of wheat pentosans.

The isolation process of water insoluble pentosans (WIP) involved saponification (0.2M NaOH, 80°C, 2 hours), neutralization and extraction of the soluble fraction. When WIP are incorporated into a sorghum based diet, a dietary level of pentosans as low as 29g/kg (2.5g WIP pentosans and the rest sorghum pentosans) shows anti-nutritive activity (diet 2 of Choct and Annison, 1990) which is due to an increase in intestinal viscosity (Choct and Annison, 1992b). The activity of WIP has been suggested to be applicable to native wheat pentosans (Annison *et al.* 1992). Wheat contains a considerable amount of pentosans (>50g/kg, Annison, 1990) and therefore, any wheat diet at an inclusion level of 800g/kg, is expected to show the activity. However, this rarely happens in practice. To verify whether the activity was caused by the extraction process, the same pentosan-rich wheat-milling by-product as used by Choct and Annison (1990) was used in this study.

II. MATERIALS AND METHODS

Wheat and casein were obtained from local commercial sources. The AME values (MJ/kg) of casein and the by-product were 15.95 (determined previously) and 5.96 (calculated from the composition). A low foaming detergent was added to combat expected viscosity. The diets (Table 1) were composed of (g/kg) wheat

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

820, casein 133, dicalcium phosphate 26, calcium carbonate 11 and mineral-vitamin premix 10. The by-product replaced wheat. Five week old broiler chickens were used in the experiment. AME was determined according to the conventional total collection method, using four replicates of five birds each. A five day acclimatization period was followed by a three day collection period.

III. RESULTS

The results appear in Table 1. The milling by-product did not affect AME of wheat. None of the excreta was unusually watery/sticky or contained any significant amount of starch according to the iodine test. There was an interaction between the milling by-product and the detergent.

Table 1. Composition of diets and AME of wheat

Diets	Composition (g/kg) Wheat	Milling by-product*	Feed intake (g/d/bird)	AME of wheat (MJ/kg DM)
1	820	0	113 (6.7)a	14.4 (0.40)a
2	720	100	104 (3.3)a	14.4 (0.40)a
3**	820	0	110 (5.7)a	14.0 (0.71)a
4**	720	100	94 (5.3)a	15.7 (0.52)b

* One hundred grams of the by-product supplied 17.3g pentosans, of which 14.6g water insoluble and 2.7g water soluble.

** Detergent (5ml/kg) was added to reduce expected intestinal viscosity.

In a column figures with unlike letters differ significantly ($P < 0.05$).

Figures in the brackets are standard deviations.

IV. DISCUSSION

The absence of watery/sticky nature of, and appreciable amount of starch in the excreta from all the diets excluded an abnormal intestinal viscosity. The detergent probably dispersed the by-product, a poorly utilised substance and thus, enhanced its utilisation, reflecting in an increase in the AME of wheat. Detergents are known to solubilize proteins and disperse lipids. As yet unknown phenomenon of the detergent involved in the observed interaction may not be ruled out.

In the present study, 14.6g/kg of supplemental insoluble pentosans from the by-product did not depress the AME of wheat; whereas 2.5g/kg of supplemental pentosans from WIP which were extracted from the by-product, depressed the AME of sorghum (diet 2 of Choct and Annison, 1990). Therefore, the reported anti-nutritive activity of WIP was very likely caused by the extraction process which made WIP viscous. A fraction similar to WIP may be obtained from any cereal. A similar fraction from oat-hulls (hemicellulose B) depressed AME as well as starch digestibility of wheat (Mollah 1982).

The level of pentosans in the present study was more than 29g/kg in all the diets which did not show any sign of anti-nutritive activity. Therefore, the level of dietary total pentosans may not be anti-nutritive as has been found by Annison

(1990, 1991). Despite the claim of the anti-nutritive activity of total pentosans (Choct and Annison, 1990), the case has not been established. The diet 2 with comparatively low level of dietary pentosans showed anti-nutritive activity, whereas the diet 6 with high level of pentosans did not.

According to Choct and Annison (1990) the anti-nutritive activity of total pentosans is indicated by the negative correlation between AME and level of pentosans of several cereals (Figure 1, the dashed line, r = -0.95). But the negative correlation indicates mainly the dilution effect of pentosans and related substances (e.g cellulose, ash, etc.). For linear regression, the data set is biased due to the inclusion of barley and rye which are known to have anti-nutritive activity. Excluding barley and rye, the regression line (Figure 1, solid line, r = -0.98), predicts the AME of barley to be 13.68 MJ/kg DM which is the normal value expected from its standard composition (i.e. without anti-nutritive activity) and thus, indicates the absence of anti-nutritive activity of total pentosans.

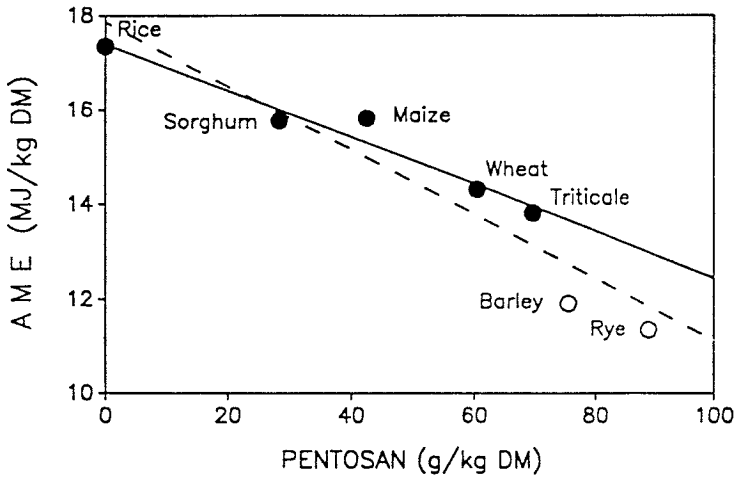


Figure 1. Regreesion of AME on pentosans of cereals.

Based on the NMR studies of several preparations of pentosans, Annison *et al.* (1992) suggested that the native wheat pentosans might have anti-nutritive activity. NMR study reveals the nature of the chemical bonds, but does not reveal the viscous property, the most important anti-nutritive factor. The data on the arabinose/xylose ratios and the molecular weights (Table 2) indicate that WSPw (see note 2 table 2) differs from WIP, WSP and WIPw. Apparently the milling by-product has lost WSPw-equivalent, perhaps, during the previous treatment(s) which might have also partially converted WIPw into WSP. Therefore, WIP or WSP do not represent WSPw, and the results of the studies with WIP and WSP (Choct, 1991; Choct and Annison, 1990, 1992a) may not apply to natural wheat.

The molecular weight distribution (Choct, 1991) indicate that WSPw may be less viscous than WIPdep (WIP depolymerized) which has little anti-nutritive activity (Choct and Annison, 1992b). This mild nature coupled with the low level of WSPw makes their activity practically negligible.

Table 2. Comparison of different pentosan preparations[1]

Pentosan[2]	Arabinose/xylose ratio	Molecular weight (Daltons)
WIP	0.59	758 000
WSP	0.59	500 000
WIPw	0.62	500 000
WSPw	0.80	Polydispersed, Very low - 758 000
WIPdep	-	Polydispersed, 5 300 - 758 000

[1] Data from Choct (1991). Viscosity data are not available.
[2] WIP = water insoluble pentosans from the wheat milling by-product;
WSP = water soluble pentosans from the by-product;
WIPw = water insoluble pentosans from wheat;
WSPw = Water soluble pentosans from wheat and
WIPdep = WIP depolymerized.
All fractions were soluble in water.

The anti-nutritive activity of wheat pentosans has been implied to be the cause of low-ME wheat (Choct and Annison, 1990). But the phenomena of isolated pentosans and low-ME wheat differ widely in their characteristics. WIP and WSP depress growth and feed intake (Choct and Annison, 1992a) and is likely to affect all birds; but low-ME wheat affects only a few birds (Rogel, 1985) and supports normal growth (if not better) at the cost of increased feed intake (Mollah, 1982). The intestinal viscosity due to pentosans is not involved in the low-ME wheat phenomenon (Mollah, 1982; Rogel, 1985). Therefore, the anti-nutritive activity of total or soluble wheat pentosans may not be the cause of low-ME wheat.

The pro-nutritive activity of wheat pentosans, obtained from wheat bran after treating it with acid plus ammonium oxalate and observed with rats (McLaren *et al.* 19769), needs to be verified with chickens. Thus, the nutritive activity of wheat pentosans appears to depend on the extraction process.

REFERENCES

ANNISON, G. (1990). Aust. J. Exp. Agric. 30: 183.
ANNISON, G. (1991). J. Agric. Food Chem. 39:1252.
ANNISON, G., CHOCT, M. and CHEETHAM, N.W. (1992). Proc. Aust Poult. Sci. Symp. 4:151 Ed. R Johnson.
CHOCT, M. (1991). Ph. D. Thesis, The University of Sydney, Sydney.
CHOCT, M. and ANNISON, G. (1990). Br. Poult. Sci. 31:811.
CHOCT, M. and ANNISON, G. (1992a). Br. J. Nutr. 67:123.
CHOCT, M. and ANNISON, G. (1992b). Br. Poult. Sci. 33:821.
MCLAREN, G.A., BANKES, P.W., PATEL, T.I., SMITH, T.R., FAHEY, G.C., WILLIAMS, J.E and NORMANI, M.Z.A. (1979). NUTR. REP. INT. 20:469.
MOLLAH, Y (1982). Ph. D. Thesis, The University of Sydney, Sydney.
ROGEL, A. M. (1985). Ph. D. Thesis, The University of Sydney, Sydney.

CHICKEN GENE MAPPING

CHRIS MORAN[1]

Summary

Molecular genetics has permitted rapid advances in the development of a gene map for the chicken. The first low-resolution map using molecular markers has recently been published. Given the short generation interval of chickens and technical developments in gene mapping, particularly the use of microsatellite markers, a more dense map will soon be produced. Then the useful application of gene maps in identification of quantitative trait loci (QTL) and marker assisted selection (MAS), as well as the cloning of genes via their proximity to characterised marker loci or positional cloning, will progress.

I. INTRODUCTION

The 1980s and 1990s are decades of discovery for those interested in genomes quite on parallel with the voyages of Columbus 500 years ago, which revealed the existence and positions of previously unknown lands. These modern microscopic voyages of discovery promise us benefits in terms of improved human health and greater animal and plant productivity. Like all progress, it has been built on many years of research and technological developments, which are now permitting the flourishing of the age of gene mapping.

Of all the domestic animal species, chickens have always had the best gene map, due to the existence of numerous morphological markers. Also, compared with large mammals, the ease and speed with which test crosses can be made provides a substantial advantage, with perhaps the rat and the mouse being the only other more amenable species. Indeed the first linkages in the chicken were recognised in the late 1920s (Dunn and Jull, 1927; Warren, 1928). The first genetic map of the chicken, with 18 loci in five linkage groups, was published in 1936 (Hutt, 1936). By 1949, 16 autosomal loci were mapped (Warren, 1949) and eight sex-linked loci (Hutt, 1949). The total had climbed to 14 Z-linked and 16 autosomal by 1971 (Somes, 1971) and by 1973, an additional 4 autosomal loci had been mapped (Etches and Hawes, 1973). By 1978, 41 loci were mapped, of which 16 were sex-linked (Somes, 1978).

Somes (1980) presented a summary prior to any substantial impact of molecular techniques, where 68 loci were assigned to 10 linkage groups. By 1984, that number had grown to 88, following the first impact of molecular biology on chicken gene mapping, mainly as a result of the assignment of endogenous viral loci and several oncogenes to linkage groups (Somes, 1984). It is noteworthy that at this stage, the genetic map of the cow consisted of 17 loci, mapped to 6 linkage groups (Womack, 1984) and in the pig, only 36 loci were mapped (Echard, 1984). The maps of these and other domestic species were so rudimentary as to not be included in Volume 1 of "Genetic Maps" published in 1980. By 1987, the number of mapped loci had grown to 101 in the chicken, but still only 10 linkage groups

[1]Department of Animal Science, University of Sydney, NSW 2006

were recognised (Somes, 1987). By mid-1992, the number of mapped loci reached about 265, although not all of these have been reliably mapped relative to each other (Bitgood and Somes, 1990, 1992: 106 loci, mainly morphological; Bumstead and Palyga, 1992: 100 loci, all RFLP; Burke, Hanotte, Gibbs and Thompson, 1992: 22 loci, all minisatellite; Crittenden, Levin, Santangelo and Dodgson, 1992: 37 loci, mainly RAPD). Bumstead and Palyga (1992) believe that 85% of the genome lies within detectable linkage of their 100 RFLP markers. The progress in the mapping of the chicken genome is plotted in Figure 1, which shows the number of loci mapped against year. Despite its half century head start over other species, it is clear that chicken gene mapping is just entering an exponential growth phase, similar to that seen in human gene mapping during the 1980s, when the number of loci mapped increased from 192 in 1980 (Lalley, 1980) to 15,023 at September 8, 1992 (Genome Data Base, Johns Hopkins University, Baltimore).

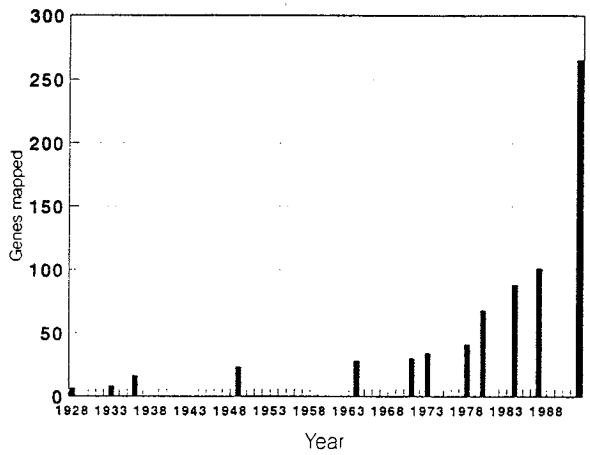


Figure 1. Development of the chicken gene map.

The number (2n = 78) and size of chicken chromosomes cause difficulties for avian gene mapping. While the five pairs of macrochromosomes, which are large and relatively easy to individually recognise, present no problems, microchromosomes are very problematical. The microchromosomes are numerous and very small. While the first four pairs of microchromosomes can be recognised with great difficulty, the remaining 30 pairs are impossible to recognise individually, using standard cytogenetic techniques, and are generally not even counted, let alone identified, in routine cytogenetic analysis. The 11 or so genes of the chicken major histocompatibility complex (MHC), which are the only genes mapped to an identified microchromosome, have very small introns, much smaller than mammalian introns, and in contrast to all other chicken genes, which have introns as large or larger than mammalian introns. Guillemot et al. (1989) have speculated that there may be a peculiarity of parcelling of avian genes on microchromosomes. The small size of the introns in such genes may explain the small size of microchromosomes or perhaps vice versa.

As an indication of the difficulty of dealing with microchromosomes, 33 years after the publication of the first chicken gene map, there was still sufficient uncertainty about the chicken chromosome number that Bloom (1969) was able

to publish what is now regarded as the definitive chromosome count for the chicken, although Yamashina had in fact correctly determined the chromosome number in 1944. Because of these difficulties in identifying chicken microchromosomes, physical mapping techniques, such as *in situ* hybridisation, cannot be easily applied to these chromosomes.

Microchromosomes create difficulty even for linkage mapping. For a genome consisting of few large chromosomes, there is a reasonably high probability that any two random marker loci will be present on the same chromosome so that even loose linkage can be recognised. However if the genome consists of large numbers of very small chromosomes, then it is quite unlikely that two markers will be present on the same chromosome. Therefore linkage will be difficult to detect. Microchromosomes comprise 35% of the physical length of the chromosomes of the chicken (Fechheimer, 1990) and coverage of that part of the genome will be difficult for genetic mappers. Ironically their eventual individual recognition will depend on construction of a well-marked map, which will permit their "genetic" identification via chromosome-specific markers, although they now represent a substantial impediment for the initial construction of a map.

The frequency of crossing-over determines the scale of a genetic map and, together with the position of chiasmata, determines the ease with which close genetic markers can be ordered. Rodionov et al. (1992) have estimated from cytogenetic analyses of female meiosis that the total map length of the chicken genome is 2950-3200 centiMorgans (1 chiasma or crossover = 50 centiMorgans). Assuming one crossover per microchromosome, less than half is macrochromosomal. The small haploid genome size of approximately 1.2×10^9 base pairs, which is 2.75 times smaller than the mammalian genome size, and the mean chiasma frequency of 59-64 per nucleus, means that each centiMorgan is equivalent to about 400 kilobases of DNA. This is considerably less than the value of 1-2 megabases of DNA per centiMorgan for mammals, which should make positional cloning easier in chickens than in mammals, although it is still a daunting task.

In many species of mammals, there is a considerable difference in the rate and distribution of recombination between the sexes. Ito et al. (1988) have shown a sex difference in recombination frequency between two loci in the Japanese quail, but this phenomenon has not been reported in the chicken.

II. METHODS AND PROGRESS IN CHICKEN GENE MAPPING

(a) Physical mapping

In the past, the only way of allocating genes or linkage groups to chromosomes was via cosegregation of chromosome markers, such as chromosome rearrangements, and genetic markers. Following Zartman's (1973) assignment of the gene for pea comb to chromosome 1 in this way, which was the first recognition of the chromosomal location of an autosomal linkage group, numerous other chromosomal rearrangements have been made and exploited. Shoffner's laboratory at the University of Minnesota (pers. comm.) has recently used a range of chemical mutagens to induce additional rearrangements in chickens, mainly translocations, to attempt to assign genes to chromosomes. This technique is not possible in many species and has been supplanted by other

methods of physical mapping for the direct allocation of genes to chromosomes, using cytological and/or cell culture techniques, supplemented by molecular technology. Another example of the use of chromosomal markers in physical mapping in the chicken was the mapping of the MHC to a microchromosome containing the ribosomal RNA gene cluster (Bloom and Bacon, 1985). In this case, the microchromosome could be recognised by the nucleolus organiser (NOR) or secondary constriction which it bore, and the MHC linkage was detected by correlating the number of MHC alleles with the number of NORs in aneuploid birds. Subsequently, cloning studies have shown that the MHC and the rRNA loci are no further than 15kb apart (Guillemot et al., 1989).

However, much more generally applicable and subtle methods of physical mapping are now available. The two main forms of physical mapping are *in situ* hybridisation and the use of somatic cell hybrids. A new form of physical mapping, which makes an immediate and direct connection with linkage mapping, is chromosomal microcloning, where individual chromosomes are manipulated and fragments from the chromosome are molecularly cloned. Clearly all such clones are assigned to a particular chromosome from the start, and linkage mapping can then be used to order the clones within a linkage group.

1. *In situ* hybridisation.

Probes are cloned fragments of DNA, which can be labelled either with radioactivity or with a non-radioactive label such as biotin or digoxigenin. These can be hybridised or made to stick to complementary DNA sequences, even when the target DNA is still present in chromosomes. Thus by using suitable detection systems, either photographic emulsion, which detects the position of the radioactivity, or a dye-labelling reaction, the position of the probe can be detected at a specific position on a specific chromosome. Initially, radioactively labelled probes only were used, but due to the very weak signals obtained, which necessitated scanning of large numbers of cells, the technique was quite error prone. Much more sensitive non-radioactive detection systems, using fluorescent dyes, are now widely used (Polak and McGee, 1991). FISH (fluorescent *in situ* hybridisation) can routinely detect single-copy DNA sequences on chromosomes.

An intrinsic limitation of *in situ* hybridisation is the resolution possible when hybridising a probe to relatively contracted metaphase chromosomes. However multiple colour dye labelling is now possible, so that several probes can be detected in the same cell and even on the same chromosome. This means that loci can not only be assigned to chromosomes, but ordered within a chromosome. A potentially important application of multiple dye labelling in chickens will be in the identification of the microchromosomes. By using probes of known location on microchromosomes, it will be possible to assign loci of unknown position by co-labelling with probes for previously assigned loci.

Numerous endogenous proviral loci have been mapped by *in situ* hybridisation (reviewed by Tereba, 1983), with five ev loci in order on chromosome 1 alone. Also, the actin gene family (Shaw et al., 1988) and the c-erb and c-myc oncogenes (Symonds et al., 1986) have been mapped in this way. Dominguez-Steglich et al. (1990) have mapped the dystrophin gene to a microchromosome. Ponce de Leon is in the process of *in situ* mapping a number of chicken cosmid clones, isolated in my laboratory by Toye, and shown to detect RFLPs in the CSIRO reference population.

2.Somatic cell hybrids.

In mammalian gene mapping, somatic cell hybrids are made between cells from two different species, for example human and mouse. These somatic cell hybrids randomly lose chromosomes from one of the species. By maintaining a panel of somatic cell hybrids and determining the presence or absence of genes from particular clones, it is possible to determine whether genes are syntenic, that is present on the same chromosome or section of chromosome. If the cell hybrids have been cytologically characterised, the syntenic groups can be assigned to chromosomes.

Kao (1973) made hybrids between chicken erythrocytes and Chinese hamster cells, which carried single chicken chromosomes, namely 6 and 7, which complemented genetic deficiencies in the Chinese hamster cells, and thus was able to map the genes for these functions in the chicken. Palmer and Jones (1986) were able to map three more loci, Pgm-2, Alb, Gc, to chromosome 6 using these cell lines. Li and Ponce de Leon (1992) have developed a more general method of maintaining chicken chromosomes in chicken by human hybrid cells, by first transfecting the chicken cells with a gene for antibiotic resistance, and then growing the hybrid cells on antibiotic-containing medium. In this way, they have constructed cell hybrids containing single macro- or microchromosomes.

3. Chromosome Microcloning.

In this technique, individual chromosomes are individually picked up with micromanipulators and a library of clones is made from a single chromosome. Development of this technique has depended on the production of sensitive micromanipulation apparatus and/or laser ablators and the ability to amplify the individual fragments of DNA produced from the chromosome. The polymerase chain reaction (PCR), which was invented in the mid 1980s (Saiki et al., 1985; Mullis and Faloona, 1987) is essential to the process of microcloning since its enables amplification of individual DNA fragments many billion fold, and thus leads to easier and more reliable cloning.

At this stage, only Mizuno's laboratory in Japan, which is concentrating on the sex chromosomes and using the laser ablation method, and Ponce de Leon's laboratory in the USA, which is using mechanical manipulation of chromosome 1, are using this technique in the chicken.

(b) Linkage Mapping

By analysis of the segregation of genes in crosses or families, the recombination frequency between genes can be estimated, linkage groups can be recognised and gene order within a linkage group can be determined. In the past, the availability of many morphological markers, affecting plumage colour and other characteristics, such as colour, shape and size of the comb, and body size, to name a few, and the ease of making crosses, meant that the chicken had the earliest linkage map for a domestic species. Since the late 1970s, the following technical developments in molecular biology have permitted the analysis of a much larger range and number of new genetic markers and a blossoming of gene mapping in many species, including humans.

1. Restriction Fragment Length Polymorphisms (RFLPs).

Variable sized DNA fragments can be detected by cutting DNA with restriction enzymes and then detecting specific fragments with a labelled probe (cloned DNA), which hybridises with (binds to) fragments of interest. The polymorphisms detected are of two major types, namely restriction site polymorphism (RSP), where restriction enzyme recognition sequences are created or destroyed by nucleotide substitutions, and minisatellite VNTRs discussed below, where the size of a fragment between two constant restriction sites varies. Both are called RFLPs. Although RSPs have contributed very significantly to genetic mapping in many species, there is a number of limitations to the technique. First, only a limited proportion of nucleotide substitutions create RFLPs. Using human sequence data, Wijsman (1984) has estimated empirically that if 71 restriction enzymes are used, only 45% of mutations are theoretically detectable, assuming that all fragments are detectable, and the practical detection rate would be expected to be somewhat lower. Secondly, a rather tedious and time-consuming procedure of screening various enzymes (but generally far fewer than 71) must occur before informative probe/enzyme combinations can be found. If distantly related populations are crossed, for example *Mus musculus* and *Mus spretus* (Avner et al., 1988), far fewer enzymes need to be screened before variants which characterise the two parent populations are recognised. Crittenden et al. (1992) have established a Red Jungle Fowl by White Leghorn backcross for this reason.

Despite these potential drawbacks, Bumstead and Palyga (1992) have created a map of 100 RFLP loci, using a cross between two partially inbred lines of White Leghorns. These authors expressed surprise at the ease with which polymorphic markers could be recognised, as 41% of their probes, which were randomly cloned fragments of DNA, detected polymorphisms. This is all the more surprising as only seven enzymes were screened and only a very limited sample of the gene pool was assayed, namely one parental bird used from each White Leghorn line. However, these inbred lines were originally selected from a diverse gene pool for resistance or susceptibility to salmonellosis, which may explain the high level of variation between them. Alternatively, chickens may be like maize, where only three enzymes are required to detect restriction fragment variants between any two commercial lines (Burr and Burr, 1991), and thus may display a high intrinsic level of restriction fragment length variation. Van Hest et al (1992) found 13 RFLPs using 17 probes for known genes and 11 enzymes, and Toye et al (1992) found that 19 from 40 anonymous, single copy cosmid probes detected RFLPs when screening with 10 enzymes on the CSIRO reference population.

2. Variable Number Tandem Repeats (VNTRs)

Variable number tandem repeats are of two types. Minisatellites (Jeffries et al., 1985) have repeating units of 9-64 base pairs, and the variation in repeat number is attributed to unequal crossing-over. Indeed, minisatellite have been proposed to be recombination signals, as they are often located in recombinational hotspots. Minisatellite variants are usually detected as RFLPs. Microsatellites (Tautz, 1989; Weber and May, 1989) have smaller repeat units, from mononucleotide repeats, but more frequently dinucleotides up to tetranucleotides and even pentanucleotides in the chicken (Moran, unpublished). Variation in repeat number is attributed to "slippage" during DNA replication.

i) Minisatellites

Minisatellite variants are detected as RFLPs, using either the core repeat sequence as a probe, which wil generate a DNA finger print, or a probe from a region adjacent to one of the sites of minisatellite repeats, which will reveal a much simpler and more easily interpretable "single locus" pattern. Minisatellite repeats are highly polymorphic, and DNA fingerprints are essentially individual specific as a result of this high level of variation. However, interpretation of DNA fingerprints is difficult and they are not widely used in gene mapping as a result. Single locus minisatellite polymorphisms are much more interpretable and amenable to gene mapping studies. A limitation of the usefulness of minisatellite loci in gene mapping in humans at least is that they tend to be clustered in telomeric locations on chromosomes (Royle et al., 1988). Hanotte et al. (1992) have recognised several new linkage groups in the chicken using minisatellite markers, but full reports of this work are yet to be published.

ii) Microsatellites

Microsatellite variation is detected via PCR, followed by electrophoretic separation of the products on gels which are able to resolve differences in size of as little as one or two base pairs. Thus sequence information bracketing the site of microsatellite repeats is a prerequisite for amplification of the PCR product. These sequence-tagged sites (STSs) can be found by searching electronic databases of published sequences for the occurrence of microsatellite repeats (Moran, unpublished) or more generally by screening libraries of clones with microsatellite repeat probes and then sequencing either side of the repeat region (Crooijmans et al., 1992; Hanotte et al., 1992). Moran (unpublished) has found that dinucleotide repeats comprise a much smaller proportion of the microsatellite spectrum in chickens than in the pig. For example, the frequency of commonly analysed AC/GT dinucleotide repeat in the chicken (0.9% of genes carry the repeat at $n > 10$) is only one quarter the frequency in the pig (3.9%). For GA/CT repeats, the frequencies were 0% for the chicken and 1.7% in the pig, but for AT repeats the figures were 0.6% for the chicken and 0% for the pig. However considering all classes of microsatellite repeats, about 10% of chicken genes and 13% of pig genes contained potentially useful microsatellite markers. All occurrences of microsatellite repeats found in the sequence databases are now being systematically evaluated for use in gene mapping.

3. Other Screening Techniques.

i) RAPDs

Randomly amplified polymorphic DNA (RAPD) is generated by using short PCR primers, usually 10 bp. Wherever primer complementary sites occur sufficiently close for PCR amplification to occur, generally less than about 1.5 kb apart, then an amplified product will be detectable on an electrophoretic gel. If one of these sites is absent, due to nucleotide substitution or deletion, then no product will be produced. Thus presence of a RAPD band is dominant and absence is recessive. Although this mode of gene action is less than ideal for gene mapping purposes, RAPDs have been widely and successfully used, particularly in plant gene mapping. Crittenden et al. (1992) have mapped a number of RAPDs in the chicken. Toye et al (1992) have found that RAPDs are quite polymorphic, but that it is difficult to obtain a repeatable and consistent pattern of bands, which makes

interpretation difficult.

ii) Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis, or more specifically temperature gradient gel electrophoresis (TGGE), permits the detection of variation in nucleotide sequence based on the differential thermostability of double-stranded DNA of only slightly different nucleotide composition. For example, substitution of a G/C base pair for an A/T base pair, will cause an increase in stability which influences the pattern of electrophoretic migration. TGGE is generally used in association with PCR to screen for nucleotide substitutions in a region of interest. The only application in domestic animals to date has been in the detection of a single nucleotide substitution in the bovine β-lactoglobulin gene (Tee, Moran and Nicholas, 1992). However, the technique has a much broader potential application for the detection of genetic variants for use in gene mapping.

III. APPLICATIONS OF GENE MAPPING IN IDENTIFYING IMPORTANT GENES

Economically important quantitative effects already have been found associated with some genetic markers in chickens and with the increasing effort in chicken gene mapping, many more quantitative trail loci will be identified. An interesting case, where a useful genetic marker causes an adverse side effect is provided by the ev-21 locus of the chicken, which is the site of insertion of an endogenous retrovirus in the slow feathering gene. This insertion is associated with effects on egg production and growth traits (Sabour et al., 1992). The adverse effects have been attributed directly to the endogenous virus, which induces immunotolerance (Crittenden et al., 1982) and thus influences the ability of chickens to mount a response to exogenous viral challenge.

Resistance to Mareks disease, attributable to the B21 haplotype of the MHC, is well known. However, genetic differences in susceptibility are known for many chicken diseases (Bumstead et al., 1991). The linkage maps being developed will greatly assist in the process of identifying and characterising these genes.

For non-disease related traits, Plotsky et al. (1990) have demonstrated an association between a DNA fingerprint band and abdominal fat and the same laboratory has subsequently identified a marker in relation to shank length. Mapping of these markers will be the first step in mapping the associated quantitative trait loci.

IV. CONCLUSIONS

Chicken gene mapping is now entering a very exciting phase, during which there will be rapid development of the map. Ultimately, genes affecting disease resistance and productivity will be mapped, identified and characterised. This will enable design of better breeding programmes and will open many possibilities for genetic engineering of chickens for enhanced productivity.

V. ACKNOWLEDGEMENTS

I wish to thank Mr Ayo Toye for valuable discussions and for providing bibliographic information and references, particularly from the Interlaken meeting,

which have greatly assisted me in preparing this review. I also thank my colleagues Frank Nicholas and Bruce Sheldon for valuable comments on the manuscript.

REFERENCES

AVNER, P., AMAR, L.,DANDALO, L. and GUENET, J.L. (1988) Trends in Genetics 4: 18-23.

BITGOOD, J.J. and SOMES, R.G. (1990) Chapter 21 in Poultry Breeding and Genetics, R.D Crawford, ed., pp 469-495, Elsevier, Amsterdam.

BITGOOD, J.J. and SOMES, R.G. (1992) Poultry Gene Mapping Workshop, XXIII International Congress on Animal Genetics, Interlaken, Switzerland.

BLOOM, S.E. (1969) J.Hered. 60: 217-220.

BLOOM, S.E. and BACON, L.D. (1985) J.Hered. 76:146-154.

BURKE, T., HANOTTE, O., GIBBS, M. and THOMSON, P. (1992) Poultry Gene Mapping Workshop, XXIII International Congress on Animal Genetics, Interlaken, Switzerland.

BUMSTEAD, N., MILLARD, B.J., BARROW, P. and COOK, J.K.A. (1991) in Breeding for Disease Resistance in Farm Animals, J.B. Owen and R.F.E Axford, eds, pp. 10-23, CAB International, Wallingford.

BUMSTEAD, N. and PALYGA, J. (1992) Genomics 13: 690-697.

BURR, B. and BURR, F.A. (1991) Trends in Genetics 7: 55-60.

CRITTENDEN, L.B., FADLEY, A.M. and SMITH, E.J. (1982) Avian Diseases 26: 279-294

CROOIJMANS, R.P.M.A., VAN DER PLOEGEL, J.J., VAN KAMPEN, A.J.A. and GROENEN. M.A.M. (1992) XXIII International Congress on Animal Genetics, Interlaken, Switzerland, p. 72.

DOMINGUEZ-STEGLICH, M., MENG, G., BETTECKEN, T., MULLER, C.R. and SCHMID, M. (1990) Genomics 8: 536-540.

DUNN, L.C. and JULL, M.A. (1927) J. Genet. 19: 27-63.

ECHARD, G. (1984) Genetic Maps, Vol. 3, pp. 392-395, ed S.J. O'Brien.

ETCHES, R.J. and HAWES, R.O. (1973) Can.J. Genet. Cytol. 15: 553-570.

FECHHEIMER, N.S. (1990) in Advances in Veterinary and Comparative Medicine: Domestic Animal Cytogenetics. Vol. 34, pp. 169-207, R.A. McFeely, ed. Academic Press, New York.

GUILLEMOT, F., KAUFMAN, J.F., SKJOEDT, K. and AUFFRAY, C. (1989) Trends in Genetics 5: 300-304.

HANOTTE, O. THOMSON, P. BRUFORD, M., MILLER, C., BUMSTEAD, N. and BURKE, T. (1992) XXIII International Congress on Animal Genetics, Interlaken, Switzerland, p.96.

HUTT,F.B. (1936) Neue Forsch. Tierz. Abstammungsl. (Fetschr.): 105-112.

HUTT, F.B. (1949) Genetics of the Fowl. McGraw-Hill, N.Y.

HUTT, F.B. (1964) Animal Genetics. The Ronald Press Company, N.Y.

JEFFREYS, A.J., WILSON, V. and THEIN, S.L. (1985) Nature 314: 67-73.

KAO, F.T. (1973)Proc. Natl. Acad. Sci. USA 70: 2893-2898.

LALLEY, P.A. (1980) Genetic Maps, Vol 1. pp. 255-264, ed S.J. O'Brien

LI, Y. and PONCE DE LEON, F.A. (1992) Poultry Science 71: 151-160.

MULLIS, K.B. and FALOONA, F.A. (1987) Meth. Enzymol. 155: 335-350.

PALMER, D.K. and JONES, C. (1986) J.Hered. 77: 106-108.

PLOTSKY, Y., CAHANER, A., HABERFIELD, A., LAVI, U. and HILLEL, J. (1990) Proc.4th World Cong.Genet. Appl. Livestock Prod. 13: 133-136.
POLAK, J.M. and McGEE, J.O. (eds) (1991) *In situ* Hybridisation Principles and Practice, Oxford Science Publishers, Oxford.
RODIONOV, A.V., CHELYSHEVA, L.A., SOLOVEI, I.V. and MYAKOSHINA, YU.A. (1992) XXIII International Congress on Animal Genetics, Interlaken, Switzerland, p.97.
ROYLE, N.J., CLARKSON, R.E., WONG, Z. and JEFFREYS, A.J. (1988) Genomics 3: 352-360.
SABOUR, M.P., CHAMBERS, J.R., GRUNDER, A.A. and GAVORA, J.S. (1992) XXIII International Congress on Animal Genetics, Interlaken, Switzerland, p. 108.
SAIKI, R.K., SCHARF, S., FALOONA, F., MULLIS, K.B., HORN, G.T., ERLICH, H.A. and ARNHEIM, N. (1985) Science 230: 1350-1354.
SHAW, E.M., GUISE, K.S. and SHOFFNER, R.N. (1988) Poultry Sci 67 (Suppl. 1): 154.
SOMES, R.G. (1971) Bull No. 420 Storrs Agr. Exp. Sta., Univ Conn., Storrs, Conn.
SOMES, R.G. (1973) J.Hered. 64: 217-221.
SOMES, R.G. (1978) J.Hered. 69: 401-403.
SOMES, R.G. (1980) Genetic Maps, Vol 1. pp. 210-217, ed S.J. O'Brien
SOMES, R.G. (1984) Genetic Maps, Vol. 3, pp.465-473, ed. S.J. O'Brien.
SOMES, R.G. (1987) Genetic Maps, Vol. 4, pp. 423-429, ed. S.J. O'Brien.
SYMONDS, G., QUINTRELL, N., STUBBLEFIELD, E. and BISHOP, J.M. (1986) J. Virol. 59: 172-175.
TAUTZ, D. (1989) Nucleic Acids Research 17: 6463-6471.
TEE, M.K., MORAN, C. and NICHOLAS, F.W. (1992) Animal Genetics 23: 401-405.
TEREBA, A. (1983) Curr. Top. Microbiol. Immunol. 107: 29-50.
TOYE, A.A., LE TISSIER, P.R., MORAN, C., NICHOLAS, F.W. and SHELDON, B.L. (1992) Proc. Aust. Assoc. Anim. Breed. Genet. 10: 252-255.
VAN HEST, B.J., MOLLOY, P. and SHELDON, B.L. (1992) Proc. Aust. Poult. Sci. Symp. 4: 133 ed. R Johnson.
WARREN, D.C. (1928) Genetics 13: 421-433.
WARREN, D.C. (1949) Genetics 34: 333-350.
WEBER, L.L. and MAY, P.E. (1989) Am. J. Hum. Genet. 44: 388-396.
WIJSMAN, E.M. (1984) Nucleic Acids Research 12: 9209-9226.
WOMACK, J. (1984) Genetic Maps, Vol. 3, p. 401, ed S.J. O'Brien.
YAMASHINA, M.Y. (1944) Cytologia 13: 270-296.
ZARTMAN, D.L. (1973) Poultry Sci. 52: 1455-1462.

PREDICTION OF BODY COMPOSITION OF QUAIL USING ELECTROMAGNETIC SCANNING

R.A.E. PYM and D.A.V. BODERO

It is generally acknowledged that genetic selection plays an important role in reducing carcass fatness and increasing lean tissue growth rate in meat chickens. The existing techniques used by commercial breeders, viz plasma VLDL concentration and calliper measure of abdominal fatness, are useful but the respective measures only account for at best about 50 percent of the variation in the proportion of fat in the body. Recent development and refinement of electromagnetic scanning, which measures total body electrical conductivity, indicates that this may be a useful non destructive technique for predicting body composition of animals and birds (eg. Walsberg, 1988). The technique has not yet been used in a selection study in birds.

An EM-Scan Model SA-2 Small Research Animal Body Composition Analyser (EM-SCAN Inc., Illinois) was used to determine the prediction of body composition of 50 male and 50 female five-week-old Japanese Quail. The dimensions of the measurement chamber of the analyser were appropriate for measurement of animals of about 200 g liveweight which limits its use for chickens.

The analyser was calibrated and the birds, restrained with rubber bands, were weighed, their length was measured and they were then placed in a standard manner in the chamber. Three measures were taken on each bird and the mean used as the SA-2 reading for that bird. Prediction equations were determined using an ad-hoc multiple regression technique since such an approach allowed the selective introduction or removal of variables on the basis of biological as well as statistical contribution to the prediction equation.

Mean five week liveweight, length, SA-2 reading and body fat proportion were: 180 and 210 g, 240 and 253 mm, 377 and 498 units and 152 and 150 g/kg in the males and females respectively. The coefficient of variation was about 20% for fat and SA-2 reading, 9% for liveweight and 5% for body length.

The prediction of body fat expressed as a proportion of body weight was maximised in both sexes by the multiple linear regression equations which incorporated all three independent variables, live weight (W,g), length (L,mm) and SA-2 reading (SA2, units). The equations for males and females were:

Fat (g/kg) = 1.49 W - 0.25 SA2 - 0.65 L + 133 $R^2 = 0.43$

Fat (g/kg) females = 1.19 W - 0.24 SA2 - 0.79 L + 219 $R^2 = 0.34$

The results of the study suggest that electromagnetic scanning may not provide greatly enhanced prediction of fatness relative to other measures in use. However, such comparison is confounded by a species difference and there was an indication that bird restraint and relaxation may be an important factor in determining the reading. We will be looking more closely at this and will report our findings at the symposium. Depending on the ultimate accuracy of prediction, our intention is to use the technique in a selection experiment for aspects of growth and body composition in Japanese Quail.

WALSBERG, G.E. Physiol. Zoology 61: 153-159.

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

INSULIN-LIKE GROWTH FACTOR-I IN THE OVARY OF THE LAYING HEN

R.D. ROBERTS*, C. GODDARD and P.J. SHARP

Summary

The presence of IGF-I and the expression of IGF-I mRNA in granulosa and thecal tissues was demonstrated by radioimmunoassay (RIA) of tissue extracts and by RT-PCR with Southern blotting analysis, respectively. IGF-I stimulated DNA synthesis in cultured granulosa and thecal cells in a dose-dependent manner. LH, but not FSH, was shown to have a dose-dependent stimulatory effect on DNA synthesis in granulosa cells; neither gonadotrophin had this effect in thecal cells. When IGF-I and LH treatments were combined, their stimulatory effects on DNA synthesis in granulosa cells were synergistic. Receptor binding studies demonstrated that both cell types possess IGF type-I receptors. The observations are consistent with an autocrine or paracrine role for IGF-I in the ovary of the domestic hen.

I. INTRODUCTION

Within the ovary of the domestic hen, pre-ovulatory follicles enter a developmental hierarchy when 8 mm in diameter and begin to accumulate yellow yolk. There are 4-7 yellow-yolky follicles in the hierarchy of a laying hen, ranked F1-Fn with the F1 follicle being the largest and next to ovulate. Follicles develop rapidly in the hierarchy for approximately 6 days, until they ovulate at a diameter of 35-40 mm (Gilbert *et al.* 1985).

The involvement of growth factors such as the insulin-like growth factors, IGF-I and IGF-II, in the development and functions of the mammalian ovary has been established. The IGF-I gene is expressed and IGF-I is present in rat granulosa cells (Hansson *et al.* 1988; Oliver *et al.* 1989) and IGF-I receptors have been shown to be present on porcine and rat granulosa cells (Baranao and Hammond, 1984; Adashi *et al.* 1985b; Davoren *et al.* 1986). The evidence for the involvement of IGF-I in the mammalian ovary is consistent with an autocrine/paracrine mode of action (Adashi *et al.* 1991b). In comparison with observations from mammalian studies, less information is available on the effects of growth factors in the avian ovary.

The present study was undertaken to determine whether the IGF-I gene is expressed and associated with IGF-I receptors in granulosa and thecal cells derived from yellow yolky follicles of the domestic hen. Further, the role of IGF-I in the growth of these cells *in vitro* was investigated using tissue culture systems.

II. MATERIALS & METHODS

a) Animals

ISA Brown laying hens (ISA Poultry Services Ltd., Peterborough, U.K.) were

AFRC Institute of Animal Physiology and Genetics Research, Roslin, Midlothian, U.K. EH25 9PS *Now at CSIRO Division of Animal Production, P.O. Box 239, Blacktown, NSW 2148, Australia.

killed by cervical dislocation between 50 and 70 weeks of age, 0 - 2 hours before a predicted ovulation. The hens used in any one study were of the same age, i.e. they had been reared from the same hatch. The birds were kept caged under a 24 hour artificial lighting pattern (14 hours light and 10 hours dark). Egg laying records were kept for each hen and only hens laying daily were selected for study.

b) Reagents

Medium 199 (M199) containing Earle's salts and sodium bicarbonate was supplemented with HEPES buffer (20 mmol/l), L-glutamine (2 mmol/l), sodium pyruvate (2 mmol/l), streptomycin (0.1 mg/ml) and penicillin (1000 u/ml). All of these reagents were obtained from Sigma Chemical Co. (Poole, U.K.). The peptides used in the experiments were human recombinant derived insulin-like growth factors I and II (Bachem, Saffron Walden, U.K.) and porcine monocomponent insulin (Novo Biolabs Ltd., Cambridge, U.K.). NIADDK-oLH-25 was a gift from NIADDK (Bethesda, Maryland., U.S.A.). All other reagents were obtained from FSA Laboratory Supplies, Loughborough, U.K, unless otherwise stated.

c) Tissue collection and cell culture

The granulosa and thecal layers were separated in sterile Dulbecco's phosphate-buffered saline (Oxoid Co., Basingstoke, U.K.), then snap frozen if used to study IGF-I gene expression or tissue concentration determinations. Tissues to be used for cell culture were maintained in sterile M199 and then dispersed with collagenase (1 mg/ml and 5 mg/ml solutions for granulosa and thecal layers, respectively). Granulosa layers were digested for 5 minutes at room temperature, the thecal layers for 90 minutes at 37°C followed by centrifugation in 40% Percoll to remove red blood cells. Both preparations were washed twice in fresh M199 and resuspended in M199 with 3% foetal calf serum (FCS) prior to plating. Cells were cultured in a humidified incubator containing 5% CO_2 at 41°C. Granulosa and thecal cells were plated at 50,000 and 250,000 viable cells per cm^2, respectively. Following an attachment period of 48 hours, the medium was removed and replaced with medium containing 0.1% FCS. This was repeated after a further 24 hours of culture and then the effects of various hormone treatments were investigated.

d) IGF-I extraction and chromatography

Tissues were extracted as described by Enright *et al.* (1989) and the extracts assayed for IGF-I using the radioimmunoassay (RIA) described by Goddard *et al.* (1988).

e) Reverse transcription-polymerase chain reaction

RNA was prepared from frozen tissues as described by Chomczynski and Sacchi (1987). The RNA was reverse transcribed and subjected to the polymerase chain reaction as described by Roberts (1992). Synthetic DNA oligo primers specific for both IGF-I and β-actin were used (Oswel DNA, Edinburgh, U.K.). The products of the PCR reactions were analysed by gel electrophoresis followed by

Southern blotting.

f) Measurement of [^{3}H]-thymidine incorporation

For the final 16 hours of treatment [^{3}H]-thymidine label (Amersham International plc, Amersham, U.K.) was added to each well (0.3 μCi/ml). The treatment was terminated by the addition of cold trichloroacetic acid (1 ml, 10% w/v) and incubation at 4°C for 20 minutes to precipitate the DNA. The cells were then solubilised with sodium hydroxide (500 μl, 0.5 mol/l), then the plates incubated at 37°C for 2 hours. The radioactivity in the samples was then measured using a scintillation counter.

g) Ligand binding studies

The method used was as described by Duclos *et al.* (1991).

III. RESULTS

Granulosa and thecal tissues were dissected from F1-F4 yellow yolky follicles and pooled. RNA was extracted from these tissues and from liver, then subjected to reverse transcription and the polymerase chain reaction with IGF-I specific primers. Distilled water was also subjected to these processes and used as a negative control. The resulting gels revealed that IGF-I mRNA was present in all these tissues. This was confirmed by Southern analysis of the gels when the experiment was repeated.

IGF-I was present in extracts of all the tissues examined with the greatest concentrations being found in the granulosa tissue in both experiments (6.42 ± 0.09 and 3.0 ± 0.03 ng/g wet weight). Lower concentration of IGF-I were measured in thecal tissue (2.8 ± 0.17 and 2.06 ± 0.02 ng/g wet weight) then the liver (1.93 ± 0.09 and 1.6 ± 0.38 ng/g wet weight), $n = 4$ in both experiments. IGF-I was not detected in the negative controls of either experiment.

Granulosa and thecal cells were cultured and then incubated with [^{125}I]-IGF-I. The radiolabelled ligand bound to both cell types and was displaced in a dose-dependent manner by the addition of increasing concentrations of unlabelled IGF-I, IGF-II or insulin. The amount of peptides required to displace 50% [^{125}I]-IGF-I bound to granulosa cells were 2.87 ± 0.27 (s.e.m.) pmol/l, 3.31 ± 0.97 pmol/l and 248.2 ± 39.4 pmol/l for hIGF-I, hIGF-II and insulin respectively; for thecal cells the amounts were 1.36 ± 0.15 (s.e.m.) pmol/l, 4.7 ± 0.74 pmol/l and 249.3 ± 31.9 pmol/l for the same ligands. Thus the order of potency with which the unlabelled peptides displaced [^{125}I]-IGF-I from both granulosa and thecal cells was IGF-I > IGF-II > Insulin.

The affinity constant (K_a) and binding capacity (B_{max}) of IGF-I for the binding site on granulosa cells were 7.36 ± 17.3 litres/nmol and 215 ± 40.1 fmol/well respectively; and for those on thecal cells, 7.7 ± 27.2 litres/nmol and 214 ± 90 fmol/well respectively (300 ng DNA per well). Comparison of one and two binding-site models for the Scatchard analysis indicated that the one binding site model was most appropriate for the data for both the granulosa and thecal cell studies.

IGF-I stimulated uptake of [^{3}H]-thymidine in a dose-dependent manner by

both granulosa and thecal cell cultures. All the cultures responded to the addition of FCS treatment (positive control) with an increased [^{3}H]-thymidine incorporation compared with untreated cells. The amount of IGF-I required for 50% of the maximum incorporation (ED_{50}) was 7.37 (±1.46), 11.61 (±7.28) and 11.83 (±2.57) ng/ml for triplicate granulosa cell cultures prepared from the three individual birds and 20.05 (±28.31), 15.16 (±4.70) and 7.05 (±0.60) for triplicate thecal cell cultures. In each experiment the granulosa and thecal tissues were obtained from the same hen, the experiments were repeated twice.

Cell cultures were treated with a range of doses of LH or FSH up to 50 ng/ml, they were also treated with IGF-I for comparison with the previous experiments. Treatment with FSH did not stimulate [^{3}H]-thymidine incorporation above the baseline level of the untreated control in either cell type. LH treatment caused a dose-dependent increase in thymidine incorporation in granulosa cells in both experiments with ED_{50} values (ng/ml ± S.E.M.) of 14.32 ± 2.57 and 10 ± 1.24, respectively, but did not have this effect on thecal cells. The granulosa cells responded to IGF-I in a similar (dose-dependent) manner to those described above, with ED_{50} values (ng/ml ± S.E.M.) of 13.57 ± 1.60 and 10.55 ± 6.01, respectively. There was good agreement between the results from the two independent experiments. The ED_{50} values of IGF-I and LH were not significantly different from each other in either experiment. The experiment was repeated with cells prepared from different birds.

Cultured granulosa and thecal cells were treated with 25 ng/ml doses of either IGF-I, LH, FSH or IGF-I with LH or FSH. All treatments were carried out on triplicate wells; the experiment was replicated. No significant difference was found in the incorporation of [^{3}H]-thymidine into thecal cells treated with IGF-I, with IGF-I and LH, or IGF-I and FSH. Similarly no differences in the incorporation of [^{3}H]-thymidine were seen in granulosa cells treated with IGF-I or IGF-I and FSH. However, [^{3}H]-thymidine incorporation into granulosa cells treated with IGF-I and LH combined was significantly greater than that into granulosa cells treated with IGF-I or LH alone or than the sum of the separate treatments, ($P < 0.001$).

IV. DISCUSSION

Previously, IGF-I has been shown to be present in the serum of chickens (Shapiro and Pimstone, 1977) and its receptors have been demonstrated in many chicken tissues (Bassas *et al.* 1988; 1989); here, the role of this growth factor in the reproductive functions of poultry was investigated. IGF-I mRNA is expressed, and the peptide is present, in ovarian follicular granulosa and thecal tissues, showing that it is produced by these cells.

The evidence for production of IGF-I by both cell types, coupled with the demonstration that IGF-I type-I receptors are present, is compatible with an autocrine or paracrine action of IGF-I in the pre-ovulatory follicle. There is considerable evidence of autocrine or paracrine IGF-I actions in other cell types including mammalian ovarian cells (Minuto *et al.* 1991; Hansson *et al.* 1988, Adashi *et al.* 1991). Thus the existence of a local ovarian IGF-I system of growth control in chickens suggested by this work is supported by previous studies.

LH treatment stimulated DNA synthesis in granulosa cells, as first described by Yoshimura and Tamura (1988), in a similar manner to IGF-I and combining both treatments was synergistic in this respect. The mechanism of the IGF-I/LH synergy

remains unknown, requiring further study for its elucidation.

Thus IGF-I is involved in the functions of the cells in pre-ovulatory follicles, possibly regulating the way in which they mature before ovulation. The results of these experiments therefore suggest an important role for IGF-I in regulating normal ovarian function and hence egg laying in poultry. Selection of poultry for rapid somatic growth may also affect the paracrine/autocrine mechanisms controlling ovarian follicular growth resulting in the increased incidence of multiple ovulation at the onset of lay in broiler breeders. This research suggests that IGF-I could be involved in these paracrine/autocrine mechanisms since IGF-I production is known to be controlled by growth hormone (Zapf *et al.* 1981) and concentrations of plasma GH are affected by selection for growth (Goddard *et al.* 1988).

REFERENCES

ADASHI, E.Y., RESNICK, C.E., D'ERCOLE, A.J., SVOBODA, M.E. and VAN WYK, J.J. (1985). Endocrine Rev. 6, 400-420.

ADASHI, E.Y., RESNICK, C.E., HERNANDEZ, E.R., HURWITZ, A., ROBERTS, C.T., LEROITH, D. and ROSENFIELD, R. (1991). In Modern Concepts of Insulin-Like Growth Factors. Ed., Spencer, E. M. Elsevier, New York. pp 267-274.

BARANAO, J.L. and HAMMOND, J.M. (1984). Biochem. Biophys. Res. Commun. 124, 484-490.

BASSAS, L., GIRBAU, M., LESNIAK, M.A., J., ROTH, J. and de PABLO, F. (1989). Endocrinol. 125, 2320-2327.

BASSAS, L., LESNIAK, M.A., SERRANO, J., ROTH, J. and de PABLO, F. (1988). Diabetes 37, 637-644.

CHOMCZYNSKI, P. and SACCHI, N. (1987). Analyt.Biochem. 162, 156-159.

DAVOREN, J.B, KASSON, B.G., LI, C.H. and HSUEH, A.J.W. (1986). Endocrinol. 119, 2155-2162.

DUCLOS, M.J., WILKIE, R.S. and GODDARD, C. (1991). J. Endocrinol. 128, 35-42.

ENRIGHT, W.J., CHAPIN, L.T., MOSELEY, W.M., ZINN, S.A., KAMDAR, M.B., KRABILL, L.F. and TUCKER, H.A. (1989). J. Endocrinol., 122, 671-679.

GILBERT, A.B., PERRY, M.M., WALKER, M.A. and WADDINGTON, D. (1985). IRCS Med. Sci. 13, 732-733.

GODDARD, C., WILKIE, R. and DUNN, I. C. (1988). Domest. Anim. Endocrinol. 5, 165-176.

HANSSON, H.A., NILSSON, A., ISGAARD, J., BILLIG, H., ISAKSSON, O., SKOTTNER, A., ANDERSON, I.K. and ROZELL, B. (1988). Histochem, 89,403-410

MINUTO, F., BARRECA, A., DEL MONTE, P. and GIORDANO, G.(1991). Acta Endocrinol. 124 suppl. 2, 63-69.

OLIVER, J.E., AITMAN, T., POWELL, J., WILSON, C. and CLAYTON, R. (1989). Endocrinol. 124, 2671-2679.

ROBERTS, R.D. (1992). PhD Thesis. Univ. Edinburgh.

SHAPIRO, B. and PIMSTONE, B.L. (1977). J. Endocrinol. 74, 129-135.

YOSHIMURA, Y. and TAMURA, T. (1988). Poultry Sci. 67, 814-818.

ZAPF, J., FROESCH, E. R. and HUMBEL, R.E. (1981). Curr. Top. Cell. Reg. 19, 257-309.

SHORT PRODUCTION PAUSES INDUCED BY FEEDING BARLEY OR OATS - EFFECTS ON PERFORMANCE OF HENS PREVIOUSLY FED UNCRUSHED GRAINS AND EFFECTS OF FEEDING LIMESTONE DURING THE PAUSE

D. ROBINSON and K.M. BARRAM

There is evidence that in many circumstances a series of production cycles separated by short pauses has economic advantages over conventional production regimens. Two experiments were conducted into factors affecting the success of the technique of inducing a short production pause by feeding uncrushed barley or oats for a period of 6-8 days.

In experiment 1, birds of two strains were fed from 22 to 72 weeks of age on either of two fully ground diets or one of ten diets composed mainly of one or more uncrushed cereal grains and oilseeds (wheat, sorghum, maize, sunflower seed, rape seed). At 72 weeks of age five pause inducement procedures were applied to an equal number of hens from each of the twelve original treatments. A sixth group in each treatment was not paused. The pausing procedures involved feeding uncrushed oats or barley for six to eight days, or barley for five days followed by one day without feed. Following the pausing procedure all birds were given a standard ground layer diet until 94 weeks of age. In the first production cycle (50 weeks) hens given ground feed laid an equal or greater number of eggs than those given diets containing uncrushed ingredients. In the second cycle (22 weeks) paused hens which had previously received ground feed produced approximately eleven more eggs/bird than the corresponding unpaused hens, while paused hens which had received diets with uncrushed ingredients produced approximately five fewer eggs/bird than the unpaused hens. The latter result was not significantly influenced by grain-type or pausing method. In the second cycle egg specific gravity and Haugh unit score were initially higher for the crushed grain/pause treatments than for the other treatments.

In experiment 2, birds which had been subjected to various rearing treatments prior to 22 weeks of age were either not paused or paused at 47 weeks of age by feeding uncrushed oats for six days, with or without supplementary limestone chips, after which they were returned to a normal layer diet. During the pause inducement period the specific gravity of eggs from each pause treatment was lower ($P<0.001$) than that of the unpaused treatment. In this period only, eggs from paused hens given supplementary limestone had a slightly higher ($P<0.05$) specific gravity than those from unsupplemented hens. For approximately three months following resumption of lay, the paused hens produced eggs showing small but often significant ($P<0.05$) improvements in specific gravity and Haugh unit score compared to the unpaused hens. Feeding limestone in the pause inducement period had no effect on subsequent egg yield.

It is concluded that the technique of inducing a short pause by replacing the normal diet with whole oats or barley is inappropriate for birds already accustomed to uncrushed grains, and that limestone supplementation during the pause inducement period has only a very temporary benefit.

Redlands Poultry Research Centre, PO Box 327, Cleveland, Qld. 4163.

OPTIMISING YOLK COLOUR WITH CHILLI AND *LEUCAENA*

D.N. SINGH and J.S. KOPINSKI

Fruits of plants of the genus *Capsicum* (pimiento, chilli and paprika) have been used overseas as yolk pigmenters. Brown (1938) demonstrated that pigments present in pimiento were deposited in egg yolk. Mackay et al (1962) reported that chilli powder in the diet of layer hens produced a reddish tinge in the yolk. The present study was undertaken to investigate the value of chilli powder as a natural source of red pigmentation to complement our earlier *Leucaena* studies (Singh and Kopinski 1992).

A total of 216 birds in each of two factorial experiments had their egg yolk colour responses examined when fed diets containing *Leucaena* at either 20 or 25 g *Leucaena*/kg diet with chilli powder at 2.5, 5 or 10 g/kg diet. The basal diet consisting of a nutritionally adequate wheat, sorghum, soybean and meatmeal formulation. This basal diet allowed the depletion of the pigment reserves of the laying hens within three weeks. Egg yolk pigmentation was then assessed following placement of the hens on the basal diet containing various levels of *Leucaena* and chilli powder. The weekly assessment being independently carried out by two observers using Roche Colour Fan (RYCF) scoring.

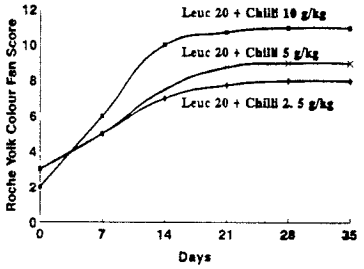


Figure 1. Egg yolk pigmentation scores using *Leucaena* (20 g/kg) and chilli powder at 2.5, 5.0 or 10 g/kg.

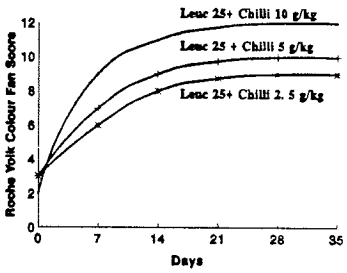


Figure 2. Egg yolk pigmentation scores using *Leucaena* (25 g/kg) and chilli powder at 2.5, 5.0 or 10 g/kg.

At the levels of *Leucaena* and chilli powder used, no differences in feed intake were observed between treatments.

The results in Figure 1 indicate that optimal natural yolk pigmentation (RYCF score of 9-12) can be achieved using *Leucaena* at 20 g/kg of diet and chilli powder at 10 g/kg of diet. Figure 2 shows that with *Leucaena* at 25 g/kg of diet both 5 and 10 g/kg of diet chilli powder usage would yield optimal yolk pigmentation. Studies are continuing on other sources of natural yolk pigmentation and on stability of such pigmentation upon storage of eggs.

BROWN, W.L. (1938). J. Biol. Chem. 122:655-659.
MACKAY, E., MOUNTNEY, G.J. and NABER, E.C. (1962). Poult. Sci. 41:32-37.
SINGH, D.N. and KOPINSKI, J.S. (1992). Aust. Poult. Sci. Symp. 4:140 ed. R Johnson.

Queensland Department of Primary Industries, Brisbane Q 4000

EFFECT OF CYCLOPIAZONIC ACID ON FERTILITY, HATCHABILITY AND PROGENY PERFORMANCE OF LAYING HENS

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

Cyclopiazonic acid (CPA) is a mycotoxin produced by some species of the genus *Penicillium* and genus *Aspergillus* (Cole, 1984). Laying hens dosed with CPA have decreased egg production and egg shell quality (Suksupath *et al.*, 1989) and the toxin is found in both egg whites and yolks (Suksupath *et al.*, 1991). The present study was designed to determine the effect of CPA on fertility, hatchability and progeny performance of laying hens.

Hens (White Leghorn x Australorp), aged 45 weeks and laying at 90 percent, were orally dosed daily *via* gelatin capsules at 0, 1.25 and 2.50 mg CPA/kg bodyweight for four weeks and then monitored during a recovery period of four weeks. Twenty hens were used for each CPA dose level. During dosing and the four-week recovery period eggs were collected daily, and those with soft and cracked shells were recorded and discarded. The remaining eggs were stored at 12°C for seven days and then placed in a forced draught incubator.

Egg production (52%) but not egg weight decreased significantly ($P<0.001$) for hens dosed with 2.50 mg CPA/kg. Unsettable egg numbers increased from 2 to 15% and embryonic mortality was significantly ($P<0.05$) increased by dosing CPA at 2.50 mg/kg, whereas fertility was not affected. Hatchability of fertile eggs decreased significantly from 92 to 58% during weeks 2-4 of the dosing period for hens dosed with 2.50 mg CPA/kg. Although the same general trends were apparent for the hens dosed with 1.25 mg/kg the effects were not significant. Feed intake of dosed hens was only decreased during the first week of dosing but increased significantly during the recovery period and was associated with a significant increase in weight of chicks at hatching from hens previously dosed with 1.25 and 2.50 mg CPA/kg. Egg production, number of unsettable eggs, embryonic mortality and hatchability all returned to control values during the first week after the cessation of dosing. Hatch weight, posthatch chick mortality and bodyweight of chicks at 10 days of age were not affected by the toxin.

The results of the study indicate that CPA increased embryonic mortality and reduced hatchability. The effects maybe due, in part to the inferior egg shell quality following the ingestion of CPA and the deposition of the toxin in eggs noted in previous studies. However, there was no effect on performance of progeny from hens dosed with CPA.

COLE, R.J. (1984). *In* : "Mycotoxins - Production, Isolation and Purification". p. 405 - 415, ed. V. BETINA (Elsevier : Amsterdam).

SUKSUPATH, S., COLE, E.A., COLE, R.J. and BRYDEN, W.L. (1989). Proc.Aust.Poult.Sci.Symp., 2 : 94 ed. D Balnave.

SUKSUPATH, S., DORNER, J.W., COLE, R.J. and BRYDEN, W.L. (1991). Proc.Aust.Poult.Sci.Symp., 4 : 70 ed. R Johnson.

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

CLEARANCE OF CYCLOPIAZONIC ACID BY LAYING HENS

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

Cyclopiazonic acid (CPA) is a mycotoxin produced by some species of *Aspergillus* and *Penicillium*. It has been found in skeletal muscle of broiler chickens within 3 h after oral dosing and it was cleared from the tissues within 48 h (Norred *et al.*, 1987). The toxin has been detected in egg white within 24 h of dosing but not in egg yolk till four days after the initial dose (Suksupath *et al.*, 1991). With in excess of 90 percent of the toxin recovered in eggs found in egg white. The present report describes the clearance of CPA from hens.

Hens were allocated to seven groups and dosed orally daily with CPA in gelatin capsules at 0, 1.25 or 2.50 mg/kg bodyweight for 1, 3 or 7 days. Egg whites and excreta were collected and analysed (Suksupath *et al.*, 1991) for CPA residues 1, 3, 5 and 7 days after the cessation of dosing. Concentration (ng/g) of CPA in egg whites (EW) and excreta (EX) of controls and hens receiving (a) 1.25 and (b) 2.50 dose is shown in the Table.

No. Daily Doses		Withdrawal time (day)							
		1		3		5		7	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
0	EW	0	0	0	0	0	0	0	0
	EX	0	0	0	0	0	0	0	0
1	EW	96	187	6	12	0.9	3	0	0
	EX	693	1,119	40	30	0	0	0	0
3	EW	206	263	12	14	0.6	3	0	0
	EX	639	1,493	22	35	0	0	0	0
7	EW	149	209	13	17	4	12	0	0
	EX	692	1,012	15	46	0	0	0	0

The results show that one day after the last dose, CPA concentration in egg whites was higher in hens dosed with either 1.25 or 2.50 mg/kg for 3, than for 1 or 7 days. Presumably reflecting the pharmocokinetics of the toxin or the induction of toxin metabolising enzymes. Concentration of CPA in egg whites rapidly decreased within 3 days and was not detectable 7 days after the cessation of dosing. The elimination pattern of CPA in excreta was similar to but more rapid than the rate in eggs. CPA in excreta was not detectable 5 days after the last dose. The results indicate that CPA is rapidly cleared by the laying hen.

NORRED, W.P., PORTER, J.K., DORNER, J.W., and COLE, R.J. (1987). J.Agric. Food Chem. 36: 113.

SUKSUPATH, S., DORNER, J.W., COLE, R.J. and BRYDEN, W.L. (1991). Proc. Aust.Poult.Sci.Symp. 3 :70 ed. D Balnave.

Department of Animal Science, University of Sydney, Camden, NSW 2570
* ARS, USDA, National Peanut Research Laboratory, Georgia, USA

EFFECT OF *EIMERIA SPP.* INFECTION AND CYCLOPIAZONIC ACID IN BROILER CHICKENS

S. SUKSUPATH, W.I. MUIR, R.J. COLE* AND W.L. BRYDEN

The mycotoxin, cyclopiazonic acid (CPA) (Smith *et al.*, 1992) and coccidiosis (Davis, 1981) are both capable of depressing the performance of broiler chickens. The present study was designed to determine the effects of giving CPA and coccidiosis alone or in combination to broiler chickens.

Day-old broiler chickens were housed in electrically heated cages. Coccidiostat-free diet and water were available *ad libitum*. At three weeks of age chickens were randomly allocated into six groups of eight birds. Birds were dosed daily with CPA in gelatin capsules containing either 0 or 2.50 mg/kg of body weight for nine days. Two days after dosing with CPA commenced half the birds on each dosing level were singly inoculated orally with either 5.5×10^3 sporulated oocyst/bird of *E.tenella* or 11×10^3 sporulted oocyst/bird of *E.acervulina*. Birds were weighed, excreta collected and oocyst output measured. A blood sample was taken and relative organ weights determined. All birds were scored for coccidal lesions (Johnson and Reid, 1970) seven days after inoculation.

Dosing with CPA and inoculation with both coccidiosis species alone significantly ($P<0.01$) depressed weight gain, and protein digestibility and feed conversion ratio ($P<0.05$) and significantly ($P<0.05$) increased liver weight. *E.tenella* significantly ($P<0.05$) increased spleen and heart weights. There were no effects on kidney and pancreas weight of dosing with CPA and coccidiosis alone or in combination. Plasma cholesterol was significantly ($P<0.01$) increased by CPA. Packed cell volume was significantly ($P<0.001$) decreased by *E.tenella*. There were no effects on plasma alkaline phosphatase, creatinine, protein, glucose, calcium, phosphate, magnesium and chloride when birds were given CPA or coccidiosis but potassium concentrations were significantly ($P<0.05$) increased by coccidiosis. Duodenal and caecal lesion scores and oocyst counts were significantly ($P<0.05$) decreased by the combination of CPA and coccidiosis.

The data from the present study demonstrate that CPA and coccidiosis adversely affect broiler performance and health. In some cases the effects of CPA and coccidiosis were additive.

DAVIS, P.J. (1981). *In* "Avian Immunology". p.361 - 385, ed. M.E. Rose., L.N. Payne, and B.M. Freeman (Edinburg, Br.Poult.Sci.Ltd)

JOHNSON, J. and REID, W.M. (1970). Exp.Parasit., 28 : 30.

SMITH, E.E., KUBENA, L.F., BRAITHWAITE, C.E., HARVEY, R.B., PHILLIPS, T.D. and REINE, A.H. (1992). Poult.Sci., 71 : 1136 - 1144.

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

THE EFFECTIVENESS OF SHRINK-WRAPPING EGG PACKS ON ALBUMEN QUALITY OF STORED EGGS

SRI SULANDARI* and S.G. TULLETT**

It is commonly accepted that an egg of high internal quality, as indicated by a high Haugh unit value, is better for poaching, boiling in the shell and cake volume (Lowe, 1955). Use of an appropriate egg pack may provide protection to the interior quality of the egg during distribution. The primary purpose of this study was to ascertain whether there were differences in the ability of six types of popular egg packs to maintain albumen quality during storage and also to indicate any beneficial effects of shrinkwrapping egg packs.

Six types of egg packs were used with 24 eggs in each type of egg pack either shrink-wrapped or not shrink-wrapped, and stored at 13 - 15°C. Half of the eggs were used for egg quality determination after one week of storage and the remainder after two weeks. At the end of the storage periods, Haugh units were determined. Measurement of Haugh units were made electronically using the measuring equipment designed by York Electronic Centre and the readings processed by dedicated microprocessor.

The results showed that the different types of egg pack had different abilities in retarding interior quality loss during storage. The highest quality was from eggs stored in pack type 3 (1-dozen, expanded polystyrene) and the worst quality from egg pack type 6 (half-dozen, pulp top and base). It did not, however, differ significantly ($P > 0.05$) from egg pack 1 (2-dozen, clear detachable plastic top with pulp base), 2 (1-dozen, clear plastic top and base), 3, 4(as 2, half-dozen pack) and 5 (as 3, half-dozen packs). One possibility for the differences observed may be the effectiveness of each egg pack type in reducing carbon dioxide and water loss from egg.

Shrink-wrappping of the egg packs resulted in significantly ($P < 0.05$) better albumen quality after storage by about 2 Haugh units. It is suggested, that the ability of shrink-wrapped egg packs to retard the loss of carbon dioxide from eggs resulted in less deterioration in albumen quality than for eggs stored in packs that were not shrink-wrapped.

LOWE, Belle (1955). 'Experimental Cookery' 4th edn (J. Wiley and Sons Inc.: New York).

---

* University of New England, Armidale, New South Wales 2351

** Present address: Bernard Mattews plc, Glebe House, Gintonham Road, Wrengingham, Norfolk

HETEROSIS FOR GROWTH AND BODY COMPOSITION IN F_1 AND F_2 PROGENY OF CROSSES BETWEEN LEAN AND FAT LINES OF CHICKENS

H. SUTEDJO and R.A.E. PYM

Commercial meat chicken breeders require information on heterotic, maternal and sex-linked effects upon growth and body composition to determine optimum cross-breeding strategies in their lines. The aim of the present study was to provide such information from F_1 and F_2 progeny of crosses between lines of chickens selected for high (line F) or low (line L) abdominal fatness.

Six males from each of the two pure lines were each mated to 12 females from each line to produce 12 FF, 30 LL, 51 FL and 39 LF progeny in the one hatch. At the same time, 4 males from each of the two F_1 crosses were each mated to 8 females from the same F_1 cross to produce 48 FLFL and 46 LFLF F_2 progeny in the same hatch. The progeny were placed in single cages with individual feeders at 25 days of age and given a broiler finisher diet containing 200 g crude protein and 13.0 MJ ME/kg. Food intake was measured to 56 days of age when the birds were weighed, measured with the abdominal fat calipers and killed for determination of abdominal fat weight. Heterotic effects were estimated in the F_1 and F_2 progeny, maternal effects in the F_1 male progeny and sex-linked effects in the F_1 female progeny after adjustment for maternal effects.

Means in the FF and LL lines for 8-week liveweight (8WW,g), food consumption (FC,g), FCR, abdominal fat (AF,g) and caliper measure (CM,mm) and heterotic (F_1 and F_2 progeny), maternal (LF-FL, i.e. F♀♀ - L♀♀) and sex-linked (FL-LF, i.e. F♂♂ - L♂♂) effects expressed in these units and, in parenthesis, as percentage change.

Line	8WW	FC	FCR	AF	CM
FF	1407	2659	2.94	25.4	5.12
LL	1491	3030	3.06	14.9	3.00
Effect					
heterotic (F_1)	40(3)	111(4)	0(0)	5.0(25)	0.68(17)
heterotic (F_2)	50(3)	169(6)	0(0)	3.9(19)	0.47(12)
maternal	-55(-4)	-144(-5)	-0.03(-1)	0.8(3)	0.28(6)
sex-linked	43(3)	19(1)	-0.08(-3)	4.0(13)	0.04(1)

In contrast to the results from an earlier comparison (Sutedjo and Pym, 1992), the present results show caliper measure (and also abdominal fatness) to exhibit substantial positive heterosis indicating the possible involvement of genotype X environment interaction. For the selected traits (AF and CM), the relative level of heterosis in the F_1 and F_2 progeny would not appear to depart significantly from expectations based on the dominance explanation for heterosis. The moderate sex-linked effect for abdominal fat was in the expected direction.

SUTEDJO, H. and PYM. R.A.E. (1992). Proc. Aust. Poult. Sci. Symp. 4: 131 ed. R Johnson.

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

CHANGES IN CHICKEN EGGSHELL ULTRASTRUCTURE WITH GROWTH OF THE EMBRYO.

L. TURNBULL*, C.E. BRACKPOOL*, J.R. ROBERTS*, S.E. SOLOMON**

Changes in eggshell ultrastructure during incubation were studied in a total of 160 eggs taken from a flock of commercial broiler breeder hens at 39 weeks of age. Twenty fertilised but unincubated eggs were sampled from the group (Day 0) and a further 20 eggs were taken from the incubator on each of days 3, 6, 9, 12, 15 and 18. The remaining 20 eggs were left to hatch. Each individual egg was examined to determine the embryonic stage of development (Freeman and Vince, 1974) and traditional eggshell quality parameters were recorded. Eggshells were viewed in transverse section and from the inner surface. The latter were plasma etched (ashed) according to the method described by Reid (1983).

Upon examination, the shells from Day 0 appeared the same as the shells of unfertilised eggs of the same strain, as did the shells from Days 3, 6 and 9 of incubation. At Day 12 of incubation, the majority of the shell area examined appeared similar to that of the Day 0 shells, although small patches of erosion were apparent in some shells. After 15 days of incubation, the eggshells were noticeably eroded away at the mammillary tips. The column edge could still be clearly defined but the membrane/calcite bond was weaker. At Day 18 of incubation, the shells showed extensive erosion, the individual column heads were less well defined and the membrane separated from the mineralised shell. In the shells from which chickens had hatched, the erosion of the shells had proceeded to the base of the mammillary layer and in some cases into the palisade layer where the individual column heads were non-existent.

The changes in the shell quality parameters and the appearance of the shell at the microscopic level over the incubation period reflect the changes occurring within the egg due to the growth and development of the embryo. The first sign of obvious morphological change in the eggshell occurs on Day 12 of incubation and corresponds to the time when the chorioallantois of the developing embryo completely lines the inside of the shell (Burton and Tullett, 1985) and transports calcium from there to the developing embryo (Crooks and Simkiss, 1974). Mineralisation of the skeleton of the embryo begins on the eighth day on incubation with the mineral content of the bone increasing to produce a hardened skeleton by the fifteenth day. The results of the study show that calcium is removed from the mammillary cones and cores rather than from the intermammillary channels.

BURTON, F.G. and TULLETT, S.G. (1985). Comp.Biochem.Physiol.82A: 735
CROOKS, R.J. and SIMKISS, K. (1974). Quart.J.Exp.Physiol. 60:55
FREEMAN and VINCE (1974) Development of the Avian Embryo Chapmen and Hall Ltd. London
REID, J, (1983). Brit.Poult.Sci. 24:233

---

* Department of Physiology, University of New England, Armidale, NSW, 2351.

** Poultry Research Group. Department of Veterinary Anatomy, University of Glasgow Veterinary School, U.K.

CHARACTERIZATION AND PRODUCTION OF RECOMBINANT CHICKEN INSULIN-LIKE GROWTH FACTOR-II FROM *ESCHERICHIA COLI*

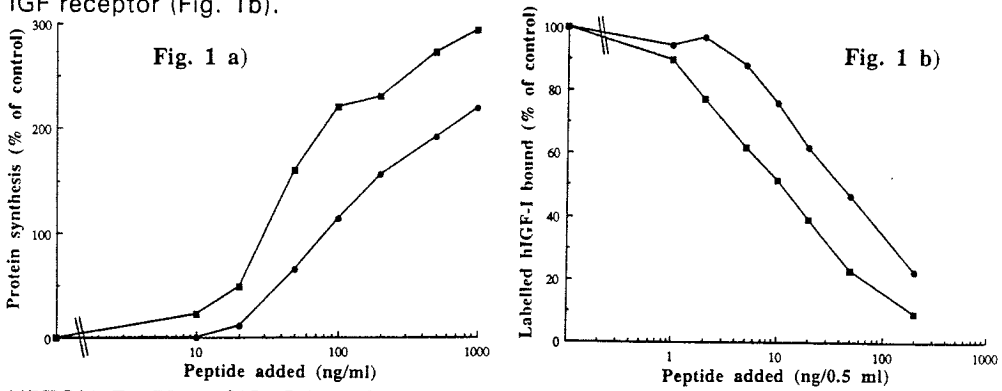
Z. UPTON, G.L. FRANCIS, J.C. WALLACE and F.J. BALLARD

We have previously described the characterization and production of recombinant chicken insulin-like growth factor-I (cIGF-I) from *Escherichia coli(E.Coli)* (Upton et al. 1992). We now report the use of a similar gene fusion system to produce recombinant chicken IGF-II (cIGF-II).

A synthetic gene for human IGF-II (hIGF-II) with nucleotides optimized for protein synthesis in *E. coli* was utilized. Using *in vitro* site-directed mutagenesis, the hIGF-II codons were replaced with codons which permit the expression of the amino acids which are different in cIGF-II (Kallincos et al. 1990, Taylor et al. 1991). The cIGF-II construct was subcloned into an expression vector which, when induced, expresses the cIGF-II as a fusion protein containing the first 46 amino acids of methionyl porcine growth hormone, followed by the amino acids Val-Asn-Phe-Ala-His-Tyr.

The cIGF-II fusion protein was isolated from bacterial inclusion bodies using a strategy similar to that reported for recombinant cIGF-I. Chicken IGF-II was released from the fusion protein using a genetically-modified form of subtilisin which cleaves on the carboxyl side of the motif Phe-Ala-His-Tyr. The cleaved peptides were then purified to homogeneity by three additional chromotographic steps.

Preliminary *in vitro* assessment of recombinant cIGF-II in cultured rat myoblasts indicates that it differs from its human counterpart, hIGF-II. Chicken IGF-II (●) was 3.5-fold less potent than hIGF-II (■) in its ability to stimulate protein synthesis (Fig. 1a). This appears to be due to a decreased affinity for the type-1 IGF receptor (Fig. 1b).



UPTON, Z., FRANCIS, G.L., ROSS, M., WALLACE, J.C. and BALLARD, F.J. (1992) J. Mol. Endocrinol. 9, 83-92.

KALLINCOS, N., WALLACE, J.C., FRANCIS, G.L. and BALLARD, F.J. (1990) J. Endocrinol. 124, 89-97.

TAYLOR, E.R., SELEIRO, E.A.P. and BRICKELL, P.M. (1991) J. Mol. Endocrinol. 7, 145-154.

Cooperative Research Centre for Tissue Growth and Repair, PO Box 10065 Gouger Street, Adelaide, SA 5000

RESPONSES OF COMMERCIAL LAYING PULLETS TO THE FEEDING OF DIETS CONTAINING PHYTASE AND LOW CONCENTRATIONS OF AVAILABLE PHOSPHORUS

NADA USAYRAN EL-KHATIB and D. BALNAVE

One of the major problems relating to environmental pollution concerns the presence of high concentrations of phosphorus in surface and ground water. Accordingly, attempts are being made to more accurately define the minimum dietary phosphorus requirements of animals commensurate with optimum production. The use of dietary supplements of phytase enzyme is also under investigation since this enzyme would be expected to convert at least part of the mainly unavailable phytase phosphorus present in vegetable feedstuffs into a utilizable phosphate form.

In the current study 45-week-old laying hens were fed diets derived solely from plant sources. Six replicates of six hens were allocated to each of nine experimental diets. The diets contained one of three levels of available phosphorus (1.2, 1.9 and 2.6 g/kg corresponding to 3.5, 4.2 and 4.9 g total phosphorus/kg respectively). A commercial fungal phytase enzyme (410 PU/g) was included at three concentrations (0, 0.5 and 1.0 g/kg) with each of the three diets. The diets were fed for a period of 12 weeks during which time food intake, egg production, egg weight and egg shell quality were determined. Results are shown in the Table.

	Food intake (g/h/d)	Egg production (eggs/h/d)	Egg weight (g)	Shell breaking strength (g)	Shell thickness (μm)
Available phosphorus (g/kg)					
1.2	106.5	0.78	60.2	2202	368
1.9	107.2	0.78	61.2	2238	368
2.6	111.4	0.79	61.5	2291	368
Phytase (mg/kg)					
0	109.0	0.78	61.1	2238	370
500	108.9	0.79	60.8	2221	367
1000	107.1	0.78	61.1	2271	366
LSD ($P<0.05$)	3.8	0.06	1.2	110	7

Dietary phosphorus concentration significantly influenced food intake but no other significant effects of dietary phosphorus or dietary phytase were observed on the production and shell quality measures. Also, there were no significant (phosphorus x phytase) interactions.

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

FUSAROCHROMANONE INDUCED TIBIAL DYSCHONDROPLASIA

P.W. VAN WEL, L.W. BURGESS*, D.R. FRASER and W.L. BRYDEN

Skeletal disorders continue to be a significant developmental problem of broiler chickens (Riddell, 1992). Tibial dyschondroplasia (TD), one such abnormality, is characterised by a persistence of a mass of avascular opaque cartilage in the metaphysis below the growth plate. The lesion can occur at different sites but the proximal tibia is the most commonly affected (Leach and Lilburn, 1992; Riddell, 1992) The incidence of TD is influenced by genotype, nutrition and management and may exceed 30% in some flocks (Leach and Lilburn, 1992). Despite 30 years of research, our understanding of the pathogenesis of TD is incomplete. Research in this area has been hindered by the lack of a reproducible model of TD. In this study we confirm previous reports (see Walser, 1987) that fusarochromanone, a mycotoxin produced by *Fusarium equiseti*, induces TD in broilers.

Cultures of seven *F. equiseti* isolates were grown on moist rice at 25°C in the dark for three weeks. Following drying, the cultures were ground and added (30 g/kg) to broiler diets. Sixteen, one day old male broiler chickens were fed each experimental diet till four weeks of age. The proximal tibiotarsus of each bird was examined and three isolates were found to induce an incidence of 79%, 49% and 6% of TD. Control chickens and chickens consuming diets containing cultures of the other four isolates, a total of 80 chickens, did not have the dyschondroplastic lesion. Analysis of the culture material by thin-layer chromatography (Lee *et al.*, 1985) showed that TD inducing isolates produced fusarochromanone.

A high correlation between the incidence of TD in broilers and the natural occurrence of fusarochromanone has been observed in Denmark (Krogh *et al.*, 1989). However the inability of most *F. equiseti* isolates to produce fusarochromanone (Wu *et al.*, 1990) suggests that this mycotoxin is unlikely to be the cause of TD in most field cases. Nevertheless, fusarochromanone-induced TD is a reproducible model in which the mechanisms of pathological bone development may be studied.

KROGH, P., CHRISTENSEN, D.H., HALD, B., HARLOU, B., LARSEN, C., PEDERSEN, E.J. and THAN, U. (1989) Appl.Environ.Microbiol. 55: 3184.

LEACH, R.M. and LILBURN, M.S. (1992). Poult.Sci.Rev. 4: 57.

LEE, Y.W., MIROCHA, C.J., SHROEDER, D.J. and WALSER, M.M. (1985). Appl.Environ.Microbiol. 50: 102.

RIDDELL, C. (1992). Aust.Poult.Sci.Symp. 4: 52 ed. R Johnson.

WALSER, M.M. (1987). Proc.Cornell Nutr.Conf. p. 69.

WU, W., NELSON, P.E., COOK, M.E. and SMALLEY, E.B. (1990) Appl.Environ.Microbiol. 56: 2989.

Department of Animal Science, University of Sydney, Camden NSW 2570
*Department of Crop Sciences, University of Sydney, NSW 2006

FOOD INTAKE AND PLASMA INSULIN AND GLUCOSE IN FAT- AND LEAN-LINE CHICKENS INFUSED INTRAHEPATICALLY WITH GLUCOSE OR LIPID

J.X. WU and R.A.E. PYM

It has been suggested that an alteration in the glucose: insulin relationship accounts for differences in fattening between lines of chickens selected divergently for body fatness (Simon and Leclercq, 1982). Wu and Pym (1992) found higher plasma levels of insulin and lower levels of glucose in a line of chickens selected for increased abdominal fatness than in their lean-selected counterparts.

In an attempt to identify the differential physiological responses of fat- and lean-line birds to specific absorbed nutrients, glucose or lipid were infused via the coccygeomesenteric vein into nine 56-day-old cockerels from each of two lines selected for high (line F) or low (line L) abdominal fatness. Birds were fasted for 18 h prior to infusion at 33 ml/h for 3 h with sterile solutions containing either 0.9% saline, 50% glucose, or 20% lipid. Birds were given the broiler finisher diet again following infusion. Blood samples were taken for determination of plasma glucose and insulin at commencement of fasting and infusion, conclusion of infusion and 30 and 180 minutes after infusion. Food intake was measured over the first 180 minutes following infusion.

There was a considerable reduction in both plasma insulin and glucose with fasting with a greater reduction in the F than L line for both insulin (0.052 vs 0.016 ng/100 ml) and glucose (31 vs 17 mg/100 ml). Whilst the F line had higher plasma insulin levels (0.15 vs 0.10 ng/100 ml) and food intake (29.0 vs 22.6 g) than the L line, there was no difference in plasma glucose levels between the lines (246 vs 247 mg/100ml). A significant interaction between line and infusate for food consumption was due to a marked differential response in the F and L lines. Relative to the saline-infused control, in the F line there was no change in intake with glucose infusion but a moderate drop with lipid infusion (7.7 g), whereas in the L line there was a moderate drop with glucose infusion (12.2 g) but a very large reduction with lipid infusion (26.0 g). Across the two lines and three post-infusion sampling times, relative to the saline-infused birds glucose infusion had no effect on plasma glucose but resulted in a mild reduction in plasma insulin (0.143 vs 0.121 ng/100 ml). In contrast, lipid infusion resulted in a marked reduction ($P < 0.05$) in both plasma glucose (251 vs 235 mg/100 ml) and insulin (0.143 vs 0.110 ng/100 ml).

The substantially higher insulin levels in the F than the L line with no difference in plasma glucose levels, supports the suggestion of a glucose: insulin imbalance in fat lines which leads to the greater degree of fattening in such lines. The glucostatic mechanism of appetite control would appear to be active in the L line birds, whereas in the F line there was no indication of such a response. Since total energy infused with lipid and glucose was similar, the food intake response of both lines to lipid infusion was not only attributable to energy-related effects.

SIMON, J. and LECLERCQ, B. (1982). J. Nutr. 112: 1961-1973.
WU, J.X. and PYM, R.A.E. (1992) Proc. 5th Int. Symp. Avian Endocrin. P20.

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