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TISSUE DISTRIBUTION OF HOMOARGININE IN CHICKENS FED GUANIDINATED CASEIN

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

Guanidinated (G) proteins, in which lysine is converted to homoarginine (HA), have been used as markers to determine endogenous amino acid losses in chickens (Siriwan *et al.*, 1994) and to estimate true ileal amino acid digestibility values. When digestibility measurements were made using the excreta of caecectomised cockerels, high concentrations of HA were observed in excreta compared to ileal digesta and it was shown that significant quantities of HA were excreted in urine (Angkanaporn *et al.*, 1994). The present study was initiated to study the fate of HA within the body after *ad libitum* feeding of G-protein by determining the distribution of HA in various tissues.

Male broiler chicks were fed five semi-purified diets containing either casein or G-casein as the sole source of protein (CP, 230 g/kg) from days 6 to 13 post-hatching. The casein-based diet was balanced to meet recommended amino acid requirements and served as the control (Diet 1). In Diets 2, 3, 4 and 5, casein was substituted by G-casein, and L-lysine was supplemented at 0, 5.6, 11.4 and 17 g/kg respectively. On day 13 post-hatching, plasma and tissue samples (brain, kidney and breast muscle) were collected and analysed for amino acids (including HA). The results are summarized in the Table.

	Plasma HA conc (μmole/litre)	Tissue HA conc (μmole/kg) Brain	Breast	Kidney	Brain lysine (μmole/kg)
Diet 1	21.6^{d}	2.3^{b}	2.1^{d}	10.6^{b}	77.1^{b}
Diet 2	963.8^{c}	95.8^{a}	550.8^{a}	777.7^{a}	23.3^{c}
Diet 3	1439.7^{b}	106.7^{a}	320.9^{b}	656.5^{a}	41.4^{c}
Diet 4	2042.7^{a}	100.8^{a}	115.2^{c}	797.4^{a}	65.7^{b}
Diet 5	1820.2^{a}	90.6^{a}	117.1^{c}	687.2^{a}	108.4^{a}

Means in the same column with different superscripts are significantly different ($P<0.05$).

Chickens fed on G-casein diets (Diets 2-5) had higher concentrations of HA in brain and kidney compared to the control, but the levels in all G-casein diets were numerically similar, indicating no competition from other amino acids in uptake in these tissues. The highest concentrations of HA were found in the kidney. Muscle HA concentrations in chicks fed Diet 2 were significantly higher and probably related to the pronounced atrophy of muscles that was observed in this group. In the lysine-deficient diets (Diet 2, 3), brain lysine concentrations were significantly reduced. It has been proposed that HA depresses plasma concentrations of amino acids of the corresponding transport class (e.g. lysine, arginine ; Tews and Harper, 1986). Therefore, in addition to the low dietary lysine intake, high plasma HA levels may also have contributed to the depression of lysine concentrations in the brain by competing for lysine transport at the blood-brain barrier. Decreased concentrations of lysine in specific area(s) of brain may be expected to provide a signal leading to behavioural effects such as reduced feed intake. The finding that the adverse effect on feed intake in birds fed G-casein diets was overcome by supplemental lysine (Angkanaporn *et al.*, 1995) lends support to this hypothesis.

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ASPECTS OF LIPID METABOLISM IN BROILER CHICKENS

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Summary

The use of exogenously administered hormones, such as growth hormone and human insulin-like growth factor-1, as a means of controlling fat deposition in broiler chickens has proved largely unsuccessful. Low concentrations of tri-iodothyronine in the diet offer some potential, the mechanism of response being associated with altered plasma insulin:glucagon ratios. Immunoneutralisation of somatostatin, while theoretically promising, has so far proved ineffective. The use of beta-agonists, although of great potential value, especially in birds having a longer growing period, is restricted because of their pharmacological activity in humans.

The value of fats and oils as feed ingredients is dependent on an accurate knowledge of their metabolisable energy (ME). Recently, equations have been derived to predict apparent ME (AME) from fatty acid composition and apparent digestibility. Currently, this approach is being extended at Camden to the measurement of true digestibility using ileal digesta and excreta and endogenous fatty acid production in broilers fed a semi-purified, low-fat diet.

I. INTRODUCTION

Aspects of lipid metabolism of major interest to the poultry industry are the control of carcass fat deposition and the use of fats as sources of dietary energy. However, the two areas are interrelated since the fatty acid composition of carcass fat may be influenced, but not wholly controlled, by the fatty acid content of the diet.

It is widely recognised that excessive fat deposition in broilers is undesirable in view of consumer preferences for leaner products. In addition, the use of dietary energy to produce fat which must be removed during processing increases production costs. Strategies to minimise carcass fat deposition, however, must be consistent with the primary aim of broiler production, which is to produce protein at minimum overall cost. This objective has driven research based on genetic selection, improved nutrition and management, and effective disease control to develop broiler chickens which grow rapidly with relatively high efficiency of conversion of nutrients into body tissue. Nevertheless, skeletal development and muscle accretion are inescapably accompanied by varying degrees of fat deposition, and as birds approach slaughter weight fat deposition becomes the major component of liveweight gain (Fisher, 1984).

The successful manipulation of carcass composition in pigs in favour of protein accretion by treatment with growth hormone (GH) (Etherton *et al.*, 1987) has stimulated interest in the endocrine control of body composition in broiler chickens (Cogburn, 1991). The effects of GH, insulin-like growth factors, the hypothalamic releasing factors for GH (GRF) and thyrotropin releasing hormone (TRH), together with the influence of thyroid hormones and circulating levels of insulin and glucagon have been extensively studied (see Cogburn, 1991), as discussed below.

Two other approaches to the problem of reducing body fat deposition in broiler chickens are treatment with adrenergic agonists (see Wellenreiter, 1991) and immunological

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control via immuno-enhancement of GH or by immuno-cytotoxicity based on the production of antibodies to adipocytes (Futter and Flint, 1990). An alternative immunological approach based on the immunoneutralization of somatostatin, the hypothalamic hormone which inhibits secretion of both GH and thyroxine (T_4), has been reported to be ineffective in poultry (see Cogburn, 1991).

High concentrations of dietary fat do not necessarily give rise to increased carcass fat (Alao and Balnave, 1984; Akiba *et al.*, 1994). However, their correct application requires an accurate knowledge of their metabolisable energy content. Also, the extent of usage of fats as energy sources in broiler diets is largely dictated by the relative costs of alternative ingredients. Factors which influence their energy value have been extensively studied (Annison, 1974; Wiseman and Lessire, 1987a, 1987b; Wiseman and Salvador, 1991). In current research at Camden the present authors are attempting to improve the precision of prediction of apparent metabolisable energy (AME) from chemical composition. This topic has been comprehensively studied by Wiseman and his colleagues (Wiseman *et al.*, 1991; Wiseman and Blanch, 1994).

II. ENDOCRINE REGULATION OF BODY COMPOSITION

This topic has been comprehensively reviewed by Cogburn (1991), and in this necessarily brief overview attention will focus on factors which influence fat deposition in broiler chickens.

(a) Growth hormone and insulin-like growth factors

Sustained efforts to reduce body fat in broiler chickens by treatment with GH have been largely unsuccessful (Cogburn, 1991), although Vasilatos-Younken and Scanes (1991) believe that significant effects of GH on the performance of growing chickens are possible.

Growth hormone would appear to play a less dominant role in avian growth and development than in mammals. In particular, the linkage between GH and insulin-like growth factor-1 (IGF-1), a key element in the mammalian response to GH, is largely absent in chickens (see Cogburn, 1991). This difference probably stems from the lack of GH receptors in the avian liver. In pigs, which are highly responsive to the lipolytic action of GH, binding to hepatic receptors is an essential step in the enhanced secretion of IGF-1 in pigs injected with GH (Etherton *et al.*, 1986).

Administration of human IGF-1 failed to improve growth rate, feed efficiency or body composition in broiler cockerels (McGuiness and Cogburn, 1987) but, as pointed out by Cogburn (1991), definitive studies will not be possible until adequate quantities of chicken IGF-1 become available, presumably by recombinant technology.

(b) Thyroid hormones

There is extensive evidence that thyroid hormones play as important a role as GH in the regulation of growth and IGF-1 production in young chickens (Cogburn, 1991). Thyrotropin releasing hormone (TRH) triggers the release of both GH and thyroid stimulating hormone (TSH). The latter hormone promotes synthesis and secretion of thyroxine (T_4), a prohormone with little metabolic activity. Conversion of T_4 into metabolically active tri-iodothyronine (T_3) occurs in peripheral tissues. Broiler chickens selected either for higher growth rates, or for leanness, have higher levels of circulating T_3 than their counterparts (Cogburn, 1991).

Administration of T_3 in feed (0.25 mg/kg) to broiler chickens from 1-54 days reduced abdominal fat by 28-66% and body fat by 26% (May, 1982). Larger and more consistent reductions in body fat have been achieved, however, by combining dietary T_3 treatment with daily injections of GH (Cogburn *et al.*, 1989). The depletion in body fat in response to the combined treatment is accompanied by a reduced molar ratio of insulin to glucagon (I/G) in plasma. The I/G ratio would appear to be an important regulator of fat deposition, since the ratio is highly correlated with the amounts of abdominal fat in broiler chickens (Cogburn, 1991).

(c) Pancreatic hormones

Insulin, in birds and mammals, is an anabolic hormone which stimulates lipogenesis and fat accretion. Glucagon, the counter-regulatory hormone, stimulates lipolysis. A high I/G ratio favours lipogenesis, whereas a low I/G ratio promotes mobilisation of body fat. Both GH and the thyroid hormones influence secretion of insulin and glucagon. Plasma insulin levels increase proportionately with age and are highly correlated with level of deposition of abdominal fat (see Cogburn, 1991). In general, treatments which increase circulating insulin levels increase body fat deposition.

Cogburn (1991) has pointed out that although glucagon is a powerful lipolytic hormone in chickens, sustained treatment results in a range of metabolic disorders which preclude its use to reduce body fat content. The effectiveness of low levels of dietary T_3 (0.25 - 1 mg/kg) in reducing fat deposition, however, is due in large part to increased glucagon secretion, and a more favourable I/G ratio.

(d) Hypothalamic releasing factors

Both GH-releasing factor (GRF) and thyrotropin releasing hormone (TRH) stimulate the release of GH, whereas somatotrophin-release inhibiting factor, somatostatin, inhibits release of both GH and thyroid-stimulating hormone (TSH).

Many studies involving short term and chronic administration of GRF and TRH to broiler chickens have failed to demonstrate consistent reductions in body fat content (Cogburn, 1991).

An alternative, theoretically attractive approach based on the immunoneutralization of somatostatin to enhance endogenous GH secretion has proved ineffective in producing leaner broiler chickens (see Cogburn, 1991).

III. ß-ADRENERGIC AGONISTS

The possible use of synthetic ß-agonists to manipulate carcass composition in poultry has been reviewed by Wellenreiter (1991). These pharmacologically active compounds interact with ß receptors present on the membranes of cells in most body tissues (see McDowell and Annison, 1991). When given orally, a number of ß-agonists, which include the compounds clenbuterol (Boehringer), cimaterol (Cyanamid) and ractopamine (Eli Lilly), increase protein accretion and reduce fat deposition. Part of the reduction in carcass fat of treated birds is accounted for by the diversion of energy to support both increased protein synthesis and a higher basal metabolic rate. The major effects of ß-agonists on adipose tissue, however, are accounted for by increased lipolysis, reduced lipogenesis and reduced triacylglyceride synthesis (Yang and McElligott, 1989).

The responses of avian species to ß-agonists are generally not as large as those observed in mammals (Buttery and Dawson, 1987). The exception is the turkey, where

significant increases in growth accompanied by reduced body fat accretion have been reported (see Wellenreiter, 1991). Results of similar feeding experiments with chickens have been less impressive, but both clenbuterol and cimaterol increased weight gain and protein accretion, with some lowering of both carcass and abdominal fat (see Wellenreiter, 1991). The effects on carcass fat were more pronounced in female birds.

The ease of administration of ß-agonists, which may be included in feed, and their effectiveness as growth promoters and repartitioning agents has led to pressure for their clearance for use in all livestock production. A major drawback to their use, however, is their pharmacological activity in humans at low concentrations. Insistence on a safe withdrawal period before slaughter will ensure consumer safety, but the preparation, distribution and usage of food containing ß-agonists will require special care.

IV. FAT DEPOSITION IN BIRDS

This topic was comprehensively reviewed by Nir *et al.* (1988), who emphasised that adipose tissue has much the same role in avian species as in mammals. Absorbed energy yielding nutrients in excess of current requirements are converted into long chain fatty acids, which after esterification to triacylglycerols are transported from the liver, the main site of lipogenesis, as lipoproteins and deposited as adipose tissue. Complete hydrolysis of triacylglycerols by the enzyme lipoprotein lipase is a pre-requisite to uptake by adipose tissue, and Griffin *et al.* (1987) showed that broiler chickens have about twenty times more lipoprotein lipase activity in their tissues than layer-strain chickens. The key role of the enzyme in the regulation of adipose tissue accretion has been reviewed by Butterwith (1988).

Adipose tissue sites develop at different rates, and involve both hyperplasia (new adipocyte production), predominant up to 14 weeks of age (see Nir *et al.* 1988), and hypertrophy (enlargement of existing adipocytes). Both processes operate simultaneously during the growth of broiler chickens, and may contribute to the diverse and unexplained differences in the responses to factors which influence adipose tissue accretion and mobilisation (see Fisher, 1984).

The fatty acid composition of adipose tissue is controlled by the relative contributions of fatty acids synthesized in the liver and those derived from the diet. In addition, the activities of enzyme systems responsible for chain-elongation (mainly conversion of palmitic acid to stearic acid) and desaturation (mainly conversion of stearic to oleic acid) influences the proportions of individual fatty acids (see Gurr and Harwood, 1991). Dietary fatty acids must include adequate amounts of the essential fatty acid, linoleic acid, and possibly small amounts of α-linolenic acid. Both fatty acids are key components of cell membrane lipids in man, and are precursors of the eicosanoids (see Gurr and Harwood, 1991), but the relative importance of the n-3 and n-6 series of polyunsaturated fatty acids in poultry remains to be defined.

V. DIGESTION AND ABSORPTION OF FAT

The pathways involved in the hydrolysis and uptake of dietary fats in the chicken appear to be closely similar to those established in the pig and other non-ruminant species (Freeman, 1984). The key step in fat digestion and absorption is the rate of formation of micelles, which consist mainly of long chain fatty acids and monoacylglycerides. Unsaturated fatty acids form micelles more readily than saturated fatty acids (Freeman, 1976), and the ability of unsaturated fatty acids to promote the formation of micelles containing both saturated and unsaturated fatty acids explains the dramatic increase in the digestibility of highly saturated fats when as little as 5% of an unsaturated fat is added to the ration (Lewis

and Payne, 1966). The interaction is the most important contributor to the "extra caloric value" of fats in poultry diets (see Summers, 1984), which is observed when relatively small amounts of unsaturated fats increase the digestibility and, therefore, AME of saturated fats. A further contributing factor to the "extra caloric" effect may be the reduced rate of passage of digesta in birds fed high fat diets (Mateos *et al.*, 1982; Summers, 1984). The capacity of fatty acids to form micelles is also related to chain length, the medium chain length acids, even when fully saturated, forming micelles more readily than their longer chain counterparts (Freeman, 1976).

The monoacylglycerides present in micelles act as amphiphilic components to stabilise micelle structure (Freeman, 1976), and in diets containing normal fats, the availability of monoacylglycerides is not rate limiting for micelle formation. At high ratios of free fatty acid to monoglyceride, however, fat digestibility is depressed (Wiseman and Blanch, 1994). This situation may occur in commercial practice when acid oils, a cheap source of fat comprising mainly free fatty acids, are used in poultry diets.

VI. PREDICTION OF METABOLISABLE ENERGY OF DIETARY FATS FROM CHEMICAL COMPOSITION

The gross energy (GE) of pure fats may be determined directly by bomb calorimetry, or calculated from bond energies (see Annison, 1974). Most of the crude fats used in poultry diets, however, contain organic matter contaminants. These include non-saponifiable material (mainly sterols), and oxidized and polymerized fatty acids. Although combustible in the bomb calorimeter, these compounds make little or no contribution to the energy needs of the bird. In fact, it is reasonable to assume that only the long chain fatty acid and glycerol contents of the fat source need be considered. From these data, determined by chemical analysis, GE values can be calculated from known values for bond energies.

In order to calculate AME values of dietary fats from GE values based on fatty acid and glycerol content, however, the digestibilities of the fatty acids need to be known. As indicated above, these are influenced by both the chain length and the degree of unsaturation of the fatty acids, and by the availability of monoacylglyceride, or equivalent amphiphile. Wiseman and Lessire (1987a,b) examined these interrelationships at length, and devised prediction equations for fatty acid availability and AME from fatty acid composition and apparent digestibility. A possible limitation of these comprehensive studies was the absence of data on endogenous fat production, which precluded the calculation of true digestibility values from digestibility values based on the fat content of excreta. Furthermore, as noted by Wiseman and Lessire (1987b), data based on excreta take no account of the possible biohydrogenation of unsaturated fatty acids in the caeca and large intestine which, if appreciable, would invalidate digestibility data for individual fatty acids.

In current studies at Camden by the present authors, the approach of Wiseman and Lessire (1987a, b) is being extended by measuring true digestibility data based on values corrected for endogenous fatty acid production determined using a semi-purified, low-fat diet (60 mg lipid/kg). In addition, true digestibilities based on digesta collected from the lower ileum of broilers fed this diet supplemented with different lipid sources are being compared with values determined using total excreta.

The nature of responses can be judged from two studies using soyabean oil (Experiment 1) and a mixed animal-vegetable acid oil (Experiment 2). Daily endogenous fatty acid productions in the ileum were 24 mg/day (Experiment 1) and 38 mg/day (Experiment 2) and the corresponding values for excreta were 44 mg/day (Experiment 1) and 53 mg/day (Experiment 2). The endogenous fatty acid profiles in the two studies consisted

primarily of palmitic, stearic, oleic and linoleic acids and were markedly different from the dietary profiles. The effects of correcting the apparent fatty acid digestibilities for endogenous production are shown in Table 1. The reduced digestibility of the fatty acids from the mixed acid oil, relative to soyabean oil, is expected with practically complete digestibility of the fatty acids being obtained from soyabean oil.

Table 1. Apparent and true fatty acid digestibilities (g/100g fatty acids) determined using ileal and excreta samples of digesta from broilers receiving supplements of approximately 43 g lipid/kg diet.

	Soyabean oil				Mixed acid oil			
	Apparent		True		Apparent		True	
	Ileum	Excreta	Ileum	Excreta	Ileum	Excreta	Ileum	Excreta
Fatty acid								
Palmitic	95.3	94.4	97.8	99.9	73.3	58.1	75.6	61.4
Stearic	93.5	91.0	98.0	99.7	59.4	43.4	63.4	48.4
Oleic	98.5	98.3	99.3	99.5	82.9	75.2	83.7	76.5
Linoleic	98.8	99.0	99.4	99.8	82.3	77.4	84.4	79.9

VII. CONCLUSIONS

The broiler chicken has proved to be less amenable to hormonal manipulation of carcass composition than has the pig. In particular, the responses to growth hormone treatment contrast markedly in these two species. Increasing dietary fat concentrations do not necessarily result in increased carcass fat but successful use of dietary fat supplements requires an accurate knowledge of the ME of the lipid source. Recent, and current, studies are providing equations by which the AME and TME of dietary fats can be predicted.

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THE EFFECTS OF DIETARY LIPID ON POULTRY PERFORMANCE AND COMPOSITION

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Summary

In recent times performance and quality of carcass, particularly fatness, of chickens have received considerable attention in both the broiler industry and among consumers. Carcass composition of broilers is manipulated by nutritional factors as well as by genetic and environmental factors. This paper describes some aspects of fatness in broilers and the effects of dietary fat sources on carcass composition.

Abdominal fat content correlates well with fat contents in carcass and edible meats, substantiating the fact that abdominal fat content is an appropriate criterion for assessing broiler fatness. In abdominal adipose tissue, adipocyte number increased extensively until 6 or 8 weeks of age, while cell volume significantly increased beyond 4 weeks in heavy-type broiler chickens. Feeding corn oil or chicken oil reduced abdominal fat deposition compared to feeding yellow grease and the decrease was associated with reduced adipocyte volume in female chickens. Medium-chain triglycerides (MCT) were utilized more efficiently than yellow grease, a source of long-chain triglycerides (LCT). Feeding MCT improved feed efficiency and edible meat yields and reduced both abdominal and subcutaneous fat content in edible meats, suggesting that utilization of MCT has advantages in broiler production. Broiler chickens fed n-3 polyunsaturated fatty acids for 2 weeks from 14 days of age reduced both plasma triglyceride concentration and hepatic fatty acid synthetase activity.

Manipulation of early nutrition is one of the feasible ways to modify growth and quality of chickens. Feeding high-fat diets for only 10 days after hatching resulted in a decrease in abdominal fat content at 63 days of age. It appears that early nutrition influences carcass composition of broilers at market-age.

1. INTRODUCTION

Considerable knowledge of avian lipid metabolism has accumulated during the last 30 years. Key aspects of lipid metabolism in avian species which are different from those of mammals are that lipoproteins synthesized in the intestinal epithelium are transported via the portal system, and that the liver, and not adipose tissue, is the main site of fatty acid synthesis (Annison, 1971; Leveille *et al.*, 1975).

Excessive fat deposition in rapidly growing broilers has been of great concern, due to the resultant tremendous financial loss to the broiler industry. Fat depot and adhering fat on edible meats are usually discarded or processed as a component of poultry by-products of low value. Most importantly, excessive fat in broiler meats conflicts with the preference of consumers for low-fat and low calorie meats.

The fatness in broilers might partly be a consequence of genetic improvement toward a large body size and/or an increase in feed intake, and could be manipulated by dietary,

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genetic, sex, age and environment factors (Lin *et al.*, 1980; Akiba *et al.*, 1986). Of nutritional factors, the manipulation by dietary protein and amino acid contents, energy:protein ratio and dietary protein sources has been greatly exploited, whereas effects of fat sources on the adiposity of broilers have been little studied apart from some work by Griffiths *et al.* (1977) and Alao and Balnave (1984). Fatty acid composition in abdominal fat tissue and edible meat is influenced by dietary fat sources. In recent years, n-3 polyunsaturated fatty acids (PUFA) have generated considerable interest in the field of human cardiovascular disease and feeding n-3 PUFA-rich oil has been reported to increase n-3 PUFA in carcass and edible meats of broilers (Hulan *et al.*, 1989).

The present paper described some aspects of fatness in broilers and manipulation of carcass composition, particularly fat content, by dietary fat sources.

II. FATNESS IN BROILERS

Along with the increase in growth rate of broilers, fatness has been emphasized. Abdominal fat content as % of body weight at market (8 weeks) rose by 0.07 and 0.11% a year for male and female chickens, respectively, over the last 25 years in Japan, suggesting the development of fatness in the latest strain of broilers (Akiba, 1988). Growth curves fitted with the Gompertz function of carcass lipid deposition in male broiler chickens subjected to feeding for 30 weeks with high protein (230 g/kg) or low protein (150 g/kg) diets which were formulated isoenergetically (13.4 MJ/kg) are illustrated in Figure 1. Carcass lipids were lower in chickens fed the high protein diet than in their counterparts on the low protein diet during 9 weeks posthatch and increased extensively beyond 4 weeks of age up to their asymptotes (1.17 kg for high protein diet and 1.55 kg for low protein diet). It is, therefore, suggested that carcass fatness in broilers, although substantial, is under dietary control.

Besides abdominal adipose tissue, considerable amounts of adipose tissue were detected on the thigh, neck, breast and back of broilers. The amount of fat in edible thigh and breast meats is, of course, crucial for consumer perceptions. The subcutaneous fat in both breast and thigh meats increased as chickens grew and amounted to approximately 45g (2.4% of body weight), indicating relatively large amounts of fat in the edible meats and, hence, the need to reduce them dietarily like that of the abdominal fat. We have observed significant positive correlationships between abdominal fat weight and subcutaneous fat weight in thigh or breast meats at marketing age of broilers (Akiba, 1992). Since it is confirmed that abdominal fat weight correlates with carcass fat weight, it seems likely that abdominal fat weight represents carcass fatness and fat contents in the edible meat of broiler chickens.

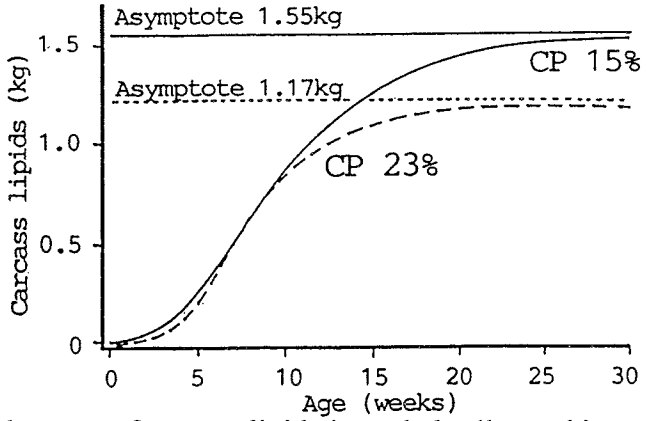

Figure 1. Growth curve of carcass lipids in male broilers subjected to high or low protein diet.

Accumulation of fat in animals is determined by hypertrophy and hyperplasia of adipose cells. Following the first report by Hood (1982), cellular growth of abdominal fat pad in broiler-type chickens has been reported (Cherry *et al.*, 1984). However, cellularity of adipose tissues of heavy-type broilers with much depot fat has been little studied. Cellularity of abdominal tissues during growth in heavy-type broiler chickens was determined using the method of Lavau *et al.* (1977). Abdominal fat weight averaged 75 g and 110 g at 8 and 10 weeks of age in both male and female chickens, respectively. As shown in Figure 2, the adipocyte number increased extensively until 6 (males) or 8 (females) weeks of age, followed by small increases thereafter. On the other hand, no significant increases in adipocyte volume were observed during the first 4 weeks after hatching, followed by drastic increases (Akiba, 1988). These growth patterns of adipocytes found in heavy-type broilers appear quite different from those reported by Hood (1982) who reported that adipocyte number increased until 14 weeks of age in light-type broilers. It is, therefore, likely that adipocyte hyperplasia proceeds extensively after hatching and attains a plateau at an early age in currently available heavy-type broilers.

Hepatic lipogenesis in chickens is inhibited by feeding unsaturated fatty acid-rich fats (Leveille *et al.*, 1975; Donaldson, 1985). Increasing fat content in isoenergetically formulated diets decreased carcass fat content in broiler chickens (Alao and Balnave, 1984). Adiposity of broilers has been reported to be modified by fat sources (Griffiths *et al.*, 1977; Alao and Balnave, 1984). Effects of dietary fat sources on performance, abdominal fat content and cellularity of abdominal adipose tissue were determined in broiler chickens in the following experiment. Arbor Acre broiler chicks were allocated into three treatments (two replicates of 110 males and 110 females) and raised for 8 weeks in floor pens in a windowless house with isoenergetic and isonitrogenous diets containing each of three fat sources at 36 - 50 g/kg, i.e. yellow grease (YG), corn oil (CO) and chicken oil (CH).

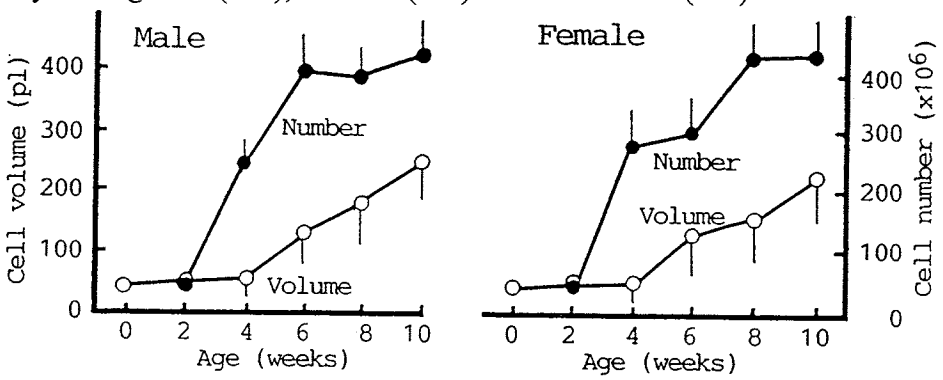

Figure 2. Cellularity of abdominal adipose tissue in broiler chickens.

III. FEEDING VARIOUS FAT SOURCES

Our data demonstrate that dietary fat sources have no significant influence on the performance of broiler chickens at 21 and 56 days of age (Table 1). This was in accord with the findings that CH and CO were utilized almost as efficiently as tallow in chickens (Griffiths *et al.*, 1977; Hulan *et al.*, 1984). Abdominal fat content at 56 days of age in chickens fed CH and CO tended to fall, but not significantly, when compared with YG. Pan *et al.* (1979) noted that abdominal fat was less in female broilers fed soybean oil than when fed tallow, corroborating our results. The descending trend in abdominal fat content by feeding CO and CH in the present experiment seemed to be mainly dependent upon decrease in the adipose cell volume in female chickens. Bourgeois *et al.*(1983) demonstrated in mice that feeding lard increased both cell size and cell volume in adipose tissue, while feeding

soybean oil increased only cell size. However, in chickens, less information is available on the effect of dietary fat sources on the growth of adipose cells.

Table 1. Effects of dietary fat sources on performance, abdominal fat content and cellularity of abdominal adipose tissue in 56 day old broilers.

Sex	Treatment	Body weight (kg)	Feed intake (kg)	Abdominal fat (g/kg BW)	Adipocytes volume (pl)	Adipocytes number ($x10^6$)
Male	YG	2.86	5.74	32	186	472
	CO	2.88	5.73	28	210	426
	CH	2.79	5.71	27	179	443
Female	YG	2.45	5.18	42	307^{a}	328
	CO	2.44	5.21	36	248^{b}	377
	CH	2.46	5.23	36	275^{ab}	357
	SEM	0.04	0.08	2.0	18	38

Abbreviations: YG, yellow grease; CO, corn oil; CH, chicken oil.
Means with different superscripts in columns are significantly different ($P<0.05$).

IV. FEEDING MEDIUM-CHAIN TRIGLYCERIDES

Medium-chain triglycerides (MCT) are composed of saturated fatty acid moieties of six to 12 carbon atoms and their effects on lipid metabolism have received considerable attention in recent years. MCT are metabolized quite differently from long-chain triacylglycerides (LCT) in that MCT are easily digested even when bile acids are in short supply. MCT are efficiently oxidized in tissues and increase lipolysis (Bach and Babayan, 1982). Geliebter *et al.* (1983) observed that feeding MCT to rats reduced carcass fat deposition. In the following experiments, digestibility and energy value of MCT in chickens and effects on fatness of broilers were evaluated.

The fat sources provided were yellow grease (YG) as a LCT source, and triacylglycerols with capric acid (C10-TG) and triacylglycerols with capric and caprylic acid (40:60, w/w, C10C8-TG) as MCT sources. Nitrogen-corrected true metabolizable energy (TMEn) and digestibility of the lipids were determined using the method of Murakami *et al.* (1994) in 3-day-old broiler chicks. Nitrogen-corrected apparent metabolizable energy (AMEn) of the fats was assayed in 3 week old male broiler chickens. The gross energies (GE) determined by bomb calorimetry of YG, C10-TG and C10C8-TG were 39.0, 35.7 and 35.1 MJ/kg, respectively. True digestibility of lipids and TMEn of C10-TG were not significantly different from those of YG as is shown in Table 2 (Akiba *et al.*, 1993). The AMEn and true digestibilities of C10-TG and C10C8-TG tended to be higher than those of YG in 3-week-old broilers. Thus, bioavailabilities of MCT are higher than those of LCT and the metabolizable energy values are almost comparable with those of LCT in chickens although GE values of MCT are less than those of LCT. This suggests that MCT can be utilized as a potential fat source in chickens.

Broiler chicks (1350 females) were allocated into 3 treatments with 3 replicates each and reared in an open-sided house for 8 weeks. C10C8-TG was provided as the MCT source and replaced 4% of YG in isoenergetic (13.2 MJ/kg) and isonitrogenous diets and the diets were fed for 4 or 8 weeks from one day of age. Body weight gains during 4 and 8 weeks

posthatch were reduced to a small extent by feeding C10C8-TG (Table 3). Feed conversion ratios for 4 and 8 weeks, and yields of edible meat were improved by feeding MCT. Feeding MCT reduced abdominal fat content at 4 and 8 weeks of age and subcutaneous fat content of

Table 2. TMEn, AMEn and true digestibility of the lipids of fat sources in 3 day old chicks and 3 week old broiler chickens.

Fat source	3 day old chicks		3 week old chickens	
	TMEn (MJ/kg)	True digestibility of lipids (%)	AMEn (MJ/kg)	True digestibility of lipids (%)
Yellow grease	39.3	94.6	32.0	94.0^{b}
C10-TG	38.5	96.3	32.0	98.3^{ab}
C10C8-TG	-	-	33.8	100.8^{a}
SEM	2.8	3.8	1.4	1.6

Means in columns with different superscripts are significantly different (P<0.05).

breast meat at 8 weeks (Table 3). These data show that MCT might be provided as an efficient fat source to improve efficiency and edible meat yields, and reduce fatness in broilers, even though it reduces body weight gain, probably because of its low palatability for young chicks. Harada *et al.* (unpublished data) observed that feeding MCT to broiler chickens reduced plasma lipoprotein concentration and lipoprotein lipase activity in adipose tissues although lipogenic activities in the hepatic tissue were higher in MCT-fed chickens. Takahashi *et al.* (1981) reported that lipolytic activity in adipose tissues was significantly higher in chicks fed coconut oil, rich in lauric acid (C12), than chicks fed lard. Thus, MCT modifies lipid metabolism and carcass composition of chickens.

Table 3. Effects of feeding medium-chain triglycerides (MCT) on performance, edible meat yield and fat deposition in 56 day old broiler male chickens.

Treatment	Feed intake (kg)	Body weight gain (kg)	Feed conversion (g:g)	Edible meat yield (kg)	Abdominal fat content (g/kg BW)	Subcutaneous fat content (mg/cm^2)
Control	5.54^{a}	2.50^{a}	2.22^{a}	0.89	36.9^{a}	220
MCT(0-4 wks)	5.32^{b}	2.43^{b}	2.19^{a}	0.91	30.9^{b}	169
MCT(0-8 wks)	5.26^{b}	2.44^{b}	2.15^{b}	0.90	32.0^{ab}	158^{b}

Means in columns with different superscripts are significantly different (P<0.05).

V. FEEDING POLYUNSATURATED FATTY ACIDS

Consumption of polyunsaturated fatty acids (PUFA) of the n-3 series, which are abundant in the lipids of fish, is of great interest in human nutrition in relation to their purported influence on coronary heart disease. Several attempts have been made to increase the n-3 PUFA content of chicken meats, as Hulan *et al.* (1989) demonstrated that n-3 PUFA contents in chicken meats were significantly increased by feeding higher levels of redfish meal or redfish oil. Dietary n-3 fatty acids inhibit not only metabolism of n-6 series fatty

acids but also the biosynthesis of prostaglandins which may have antilipolytic effects. It, therefore, seemed pertinent to find out the possibilities of modifying lipid metabolism by feeding n-6 or n-3 fatty acids to chickens.

Fourteen-day-old male broiler chickens were allocated into 3 groups with 4 replicates of 4 birds each. Safflower oil (rich in n-6 C18:2 acid), γ-linolenic acid-rich oil (rich in n-6 C18:3) or linseed oil (rich in n-3 C18:3) was supplemented at 60 g/kg in isoenergetic (13.7 MJ/kg) and isonitrogenous (220 gCP/kg) diets and the diets were fed for 14 days. No significant differences among treatments were observed in the abdominal fat content of chickens. Triacylglyceride concentrations in plasma and liver were lower in chickens fed linseed oil than in chickens fed safflower and γ-linolenic oil (Table 4). The decrease in triacylglycerides in chickens fed linseed oil appeared to be associated with a decrease in fatty acid synthetase activity in hepatic tissue. Hormone-sensitive lipase activities in the adipose tissues were not influenced by dietary PUFA sources in the present experiment. These findings are in harmony with Wong *et al.* (1984) that n-3 fatty acids reduced triacylglycerol production in cultured rat hepatocytes. Since feeding n-3 PUFA has been shown to increase n-3 fatty acid concentration in edible meats, it is presumed that the n-6/n-3 ratio in the muscle lipids in this experiment was improved by feeding linseed oil. Whether dietary fat sources, in particular PUFA, modify lipid metabolism in liver and adipose tissues and regulate prostaglandin production in chickens awaits further study.

Table 4. Effects of polyunsaturated fatty acids (PUFA) on lipid metabolism in liver and adipose tissue of growing broiler chickens.

PUFA source	Plasma triglyceride (mg/100ml)	Hepatic fatty acid synthetase (units/mg protein)	Adipose tissue	
			lipoprotein lipase (μEq FFA/mg protein)	hormone-sensitive lipase
Safflower oil	50^{a}	8.29^{a}	5.39^{b}	1.35
γ-Linolenic oil	42^{ab}	6.92^{ab}	7.30^{a}	1.40
Linseed oil	28^{b}	5.17^{b}	5.18^{b}	1.38

Means in columns with superscript letters are significantly different ($P<0.05$).

VI. FEEDING A HIGH FAT DIET DURING THE EARLY GROWTH PHASE

Growth and metabolism immediately after chickens hatch have attracted attention as nutritional density during the postnatal period (early nutrition) has been reported to modify the subsequent growth and metabolism of experimental rodents. The recent trend that broiler chickens have been selected toward precocity and, thereby, early marketing age, highlights the importance of growth and early nutrition in chickens. However, comparatively few detailed reports have been made on nutrition and metabolism during the first week after hatch in broiler chickens, apart from some work by Zelenka (1968) and Nitsan *et al.* (1991). We previously reported that metabolizability of dietary energy and digestive enzyme activities were low during 2 days posthatch and rise thereafter (Akiba *et al.*, 1990; Murakami *et al.*, 1992, 1994). Taking into account these metabolic characteristics in newly-hatched chicks, possible nutritional manipulation during the early growth phase to modify the growth and fatness of chickens is still under debate.

Table 5. Effects of dietary fat content during early growth phase (10 days posthatch) on performance and fatness in male broilers.

Dietary fat	Body weight (kg)		Abdominal fat content (g/kg)		Abdominal cellularity volume	Abdominal cellularity number
(% of total ME)	10day	63day	10day	63day	(pl)	(10^6)
2.6	0.19^{a}	3.09	7.1^{a}	29.7^{ab}	273	332^{a}
35.0	0.20^{a}	3.14	5.6^{ab}	32.1^{a}	262	358^{a}
62.2	0.16^{b}	3.06	4.2^{b}	26.4^{b}	263	300^{b}

Means in columns with different superscripts are significantly different ($P<0.05$).

In order to investigate the effects of early nutrition on growth and fatness of broilers, newly-hatched male broiler chicks were fed for 10 days with 3 kinds of diets which were formulated to include fat at 2.6, 35.0 and 62.2% of the metabolizable energy. Thereafter all birds were given the same diet up to 63 days of age (Akiba, 1988). Yellow grease was used as a major fat source. Body weights at 63 days of age were not different among the treatment groups, despite the lower body weights at 10 days of age in chicks fed the highest level of fat (Table 5). Abdominal fat content at 10 days of age (at the conclusion of feeding the experimental diets) declined with the increase in dietary fat content and the chicks fed the highest fat level (62.2%) maintained significantly lower abdominal fat even at 63 days of age. With regard to adipose cellularity at 63 days of age, while no significant differences were found in the cell volume, the cell numbers were significantly lower in chicks fed the highest fat diet for the first 10 days. These results may indicate that early nutrition, particularly dietary fat content, modifies carcass fatness in broiler chicks. Feeding high fat diets for 10 days posthatch reduces fat deposition at marketing age (63 days) with no detrimental effects on performance. Jensen *et al.* (1987) and Bartov *et al.* (1987) reported that growth and fat deposition of broilers at market weight were influenced to a small degree, or not modified, by changing early nutrition. A major feature of our study was that fat accounted for over 60% of dietary energy, in order to modulate nutritional status during the first 10 days. This may explain why in our study fatness at 63 days of age was influenced by early nutrition. Since feed intake for 1 week after hatch contributes less than 3% of total feed intake for 56 days, it is possible to manipulate nutritional status during the first week at low cost.

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THE EFFECT OF HOMOARGININE ON FEED INTAKE IN CHICKENS

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Dietary proteins, in which lysine is converted to homoarginine through guanidination, have been used for the measurement of endogenous amino acid secretions in animals fasted overnight and fed a single meal of diets containing guanidinated protein (G-protein) as the only source of protein (Schmitz *et al.*, 1991; Siriwan *et al.*, 1994). However, a marked reduction in feed intake has been reported in starter pigs fed G-proteins for three days (Imbeah *et al.*, 1994). In the present study, G-protein was fed to young chicks for an extended period to represent normal feeding conditions with the object of investigating the effects of G-protein and supplemental lysine on feed intake and growth performance.

Male broiler chicks were fed five semi-purified diets containing either casein or G-casein as the sole source of protein (CP, 230 g/kg) from days 6 to 13 post-hatching. A casein-based diet, balanced to meet recommended amino acid requirements, was used as the control (Diet 1). In Diets 2, 3, 4 and 5, casein was substituted by G-casein, and L-lysine was supplemented at 0, 5.6, 11.4 and 17 g/kg, respectively. Feed intake was recorded daily and birds weighed on days 6, 8,10 and 13 post-hatching. Plasma and kidney were collected for analyses of amino acids (including homoarginine) and kidney arginase activity, respectively at day 13 post-hatching. The results are shown in the Table.

	Feed intake (g/bird/day)	Weight gain (g/bird/day)	Plasma lys/arg ratio	Kidney arginase (μmole urea/mg prot/h)
Diet 1	29.1^{a}	19.5^{a}	2.85^{b}	70.6^{b}
Diet 2	6.3^{e}	-0.9^{e}	0.65^{c}	130.3^{ab}
Diet 3	12.7^{d}	4.4^{d}	0.84^{c}	139.1^{ab}
Diet 4	24.2^{b}	15.3^{b}	2.62^{b}	153.3^{a}
Diet 5	20.5^{c}	11.6^{c}	5.55^{a}	183.2^{a}

Means in the same column with different superscripts are significantly different ($P<0.05$).

Feed intake and weight gain of chicks fed the G-casein diet without added lysine (Diet 2) were markedly depressed, but this depression was largely overcome with increasing levels of supplemental lysine. Interestingly, the intake and gains of birds on Diet 4 (11.4 lysine/kg) were better than those on Diet 5 which had the same dietary lysine level as the control diet. The feed intake and growth of birds on Diet 4, however, were lower than those given the control casein diet. It appears that the excess lysine added to Diet 5 interfered with the plasma lysine/arginine balance since the observed ratio was substantially higher than those obtained for Diets 4 and 1. It has been suggested that absorbed homoarginine can be transformed to lysine by arginase in kidney and liver (Stevens and Bush, 1950) and it is possible that part of the homoarginine from the G-diets was transformed into lysine post-absorption. Arginase activity was found to be greater in the kidney of chickens fed G-casein diets. The data suggest that feed intake and weight gain are markedly depressed in chickens fed G-casein diets *ad libitum* and that both lys/arg imbalance and homoarginine *per se* are responsible for these adverse effects. Thus, an appropriate level of lysine supplementation is necessary to maintain feed intake if G-proteins are to be fed continuously.

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ENZYMES AND THE NUTRITIVE VALUE OF LUPINS

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Summary

Two enzymes (Biofeed Plus CT and Energex MG) were added at graded levels to lupin-containing diets. The first enzyme caused an increase in the calculated apparent metabolisable energy (AME) of the lupins from 10.01 to 11.65 MJ/kg DM but no improvement in bird performance. The second enzyme had no affect on bird performance or AME of the diet but it increased the solubilization of non-starch polysaccharides (NSP) in the ileum which resulted in an increase in ileal digesta viscosity.

I. INTRODUCTION

Lupins are used as a protein source in broiler diets. There is anecdotal evidence, however, that even at moderate levels of inclusion (>10%) performance suffers. Lupins contain a-galactoside oligosaccharides (7-8%) but there is little data on their nutritive effects. The non-starch polysaccharides (NSP) of cereals are known to be anti-nutritive in some cases and lupins contain NSP consisting of a pectin-like main chain (containing rhamnose and galacturonic acid) with extended side chains of galactose and arabinose. Such branched structures are different from the cereal NSP which are more linear molecules. It has not been shown whether the NSP of lupins or other legumes possess anti-nutritive activity.

One experimental approach used to demonstrate the anti-nutritive activity of NSP is to destroy the activity of the NSP by using glycanase enzymes added to the feed. Brenes *et al.* (1993) found that adding a number of enzymes to a lupin/maize diet improved the weight gain of broilers between the age of 7 and 21 days but, interestingly, the enzyme with the most protease acitivity was the most effective.

This paper describes a study in which two enzyme preparations (Biofeed Plus CT and Energex MG) were added to lupin-containing diets to determine their effects on energy metabolisability and ileal NSP levels.

II. MATERIALS AND METHODS

(a) Experimental diets, bird husbandry,and AME trials.

Two identical AME feeding trials were carried out in succession to examine the efficacy of the feed enzymes Biofeed Plus CT (Enzyme A with hemicellulase, xylanase, pentosanase and cellulase activities) and Energex MG (Enzyme B with pectinase, hemicellulase, β-glucanase and endo-glucanase activities) (kindly supplied by Novo Nordisk, Denmark). In each trial seven experimental diets were formulated (Table 1). In both trials the *Lupinus angustifolius* var. Gungarru and sorghum came from the same batches. Diets 1 and 2 were controls to test the effects of enzyme on the basal ingredients and to allow the calculation of the direct effects of enzymes on the lupin.

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Table1. Composition of Diets.

Ingredient (g/kg)	Diets						
	1	2	3	4	5	6	7
Sorghum	800	800	543	543	543	543	543
Casein	134	134	91	91	91	91	91
De-hulled lupin	-	-	300	300	300	300	300
Vitamin and mineral mix	46	46	46	46	46	46	46
Marker	20	20	20	20	20	20	20
Enzyme (g added per kg)	-	1.0	-	0.25	0.5	0.75	1.0

Commercial broiler chickens (Ingham TM98) were raised on commercial starter (to 21 d) and finisher feeds (to 24 d) prior to the classical AME bioassay (total collection). The birds were allocated to diets in groups of 5 in metaobolism cages. There were 6 replicates for Diet 1 (AME basal diet) and seven replicates for each of the other diets. Experimental diets were fed for 7 days. The first 3 days enabled the chickens to adapt to cages and feed. The last 4 days was a collection period during which all excreta were pooled and feed intake was monitored. The gross energies of the diets and the excreta were determined by bomb calorimetry and the AME of each diet was calculated. The AME of the lupins was calculated assuming an additive model (Annison *et al.*, 1994). Bird weight was also monitored over the trial period.

(b) Ileal viscosity and NSP determinations

After the final collection of excreta, birds from each replicate (2 birds in Trial 1 and 3 birds in Trial 2) were killed by injection with pentobarbitone. Ileal digesta between the vitelline diverticulum and 4cm above the ileo-ceacal junction were removed by gentle extrusion. Samples from each replicate were pooled and stored on ice for analysis. Ileal contents were centrifuged (10,000*g*, 15 min) and the viscosity of the supernatants detemined (0.5 mL aliquot) using a Brookfield viscometer equipped with a cone and plate head. Ethanol (4mL) was added to a further aliquot to preciptate polysaccharides. The precipitate was washed (2 x 2mL of 80% ethanol) and dried (acetone, 2mL). The residue was treated with H_2SO_4 (12M, 35°C, 1 h; 1M, 100°C, 2 h) and the released sugars were derivatised and quantified by GLC as the alditol acetates.

III. RESULTS

The effects of adding Enzyme A and Enzyme B to the diets are shown in Tables 1, 2 and 3.

In the first experiment Enzyme A caused a small but significant improvement in feed efficiency and AME of the basal sorghum/casein diet. Growth was unaffected. Birds fed the lupin diets grew as well as those fed the basal diets. The AME of the lupin diet without enzyme supplementation was significantly ($P<0.05$) lower than the basal diets. Enzyme A significantly ($P<0.05$) increased the AME of the lupin diets with maximum effect achieved at 0.5g/kg (Diet 5-7) and over. In these diets the AME of the lupins was calculated to have increased to >11.50MJ/kg DM from a value of 10.01MJ/kg DM in the lupin control diet (Diet 3). The ileal digesta of birds fed the basal diets contained low levels of soluble NSP and were not viscous. The inclusion of lupins resulted in higher levels of NSP and a greater viscosity. Enzyme A caused a significant decrease in ileal viscosity.

Table 2. Effect of Enzyme A on the AME values of the basal and lupin-containing diets and on bird performance (means ± S.E.).

Diet	Enz. g/kg	Wt gain (g/bird)	FCR	AME_{diet} (MJ/kg DM)	AME_{lupin} (MJ/kg DM)
1. Basal	0.00	1015 ± 29	2.15 ± 0.05^{a}	15.35 ± 0.13^{b}	-
2. Basal	1.00	998 ± 45	1.96 ± 0.03^{c}	15.59 ± 0.06^{a}	-
3. Lupin	0.00	995 ± 36	2.05 ± 0.07^{bc}	13.40 ± 0.08^{e}	10.01 ± 0.28^{a}
4. Lupin	0.25	1032 ± 28	2.12 ± 0.08^{ab}	13.71 ± 0.22^{d}	11.05 ± 0.72^{b}
5. Lupin	0.50	1006 ± 54	2.06 ± 0.06^{ab}	13.89 ± 0.13^{c}	11.65 ± 0.44^{c}
6. Lupin	0.75	1027 ± 31	2.06 ± 0.06^{abc}	13.90 ± 0.08^{c}	11.65 ± 0.26^{c}
7. Lupin	1.00	993 ± 45	2.05 ± 0.14^{abc}	13.85 ± 0.17^{cd}	11.50 ± 0.58^{ab}

^{a,b,c} Values with unlike superscrripts differ significantly ($P<0.05$).

Enzyme B had no effect on the performance of birds fed either the basal diet or the lupin diets. Enzyme B also did not affect the AME of the basal diet and had no major effect on the AME of the lupin diets, although a small, significant depression in AME values was detected in birds fed Diet 4 (Enzyme B, 0.25g/kg). Enzyme B caused a significant increase in the levels of NSP in the ileal digesta and substantial increases in digesta viscosity.

Table 3. Effect of Enzyme B on the AME values of the basal and lupin-containing diets and on bird performance (means ± S.E).

Diet	Enz. g/kg	Wt. gain (g/bird)	FCR	AME_{diet} (MJ/kg DM)	AME_{lupin} (MJ/kg DM)
1. Basal	0.00	710 ± 16	2.06 ± 0.13	15.37 ± 0.11^{a}	-
2. Basal	1.00	704 ± 16	1.98 ± 0.21	15.44 ± 0.11^{a}	-
3. Lupin	0.00	716 ± 30	1.99 ± 0.21	13.32 ± 0.12^{bc}	9.68 ± 0.39^{ab}
4. Lupin	0.25	735 ± 37	2.04 ± 0.18	13.13 ± 0.23^{c}	9.04 ± 0.79^{b}
5. Lupin	0.50	714 ± 19	1.92 ± 0.08	13.34 ± 0.20^{b}	9.75 ± 0.67^{ab}
6. Lupin	0.75	728 ± 35	1.94 ± 0.13	13.38 ± 0.23^{b}	9.88 ± 0.79^{a}
7. Lupin	1.00	716 ± 23	2.00 ± 0.15	13.42 ± 0.19^{b}	10.03 ± 0.63^{a}

^{a,b,c} Values with unlike superscripts differ significantly ($P<0.05$).

Table 4. Ileal digesta NSP levels in birds fed basal and lupin diets (means ± S.E).

		Trial 1 (Enzyme A)		Trial 2 (Enzyme B)	
Diet	Enz. g/kg	Ileal soluble NSP (mg/mL)	Ileal vis. (mPa.s)	Ileal soluble NSP (mg/mL)	Ileal vis. (mPa.s)
1. Basal	0.00	4.76 ± 0.25^{a}	3.3 ± 0.4^{a}	6.40 ± 0.32^{a}	2.9 ± 0.3^{a}
2. Basal	1.00	4.44 ± 0.35^{a}	2.4 ± 0.4^{a}	6.53 ± 0.35^{a}	2.8 ± 0.3^{a}
3. Lupin	0.00	16.90 ± 1.37^{b}	9.5 ± 5.6^{c}	19.21 ± 1.54^{b}	11.4 ± 6.3^{a}
4. Lupin	0.25	15.49 ± 0.61^{b}	6.8 ± 2.0^{b}	28.78 ± 0.95^{c}	31.3 ± 8.0^{b}
5. Lupin	0.50	14.70 ± 0.94^{b}	5.2 ± 1.2^{ab}	31.58 ± 0.31^{cd}	30.9 ± 11.6^{b}
6. Lupin	0.75	14.97 ± 1.06^{b}	4.8 ± 0.6^{ab}	34.76 ± 1.66^{de}	33.1 ± 14.9^{b}
7. Lupin	1.00	14.80 ± 0.49^{b}	4.4 ± 0.8^{ab}	35.77 ± 1.35^{e}	34.2 ± 11.0^{b}

^{a,b,c} Values with unlike superscripts differ significantly ($P<0.05$).

IV. DISCUSSION

The broiler chickens grew less well in the second experiment compared to the first. The reasons for this are unclear as the chickens were from the same stock and hatchery. Nevertheless, the data from the trials are probably comparable as there was good agreement in the AME of the control diets (Diets 1 and 3 in each trial).

These trials were designed to show the effects of the enzymes on the lupin component of the diet only. Thus, the basal diets were composed of sorghum and casein which are both highly digestible. Therefore, it is interesting to note that Enzyme A had a small but significant effect on the AME of the basal diet. It is possible that Enzyme A cleaves the arabinoxylan polysaccharide from the sorghum although if this is the case it is not evident from the ileal NSP data.

Enzyme A increased the AME of the lupin diets and, as the effects on the basal components are minimal, the improvement in AME appears to result from action directly on the lupins. Considerable amounts of lupin NSP become soluble when ingested by the chicken, as shown by the increase in the ileal soluble NSP levels. The level of the soluble NSP in the ileal digesta of the birds fed the lupin diets appeared unaffected by Enzyme A but the drop in ileal digesta viscosity indicates that at least part of the NSP is cleaved by the glycanase activity of Enzyme A. The results of the second trial suggest that this is not part of the mechanism of action in the improvement of the AME of the lupins.

Enzyme B (Energex MG) had no effects on bird performance, or on the AME value of the diets or lupins. There was, however, a great increase in ileal digesta viscosity which was caused by a substantial release of lupin NSP into the aqueous phase. Enzyme B possibly attacks part of the cell wall to which the NSP bonds, or the bond itself to release, the NSP. The NSP had high levels of galactose (data not reported) which confirms that the NSP are from the lupins which had high levels of a galactan-containing NSP. Enzyme B had no additional glycanase activities to cleave the polysaccharide and thus the viscosity of the digesta is increased.

Previously it was thought that increases in the viscosity of intestinal digesta results in a depression of nutrient digestibility, which in this study did not occur. Thus, viscosity may not be the sole property of NSP to contribute to its anti-nutritive activity. The viscous cereal polysaccharides (arabinoxylans and β-glucans) may have other nutritionally important activities (ie surface active properties, ion binding) which the lupin polysaccharides do not share.

This study has demonstrated that the nutritive value of lupins can be improved by addition of enzymes to diets. Similar results have been reported previously (Bryden *et al.*, 1984). The mechanism of improvement remains unclear. Correct targeting of enzymes against substrates is essential as not all enzymes have the same effects and indeed some can have effects opposite to those predicted.

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CONDITIONED IMMUNOSUPPRESSION IN CHICKENS

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There is growing interest in the interactions between the central nervous system (CNS) and the immune system which may occur through the direct innervation of lymphoid compartments, by paracrine means through the release of mediators from nerves situated in close proximity to lymphocytes, or by neuroendocrine signals in the form of hypothalamic, pituitary and peripheral endocrine hormones. One of the most interesting examples of CNS-immune interactions is the demonstration of conditioned immunomodulation using Pavlovian conditioning techniques. Pavlovian conditioning is a familiar concept, and in general terms consists of the presentation of a neutral sensory stimulus (the conditioned stimulus) paired with an event or substance which provokes a physiological response (termed the unconditioned stimulus) (Pavlov, 1927). On subsequent exposure to the conditioned stimulus alone the physiological response to the unconditioned stimulus is re-enlisted. We have previously shown in rats (Husband *et al.*, 1993) that if the stimulus has immunomodulatory properties, the immune changes can be re-enlisted as a conditioned response after stimulus re-exposure. The aim of the present study was to determine if taste aversion conditioning is effective in enlisting conditioned immunosuppression in chickens.

Preliminary experiments indicated that four-week old chickens were able to discriminate between saccharin and water, showing a preference for a dilute saccharin solution over water on first exposure but avoiding saccharin on re-exposure. Thus, saccharin was used as the conditioned stimulus and cyclophosphamide was used as an immunosuppresive agent. Twenty-four chickens were habituated to a water deprivation schedule over a 7 day period (3 h water deprivation after which two bowls were offered and intake recorded over a 10 min period). At the commencement of the trial they were randomly allocated to four groups in which the birds received the appropriate conditioned stimulus, water or saccharin paired with either an injection of cyclopospham ide or a sham injection with saline. Conditioned birds were given cyclophosphamide paired with saccharin and control birds were injected with saline. Ten days later they were re-exposed to saccharin or water respectively. White cell counts (WCC) were performed in blood samples taken on Day 0 and at 24 h after conditioning (Day 8), and test or re-exposure day (Day 18). The results indicated that conditioned birds ingested less saccharin on re-exposure (i.e. taste aversion behaviour). They also displayed a 30% depression in WCC relative to the control group. Differences between conditioned and control groups were significant ($P < 0.001$).

This study demonstrates that taste aversion conditioning can be used to re-enlist immunosuppression in chickens. This paradigm will form the basis for more thorough investigations of conditioned immunological responses in this species, including immunostimulation.

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PERFORMANCE OF AUSTRALORP CHICKENS CHOICE FED CASSAVA, SWEET POTATO AND MAIZE AS ENERGY FEED SOURCES IN VILLAGE CONDITIONS

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The Australorp chicken is a dual-purpose bird which was introduced into, and has been promoted in, Papua New Guinea since the 1960s as a "village-type" chicken. However, recent studies (Bakau and Colombus, 1992) have indicated that the bird has problems in adapting to village conditions, often being 42 to 53 wk old at first egg. The underlying cause of such poor performance is an inadequacy of feed sources, particularly energy, in village conditions.

Five farmers, one each from five different villages, were provided with twenty 8-week old Australorp pullets. The first farmer was supplied with commercially-formulated pullet rations (pullet developer and layer crumbles) and was advised to rear them according to commercial practice. The second, third, fourth and fifth farmers were advised to offer their birds cassava, sweet potato, maize or a mixture of the three ingredients as feed energy sources, respectively, in addition to a 350 g crude protein/kg concentrate supplement. The results of the study are shown in the Table (Mean value ± SE).

Farmer/ Treatment	Initial weight (g)	Weight gain (g) after					Pelvic gap (mm)			Point of lay
		12 wk	16 wk	20 wk	22 wk	24 wk	16 wk	22 wk	24 wk	(wk)
Commercial	650	316	670	950	1086	1161	11.3	23.5	24.0	24
		(60.1)	(68.1)	(72.1)	(73.1)	(81.0)	(0.81)	(1.46)	(0.89)	
Cassava	350	100	666	660	930	1054	11.1	12.6	20.4	25
		(6.0)	(74.9)	(74.9)	(50.3)	(64.7)	(0.52)	(0.67)	(1.22)	
Sweet potato	380	150	723	855	1000	933	11.9	15.6	20.4	22
		(50.0)	(108.9)	(67.9)	(51.6)	(67.3)	(0.57)	(1.67)	(2.11)	
Maize	660	180	620	619	686	680	12.0	13.4	21.4	26
		(37.4)	(91.5)	(91.2)	(60.1)	(62.1)	(1.24)	(0.47)	(1.53)	
Cassava + Maize + Sweet potato	360	200	606	660	976	86.5	11.4	13.3	18.7	28
		(57.7)	(72.2)	(96.9)	(40.8)	(35.5)	(0.47)	(1.04)	(0.83)	

All birds except those offered commercial rations experienced a slight drop in growth rate upon switching to the test diets. They recovered quickly and grew and matured at a similar rate, as confirmed by less variation in distance between the pelvic points and reduced variation in the point-of-lay ages between the birds managed by each farmer.

These data indicate that the growth performance of village chickens can be improved significantly by improving their nutrition. This can be achieved by utilizing some of the locally grown energy feeds and allowing birds to select nutrients according to their needs.

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EFFECTS OF SOLID SIDES IN CAGES ON THE WELFARE OF LAYING HENS

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The effects of solid sides in cages on stress physiology and condition of Tegel Tint hens were examined in a factorial experiment. The 4 factors were cage sides (open or solid {1.5 mm grey polypropylene replaced the mesh cage sides}), tier (upper or lower), hens (1 or 2/cage) and age (35 and 60 weeks). At each of the 2 age periods 6 cages were sampled, giving a total of 48 cages/factor. The cages measured 32 x 47 x 43 cm (w x d x h) and were in banks of 6 cages in two back-to-back rows of 72 cages/row. Each bank was randomly allocated to position within a tier and was comprised of one treatment. All experimental cages had neighbouring cages of a similar treatment. Overall results of the effects of solid sides and hens/cage on stress physiology and condition are in the Table.

Parameter	TREATMENT				
	SIDES		NUMBER		LSD
	Open	Solid	1	2	(P=0.05)
B 'at rest'[1,2]	-0.02[b]	-0.99[a]	-0.32[b]	-0.87[a]	0.51
B response to ACTH[1]	65.78	72.74	73.06	65.46	10.54
Immunological response[3]	66.5	183.1	181.7	168.0	29.1
H/L ratio[4]	1.66[b]	1.36[a]	1.52	1.50	0.28
Total plumage score[5]	3.18[a]	3.33[b]	3.35[b]	3.16[a]	0.11
Claw length (mm)	25.1	26.0	25.7	25.4	1.1

[1]B = corticosterone concentration ($nmol^{-1}$); [2]log_e transformed data; [3]% increase in wattle thickness 24 h post-injection of a kidney bean extract; [4]H/L = heterophil to lymphocyte;[5]4-point scale (1 = poor, 4 = good condition/cover); [ab]different letters denote a within factor difference ($P<0.05$).

Feather condition was improved in cages with solid sides, predominantly due to better condition of the tail and surround with no differences between other areas. There was evidence of a reduced level of stress in cages with solid sides, based on lower corticosterone concentrations (P=0.003) and H/L ratios (P=0.04) and an increased cell mediated immunological response (P=0.27). Corticosterone concentrations were higher and feather condition was better in 1-bird cages, as previously observed (Barnett, 1994).

Solid sides in cages may improve welfare by decreasing the level of stress, perhaps by modifying social behaviour and they also improve feather condition of the tail and its surrounds. However, data from another experiment indicated increased mortality in hot weather in a solid sides treatment in a naturally ventilated shed (unpublished).

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FACTORS THAT IMPROVE EGGSHELL QUALITY: EFFECT ON EGGSHELL ULTRASTRUCTURE.

C.E. BRACKPOOL and J.R. ROBERTS

Summary

This study examined the effects of six factors, reported to improve eggshell quality, on the ultrastructure of eggshells. The results indicate that only some of the factors significantly improved eggshell quality and these supposedly beneficial factors had varying effects on the ultrastructure of eggshells. Further work is required to gain a better understanding of the relationship between factors that improve eggshell quality and eggshell ultrastructure.

I. INTRODUCTION

Eggshell quality is an important aspect of egg industries throughout the world. The present study investigated the effect of some factors reported to improve eggshell quality on the ultrastructure of eggshells. Solomon (1991) identified and described several abnormalities found in the mammillary region of eggshells. Further work by Solomon and co-workers has attempted to classify some of these features in terms of their function in the eggshell. Stress analysis of the eggshell revealed that the mammillary knobs on the innermost surface of the eggshell provide the sites for crack initiation (Bain, 1992). However, catastrophic failure of the eggshell does not result unless one or more of the minor cracks which radiate out from the crack initiation site become unstable and propagate out from the site of trauma (Bain, 1992). Ultrastructural features that increase the shell's resistance to crack propagation, and thus improve the shell strength, include early fusion, cuffing and confluent mammillae (Bain, 1992). The beneficial property of the first two features is that they reduce the inter-mammillary spaces while confluence increases the contact between the calcified shell and the outer shell membrane. At the same time these features also increase the eggshell's resistance to bacterial penetration (Solomon *et al.*, 1994). Confluent mammillae have also been demonstrated previously in the eggshells of stressed birds and slab-sided eggs (Solomon, 1991). Late fusion and aragonite tend to open up the framework of the mammillary layer, whereas alignment of the mammillae creates long grooves, all of which reduce the shell's resistance to crack propagation (Bain, 1992), and bacterial penetration (Solomon *et al.*, 1994). Cubics and changed membrane are other ultrastructural features that reduce an eggshell's resistance to bacterial penetration (Solomon *et al.*, 1994) and have been associated with poor quality eggshells (Watt, 1989).

II. MATERIALS AND METHODS

Eggshells were collected from six trials conducted to examine the effect of factors which have been reported to improve eggshell quality, upon the eggshell ultrastructure. The first trial, in collaboration with Mr. R. Hughes at the South Australian Department of Agriculture, examined the possibility that the inclusion of sodium bicarbonate in the diet alleviates the deleterious effect of high dietary phosphorus on eggshell quality. The second trial, also in collaboration with Mr. R. Hughes, examined the provision of limestone chips

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as varying percentages of the dietary calcium supplementation. The third trial, in collaboration with Dr. R.B. Cumming at the University of New England, investigated the effect of choice feeding of laying hens. Trial 4, in collaboration with Dr. P. Glatz and Mr. R. Hughes, investigated the possibility that cool drinking water alleviates the deleterious effects of heat stress on eggshell quality. The fifth trial examined the effect of an induced moult on birds nearing the end of their first year of lay, on eggshell quality and ultrastructure. Moult was induced by feeding the hens whole grain barley and was conducted at the poultry farm at the University of New England. In Trial 6, the possibility that zinc supplementation in the drinking water can alleviate the deleterious effects of saline drinking water on eggshell quality was investigated, in collaboration with Associate Professor D. Balnave at the University of Sydney. All ultrastructural examinations were conducted on equatorial pieces of eggshell that had been prepared for viewing under a JEOL JSM35 Scanning Electron Microscope. Ultrastructural observations were compared by contingency table analysis using X^2 test. Values for the traditional eggshell measurements were compared by analysis of variance. For all statistical analyses significance was assumed at $P<0.05$.

III. RESULTS

The results are summarised in Table 1. In the present study high levels of phosphorus or the inclusion of sodium bicarbonate in diets high in phosphorus, and the provision of limestone chips as the calcium supplement in the diet, had no significant effects on the traditional measures of eggshell quality. Of the two strains of birds examined in the choice feeding trial, only one strain produced heavier and thicker shells and that was when the birds were 60 weeks of age. Cool drinking water (5°C and 10°C) offset the deleterious effects of hot temperatures on eggshell quality. Birds receiving the cool drinking water produced eggs with significantly thicker shells and higher shell weight to egg weight ratios. Interestingly, birds receiving drinking water at 17°C produced the poorest quality eggshells. Inducing a moult in birds nearing the end of their first year of lay resulted in an improvement in eggshell quality. After the moult the eggs had significantly heavier and thicker shells, higher shell weight to egg weight ratios and a greater shell weight per unit of egg surface area. For the zinc supplementation trials, no differences in the traditional parameters of eggshell quality were recorded although shell breaking strength tended to be lower in the group receiving the saline drinking water (Balnave and Zhang, 1993).

The ultrastructure of eggshells was not significantly affected by high levels of phosphorus or the inclusion of sodium bicarbonate in high phosphorus diets and the provision of limestone chips as the calcium supplement in the diet. The eggshells of birds in which choice feeding had improved the shell quality had a high incidence of changed membrane. The size of the mammillary caps was also noted to be variable. Heat stressed birds receiving cool drinking water (5°C and 10°C) produced eggs with less early fusion. A positive correlation existed between drinking water temperature and the percentage of shells containing aragonite. Eggshells from birds receiving 5°C drinking water contained no aragonite whereas 7%, 29% and 36% of shells from birds receiving 10°C, 17°C and 30°C, respectively, had an isolated incidence of aragonite. Ultrastructurally, birds after an induced moult produced shells with significantly less alignment. $ZnSO_4$ in saline drinking water had no effect on eggshell ultrastructure, whereas Zn-EDTA and Zn-methionine had varying effects on the ultrastructure of eggshells. Zn-EDTA in saline drinking water

Table 1. Significant effects on traditional shell quality parameters and on eggshell ultrastructural features.

Trial	Traditional shell quality measurements		Ultrastructural features	
	Improvement	Deterioration	Beneficial	Adverse
High phosphorus + bicarbonate	-	-	-	-
Limestone chips	-	-	-	-
Choice Fed - BB @ 50 wks	-	-	↓ changed membrane	-
BB @ 60 wks	↑ shell weight ↑ shell thickness	-	-	↑ changed membrane ↑ variability of MCS
BB @ 70 wks	-	-	-	-
BW @ 50 wks	-	-	-	-
BW @ 60 wks	-	-	↓ changed membrane	-
BW @ 70 wks				
Heat stress + 5°C water	↑ shell thickness ↑ % shell ↑ shell wt/SA	-	↓ aragonite	↓ early fusion
10°C water	↑ shell thickness ↑ % shell ↑ shell wt/SA	-	-	↓ early fusion
17°C water	-	↓ shell thickness ↓ % shell ↓ shell wt/SA	↑ early fusion	-
30°C water	-	↓ shell wt/SA	↑ early fusion	↑ aragonite
Induced Moult	↑ shell weight ↑ shell thickness ↑ % shell ↑ shell wt/SA	-	↓ alignment	-
Saline drinking water + $ZnSO_4$	-	-	-	-
Zn-EDTA	-	-	↑ cuffing ↓ late fusion	↑ changed membrane ↑ cubics
Zn-Methionine	-	-	↓ changed membrane ↑ confluence	↓ cuffing ↑ cubics

BB-Baiada Black; BW-Baiada White; MCS-mammillary cap size; SA-surface area

resulted in birds producing eggshells that had an increase in cuffing, cubics and changed membrane and a reduction in the amount of late fusion.

IV. DISCUSSION

The relationship between factors reported to be beneficial to the quality of eggshells and eggshell ultrastructure is not a simple one. From the results of this study it would appear that some factors reported to improve eggshell quality did not significantly affect eggshell quality and eggshell ultrastructure (bicarbonate and limestone chips). Choice feeding of laying hens resulted in some improvement of eggshell quality. However, ultrastructurally the eggshells had a higher incidence of changed membrane, a feature that is thought to have an adverse affect on eggshell quality. In contrast, eggshells after birds were moulted had less alignment, a feature that is adverse to eggshell function, and the overall eggshell quality was improved. Providing cool drinking water during heat stress improved eggshell quality. Ultrastructurally the eggshells had a reduction in the incidence of aragonite (a feature thought to be detrimental to eggshell quality) but a lower incidence of early fusion (a feature thought to be beneficial to shell strength). Zinc supplementation in the drinking water had no effect on shell quality parameters and, depending on the zinc compound, had varying effects on the ultrastructure of eggshells. However, the zinc supplementation trials were conducted on old hens and it is possible the adverse effect of age on eggshell ultrastructure (Brackpool *et al.*, 1993) masked the effects of zinc.

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ROLE OF ALBUMEN QUALITY IN EARLY EMBRYONIC DEVELOPMENT AND HATCHABILITY

J. BRAKE, C. E. BENTON, JR. and T. J. WALSH

Summary

There appear to be certain obligatory changes in albumen quality of hatching eggs during preincubation storage. An increase in pH appears to develop a necessary trans-vitelline membrane potential and a decrease in albumen height removes a barrier to gaseous diffusion. Initiating incubation before these changes occur increases the probability of early embryonic death. These changes appear to occur independent of weight loss. There are significant strain and age effects on albumen quality which probably govern the rate at which these obligatory preincubational changes occur.

I. INTRODUCTION

There is considerable evidence that a certain amount of preincubation egg storage is beneficial but excessively long storage results in decreased hatchability. Asmundson and MacIlraith (1948) found that turkey eggs stored for 4 or 8 days hatched better than those set fresh without storage. Hodgetts (1988) indicated that the minimum storage time for chicken eggs was 2 days. This effect appears to be temperature dependent as Kirk *et al.* (1980) found that chicken eggs stored for 2 days hatched better when held at 18°C than at 15°C while El Jack and Kaltofen (1969) found that hatchability of chicken eggs stored for 3 days at 15°C was significantly lower than that of eggs held the same time at 29.5°C or at 32°C. Conversely, chicken eggs that were stored for 8 days at 15°C hatched better than eggs stored at 18°C (Kirk *et al.*, 1980). As Fasenko *et al.* (1994) found no turkey embryo development below 17.4°C, it is unlikely that all of the above temperature effects can be fully explained on the basis of changes in the embryo during storage. However, changes in albumen quality are very temperature dependent within these ranges (Walsh, 1993). Thus, a study of how egg albumen could influence embryo development and hatchability was undertaken.

II. METHODS

In Experiment 1 broiler hatching eggs from a 43-week-old flock were incubated after being stored at 18°C and 75% RH for 0, 4, 8, or 12 days. At 2, 24, 48, and 66 hours of incubation, eggs were removed from the incubator, weighed and broken open for determination of thick albumen height and pH and weight loss.

In Experiment 2 broiler hatching eggs from a 30 (young) or 50 (old) week old flock were incubated after being stored at 18°C and 75% RH for 0 (fresh) or 5 (stored) days. Egg weight loss, albumen height and pH were determined as for Experiment 1.

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In Experiment 3 broiler hatching eggs from virgin or artificially inseminated hens were stored for 4 days at 18°C and 75% RH. Eggs were broken open daily for determination of albumen height and pH. This experiment was replicated three times.

In Experiment 4 eggs were taken from six commercial strains of broiler breeders housed and fed together at 47 weeks of age. The eggs were opened and albumen height measured either on the day of oviposition or after 4 days of storage at 18°C and 40% RH.

In Experiment 5 two of the commercial strains used in Experiment 3 were further evaluated at 40, 53, and 64 weeks of age after 5 days of storage at 18°C and 70% RH.

III. RESULTS AND DISCUSSION

Albumen height declined during the first 24 hours of incubation to a level equivalent to that achieved during the first 4 days of storage regardless of the age of the flock (Figures 1 and 2). The layer of thick albumen has been identified as a significant barrier to gaseous diffusion during the early stages of incubation (Meuer and Baumann, 1988). This would be expected to impede oxygen availability to the rapidly dividing embryo. Albumen liquefaction probably liberates numerous nutrients essential to embryo development. Glucose is abundant in albumen (Burley and Vadehra, 1989) and has been shown to be a substitute for albumen in early embryo culture (Spratt, 1948). An overly viscous albumen may impede the movement of nutrients such as glucose out of the albumen and across the vitelline membrane. Albumen height of the fresh eggs from the old flock (Figure 2) was initially lower than that of the young flock which might explain the common field observation that eggs from older flocks can be set sooner after lay.

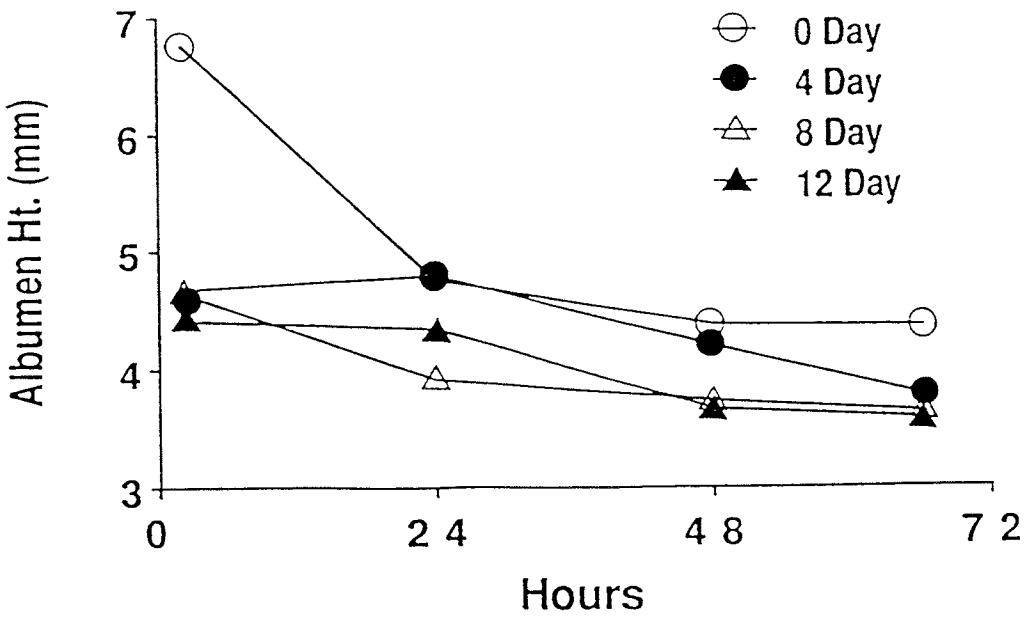

Figure 1. Albumen height of eggs stored 0, 4, 8, or 12 days during the first 66 h of incubation in Experiment 1. SE ranges are within symbols.

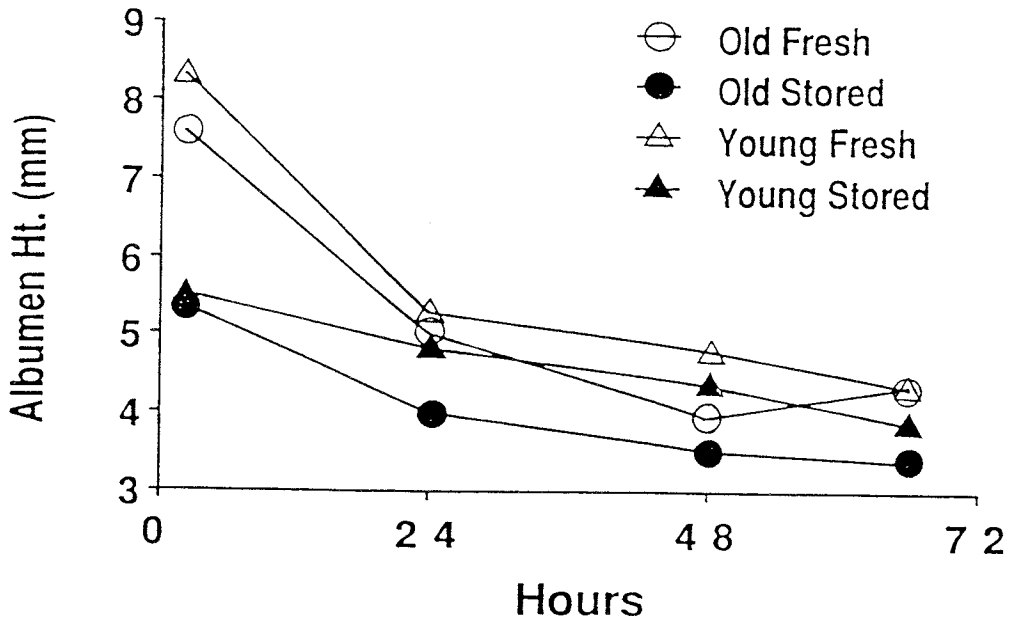

Figure 2. Albumen height of eggs from 30 (young) or 50 (old) week old hens set after 0 (fresh) or 5 (stored) days of storage in Experiment 2. SE ranges are within symbols.

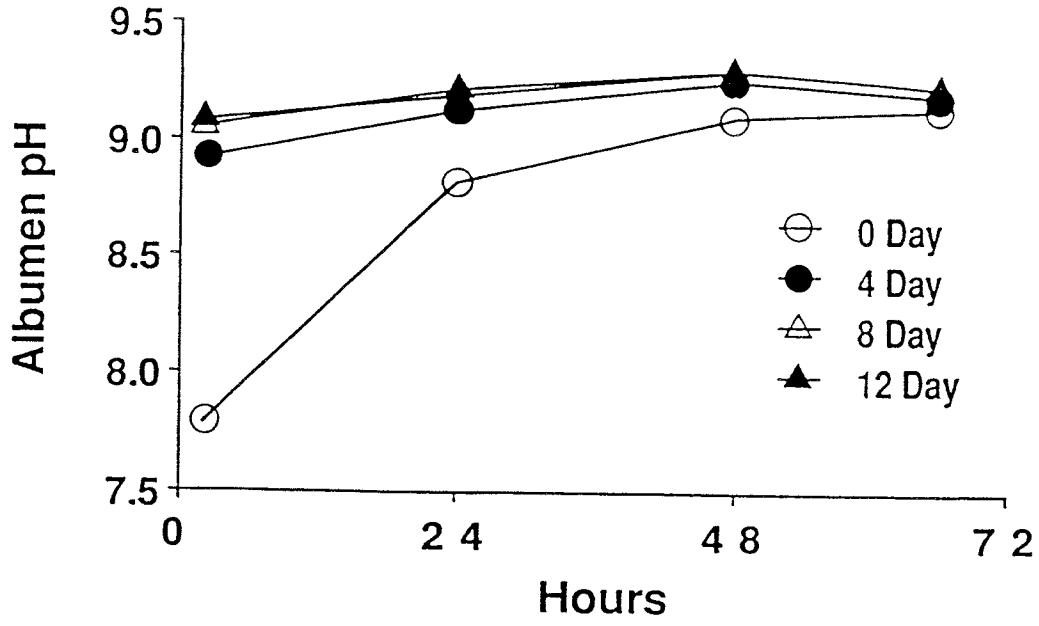

Figure 3. Albumen pH of eggs stored 0, 4, 8, or 12 days during the first 66 hours of incubation in Experiment 1. SE ranges are within symbols.

Albumen pH was not affected by flock age (data not shown) but was affected by day of storage (Figure 3). A full 48 hours was required for the pH of the fresh egg to increase to the level of the stored eggs. The slow increase in pH of the eggs set without storage may prevent the establishment of an essential asymmetric ion-exchange gradient across the vitelline membrane (Rymen and Stockx, 1974). This gradient may be essential for the transport or diffusion of essential nutrients (Burley and Vadehra, 1989; Stern, 1991). The absence of this gradient may increase the probability of early embryonic death.

Table 1. Effect of broiler breeder strain and 4 days of storage at 18°C and 40% relative humidity on albumen height of 47-week-old hens.

Broiler breeder strain	Albumen height (mm)[1]		%
	Fresh	4 days of storage	Decrease
1	8.5 ± 1.0	6.2 ± .7	27.1
2	8.4 ± 1.4	6.8 ± 1.1	19.0
3	8.1 ± 1.4	6.7 ± 1.5	17.3
4	7.8 ± 1.1	7.1 ± .1	9.0
5	7.8 ± 1.0	5.4 ± 1.2	30.8
6	7.7 ± 1.2	4.2 ± 1.9	45.5

[1] Mean ± SD for n = 6.

Egg weight loss during the first 66 hours of incubation does not appear to be affected by storage or flock age (Figure 4). Thick albumen quality may not affect exchange of water from the outer thin albumen, and oxygen across the shell, but this does not imply that this oxygen can then freely diffuse across the thick albumen to the embryo.

Although albumen degradation occurs in the absence of an embryo, it is evident that the embryo contributes to and accelerates this process (Figure 5). This can be attributed to ammonia produced by the embryo in what may be a preparatory process for incubation.

Since albumen quality during storage is sensitive to both temperature and relative humidity (Walsh, 1993), the above data imply storage of eggs from a young flock at a higher temperature and lower humidity will reduce early embryonic mortality while increasing the same for eggs from old flocks. This was demonstrated by Brake and Walsh (1993; see Figure 3) who further showed that a low temperature and high relative humidity decreased early embryonic deaths in eggs from old flocks which are known to exhibit poor albumen quality.

Strain differences with respect to albumen quality may be important to the design of optimum egg storage practices. Considerable differences in albumen height on day of lay and after 4 days of storage at 40% RH were found among 6 commercial strains of broiler breeders at 47 weeks of age (Table 1) which represents the age of onset of a rapid decline in albumen quality. This has broad implications for the design of optimum egg storage and nutrition programs. A further study of two of these strains (Table 2) suggests that much of the difference in egg weight between these two strains is due to a greater volume of thick albumen in strain 1. This implies a lower hatchability for strain 1 unless storage conditions are adjusted to provide for a greater decline in albumen quality before incubation. This is indeed what has been observed commercially. These data also imply that nutrients such as

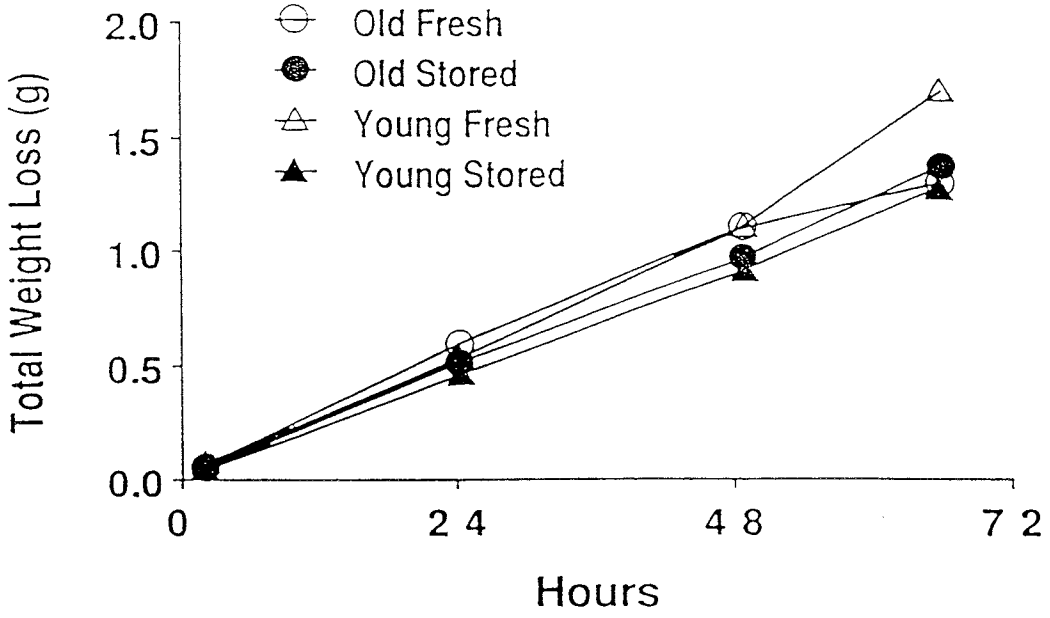

Figure 4. Total egg weight loss of eggs from 30 (young) or 50 (old) week old hens stored 0 (fresh) or 5 (stored) days during the first 66 hours of incubation in Experiment 2. SE ranges are within symbols.

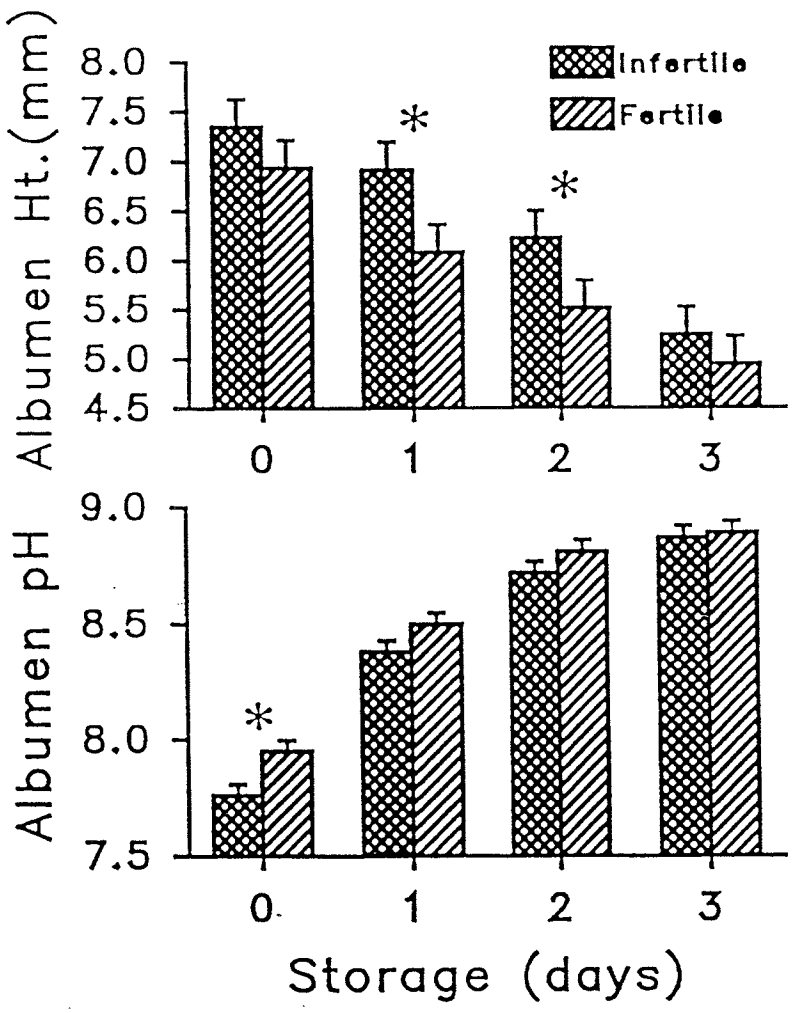

Figure 5. Albumen height and pH of infertile and fertile eggs during 4 days of storage at 18 C and 75% RH in Experiment 3. * represents $P<0.05$.

Table 2. Strain comparison of albumen quality after 5 days of storage at 18°C and 70% relative humidity.

Strain	Flock age	Fresh egg weight	5-day storage weight loss	5-day thick albumen height	Thick albumen	Thin albumen	Total albumen
	(wk)	(g)	(%)	(mm)	(mL)	(mL)	(mL)
1	40	63.11^{b} ±.90	$.42^{A}$ ±.01	5.37^{a} ±.18	21.4^{a} ±.6	14.0^{a} ±.5	35.4^{a} ±.7
3	40	60.89^{a} ±.64	$.46^{B}$ ±.01	5.27^{a} ±.16	20.6^{a} ±.6	12.8^{b} ±.5	33.4^{b} ±.5
1	53	65.07^{B} ±.59	$.43^{A}$ ±.01	4.71^{a} ±.16	22.1^{A} ±.7	13.9^{a} ±.5	36.0^{A} ±.5
3	53	62.18^{A} ±.60	$.48^{B}$ ±.01	4.52^{a} ±.20	19.5^{B} ±.7	13.8^{a} ±.5	33.3^{B} ±.5
1	64	69.77^{b} ±.70	$.45^{a}$ ±.01	4.45^{a} ±.17	24.0^{A} ±.6	14.4^{a} ±.5	38.5^{A} ±.5
3	64	67.57^{a} ±.70	$.47^{a}$ ±.02	$4.06^{a\text{-}1}$ ±.17	21.2^{B} ±.5	14.6^{a} ±.6	35.8^{B} ±.5
Significant effects overall							
Age		***	NS	***	**	.07	***
Strain		***	***	.10	***	NS	***
Age & Strain		NS	NS	NS	NS	NS	**

n = Approximately 30 for all samples.
a,b Means ±SE within an age group with different superscripts are significantly different ($P<0.05$).
AB Means ±SE within an age group with different superscripts are significantly different ($P<0.01$).
a-1 Differences approach significance ($P<0.10$).
*** $P<0.001$
** $P<0.01$

ascorbic acid (Peebles and Brake, 1985), which increase albumen quality, can improve hatchability on older flocks, particularly those with poor albumen quality. The common practice of decreasing dietary protein intake on older breeder flocks can also be questioned as this probably affects albumen quality adversely.

It is suggested that strain, flock age, and nutrition, as well as storage length, temperature, and humidity, all of which impact albumen quality, must be considered when designing egg handling programs for modern broiler breeders.

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OPTIMUM BROILER BREEDER FERTILITY REQUIRES ADEQUATE DIETARY PROTEIN FOR FEMALES DURING THE GROWING PERIOD

J. BRAKE and T.J. WALSH

Summary

In three experiments broiler breeder females were reared on various diets ranging in crude protein (CP) from 110 to 200 g/kg. In all experiments males were reared on a diet containing 170 g CP/kg and males and females were intermingled and photostimulated at 140 days of age. Fertility was generally increased by rearing diets containing 155 g CP/kg and greater, irrespective of the fact that all females possessed equivalent body weights at photostimulation. These data suggest that an increased protein intake during rearing of broiler breeder females will increase fertility during the laying period.

I. INTRODUCTION

Broiler breeder females have been generally reared on the basis of achieving a recommended weekly body weight. It is apparent that the recommended body weights can be achieved by using a variety of dietary formulations and that the severity of the restriction of the intake of metabolizable energy (ME) has increased during recent years which has had the additional effect of a reduction in protein intake relative to genetic potential. Lilburn (1991) fed diets ranging from 180 to 300 g CP/kg in a continuous light environment and showed that the delayed onset of egg production in Japanese quail selected for high 4 week body weight can be eliminated by feeding a dietary protein level during rearing (300 g CP/kg) which was commensurate to that fed during the selection process. Since modern broiler stock are genetically selected for protein accretion on high protein diets, it was surmised that protein intake during rearing might influence post-photostimulation reproductive performance.

II. METHODS

Three experiments were conducted. All birds were reared in light controlled facilities with an 8-hour photoperiod. Males and females were grown separately in all experiments and photostimulated at 140 days of age with a 14-hour photoperiod which was subsequently increased to 16 hours. Feeding programs resembled those for the "low" treatment of Brake (1993). Sexes were fed together in Experiment 1 and separately in Experiments 2 and 3 during the laying period. Males and females received 1987 kJ ME per day at maximum intake in Experiment 1. Females received the same amount in Experiments 2 and 3 while males received about 1465 kJ ME per day. It is common practice in the USA to feed a single diet throughout the growing period. The range of dietary CP selected included and extended outside the normal range.

In Experiment 1 Arbor Acres slow-feathering females were given diets analyzed to contain 110, 140, 170, or 200 g CP and 12.24 MJ of ME/kg from hatch to 18 weeks of age. Feed intakes were adjusted to give essentially equal body weights (~1750 g) at 18 weeks of age. From 19 to 24 weeks all birds received the 170 g CP/kg diet

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followed by a 160 g CP/kg breeder diet to 64 weeks of age. There were four replicate pens of 60 females plus 6 males per treatment. Further details are provided by Brake (1993).

Arbor Acres slow-feathering females were given diets analyzed to contain 140 and 170 g CP and 12.10 MJ of ME/kg from hatch to 24 weeks of age in Experiment 2. Body weights were equivalent at 24 weeks of age (~2500 g). Males received the 170 g CP/kg diet. From 25 to 64 weeks of age all birds received a 160 g CP/kg breeder diet. There were 6 pens of 200 females plus 20 males per treatment.

In Experiment 3 Cobb 500 fast-feathering females were given diets analyzed to contain 140, 155 and 170 g CP and 12.10 MJ of ME/kg from hatch to 20 weeks of age. Body weights were equivalent at 20 weeks of age (1950 g). All females were fed a 180 g CP/kg prebreeder diet to 25 weeks of age and a 160 g CP/kg breeder diet thereafter. Males were fed the 170 g CP/kg diet to 25 weeks of age and the breeder diet thereafter. There were 2 pens of 200 females plus 20 males per treatment.

III. RESULTS AND DISCUSSION

The percentage fertilities exhibited by females grown on the various diets are shown in Tables 1, 2, and 3 and Figure 1. There was a positive dose response of increased fertility with increased grower dietary protein concentration in several portions of the laying period during the three experiments. It is noteworthy that only in Experiment 2 was there a significant effect at the onset of lay. This was the only experiment where the growing diets were continued to point of lay instead of being discontinued by the imposition of a 170 or 180 g CP/kg prebreeder diet. This implies that even severe growing protein deficiencies can be ameliorated and masked with a prebreeder diet for a period of time but that these effects again become apparent later in lay. The obviously deficient 110 g CP/kg group in Experiment 1 exhibited excellent fertility through 36 weeks of age before experiencing a precipitous decline. Furthermore, the 200 g CP/kg growing diet in Experiment 1 produced hens which exhibited fertility of over 94% to 64 weeks of age even though the sexes were fed together with no attempt to control male body weight.

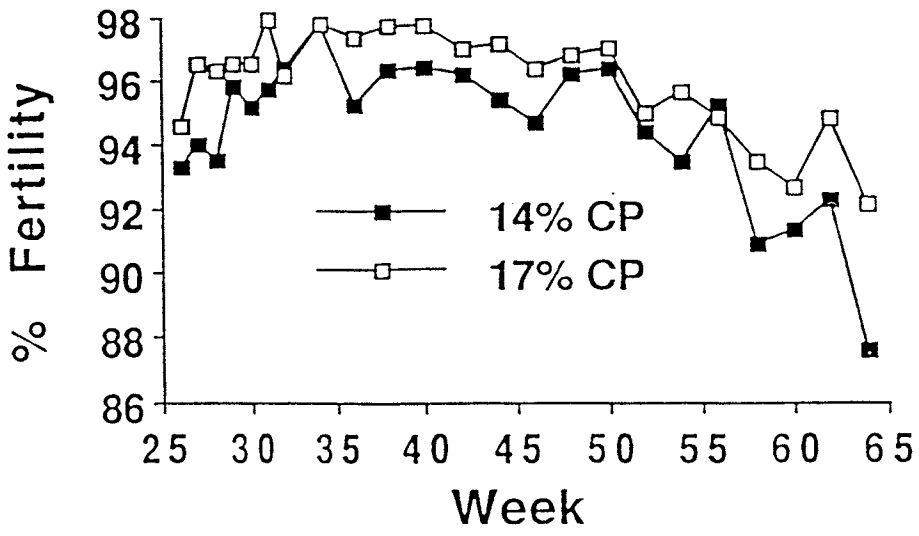

Figure 1. Graphic representation of fertility of broiler breeders when females were grown on diets containing either 140 or 170 g CP/kg in Experiment 2.

Table 1. Percentage fertility during the laying period of Arbor Acres females as affected by diet from hatch to 18 weeks of age in Experiment 1.

	Weeks of age			
Female growing diet (% CP)	28-36	37-46	47-56	57-64
	(% fertility)			
11	96.4[a]	90.1[b]	88.2[b]	82.1[b]
14	97.2[a]	94.7[a]	95.8[a]	91.6[ab]
17	97.9[a]	98.1[a]	94.9[a]	92.1[ab]
20	97.6[a]	98.1[a]	96.5[a]	94.4[a]

[a,b] Statistically significant differences ($P < 0.05$).

Table 2. Percentage fertility during the laying period of Arbor Acres females as affected by diet from hatch to 24 weeks of age in Experiment 2.

	Weeks of age			
Female growing diet (% CP)	26-34	35-44	45-54	55-64
	(% fertility)			
14	95.1[b]	96.0[b]	95.4[a]	92.4[a]
17	96.7[a]	97.4[a]	96.2[a]	93.9[a]

[a,b] Statistically significant differences ($P < 0.05$).

Table 3. Percentage fertility during the laying period of Cobb 500 females as affected by diet from hatch to 20 weeks of age in Experiment 3.

	Weeks of age			
Female growing diet (% CP)	28-36	37-46	47-56	57-64
	(% fertility)			
14.0	97.2[a]	96.6[b]	94.4[a]	81.4[b]
15.5	97.4[a]	97.5[a]	95.4[a]	92.8[a]
17.0	97.3[a]	97.9[a]	94.6[a]	89.1[ab]

[a,b] Statistically significant differences ($P < 0.05$).

This effect is most likely due to altered sperm storage capacity of the utero-vaginal glands which has been shown to decline in broiler parent stock selected for a high 42-day body weight (VanKrey and Siegel, 1974). Those birds were grown on a 200 g CP/kg diet to 8 weeks of age, a 140 g CP/kg diet to 20 weeks of age, and were then fed a 160 g CP/kg diet thereafter. These diets were within the range of the present study and the birds were exposed to a 14-hour photoperiod from 18 weeks of age onward.

Inspection of the data within the 140 to 170 g CP/kg range suggests that fertility was improved throughout lay at levels of 155 g CP/kg and above even though statistical

differences were not apparent due to increased variability with age. It is proposed that an adequate dietary protein intake prior to puberty provides for the fulfillment of metabolic requirements for optimum spermatozoal storage independent of body weight requirements.

These data suggest a specific dietary protein requirement during the rearing period of broiler breeder females to maximize fertility. It is further suggested that the reported decrease in fertility due to genetic selection for growth (Soller and Rappaport, 1971) is simply a result of an increased nutrient requirement for fertility not met by a given nutritional and feeding program aimed at controlling body weight and maximizing egg production.

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THE CASE FOR ANTIOXIDANT TREATMENT OF RENDERED BY-PRODUCTS FOR POULTRY FEED

CHAO LIANG

Summary

Autoxidation is an inevitable process in rendered products and poultry feed. It has many detrimental implications for poultry feed. In order to prevent autoxidation, choosing an effective antioxidant product is critical. An effective antioxidant product should have the following characteristics: (1) be small in particle size, or easily dispensed if it is in liquid form, (2) contains several antioxidants, (3) has mineral ion chelators and (4) is made from a synergistic-blend process.

I. INTRODUCTION

When fats or oils are kept too long, they smell badly or become rancid. This is one of the examples of autoxidation in daily life and the unpleasant odour is due to autoxidation products called carbonyls, which give a bad smell at very low concentrations.

Another example of autoxidation is vitamin A degradation during storage. Klopfenstein and Zhuge (1986) found that the potency of vitamin A in a premix decreases regardless of the temperature of storage.

These two observations, although seemingly unrelated, are the result of the same chemical reaction, autoxidation. In this reaction, unsaturated portions of fat or vitamin A are attacked by a special chemical species -- free radicals, to become unstable compounds. These unstable compounds react with oxygen in the air to form peroxides, followed by a serious degradation process to produce various small compounds such as carbonyls. A simplified mechanism for the autoxidation reaction suggests two important aspects of the mechanism are: (1) free radicals which are the key to the initiation of the reaction. Antioxidants can be used to prevent autoxidation by actually absorbing free radicals in a feed system. (2) some minerals are the catalyst for the formation of free radicals. This is why chelator agents are needed to interrupt the consistent supply of free radicals in order to make an antioxidant more effective.

There are many ways to measure the autoxidation reaction in poultry feed. The simplest and most practical method is sensory evaluation. An experienced producer can easily tell the difference between fresh and rancid feed. Traditionally, peroxide value is used to evaluate the quality of feed or fats in terms of their degree of autoxidation. Assaying of special components such as vitamin A or pigmenters is also employed to monitor the stability of poultry feed. Recently, gas chromatography-mass spectrometry (GC-MS) has also been used to analyse autoxidation products.

Although these methods are commonly used, the results of these tests usually do not reveal the actual conditions of poultry feed in terms of autoxidation. These methods use a static approach (one point sampling) while autoxidation is a dynamic process which requires a kinetic approach (time-course study) to reveal the changes in the system. A kinetic methodology is the activated oxygen method (AOM), an official method for checking oil or fat quality. With this

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test, oil samples are incubated at 98°C while being oxygenated with compressed air. Peroxide values are assayed in a time-course fashion. Another kinetic methodology is known as the oxygen bomb test which is specially designed for dry samples, such as animal feeds or vitamin premixes. In this test, samples are loaded into a special container and pressurized with pure oxygen. The oxygen pressure is monitored as the indicator of the degree of autoxidation. These kinetic studies provide reliable results of the stability of samples and give a quick assessment of the efficacy of antioxidant products.

II. AUTOXIDATION AND POULTRY PERFORMANCE

Not only can an experienced producer tell the difference between fresh and rancid feed but so does the animal. Broiler trial results indicate that high peroxide values in broiler feed (54 meq/kg) can reduce feed intake by 21%, weight gain by 33% and feed efficiency by 10% (Seemann, 1990).

Cabel *et al.* (1988) concluded that the products of autoxidation in poultry feed, even at a low level, could be detrimental to the performance of broilers. When broilers were given feed containing 7 meq peroxide/kg, feed conversion ratio (FCR) increased in 49 and 21 day feeding trials by about nine points and more than 20 points, respectively.

Autoxidation products also have a detrimental effect on laying hens. Voreck and Kirehgossner (1981) reported that eggs per hen per day declined from 0.9 to 0.18 when the layer feed was changed from a normal maize diet to a diet containing oxidized fat. Further study indicated that the detrimental effect from oxidized fat could not be overcome by the addition of vitamin D up to 5,000 units.

As for the explanation of these negative implications of autoxidation on poultry performance, one of the hypotheses is that the products from fat oxidation are carcinogenic or cyto-toxic to the animal. This hypothesis was supported by Mortia *et al.* (1983) who demonstrated a relationship between the degree of DNA damage in the experimental system and the level of autoxidation of linoleic acid. The higher the degree of autoxidation of linoleic acid, the more the DNA was destroyed.

More recently, another hypothesis has been proposed which suggests that the products of autoxidation are inhibitors of animal digestive enzymes (Smith *et al.,* 1993). Their *in vitro* digestion study indicated that there was a significant decrease in dry matter digestibility when the tested grains were mixed with 4% oxidized fat.

III. HOW TO PREVENT AUTOXIDATION IN POULTRY FEED

In order to maintain good quality poultry feed, choosing a good antioxidant is an important step in a whole management strategy. Howere, there are some technical considerations for selecting an effective antioxidant.

(a) Particle size of antioxidant

An antioxidant must have direct contact with free radicals in order to react with them. Because free radicals are tiny molecules with a very short life span (less than 1 msec.), the number of active particles of antioxidant becomes the most critical factor for inhibiting autoxidation. Solid geometric principles indicate that for the same volume of material, if the particle size becomes smaller, the number of particles will increase dramatically. For example, if a one meter cube is divided into 1 mm cubes, the number of cubes will increase one billion

times. This is why a specially made antioxidant product works much better than antioxidant commodities, which are in the form of crystals such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The specially made product contains much more active particles than these crystal BHA and BHT products at the same weight.

(b) Synergism

The longer protection time resulting from the use of two antioxidants together is greater than the sum of protection time obtained from using these two antioxidants separately. One of the classical examples of synergism among different antioxidants is shown by the work of Lundberg *et al.* (1962). They discovered that BHA and ethoxyquin (EQ) together provided an additional 24 h stability for lard compared with the sum of the stability times from using BHA and EQ separately. There are many examples of synergism among antioxidants (Kanemalsu, 1983; Hudson, 1984). Kurechi and Kato (1983) proposed an hypothesis to explain the mechanism. They suggested that one antioxidant could be regenerated in the presence of another antioxidant due to differences in their hydrogen donating capabilities.

Another advantage of using a combination of antioxidants is the broader protection spectrum that can be achieved because the strength and weakness of individual antioxidants compensate each other. One of the studies reported by Eastman Kodak (1990) demonstrated that tertiary-butyl hydroquinone (TBHQ) is a better antioxidant for protecting peanut oil than BHA. However, BHA was shown to be a better choice than TBHQ for protecting pecan oil. In the light of the huge variation and diversity of poultry feeds and their ingredients, a combination of several antioxidants should be a better strategy for protecting feed from autoxidation.

(c) Chelators

Marcuse and Fredrilesson (1971) discovered that the higher the concentration of copper ions, the more linoleic acid was oxidized. The reason for the involvement of mineral ions in autoxidation is due to the participation of mineral ions in the regeneration of free radicals in the autoxidation reaction. Therefore, in order to make the antioxidant product more effective, the inclusion of ion chelators is very important since they bind mineral ions.

(d) Synergistic-blend or dry mix

There are two kinds of processed antioxidants, dry-mix and synergistic-blend. For dry-mix, antioxidants are mixed with a non-active carrier. This has two major technical problems: (1) it is not one hundred percent active, and (2) it is not synergistic because antioxidants are still in the crystal form. For synergistic-blend products, the antioxidant crystals are dissolved first in a solvent and then sprayed onto a carrier. This process makes the particles in the product one hundred percent active and the synergism can take place because different antioxidants and chelators are in solution and can react together.

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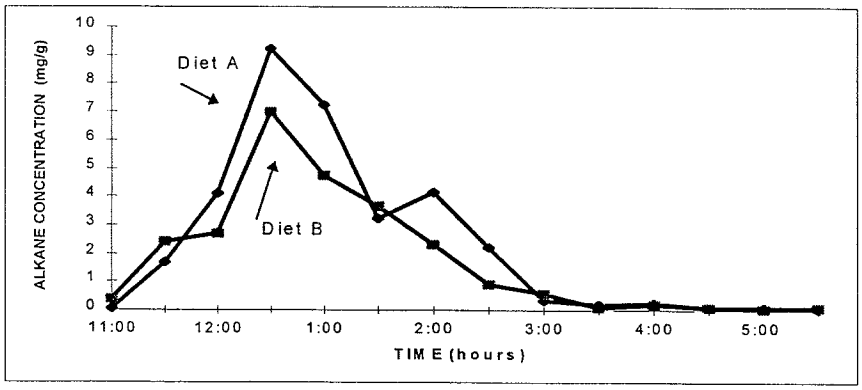
HYDROCARBON MARKER: A NEW TOOL FOR TRANSIT TIME STUDIES

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

Feed transit time is the amount of time feed components are retained in the gastrointestinal tract and is often measured by giving a known amount of marker-containing diet and determining the first appearance of the marker in the faeces. Most markers used in digestibility studies are insoluble substances such as Cr_2O_3 and acid-insoluble ash which must be incorporated into the diets in substantial quantities for sufficient accuracy in subsequent determinations. Feeding birds a marker-containing diet in a given amount of time is difficult and inaccurate, and force-feeding birds using the Sibbald technique is tedious and not suitable for young broilers. The current paper describes a new fat-soluble marker, a long-chain alkane ($C_{36}H_{74}$), which can be easily and accurately administered to chickens orally.

The long-chain hydrocarbons are neither absorbed in the GI tract nor utilised by the gut microflora (Dove and Mayes, 1991). In the current study, Diets A and B were each fed to a group of eight 23-d-old chickens (~700 g body weight) for three days. Each bird was then given 20 mg of alkane (10 mg $C_{36}H_{74}$ per mL cooking oil) orally using a Gilson pipette at 0830 h. Excreta from each bird were collected every 30 min over the next 9 h and excreta from two birds were pooled within treatments, dried at 80°C, and the weight recorded. The alkane levels were determined using an internal standard method on a gas-chromatograph fitted with a capillary column. The patterns of excretion of the alkane marker are shown in the Figure.



The marker excretion patterns were identical for both diets, with the alkane concentration peaking 4 h after the administration of the marker. It is recommended that the maximum concentration of the marker, rather than the first appearance of it, in the excreta be taken as the indicator of the transit time. Dietary factors, such as the presence of viscous non-starch polysaccharides, will be examined in further experiments.

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FEED ENZYMES ELIMINATE THE ANTI-NUTRITIVE EFFECT OF NON-STARCH POLYSACCHARIDES AND MODIFY FERMENTATION IN BROILERS

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Summary

A study was undertaken to examine the effect of a glycanase product on fore- and hind-gut fermentation in soluble non-starch polysaccharide (NSP)-enriched broiler diets. The enzyme product increased ($P<0.01$) weight gain, feed efficiency and apparent metabolizable energy (AME), and was highly effective in reducing viscosity along the gastrointestinal (GI) tract. Comparing viscosities (mPa.s) between birds fed the NSP-enriched diet and the same diet supplemented with enzyme showed 11.9 vs 2.3 in the duodenum; 78.3 vs 4.4 in the jejunum and 409.3 vs 10.8 in the ileum, respectively. Caecal volatile fatty acid (VFA) production was markedly ($P<0.01$) elevated by enzyme supplementation, whereas ileal fermentation was inhibited. The current results suggest that extensive fermentation in the small intestine may be detrimental to birds as the ileal VFA levels and the AME values were negatively correlated ($r=-0.83$). Adding a synthetic antibiotic (Amoxil) in the drinking water had no beneficial effect on bird performance.

I. INTRODUCTION

Non-starch polysaccharides have recently become the focus of attention in monogastric nutrition, perhaps due to the fact that they are one of the most under utilised by-products in animal feed and also because the soluble NSP have anti-nutritive activities which impair nutrient digestion (Fengler and Marqaurdt, 1988; Bedford and Classen, 1992). The anti-nutritive activity of NSP is multifaceted. They can change gut physiology and influence gut microflora by increasing digesta viscosity (Choct, 1992). The direct effect of viscosity has been well elucidated, but the mechanism by which the gut microflora are involved in the anti-nutritive effect of viscous NSP is largely speculative. Supplementation of rye-based broiler diets with antibiotics often resulted in marked improvement in bird performance (Misir and Marguardt, 1978), which coincided with large increases in ileal anaerobic counts (Wagner and Thomas, 1978). The current study investigated the role of gut microflora in birds fed a diet with chemically well-defined soluble NSP by feeding it intact and supplementing it with enzymes or an antibiotic.

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II. MATERIALS AND METHODS

(a) AME trial, digesta collection and gross energy determination

Day-old mixed sex broiler chicks were obtained from a local hatchery and raised on commercial starter diet to 14 d and then on finisher diet to 21 d. An 8-d classical AME trial was conducted using individual ME cages with a 4-d adaptation period and a 4-d collection period. Four diets were prepared as shown in Table 1. Each diet was fed to 8 birds. At the end of the AME trial the birds were killed by intravenous injection of Nembutal. The contents of the duodenum, jejunum and ileum (from Meckel's diverticulum to 4 cm above the ileo-caecal junction) were collected in pre-weighed containers and the fresh weights recorded. After centrifugation (see below) the supernatants and the pellets were immediately separated and frozen at -21°C for viscosity and volatile fatty acids determinations. Gross energy was determined using a Parr isoperibol calorimeter.

Table 1. The composition of the experimental diets (g/kg).

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Sorghum	614	614	614	614
Meat and bone meal	76	76	76	76
Soybean meal	170	170	170	170
Tallow	40	40	40	40
NaCl	2.5	2.5	2.5	2.5
L-lysine	2.5	2.5	2.5	2.5
DL-methionine	3.2	3.2	3.2	3.2
Premix	5.0	5.0	5.0	5.0
Choline chloride(50%)	0.8	0.8	0.8	0.8
Celite	20	20	20	20
Cellulose	66	0.0	0.0	0.0
Isolated NSP[1]	0.0	66	66	66
Enzyme[2]	-	-	+	-
Antibiotic (Amoxil)	-	-	-	+

[1] NSP was isolated from a wheat milling by-product using the alkaline extraction procedure described by Choct and Annison (1992). The isolate contained approximately 60% soluble NSP.

[2] Added 1 g per kg diet. It had activities of arabinoxylanase, ß-glucanase and pectinase.

(b) Viscosity, volatile fatty acids and data analyses

Approximately 2 g of fresh digesta were centrifuged (12,000 *g* 10 min at 20°C). Viscosity was determined on a 0.5 mL supernatant using a Brookfield viscometer at 25°C.

Volatile fatty acids (VFA) were measured using the method of Illman and Topping (1985). After the determination of viscosity and VFA, the supernatant was re-combined with the solid and freeze-dried for further analyses.

All data were analysed using one-way ANOVA and simple regression. Multiple comparisons were made using the Duncan's test.

III. RESULTS

Addition of an equivalent of 40 g soluble NSP/kg to a commercial-type broiler diet depressed weight gain, feed conversion and AME by 28.6%, 27.0% and 21.2%, respectively. Supplementation of the NSP-enriched diet with a commercial enzyme product (Avizyme TX) eliminated the effect of the NSP on bird performance. Addition of a synthetic antibiotic (Amoxil, 500 mg/L) in the drinking water did not reverse the adverse

Table 2. Effects of a glycanase and antibiotic on weight gain (WG; g/bird/wk), feed intake (FI; g/bird/wk) and feed conversion ratio (FCR) of broilers fed NSP-enriched diets. AME (MJ/kg dry matter) values are also shown (n=8; means ± SE).

Diet	WG	FI	FCR	AME
Control	475 ± 20^{a}	843 ± 28^{a}	1.790 ± 0.074^{bc}	13.78 ± 0.17^{a}
NSP	339 ± 27^{b}	789 ± 47^{a}	2.453 ± 0.252^{b}	10.86 ± 0.57^{b}
NSP + enzyme	488 ± 11^{a}	779 ± 28^{a}	1.602 ± 0.071^{c}	14.13 ± 0.23^{a}
NSP + antibiotic	233 ± 29^{c}	747 ± 34^{a}	3.539 ± 0.403^{a}	10.11 ± 0.81^{b}

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

Table 3. Effects of a glycanase on digesta viscosities (mPa.s) (n=8; means ± SE).

Diet	Duodenal	Jejunal	Ileal
Control	1.4 ± 0.1^{c}	1.5 ± 0.1^{c}	2.4 ± 0.2^{c}
NSP	11.9 ± 1.9^{b}	78.3 ± 10.8^{b}	409.3 ± 61.5^{b}
NSP + enzyme	2.3 ± 0.1^{c}	4.4 ± 0.4^{c}	10.8 ± 1.4^{c}
NSP + antibiotic	17.5 ± 2.6^{a}	134.1 ± 33.4^{a}	711.6 ± 200.8^{a}

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

Table 4. Volatile fatty acid (VFA) levels (μmol) in the ilea and caeca of broilers fed NSP-enriched diets with and without enzyme or antibiotic (n=8; means ± SE). The relationship between ileal VFA and AME is also shown.

Diet	Ileum	Caeca
Control	8.3 ± 1.8^{b}	312.3 ± 77.4^{b}
NSP	118.2 ± 33.4^{a}	369.0 ± 90.2^{b}
NSP + enzyme	5.4 ± 1.4^{b}	930.0 ± 194.7^{a}
NSP + antibiotic	178.9 ± 32.8^{a}	413.5 ± 101.3^{b}

Correlation between AME and Ileal VFA: $r = -0.83$, $r^2 = 0.69$.

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

effect of NSP (Table 2). Gut viscosities of the birds were markedly increased when soluble NSP were added to the diet, but they were effectively reduced by the enzyme. The antibiotic had an adverse effect on viscosities (Table 3). The caecal VFA level was influenced neither by elevated amounts of soluble NSP nor by addition of the antibiotic, but it was significantly increased by enzyme supplementation. In contrast, the ileal VFA level was significantly higher in birds fed diets with soluble NSP compared with those fed the control or the enzyme supplemented diet. Furthermore, there was a highly significant

negative relationship ($r = -0.83$; $r^2 = 0.69$) between the ileal VFA levels and AME values (Table 4).

IV. DISCUSSION

Addition of an equivalent of 40 g soluble NSP/kg to a commercial-type broiler diet significantly impaired bird performance and increased digesta viscosity. These adverse effects, however, were largely eliminated by the addition of a NSP-degrading enzyme product to the diet. The anti-nutritive effect of soluble NSP may be related to gut microflora as supplementation with antibiotics alleviates the adverse effects of rye in poultry (Misir and Marquardt, 1978). The positive effect of antibiotics appears to be related to the elimination of fermentative microorganisms (mainly butyric acid producers) from the small intestine. Thus, Wagner and Thomas (1978) reported that the anaerobic counts were two to three logarithmic cycles higher in the ilea of birds fed rye or pectin-enriched diets compared with those fed a maize-soy diet, and they were reduced by five logarithmic cycles when penicillin was added to the diets. The results for the antibiotic in the current study, however, were unexpected. The synthetic antibiotic (Amoxil) added in the drinking water of the birds reduced weight gain and had a negative effect on feed conversion. The failure of the product to suppress the fermentative microorganisms was clearly suggested by the presence of large amounts of VFA in the caeca as well as in the ilea. A total loss of activity of the antibiotic, however, was not a likely explanation as digesta viscosities were constantly higher in birds given the antibiotic. Amoxil is a synthetic penicillin which is supposed to have a wide antibacterial spectrum but it is destroyed by penicillinase which is produced by bacteria such as *Staphylococcus aureus*. In general, it is much less active than benzyl penicillin against most Gram-positive cocci (Ross and Peutherer, 1987).

Variable amounts of volatile fatty acids (VFA) are present in all regions of the digestive tract of birds, with the caeca being the major site (Annison *et al.*, 1968). In the current study, the VFA production in the caeca was similar in all treatments except for the much higher production in the caeca of the enzyme-supplemented group. Conversely, birds fed the NSP-enriched diet and that diet with the antibiotic had significantly higher amounts of VFA in the ilea compared to birds fed the control and the enzyme-supplemented diets. This was indicated by a strong negative correlation ($r = -0.83$) between the ileal VFA levels and the AME values. Under normal circumstances, facultative anaerobes dominate the small intestinal microflora and strict anaerobes make up nearly the entire caecal microflora of the chicken (Salanitro *et al.*, 1978). However, when a large amount of viscous NSP is present in the diet the condition of the small intestine may become more fermentative. It is probable that the colonisation of the small intestine by strict anaerobes increases because of the delayed transit time caused by highly viscous digesta.

The current study demonstrated a close negative correlation between the VFA levels in the ileum and the AME values, which may indicate that the effect of viscous NSP on bird performance is indirectly related to proliferation of fermentative microflora in the small intestine.

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THE VALUE OF FLOCK SURVEILLANCE AND POST-MORTEM ANALYSIS FOR HUSBANDRY AND HYGIENE MANAGEMENT

R. C. CHUBB, R. B. CUMMING and W.J BALL

The demise of the poultry extension officer, and the implementation of full 'user pays' principles within the State Veterinary Services, highlight the need of the poultry industry, especially the egg industry, to evaluate methods of health/husbandry surveillance systems directed to individual breeds of hens and husbandry practices. The layer and broiler breeding companies usually have veterinary services, whereas the egg producers tend to be owners, perhaps associated with a co-operative which may provide some services. The poultry health liaison committees of each state are probably the means by which the Departments of Agriculture seek information which is used to set up remedial programmes. Without collective and comparative data from the egg industry, however, these programmes will tend to be directed to problems of the large integrated operations, rather than the small egg producer.

Post-mortems were performed on all hens dying during a comparative laying trial at the University of New England's poultry farm "Laureldale". Three strains of hens were fed on four separate diets. The resulting data highlights the value of full post-mortem recording coupled with productivity recording. A comparative loss in egg production was seen with one breed of hen on one diet. This breed had a watery droppings syndrome also associated with this diet. In two breeds of hens on this particular diet, enhanced mortality from cannibalism and vent pecking was seen, as well as a deterioration in feathering. This was not reflected in Marek's disease mortality. The mortality could have been associated with a phosphorus deficiency. One of the breeds had both Marek's disease and lymphoid leucosis, which showed as a biphasic mortality curve (this breed also had an endothelioma condition). The third breed did not have any major problem on any diet, and had good production, although downgrading of eggs from blood spots periodically occurred, showing the need for egg quality surveillance.

This data from a comparative nutrition trial using three breeds of laying hen illustrates the need for continuing comparisons across breeds under Australian conditions, especially as the imported breeds come into production. Also, there is a need to scrutinise suggested changes in husbandry and nutrition on a comparative basis. Each breed performed differently in the comparisons described here.

A serological survey for infectious bronchitis was carried out on four laying farms in the Tamworth district in 1971. This showed quite clearly that rearing practices on multi-aged laying farms affected the need for IBV vaccination, and highlighted the value of intercurrent infections on the farms. The survey showed the need for surveillance of husbandry and hygiene practice, the keeping of productivity records, and a serological, or some form of infectious status, survey.

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THE RELEVANCE OF INTESTINAL VISCOSITY ON PERFORMANCE OF PRACTICAL BROILER DIETS

W.D.COWAN

Summary

Feeding trials of wheat-based broiler diets have indicated that intestinal viscosity is often found to be between 10 and 15 centipoise (cp) in the absence of added microbial enzymes. Addition of microbial enzymes improves performance and reduces intestinal viscosity. Two factors, viscosity reduction and nutrient release, are involved in improving performance. It has also been found that at low viscosity levels (<10cp) further reduction in viscosity does not result in further performance gains. Microbial xylanases for broiler nutrition should, therefore, contain enzyme activity against both soluble and insoluble arabinoxylans, for viscosity reduction and nutrient freeing respectively.

I. INTRODUCTION

Microbial enzymes are becoming a more common component of broiler rations and their successful utilisation depends upon an understanding of their modes of action and the role they are to perform.

In wheat and barley there are considerable differences between gross and metabolisable energy and the presence of anti-nutritional factors such as pentosans and beta-glucans have been associated with part of this difference, through interference with nutrient absorption . While the concept of the role of soluble non starch polysaccharides (NSP) being the causative agent of this energy difference (Annison, 1991; Annison and Choct, 1993) is now generally established, some investigators have been unable to observe this correlation (Veldman and Vahl, 1994; Wiseman *et al.*, 1994).

The anti-nutritive effect of wheat NSP's has been associated with raised intestinal viscosity (Bedford and Classen, 1992) and with the masking of nutrients by insoluble cell wall material (Hesselman and Åman, 1985). It is now assumed that both factors are involved and that their relative importance may vary from situation to situation.

In cases of high intestinal viscosity (>250cp), the overriding factor will most probably be reduced nutrient (feed starch, protein and fat) diffusion, whereas at low viscosity, diffusion is not affected and nutrient availability becomes more important. Indeed, Bedford and Classen (1992) observed that beyond a certain point further reduction in viscosity is not reflected in performance improvements.

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Feeding trials made with wheat-based diets have indicated that in many cases intestinal viscosity of broiler diets does not exceed 15 cp (Veldman and Vahl, 1993; Cowan *et al.*, 1994). It was therefore decided to examine the effect of enzyme supplementation in this area in order to determine the relative importance of the two modes of action of exogenous microbial enzymes under conditions encountered in broiler nutrition today.

II. MATERIALS AND METHODS

(a) Characterisation of Enzyme Types

Viscosity reducing xylanases will exert their effect primarily on the water-soluble portion of the wheat NSP fraction, whereas, "nutrient freeing" enzymes will exert their effect on the insoluble portion making up the endosperm cell walls. In order to characterise enzymes by their ability to degrade these two substrates types an assay of enzyme activity was carried out using a standard endoxylanase method (FXU) and a reducing sugar method based on the release of reducing sugars from soluble or insoluble wheat arabinoxylan. Six different xylanase enzyme products were assayed in this characterisation. Three of these were multi enzyme products, ie a single fermentation but yielding an enzyme product containing more than a single activity, and three were single activity products produced by R-DNA technology.

The standard FXU assay for endoxylanase was carried out at pH 6.0 and 50 °C using a remazol brilliant blue substrate. The reducing sugar assays were carried out using a micro titre plate system with p-hydroxy-benzoic acid hydrazide in a pH 6.0 buffer at 40 °C. For the other two assays, water insoluble pentosan (WIP) (arabinose/xylose ratio ~ 0.75) and water soluble pentosan (WSP) (arabinose/xylose ratio ~ 0.84-0.94) were prepared by enzymatic digestion (amylase and protease) of ground wheat followed by washing out of the low molecular weight sugars by ultrafiltration. Separation and purification of the two fractions was followed by freeze drying. Each enzyme preparation was assayed by the three methods and the levels of activity against each of the three substrates determined.

(b) Feeding Trials

Two xylanases from the characterisation programme (A and F), were selected for feeding and in vivo viscosity studies. The birds received a diet high in wheat (see Table 1) to accentuate viscosity effects and the pelleting temperature was resticted to 65 °C. Enzymes were dosed at 0, 400, or 800 FXU/kg feed, with six replicates per treatment and nine chickens per replicate. Measurements were made of feed intake, weight gain and foregut viscosity. For viscosity determinations, three chickens per replicate were removed, sacrificed and the viscosity of the centrifuged gut contents determined on a Brookfield LVTD-II viscometer.

III. RESULTS

The results from the enzyme characterisation are shown in Table 2. As the FXU contents of the different xylanase preparations were not the same the results are presented as the ratio between the number of FXU units in the sample compared to the number of reducing sugar units (expressed as μmole/min/mg) from either the WSP or WIP substrates. There were considerable differences between the xylanase preparations in their action patterns as revealed by their relative activity against the two wheat pentosan substrates.

Xylanases A and F were chosen for further study as they had similar levels of activity against WIP but xylanase F showed more activity against WSP.

Table 1. Feed composition for feeding trial (0-3 weeks).

Component	Inclusion (g/kg)	Component	Inclusion (g/kg)
Wheat	810	Lysine (40 %)	2
Fish meal	78	Threonine (50 %)	1
Meat and Bone meal	69	Vitamin/Minerals	6
Soyabean oil	13	Calculated content	
Calcium carbonate	8	ME/(MJ/kg)	12.74
Dicalcium phosphate	12	Protein (g/kg)	191
Methionine (40 %)	1	Fat (g/kg)	47

Table 2. Ratio between Reducing Sugar units and FXU units for different xylanases.

Enzyme	Water Soluble Units : FXU	Water Insoluble Units : FXU
A	2.60	0.68
B	1.97	0.54
C	2.80	6.31
D	3.06	7.02
E	2.33	0.64
F	6.29	0.76

The feeding trials indicated a clear dosage response for the two xylanase preparations (Figure 1), with the maximum effect being achieved at 400 FXU/kg. As birds were removed for viscosity determination at 3 weeks of age, performance figures are only shown for this period.

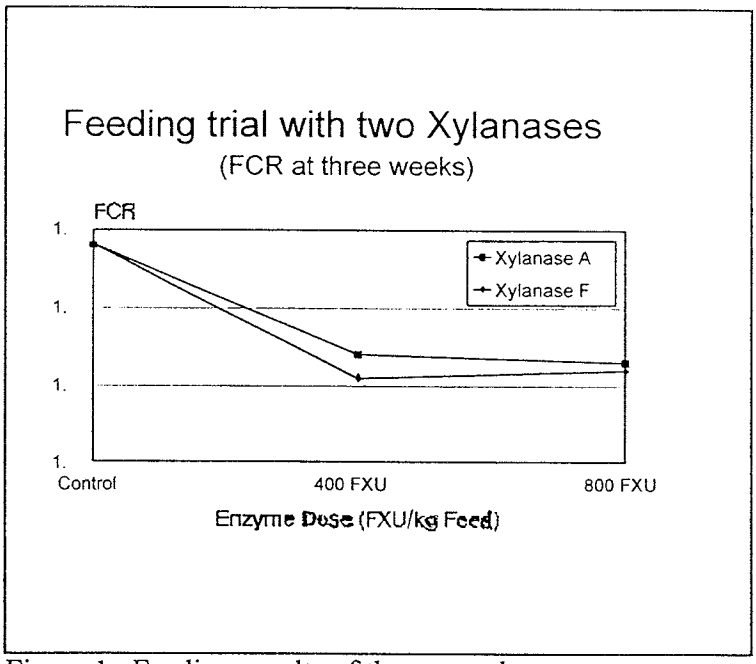

Figure 1. Feeding results of the two xylanases

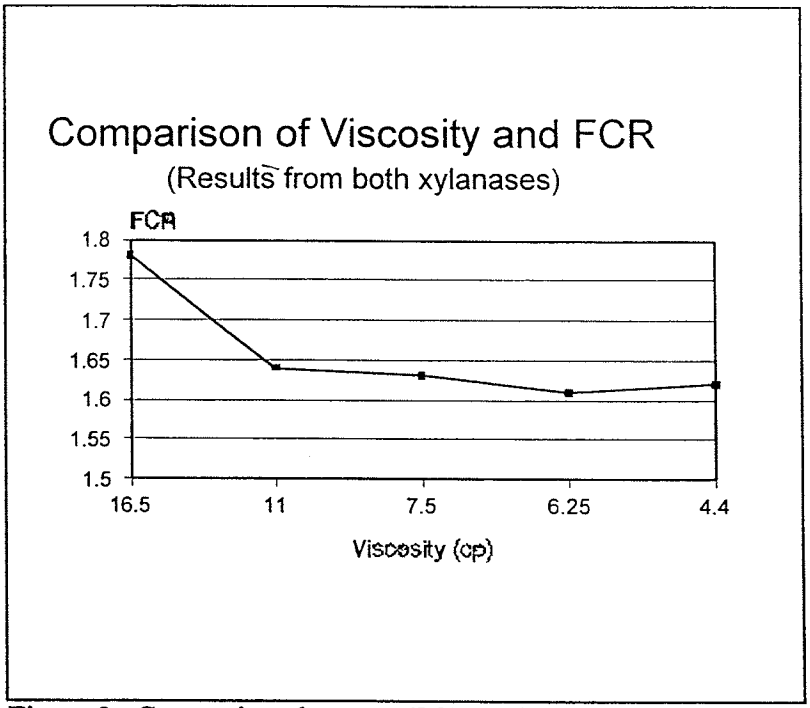

Figure 2. Comparison between FCR and Intestinal Viscosity

Viscosity determinations revealed that a dosage of 800 FXU of either enzyme reduced viscosity to its lowest level but that at 400 FXU/kg feed the FCR had already reached its minimum value. Comparison of viscosity reduction and FCR indicated that once viscosity had fallen below approximately 10 cp, further reductions did not improve performance. (Figure 2).

IV. CONCLUSIONS

In practical broiler diets intestinal viscosity is often in the range 8-12 cp and seldom exceeds 15-20 cp for wheat-based diets. Xylanase enzymes may be classified according to their affinity for soluble and insoluble pentosans as well as by endo or exo activity. As performance has been shown not to be solely related to viscosity reduction, it is necessary to use xylanases that contain both viscosity reducing and nutrient freeing abilities. It may be hypothesised that under conditions of high viscosity, such as may be encountered with diets containing large amounts of barley or rye, viscosity reduction is of greater importance than nutrient freeing. Conversely, for mash broiler diets or those pelleted at low temperatures which would allow the functioning of endogenous wheat xylanase, a viscosity reducing endoxylanase would not improve performance. For practical diets subjected to high pelleting temperatures an enzyme containing both types of activity would be required. The new technique of R-DNA manipulation will allow for the selection of specific enzyme types which can be recombined in order to produce the optimum enzyme profile for each situation.

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HUMAN FACTORS AFFECTING THE BEHAVIOURAL RESPONSE TO HUMANS AND THE PRODUCTIVITY OF COMMERCIAL BROILER CHICKENS

P.H. CRANSBERG, G.J. COLEMAN* and P.H. HEMSWORTH

Our present research program is testing the proposal that the attitudes and consequent behaviours of stockpersons towards broiler chickens will affect the birds' fear of humans which, in turn, will affect the birds' performance and welfare. The mechanism whereby fear may affect performance is likely to be a stress response. Previous research in the pig and egg industries strongly support these interrelationships between human attitude and behaviour, and animal behaviour, performance and welfare (Barnett *et al.*, 1994; Hemsworth *et al.*, 1994a). Recent research in the broiler chicken industry revealed significant negative correlations between level of fear of humans and feed efficiency of broiler chickens (Hemsworth *et al.*, 1994b).

The aim of the present study was to examine between-farm relationships concerning human attitude and behaviour and bird behaviour and performance at 25 commercial broiler farms. At each farm the behaviour of stockpeople in the sheds was observed by two experimenters, one recording the speed of movement of the stockperson, and the other recording fear-provoking behaviours exhibited by the stockperson, such as waving buckets or arms. The level of fear of humans by the chickens was assessed at 35 days of age using two behavioural tests, both examining the withdrawal responses of birds to humans. Productivity results were supplied by the controlling company. A significant positive correlation was found between the stockperson's speed of movement and level of fear of humans by birds ($r^2=0.44$, $P<0.01$). Significant correlations were also found between human behaviour variables and productivity of birds. For example, speed of movement by the stockperson was associated with first week mortality ($r^2=0.38$, $P<0.05$). Furthermore, fear of humans by birds was significantly correlated with productivity. For example, level of fear of humans was correlated with first week mortality ($r^2=0.49$, $P<0.01$).

The existence of these significant interrelationships between human behaviour and bird behaviour and performance demonstrates the need to examine whether or not these relationships are "cause and effect" relationships. Our present research is studying this aspect by examining the effects of manipulating human behaviour on bird behaviour and performance. If these relationships are shown to be "cause and effect" relationships, the next phase of the project will involve the development and implementation of a training program aimed at improving stockpersons' attitudes and behaviour in order to improve the productivity of commercial broiler chickens.

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A COMPARISON OF THE SIZE OF THE GIZZARDS OF END-OF-LAY HENS FED A COMPLETE DIET OR FREE CHOICE FED ON WHOLE WHEAT

R.B. CUMMING and W. BALL

In the modern laying industry, rations are generally fed as mash or crumbles, with the grains and protein concentrate materials ground and mixed together. Similarly completely ground diets, usually in the crumble form, are fed to modern broiler chickens, and one of the results is that the gizzard of the modern broiler tends to be atrophied, acting as a transit rather than a grinding organ (Hill, 1971). Studies by Mastika (1981, 1987) have shown that broilers fed the same ingredients free choice (whole grain and protein concentrate) develop gizzards that are significantly larger than those of broilers fed complete diets.

In this study the gizzard sizes of 74-week old commercial crossbred (White Leghorn x Black Australorp) hens that had been fed a commercial complete diet were compared with those of similar hens that had been free choice fed (whole wheat, protein concentrate and particulate calcium). The free choice hens had been fed in this way from eight weeks of age. There were 500 hens in each group and 20 hens, visually in lay, were selected at random from each. The hens were starved overnight, killed by cervical dislocation and group weighed. The gizzards were removed, emptied and weighed. The results are shown in the Table. The reproductive status of each hen was also recorded.

Parameter	Complete	Choice
n	20	19
Body weight (g)	2209	2366
SE	77.2	67.1
Gizzard weight (g)	26.9	56.5
SE	0.73	1.85

The gizzards of the free choice hens were significantly ($P<0.001$) larger than those fed the complete diets. Whether this larger (and more normal) size reflects a more normal function of the gizzard in the choice fed hens requires further investigation. The retention of particulate limestone or oyster shell in the gizzard may be one of the advantages of this larger gizzard. Rao and Roland (1989) reported that calcium particles smaller than 0.8mm were not well retained in the gizzards of laying hens fed complete diets.

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IMPROVED METHOD FOR THE ROUTINE DETERMINATION OF XYLANASES IN FEED

N. ELVIG and P. B. RASMUSSEN

Traditionally, endoxylanases are determined by a colour release method. Remazol-dyed xylane (Biely *et al.*, 1985) has been used widely. Recently, Novo Nordisk has suggested a new In-Feed analysis method, the substrate being a cross-linked Azurine coloured wheat pentosan in tablet form, Xylazyme AX from Megazyme (Aust) Pty.Ltd. (McCleary, 1991) applicable to a wide range of xylanases.

In principle, 10 g of ground enzyme-containing feed sample is extracted into pH 6 buffer for 30 minutes. The suspension of buffer and feed is then centrifuged at 1700 g for 10 minutes. For each determination, 2 mL of the centrifugate is added to a tablet, without stirring. After 2 h reaction at 50 °C, 5 mL reagent is added to stop the reaction and the suspension is mixed and centrifuged. The absorbance of the supernatant is measured at 590 nm. Activity is determined by reference to a standard curve. The day to day variation is only minor, as can be seen from Figure 1.

The enzyme reaction is affected by the components present in the feed. Therefore, it is necessary to use a control feed to produce the standard curve. This control feed has to be the same as the sample feed, with respect to composition and physical treatment. Different feeds will result in different slopes in the standard curves (Figure 2), so the same feed must be used both for test and control determinations.

Several types of reagents have been tested to stop the reaction. Acidified 99% ethanol was found the most applicable for the In-Feed analysis, as all long chain polymers are precipitated, resulting in a clear supernatant for the spectrometer measurement. As only small coloured dextrins stay in solution, a relatively long incubation time is needed. Alternatives such as NaOH or Tris buffer (resulting in a pH of 8.2 in solution) produce an unclear supernatant which is difficult to analyse and does not stop the reaction. NaOH continues to release colour and pH 8.2 at ambient temperature does not totally inactivate the enzyme. During the subsequent filtration a systematic error may be introduced.

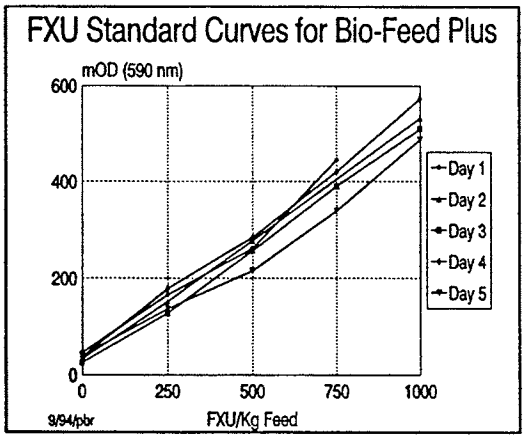

Figure 1

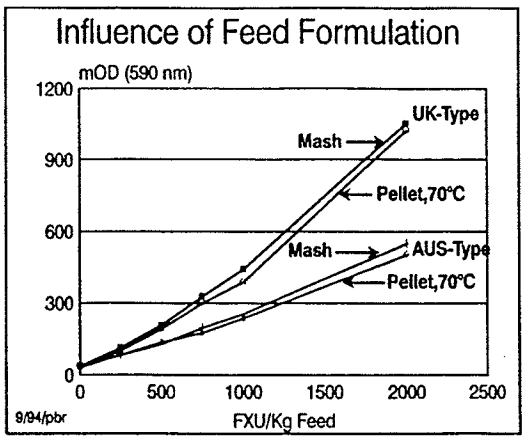

Figure 2

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RESPONSES BY GROWING CHICKENS TO DIETS WITH WHEAT BRAN WITH OR WITHOUT A FEED PHYTASE

D.J. FARRELL* and R. GOUS**

Previous results (Farrell *et al.*, 1993) with chickens showed beneficial effects on production parameters of Natuphos, a feed phytase (Gist-brocades) in diets deficient in available phosphorus (P) and based on soybean and sorghum. In this study the diets were based on maize (500 g/kg) and soybean meal (300 g/kg) with wheat bran providing phosphorus (12 g P/kg) in Diets 2 and 3 (Table). Monocalcium phosphate contributed similar amounts of P to that in the wheat bran to Diets 4 and 5. Diets were with (+) or without (-) phytase (900 U/kg). Eight broiler chicks x 3 replicates per treatment were grown on the 10 diets from 3-21 days in heated brooders.

Wheat bran and monocalcium phosphate inclusions in diets and results without (-) or with (+) a feed phytase supplement.

Diet	1	2	3	4	5
Wheat bran (g/kg)		60	120		
Monocalcium phosphate (g/kg)	1	1	1	3.8	6.7
Total P (g/kg)	3.8	4.1	4.8	4.1	5.0
Ca (g/kg)	8.0	8.0	8.4	8.3	9.0

Results

Diet	1		2		3		4		5		LSD
	-	+	-	+	-	+	-	+	-	+	(P>0.05)
Liveweight gain (g/d)	15.4	16.9	21.0	15.0	14.9	15.4	17.4	18.2	18.2	19.8	3.14
FCR (g/g)	2.01	2.11	1.90	2.07	2.11	2.12	1.90	1.89	1.90	1.90	0.184
Bone ash (g/kg)	413	456	452	481	460	490	441	485	488	510	13.9
Bone P (g/kg)	148	151	150	149	150	156	155	152	151	154	2.6
Bone Ca (g/kg)	337	330	335	347	323	337	336	340	335	333	5.4
Faecal P (g/kg)	6.6	4.7	7.3	6.7	7.4	6.8	7.2	6.0	7.9	7.1	0.7
Faecal Ca (g/kg)	16.3	13.6	15.0	14.1	13.4	11.3	18.1	15.2	16.0	15.8	2.8

The results are shown in the Table. Growth rate and FCR were variable and small differences could not be readily explained by treatment. There was a consistent response ($P<0.01$) to feed phytase for bone ash, bone P and bone Ca with a significant enzyme x diet interaction ($P<0.01$). Mean faecal P and Ca were reduced ($P<0.01$) due to enzyme treatment from 7.3 to 6.2 g/kg and from 15.7 to 14.0 g/kg respectively. Responses were greatest on Diets 1-3 although, surprisingly, smaller significant responses were also seen on Diets 4 and 5 even though they contained a significant level of inorganic P from monocalcium phosphate.

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THE ENRICHMENT OF POULTRY PRODUCTS WITH THE OMEGA (n)-3 POLYUNSATURATED FATTY ACIDS: A SELECTED REVIEW

D J FARRELL

Summary

Changes in dietary habits have resulted in large imbalances in dietary omega(n)-6 and omega(n)-3 polyunsaturated fatty acids (PUFA). Enrichment of poultry meat and eggs provides an alternative dietary source to fish of n-3 PUFA. Flax seed and canola seed contain significant amounts of α-linolenic acid (ALA). Feeding these seeds to hens can increase ALA, docosapentaenoic (DPA) and docosahexaenoic (DHA) acids in breast and thigh meat and in egg yolk. The important long chain (LC) PUFA eicosapentaenoic acid (EPA) is difficult to increase significantly in poultry products since the hen converts ALA to DPA and DHA directly; fish oil and fish meal are only moderately effective in increasing EPA. Enriched chicken meat can provide the same amount of n-3 PUFA as cod on an equal weight basis and enriched eggs significantly more. Off-flavours are often associated with n-3 enrichment of meat and eggs but can be depressed by using a combination of antioxidants. Storage life of enriched eggs appears to be similar to ordinary eggs held under similar conditions. For chicken meat storage life may be reduced.

I. INTRODUCTION

There are two distinct families of polyunsaturated fatty acids (PUFA); both are essential in our diet. Until very recently most nutritionists and dietitians did not distinguish between the n-6 and n-3 families mainly because they have several chemical features in common. Both have at least 18 carbon atoms and two double bonds. It is the location of these double bonds that is their main distinguishing feature. In the n-3 family the first double bond in the chain is located three carbon atoms adjacent to the terminal methyl group; in the n-6 family the first double bond is located at carbon 6 adjacent to the methyl group. The precursors of the biologically active 20 carbon or more long chain (LC) PUFA are linoleic acid (LA, n-6) and α-linolenic acid (ALA, n-3) (Figure 1). Man and animals cannot convert n-6 PUFA to n-3 PUFA or vice versa but with some exceptions they can extend the chain length by elongation and desaturation to form the appropriate a LC PUFA. The D-6 desaturase enzyme is the rate limiting step, and there is competition between the n-6 and n-3 PUFA for this enzyme. Thus, a diet with high amounts of LA may reduce the synthesis of eicosapentaenoic acid (20:5, EPA) and docosahexaenoic acid (22:6, DHA) from ALA (Simopoulos, 1991; Sinclair, 1991). An important LC PUFA is docosapentaenoic acid (DPA, 22:5, n-3); this fatty acid is an intermediate in the retro- conversion of DHA to EPA (Gaudette and Holub, 1991).

It is thought that the elderly (Sinclair, 1991) and the very young (Leaf *et al.*, 1992) may not have the necessary activity of desaturase and elongase enzymes to synthesise EPA and DHA from ALA. Consequently these vulnerable groups may require a dietary source of EPA and DHA. The LC PUFA occur mainly in fish but the amounts will vary greatly depending on species, season and water temperature. Some vegetable oils such as soybean,

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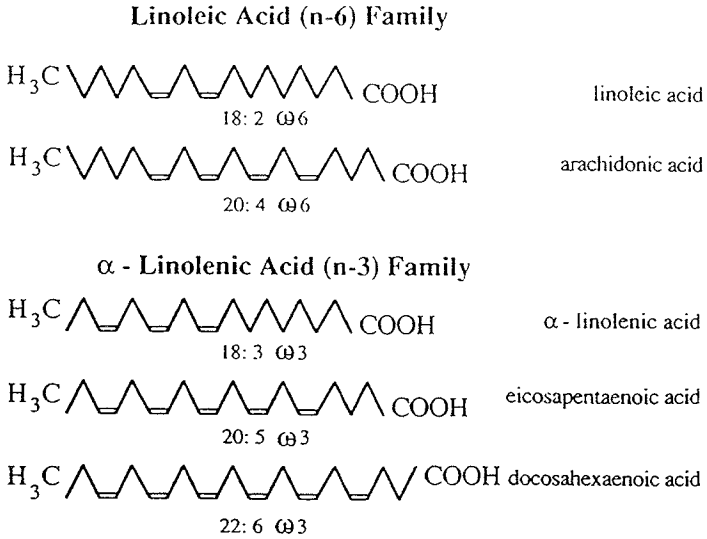

Figure 1. Structural formulas for n-6 and n-3 fatty acids. Key: First figure gives the number of carbon atoms in the molecule, figure after colon gives the number of double bonds and figure after "w" or "n" gives the position of the first double bond, counted from the methyl end of the molecule.

canola and linseed contain significant amounts of ALA.

Animals that graze on green leafy material obtain a source of both LA and ALA, and these they can convert to the appropriate LC PUFA. Shown in Table 1 is a comparison of the fatty acid profile of wild and domestic bovids (Crawford *et al.*, 1981). Clearly only the former group has meat that is significantly enriched with the n-6 and n-3 PUFA.

Grass seeds also contain significant amounts of ALA (Lamberston *et al.*, 1966) and the eggs of wild pheasants, partridge and grouse contain significant amounts of ALA of 7, 10 and 30% respectively of total FFA (Hubbard and Poklington, 1968).

It is only comparatively recently, through dietary change, that there has been a significant increase in both our total dietary fat intake and in the ratio of n-6:n-3 PUFA (Skjervold, 1992) from about 3:1 to 30:1 in Australia, for example (Sinclair, 1991). This insufficiency of dietary n-3 and the imbalance in the n-6:n-3 ratio has resulted in a number of `modern' diseases in western countries, in particular cardiovascular disease (Simopoulos, 1991; Sinclair, 1991; Sanders, 1993). Since the main dietary source of LC PUFA (EPA + DHA) is fish whose consumption is declining in many countries (Barlow and Pike, 1991), attention has turned to alternative food sources and, particularly, to poultry meat and eggs (see Hargis and Van Elswyk, 1993; Farrell, 1993, for reviews).

Table 1. Percentage fatty acid composition (SEM) of meat from wild and domestic bovids (Crawford *et al.*, 1981).

Saturated and monounsaturated	Wild (n=10)	Domestic (n=10)
16:0	16.0(1.2)	28.0(0.6)
18:0	20.0(1.0)	12.0(1.1)
18:1	21.0(1.8)	40.0(1.6)
Polyunsaturated		
18.2(n-6)	16.0(1.6)	2.1(0.6)
18.3(n-3)	5.0(0.8)	0.8(0.1)
20.4(n-6)	8.2(0.6)	0.7(0.2)
22.5(n-3)	3.2(0.4)	0.8(0.1)

II. POULTRY MEAT

The fatty acid composition of chicken meat is closely related to the fatty acid content of the diet but composition varies according to the tissue (Walisundera *et al.*, 1989). Changing the fatty acid profile of poultry meat is, therefore, not difficult (Yau *et al.*, 1991). Analysis of the carcass of chickens grown in Australia yields typical values (of total FFA) that range from 30-40% saturated fat, 49-54% monounsaturated and 10-20% PUFA of which n-3 ranges from 0.2-1.4% (D.J. Farrell, unpublished results, 1991). Barlow and Pike (1991) summarised data, mainly from South Africa, on the effects of feeding chickens different amounts of fish meal from different fish sources on carcass lipid profile. Although there was considerable enrichment of EPA and DHA in chicken lipid and, to a lesser extent, in edible meat portions, the overall capture of EPA was much less than of DHA. It has been suggested that chicken meat contributes substantially to the n-3 LC PUFA in the diets of people in the United States (Hulan *et al.*, 1988).

Attempts to enrich the carcass fat of poultry meat have been based on fish meal and fish oil. In Canada, Hulan and his group have focussed on red fish (*Sebastes* sp.) meal (100 g lipid/kg) and red fish oil (6.0% EPA). In some cases a decrease in feed consumption and an increase in feed conversion ratio (FCR) have been observed from broiler diets containing fish meal and fish oil (Hulan *et al.*, 1988). Enrichment of thigh and breast meat with n-3 LC PUFA did not parallel the increase in dietary fish meal or oil. The content of DHA was generally twice as high as EPA in meat. Thigh meat had twice as much n-3 LC PUFA as breast meat in chickens (Walisundera *et al.*, 1989) and pullets (Huang *et al.*, 1990). Adipose tissue had less LC PUFA than either white or brown meat (Huang *et al.*, 1990). White meat contains about 1% fat, thigh meat > 2% and skin 27-37% (Walisundera *et al.*, 1989). However, much of the lipid in breast meat is in the form of phospholipids (50-70%); these are only present at about 30% in dark meat and there is none in skin where the lipid is all as triglycerides (Walisundera *et al.*, 1989). The latter would be expected to contain only small amounts of LC PUFA. Interestingly, as the dietary incorporation of fish meal increased from 10 to 80 g/kg, the saturated fatty acid content of the tissues increased and total LC PUFA decreased in the three tissues examined in both male and female broiler chickens (Walisundera *et al.*, 1989).

Huang *et al.* (1990) killed laying pullets after 4 weeks on diets with 0, 10, 20 and 30 g menhaden oil/kg. This oil contained 160 g of EPA and 90 g DPA + DHA/kg. Both EPA and DHA increased with increasing level of fish oil in both thigh meat and in adipose tissue taken from the thighs. Thigh meat contained much more n-3 PUFA than adipose tissue. The content of DHA in thigh meat was much greater than EPA but the reverse was true for adipose tissue (Table 2).

Table 2. Distribution (%) of EPA (20:5, n-3) and DHA (22:6, n-3) in thigh meat and adipose tissue of pullets after 4 weeks on experimental diets with 0-30 g menhaden oil/kg (Huang *et al.*, 1990).

	EPA		DHA	
Oil (g/kg)	Thigh	Adipose	Thigh	Adipose
0	0.00 (0.00)	0.00 (0.00)	0.36 (0.24)	0.00 (0.00)
10	0.14 (0.09)	0.06 (0.01)	0.96 (0.08)	0.05 (0.01)
20	0.27 (0.15)	0.26 (0.08)	0.92 (0.08)	0.11 (0.11)
30	0.43 (0.38)	0.36 (0.04)	1.43 (0.30)	0.19 (0.05)

Hulan *et al.* (1988, 1989) concluded that edible meat from chickens fed diets with red fish meal (80 g-120 g/kg) provided the same n-3 PUFA as the same serve of white fish (cod).

Sim (1987) has used full-fat flax seed (420 g oil/kg) rich in ALA at 150 g and 250 g/kg in broiler diets. Canola seed (250 g/kg diet) was included in one treatment. Both oil seeds depressed growth rate, especially flax seed at 250 g/kg. The n-3 PUFA of breast meat is shown in Table 3.

Table 3. The n-3 fatty acid content (% total FFA) of breast meat of broiler chickens on diets with flax seed, canola seed and a control diet (Sim, 1987).

DIET	18:3 n-3	20:5 n-3	22:5 n-3	22:6 n-3	Total n-3
Soybean meal control	2.06	1.20	1.19	1.16	5.60
15% flax seed	12.05	3.38	3.22	2.17	20.82
25% flax seed	19.52	2.41	2.44	1.81	26.18
25% canola seed	5.44	1.30	2.38	2.22	11.34
SEM	0.28	0.17	0.09	0.10	0.21

There were substantial increases in all the n-3 PUFA in broilers fed the diets with the oil seeds compared to the soybean meal control diet. Canola seed contains about 110 g ALA/kg; total n-3 PUFA was increased two fold, but EPA was not increased on the canola seed-based diet in contrast to flax seed (Table 3).

Farrell (1991) demonstrated that ducks given diets, initially at 22 days of age containing 35 g of linseed or 35 g of fish oil (MAXepa)/kg and then increased to 75 g/kg at 37 days of age, when killed at 44 days showed considerable enrichment of carcass lipid with n-3 LC PUFA. Levels of EPA (1.4%) and DHA (1.3%) were similar in the carcasses of ducks on the MAXepa diet. On the diet with linseed oil, only ALA was significantly elevated compared with the controls. Similar observations on broiler chickens fed diets with 50 g cod liver, canola or linseed oils/kg from 20-39 days of age increased total n-3 PUFA in carcass mince from 0.1% (controls) to above 3%. Only cod liver oil increased EPA and DHA significantly (Farrell, 1990).

III. ENRICHED EGGS

The hen's egg usually contains about 1% n-3 PUFA when fed a conventional commercial diet (Table 4). Like poultry meat it is not difficult to manipulate egg lipid (Noble *et al.*, 1991) and to enrich the hen's egg with n-3 PUFA through dietary change. Both Caston and Leeson (1990) and Cherian and Sim (1991) in Canada fed hens on diets with flax seed and showed considerable enrichment of ALA in egg yolk. However, the hen has the remarkable ability to convert ALA rapidly to DPA and DHA in significant amounts but not to EPA.

Jiang and Sim (1994) fed hens a diet containing 150 g flax seed/kg. By calculation this contributed about 63 g of oil containing 33 g ALA/kg diet. The eggs contained 9.1% of total n-3 PUFA of which 5.8% was ALA, 0.3% EPA, 0.3% DPA and 2.7% DHA. An egg contains about 10% lipid; an enriched 60 g egg would, therefore, contain 540 mg total n-3 PUFA. Assuming a daily intake of 110 g feed/day and an egg mass of 50 g/day, this would mean a capture of 13% dietary n-3 PUFA on a diet with 150 g flax seed/kg. A problem with feeding flax seed is that it may decrease egg production and those who have experimented with flax seed have been reluctant to give details of production responses.

Interestingly, Ferrier *et al.* (1994) reported identical n-3 PUFA enrichment of eggs from hens fed diets with 200 g flax seed/kg to that of Jiang and Sim (1994) who fed only 150

g flax seed/kg. Sim (1987) found that when hens were fed diets containing 80 or 160 g flax seed/kg, enrichment of egg yolk with DHA was similar to, and not different from, a diet containing 160 g canola seed/kg. Kennedy *et al.* (1994) and Aymond *et al.* (1994) found that hens, when fed dietary flax seed which contains diphenolic compounds which can be converted by microbial synthesis to lignans, produced a high number of eggs with double yolks. Oviduct and follicle weights decreased on diets containing flax seed and circulating estradiol was also significantly reduced compared to hens on a control diet.

Farrell *et al.* (1991) showed that with different combinations of fish oils and vegetable oils it was possible to predict the enrichment of hens' eggs with n-3 PUFA (Table 4). As stated, significant enrichment of egg yolk with EPA will occur only on diets containing fish oil or fish meal. However, rate of capture of n-3 PUFA declines with increasing inclusion of fish oil in the diet. In Table 4, it is evident that 60 g fish oil/kg feed will provide an egg containing about 1% EPA and very little ALA. However, cod liver oil contains about 2% ALA, and mackerel oil > 0.5% ALA, thus the difference in the ALA content of eggs from hens fed diets containing these two fish oils (Table 4). Fortification of diets with the amounts of oils shown in Table 4 could enrich 60 g eggs with typically > 400 mg PUFA of which 200 mg is n-3 LC PUFA. Many fish varieties on an equal cooked weight basis would contain much less n-3 LC PUFA than this.

Table 4. Fatty acid composition of eggs from hens given diets with different vegetable and fish oils (Farrell *et al.*, 1991).

Diet	Oil (g/kg)	Fatty acid (%)						Total n-3	Ratio n-6:n-3
		16:0	18:1	18:2	18:3	20:5	22:6		
Commercial		25.1	46.7	12.7	0.2	0.2	0.6	1.2	13.0
Cod liver	60	26.9	42.5	11.3	0.6	0.9	4.3	6.3	1.9
Mackerel	60	27.8	38.5	9.9	0.3	1.1	5.8	7.9	1.4
Mackerel	40	27.2	40.1	12.5	0.4	0.7	4.4	6.0	2.3
Mackerel	20	27.9	40.5	11.8	0.4	0.4	3.4	4.6	2.8
Linseed/	20								
Mackerel	20	24.5	43.2	12.5	3.3	0.5	3.3	7.5	1.8
Canola/	20								
Mackerel	20	25.6	42.3	13.1	0.8	0.4	3.1	4.6	3.1
Canola/	20								
Linseed	20	21.4	46.7	14.1	3.3	0.3	2.0	5.9	2.6
SEM		0.43	0.60	0.26	0.23	0.06	0.27	0.36	0.65

Hargis *et al.* (1991) gave hens a diet containing 30 g menhaden oil/kg. DHA increased from about 30 mg to 170 mg in egg yolk. EPA increased from almost zero to about 25 mg after 18 weeks on the diets.

Huang *et al.* (1990) fed diets containing 0-30 g menhaden oil/kg to laying hens. This fish oil contains 15.9% EPA, 2.3% DPA and 6.8% DHA. After hens had been on the diet for two weeks, EPA and DPA reached maximum enrichment in eggs. Concentrations are shown for n-3 LC PUFA after four weeks (Table 5). Enrichment with EPA was low, but significant, despite high amounts in the oil.

Oh *et al.* (1991) produced eggs from hens fed diets containing 100 g MaxEPA fish oil/kg. This prepared oil contains 16.3% EPA, 2.4% DPA and 11.7% DHA. The eggs contained 2% EPA and 11% DHA; these are exceptional concentrations but again show that most of the EPA is deposited as DHA in the yolk.

Table 5. Fatty acid content (%) in egg yolk of hens given diets with menhaden fish oil for 4 weeks (Huang *et al.*, 1990).

Oil (g/kg)	EPA (20:5)	DPA (22:5)	DHA (22:6)
0	0.03	0.10	0.75
10	0.25	0.33	2.72
20	0.40	0.42	4.21
30	0.59	0.53	3.83

Purslane is a plant containing high concentrations of ALA (4 mg/g). Simpopulos and Salem (1992) found that hens free ranging in areas where this plant was plentiful, produced eggs with 10 times the content of n-3 PUFA of eggs from an American supermarket. In this case ALA comprised 40 per cent of the total n-3. The authors concluded that insects and other green material also contributed to the hens' n-3 PUFA intake.

IV. SENSORY QUALITIES AND STORAGE OF ENRICHED MEAT AND EGGS

Significant off-flavours have been reported for poultry meat and eggs that have been enriched with n-3 LC PUFA (Hargis and Van Elswyk, 1993). Use of suitable combinations of antioxidants have been used to suppress these off-flavours. Farrell and Thomson (1994) reported results from 78 untrained persons who were unable to detect differences in flavour, taste, colour and texture between enriched and commercial eggs. Van Elswyk *et al.* (1992) found that eggs from hens on a diet with 30 g menhaden oil/kg were different from commercial eggs when sensory-evaluated but only when scrambled and not hard cooked. Ferrier *et al.* (1994) and Jiang and Sim (1994) reported that eggs from hens on diets containing 150-200 g flax seed/kg had a "fishy or fish-product related flavour" when evaluated by a panel. This was subsequently overcome by adding a natural antioxidant to the diet (Jiang and Sim, 1994). Storage of n-3 enriched eggs appears to be no different from ordinary eggs held at the same temperature (Farrell and Thomson, 1994; Ferrier *et al.*, 1994). For enriched chicken meat there is increased lipid oxidation during storage (Ahn *et al.*, 1994).

Significant off-flavours are often found in meat from chickens fed diets with fish oil and fish meal (Hargis and Van Elswyk, 1993) although these flavours may not be objectionable (Hulan *et al.*, 1984). However, Walisundera *et al.* (1989) found that meat from chickens fed red fish meal-enriched diets showed no consistent off-flavours and stated that 120 g fish meal/kg gave acceptable meat. Hargis and Van Elswyk (1993) concluded that fish meal may give a much lower taint in meat than fish oil when added to broiler diets.

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EFFECT OF CAGE MODIFICATIONS ON INCIDENCE OF CRACKED AND DIRTY EGGS

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

Conventional laying cages have been criticised as providing a barren environment which can be associated with abnormal hen behaviour and compromises of welfare. To improve welfare of hens the cage environment has been enriched by incorporating perches (Tauson, 1984a; Duncan *et al.*, 1992) and solid sides (Tauson, 1984b) resulting in a range of welfare and production advantages for the hen. However. a consistent disadvantage of including perches has been an increase in the numbers of cracked and dirty eggs (Appleby *et al.*, 1992). This study examines the influence of perches and solid sides on the incidence of cracked and dirty eggs from commercial Australian laying strains housed in a naturally ventilated cage layer shed.

There were 40 replicates of four treatments in the experiment comprising 4 cage modifications; control, perch, solid side and perch + solid side. The experimental cages (450 x 450 x 400mm) were modified by installing a rectangular wooden perch, 45mm wide and 35mm deep, 75mm above the cage floor in the middle of the cage and parallel to the feed trough. In the solid side treatment, sheets of polypropylene (1.5mm thick) were fixed to the sides of cages. When hens were 40 and 70 weeks of age, all eggs were candled over 5 consecutive days to determine the incidence of dirty eggs and cracks recorded either as pinhole cracks, star cracks or line cracks. The percentages of cracked and dirty eggs for each treatment were averaged over 40 and 70 weeks and are shown in the Table.

Cage type	Total cracks (%)[1]	Line cracks (%)	Star cracks (%)	Pinhole cracks (%)	Dirty eggs (%)
Perch	13.0^{a}	8.2^{a}	3.1^{ab}	1.7^{a}	14.2^{a}
Side+perch	12.6^{a}	7.0^{a}	3.3^{a}	2.3^{a}	11.4^{ab}
Side	5.4^{b}	2.7^{b}	2.1^{b}	0.6^{b}	11.6^{ab}
Control	6.0^{b}	3.0^{b}	2.6^{b}	0.4^{b}	10.1^{b}

[1] % of all eggs candled.

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

Hens were quite often seen laying eggs from perches; this accounts for the significantly higher percentage of cracked eggs from hens on perches. To support this observation, the percentage of line cracks caused by eggs colliding with the rigid cage floor from perch treatments was more than double that observed from non-perch cages. There were certain areas in perch cages which were not used by birds for standing resulting in an accumulation of manure. This contributed to a higher percentage of dirty eggs from the perch treatment compared with the control.

Quite clearly perches contribute to an increase in cracked and dirty eggs, a problem that might be overcome by fixing perches to the cage floor or providing a nest site.

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MOTILITY OF THE DIGESTIVE TRACT OF CHICKENS

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Summary

The digestive tract of the chicken is greatly different to that of the much more studied mammals. It has many adaptations which profoundly influence the type of diet that may be eaten and the digestion of that diet (e.g. lack of teeth). This review examines the major motility patterns noted in the chicken digestive tract. Much of the data in the literature has been gathered from turkeys, but it is assumed that these birds have similar characteristics to chickens.

I. OESOPHAGUS AND CROP

The oesophageal musculature is predominantly smooth muscle, with an outer longitudinal layer and an inner circular layer in the muscularis. The crop is simply an outpouching of the oesophagus and possesses a similar musculature.

The amount of time material stays in the crop ranges from 2-17h, depending largely on the consistency, hardness, water content and amount of material consumed (Dansky and Hill, 1952). The first bolus swallowed by a fasted bird enters the crop. However, food may also bypass the crop and go directly to the gizzard. Food that has reached the proventriculus may also be returned to the crop (Vonk and Postma, 1949). The state of contraction of the gizzard at the time of deglutition appears to determine whether the bolus enters the crop. In fact, there is substantial coordination between the activity of the crop and that of the gizzard (Pastea *et al.*, 1968). When the crop is distended, the motility of the gizzard is inhibited and the output of acid by the proventriculus is increased (Hill and Strachan, 1975).

Emptying of the crop is achieved by contractions of the crop and oesophagus which causes boli to be extruded and directed aboradly. With fasting the frequency of crop contractions increases (Macowan and Magee, 1932). The crop is largely innervated by parasympathetic excitatory fibres from the vagus nerve. Early studies suggest that the left vagus may be more important than the right in controlling crop motility (Sturkie, 1976).

II. PROVENTRICULUS AND GIZZARD

It is necessary to group the motility patterns of the gizzard and proventriculus as these two organs contract as part of a cyclic rhythm which also involves the more orad parts of the intestine.

Food normally passes rapidly through the proventriculus to the gizzard. The prime function of the proventriculus is simply to produce a secretion rich in pepsinogen and HCl.

The gizzard shows massive muscular development, with two pairs of opposing muscles. These muscles are simply termed thick and thin pairs (Dziuk and Duke, 1972). The thick muscles are about 8mm, whilst the thin muscles are about 2mm, thick. These muscles are circular smooth muscle arising from a central aponeurosis.

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The contraction cycle begins with a contraction of the pair of thin muscles of the gizzard. This is followed by relaxation of these muscles with an opening of the pylorus. duodenum then begins a strong peristaltic movement. Immediately after the initiation of the duodenal movement the thick muscles of the gizzard contract. This contraction pushes some gizzard chyme into the duodenum. The pylorus then closes and the isthmus opens, allowing orad movement of the digesta into the proventriculus. As the gizzard muscles relax the proventriculus contracts moving the contents back to the gizzard. The isthmus then closes. This cycle repeats about every 20 seconds (Duke, 1986).

As expected the highest intraluminal pressures are recorded during contraction of the thick muscles of the gizzard. Interestingly the pressures recorded by some workers are much higher than others and probably reflect differences in diet. Gizzards from birds fed whole grain feeds produce much higher pressures than those from birds fed mash (Duke *et al.*, 1972; Sturkie, 1976). This is also reflected in the muscular development, with birds fed pelleted diets having poorly developed gizzards compared to those fed whole grain-based rations (R.B. Cumming personal communication).

An additional movement that occurs about every 30 minutes is intestinal reflux. This movement involves all of the duodenum and the upper half of the ileum in a powerful antiperistaltic movement (Dziuk and Duke, 1972).

Regulation of Proventricular and Gizzard Motility

Cephalic Phase - During this phase, the sight of food and the anticipation of eating increase the frequency of stomach contractions. The mechanism is uncertain but is grossly attenuated by sectioning the vagal and sympathetic supply to the proventriculus and gizzard. It is also suggested that an unidentified humoral component may also be involved (Chaplin and Duke, 1988).

Gastric Phase - Upon eating food there is a greatly increased frequency of stomach contraction cycles. Following selective denervation with benzalkonium chloride, Chaplin and Duke (1990) have shown that pacemaker activity for the stomach-duodenal contraction cycle is derived from an area located near the isthmus, probably near the termination of the vagus nerve at the medio-dorsal isthmus. A secondary pacemaker with a lower frequency also probably exists elsewhere in the gizzard. An intact myenteric plexus is necessary for propagation of the pacemaker potentials to the areas involved in the contraction sequence (Duke, 1992).

Intestinal Phase - The chemical composition and volume of duodenal chyme regulates stomach motility via the enterogastric reflex. If the stimulus is severe enough, the activity of this reflex may also result in reflux of intestinal contents back into the gizzard and proventriculus. Hypertonicity, hyperacidity, amino acids, lipids and distension all inhibit stomach motility. Part of this inhibition may be due to the actions of cholecystokinin (Savory *et al.*, 1981) and avian pancreatic polypeptide (Duke *et al.*, 1985). These are the only two gastrointestinal hormones that have been studied in this area of physiology.

There is significant diurnal variation in the motility of the gizzard and proventriculus. At night, as a consequence of reduced vagal tone, there is a lower frequency of contraction and an increase in retroperistalsis. This difference occurs in both fasted and fed birds in anticipation of changes in illumination, not in response to them (Duke and Evanson, 1976). Day-old chicks and mature laying hens have a smaller diurnal difference in motility, and it is speculated that this difference may reflect increased

metabolic needs for calcium and/or energy (Roche and Decerprit, 1977). Vagotomy also results in disappearance of the diurnal variation (Ruckebusch *et al.*, 1991).

III. SMALL INTESTINE

A migrating myoelectric complex (MMC) similar to that found in mammals has been demonstrated in chickens (Clench *et al.*, 1989). This myoelectric complex correlates with a broad band of contraction that moves slowly along the intestine at about 0.6 cm/min, with contractions occurring about 6 times/min.

Recently a uniquely avian myoelectric pattern has been demonstrated (Clench and Mathias, 1993). Termed the rhythmic oscillating complex (ROC), this myoelectric complex occurs during fasting and involves a very rapidly propagated (25cm/s) series of spike bursts that periodically change direction in a highly organised way. About 37% of these spike bursts are orad with the remainder being aborad. A ROC lasts about 7.5 min. Immediately after a ROC, normal fed pattern MMCs recommence, suggesting that the purpose of these movements is to move caecal digesta back to the duodenum when an animal is hungry.

IV. CAECA

The caeca show two types of contraction. A low amplitude contraction (minor) that occurs about every 25 sec and a stronger contraction (major) occurring about every 50 sec (Duke *et al.*, 1980). The minor contractions appear to be for mixing whilst the major contractions propel digesta both orad and aborad. These movements are myogenic (Hodgkiss, 1984) and their control is unclear.

V. RECTUM

The most interesting aspect of rectal motility is its almost continual orad peristalsis, with strong contractions occurring about 15 times per minute, the only interruption being for a short period before, during and after defaecation (Lai and Duke, 1978).

VI. CONCLUSION

The gut is a highly adaptable organ and is also subject to strong evolutionary change. As an example, there are often unexplained large differences in gut structure and function between closely related species, eg. owls have well developed caeca, yet hawks have no caeca!

Much remains to be learnt about the control of gut motility in poultry. Nearly all the knowledge about this subject has been derived from studies conducted in one laboratory (Prof.G.E.Duke, University of Minnesota). Alteration of gut motility patterns in most species are known to have large effects on the rate of passage of digesta. The importance of passage rate on the digestion and absorption of nutrients is well known. Both antibiotics and coccidial infection also decrease motility and hence reduce passage rate. Do modern diets and management practices produce optimal motility for efficient digestion and absorption? Given the selection pressures, bought about by modern dietary changes, it is essential that more is learnt about this prerequisite process for digestion, so that this question may be answered.

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EFFECT OF AGE TO SLAUGHTER WEIGHT ON THE BREAST MEAT YIELD OF BROILERS

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Investigations of the effects of dietary amino acid concentrations on the breast meat yield of broilers may involve the use of dietary formulations that result in differing rates of bodyweight gain. A study was conducted to determine whether the breast meat yield of male and female broilers was influenced by the age at which the desired slaughter weight was reached.

Forty male and forty female broilers of the Ingham TM98 strain were housed in deep litter floor pens with free access to town-water. Birds had free access to commercial starter crumbles (CP 220 g/kg) from 0-21 d of age and commercial finisher pellets (CP 190 g/kg) from 21 d to termination. Individual birds were sacrificed upon reaching the desired slaughter weight of approximately 2.2 kg. Birds were killed by cervical dislocation and eviscerated by removing the gastrointestinal tract, liver, spleen and abdominal fat. The resulting carcass was weighed to provide an eviscerated carcass weight before the removal and weighing of the breast meat. Breast meat yields were then calculated as a proportion of the eviscerated carcass weight.

The male broilers in this study ranged in liveweight from 2151 to 2312 g with eviscerated weights from 1674 to 1926 g. These weights were obtained from 40 to 55 d of age. The female broilers ranged in liveweight from 2124 to 2248 g with eviscerated weights from 1665 to 1878 g, and an age at slaughter ranging from 45 to 56 d.

The following equations were obtained by linear regression of breast meat yield against age taken to reach slaughter weight :

Males : Breast yield = 20.8 - 0.0714 x Age
$R^2 = 0.01$ P = 0.263

Females : Breast yield = 17.0 + 0.0370 x Age
$R^2 = 0.00$ P = 0.587

Linear regressions for each sex showed no significant relationship between breast meat yield and age at slaughter. Furthermore, there were no significant relationships between breast meat yields and eviscerated weights within the weight ranges studied (R^2 = 0.012 and 0.000 , and P = 0.278 and 0.480 for males and females, respectively). These results indicated no effect of the age taken to reach a defined slaughter weight on the yield of breast meat within the age ranges occurring in this study. Furthermore, the lack of any significant relationship between breast meat yields and eviscerated weights indicates that the weight ranges occurring in this study were narrow enough to prevent bodyweight at slaughter from influencing the yield of breast meat.

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METHODS OF EVALUATING SIMULATION MODELS

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Summary

The development and use of simulation models is becoming more widespread among poultry scientists and producers, and as the models become increasingly complex, it is useful to reflect on procedures available for evaluating such models. The principles involved in testing a model should be similar to those used by scientists to test a theory or an hypothesis. It is not possible ever to prove a theory correct (or to validate a theory): falsification carries more weight than verification. In this paper, six criteria are presented which are effective in evaluating models. These are whether the model is useful, whether the theories incorporated into the model have been tested, whether the pattern and the magnitude of the simulated response is sensible, whether the model outputs are realistic, and whether the user-interface is friendly. Examples are given of how these criteria can be applied to the evaluation of a broiler growth model developed by the author.

1. INTRODUCTION

A simulation model is a theory, or a combination of theories, that describes a system. Such models are usually applied to particular problems in an effort to solve those problems, and are not necessarily dependent on computer technology, having been used extensively long before the advent of computers. But the scope and complexity of the models that are now being produced is far greater than ever before, mainly because of the availability and power of personal computers. As the complexity has increased, so the evaluation of these models has become more difficult; and because it is essential that models are tested, it is worth analyzing the procedures used for testing them.

Models are becoming more abundant. Some are used to predict the position of the planets in the solar system, some determine the length of time that a traffic light remains green before changing to red, while there are others that are used to assist game wardens in determining the optimum stocking densities of antelope and predators in a game reserve. In the poultry industry, models are used in many different ways: to calculate the least-expensive combination of feedstuffs that will contain the desired concentrations of all the essential nutrients; to determine the optimum time to cull a laying flock; and to predict the food intake and growth rate of broilers subjected to different feeds and different environments. Each of these models needs to be tested before faith can be put in their predictions, but there is more to evaluating a model than determining whether the predictions are realistic.

Emphasis will be placed in this paper on the evaluation of a model that simulates the food intake and growth rate of broilers. It is a model that has been produced within our Department, and is based on the theories propounded by Emmans in a number of publications, including Emmans (1981; 1989), Emmans and Fisher (1986) and Emmans and Oldham (1988). This model represents a biological system that is very complex, in spite of the relative simplicity of the theories that have been incorporated into the model.

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This complexity limits the usefulness of experimentation, the traditional approach, in solving nutritional problems, as the many interacting factors that contribute to the growth rate and food intake of a growing broiler are difficult to describe and control. Evidence abounds of apparently similar experiments each yielding very different results; the difficulty is that we often attribute observations to incorrect nutritional or environmental variables, i.e. we frequently do not know or understand what experiment we have done. Simulation models can assist greatly in overcoming these problems, but the models can also appear to have been falsified by these same experiments.

The principles involved in testing or evaluating a model are the same as those used by scientists to test a theory or an hypothesis. Models, like theories, are not verifiable; it is not possible ever to prove a theory correct (or to validate a theory). The process of induction can be used to provide support for an hypothesis, in the same way as a model can be shown to pass a test, but this process lacks the ability to guarantee its conclusions. Popper (1983) believes that the correct way to test a new theory is to test how far the new consequences of the theory stand up to the demands of practice, or to practical technological applications. This is accomplished by making predictions that can be deduced from a previous theory and then selecting, from these predictions, statements which the current theory contradicts. If, by comparing these predictions with the results of specific experiments or practical applications, it is possible to verify the new theory, then there is no reason to discard the new theory; it has been corroborated but not validated. But if the conclusions have been falsified, then the theory has also been falsified. This does not usually lead to the rejection of the theory, but rather to a search for a plausible reason for the failure or to a slight modification or refinement of the theory.

The evaluation of models is not easy, especially when they are complex and contain many interacting theories. Fisher (1991) listed six criteria on which the value of a model should be judged, and these have been used as the basis for the discussion which follows. These criteria are whether the model is useful, whether the theory has been tested, whether the pattern of response and the magnitude of response are sensible, whether the model outputs are realistic and, finally, whether the user interface is friendly. Each of these is discussed in turn.

II. IS THE MODEL USEFUL?

There are a number of objectives of modelling. One is purely scientific, as an intellectual tool: a model can be used as a means of discovering how a system works, or how different systems interact. Such models can be useful in that they may lead to improved theories, or be the basis of important and innovative experimentation. Other models, developed purely for commercial purposes, can be as useful but in an entirely different way. The purpose of modelling is to solve problems, and these problems should be clearly stated at the start of the modelling process. On the basis of the problem that is being addressed, it is likely that a model will always be useful, although sometimes only to a very limited audience.

More specifically, the model should answer the questions that are likely to be asked by the users of such a program. For example, in a broiler simulation model, inputs likely to be required by a user would encompass different breeds and sexes, different feeding programmes (on a time basis or on an amount-fed basis, with feed intakes either being *ad libitum* or on a restricted basis) and different environments. The outputs that would be useful to most users would be the effects of the above inputs on food intake, growth rate,

carcass composition (both chemical and physical), as well as a listing of the factors limiting the potential on each day of the growing period. A financial summary would clearly be particularly useful to those users having to make economic decisions about the most profitable genotypes, feeds, feeding programmes and environmental conditions to be used under their particular circumstances.

With further-processing of broiler meat becoming ever more popular, the degree of innovation of processors in the range of portions and cuts being produced is staggering. In the South African market, for example, there has been an increasing awareness of the demand for heads and feet, and producers are interested in predicting what weight of these components might be produced by broilers of different ages and sexes, and whether the weights can be changed significantly by feeding broilers differently. Clearly, the model inputs and outputs should be sufficiently comprehensive and versatile to allow users to determine the value of a combination of different cuts, which might be put together in a value-added pack. What is important is that the model should address the needs of the user.

III. HAS THE THEORY BEEN TESTED?

Most models are based on a theory or a set of theories, and many of these theories have been corroborated by research. However, there are good theories as well as poor theories and it is imperative that the model user is satisfied that the theory incorporated into the model is believable. Now that simulation models have become a marketable commodity, many of the theories incorporated into them are deliberately kept hidden, which I believe is dishonest. It is essential that the theoretical structure of each part of the model should be explicit and should be one that can be examined professionally. Some examples of the testing of theories incorporated into our broiler model are given below, to illustrate the process of testing.

(a) Growth is well described by the Gompertz growth curve

Emmans (1989) has asserted that the growth of animals is well described by a Gompertz growth curve and because of the virtues of this equation, for example, in determining allometric relationships between body components, the simulated potential growth rate of broilers in the model is based on this equation. The accuracy of this equation in predicting the growth rate of laying-type pullets (Martin *et al.*, 1994) during the first 13wk of growth is evident in Figure 1(a). Apart from justifying the use of the Gompertz equation, these results illustrate the effectiveness of a low density feed on growth restriction after 13 wk of age.

This accurate prediction of the growth of laying pullets should be compared with that for a broiler female, Figure 1(b), from another experiment in our Department. In this case, the observed growth rate in the first three weeks appears to follow a different Gompertz curve to that thereafter. This is not an isolated observation - in no instance has the growth rate of male broilers been shown to be greater than about 80 g/d, although the Gompertz equation fitted to the growth rate during the first three weeks indicates that broilers have the potential to grow considerably faster than has been observed.

This does not disprove the Gompertz equation as being a predictor of the potential growth rate of broilers, but what it does illustrate is that some, as yet unknown, factor(s) is preventing broilers from achieving the growth rates of which they are genetically capable. It is unlikely that the factors responsible are feed- or environmentally-related, but they may

be associated with a physiological constraint in the modern broiler which is manifested in conditions such as sudden-death syndrome and ascites. It is worth ensuring, by further experimentation, that it is not the food or the environment that is the limiting factor.

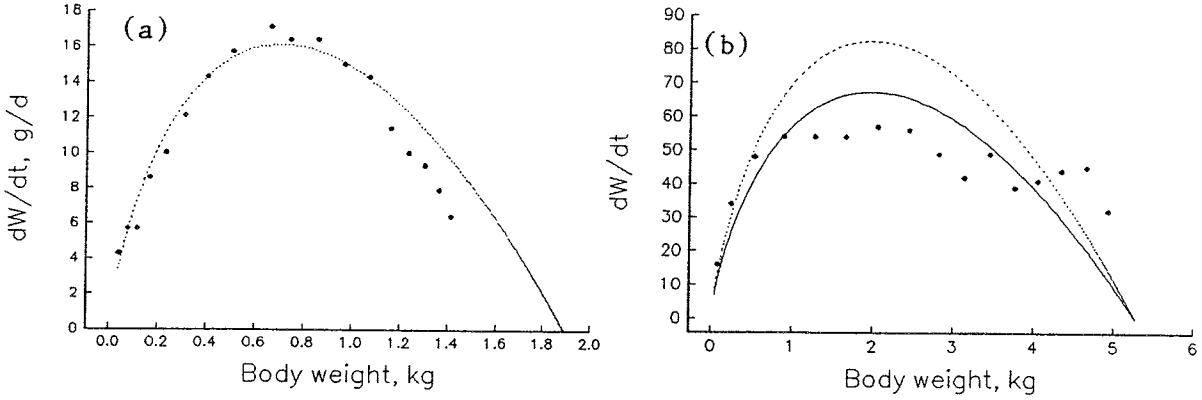

Figure 1. Observed body weight gains of laying type pullets (a), and broiler females (b), during the growing period, plotted against their body weights. Gompertz growth curves have been fitted to the growth data. In (b), two growth curves have been fitted, to illustrate the apparent potential growth rate (—) and the potential based on their early growth rate (----).

(b) <u>There is a genetically-determined degree of fatness in an animal towards which the animal will tend, if given the correct conditions</u>

For purposes of prediction it is useful to assume that under non-limiting conditions the chemical components of an animal are allometrically related, and that the weights of the different components can, therefore, be predicted from the body protein weight. This applies to the lipid component as well as to the water and ash components. It is known that the actual fatness of an animal is sensitive to its treatment (Emmans, 1989), with departures from non-limiting conditions causing fatness to be either above or below that of potential growth. An important theory incorporated into the model states that animals made nutritionally fat or lean will always attempt to revert to their genetically-determined fatness, which is related to the body protein content at the time, if given the opportunity to do so.

To test the hypothesis that an animal will at all times attempt to retain its genetically-determined lipid to protein ratio, two experiments were conducted (Gous *et al.*, 1992) in which broilers were fed from day old on feeds of either low or high protein content, until they weighed 1000g. At that weight, in the first experiment, they were offered one of four feeds differing in protein content (310, 248, 186 and 124 g protein/kg food), while in the second experiment, involving lean and fat lines, only the two feeds with the highest protein contents were offered. The subsequent feed efficiency was greater for those from the low-protein feed, particularly where they were subsequently given feeds of high protein content; on the feeds with the two highest protein contents the improvement in efficiency in the first experiment was 20 percent. In the second experiment, the improvement in feed conversion efficiency was 35 and 17 percent higher in males and females, respectively, where they had previously been on the low-protein feed. The reason for this improved efficiency was that the birds utilized their excess fat reserves as an energy source, thereby requiring lower intakes especially of the high-protein feeds.

(c) The effective energy scale is more accurate than the metabolizable energy scale in predicting the amount of feed energy available for productive purposes

The metabolizable energy (ME_n) supplied by a feed comes from three digestible components, protein, fat and carbohydrate. Only if two conditions are met will the ME_n scale be sufficiently accurate. These are: (1) the Me_n from each of the three digestible components has equal value, and (2) there is no effect of the organic matter that is not digested on the energy yielded to the animal by the feed. De Groote (1974) pointed out that the first assumption is not likely to be true: protein energy is less valuable than that from carbohydrate energy, and, for lipid retention, fat is more valuable. Emmans (1994) has shown that the ME from feeds of low digestibility is less useful than that from feeds of high digestibility. Adjustments to the ME_n scale are, therefore, necessary in order to improve the accuracy of prediction of the energy available to the animal from the feed. The effective energy system (Emmans, 1994), which has been incorporated into our broiler model appears to accomplish this, as is evidenced by the independent assessment conducted by Emmans in his paper.

IV. IS THE PATTERN OF RESPONSE SENSIBLE?

It is easy to find any number of individual treatments that can be predicted accurately by just about any simulation model. The performance of flocks of commercially reared broilers, or single treatments lifted from scientific papers, can provide many such examples that can be used to impress. This is because there are usually a sufficient number of variables in a model that can be manipulated in order to produce the desired result. A more rigorous test of a model is one in which an evaluation is made of the results of a response experiment, in which there is a logical structure to the set of treatments, as it is critical that the model accurately predicts the pattern of response. Simulation models should, therefore, be judged according to their ability to match the results of well conducted response experiments.

The types of responses which could be used to evaluate simulation models are those in which responses have been measured to different amino acid and protein concentrations, to different nutrient densities, to different environmental temperatures and genotypes, to experiments in which fat is substituted for starch, and to feed restriction experiments. It is in the evaluation of experiments such as these that great circumspection is needed before the model is rejected outright.

Most experiments are not comprehensively reported in the literature. Geneticists tend to describe the genotypes well but gloss over a description of the feed and the environment; nutritionists tend to describe the feed in considerable detail, but fail to describe the genotype or the environment sufficiently; and environmental physiologists describe only the environment in detail. A good example of this tendency is the series of papers by Leenstra and Cahaner (1992) in which five different broiler crosses were subjected to three temperatures (described in the paper) - an ideal experiment to use for evaluating growth models. Growth rate, feed efficiency, breast meat yield and abdominal fat were all reported, but the only reference to the feed was that it contained 3203 kcal ME and 215g crude protein/kg diet. This information is insufficient to allow the growth of these genotypes to be simulated. As the use of models becomes more widespread, and if they are to be evaluated satisfactorily, it becomes increasingly valuable and important to

have available more comprehensive details of the genotype, the feed and the environment used in each experiment.

In the absence of comprehensive details it is difficult, but possible, to make use of some experiments in model evaluation, depending on the details that are given. A number of response experiments have been chosen from the literature and from theses published in our Department as examples with which to evaluate our simulation model. In general, these responses, either to a single amino acid or to balanced protein mixtures, are accurately predicted by the simulation model. Comprehensive evaluations have been conducted on six response experiments reported by Maclachlan (1982), Clark (1982) and Han and Baker (1993), but due to lack of space only one such example is given here to illustrate an important point.

Two simulated results are presented in Table 1, for the same set of data from the experiment of Maclachlan (1982), one of which resulted in a significantly better fit to the observed data than the other. In the initial simulation, a constant temperature of 31°C was used, this being the temperature reported in the thesis. The simulated results were considerably improved by reducing the temperature as the experiment progressed, the reduction being proportional to the dietary lysine concentration. This correction can be justified on the grounds that in tiered brooder cages, broilers may choose to move away from the heat source into a cooler, more comfortable environment, if this results in their being able to eat more of a feed low in protein.

Table 1. Observed and simulated results of the response of broilers to dietary lysine (from Maclachlan, 1982).

Lysine concentration	Observed		Predicted Constant Temperature		Predicted Comfort Temperature	
g/kg	Growth g/d	Food in g/d	Growth g/d	Food in g/d	Growth g/d	Food in g/d
14.9	22.2	36.7	22.3	33.0	22.3	33.0
12.3	22.3	37.0	22.5	34.0	22.5	34.0
9.7	19.6	37.3	18.0	30.9	19.3	33.3
7.0	17.0	36.7	9.9	22.4	17.1	38.3
4.4	11.1	31.6	4.1	14.3	9.8	32.5

In all the experiments mentioned above, the pattern of response, in both weight gain and food intake, closely parallel the observed results when corrections are made to the environmental temperature. No simple theory based on empirical equations would be capable of matching the pattern of food intakes in the three studies (six experiments) mentioned above, as accurately as did this model, hence the value of using the pattern of response as a measure of the usefulness of a model.

V. IS THE MAGNITUDE OF RESPONSE SENSIBLE?

If models are to be useful as a basis for economic analysis they need to predict accurately the magnitude of response, since small differences in animal performance can have large economic consequences. In general, however, models will predict the rate of response or a difference between two treatments more accurately than the actual level of performance (Fisher, 1991). The types of experiments that are useful in evaluating

whether models accurately predict the magnitude of response are exactly the same as those used for evaluating the pattern of response.

In the evaluations of the experiments reported above, the magnitude of response is sufficiently accurate to ensure that sensible economic decisions could be made of the optimum lysine or protein concentrations to be included in feeds for broilers. The major advantage of such a model over a response experiment is that, whereas the experiment indicates the optimum intake of lysine under the conditions imposed, but not always recorded, by the researcher, the model is able to predict the optimum dietary concentration of lysine over a wide range of conditions.

Although a nutritionist or a producer may not be interested in the consequences of deficient feeds or of restricted food intake on performance, nevertheless, such treatments provide more powerful tests of the models and can increase the confidence of the user in models if they pass such tests by accurately predicting the magnitude of the response to such treatments.

VI. ARE THE MODEL OUTPUTS REALISTIC?

It is to be expected that the model will predict higher levels of performance and lower food intakes than are observed in the field. Many factors outside those addressed by the model have an influence on performance: feed wastage, sub-clinical disease, vitamin or trace mineral deficiencies, social competition, and poor husbandry, such as inadequate ventilation. Also, small pen trials usually give better results than do trials conducted in commercial operations, in many cases because of one or more of the factors listed above. But, if economic decisions are to be made on the basis of the outputs from the model, then these outputs need to be realistic.

The only solution to such a dilemma is to use appropriate adjustment factors. Users of models are justifiably suspicious of such factors. Their use can only be vindicated if the model has passed all the other tests, if the factor is a scaling of one or two simple practical measures, such as weight for age or feed conversion efficiency, and that the theory behind the adjustment factor itself is explicit and that its feasibility can be tested (Fisher, 1991). If the `fudge factors' are hidden at different places in the model, then their use is not justified.

VII. IS THE USER-INTERFACE FRIENDLY?

The last of the criteria listed by Fisher (1991) for evaluating models is an important issue. The ease with which inputs to models are manipulated is no guarantee of the accuracy of such models. However, if a model has passed all the other tests but is user-unfriendly, there will be a reluctance on the part of the user to make full use of the model.

Some of the more useful attributes of a model are the following. The interface should consist of pick lists from which the various options can be chosen; the manipulation of variables should be easily accomplished, with checks being made of the magnitude of the values being entered; it should be possible to alter the units of measurement to suit the user's preferences; comprehensive records of input files and the corresponding output files should be saved for later interrogation; graphical outputs of results are useful, especially if the results of different simulations can be compared in this way; and context-sensitive help should be available throughout.

With the advent of computers with greater capacity and faster processing capabilities, it is now possible to make models even more user-friendly by offering the facilities to simulate a number of different treatments, be they different genotypes, feeds, feeding schedules or environments, or combinations of these, in a single run. Also, whereas many simulation models are at the level of the individual animal, their usefulness can be improved by simulating a population of animals simultaneously. This cannot be accomplished by changing the efficiencies of response in the model, as is done in some models, but can be done by introducing stochastic elements into the model, running the model a number of times, thereby simulating different elements in the population and hence obtaining an average response to the treatment imposed. This latter option could only be done automatically, and with a large processor - it would be extremely laborious if a user had to run the model a large number of times to accomplish this result. Models have not yet achieved this level of sophistication, but will soon be there, making their evaluation even more challenging.

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DOES INGREDIENT PARTICLE SIZE AND FEED FORM INFLUENCE THE PERFORMANCE OF BROILER OR ROASTER CHICKENS,TURKEY BROILERS OR LAYING HENS?

R. M. G. HAMILTON

Summary

A series of experiments was done in which commercial broiler or roaster chickens, turkey broilers or laying hens were given mash [M], pelleted [P] or crumbled [C] feeds that contained fine [F], coarse [C] or very coarse [VC] ground grains. Compared to the FM feeds, pelleting [FMP or CMP] of the mash feeds increased the 21d body weights of the broiler and roaster chickens by 7.8-9.2% and 26.5-32.7%, respectively and the market body weights 8.7-9.8% (42d) and 11-11.7% (63d), respectively, but had little influence on the differences in 28d or market weights (84d) of the turkeys. While the intakes of the pelleted feeds were increased 3.6-6.9% for the broilers and 7.3-10.55% for the roaster, pelleting had little effect (-1.7 to 3%) on those for the turkeys. Feed utilization efficiency was 2-2.6% and 17.8-20.1% higher for the broilers and roasters, respectively given the pelleted feeds relative to those fed the FM feeds during the starter 1-21d period. Pelleting of the feeds for turkeys reduced their overall 1-84d intakes by 10.1%, but feed efficiencies were about 10.2% higher. In the case of laying hens, both pelleting [FMP or CMP] and crumbling [FMC or CMC] of the feed reduced egg production (2-4.6%), feed utilization efficiency (1-1.6%) and monetary returns (10.5-17.4%) compared to the FM feed. Feeds for broiler and roaster chickens and turkey broilers should be pelleted for best performance and economic returns, but mash feed is more desirable for laying hens.

I. INTRODUCTION

Routinely cereal grains are passed through either hammer or roller mills to reduce the size of the whole kernels to smaller sized particles (Church, 1991) and to rupture the seed coat. This grinding improves the productive performance of poultry through an increase in nutrient utilization (Schaible, 1970).

The energy required to grind dietary grains depends on the equipment used and the extent of the particle size reduction. In a comparison of milling equipment, Appel and Behnke (1988) found that the net energy required to reduce the particle sizes of corn, depending on the fineness of grind, was 7-69% less for a roller mill than for a hammer mill. Furthermore, the rate at which dietary grains can be ground is inversely related to the size of the screen used in a hammer mill for particle size reduction. Reece *et al.*(1986) reported that the grinding rate was increased by 27% when the hammer mill screen openings were increased from 4.76 to 6.38 mm. Thus,the costs to produce coarse ground grains are less than those to produce fine ground grains for feed manufacture, particularly if roller mills are used.

Most of the feeds for poultry are pelleted (Church, 1991) and, in the case of those for young birds, crumbled. While the feeding of pelleted, compared to mash, feeds increases feed intakes and body weight gains, and improves feed conversion for broiler

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chickens (Calet, 1965; Slinger, 1973), the extent of these increases or improvements vary over a wide range (Jones, 1979). The response of egg stocks to pelleted feeds also is inconsistent (Hamilton and Proudfoot, 1994).Furthermore, pelleting of feed is an energy costly operation (McEllhiney, 1985).

The extent of grinding of dietary grains and pelleting of mixed feeds are major cost factors in the production of poultry feeds because of the energy and time required to perform these operations. Therefore, a series of experiments was done to determine the effects of ingredient particle size and feed texture on the performance and profitability of broiler and roaster chickens, turkey broilers and laying (Leghorn) hens.

II. METHODS

Two experiments were done with each of broiler and roaster chickens and laying hens, and one was done with turkeys. The experiments contained 2000 broiler chickens, 1600 roaster chickens, 2400 turkey broilers or 1536 pullets; all birds were from commercial hatcheries. Equal numbers of female and male broiler chickens and turkeys, but only male roaster chickens were used. Table 1 summarizes the duration of the experiments and the crude protein and metabolizable energy (ME) contents of the diets for the meat birds. The feeds for the laying hens were fed between 140 to 490 days of age and contained (/kg): 190 g crude protein, 11.70 MJ ME, 35 g calcium and 6.7 g total phosphorus.

Table 1. Growth periods, and crude protein (CP) and metabolizable energy (ME) content of the diets for chickens and turkeys.

Type of Bird and Factor	Period		
	Starter	Grower	Finisher
Broiler chickens			
Age, days	1 - 21	-	22 -42
CP, g/kg	240	-	160
ME, MJ/kg	12.54	-	13.38
Roaster chickens			
Age, days	1 - 21	22 - 49	50 -63
CP, g/kg	160	200	160
ME, MJ/kg	12.54	12.96	13.59
Turkey broilers			
Age, days	1 - 28	29 - 63	64 - 84
CP, g/kg	290	240	170
ME, MJ/kg	12.54	12.96	13.38

The ingredient particle sizes and feed textures used in the experiments with the meat chickens and turkeys included: fine mash [FM], FM pelleted [FMP], coarse mash [CM], CM pelleted [CMP] or very coarse mash [VCM]. During the starter period, the pelleted feeds [FMP and CMP] were fed to the broiler and roaster chickens (1-21d) and turkeys (1-28d) as crumbles. With the laying hens, the VCM feed was omitted, but crumbles from the FM and CM feeds [FMC and CMC] were added to the combinations used with the meat birds. Corn, wheat and soybean meal (47% CP) were the major ingredients in the experimental feeds. Details of the management and formulations of the

broiler and roaster chicken diets were published previously by Hamilton and Proudfoot (1994a) and Proudfoot *et al.* (1989), respectively, for the turkey broilers by Proudfoot *et al.* (1990) and/or the laying hens by Hamilton and Proudfoot (1994b).

The traits recorded or calculated for the meat birds included: body weights at the end of and feed intakes during the starter, grower (except broiler chickens), and finisher periods; mortality as it occurred; feed to gain ratios from housing to the end of the starter, grower (except broiler chickens) and finisher period. Monetary returns were calculated from the revenue for the weight of live chickens or turkeys sold for processing minus the cost of the chicks or poults and the feed they consumed. Traits measured for the laying hens were: 140 and 490 d body weights, daily egg production, feed intakes over 28-d periods, egg quality [egg weight, specific gravity, albumen height and blood spots] at 195-196d and 484-485d, and egg grades of one day's collection weekly until 228d and then biweekly thereafter. Monetary returns were calculated from the weekly prices of eggs to the producer, as set by the provincial egg board, of eggs laid less the cost of the pullets and feed consumed by the hens. The cost for pelleting/crumbling was set at $13.00/tonne.

Modules from the statistical computer language Genstat 5 (Genstat 5 Comm., 1987) were used for the ANOVA analyses. Single degree of freedom contrasts were used to test for differences between mash versus crumbles and pellets, and between crumbles and pellets. The data from the two experiments with broiler and roaster chickens and laying hens were pooled for statistical analyses because preliminary analyses of the data from the individual experiments indicated that the ratio of the experimental errors were within a factor of 2 of each other for most traits.

III. RESULTS and DISCUSSION

The methods used to produce the different particle sizes of the cereal grains are shown in Table 2. Particle size distribution analyses of the broiler chicken and laying hen feeds indicated that the majority of the particles in the mash feeds were <1.0 mm in size, while $>$ 85% of the particles in the pelleted feeds were $>$ 2.83 mm. As the fineness of grind decreased there was a corresponding decrease in the portion of particles <0.71 mm in size in the mash diets.

Pelleting, and crumbling in the case of the starter feeds, of either the FM or CM diets, for the broiler or roaster chickens significantly ($P<0.001$) improved body weights at 21 days and at market, feed conversion over the starter and overall experimental periods, and monetary returns compared to those given the mash feeds. These data have been reported in more detail elsewhere (Hamilton and Proudfoot, 1994a; Proudfoot *et al.*, 1990). In the case of the turkey broilers, pelleting the mash diets (FM or CM) influenced only their body weights at the end of the starter period ($P<0.05$) and feed conversions ($P<0.001$) during the starter (1 - 28 d) and overall (1 - 84 d) periods (Hamilton, unpublished results). With the laying hens, birds fed the FM or CM feeds had higher egg production ($P<0.05$), feed intakes ($P<0.001$), monetary returns ($P<0.001$) and numbers of medium and small sized eggs ($P<0.05$), and lower specific gravity of eggs laid at 195-196 days of age than those given the FMP or CMP feeds (Hamilton and Proudfoot, 1994b). Regardless of the type of bird, ingredient particle size and feed texture had little affect on mortality.

Table 2. Operations used to produce different particles sizes of cereal grains for experimental diets.

Ingredient paticle size	Mill used	Screen or roller opening (mm)	
		Corn	Wheat
Fine	Hammer	3.2	4.0
Coarse	Hammer	5.6	-
	Roller	-	1.6
Very coarse	Roller	3.2	2.4

Table 3 summarizes the performance results for the chickens and turkeys fed the FM diets. These diets have been arbitrarily defined as controls. To compare the results from the experiments with broiler and roaster chickens and turkeys, relative differences between the values for the birds given the FM feeds and the other mash (CM or VCM) or pelleted (FMP or CMP) feeds were calculated and are expressed as percentage change. The effects of ingredient particle size and feed form on the relative differences for body weights, feed intakes and feed-to-gain ratios, and monetary returns for the meat chickens and turkeys are presented in Tables 4-6, respectively.

Table 3. Body weights, feed conversions and monetary returns for meat birds given the fine mash diets.

	Broiler chickens	Roaster chickens	Turkey broilers
Body weights, g			
at end of starter period	705	510	764
at market	1942	3296	4772
Feed conversion, g feed/g gain			
for starter period	1.401	1.852	1.408
overall	1.913	2.213	2.265
Mortality, %	3.0	4.4	2.5
Monetary returns, $/bird	0.781	1.500	1.780

When the mash feeds (FM or CM) were pelleted, the greatest improvement in body weights occurred during the starter period for the roaster chickens as evident by increases of 26.5 and 32.7% in the relative 21d body weights (Table 4). The 63d body weights of the roasters were also 11 - 12% higher for the pellet- than mash-fed birds. Pelleting of the mash feeds produced a 7.8 - 9.8% improvement in the 21 and 42d body weights of the broiler chickens. In contrast, pelleting of the mash feeds for the turkeys had little or no effect on the relative differences for the 28 or 84d body weights. Relative differences for the 21 and 63d body weights of the roaster chickens were progressively higher for the birds given the CM or VCM feeds than for those fed the FM feeds. Likewise, the relative 42d body weight differences for the broilers increased with coarseness of grind in the mash diets [CM and VCM]. There were no significant differences in the body weights of the turkeys given the CM feeds compared to those receiving the FM feeds.

As shown in Table 5, the relative differences for the feed intakes of the broiler chickens were higher (3.6 - 6.9%) for the birds given the pelleted feeds than for those fed the FM feeds. Feed ulitization efficiencies were 2.1 - 4.9% better for the broilers receiving

the pelleted feeds relative to those given the FM feeds. The increases in the relative 21 and 63d body weights of the roaster chickens to pelleting of the mash (FM or CM) feeds (Table 4) were associated with higher intakes (7.3 - 10.5%) of the pelleted feeds (FMP and CMP) than the FM feeds (Table 5) during either the 1-21d or the 1-63d periods. Furthermore, pelleting of the starter feeds increased the feed utilization efficiency of the roasters by 17.8 - 20.1% for the chickens fed the FMP or CMP feeds compared to those given the FM feeds. These differences were, however, not reflected in feed efficiency over the 1-63d period. In contrast, pelleting of the mash feeds given to the turkeys reduced their relative feed intakes by 10%, due principally to differences over the 28-84d period of the experiment. However, pelleting of the mash feeds improved the feed utilization efficiency 10.2% for the turkeys given the pelleted feeds (FMP or CMP) compared to those receiving the FM feeds. As the ingredient particle size of the mash feeds increased, the intakes of the CM and VCM feeds by the broiler chickens relative to the FM feeds also increased (2.2 - 7.9%), particularly during the 1-21d starter period. The negative values (-0.2 to -6.7%) for the relative differences in feed-to-gain ratios, however, indicated that these mash feeds were not as well utilized as the FM feeds.

Table 4. Effects of ingredient particle size and feed form on the differences (%) in body weights of meat chickens and turkeys relative to the fine mash control diets.

	Broilers		Roasters		Turkeys	
	21d	42d	21d	63d	28d	84d
FMP	9.2	9.8	26.5	11.0	3.0	-0.7
CM	2.1	2.1	2.0	2.7	-0.5	-0.2
CMP	7.8	8.7	32.7	11.7	1.8	-1.7
VCM	0.7	3.2	4.9	4.1	-	-

Pelleting of the mash feeds increased the monetary returns for the broiler chickens by 13.2 - 14%, for the roasters by 6.7 - 8.0%, and for the turkeys by 6.7 - 7.9% (Table 6). Increases in the ingredient particle size of the mash from the FM to the CM diets had a greater influence on the relative monetary returns of the roaster chickens and turkeys than for the broiler chickens.

The performance of the laying hens fed the FM feed over the 350d experimental period was as follows: egg production, 79.7% hen-day %; feed conversion, 1.453 kg/12 eggs; monetary returns, $11.14/hen; and the distribution of eggs into extra large, large and other (medium, small and pee-wee) grades, 31.8, 47.2 and 21.0%. Hens given the FM and CM feeds had higher egg production ($P<0.05$), feed intakes ($P<0.001$), monetary returns ($P<0.001$) and numbers of medium and small sized eggs, and lower ($P<0.001$) egg specific gravity at 195-196d, than the birds given crumbled (FMC or CMC) or pelleted (FMP or CMP) feeds.

Table 5. Effects of ingredient particle size and feed form on the differences (%) in feed intakes and feed-to-gain ratios for meat chickens and turkeys relative to the fine mash control diets.

	Broilers		Roasters		Turkeys	
	1-21d	1-42d	1-21d	1-63d	1-28d	1-84d
Feed intake						
FMP	6.9	5.4	7.3	10.5	0.3	-10.1
CM	5.2	2.2	-6.1	1.8	-2.2	-5.4
CMP	5.1	3.6	10.5	10.2	-1.7	-10.1
VCM	7.9	3.6	0.9	4.1	-	-
Feed-to-gain						
FMP	-2.1	-4.1	-17.8	-1.0	-3.0	-10.1
CM	2.9	0.2	-8.8	-1.0	-1.9	-6.3
CMP	-2.6	-4.9	-20.1	-2.1	-3.8	-10.3
VCM	6.7	0.4	-3.9	1.0	-	-

Table 6. Effects of ingredient particle size and feed form on the differences (%) in monetary returns from meat chickens and turkeys relative to the fine mash control diets.

	Broilers	Roasters	Turkeys
FMP	14.0	6.7	6.7
CM	2.7	6.7	8.4
CMP	13.2	8.0	7.9
VCM	4.0	6.0	-

The effects of ingredient particle size and feed texture on egg production, feed conversion and monetary returns relative to the FM feed are summarized in Table 7. Crumbling (FMC and CMC) or just pelleting (FMP and CMP) the feeds for laying hens depressed egg production, feed conversion and monetary returns relative to the values for the hens given the FM feed. Hens given the crumbled or pelleted feed, however, produced a higher proportion of extra large eggs. The differences in the relative values for eggs from hens at 195 and 484 d given the FM and CM feeds, pelleted (FMP or CMP) or crumbled (FMC or CMC), were small for egg weights (-0.8 to +1.2%), specific gravity (-0.8 to +1.9%) and Haugh units (-0.1 to +2.0%).

In conclusion, feeds for broiler and roaster chickens and turkey broilers should be pelleted for best performance and economic returns, but mash feed is more desirable for laying hens. Particle size of the grain components has little effect when used in pelleted feeds, but can influence the performance, especially of turkeys, when used in all-mash feeds.

Table 7. Effects of ingredient particle size and feed form on the differences (%) in egg production, feed conversion, monetary returns and egg grades from Leghorn hens relative to the fine mash control diet.

	Egg production	Feed conversion	Monetary returns	Egg grades		
				Extra large	Large	Other
FMC	-2.0	1.0	-10.5	2.2	-1.9	1.0
FMP	-4.6	2.9	-17.4	15.8	-3.0	-17.2
CM	-1.3	2.2	-7.2	14.9	-4.7	-12.2
CMC	-2.3	1.1	-10.7	10.2	-2.5	-9.9
CMP	-2.4	1.6	-10.7	13.4	-1.0	-18.1

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PERFORMANCE OF BROILERS AND SENSORY ATTRIBUTES OF MEAT FROM CHICKENS FED STARTER AND FINISHER FEEDS CONTAINING `Cavena®' (NAKED OATS)

R.M.G. HAMILTON*, L.M. POSTE** and G. BUTLER**

Summary

Female and male commercial broiler chickens were given starter (1-21d) and finisher (22-38d) feeds that contained 0, 125, 250 or 500 and 0, 250, 500 or 750 g Cavena (naked oats)/kg, respectively. In addition, the starter feeds either did not or did contain neomycin (80 mg/kg). Body weight gains decreased ($P<0.001$) during both the starter and finisher periods and the 1-38d feed-to-gain ratios became less efficient ($P<0.01$) as dietary Cavena levels increased. Feed intakes were higher ($P<0.01$) for the birds fed the neomycin-supplemented starter feeds. Male birds had higher ($P<0.001$) body weight gains and feed intakes than the female chickens. Juiciness mean scores showed a clear decline in juiciness at the 500/750 g/kg Cavena levels ($P<0.05$). As the inclusion of Cavena increased to 500/750 g/kg, the chewiness scores and chew counts decreased ($P<0.05$). The performance indicators and sensory attributes were not influenced by incorporating 125/250 or 250/500g Cavena/kg, respectively into the starter and finisher feeds of broiler chickens. Further research is being done to determine the dietary Cavena levels needed to give optimum performance in terms of economic returns and sensory quality.

I. INTRODUCTION

There is much interest in Canada in the production of Cavena (naked oats, *Avena nuda*) as a feedingstuff for livestock and poultry as well as for use in the cosmetic industry and in the production of "gasohol" for fuel. As a result, new Cavena varieties have been developed, especially from the oat breeding program at Agriculture and Agri-Food Canada's Plant Research Centre in Ottawa. Recently the name "Cavena" was registered as the trade mark for naked oats developed in Canada and is derived from Canada and the botanical name of oats, Avena.

The available energy content of the current Cavena cultivars for poultry are similar to maize (16.2 - 16.9 MJ TME_n/kg). The crude protein levels are higher (160 - 180 g/kg) and the amino acid balance is better for Cavena than for other cereal grains (Burrows *et al.*, 1993). Because the papery hull separates from the groat during the threshing operation, the crude fibre content of Cavena® is similar to maize (approx. 25 g/kg), but lower than that for hull oats (about 130 g/kg).

Initial studies of Cave and Burrows (1985) indicated that there was an inverse relationship between the body weight gains of broiler chickens during the starter (1 - 21 d) period and the Cavena content of their feed, but not during the finisher (22 - 49 d) period. This reduction in body weights was due to the β-glucan content of the Cavena and could be modified by supplementing broiler starter feeds with the enzyme, β-glucanase; the antibiotic, neomycin; bile salts and/or water miscible fat-soluble vitamins (Cave *et al.*,

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1990). Rotter *et al.* (1989) found that the presence of dietary β-glucans decreased the nutrient intakes of chickens during the starter period by increasing the viscosity of the intestinal contents which, in turn, decreased the rate of movement of dietary residues through the bird's intestinal tract.

Results obtained from experiments done at the Kentville Research Centre indicated that there was no relationship ($P>0.05$) between dietary Cavena levels and body weight gains or feed conversions of broiler chickens during the 1 - 21 d starter period (Hamilton, unpublished). The cultivar of Cavena used also did not influence these traits. Similarly, Maurice *et al.* (1985) reported that the growth of broiler chickens during the starter period was not influenced by dietary naked oat levels. Furthermore, a feeding trial with turkey poults showed that the incorporation of up to 300 g Cavena/kg into their starter feeds had no significant effects on their performance from 1 - 28 d of age (Hamilton, 1994).

The results being presented are from an experiment designed to examine the relationship between the Cavena levels of the starter and finisher feeds and the performance of and sensory attributes of meat from broiler chickens. In addition, the effects of adding the antibiotic, neomycin, to broiler starter feeds that contained Cavena was examined.

II. METHODS

Female and male commercial broiler chickens (1200 of each sex) received feed and water *ad libitum* during the starter (1-21 d) and finisher (22-38 d) periods. For the first 72 hours after housing they received a 24L:0D photoperiod followed by 4 cycles/day of 4L:2D until the completion of the experiment. The starter and finisher feeds contained 0, 125, 250 or 500 and 0, 250, 500 or 750 g Cavena/kg, respectively. The starter feeds either did not or contained supplemental neomycin (80 mg/kg). In the Cavena feeds, naked oats replaced maize and soybean meal used in the control feeds. All the feeds contained 50 g canola meal/kg and 7 - 18g added poultry fat/kg. These feeds were fed to groups of 25 birds; there were 6 groups of birds/treatment.

All birds were weighed at 1, 21 and 38 d and feed weigh-backs were done at 21 and 38 d. Mortality was recorded as it occurred. Monetary returns were calculated from the returns from the sale of live birds minus the cost of the chicks plus their feed.

At the completion of the experiment, one bird from each pen was "custom" killed and eviscerated. The carcasses of these birds were used for sensory testing which included the following attributes: chewiness and chew count (tenderness), initial and sustained juiciness, flavour (`chickeny' or `meaty' for light and dark meat, respectively), and adhesiveness (for the dark meat only). A 15 cm unstructured line scale was utilized by trained panellists to score the perceived intensities of these attributes (Poste *et al.*, 1991).

III. RESULTS AND DISCUSSION

The Cavena used in this experiment was from the variety AC Lotta (Burrows, 1992) and was grown on Prince Edward Island in 1993. There was no evidence of feed refusal or the occurrence of wet litter during the 38-day experimental period. Necropsy examination of the birds which died, by a veterinary poultry pathologist, indicated the causes were not related to the dietary treatments.

As indicated in Table 1, there was a significant decrease ($P<0.001$) in the 1-21 and 22-38 day body weight gains and the 1-21 day feed intakes as the Cavena content of the feeds increased. Feed-to-gain ratios over the 1-38 day period increased ($P<0.001$) as the

dietary Cavena levels increased. Intakes were higher ($P<0.01$) when the starter feeds were supplemented with the antibiotic, neomycin. As expected, body weight gains and feed intakes were higher ($P<0.001$) for the male than the female birds. The growth results

Table 1. Effects of dietary Cavena level, neomycin supplementation and sex on the performance and monetary returns from boiler chickens.

Cavena Starter/ Grower	Body weight gain[1] 1-21d g/bird	22-38d g/bird	Feed intake 1-21d kg/bird	22-38d kg/bird	Feed/gain 1-21d g/g	1-38d g/g	Monetary return c/bird
(g/kg)	***LQ	***L	**L	ns	ns	***L	ns
0/0	717	1273	1.26	2.59	1.639	1.985	46.9
125/250	716	1280	1.28	2.62	1.661	1.991	52.5
250/500	711	1221	1.27	2.60	1.647	2.037	50.4
500/750	668	1200	1.20	2.60	1.655	2.103	48.4
SEM[3]	6	10	0.02	0.03	0.024	0.019	3.0
Neomycin[2,4]	ns	ns	**	ns	ns	ns	ns
-	698	1240	1.22	2.60	1.619	2.036	49.7
+	798	1247	1.29	2.61	1.682	2.039	49.4
SEM[3]	4	7	0.01	0.02	0.017	0.013	2.1
Sex[2]	***	***	***	***	ns	ns	***
Female	668	1139	1.17	2.38	1.614	2.030	40.4
Male	738	1349	1.33	2.83	1.687	2.046	58.7
SEM[3]	4	7	0.01	0.02	0.017	0.013	2.1

[1] Average body weight at housing was 45.6 g.

[2] Probabilities for main effects: ns, not significant; *($P>0.05$); **($P<0.01$); ***($P<0.001$); L = linear and Q = quadratic.

[3] Standard error of mean (df = 48, 24, 48 respectively).

[4] - or + : Antibiotic not added or added to starter feeds.

support the previously published results of Cave and Burrows (1993) that dietary Cavena levels influence the growth of broiler chickens in an inverse manner. The effect of neomycin supplementation, however, do not support the previous published findings of Cave *et al.* (1992). While the relationship between dietary Cavena level and monetary returns was not significant, the best returns were received from the broilers given the 125/250 g Cavena/kg combination of starter and finisher diets,respectively. Since the dietary Cavena levels were fixed between the starter and finisher diets, it is not possible to predict the Cavena combinations that should give the optimum performance of broiler chickens, particularly in terms of monetary returns.

The sensory results are summarized in Table 2. Dietary Cavena level influenced juiciness of the white meat ($P<0.01$) and the chewiness of the dark meat ($P<0.05$). There was a clear decline in the juiciness of the white meat at the 500/750 g/kg (starter/grower) dietary Cavena levels; differences among the first three dietary Cavena levels were not significant ($P>0.05$). As the inclusion of Cavena increased to 500/750 g/kg, the chewiness scores and chew counts for the dark meat decreased. With the white meat, there were interactions between dietary Cavena level and sex of the birds which affected both

measures of tenderness; chewiness ($P<0.01$) and chew count ($P<0.05$). The flavour scores for the dark meat of the neomycin supplemented chickens were higher ($P<0.01$) than for the meat from the non-supplemented birds.

Table 2. Effects of dietary Cavena on sensory characteristics of chicken.

Cavena Starter/ Grower	White Meat			Dark Meat		
(g/kg)	Chewiness[1]	Juiciness[1]	Flavour	Chewiness	Juiciness	Flavour
0/0	5.6[2]	5.5	7.4	4.3	5.9	8.3
125/250	6.2	5.5	7.3	4.1	6.3	7.8
250/500	5.4	5.8	7.5	3.9	6.3	8.1
500/750	6.1	4.8	7.2	3.6	6.4	8.2
SEM[3]	0.3	0.2	0.2	0.2	0.2	0.2
Sign.[4]	ns	Diet*Q	ns	Diet*L	L	ns

1 Scales from tender to tough (chewiness) or slightly juicy to very juicy (juiciness); the lower the score the more tender or less juicy, respectively.
2 Mean of 7 judgements of 16 birds.
3 Standard error of the mean (df = 48).
4 Significance of diet effects: ns, not significant ($P>0.05$); *($P<0.05$); linear (L) or quadratic (Q) contrasts for diet effects ($P<0.05$).

In conclusion, performance and sensory attributes of the meat from broiler chickens were not influenced by incorporating up to 250 and 500 g Cavena/kg into their starter and finisher feeds, respectively. However, further research is being done to determine the dietary Cavena levels needed to give optimum performance in terms of economic returns and sensory quality.

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RELATIVE BIOAVAILABILITY OF TRACE MINERALS FOR POULTRY

P. R. HENRY and C. B. AMMERMAN

Summary

Knowledge of the bioavailability of trace minerals in feedstuffs and supplemental sources is important for economic feed formulation to support optimal animal performance. Bioavailability should be thought of as an estimated value which reflects absorption and utilization of a mineral under conditions of an experient rather than a specific inherent property of a feedstuff or supplemental compound. With current technology, determination of bioavailability within its strictest definition is often difficult to impossible for some elements, so acceptable compromises must be made.

Manganese sulfate along with the protein and amino acid complexes are highly available forms of manganese for poultry, and the carbonate and monoxide are less well utilized. The protein and amino acid complexes of copper are well utilized, as are the sulfate, acetate, chloride and cuprous oxide forms. Cupric oxide is unavailable and cupric carbonate is less available than cupric sulfate. Highly available forms of zinc include the sulfate and amino acid complexes with the oxide, carbonate and zinc metal less well utilized. The iron mono- and heptahydrate sulfates, ferrous chloride, ferrous ammonium sulfate, and ferric ammonium citrate are all available sources of iron with ferriic chloride and reduced iron less available. Ferric oxide, ferrous carbonate and sodium iron pyrophosphate are essentially unavailable. Sodium selenite and selenate are both available forms of selenium and potassium and sodium iodide, calcium iodate and cuprous iodide are well utilized by poultry as supplemental sources of iodine.

I. INTRODUCTION

Diets for poultry consisting of natural feed ingredients are frequently deficient in one or more mineral elements; therefore, these elements must be provided to the animal in a supplemental form. Thus, it is important to know the bioavailability of mineral elements both in dietary ingredients and in dietary supplements. At the present time, more information exists on the utilization of minerals commonly used as supplements than on those in practical dietary ingredients. The term "bioavailability" has been defined as the degree to which an ingested nutrient can be absorbed and is utilized in metabolism by the normal animal (Fox *et al.*, 1981) . Thus the nutrient is "available" at the tissue level rather than just at the dietary level. Other terms which have been used to express this degree of utilization include "biological availability", "bioactivity", "biopotency" and "bioefficacy". With regard to certain minerals, actual measurement of utilization of the element is difficult and researchers have frequently relied on techniques which do not meet the strictest definition of bioavailability. Consequently, many researchers have altered the definition to be the degree to which an ingested nutrient is absorbed and can be utilized in metabolism (Forbes and Erdman, 1983; Southgate, 1989).

Bioavailability should not be considered as an inherent property or characteristic of the material being assayed, but rather, an experimentally determined estimate which reflects the absorption and utilization under conditions of the test (Fairweather-Tait, 1987). With current technology, attempting to determine "the universal bioavailability value" of a source can be considered somewhere between frustrating and misleading.

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II. METHODOLOGY

Bioavailability values can be influenced greatly by method of mathematical calculation used to derive the estimate. Data must meet certain criteria for valid tests. As examples, data must be linear or transformable to linear for a multiple linear regression to yield valid results; whereas, for a parallel line assay, data must conform to parallelism as well as linearity. A control group displaying either no response or a limited response must also be included in an experiment to determine whether some measurable response to a test source has, indeed, occurred. In a situation where feed intake differs among treatments, either because the control diet was deficient in the element or some diets had elevated concentrations of the element, the total amount of the element consumed rather than dietary concentration should be used as the independent variable in regression. Most importantly, if a value makes no sense biologically, discard it. Experimentation is not perfect, and sometimes unknown factors will radically influence results. Accepting results of all experiments may lead to costly errors in diet formulation.

A bioavailability method suitable for one element may be totally unsuitable for another element and yield erroneous results. An example would be comparison of tissue retention of selenium from selenomethionine *vs* sodium selenite. Selenomethionine can be incorporated directly into body proteins, whereas selenite is metabolized through a different pathway and is not stored in this manner.

Absorption, either apparent or true, of a mineral has been used as an indicator of its bioavailability. Absorption, however, cannot always be equated to bioavailability as is the case with the selenoamino acids. For the trace elements, requirement levels are generally small and/or absorption so limited as to render balance experiments unreliable. For some elements in various forms, however, apparent absorption, or especially true absorption, can provide very useful information with regard to bioavailability.

Animal body weight gain and bone development have been used as primary criteria for functional assays of several trace mineral elements. The day-old chick with its limited nutrient stores and its genetic potential for rapid growth is an ideal choice as an assay animal for several trace minerals. In the case of iron or zinc fed at elevated dietary concentrations, feeding should be delayed until 3 to 5 days of age to avoid severe growth depression (Henry and Ammerman, unpublished data). Metabolically essential compounds or enzymes have been used as criteria in several mineral bioavailability studies. Perhaps one of the earliest uses of such a compound is that of hemoglobin and its response to iron. More recently measurement of glutathione peroxidase activity has been used to assay selenium sources.

Accumulation of a mineral element in various target organs has been used as a response criterion for bioavailability. Early observations, including those by Nesbit and Elmslie (1960) with rats and Bunch *et al.* (1961) with swine, indicated that biological availability of iron and copper from various compounds was related to tissue concentrations of the elements. Watson *et al.* (1970) fed semipurified diets (4 ppm manganese) to chicks and reported that bone manganese concentrations were more directly related to supplemental levels than were growth rate or leg development. The authors suggested that "...a biological assay for manganese may be developed using manganese sulfate as a reference standard and bone manganese levels along with growth rate and leg development as the response criteria." Years later this suggestion was followed to the extent that Black *et al.* (1984 a, b) used bone manganese concentrations along with concentrations of the element in other tissues as indicators of bioavailability.

Traditionally, bioavailability values have been determined at dietary levels below the requirement. These studies technically do not meet the definition of bioavailability in that the animal is no longer "normal" if consuming a deficient diet. Some dietary mineral deficiencies can affect other areas of metabolism indirectly through numerous complex interrelatonships.

A method to estimate the biological availability of the micro mineral elements for poultry based on tissue uptake of the element following high-level, short-term supplementation has been developed at the University of Florida (Henry *et al.*, 1986). As stated by Combs and Combs (1986), with regard to the availability of selenium, this method measures the element in both its "adventitious" form, as well as in its "critical" or metabolically active form. One of the biggest advantages of this method is that it takes far fewer animals to detect statistically significant differences when greater dietary concentrations of the particular element are fed and the degree of response is proportionately greater. This factor may become increasingly important in light of the animal rights movement. The use of high-level, short-term supplementation suggests that levels of the mineral element fed can approach what would be considered the toxic range for longer term supplementation. It is important, however, that significant reductions in feed intake, and weight gain do not occur. The use of elevated dietary concentrations allows formulation of diets with natural ingredients which meet the animal's nutrient requirements and allow them to grow at their maximal genetic potential. Diets containing such ingredients are less expensive and, in general, more palatable than are diets containing purified ingredients. Also, contamination of either diet or tissue samples with the mineral element being tested is of less consequence when high dietary levels of the element are being fed as opposed to having the diet as completely free as possible of the element. In studies of this kind, slope ratios are used in comparing responses from test sources with that of the standard source.

III. MANGANESE

Except for recognition that rhodochrosite was an ineffective dietary source of manganese, few differences in bioavailability among manganese sources were observed when chicks were fed purified diets and body weight change and leg abnormalities served as criteria. More recently, with supplemental dietary manganese levels of 1000 to 4000 ppm, manganese carbonate and manganese monoxide have been found to be less effective than manganese sulfate as a source of the element for poultry . Researchers contributing to these findings include Southern and Baker (1983), Black *et al.* (1984b), Henry *et al.* (1987b) and Wong-Valle *et al.* (1989) . A manganese-protein complex was shown to have greater bioavailability than manganese sulfate as a source of manganese for chicks in one study by Baker and Halpin (1987). Manganese methionine was equal to manganese oxide for chicks in one study (Scheideler, 1991) and superior to manganese oxide in another study (Fly *et al.*, 1989). The methionine form was equal to or more effective than manganese sulfate (Henry *et al.*, 1989).

IV. COPPER

An early review of the literature (Ammerman and Miller, 1972) indicated that copper as cupric sulfate had been found to be the most available form for both domestic and laboratory animals. Copper as cupric oxide was absorbed to a lesser degree than that from cupric sulfate and cupric carbonate was intermediate in response between the oxide and sulfate forms. Considerably more information on the bioavailability of various sources has been generated in recent years. Most studies contributing to this information have used liver copper deposition as the criterion and, frequently, supplemental dietary copper levels have extended to 300 to 400 ppm.

The cupric form of acetate was shown (Ledoux *et al.*, 1991) to be equal to cupric sulfate as sources of copper. In general, cupric chloride has been shown to have a greater bioavailability than cupric sulfate (Norvell *et al.*, 1974, 1975). Although cupric oxide has been used as a supplemental source of copper in the livestock and feed industry, more recent

research has suggested it has essentially zero bioavailability for poultry (Norvell 1974, 1975; Baker *et al.*, 1991; Ledoux *et al.*, 1991). Baker *et al.* (1991) found that copper from cuprous oxide was well utilized by the chick. Supplemental copper in the organic form, including copper lysine, copper methionine and copper proteinate, was absorbed equal to or greater than that from cupric sulfate (Kincaid *et al.*, 1986; Baker *et al.*, 1991).

Ledoux *et al.* (1991) fed cupric carbonate and cupric sulfate to chicks at supplemental copper levels of 150, 300 and 450 ppm. The basal diet contained 11 ppm copper. Liver copper concentrations were examined by the slope ratio method and relative bioavailability of the copper as cupric carbonate was 68%. Zanetti *et al.* (1991) conducted a similar experiment with chicks in which supplemental copper levels of 5, 10, 15 and 20 ppm were added to a diet containing 5 ppm copper. The relative bioavailability of copper in the carbonate form in this study in which dietary copper additions were much closer to requirement was very similar at 66%.

V. ZINC

Tucker and Salmon (1955) demonstrated the relationship between zinc deficiency and parakeratosis in swine, and soon thereafter the potential need for supplemental dietary zinc was established in domestic species. Following these observations, several bioavailability studies were conducted in which inorganic zinc compounds were evaluated as sources of the element for chicks or poults (Ammerman and Miller, 1972). Most of the studies used growth as the response criterion and few differences were observed among the supplemental sources of zinc tested.

There has been an increased interest recently in determining the bioavailability of zinc supplements. Chick zinc bioassays were conducted with a soy isolate-dextrose diet containing 13 ppm zinc to which 7.5 or 15 ppm were added from either feed grade zinc oxide or feed grade zinc sulfate (Wedekind and Baker, 1990). When weight gain was regressed on supplemental zinc intake the relative bioavailability of zinc as zinc oxide was 61% when the sulfate form was assigned a value of 100%. A similar comparison based on tibia zinc gave a value for zinc oxide of 44%. However, zinc concentraton of the basal diet and feed intake were not accounted for in this calculation. Wedekind *et al.* (1992) reported on studies with chicks in which the bioavailability of zinc-methionine was compared to that of feed-grade zinc sulfate using three different diets described as purified (crystalline amino acid), semipurified (soy isolate), and complex (corn-soybean meal) diet. Zinc from zinc methionine was absorbed significantly greater than that from zinc sulfate with the purified diet and the difference in degree of bioavailability was even greater with semipurified and complex diets. The authors suggested that metabolism of the zinc methionine complex differed from that of inorganic sources as influenced by phytate and fiber in the diet.

The potential for using elevated dietary levels of zinc with practical dietary ingredients was examined by Henry *et al.* (1987a). These investigators fed a basal corn-soybean meal diet containing 74 ppm zinc to chicks and supplemented it with 0, 500, 1000 and 1500 ppm zinc as reagent grade zinc sulfate. Feed intakes and body weight gains were depressed, especially at the greatest concentration of zinc, when chicks were fed for 1 week. Highly significant increases in tissue zinc occurred and the authors suggested that this technique might be useful as a measure of zinc bioavailability. Of the tissues examined, bone was most sensitive to dietary zinc followed by liver and kidney. Sandoval (1992) used this technique with chicks and when zinc sulfate served as the control, zinc carbonate, zinc oxide and zinc metal were lower in bioavailability in the order listed.

VI. IRON

Although iron deficiency is not generally a practical problem in poultry nutrition, the chick has been used extensively as a model for human bioavailability studies. Hemoglobin regeneration following the feeding of an iron-deficient diet for several weeks has generally been used to measure availability of sources in relation to that of ferrous sulfate heptahydrate. Ferric oxide was reported to be 17% as available as ferrous sulfate in very early studies (Elvehjem *et al.*, 1929). Pla and Fritz (1970) depleted chicks for 2 wk, then fed diets supplemented with 5, 10, 15, or 20 ppm iron for 2 wk and measured hemoglobin regeneration. Ferrous chloride, ferric chloride and ferrous carbonate were 106, 78 and 5%, respectively, as available as ferrous sulfate. In similar studies Fritz *et al.* (1970) reported values of 107, 99, 98, 44, 37, 4 and 2% that of ferrous sulfate for ferric ammonium citrate, ferrous ammonium sulfate, ferrous chloride, ferric chloride, reduced iron, ferric oxide and ferrous carbonate, respectively. A basal diet containing 7 ppm iron was fed for 3 wk, then experimental diets supplemented with from 10 to 60 ppm iron were fed for 2 wk, and slope ratios of hemoglobin regressed on iron dose were calculated (Amide *et al.*, 1972). Relative values were ferrous sulfate 100, ferric orthophosphate 27, sodium iron pyrophosphate 4, reduced iron 49, and ferrous carbonate 2. Poitevint (1979) reported that ferrous sulfate monohydrate was 103% as available as the heptahydrate form of the element.

More recent work by Chausow and Czarnecki-Mauldin (1988) reported relative iron bioavailability estimates based on hemoglobin regeneration and compared to ferrous sulfate of 96% for sesame seed meal, 77% for rice bran, 65% for alfalfa meal, 45% for soybean meal and 20% for corn. Relative bioavailabilities of feedstuffs of animal origin were poultry by-product meal, 68%; meat and bone meal, 48%; feather meal, 39%; fish meal, 32%; and blood meal, 22%. Based on hemoglobin regeneration in chicks fed a purified iron-deficient diet, iron in ferrous sulfate monohydrate was 102%, while that in monocalcium/dicalcium phosphate and defluorinated phosphate was 67 and 48%, respectively, compared with 100% for ferrous sulfate heptahydrate (Ammerman *et al.*, 1993).

VII. SELENIUM

The bioavailability of selenium is somewhat of a moot point as government regulations in numerous countries prohibit use of supplemental sources with the exception of sodium selenite and sodium selenate. Combs and Combs (1986) compiled a table which summarized the 291 inorganic and organic selenium sources which were evaluated by Schwarz and coworkers for their efficacy in prevention of liver necrosis in vitamin E-deficient rats. With regard to poultry, three approaches have been taken to estimate bioavailability of selenium in feedstuffs and supplements: (a) prevention of various selenium-responsive diseases; (b) functional assays measuring glutathione peroxidase (GSH-Px) activity; and (c) tissue accumulation of selenium. Unfortunately, estimates derived by these different methods are often dissimilar. For example, selenomethionine was not as well utilized as sodium selenite for prevention of exudative diathesis in the vitamin E-deficient chick, but was valued at approximately 350% compared with 100% for sodium selenite for prevention of nutritional pancreatic atrophy in vitamin E-fed chicks. Extensive work at Cornell University using prevention of exudative diathesis in chicks indicated that availability of selenium in animal by-product sources was low, generally 9 to 25%, while that in plant feedstuffs was of the order of 79% of that in sodium selenite. Selenium in sodium selenate has been estimated to be 120% that in sodium selenite (100%) based on tissue uptake in chicks from a 6 ppm addition of the element in a practical diet (Echevarria *et al.*, 1988). In turkeys fed a selenium-deficient diet, relative availability of sodium selenate was 141% that of sodium selenite based on plasma selenium concentration and 220% based on plasma GSH-

Px activity (Cantor and Tarino, 1982). Estimates for selenomethionine in another experiment with turkeys were 124 and 97%, respectively, based on the same variables (Cantor *et al.*, 1982). High selenium yeast products contain most of their selenium in the form of selenomethionine and would, therefore, have similar bioavailability. Calcium selenite was tested in several experiments at the University of Florida and was found to be equal to sodium selenite for chicks (Tarla *et al.*, 1991).

VIII. IODINE

Potassium iodide and calcium iodate were found to have similar value for chicks as sources of iodine based on maintenance of normal thyroid weight and histological examination (Hixson and Rosner, 1957). From extrapolation of studies with other species (Ammerman and Miller, 1972), it would appear that sodium iodide and cuprous iodide would also be highly available sources of the element for poultry.

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ALGAE AS A POULTRY RATION SUPPLEMENT FOR THE PRODUCTION OF OMEGA-3 FATTY ACID RICH EGGS

S. M. HERBER and M.E. VAN ELSWYK

Omega-3 fatty acids (n-3 FA) found in fish are proposed to reduce the risk of coronary heart disease (Kromhout, 1992). Unfortunately, American consumption of fish is below that suggested to achieve cardiovascular benefits (USDA, 1992). Modification of egg yolk fatty acid content through dietary manipulation of laying hen rations may result in a viable alternative to fish for consumption of these healthful fatty acids (Hargis and Van Elswyk, 1993). The purpose of the current study was to investigate a novel source of n-3 FA for use in poultry rations. Heterotrophic algae in the form of OmegaFeed™ was fed for four weeks to single-combed White Leghorn hens (n=24/treatment). The two levels of OmegaFeed™ investigated supplied 200 mg and 400 mg n-3 FA/day, primarily as docosahexaenoic acid (DHA, 22:6n-3). OmegaFeed™ treatments were compared to a typical corn-soy laying hen ration. Egg production was recorded daily. Yolk fatty acids were analyzed weekly. Egg yolk color was evaluated using the L*a*b* color notation system.

At the end of four weeks, eggs from hens fed 200 mg and 400 mg of DHA as OmegaFeed™ contained 6.8 mgDHA/g yolk and 9.0 mgDHA/g yolk, respectively, as compared to control eggs which contained 1.4 mgDHA/g yolk. As yolk DHA increased, yolk omega-6 fatty acids (n-6 FA) decreased. Specifically, control eggs contained 5.8 mg arachidonic acid (20:4n-6)/g yolk while eggs from hens fed 200 mg and 400 mg of DHA as OmegaFeed™ contained 3.6 mg/g yolk and 3.2 mg/g yolk, respectively. Canthaxanthin, a carotenoid naturally occurring in OmegaFeed™, increased yolk a* values as levels of OmegaFeed™ increased, resulting in an enhancement of the yellow-orange color of yolk at the fourth week of feeding. These data indicate that algae, in the form of OmegaFeed™ , are useful in laying hen rations for yolk n-3 FA incorporation and enhancing yolk pigmentation.

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ENZYME SUPPLEMENTATION IMPROVES ILEAL AMINO ACID DIGESTIBILITY VALUES OF WHEAT FOR BROILERS

L.I. HEW, V.RAVINDRAN, Y.MOLLAH, R.J.GILL and W.L.BRYDEN

Marked improvements in the nutritive value of wheat by the addition of xylanase enzymes has been reported (Annison,1992). While this improvement is generally attributed to enhanced energy metabolizability, it is possible that the added enzymes may also act through their influence on the digestibility of amino acids. The objectives of the present study were to evaluate the effects of two commercial carbohydrase preparations (Enzyme 1 - Avizyme 1300, Finnfeeds International Ltd., UK; Enzyme 2 - Bio-Feed^{TM} Plus, Novo Nordisk, Denmark) on the apparent metabolizable energy (AME) and apparent ileal amino acid digestibilities of wheat for broiler chickens.

The basal diet contained wheat as the only source of protein and was used with or without the two feed enzymes. Celite, a source of acid-insoluble ash, was added at 20 g/kg to all diets as an indigestible marker Each of the three dietary treatments were randomly assigned to four pens (4 birds/pen) of male broilers from 35 to 42 days of age. The AME values were determined using a classical total collection method (Mollah *et al.*, 1983). At the end of the trial, ileal contents were collected and processed (Siriwan *et al.*, 1993). Samples of diets and digesta were assayed for amino acids and acid-insoluble ash, and the apparent digestibility values were calculated. The results are summarized in the Table.

Parameter	Wheat	Wheat + Enz 1	Wheat + Enz 2	SEM
AME, MJ/kg DM	12.35^{a}	13.91^{ab}	14.66^{b}	1.05
Apparent ileal digestibility, %				
Threonine	82.0^{b}	86.8^{a}	85.4^{a}	1.56
Valine	88.3^{b}	91.8^{a}	91.5^{a}	1.43
Methionine	92.7^{b}	95.3^{a}	95.1^{a}	0.95
Isoleucine	89.9^{b}	93.0^{a}	92.8^{a}	1.16
Leucine	91.7^{b}	94.2^{a}	94.1^{a}	1.07
Phenylalanine	89.8^{b}	93.4^{a}	93.2^{a}	0.48
Histidine	90.0^{b}	93.0^{a}	93.0^{a}	1.16
Lysine	87.4^{b}	91.9^{a}	91.6^{a}	1.55
Arginine	90.5^{b}	94.0^{a}	94.1^{a}	1.35

^{a,b} Means in a row bearing different superscripts are significantly different ($P < 0.05$).

Both enzymes improved the AME and apparent ileal amino acid digestibility values of wheat for broilers. Improvements in digestibility were consistent for all essential amino acids and ranged from 3 to 4 percentage units. he reaction between the enzymes and wheat enhanced amino acid utilization, possibly by lowering the viscosity of digesta and thereby exposing the proteins to intestinal proteolytic enzymes, and/or by reducing endogenous amino acid losses.

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RESPONSES OF LAYING HENS TO FORCED-MOLTING

S. HURWITZ

Summary

Forced-molting in layers over 500 days of age, accelerated egg production rate and diminished its age-dependent decline. Except for some induced oscillations, egg weight was little affected by forced molting. Shell quality was markedly improved and its time-dependent decline was retarded, resulting in a reduction in egg breakage. The various production traits were influenced differently by variables of forced-molting such as the length of the "rest" period, and the elimination of artificial illumination. Since forced molting involves a temporary loss of product and since the effects of forced molting are time-dependent, it is concluded that applicability of forced-molting, and decisions regarding treatment variables, must be evaluated together with the length of the production period.

I. INTRODUCTION

Despite the size and importance of the egg industry, many management decisions remain intuitive or are based on educated guesses. A major problem concerns the determination of the optimal length of the production period and the decision regarding its termination or application of forced molting. As stated by Wakeling (1977), the benefits of forced molting depend on market prices of eggs, direct production costs and other economic factors. Since these are constantly subject to change, management decisions are also subject to changing market conditions. Further complications of the decision process are the different responses to various forced-molting techniques, and the variables within each of the techniques such as the length of the imposed "rest" period. Consideration of all these complicating factors may exceed the capacity of the human intellect, and the aid of a computer should be sought.

The earliest description of the forced-molting technique was given by Rice (1905). Since that time, several approaches have been developed. The classical forced molt technique involves a period of starvation followed by a "rest" period during which time the birds receive quantities of diet sufficient to maintain the low body weight. The birds are also deprived of artificial illumination during that time. "Chemical" means of forced molting include the feeding of low calcium (Begin and Johnson, 1975; Gilbert and Blair, 1975; Hurwitz *et al.*, 1975), low sodium (Bornstein *et al*, 1979; Nesbeth *et al.*, 1976; Ross and Herrick, 1981; Whitehead and Shannon, 1974;) or high zinc (Brake, 1993; Shippee *et al.*, 1979) diets. McCormick and Cunningham (1987) concluded that the efficacy of a high zinc diet resulted from its ability to depress feed intake. Another popular forced molt technique is the feeding of a low protein diet (Christmas *et al.*, 1985; Wakeling 1977).

Using the classical techniques of forced-molting, Cunningham and McCormick (1985) and Koelkebeck *et al* (1992) showed that performance (production rate, feed efficiency) following forced molting changes with the duration of starvation or "rest" periods. Although no general agreement exists, performance improves in proportion to the starvation/rest periods. However, the economic benefit of this improvement may be offset by the loss of

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income during the length of the molt period. This trade off emphasizes the need for a good algorithm for decision making.

Some attempts have been made to characterize the forced molt variables. McCormick and Cunningham (1987) showed that the minimum period for force molting was the 10 days required for ovary degeneration. Baker *et al* (1983) and Koelebeck (1992) found that a loss of approximately 30% of body weight during the forced molt period is required for optimal expression of force molting, especially with regard to egg shell quality.

As summarized by Wakeling (1977), forced molt results in improved egg weight, and egg and egg shell quality. Many studies including ours (Hurwitz *et al.*, 1975) show also a significant improvement in the rate of egg production. Roland and Brake (1982) found a correlation within a flock between pre-molt and post-molt production rates. Greatest improvement was found in the less productive layers. Egg weight and shell quality were improved equally well in all groups of birds.

In reviewing the literature, an unavoidable conclusion is that despite the abundance of publications on forced-molting, the detailed information needed for modeling the responses to forced molting is rather incomplete. Due to the large volume of data in any detailed layer studies, computer-aided processing of experimental results appears to be instrumental in evaluation of the kinetics of responses to forced molting. This communication presents a preliminary account of experiments in which the significance of the duration of the "rest" period and the lighting regimen have been evaluated in detail.

II. EXPERIMENTAL

Experiments were conducted with White Leghorns (Lohman), 540-580 days old, housed in individual laying cages. The hens were distributed into similar experimental groups on the

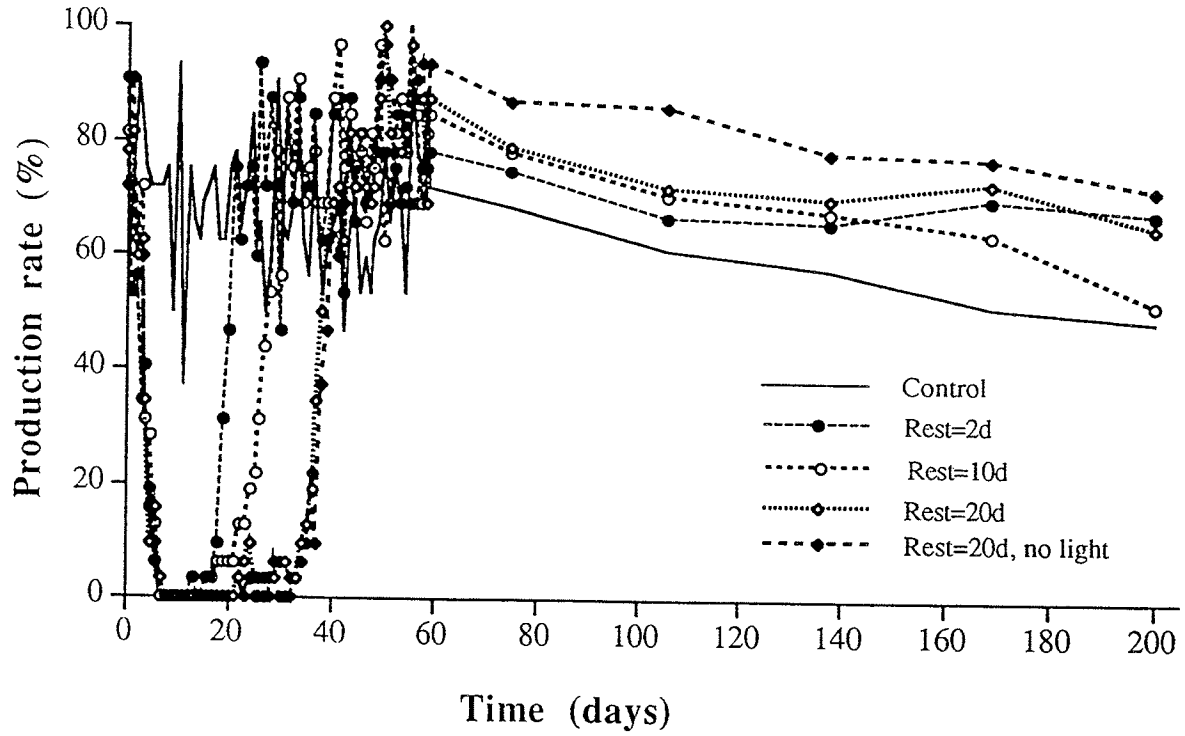

Figure 1. Egg production rate of layers subjected to forced-molt with a variable length of "rest" period (indicated in graph), with one treatment in which artificial illumination was discontinued during treatment (no light).

basis of previous performance. During the experiments, the birds received a diet containing 11.92 MJ of ME and 167 g crude protein/kg. In all cases, the forced-molting treatment consisted of 8 days of starvation, followed by a "rest " period during which time the birds received 60 g/day of a maintenance diet containing (/kg): 13.10 MJ of ME, 82.5 g crude protein, 12 g calcium and 4.5 g available phosphorus. The "rest" period was followed immediately by the feeding of the regular layer diet *ad libitum*.

In one trial, the effects of the length of the "rest" period was evaluated in hens forced molted while maintaining artificial illumination (16 h of daylight), and compared with a single treatment with a 20 day "rest" period and no artificial illumination (12 h of daylight). In another trial in which all treatments, except control, included discontinuation of artificial illumination, the "rest" periods ranged between 0 and 42 days.

II. EFFECTS OF FORCED- MOLTING

(a) Egg production rate

Results suggest that during the return to egg production, the peak production of the molted birds exceeded that of the control group (Figure 1). Furthermore, the rate of decline in egg production appeared to have been diminished by the molt treatment. If the last two points of the 2-day rest group are ignored, the benefit of forced molting to rate of production appears to be progressive. Furthermore, the full expression of treatment was obtained only when the birds were deprived of artificial illumination during molting. The impact of the length of the "rest" period was re-evaluated in another study, not detailed here but regressions calculated for the period following peak production are given in Table 1. Clearly, forced molting resulted in improved initial production as suggested by the change of the intercept, and progressively slowed down the age-dependent decline in production rate.

Table 1. Decline of egg production rate as a function of the "rest" period in a conventional forced-molt applied at the age of 550 days.

Rest Period Days	Decline %/Day	Intercept %
No molt	-0.132	86.5
0	-0.135	96.8
7	-0.110	97.3
14	-0.109	99.6
21	-0.086	98.6
28	-1.110	101.4
42	-0.046	94.5

(b) Egg weight

From the practical viewpoint, forced-molting had little effect on egg weight. It can, however, be mentioned that forced molting introduced a marked oscillatory behavior which was dependent on the length of the rest period. After molt, egg production resumed with small eggs, and continued with an increase towards normal during the first month. The weight of the first egg was reduced, and the amplitude of oscillations was higher, as the length of the

rest period increased. In contrast to production rate, induced changes of egg weight were not modified by discontinuation of artificial illumination.

(c) Shell quality

Shell weight per unit surface area also exhibited an oscillatory behavior and a marked improvement in response to forced molting. Neither illumination nor the length of the rest period significantly affected the initial response. However, the rate of decline in shell quality with time was reduced when the "rest" period exceeded 20 days. Results of laboratory tests of shell quality, such as shell thickness or shell density as measured here (shell weight per unit surface area), are poor estimates of the effective shell quality and usually do not correlate well with the actual proportion of damaged eggs. It is out of the scope of this presentation to review all the possible factors contributing to this discrepancy but two should be mentioned. Firstly, a large fraction of the eggs (obviously those with the poorest egg shells) are broken, and are not collected for analysis. The determined shell quality values are therefore overestimates of the true quality. Secondly, no damage to eggs is expected within the wide upper range of shell quality. Some proportionality of damage may be observed when shell quality falls below a certain threshold level, usually around 70 mg/cm^2. For evaluation of benefits of forced molting, actual proportion of damaged eggs is important. Results (Figure 2) clearly show an initial decline in the proportion of broken eggs as a result of forced-molting, and a decline in the rate of the temporal increase in proportion to the length of the rest period.

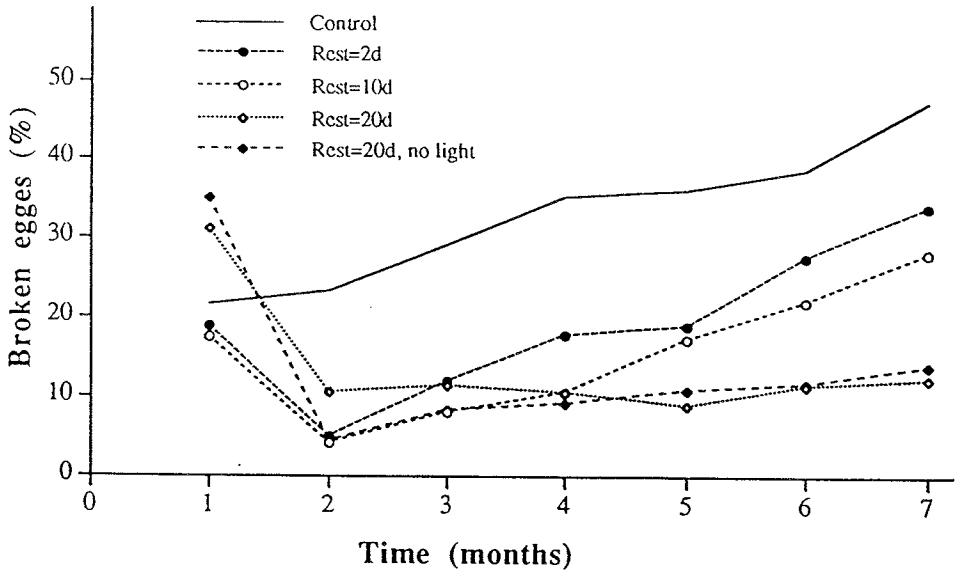

Figure 2. Breakage of eggs from hens subjected to forced-molt with a variable length of the "rest"" period, and one treatment (no light) where artificial illumination was discontinued during treatment.

(d) Body weight

During the starvation period, the hens lost approximately 500 g from an initial weight of about 2000 g. The low body weight remained unchanged during the rest period. When the treatment was lifted body weight increased rapidly to values exceeding those of the untreated controls. The mechanism of this overshoot is not known. It may, however, be mentioned, that

the higher body weights were maintained until the termination of the experiment, about 6 months after treatment.

(e) Feed intake and feed efficiency

As expected, feed intake increased considerably after forced molting as a result of both increased production and body size, and feed efficiency was markedly improved due to stimulation of production.

III. CONCLUSIONS

Most important production traits are changed by forced molting. These changes are not parallel to each other and should be considered independently. Furthermore, since rates of change in the various variables are influenced by treatment variables, and since variable numbers of eggs are lost during the rest periods, the efficacy of a treatment and its economic benefits change with the length of the period of production following treatment. Therefore, attempts to compare overall results of treatments within a single experimental period, as commonly found in the literature, are to a large extent futile. Time-dependent relationships should be calculated (Bell and Adams, 1992) and the practical conditions of forced-molting should be determined together with the estimation of the desired production period. It also appears important to establish the biological basis for the changes induced by forced molting in order to improve the flexibility of implementation of research results.

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IMPACT OF SPECIFIC ENZYMES ON THE METABOLISABLE ENERGY OF SELECTED FEEDSTUFFS IN BROILER DIETS

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Summary

Supplementation with the commercial carbohydrase preparation (Bio-Feed® Plus CT) increased the apparent metabolisable energy (AMEn) of wheat, wheat shorts and rapeseed meal by 9%, 10.8% and 15.8%, but had no significant effect when added to diets based on maize, soyabean meal or peas. The other commercial carbohydrase preparation (Energex® MG) increased the AMEn of maize, soyabean meal, rapeseed meal and peas 2.9%, 7.2%, 30.6% and 6.5%, respectively. For wheat and rapeseed meal based diets the relation between the AMEn value and the dosage of the two enzymes was linear in the range 0 - 500 mg enzyme per kg diet. These results should be taken into account when formulating diets containing carbohydrases.

I. INTRODUCTION

It is well known that specific exogenous enzymes can improve the AMEn of selected feedstuffs and diets (Leong *et al.*, 1962; Rotter *et al.*, 1990; Annison, 1992). Applying this information in dietary formulations is not new (Cowen, 1990; Rotter *et al.*, 1990).

In order to examine the effects of two carbohydrase products on the AMEn of selected feedstuffs a joint project was started between The Research Station for Small Stock Husbandry, Belgium and Novo Nordisk A/S, Denmark. This paper reports the results of 3 trials (Huyghebaert, 1993ab, 1994). The application of the results to least-cost diet formulation is discussed.

II. MATERIALS AND METHODS

One-day-old male Ross broiler chicks were obtained from a local commercial hatchery. From days 1 to 16 they were fed a commercial chick starter mash diet. On day 16, the birds were weighed and birds having relatively high or low body weight were discarded. Groups of four birds were then assigned randomly to pens housed in battery cages. The adaptation period lasted from day 16 to 21. From days 21 to 25 AMEn was measured by the procedure of Bourdillon *et al.* (1990). For each treatment five replicates were used.

The composition of the basal diet was: sorghum 56 %, soybean meal 32 %, animal fat 6 %, vegetable oil 1 %, minerals, vitamins, trace elements and amino acids 5% . The nutrient content was in accordance with NRC (1994). The inclusion levels of the feedstuffs under study were 50 % for cereals, 30 % for cereal by-products and 25 % for protein feedstuffs. The inclusion was at the expense of all ingredients of the basal diet.

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The respective enzyme supplementation was 0 and 500 mg/kg for the basal diet, wheat middlings, maize, soyabean meal and peas based diets. For the wheat and rapeseed meal based diets the supplementation was 0, 250 and 500 mg/kg.

The *Humicola insolens* enzyme preparation (Bio-Feed Plus CT) contained the following main enzyme activities: Xylanase (800 FXU/g), Pentosanase, Hemicellulase, Fungal β-glucanase, Endoglucanase and Cellulase. The *Aspergillus sp.* enzyme preparation (Energex MG) contained the following main enzyme activities: Fungal β-glucanase (75 FBG/g), Hemi-cellulase, Pectinase, α-galactocidase and Endoglucanase.

The chicks were fed the respective mash diets at a level of 90 % of *ad libitum* and their excreta were quantitatively collected daily. The excreta were blended, freeze-dried, equilibrated and finally ground before analysis. Samples of feeds and freeze-dried excreta were analyzed for gross energy (GE) and nitrogen.

The AMEn-contents of the experimental diets were calculated from their respective excreta/feed ratios as well as their corresponding GE-contents. A correction for N-retention to zero was made by using an energy equivalent of 34.36 kJ/g N-retained.

The results were analyzed by factorial analysis of variance. The significant treatment differences were identified by a LSD-multiple range test, according to Statgraphics version 5 (1991). The AMEn-values for the feedstuffs under study were calculated by difference, assuming additivity for AMEn. The effect of the enzyme was only ascribed to the AMEn-values of the test ingredients. On wheat and rapeseed meal the dosage/response of the exogenous enzymes was calculated by means of linear regression analysis.

III. RESULTS AND DISCUSSION

Neither of the two enzyme preparations had a significant effect on the basal diet (Tables 1 and 2), a finding consistent with the absence of enzyme-susceptible substrates in this diet.

Dietary supplementation with Bio-Feed Plus CT resulted in a distinct increase in the AMEn of wheat (varieties 1, 2, 3) of 6.8%, 8.1% and 12.0%, respectively. Also the effect on wheat middlings was high (10.8%). This effect on wheat and wheat byproducts was to be expected as Bio-Feed Plus contains a high amount of xylanase activity.

The effect on the AMEn of maize was low with both carbohydrase products. This was anticipated as the level of soluble non-starch polysaccharides (NSP) is low. However, Energex MG did have a significant effect on the AMEn of maize. For soyabean meal (SBM), rapeseed meal (RSM) and peas it is clear that the different enzyme products had a different effect on AMEn. This group of feedstuffs contains a high content of pectic substances. Not surprisingly Energex MG gave the best result in this group as it contains pectinase activity. The results on rapeseed meal was surprisingly high for both enzyme products - 15.8% and 30.6% for Bio-Feed Plus CT and Energex MG, respectively.

The regression analysis showed a linear connection between the dosage of carbohydrase and the AMEn of the wheat and rapeseed meal from 0 to 500 mg/kg diet (Table 3).

There are several ways of using the information from Tables 1, 2 and 3 in practical diet formulation to take into account the energy liberated by the enzyme product e.g:

* An energy value could be added to the enzyme product when included in the diet. The energy value of the enzyme on wheat and RSM can be derived from the slope of the model (Table 3)
* The total energy specification of the diet could be reduced by 3-4%
* The energy specification of the feedstuffs could be regulated according to quality and quantity of enzyme products added.

The first two ways are not very accurate as they do not take into account the differences in the effect of the enzyme from diet to diet. The observed AMEn-results favour the last way of application in practical diet formulation.

Table 1. Effects of Bio-Feed Plus CT on AMEn of selected feedstuffs. The enzyme dosage is 500mg/kg feed (modified after Huyghebaert, 1993ab,1994).

Diet	AMEn-diet MJ/kg	AMEn-feedstuff MJ/kg	Relative
Basal diet (B)	11.79±0.19	-	100.0[a]
B+enzyme(e)	11.82±0.12	-	100.3[a]
B+wheat 1	11.76±0.18	11.28	100.0[a]
B+wheat 1+e	12.15±0.21	12.05	106.8[b]
B+wheat 2	11.51±0.33	10.77	100.0[a]
B+wheat 2+e	11.94±0.24	11.64	108.1[b]
B+wheat 3	11.23±0.23	10.67	100.0[a]
B+wheat 3+e	11.87±0.25	11.95	112.0[b]
B+wheat middlings	10.15±0.34	5.26	100.0[a]
B+wheat middling+e	10.32±0.21	5.82	110.8[b]
B+maize	13.12±0.07	13.50	100.0[a]
B+maize+e	13.16±0.10	13.59	100.7[a]
B+soyabean meal	11.10±0.19	9.01	100.0[a]
B+soyabean meal+e	11.18±0.22	9.35	103.7[a]
B+rapeseed meal	10.77±0.12	4.88	100.0[a]
B+rapeseed meal+e	10.96±0.90	5.65	115.8[b]
B+peas	11.29±0.22	9.80	100.0[a]
B+peas+e	11.35±0.15	10.02	102.3[a]

[ab] Enzyme comparison of individual feedstuffs with unlike superscripts are significantly different $P < 0.05$.

The results confirm that carbohydrases are highly substrate specific and that their effects are dose related. However, as substantial variation exists in field conditions it is recommended that improvements in metabolic energy are used conservatively. Feeding trials with these conservative energy improvement factors for feedstuffs indicate that in

practice the concept can be used to reformulate diets with greater cost effectiveness (data not reported).

Generally, the addition of the enzyme products increased the N-retention in the broilers (Huyghebaert, 1993ab,1994). This suggests an increase in amino acid digestibility. Further studies are now underway to determine this.

Table 2. Effect of Energex MG on AMEn of selected feedstuffs. The enzyme dosage is 500mg/kg feed (modified after Huyghebaert, 1993b,1994).

Diet	AMEn-diet MJ/kg	AMEn-feedstuff MJ/kg	Relative
Basal diet (B)	11.79±0.19	-	100.0[a]
B+enzyme(e)	11.81±0.30	-	100.2[a]
B+maize	13.12±0.07	13.50	100.0[a]
B+maize+e	13.31±0.03	13.89	102.9[b]
B+soyabean meal	11.10±0.19	9.01	100.0[a]
B+soyabean meal+e	11.26±0.20	9.66	107.2[b]
B+rapeseed meal	10.77±0.12	4.88	100.0[a]
B+rapeseed meal+e	11.14±0.20	6.37	130.6[b]
B+peas	11.29±0.22	9.80	100.0[a]
B+peas+e	11.45±0.03	10.43	106.5[b]

[ab] Enzyme comparison of individual feedstuffs with unlike superscripts are significantly different ($P < 0.05$).

Table 3. The relationship between the dosage (x) (mg/kg) of enzyme and the AMEn (y) (MJ/kg) of the wheat and rapeseed meal based diets (modified after Huyghebaert,1993ab).

Feedstuff	Enzyme	Dosages (mg/kg)	Model (complete diet)
Wheat 1	*Humicola insolens*	0, 250, 500	y = 11.75 + 0.00077x
Wheat 2	*Humicola insolens*	0, 250, 500	y = 11.54 + 0.00087x
Rapeseed meal	*Aspergillus sp.*	0, 250, 500	y = 10.78 + 0.00075x
Rapeseed meal	*Humicola insolens*	0, 250, 500	y = 10.78 + 0.00039x

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HAEMATOLOGY OF BROILERS SUSCEPTIBLE TO ASCITES

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Differences between broiler chicken strains have been observed in susceptibility to the ascites syndrome which may be related to lower oxygen consumption by the bird. Recent overseas reports have focused on differences in haematology and blood enzyme levels in broilers grown in environments conducive to the syndrome, such as high altitude (Witzel *et al.*, 1990) or low atmospheric oxygen content (Maxwell *et al.*, 1990) when compared to broilers grown under 'normal' conditions. Previous work using hyperoxic environments (Jones, 1995) has shown that broilers susceptible to ascites may be limited by the 'normal' environmental oxygen content in their genetic capacity for growth. This paper examines the haematological differences between broiler strains grown in normal or hyperoxic environments.

Broilers susceptible (Strain S) or non-susceptible (Strain N) to ascites were housed in 4 replicate groups of 6 birds in each of two environmentally controlled cabinets at 4 d of age. One of the cabinets was maintained at 23% oxygen by infusion of industrial grade oxygen. The birds were fed broiler starter crumbles and at 28 d of age were bled from a wing vein for haematological comparison, the results of which are shown in the Table.

	Strain N		Strain S		SED	
	21%O_2	23%O_2	21%O_2	23%O_2	Strain	Strain x Oxygen
RBC (10^6 / mm^3)	2.38	2.38	2.35	2.31	0.05 NS	0.07 NS
Hb (g / 100ml)	11.67	11.95	11.70	11.08	0.23 NS	0.32 NS
Hct (%)	39.2	38.6	37.9	36.6	0.60 **	0.85 NS
MCV (μm^3)	150.7	153.7	153.3	151.3	1.16 NS	1.64 NS
MCH (pg)	49.1	50.3	50.1	48.1	1.25 NS	1.75 NS
MCHC (%)	29.8	31.0	30.9	30.4	0.77 NS	1.09 NS

The design of the experiment precluded a comparison between oxygen levels. However, strain comparisons revealed that broilers susceptible to ascites had lower ($P<0.01$) haematocrit (Hct) values than Strain N birds as well as a tendency ($P=0.09$) towards a lower haemoglobin (Hb) concentration. No other differences were observed in haematology between the strains.

The susceptibility of the Strain S broilers may not be related to blood viscosity as evidenced by the haematocrit data shown here. The lack of inter-strain differences in the other parameters is in agreement with other work. Differences between the strains examined here with respect to susceptibility to ascites may not be related to haematology although these birds were not subject to hypoxic conditions.

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THE USE OF CRACKED GRAIN TO PRODUCE AN EARLY-LIFE GROWTH RETARDATION IN BROILER CHICKENS

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The use of early-life growth retardation of broilers, either by food restriction (Jones and Farrell, 1992) or by light manipulation, has been used to improve carcass quality and food conversion efficiency and to decrease problems and mortalities associated with metabolic and physiological disorders. The use of early-life food restriction, although having been shown to be effective, is limited in commercial practice due to an increase in the excitability of birds and crushing at feeders. The experiment reported here examines the use of feeding cracked grain to produce a similar growth retardation to that obtained by early-life food restriction.

Unsexed broilers were fed broiler starter crumbles until 7 d of age when they were divided into 6 replicate groups of 8 birds and fed either cracked wheat, barley, maize, oats or sorghum *ad libitum* or maintained on starter crumbles for 4 d. All groups were then fed starter crumbles to 28 d of age and a broiler finisher feed to 42 d of age. The results are shown in the Table.

	Control	Wheat	Barley	Maize	Oats	Sorghum	LSD P=0.05
Bodyweight @ 7 d (g)	147	147	146	147	150	145	3.8
@ 11 d (g)	275	157	161	153	144	152	3.3
@ 42 d (g)	2078	1921	1883	1882	1885	1882	66.9
Feed intake 7 - 42 d (g)	3620	3185	3227	3266	3212	3262	122.1
FCR 7 - 42 d	1.74	1.66	1.71	1.73	1.70	1.73	0.03
Abdom. fat pad (g/kg)	13.6	13.6	13.3	13.9	12.6	13.1	0.50

The feeding of the cracked grains induced a growth retardation similar to that achieved by previous, successful food restrictions (Jones and Farrell, 1990). However, bodyweight was significantly decreased at slaughter and in only two treatments was food conversion ratio (FCR) significantly improved. Abdominal fat content was significantly lower in broilers fed cracked oats.

The lack of success of the cracked grain treatments examined here when compared to food restriction used in previous studies (Jones and Farrell, 1992) may be accounted for by the energy : protein (E : P) ratio of the grains. The E : P ratio of the grains is markedly higher than the previously used starter crumbles and protein intake may be insufficient to maintain lean tissue growth during the retardation phase. Similarly, excess energy consumption during this phase may not result in a decrease in body fat at slaughter. Hence, these data indicate that the feeding of cracked grain is not a viable alternative to food restriction for growth retardation of broilers.

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THE MANAGEMENT OF ASCITES IN BROILER CHICKENS BY PHYSIOLOGICAL MANIPULATION

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Summary

Three experiments examining the effects of physiological manipulation of broilers are described which were designed to decrease the occurrence of the ascites syndrome. Increases in the incidence of the syndrome have been related to breeding strategies such that the syndrome is now common in all broiler flocks. It has been proposed that alteration of the growth and performance of the organs and hormone systems of the bird may alleviate ascites. Increasing the development of the internal organs relative to total body growth has been shown to decrease ascites. However, manipulation of the activity of the thyroid gland had no effect. Attempts to decrease blood viscosity with dietary salicylic acid have met with no success.

I. INTRODUCTION

Intensive genetic selection for growth and food conversion efficiency in the broiler chicken has led to the development of metabolic and physiological consequences such as skeletal problems, but more importantly, ascites or right ventricular failure. This syndrome, once common only in hypoxic environments, now occurs universally in broiler production. In Australia, mortalities are generally 2-3% of flocks but can reach 10% (Groves, 1994).

The largest contributing factor to the increased incidence of ascites has been genetic selection for improved food conversion, and an associated decrease in oxygen consumption (Scheele *et al.*, 1991) and increase in pulmonary arterial pressure (Jones, 1994). Numerous measures have been proposed to reduce the severity of the syndrome, largely aimed at altering the growth rate and growth pattern of the broiler. This paper examines three approaches that consider strategies to reduce ascites in the broiler chicken by physiological manipulation.

II. GROWTH RETARDATION

There have been suggestions that early-life growth retardation, either by food restriction or light manipulation, decreases the incidence of ascites. It is well accepted that the major cause of ascites at low altitude is rapid growth rate (Julian, 1993) and, thus, it seems contrary that growth retardation followed by a period of compensatory growth could decrease ascites. However, it appears that organ development relative to total body growth is enhanced by a period of early-life growth retardation.

When broilers are classified as ascitic or non-ascitic, as determined by the arterial pressure index (Huchzermeyer *et al.*, 1988) and the growth and development of the heart, lungs and liver compared (Table 1), it can be seen that the development of the lungs and liver occurs later with respect to the whole body in ascitic broilers. The use of a period of growth retardation via early life food restriction (Jones, 1995) increases the rate of

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development of the heart and liver such that these organs are better able to cope with the increased growth rate of the bird that normally occurs after 21 d of age. (Table 2).

Table 1. Allometric growth equations of the heart, liver and lungs of ascitic (A) and non-ascitic (N) broilers.

Organ	Group	Equation	R^2	SE	
				Intercept	Slope
Heart	A	Y = -1.14 + 0.638X	0.423**	0.52	0.17
	N	Y = -1.15 + 0.631X	0.586***	0.35^{NS}	0.11^{NS}
Lungs	A	Y = -1.06 + 0.652X	0.572***	0.40	0.13
	N	Y = -0.85 + 0.575X	0.611***	0.30^{NS}	0.10***
Liver	A	Y = -0.32 + 0.620X	0.470**	0.46	0.15
	N	Y = -0.18 + 0.557X	0.475***	0.38*	0.12***

Table 2. Allometric growth equations of the heart and liver of normal (N) or growth retarded (GR) broilers.

Organ	Group	Equation	R^2	SE	
				Intercept	Slope
Heart	N	Y = -1.89 + 0.884X	0.555***	0.50	0.16
	GR	Y = -1.18 + 0.658X	0.558***	0.37^{NS}	0.12***
Liver	N	Y = -0.67 + 0.706X	0.469***	0.47	0.15
	GR	Y = -0.14 + 0.557X	0.381***	0.44***	0.14***

III. THYROID MANIPULATION

The relationship between oxygen consumption and ascites susceptibility may be linked to the activity of the thyroid gland (Julian, 1993) i.e. a decrease in thyroid activity may cause a decrease in oxygen use and increase ascites. This theory was examined in an experiment (Jones and Reynolds, unpublished) where male broilers known to be susceptible to ascites were fed diets containing thiouracil or iodinated casein, these chemicals being anti-thyroidic and thyroactive respectively. The influence of these substances is shown in Table 3.

Although no effects on bodyweight were observed (Table 3), thiouracil addition caused a decrease ($P<0.05$) in FCR with a concomitant increase ($P<0.05$) in pulmonary arterial pressure as shown by the arterial pressure index (API) data. The addition of iodinated casein to the diet produced no effects on the broilers used in this study. The haematology data (Table 3) revealed an increase ($P<0.05$) in haemoglobin concentration in the blood of the broilers fed thiouracil. These data indicate that the birds may be attempting to increase blood oxygenation, caused by genetic demands for growth, but retarded by reduced thyroid activity. Bird mortality due to right ventricular failure (RVF) was positively related to API (RVF = -34.0 + 1.46 API; R^2 = 0.729*). The response of the thiouracil fed broilers can be equated to birds selected on the basis of improved food conversion efficiency i.e. increasing efficiency increases the arterial pressure index. However, it seems unlikely that a reduced thyroid activity is responsible for the increased susceptibility to ascites as the addition of iodinated casein did not affect bird performance

or haematology. It may be that the genetic potential for growth of the bird has not only outstripped heart and lung capacity but that maximum thyroid activity is also inadequate.

Table 3. Growth performance and haematology of 42 d old male broilers fed anti-thyroidic and thyroactive diets.

	Control	Thiouracil		Thyroactive Casein		SED[1]
		100 mg/kg	200 mg/kg	200 mg/kg	400 mg/kg	
Bodyweight @ 42d (g)	1897	1876	1828	1946	1830	47.5
FCR (7 - 42 d)	2.31	2.24	2.27	2.30	2.33	0.034
Arterial Pressure Index (%)	24.3	27.1	29.5	24.3	25.0	1.06
Red blood cells (10^6/mm^3)	2.28	2.52	2.39	2.32	2.38	0.147
Haemoglobin (g/100 ml)	11.38	12.15	12.16	11.34	11.81	0.38
Haematocrit (%)	29.3	32.9	31.0	29.7	30.3	2.01

[1]Standard error of difference of means.

IV. SALICYLIC ACID

The incidence of ascites is associated with increases in blood viscosity as the broiler attempts to increase blood oxygen concentration. It has been suggested in humans that dietary salicylic acid intake may decrease susceptibility to heart disease and high blood pressure and this theory was tested in broilers susceptible to ascites.

Commercial, unsexed broilers were fed salicylic acid incorporated into the diet at 0, 5, 10 or 20 mg/kg and grown to 42 d of age. The results are shown in Table 4.

Table 4. Dietary inclusion of salicylic acid in commercial broiler diets and its influence on broiler growth and haematology.

	Salicylic Acid (mg/kg)				LSD
	0	5	10	20	P = 0.05
Bodyweight @ 7 d (g)	117	117	117	117	0.67
@ 42 d (g)	1762	1775	1782	1779	77.4
FCR 7 - 42 d	2.10	2.10	2.08	2.11	0.078
Arterial Pressure Index (%)	25.0	24.7	24.7	24.7	1.24
Red blood cells (10^6/mm^3)	2.73	2.84	2.55	2.69	0.16
Haemoglobin (g/100ml)	12.00	12.17	11.87	11.73	1.04
Haematocrit (%)	35.7	37.1	34.0	35.5	1.95

There was no improvement in bodyweight gain by salicylic acid inclusion and no effect on FCR or the arterial pressure index of the birds. Red blood cell number and haematocrit tended to decrease when 10 mg/kg salicylic acid was included in the diet. However, this was not significantly different when compared to control values (Table 4).

V. CONCLUSIONS

Manipulation of the organs of the broiler with respect to total body growth may reduce the incidence of ascites. The increase in ascites in broiler flocks does not appear to be linked to abnormal thyroid activity and the data obtained indicate that genetic selection for organ size i.e. heart, lungs, liver and thyroid may reduce ascites in the long term. Attempting to decrease blood viscosity does not appear to be a satisfactory solution as salicylic acid may not have any effect *per se* or the physiological need of the broiler for oxygen may override any benefit obtained.

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THE INCIDENCE OF BONE FRACTURES AND OSTEOPOROSIS IN THE LAYING HEN

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Osteoporosis and bone fractures in caged laying hens are a great welfare concern. The objective of the study presented in this abstract was to establish the incidence of osteoporosis and bone fractures in spent laying hens (68-72 weeks of age) in Victoria, Australia. The incidence of bone fractures is expected to be higher in flocks with high levels of osteoporosis than in flocks with low levels of osteoporosis and stronger bones. Several flocks of laying hens were examined, either at the farm or at the abattoir.

The hens examined in this survey (three different breeds) were obtained from commercial egg producers. Hens were removed carefully from cages and euthanased using CO_2 to avoid induction of any fractures. Hens that were collected from the abattoir had been commercially depopulated and transported. They were collected from the processing chain after stunning and bleeding. The carcasses were taken to the laboratory where they were weighed, dissected and examined for bone fractures. Rib abnormalities (concave ribs and nodular connections between ribs) were used as indicators of osteoporosis.

The incidence of bone fractures and osteoporosis (as measured by rib abnormalities) of caged laying hens collected on four farms.

Flock No. - Breed	1 - a (n=20)	2 - b (n=50)	3 - c (n=48)	4 - c (n=50)
Mean body weight (kg)	1.93	1.87	2.34	2.08
Rib abnormalities (%)	25	18	12.5	34
Bone fractures (%)	10	8	16.5	8
Osteoporotic with fracture (%)	0	11	33	12

In the experimentally depopulated cage hens the observed fractures were mainly of the tips of the pubis. The consequences of these fractures on the welfare of the hens are unknown. The average incidence of fractures at the abattoir in three other commercial depopulated flocks was 34%. Although the observed fractures at the abattoir were more severe it is not clear what proportion of the fractures was induced during depopulation and transport and which fractures were induced post mortem. Although osteoporosis appeared to contribute to the level of fractures in most flocks (Table), the variation between farms indicates that management and handling are the main contributors to bone fractures in caged laying hens. The significance of osteoporosis to the welfare of the hen and the consequences for egg shell quality and production remain to be clarified.

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A COMPARISON OF A LOCAL AND AN IMPORTED LAYER STRAIN UNDER AUSTRALIAN SUMMER CONDITIONS

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A number of overseas layer genotypes have been introduced into Australia during the 1990's. The Australian environment exposes these birds to stresses not experienced in more temperate climates. The present study was conducted to compare the production responses of an introduced coloured layer strain with a local coloured genotype under typical Australian summer conditions.

Pullets (150) from both strains were brooded to 4 wk of age in electrically heated brooders in the same room. The local strain was vaccinated for Marek's disease at d-old using Webster's H.V.T. vaccine while the introduced strain received Maravac plus double H.V.T. vaccine at d-old. Subsequent vaccinations for infectious bronchitis, fowl pox, infectious largngotracheitis and avian encephalomyelitis were carried out according to the breeders' recommendations. At 4 wk the pullets were transferred to temperature controlled rooms and the strains grown separately to 18 wk of age in diurnal temperature ranges of 10-20°C or 25-35°C. Between 10 and 18 wk the food intake was controlled so as to achieve the recommended body weights at 18 wk. The birds were fed commercial starter (12.0 MJ of ME and 196 g CP/kg) and grower (12.0 MJ of ME and 180 g CP/kg) diets between hatch and 10 wk and between 10 and 18 wk respectively. At 18 wk, 4 replicates of 6 pullets from each strain and growing temperature combination were housed in the 25-35°C diurnal temperature regimen and 2 replicates fed one of two diets containing respectively 11.5 MJ of ME and 170 g CP/kg (Diet 1) and 12.1 MJ of ME and 214 g CP/kg (Diet 2). A 16 h photoperiod was used throughout the study. Data were analysed as a 2^3 factorial ANOVA allowing 8 replicates of 6 pullets for comparison of main treatment effects of strain, growing temperature and laying diet.

Mean age of sexual maturity for the introduced strain (136.3 d) was significantly different to that of the local strain (151.9 d). Food intake during lay was similar with both strains (109 and 105 g/d) but the introduced strain had a significantly better hen-day production (83 vs. 75%) between 18 and 50 wk. This was offset by a significantly greater mortality (25 vs. 6.3%) due mainly to Marek's disease. This resulted in the hen-housed production (71 vs. 73%) and the mean daily egg mass over the 32 wk study (40.9 vs. 40.2 g egg/d) being similar with both strains. The experiment was terminated at 50 wk because of the high mortality observed with the introduced strain. The introduced strain laid significantly larger eggs (57.8 vs. 55.1 g) and had a significantly better feed conversion (2.18 vs. 2.42 g food/g egg). The only significant effects of the growing temperature were a delay in the age at sexual maturity (145.7 vs. 142.6 d) and an increase in % egg shell (9.22 vs. 8.92%) with hens raised in the hotter temperatures. The only significant effects of layer diet were on egg composition with Diet 1 giving greater % egg shell (9.14 vs. 9.00%) and % yolk (27.6 vs. 26.7%) and reduced % egg white (63.2 vs. 64.2%). Significant ($P<0.001$) strain x growing temperature x layer diet interactions were observed with % egg shell and shell thickness.

A separate evaluation of hens given municipal and saline (2 g NaCl/L) drinking water showed a similar incidence of egg shell defects from both strains receiving tap water (3.1 and 4.9%) but a much greater incidence for the introduced strain receiving saline drinking water (13.8 vs. 7.4%).

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THE ROLE OF ALDOSTERONE IN STRESS IN CHICKENS

A. LEARY and J.R. ROBERTS

A number of physiological changes are known to occur when an animal is subjected to stress. Stimulation of the sympathetic nervous system results in a rapid release of catecholamines (dopamine, noradrenaline, adrenaline) from the adrenal glands and sympathetic nerve terminals (Harvey *et al.*, 1984). Increased activity of the adrenal cortex has also been demonstrated, resulting in increased plasma levels of corticosterone (Holmes and Phillips, 1976). Another hormone from the adrenal cortex, aldosterone, may also increase as the result of stress caused by dehydration and heat stress (Arnason *et al.*, 1986). This study investigated two aspects of the role of aldosterone. Firstly, whether the plasma levels of aldosterone increased during osmotic stress imposed by intravenous infusion of hypertonic salt solution (1M NaCl) and secondly, the effect of intravenous infusion of aldosterone on kidney function in birds. In mammals, it has been well established that aldosterone increases the reabsorption of sodium and secretion of potassium from the distal nephron. However, this function has not been directly demonstrated in avian species.

In the first experiment, birds were anaesthetised, blood vessels cannulated for infusion of renal function markers and the hindgut tied off to allow collection of urine uncontaminated by faeces. Initially, birds were infused with an istonic solution of NaCl at 0.2 mL $min^{-1}kg^{-1}$ for a period of 1 h. Two urine collections, each of 30 min, were made and blood samples were collected every 30 min The infusate was then changed to hypertonic (1M) NaCl and this was infused for a total of 1.5 h. Urine and blood samples were collected every 30 min. Blood samples were centrifuged and the plasma reserved to allow for assays for the hormones aldosterone and prolactin, chemical analyses of the renal function markers and analyses for the electrolytes sodium, potassium, chloride and total osmolality. In the second experiment, birds were prepared as described for Experiment 1. A control group was infused with isotonic NaCl at 0.2 mL $min^{-1}kg^{-1}$ for a total period of 8.5 h. In the experimental group, isotonic NaCl was infused for 30 min and then the infusate was changed to isotonic saline plus aldosterone (12.5 μg mL^{-1}). Blood and urine samples were collected every hour and analysed as described for the first experiment.

The hypertonic saline load did not elevate the plasma levels of either prolactin or aldosterone despite the increases in the concentrations of plasma electrolytes. Glomerular filtration rate decreased and the rate of excretion of electrolytes increased. The infusion of exogenous aldosterone produced plasma levels in excess of 1 ng mL^{-1}. However, these high aldosterone levels had only minor effects on renal function.

These findings indicate that the role of aldosterone in the stress response of birds and the effect of this hormone on avian kidney function require further investigation.

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PERFORMANCE AND FEARFULNESS OF WHITE LEGHORNS AS INFLUENCED BY CAGE DENSITY

K. LEE and C. W. MOSS

Placing more birds into a given space decreases floor space allowance per bird and increases group size, which results in increased area density. Lee (1989) reported that floor space allowance, group size, or area density (a combination of the two) had no significant effect on the performance and fearfulness of birds housed in litter floor pens. However, since a floor pen environment is quite different from a cage environment, and since most U.S. producers currently use "high" cage density as compared to European producers who use "low" cage density (Craig and Swanson, 1994), there has been a growing public concern about the well-being of caged layers.

In this study, three experiments were conducted to examine the effects of cage density on layer performance and egg traits. Fearfulness of the birds was investigated in Experiment 3. In all experiments, 20-week old pullets were housed in 30.5 x 50.8 cm cages at the rate of 1, 2, 3, or 4 birds per cage. Each of these 4 treatments was replicated 6 times. Feed and water were given *ad libitum* for a period of 32 weeks under a 16-hour/day lighting regime. All eggs that were collected for two consecutive days every 8 weeks starting at 28 weeks of age were used to measure egg traits. In Experiment 3, fearfulness of the birds was determined by the duration of induced tonic immobility (TI) at 52 weeks of age, induced according to the method described by Craig *et al.* (1984).

The lowest percent hen-day egg production and the poorest feed efficiency were recorded from birds housed at the rate of 4 birds per cage in all experiments. The r values between the cage density and the egg production were, -0.87 ($P<0.01$), -0.72 ($P<0.01$), and -0.66 ($P<0.01$) for Experiments 1, 2, and 3, respectively. The r values between the cage density and the feed consumed to produce a dozen eggs were, 0.56 ($P<0.01$), 0.59 ($P<0.01$), and 0.42 ($P<0.05$) for Experiments 1, 2, and 3, respectively. Final body weight, mortality, and egg weight (4-period average) were not significantly ($P>0.05$) influenced by the cage density in any experiment. Albumen height (4-period average) was significantly ($P<0.05$) lowered by having more than one bird per cage in Experiment 3. There was no significant ($P>0.05$) difference in the duration of TI among treatments.

Results of this study showed that increasing cage density decreased bird performance and that cage density did not elevate fearfulness of the birds.

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SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE IN THE UPPER BEAK OF THE CHICKEN WITH PARTICULAR REFERENCE TO THE SALIVARY GLANDS

C A LUNAM* and P C GLATZ**

Summary

Both neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) co-existed in free nerve endings near the epidermis of the upper beak of the hen; these nerve fibers are considered to function as nociceptors. Our results indicate that the salivary glands of the chicken receive dual innervation, the major innervation being intrinsic parasympathetic nerves containing SP and a minor input from extrinsic CGRP-IR sensory afferents. The dense innervation of these extensive glands highlights the importance of salivary secretion in feeding. Effects of perturbation of the glands either by their direct removal or via damaging the nerves innervating them during severe beak-trimming is discussed.

1. INTRODUCTION

The beak of grain eating birds is a complex organ that contains sensory receptors and extensive mucous salivary glands. These structures are critical to the physiological and social activities of fowls. Herbst and Grandry corpuscles are sensory receptors which enable the bird to respond to external stimuli of touch, temperature and tactile discrimination whereas the secretion of mucous from the salivary glands is important to feeding.

The precise innervation of these structures, and hence the nature of neural control of the sensory receptors and salivary glands, is not known. Nerve tracing studies have revealed that the upper beak of the domestic fowl receives sensory innervation via the ophthalmic nerve from the trigeminal ganglion (Noden, 1980). Parasympathetic innervation of the salivary glands has been assumed to be from the ethmoid and sphenopalatine ganglia.

Immunohistochemistry has shown that in mammals particular classes of neurons contain specific combinations of chemicals. Furthermore, each class of neuron projects to a specific target. For example, the neuropeptides CGRP and SP co-exist in small diameter sensory neurons that project to the dermal-epidermal region of the skin; these are engaged in nociception (Fitzgerald, 1983).

A knowledge of how the structures are innervated is a first step in understanding how they function. Using double-labelling immunohistochemistry we investigated whether CGRP and SP co-existed in nerves in the upper beak of the hen. In addition, we examined the distribution of these neuropeptides in nerves associated with Herbst and Grandry corpuscles, and nerves innervating the salivary glands.

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II. MATERIALS AND METHODS

Upper beaks of a commercial laying strain (White Leghorn x Australorp) were removed immediately after euthanasia by cervical dislocation. Beaks had not been trimmed previously. The presence and distribution of CGRP and SP were compared between beaks from four birds killed on the day of hatch and four birds killed at ten days of age.

Beaks were fixed in a solution of 2% formaldehyde and 15% saturated picric acid in 0.01M sodium phosphate buffer, pH 7.1 at 4°C, followed by decalcification in ethylenediaminetetra-acetic acid (EDTA). After clearing in dimethyl sulphoxide, the tissue was washed in 0.01M phosphate buffered saline (NaCl, 8.5g/L), and 10 micrometer - thick frozen sagittal sections collected.

A double-labelling immunohistochemistry technique for visualisation of the co-existence of two antigens within a single nerve fiber was employed as described previously (Lunam, 1993). Primary antibodies were rat anti-SP (1:300) and rabbit anti-CGRP (1:2000). Secondary antibodies were goat anti-rat TRITC- conjugated, Cappel at 1:80 and sheep anti-rabbit FITC-congugated, Silenus Australia at 1:160. Cross reactivity and pre-absorption test were conducted to check that no nonspecific binding of the antibodies had occurred.

lll. RESULTS

No differences were observed in either the distribution or amount of the two peptides in the beaks of day-old chicks compared to that in the beaks of ten-day-old chicks. Both CGRP-immunoreactivity-(IR) and SP-IR were present in large nerve bundles. Although not quantitated there appeared to be considerably fewer fibers labelling for CGRP than for SP. Fluorescent nerve fibers were found throughout the dermis including at the tip of the beak.

Free nerve endings at the dermal-epidermal border labelled for both peptides: SP and CGRP was found to co-exist in the same nerve varicosity of these fibers.

SP-IR fibers were situated along the walls of arteries and veins, as well as being associated with numerous capillaries in the dermal papillae along the ventral margin of the beak. Although CGRP was also present in fibers around the blood vessels, the two peptides were not detected within the same nerve fiber.

Intensely fluorescent nerve fibers were particularly abundant in the maxillary and palatine salivary glands. SP-IR nerve fibers were present around both the secretory acini and salivary ducts. Nerve cell bodies in ganglia amongst the salivary acini demonstrated SP-IR. In contrast to the distribution of SP, only a few CGRP-IR fibers were found within the salivary glands and no nerve cell bodies contained CGRP-IR.

Nerves innervating the sensory receptors Grandry and Herbst corpuscles did not label for either SP or CGRP.

Pretreatment of the beaks with EDTA did not diminish peptide reactivity. Controls indicated that no inappropriate binding of the antibodies had occurred.

IV. DISCUSSION

Our results confirm and extend our previous reports of the SP-IR in the beak of the domestic chicken. In addition we describe the distribution of CGRP in the beak and its co-localisation with SP.

We had previously argued that the free nerve endings labelling for SP in the beak are likely to function as nociceptors (Lunam and Glatz, 1993). The finding that these fibers also contain CGRP adds further support to this hypothesis. In mammals, nerve fibers involved in pain transmission contain both CGRP and SP (Fitzgerald, 1983). Clarification of the function of these nerves in the beak requires correlation of anatomy with electrophysiological and behavioural responses to a variety of mechanical and noxious stimuli. It is of interest that the fibers labelling for both CGRP and SP were sparse.

The lack of nerves containing either CGRP or SP innervating the Herbst or Grandry corpuscles is consistent with the distribution of these peptides innervating analagous structures in mammals, that is Pacinian and Meissners corpuscles respectively. In both birds and mammals these rapidly adapting mechanoreceptors are innervated by large diameter myelinated nerve fibers that do not contain CGRP and SP. In sensory mammalian nerves CGRP and SP are mostly confined to the small diameter nerve fibers not associated with encapsulated receptors, as is the case in hens. This confinement of specific neuropeptides to different functional classes of sensory afferents in mammals and aves demonstrates that sensory innervation is highly conservative across vertebrate classes suggesting resistance to evolutionary pressure for change.

Secretion of salivary mucous is particularly important to grain eating birds. To assist feeding, the beak of the domestic fowl has extensive salivary acini that produce large amounts of mucous. The grain, after coating with saliva, is thrown onto the palate to form a bolus. The abundance of SP-IR nerve fibers within the salivary glands suggests that these nerves control salivary secretion. This is consistent with most mammalian species where salivary secretion is controlled by parasympathetic nerves that label for SP. In fowl, nerve cell bodies that project to the salivary glands of the upper beak also have been considered to be parasympathetic, with their cell bodies lying at some distance from the glands in the ethmoid and sphenopalatine ganglia. However, our finding of groups of nerve cell bodies labelling for substance P within the salivary glands suggest that these neurons may be the source of parasympathetic innervation. In support of this notion, the submandibular salivary gland of certain mammalian species is considered to be controlled by nerves projecting from local parasympathetic ganglia (Robinson *et al.*, 1980).

That SP and CGRP were never found in the same nerve fiber suggests that the salivary glands of the upper beak are innervated by at least two different types of nerves. We speculate that salivary secretion may be under duel control; by instrinsic local SP-IR parasympathetic nerves and nerves labelling for CGRP. As CGRP was not found in cell bodies within the beak, CGRP-IR fibers must be extrinsic. As the sensory input to the beak is solely via the ophthalmic nerve (Noden, 1980) the CGRP-IR fibers are likely to be sensory projections with their cell bodies within the ophthalmic lobe of the trigeminal ganglion.

Thus, we report that the salivary glands are not only densely innervated but that the salivary glands may be under dual local parasympathetic and extrinsic sensory control. Such neural control highlights the importance of salivary secretion in feeding. Removal or excessive perturbation of the salivary glands resulting from severe beak trimming could decrease feeding ability by interfering with salivary secretion.

In support of this hypothesis the rate of food consumption was markedly reduced in 70-week-old hens that had been severly trimmed at hatch (remaining upper beak 10mm; feeding rate 10.6 mg/sec) versus (remaining upper beak 15mm; feeding rate 21.6 mg/sec) (P.C. Glatz and L. Murphy, unpublished obervations). In addition, hens with the shorter beaks made twice the number of pecks at the water nipple compared to those hens with longer beaks. One explanation for these phenomena is that decreased production of saliva

results in greater time required for bolus production. The increased pecking at the nipple may simply be an attempt to increase moisture levels to assist bolus formation.

The above hypothesis is an alternative to that suggested by many workers that the reduced feeding ability of severely beak-trimmed hens results from either chronic pain or sensory deficiency after removal of sensory receptors. It is of interest that Workman and Rogers (1990) reported beak-trimmed chickens swallowed less than untrimmed chickens. A decrease in the rate of swallowing is consistent with a greater time required for bolus formation. Further experiments need to be conducted to determine which, if any, of these hypotheses are correct.

V. ACKNOWLEDGMENTS

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NEUROMA FORMATION IN BEAK TRIMMED HENS

C A LUNAM* and P C GLATZ**

Summary

Persistent traumatic neuromas were present in the upper beak of adult hens (70 weeks of age) that had been severely trimmed (two-thirds removed) and cauterised at hatch. In contrast neuromas were not found in the upper beaks of adult hens that had one-half of the beak removed also at hatch with brief cauterisation. These data show that the severity of beak trimming, that is the amount of beak removed and cauterisation time, is a major determinant of development of persistent neuromas. The potential impact of neuromas on the welfare of hens is addressed briefly.

I. INTRODUCTION

Beak trimming is performed in commercial hens to prevent cannibalism and feather pecking. This involves partial removal of the upper and lower beak using an electrically heated blade. This process has remained unchanged since the introduction of the Lyon electric debeaker in 1942. In recent years beak trimming has come under scrutiny by animal welfare groups. Contraindications to the continuation of beak trimming include removal of sensory receptors with a subsequent reduction in feed intake (Glatz and Lunam, 1994) and permanent loss of temperature and touch responses (reviewed by Gentle, 1986b).

A major concern is that beak trimming may result in chronic pain. Neuroma formation in the beak stump after beak trimming has been implicated as a cause of chronic pain in commercial hens (Gentle, 1986ab; Breward and Gentle, 1985). Gentle (1986a) observed that the stages of neuroma development in the beak stump of chickens trimmed at 5 weeks of age was similar to that described in mammals.

An interesting phenomenon in mammals is that persistent neuromas were found to occur less frequently in injured nerves of neonatal rats compared to similar nerve injury in adult rats (Fried and Frisen, 1990). As injured mammalian and avian nerves regenerate and form neuromas in a similar fashion, we speculated that beak trimming at hatch may avoid or at least lessen the incidence of persistent neuromas.

In this study we examined the beaks of adult hens trimmed at hatch for anatomical evidence of neuromas. In addition, we examined the effects of removal of one-half compared to two-thirds of the upper beak, as well as altering cauterisation time on the occurrence of neuromas.

II. METHODS

Beaks were examined from a commercial laying strain (White Leghorn x Australorp). All hens, except intact control hens, had been beak trimmed at hatch, reared and maintained at the Parafield Poultry Research Centre with food and water provided *ad libitum*. Beak trimming was conducted using a Lyon electric machine utilising a heated

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blade that both cuts and cauterises the tissue. At hatch the chickens were divided into three groups. One group of chickens were beak trimmed by an experienced operator; these chicks had half the upper and one-third of the lower beak removed. The second group of chickens were trimmed by an untrained operator with the result that often two thirds of the upper beak and half of the lower beak was removed. The duration of cauterisation of the second group (2-4 seconds), was considerably longer than that of group 1 (2 seconds). A third control of chickens were reared under identical conditions as groups 1 and 2, except these chickens were not beak trimmed.

At 70 weeks of age hens from each of the three groups were euthanised by cervical dislocation for histological assessment of neuromas in the beak. Selection of hens was based on visual deformity of the beak. Group 1; three hens were selected at random with no swellings on their beak, none of the hens trimmed by the trained operator revealed any deformities. Group 2; three hens with abnormal swellings on the tomial margins of their upper beaks. Group 3; 2 control hens that had not been beak trimmed.

Immediately after killing the upper beaks were excised and fixed by immersion in a solution of 2% formaldehyde and 15% of saturated picric acid in 0.01M sodium phosphate buffer, pH 7.1 at 4°C. After decalcification in ethylenediaminetetra-acetic acid (EDTA). beaks were washed in 0.01M phosphate buffered saline (NaCl: 8.5g/l), and 40 micrometer-thick frozen consecutive sagittal sections collected at 400 micrometer intervals through each beak. Nerve fibers in the tissue sections were stained using a triple silver impregnation technique. Sections were dry mounted onto slides, coverslipped and viewed with white transmitted light using an Olympus BH-2 microscope.

lll. RESULTS

(a) Intact beaks from control hens

Nerve fibers in the intact beaks from the two control hens mostly ran in straight bundles, with nerve fibers lying parallel to one another. Scattered nerve fibers were found within the dermis, particularly near the tomial margins and at the tip of the beak. Many fibers were present around the blood vessels and occasionally a single fiber could be seen innervating Herbst and Grandry corpuscles. Nerve fibers were also found around the salivary glands.

(b) Beak trimmed: removal of one-half upper beak

In the proximal beak, towards the nares, nerve fibers mostly formed large bundles of parallel fibers. The distributions of fibers around blood vessels and in the dermis were similar to those found in the intact control beaks. Individual fibers were observed near the epidermis, particularly in the tomial margins and in the dermis of the distal beak. Although the numbers of Herbst and Grandry corpuscles were markedly less than in the intact beaks nerve fibers clearly innervated these sensory receptors. There was no evidence of neuromas in any of the beaks examined.

(c) Beak trimmed: removal of two-thirds upper beak

Neuromas were found in the dermis beneath the swellings on both beaks. The neuromas consisted of large tangled masses of nerve fibers. Nerve fibers that branched

from the central neuroma mass often had a crimped wavy appearance. Other small bundles of nerve fibers ended in small encapsulated swellings scattered in the dermis of the distal and tomial margins of the beak. Although not quantified, there were considerably more nerve fibers in these beaks compared to the other two groups. Nerve fibers were often found within the epidermis.

IV. DISCUSSION

Removal of two-thirds of the upper beak at hatch resulted in the formation of neuromas that persisted to seventy weeks of age. In contrast, neuromas were not observed in adult hens that had one-half of their upper beak removed at hatch. These results indicate that there is a critical amount of beak tissue that can be removed, beyond which neuromas will persist.

It is of interest that the abnormal swellings on the beak were the sites of extensive neuromas. Neuromas also exist in the beak stump 70 days after removal of one-third of the upper beak of 5-week-old chickens (Gentle, 1986a). Thus, the proportion of beak tissue that can be removed to minimise the potential for development of persistent neuromas may decrease with the age of the chicks. However, in his study Gentle (1986a) reported that cauterisation caused significant damage to the beak stump. Thus, in this case effectively more than one-third of the upper beak may have been removed.

Although we found no evidence of neuromas in hens in which one-half of the upper beak had been removed at hatch, this does not exclude the possibility that neuromas may have developed initially and then been reabsorbed into the developing beak tissue. Our rational for this is that in mammals all peripheral severed axons form neuromas as part of normal regeneration. This involves sprouting of the injured axons to form foci of axon tangles. However, these are usually transient and the sprouting axons eventually reinnervate their targets, with culling of the excess abnormal sprouts (Devor and Rappaport, 1990).

A major difference between the chicken beak and mammalian tissue is the ability of the chicken beak to regenerate. For instance, upper beaks regrow 5mm by 54 weeks of age following retrimming of 14 week-old birds (Glatz, unpublished observations). Although the regenerated beak has been reported to be abnormal with no receptors (Gentle, 1986a,b) we found Herbst and Grandry corpuscles in the regenerated beaks of hens in which half of the upper beak was removed at hatch. Although these were considerably fewer in number compared to intact control beaks, these receptors were innervated by nerve fibers, indicative of normal function. Thus, we conclude that conservative beak trimming at hatch, the time of maximal tissue regeneration, allows sufficient peripheral targets to accommodate the regenerating axon sprouts, thereby minimising the likelihood of the formation of persistent traumatic neuromas.

The presence of neuromas in chicks has been linked with chronic pain after beak trimming (Gentle, 1986a,b). The basis for this is that neuromas have been found to generate spontaneous neural activity in hens (Breward and Gentle, 1985) and such discharges can be a cause of chronic pain in mammals (reviewed by Devor and Rappaport, 1990). However, it should be emphasised that the presence of neuromas are not in themselves evidence of chronic pain.

To evaluate the effects of beak trimming on the welfare of commercial hens anatomical findings need to be correlated with behavioural and physiological data.

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PRODUCTION AND NUTRITION OF EMUS

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Summary

The farming of emus for their meat, leather and oil products is a relatively new and growing primary industry. The continuing demand for stock is sustaining economic viability while the industry establishes markets for its products.

Emus are seasonal breeders and already their potential to increase fecundity under improved management is apparent. Marked seasonal variations in voluntary feed intake occur which may be detrimental to both egg production and growth rate. Relative to liveweight their dressed carcass weight is only 70% that of broilers but their fat yield is nine times greater.

The structure and function of the alimentary tract of the emu are similar to those of poultry except that in emus the crop is absent and the paired caeca have only a limited function. The overall length of the alimentary tract relative to liveweight is much less than in the domestic fowl. Microbial degradation of cellulose and lignin is greater in the emu. While emus in the wild have been observed to eat nutritious food little is known of their nutrient requirements. On a metabolic liveweight basis their daily maintenance requirements for energy and protein are much lower than for chickens.

An experiment to determine the productive responses to lysine of emus from 23 to 65 days of age is described. From fitted quadratic models it was determined that the lysine levels corresponding to the calculated maximum growth rate and minimum FCR were 0.900 and 0.825 g lysine/MJ ME respectively.

1. INTRODUCTION

The farming of emus is an emerging primary industry which has developed considerable interest in Australia. It was first attempted here in 1970 in Western Australia but was not officially sanctioned until 1987 (O'Malley, 1989). The current size of the industry nationally extends to nearly 650 licensed farms with a population of 71 000 emus and an estimated annual production of 78 000 emu chicks. A major condition for the granting of approval by state governments to farm emus has been that only captive or farm-reared stock are permitted to be farmed. As a result, the sale of breeding stock to new and expanding farms has been a significant factor in the viability of the industry thus far. Sustainable domestic and export markets for emu products, principally meat, oil and leather are only in their infancy. In the short to medium term any expansion of these markets will be met by increases in stock numbers; in the longer term, increases in the productivity of emus through the application of genetic selection and improved management and nutrition will become significant.

II. REPRODUCTION

In common with the domestic hen, female emus have only a left side functional ovary and oviduct. They attain sexual maturity at between 18 and 21 months of age and

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males at about 12 months of age. Their breeding season of approximately 120 days falls within the period March to October. Eggs are laid in clutches of from five to 20 eggs with individuals producing up to four clutches per season. Females in their first breeding season may each average nine to 10 eggs. In their second season birds can average more than 20 eggs each, with some individuals laying as many as 50 eggs if eggs are regularly removed from the nest. Hens usually lay one egg every 2 to 3 days but good layers may produce an egg every day for several days (Tan, 1991). The regression of follicles is known to occur.

Emu eggs weigh about 600 g of which 76 g is shell (Beutel *et al.*, 1983). It is estimated an egg contains 59 g protein and 66 g fat (Angel, 1993).

III. GROWTH AND FEED INTAKE

An examination of the growth curves of five flocks (Figure 1) shows a reasonably constant growth rate of about 111 g/bird/day to 20 weeks of age followed by a lesser rate of 88 g/bird/day to 64 weeks of age. However, in short term trials with a high plane of nutrition growth rates approaching 130 g/bird/day have been attained (Kent and Mannion, 1993). Despite having unlimited access to compounded grower diets emus exhibit marked variations in liveweight gain during the year even though well below their mature liveweight of approximately 55 kg (Grzimek, 1972). This appears to be associated with seasonal effects on voluntary feed intake (Figure 2). Seasonal variation in feed intake is also exhibited by sexually mature emus. Intakes of 350 to 450 g/day during the breeding season, rising for a period of 2 to 3 months to 1500 to 1600 g/day in spring-early summer have been reported (Dingle, 1993a; Angel, 1994). Reductions in liveweight of 20 % in females post-egg laying and up to 50 % in males during natural incubation have been reported (Dingle, 1993a).

IV. CARCASS YIELD

The emu (*Dromaius novaehollandiae*) belongs to a group of birds known as ratites characterised by a noncarinate sternum (ventrally convex, lacking a keel). As a consequence of this and being flightless, the breast of the emu is devoid of muscle and thus meat yield is predominantly derived from the rump and leg muscles. Body fat is largely subcutaneous, abdominal and mesenteric.

Table 1. Carcass yield (% of liveweight) from commercially slaughtered emus[1].

Liveweight (kg)	Dressed weight (%)	Cut up meat weight (%)	Fat weight (%)	Mean number of emus per trial
39.8	47.0	31.9	19.3	16

[1] Dingle (1993 a,b).

Mean carcass yields calculated from five slaughtering trials (Table 1) show that emus have a lower dressing percentage and meat yield, but a markedly higher fat yield, than meat chickens at typical slaughter weight; respective values for broilers are 67%, 52% and 2 to 3% (Moran and Bilgili,1990).

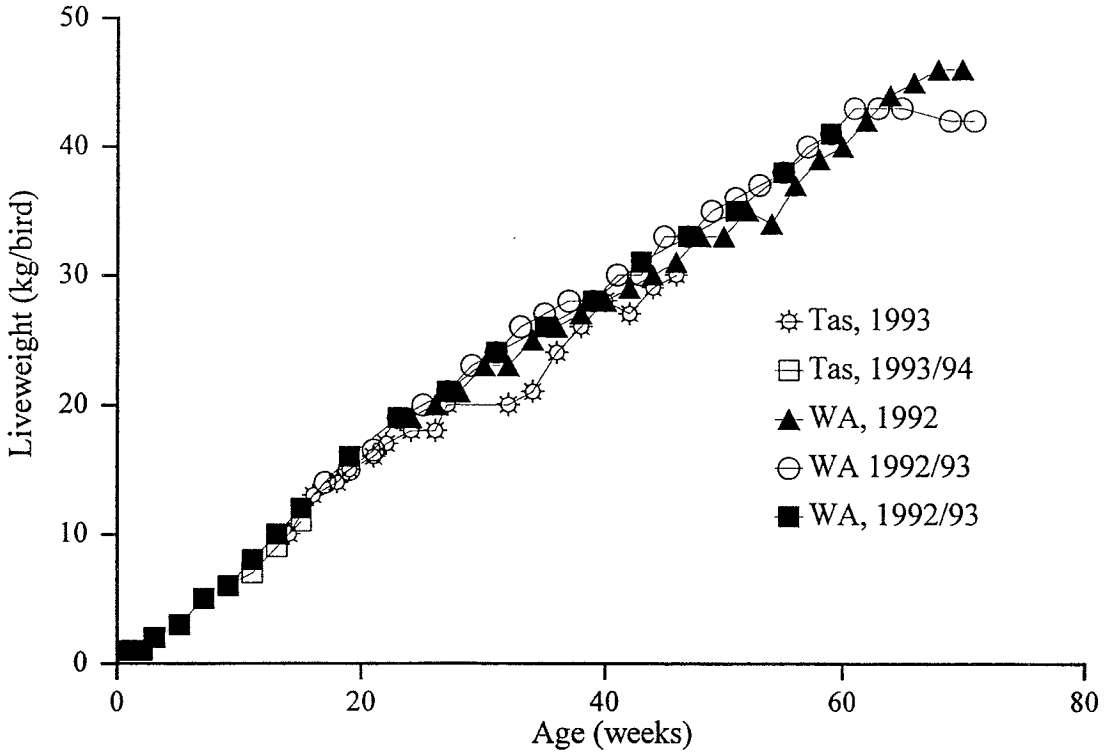

Figure 1. Liveweight data for five flocks (Auckland 1993, O'Malley 1993)

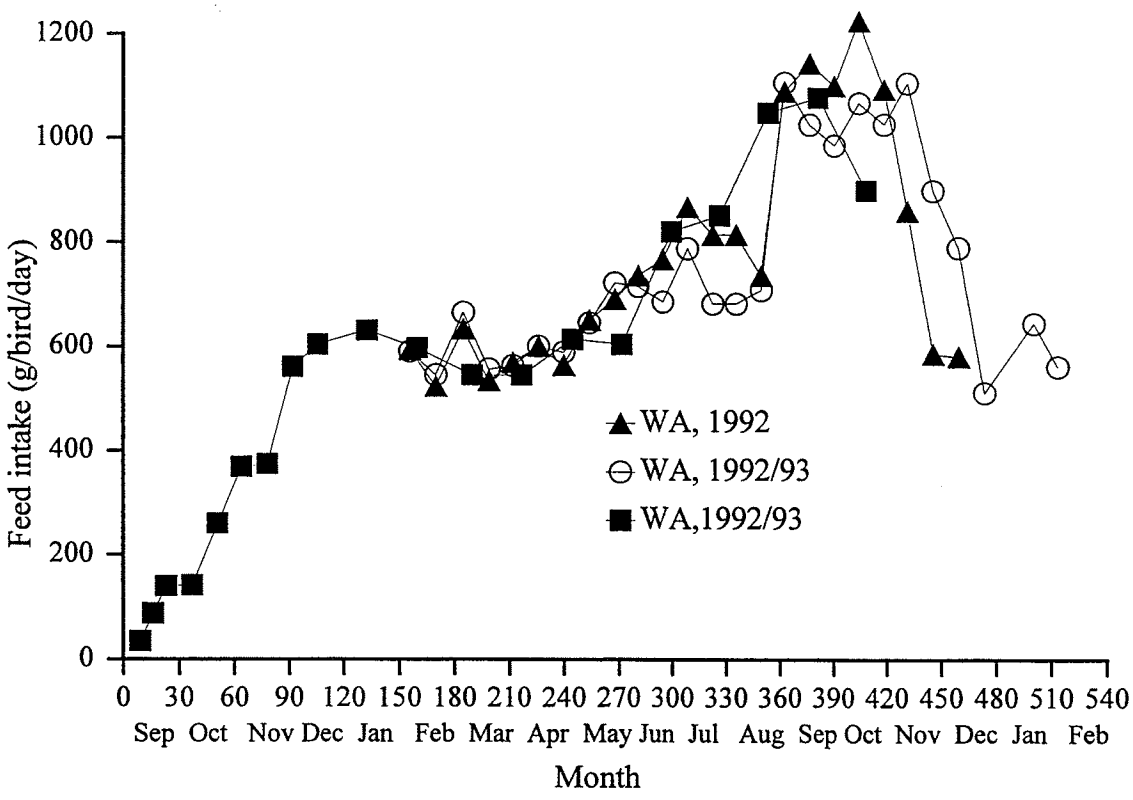

Figure 2. Feed intake data for three flocks aged at commencement:
▲ 27 weeks; ○20 weeks and ■1.6 weeks (O'Malley 1993)

Fat deposition occurs largely in the latter growth phase. Dingle (1993 a,b) was unable to harvest any fat from emus slaughtered at 30 weeks of age but at 40 weeks the fat yield was 7.1% of liveweight.

V. ALIMENTARY TRACT AND DIGESTION

In common with poultry, emus have a monogastric digestive system comprising an oesophagus, proventriculus, gizzard, duodenum, jejunum, ileum, caeca, colon and cloaca (Cho *et al.*, 1984). They have no distinct crop although the proventriculus is quite distensible and possibly could serve as an organ for food storage. The length of the small intestine and of the entire alimentary tract, in proportion to liveweight, are both much less in the emu than in the domestic fowl (Table 2.). The relative proportions of the different regions of the alimentary tract are similar in young and mature emus (Fowler, 1991). The paired, short caeca of the emu have only a limited apparent function (Fowler, 1991). The cloaca is the common site for excretion by the urinary and digestive tracts.

Table 2. Comparative lengths of the alimentary tract of emus and domestic fowl[1].

	Emu		Domestic fowl	
Region	% of total length	Length (mm/kg LW)	% of total length	Length (mm/kg LW)
Oesophagus	16.8	19.8	15.2	140
Proventriculus	3.1	3.6	) 7.4	) 68
Gizzard	2.4	2.9	)	)
Duodenum	11.1	13.1	13.0	120
Jejunum	27.3	32.1	45.2	416
Ileum	31.5	37.1	7.0	64
Caeca (one only)	1.3	1.6	7.4	68
Colon	6.4	7.6	4.7	44

[1] Herd and Dawson (1984); Koch (1973); Sturkie (1965).

A detailed account of the histology of the emu alimentary tract (Herd, 1980) indicates that the various regions function in a similar way to those of poultry (Herd and Dawson, 1984).

The rates of passage of digesta in the emu of both plant particulate matter (5.5 h) and the liquid phase (4.1 h) (Herd and Dawson, 1984) are similar to those in the domestic hen. Despite this relatively rapid rate of passage and the lack of any apparent anatomical specialisations, emus are able to digest 35 to 45% of the neutral detergent fibre (NDF) in their diet. The digestion of hemicellulose accounts for much of this (50 to 60% digested) but up to about 20% of dietary cellulose and lignin (the two fractions of acid detergent fibre) may also be digested (Herd and Dawson, 1984). Although the disappearance of NDF from the gut does not necessarily mean that the emu actually derives energy from the degraded fibre, the production of significant quantities of VFA from microbial fermentation, particularly in the ileum but also in the colon and cloaca, suggests that this may contribute to its energy needs. Theoretically, if the energy derived from the digested NDF was all utilised this would represent up to half the emu's maintenance requirement for energy. Microbial degradation of food also occurs in the domestic fowl (Barnes, 1972; Sturkie, 1965) and levels of digestibility of NDF comparable with that of emus have been

reported (Moran and Evans, 1977). Acid detergent fibre, however, is degraded to a much lesser extent in the domestic hen (only 2 to 3% digested).

The occurrence of age-related changes in the digestibility of NDF and fat, and in measures of dietary metabolizable energy (ME) content, have been demonstrated in ostriches (Angel, 1993), with increases in each component up to 17 weeks of age but stabilising thereafter. Data for the emu are lacking.

VI. NUTRITIONAL REQUIREMENTS

The nutritional requirements of emus have not been determined and as a result many of the diets formulated for them are based on limited data or previous experiences with quite unrelated species. Nevertheless emus will grow and reproduce well on low energy poultry type diets which conform in other respects to standard broiler and layer specifications, respectively (O'Malley, 1993).

Estimates of their daily maintenance requirement for energy and nitrogen, adjusted for liveweight, show that they are much lower than those determined for poultry (Dawson and Herd, 1983):

	Maintenance requirement	
	Emu	Domestic fowl
Nitrogen (g/kg $^{0.75}$/day)	0.09	0.34
ME (kJ/kg $^{0.75}$/day)	284	405-425

For an emu of 30 kg liveweight this translates to a daily maintenance requirement of 3.6 MJ of ME and 7.2 g protein. A 4 kg rooster, by comparison, requires 1.2 MJ of ME and 6.0 g protein per day. The low energy requirement appears to be associated with the low basal metabolism of emus (Calder and Dawson, 1978) and the very low nitrogen requirement may reflect some form of recycling of urinary nitrogen (Dawson and Herd, 1983). These data are from a single study with four emus and further verification of these values is warranted.

Of greatest need are data relating growth and egg production responses to a range of intakes of dietary energy and individual amino acids. Unfortunately, these data are absent from the literature. Theoretical estimates of such responses derived from growth models and carcass composition data are similarly unavailable at present although work in this area is in progress (O'Malley, 1993). The experiment reported below examines the responses of young growing emus fed diets differing in lysine content.

(a) Experimental Methods

Five diets (Table 3) ranging in lysine content from 11.50 g/kg (A) to 5.75 g/kg (E) were fed to unsexed emus from an average age of 23 days for a period of 6 weeks. The diets were formulated to contain the same calcium, phosphorus and calculated ME (11.50 MJ/kg) concentrations. It was hoped that lysine would be the first limiting amino acid. To test this assumption an additional diet, F, was fed based on diet E to which additional synthetic lysine was added to provide a total lysine content in diet F equivalent to that in diet C.

Each of the six experimental diets was fed *ad libitum* in mash form to five pens each of five emus. A randomised block design was used. Each emu was individually

weighed at fortnightly intervals and feed residues were weighed on a pen basis at each of these times.

Proximate analyses were conducted by methods described by Moir *et al.* (1980). Amino acids were analysed by ion-exchange chromatography (Waters HPLC) after hydrolysis with 6 M HCI at 110°C for 18 h under either sealed tube or reflux conditions for materials below or above approximately 200 g/kg crude protein respectively. Cystine and methionine were determined as cysteic acid and methionine sulfone respectively, following performic acid oxidation. Tryptophan was not determined.

Analysis of variance was used to test the effect of treatments, and treatment means were compared using the protected lsd test. Production responses to lysine levels were modelled as quadratic regressions.

Table 3. Composition of the experimental diets (g/kg).

	A	B	C	D	E	F
Sorghum	444.70	493.50	542.30	591.10	640.00	640.00
Cottonseed meal	25.30	31.50	37.70	43.90	50.00	50.00
Soyabean meal	223.40	176.70	130.00	83.40	36.70	36.70
Sunflower meal	220.00	191.90	163.70	135.50	107.30	107.30
Lucerne meal	-	12.50	25.00	37.50	50.00	50.00
Soyabean oil	23.10	22.40	21.60	20.80	20.00	20.00
Dicalcium phosphate	24.40	25.00	25.50	26.10	26.60	26.60
Limestone	23.30	23.70	24.10	24.50	24.80	24.80
Salt	4.10	4.00	4.00	4.00	4.00	4.00
DL-Methionine	4.00	3.40	2.80	2.20	1.60	1.60
L-Lysine HCL	-	-	-	-	-	3.69
L-Isoleucine	0.40	0.30	0.20	0.10	-	-
L-Leucine	0.86	0.65	0.43	0.22	-	-
L-Threonine	1.06	0.80	0.53	0.27	-	-
Diatomaceous earth	-	8.27	16.76	25.03	33.62	29.93
Mineral Premix	2.10	2.10	2.10	2.10	2.10	2.10
Vitamin premix	3.08	3.08	3.08	3.08	3.08	3.08
Antioxidant	0.20	0.20	0.20	0.20	0.20	0.20
Analysis						
Lysine	11.50	10.06	8.63	7.19	5.75	8.63
Methionine	7.59	6.64	5.69	4.74	3.80	3.80
Meth+Cys	11.27	10.04	8.76	7.42	6.04	6.04
Isoleucine	9.49	8.39	7.28	6.13	4.96	4.96
Threonine	8.86	7.75	6.64	5.53	4.43	4.43
Calcium	16.00	16.25	16.50	16.75	17.00	17.00
Total phosphorus	9.75	9.49	9.24	8.98	8.72	8.72

(b) Results and Discussion

The effects of the treatments on average daily liveweight gain (ADG), feed intake and feed conversion ratio (FCR) are given in Table 4. There were no significant differences between treatments for liveweight at the start of the experiment (mean $\pm$ SD = 1615 $\pm$ 45.9 g/bird). The results show that feeding diets low in lysine resulted in a significantly reduced ADG ($P<0.05$) and feed intake ($P<0.05$), and a poorer FCR

($P<0.05$). That the observed response was due solely to lysine is supported by the finding that the performance of emus fed diet F was significantly ($P<0.05$) greater than that of emus fed diet E but statistically indistinguishable ($P>0.05$) from treatment C. This has been interpreted as confirming lysine to be the first limiting nutrient.

Table 4. Average daily gain (ADG), feed intake and feed conversion ratio for emus grown from 23 to 65 days on diets differing in lysine content.

Diet	Lysine (g/kg)	ADG (g/bird)	Feed intake (kg/bird)	FCR (g/g)
A	11.50	126.4	14.98	2.90
B	10.06	126.6	14.77	2.83
C	8.63	123.5	14.11	2.72
D	7.19	105.4	12.66	2.90
E	5.75	78.1	11.31	3.48
F	8.63	120.2	13.53	2.75
LSD (P=0.05)		10.86	1.73	0.329

Regression analyses of the treatment mean data, measured over the full period of the experiment and excluding treatment F, show that a quadratic model fits the data well:

ADG (g/bird/day) = -129 + 574 ($\pm$ 51) X - 320 ($\pm$ 34) X^2, R^2 = 0.996

Feed intake (kg/bird) = 0.42 + 28.67 ($\pm$ 4.09)X - 14.07 ($\pm$ 2.72) X^2, R^2 = 0.995

FCR = 7.6 - 11.9($\pm$ 2.6) X + 7.3 ($\pm$ 1.7) X^2, R^2 = 0.94

where X = dietary lysine content, g lysine/MJ of ME.

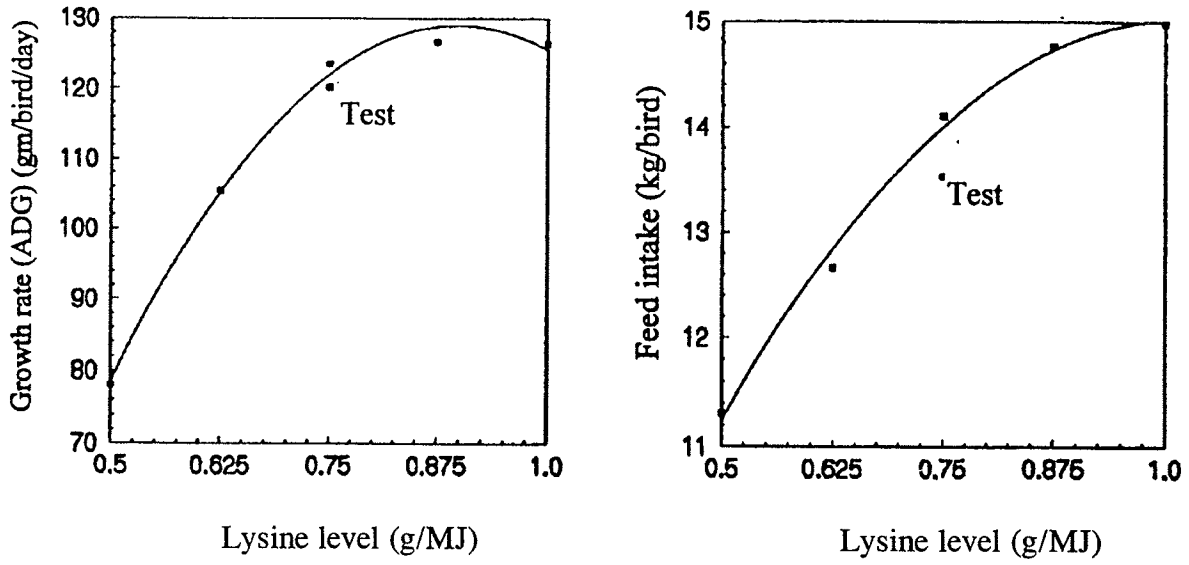

Figure 3. Growth and feed intake responses of emus fed diets differing in lysine content.

The quadratic regressions for average daily gain and feed intake were highly significant ($P<0.01$) but both the linear and quadratic components for FCR just failed to reach significance ($P=0.06$). Figure 3 illustrates that the growth rate response reached a clear plateau but feed intake continued to rise over the range of lysine content. The emus showed no evidence of an increase in feed intake when fed diets marginally deficient in an essential amino acid, as is often seen in chickens. The lysine levels corresponding to the

calculated maximum growth rate and minimum FCR were 0.90 and 0.825 g lysine/MJ ME, respectively. These data provide a basis upon which the economic optimum level of lysine inclusion can be calculated.

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THE RELATIONSHIP BETWEEN AGE OF DUCKLINGS AND CHICKENS AND THE APPARENT METABOLISABILITY OF OIL AND ENERGY IN RICE BRAN

E. MARTIN and D.J. FARRELL*

Previous studies with chickens showed that the apparent metabolisability of oil in rice bran (Warren and Farrell, 1990) and other seed meals (Askbrant and Farrell, 1987) increased with age of bird. Here we investigated further these changes with chickens and ducklings given diets with 0 or 400 g rice bran/kg. Feed intake and excreta output were measured over 5 days in four replicate groups of two chickens or three replicate groups of two ducklings at 3-7, 10-14, 17-21 and 23-27 (chickens only) days of age. The results are given in the Table.

Apparent metabolisable energy (AME) of rice bran and metabolisability of oil in rice bran measured with broiler chicks and ducklings at different ages.

Age (days)	AME (MJ/kg DM)		Oil metabolisability (%)	
	Ducklings	Chickens	Ducklings	Chickens
3-7	15.6[a]*	10.5[a]	69.9[a]	26.9[a]
10-14	16.8[b]	12.7[b]	80.8[b]	45.2[b]
17-21	17.9[c]	13.0[b]	94.0[c]	64.0[c]
23-27		14.2[c]		79.1[d]
SEM	0.32	0.31	0.99	1.6

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

Regression equations were calculated to predict metabolisability (M) of oil (%) and AME (MJ/kg DM) of rice bran from age (days) of chickens (c) and ducks (d):

$$\text{Oil } M_c = 15.0 + 2.5 \text{ age}, \quad \text{RSD} = 3.2, R^2 = 0.98, n = 16 \quad (1)$$

$$\text{Oil } M_d = 60.9 + 1.7 \text{ age}, \quad \text{RSD} = 1.7, R^2 = 0.98, n = 9 \quad (2)$$

$$AME_c = 10.1 + 0.16 \text{ age}, \quad \text{RSD} = 0.71, R^2 = 0.78, n = 15 \quad (3)$$

$$AME_d = 14.9 + 0.16 \text{ age}, \quad \text{RSD} = 0.51, R^2 = 0.80, n = 8 \quad (4)$$

Clearly ducklings, even at an early age, have a much greater capacity to metabolise oil in rice bran than chicks at a similar age. In both cases there was a concomitant increase in the AME of rice bran with age. However, values for ducklings were always considerably higher than for chickens. At 17-21 d of age the AME of 17.9 MJ/kg DM for ducklings is higher than anticipated but reflects the very high metabolisability of the oil (94%).

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THE EFFECTS ON DUCKLING PERFORMANCE OF PROTEIN SOURCE AND FEED PHYTASE IN DIETS WITH OR WITHOUT RICE BRAN

E. MARTIN AND D.J. FARRELL*

Summary

Groups of 5 ducklings were given formulated diets with 0 or 600 g rice bran and 3.9 g available phosphorus (P)/kg with or without fish meal and with or without a microbial phytase. Rice bran addition reduced growth rate and feed efficiency but fish meal overcame these depressions. Feed phytase increased feed intake. Tibia ash responded to phytase in diets with rice bran only even though calculated available phosphorus was 3.8 g/kg and fish meal was included. Availability of amino acids and P may be depressed in the diet of ducklings when rice bran is included in high amounts.

I. INTRODUCTION

Martin and Farrell (1994) showed the beneficial effects of supplementing rice bran-based duckling diets with a feed phytase (Natuphos) produced from *Aspergillus niger* (Gist-brocades, The Netherlands). Subsequent work has indicated that an all-vegetable diet in combination with rice bran may not support maximum duckling performance. Phytase, in addition to increasing available phosphorus (P), can increase nitrogen (N) retention and metabolisable energy (ME) of diets deficient in available P (Farrell *et al.*, 1993). Here, the effects on growth and feed conversion ratio (FCR) of ducklings was examined using the same feed phytase, initially at 1000 U/kg (see Martin and Farrell, 1994), in conventional, pelleted diets with or without animal protein (fish meal) and with or without rice bran.

II. MATERIALS AND METHODS

Groups of five ducklings (4-d old) in heated brooder cages were allocated to one of eight diets containing either 0 or 600 g rice bran/kg with or without animal protein (fish meal) and with or without feed phytase. Phytase inclusion commenced at 1000 U/kg feed and was increased to 1500 U/kg feed when the ducklings were aged 15 d. An additional all-vegetable diet with increased crude protein (CP) (230 g vs. 211 g/kg) from soybean meal was included. Thus, there were 9 formulated diets x 3 replicates x 5 ducklings. For all diets non phytate phosphorus (P) was calculated to be 3.9 g/kg feed. Ducklings were weighed and feed intake recorded at 4, 15 and 22 d of age. All excreta were collected and feed consumption recorded between d 4 and d 11. Acid insoluble ash in feed and excreta (Van Keulen and Young, 1977) was used to estimate excreta output between d 15 and d 22. Analyses of samples of feed and excreta followed AOAC (1990) standard methods. Tibia ash was determined as described by Farrell *et al.* (1993). Treatment effects and interactions were measured using analysis of variance. Differences between means were subjected to the Least

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Significant Difference (LSD) test with significant differences ($P<0.05$) between means being denoted by the use of different superscripts in Tables 2-4.

III. RESULTS AND DISCUSSION

Data were analysed separately at 4-15 d (age I) and 15-22 d (age II). Performance parameters of ducklings at the two ages are shown in Table 1. Significant effects of treatment

Table 1. Main effects of diets with or without rice bran, with vegetable (V) protein or vegetable (V) + animal (A) protein, with (+) or without (-) a feed phytase on growth rate, feed intake and feed conversion ratio (FCR) of ducklings between 4 and 15 d (I), 15 and 22 d (II) and 4-22 d (I & II) of age (NS = not significant, *$P<0.05$; **$P<0.01$).

Rice bran (g/kg)	Protein source	Phytase	Growth rate (g/d)		Feed intake (g/d)		FCR (g/g)	
			I	II	I	II	I	II
0	V	-	45.0	83.9	72	162	1.59	1.93
	V	+	46.9	86.2	76	156	1.63	1.82
	V+A	-	54.9	86.1	78	161	1.42	1.87
	V+A	+	54.9	84.5	80	156	1.45	1.85
600	V	-	41.8	71.6	67	143	1.60	2.00
	V	+	43.3	71.8	68	152	1.57	2.12
	V+A	-	50.6	86.4	76	168	1.51	1.95
	V+A	+	52.3	92.5	80	184	1.52	1.99
	V(23% CP)	-	43.4	82.7	68	163	1.61	1.98
	LSD (P=0.05)		2.71	6.54	5.22	8.38	0.06	0.16

MAIN EFFECTS

	Growth rate			Feed intake			FCR		
Rice bran									
	I	II	I&II	I	II	I&II	I	II	I&II
0	50.4	85.2	63.9	77	159	108	1.52	1.86	1.70
600	47.0	80.6	60.0	73	162	107	1.55	2.02	1.79
LSD (P=0.05)	1.32	3.36	1.90	2.60	2.79	2.16	0.03	0.08	0.04
Significance	***	*	***	**	*	NS	NS	**	***
Protein source									
V	44.2	78.4	57.5	71	153	103	1.60	1.97	1.79
V+A	53.2	87.4	66.5	78	167	113	1.47	1.91	1.70
LSD (P=0.05)	1.32	3.36	1.90	2.60	2.79	2.16	0.03	0.08	0.04
Significance	***	**	***	***	***	***	***	NS	**
Phytase									
-	48.1	82.0	61.3	73	158	106	1.53	1.94	1.74
+	49.3	83.7	62.7	76	162	109	1.54	1.94	1.75
LSD (P=0.05)	1.32	3.36	1.90	2.60	2.79	2.16	0.03	0.08	0.04
Significance	NS	NS	NS	*	*	**	NS	NS	NS

were seen for the three parameters at both ages. Additional vegetable protein improved growth rate ($P<0.05$) at age II only when compared with the corresponding diet containing a calculated 212 g CP/kg. However, the determined values of the other diets were all about 226 g CP/kg versus 232 g CP/kg.

With the high crude protein diet excluded, main effects of rice bran, protein type and phytase are also given (Table 1). Rice bran at this very high level depressed ($P<0.05$) growth rate at ages I and II and feed intake at age I but not feed intake when ages were combined (I and II); at age II and when combined, FCR was increased ($P<0.01$). The addition of fish meal to the diet enhanced performance at all ages; FCR was unchanged only at age II. Phytase increased feed intake at all ages but had no effect on growth rate or FCR.

Table 2. The interaction of rice bran and protein source (V, vegetable, A, animal) on growth rate and feed intake and of phytase addition on feed intake (4-22 days).

Rice bran (g/kg)	Protein source	Growth rate (g/d)	Feed intake (g/d)
0	V	61.2[b]	107[b]
	V+A	66.7[a]	110[b]
600	V	53.9[d]	99[c]
	V+A	66.2[a]	116[a]
LSD (0.05)		2.69	1.02

Rice bran (g/kg)	Phytase	Feed intake (g/d)
0	-	108[a]
	+	108[a]
600	-	104[c]
	+	110[a]
		3.06

There were significant interactions between rice bran inclusion and protein source for growth rate and feed intake and between rice bran and phytase inclusions for feed intake for the two periods combined (Table 2). Animal protein addition improved feed intake on the diets with rice bran, but growth rate was increased ($P < 0.05$) irrespective of rice bran level. These interactions suggest that despite the adequate calculated crude protein, methionine + cystine and lysine contents of the diets of 211, 7.4 and 11.2 g/kg respectively, feed intake and growth rate on diets with or without rice bran were not maximised at either age until fish meal was added. Hejgaard and Mose (1974) recommended the addition of 3-4% of the dietary protein supplement to be in the form of animal protein of high biological value for starter ducks. Phytase increased feed intake only on diets with rice bran (Table 2). The level of phytase used here was higher than that recommended (700 U/kg).

The significant effects ($P<0.01$) of dietary treatment on tibia ash of ducklings when killed at 23 d of age are given in Table 3. Despite the high calculated available P of 3.9 g/kg diet, feed phytase had a significant effect on bone ash when expressed as g or as a percent of dry, fat-extracted bone. The latter measure is probably more meaningful because the former is influenced by bird weight and possibly by a number of factors other than mineral availability. Interestingly, phytase addition elicited a response most convincingly on diets with rice bran. The diet with the additional soybean meal gave the lowest bone ash values (Table 3). Previously it was shown that the available P in soybean meal for chickens was >50% (Farrell *et al.*, 1993).

The addition of fish meal to the diet with rice bran increased tibia ash (g) compared to the same diet with vegetable protein only. This suggested that the fish meal was contributing some available P. It was surprising therefore to see the large further response to phytase addition in these diets (Table 3).

Table 3. Effect of treatment with (+) or without (-) phytase in diets with vegetable (V) protein or vegetable (V) + animal (A) protein on tibia ash of ducklings.

Rice bran (g/kg)	Protein source	Phytase	Tibia ash (g)	Tibia ash (%)
0	V	-	1.73^{a}	49.6^{a}
	V	+	1.82^{a}	49.1^{ab}
	V+A	-	1.67^{ab}	46.7^{c}
	V+A	+	1.76^{a}	48.1^{b}
600	V	-	1.28^{c}	45.4^{cd}
	V	+	1.52^{b}	47.7^{b}
	V+A	-	1.53^{b}	46.3^{c}
	V+A	+	1.84^{a}	49.0^{ab}
	V (23% CP)	-	1.35^{bc}	45.0^{cd}
	LSD (0.05)		0.19	1.31

Mitchell and Edwards (1994) observed a better response to dietary phytase when diets had higher levels of Ca and lower levels of P than normally used. The combination of 1,25-dihydroxycholecalciferol and phytase was more effective in promoting Ca and P utilization than when either was used alone (Edwards, 1994).

Feed phytase from different producers may differ in potency. Recent observations by Perey *et al.* (1993) failed to show any beneficial effect on broiler chicken performance of a different commercial phytase to that used here in a maize/soybean diet. The crude preparation, obtained also from *Aspergillus niger,* was included in amounts of up to 15 g/kg feed and provided 750 U/kg. They did show a beneficial effect on tibia ash and P retention but the results were largely equivocal.

In contrast, Korengay *et al.* (1994) calculated that Natuphos increased phytate P release in a maize/soybean diet with increasing dietary concentration to a maximum of 43% at 1000 U Natuphos/kg feed.

It is concluded that phytase in rice bran-based diets appears to have additional benefits beyond that of releasing phytic acid P from the dietary ingredients.

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EGGS: A DIETARY ALTERNATIVE TO FISH?

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Growing evidence of the role of dietary omega-3 (n-3) fatty acids in cardiovascular disease reduction and in maternal and infant health have promoted recommendations to increase consumption of these fatty acids (Kromhout, 1992; Nettleton, 1993). Shell eggs have been targeted as an alternative food source for consumption of these n-3 fatty acids found in fish. Supplementation of laying hen diets with a marine source of these fatty acids readily results in the production of eggs containing a similar n-3 fatty acid content to a 100 g serving of lean fish (Hargis and Van Elswyk, 1993). Whether consumption of these eggs will result in similar health benefits as fish, however, remains to be determined. Furthermore, n-3 fatty acid-enriched egg potency may vary depending on the n-3 fatty acid source used in the laying hen ration, i.e., marine oil vs. terrestrial sources. Therefore, the following study was designed to assess the influence of n-3 fatty acid-enriched shell egg consumption on biochemical markers of cardiovascular disease risk.

In the current study 32 volunteers, male and female, were asked to consume four eggs/week during three six-week study periods. Eggs were from hens fed diets containing either 15 g menhaden fish oil/kg, 50 g flaxseed/kg, or a typical layer ration. Each test period was followed by a 4 week "wash out" period. All volunteers consumed all egg treatments. Blood samples were collected at the beginning of each test period, at week 3 and at week 6 for the determination of plasma cholesterol and triglyceride levels. Platelet-rich plasma preparations were stimulated with collagen to assess platelet aggregation. Omega-3 fatty acids are believed to reduce platelet aggregation which contributes to plaque formation. Our preliminary data indicate that consumption of eggs from hens fed fish oil results in decreased serum triglycerides without concomitant increases in serum cholesterol. Changes in platelet aggregation were noted in response to eggs from hens fed either n-3 fatty acid source and were consistent with suppression of platelet responsiveness. These data indicate that n-3 fatty acid-enriched eggs may serve as an effective substitute for fish for the health conscious consumer.

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FEED MANAGEMENT OF BROILER BREEDERS

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Summary

Today's broiler has an exceptionally high genetic potential for fast growth and feed efficiency. This has been accomplished through intensive selection for a high level of feed consumption. The broiler breeder parents share this attribute. The broiler breeder hen presently used by the industry at age 20 weeks must be equivalent in body weight to their offspring at approximately 6 weeks of age. It is essential that broiler breeder body weight is controlled by feed restriction at a very early age. Without a feed restriction program, breeders deposit large amounts of fat and reach sexual maturity at an earlier age. This leads to reduced fertility, hatchability and total egg production. Over-restriction of body weight also suppresses total egg production.. In general, feed restriction delays the development of certain attributes (bursa of Fabricius, abdominal fat pad, pubic bone spread and comb size) associated with sexual maturity. The delay in maturity of these attributes due to feed restriction occurs without altering their ultimate physiological function. Severe feed restriction is reflected in less body weight and a shorter shank length following sexual maturity and alters the physiological function of these two attributes.

Overweight breeders caused by early rapid growth during the rearing period must be placed on an administrative (sometimes severe) feed restriction to slow down growth. Even if the broiler breeder's weight is brought back to the body weight standard prior to the onset of egg production, problems associated with low egg production peaks can be related to a flock that was overweight at various ages during the rearing period. In that case, improving performance near peak egg production through feeding management becomes very important.

I. INTRODUCTION

Numerous feed restriction programs (skip-a-day, five-out-of-seven, every day, *etc.*) are available to control broiler breeder pullet weight during the rearing period. The amount of feed required to attain a desired body weight is influenced by many factors. Pullet body weight, temperature and dietary energy level are the most important. The largest part of the energy required by the bird is used for maintenance. The pullet must be allocated enough feed to satisfy her maintenance requirement and have enough remaining to permit the desired small increment in daily weight gain. The growth curve should be smooth without the commonly seen "sawtooth" effect. During the rearing phase, the proper consumption of protein (amino acids), minerals, vitamins and other nutrients is essential to ensure proper weight gain and skeletal development. The amount of fleshing is important at first egg and a properly fleshed bird with good management should peak adequately. Normally, feed restriction during the growing period limits energy. Dietary protein levels can range from 170 - 210 g/kg from 0 - 3 weeks of age and from 140 - 160 g/kg from 4 to 20 weeks of age.

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The first 10 weeks following onset of egg production is critical to the flock's productivity as breeder hens. Many flocks with the potential for a high peak in egg production never peak above 75% due to feeding mismanagement. It is essential that breeder hens be allowed to make adequate body weight gains during this period. Harms (1984) summarized egg production and body weight records from approximately 200,000 broiler breeders from 49 flocks. Hens making steady and adequate weight gains peaked at a significantly higher rate of egg production and maintained a higher rate of lay after peak than those with inadequate gains. Simply stated, if birds coming into production do not gain adequate body weight through peak production, the flock will not peak adequately. Broiler breeders must not experience a weekly body weight loss from day of age until the end of the laying cycle. The rate of change in body weight gain corresponds to the different stages of maturity and egg production.

Three critical feeding phases exist during the life of the broiler breeder:

Phase 1: This period begins at day of age and ends at first egg. During this time, the birds should be given the amount of feed needed to attain the recommended target body weight in the breeder guide. Beginning at 17 -18 weeks, weight gain is stepped up in order to provide adequate nutrient stores and oviduct growth. The gain allowed from 17 - 18 weeks until first egg is a critical factor in determining the average weight of the flock during the laying period.

Phase 2: This period begins at first egg and ends shortly after peak production. It is essential that the hen continually gain weight in a steady manner during this period. Normally, breeder guides require between 80 to 100 grams of gain per bird per week during this period. Harms (1992) proposed a weekly gain of 90 grams per bird during this phase in order to achieve the necessary body weight. As the flock approaches peak, the body weight gain is reduced. Egg mass output (production x egg weight) is greater at the beginning of this period than towards the end. Therefore, during this time there is a greater need for feed.

When 40 - 50% production has been reached, "challenge feeding" can be implemented. The body condition of the hens should be carefully considered before a decision to challenge feed is made. Challenge feed to peak production, provided that the average flock body weight is within the suggested range and there is not an obesity problem. An additional 4.5-13.6 g/bird can be fed on two nonconsecutive days per week. Continue challenge feeding while egg production is above 80% and there is no excessive body weight. Feed challenge must be discontinued if there are no increases in egg production, production falls below 80% or if excessive body weight is recorded.

Phase 3: This period begins once the flock has reached peak egg production. The amount of feed should be kept somewhat constant for 2 - 3 weeks after peak. Controlled feeding should continue with body weight and egg production monitored continuously during this period. Normally, 2 - 3 grams of body weight gain per bird per week is recommended. As in Phase 2, energy intake is the factor most closely related to egg numbers and egg weight. Some producers feed higher protein levels than necessary while limiting energy. With limited energy intake, excess protein puts an unnecessary

stress on the hen because of the additional energy demand required to rid the body of excess nitrogen as uric acid. In hot weather, this stress would be compounded.

II. METHODOLOGY

Feed restriction of broiler breeder hens reduces the rate of follicle maturation and as a result reduces erratic ovulation (Hocking *et al.*, 1987; Yu *et al.*, 1992). Broiler breeder hens that are obese as a result of being full-fed are known to have higher numbers of mature or yellow follicles than hens fed on a feed-restriction program. In order to maximize the number of placeable chicks per broiler breeder hen housed over a normal production period, the growth curve and corresponding feeding program for any strain must be known. In an experiment designed to evaluate a breeder's recommended growth curve in relation to various restrictive feeding programs, Fattori *et al.* (1991) allocated amounts of feed which were 8% above standard, standard, and 8%, 16% and 24% below the standard recommended by the breeder. Proportional decreases in feed allocation resulted in corresponding decreases in body weight, frequency of double-yolked eggs and number of days in production. Egg weight, fertility, hatchability and female mortality to 64 weeks of age were not significantly affected by the reduction in feed intake. Shell quality was significantly improved with the 16% and 24% below standard feed treatments. Egg production was reduced in the hens that were 16% and 24% underweight in reference to the standard.

Consistent feed restriction of the broiler breeder is essential in order to prevent obesity and to obtain a high level of egg production. The hen has a very quick response time to high levels of feed consumption (Robinson *et al.*, 1991). When hens were restrictively fed to 40 weeks of age and then full-fed, feed consumption increased by 60% within 24 hours and held at a level which was 46% more than hens maintained on a restriction program. Following 14 days of full-feeding, the average number of yellow follicles per hen increased. This emphasizes the need to continually restrict feed throughout the laying cycle. Even short periods of over consumption have an impact on the hen's physiology.

Manipulation of broiler breeder body weight during the rearing phase affects the hen's preparation to lay and ability to produce eggs (Wilson and Robinson, 1991). Pullets were reared on one of three body weight curves (early light, standard and early heavy). All pullets were brought to a common standard body weight by 24 weeks of age. Hens that were light in weight in the early part of the rearing phase used more feed after 16 weeks of age in order to deposit more body fat than those on the standard weight program. The heaviest hens during the early rearing period had the least percentage of body fat. The hens maintained at standard and heavy body weights during early rearing had better egg production compared to the early light weight hens.

Body composition of the broiler breeder changes during the rearing phase and is influenced by the dietary protein level (Miles *et al.*, 1987). These researchers used a step-down protein feeding program based on a maize-soybean meal basal diet containing no supplemental DL-methionine. The crude protein levels were 140, 170, 190 and 200 g/kg. The 140 g/kg diet was fed continuously to one group of hens from weeks 3 through 24. The crude protein concentration in each of the three remaining diets was decreased by 10 g/kg each two weeks during weeks 3 through 24. Carcass lipid concentration at 14 and 22 weeks of age was inversely related to dietary crude protein level. Pullets fed the 140 g

crude protein/kg diet had the most fat at 14 weeks of age, but had the least body fat at 22 weeks of age.

There have been numerous studies conducted to investigate the mechanisms involved in the neuroendocrine initiation of sexual maturity (age at which egg production begins). Both minimum body weight and chronological age as well as maximum body fat were postulated by Brody *et al.* (1980) to be necessary for sexual maturity. An important critical stage during the development of breeder hens has been identified as the pullet-layer transition period (McDaniel, 1983; Cave, 1984; Brake *et al.*, 1985). The importance of targeting sexual maturity is a practical issue in which economic considerations are usually of greater concern than the need to understand the physiological changes that occur during this time (Fattori *et al.*, 1993). The physiological changes must be understood and used appropriately if sexual maturity is to be targeted at a certain date.

In order to characterize how the physical attributes associated with sexual maturity would be affected by different amounts of feed restriction in broiler breeders, Fattori *et al.* (1993) conducted an experiment with broiler breeder pullets as they passed through the pullet-layer transition period. Treatments consisted of five feeding programs or body weight goals, 8% above standard, standard, and 8%, 16% and 24% below standard during the period from 2 to 20 weeks of age. Characteristics measured were body and fat pad weights, pubic spread, comb development, head (comb and wattle appearance), shank length, total plasma lipid concentration, ovary weight, oviduct weight, bursa of Fabricius weight and age at sexual maturity. The generalized effect of increased severity of the feed restriction program was to delay the bird's development without altering it's ultimate physiological values. Relative body weight and shank length after maturity were reduced in the most severely restricted birds.

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ADVANCES IN THE DEVELOPMENT AND APPLICATION OF FEED ENZYMES

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Summary

The viscosity (intestinal, broiler) of six wheats was analysed and shown to range from 6.73 - 18.38 cps (Mean, 10.41; SD, 4.20). With the addition of a xylanase from *Trichoderma longibrachiatum* the range was reduced to 2.66 - 6.85 cps (Mean, 4.08; SD, 1.62). The variability in intestinal viscosity is discussed in relation to variable wheat quality (AME). The correlation between viscosity and FCR for broilers fed wheat and barley diets was shown to be good ($R^2 = 0.645$; $P = 0.009$) even when the starting viscosity is relatively low (< 10 cps). This reinforces the importance of viscosity as a major constraint on digestibility and contradicts the view that there is a threshold below which viscosity is not important. Evidence is presented suggesting that the constraints imposed by viscosity on digestibility can be partly overcome without viscosity reduction by supplementation of the diet with the types of activity found in pancreatin e.g. lipase and protease. Significant ($P < 0.05$) effects of lipase on FCR in a wheat-based diet were demonstrated. Finally, preliminary results are presented indicating that enzyme supplementation of wheat diets significantly ($P < 0.005$) reduces growth depression due to coccidiosis in pullets. The data suggests that intestinal viscosity may be involved and is itself significantly ($P< 0.005$) reduced by coccidiosis, at least in broilers.

I. INTRODUCTION

Wheat is one of the main feed ingredients used in Australia and other countries around the world. It is well known that apparent metabolizable energy (AME) values for poultry for different wheats can be highly variable with reported values ranging from 10.4 MJ/kg to 15.9 MJ/kg (Choct *et al.*, 1994) and that problems of wet litter are sometimes seen in broilers fed high levels of wheat.

The endosperm cells walls of wheat, triticale and rye contain a high level of non-starch polysaccharide (NSP) consisting mainly of arabinoxylans and some mixed-linked β-glucan. It has been suggested that the cell walls of wheat endosperm cells make nutrients such as starch and protein less available for digestion. Indeed, one of the distinctive characteristics of wheat appears to be an aleurone layer with a relatively thick cell wall structure (Parkkonen *et al.*, unpublished).

However, at least part of the arabinoxylan content of wheat endosperm cell walls is soluble. The recognition by Classen *et al.* (1985) that soluble β-glucans of barley endosperm increase intestinal viscosity and thereby reduce digestibility and increase faecal moisture content suggested that the arabinoxylans of wheat and rye might exert similar effects. More recently, Bedford and Classen (1992) have established that there is a significant linear relationship between intestinal viscosity measured *in vivo* (broilers) and performance parameters such as body weight gain and feed conversion. In the case of wheat and rye-based diets fed to poultry, Bedford and Classen (1992) have shown that as much as 70-80% of the variation in body weight gain and FCR can be described by intestinal viscosity alone. This

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demonstrates the importance of viscosity in diets for broilers and raises the possibility that the basis for wheat quality variability might be related to this phenomenon.

The relationship between variable wheat quality and intestinal viscosity is currently a key focus of research. This paper examines the variation between wheats in terms of viscosity (intestinal, broiler), the effects of enzyme supplementation (mainly xylanase and lipase), the modes of action of such enzymes, and the relationship between intestinal viscosity, performance and variable wheat AME. In addition, results are presented from a preliminary study to investigate the effect of diet and enzyme supplementaion on growth depression due to coccidial infection and the effect of coccidiosis on intestinal viscosity.

II. MATERIALS AND METHODS

(a) Intestinal Viscosity - Wheat Variability

Viscosities of different wheats were measured *in vivo* in broilers at 21 - 22 days. In separate experiments and at different locations, birds were fed diets (60 - 67 % wheat, pelleted or mash) for 21 - 22 days with and without Avizyme XP 1300 (0.1%), an experimental product containing a xylanase from *Trichoderma longibrachiatum*. Four - six birds per treatment were sacrificed and their intestinal contents assayed for viscosity by the method of Bedford *et al.* (1991). All viscosity measurements are given as centipoise (cps). A material requiring a shear stress of one dyne per square centimetre to produce a shear rate of one reciprocal second has a viscosity of one poise or 100 centipoise.

(b) Relationship between Intestinal Viscosity and Performance in a Diet with Relatively Low Starting Viscosity

Broilers (Ross 1, male) were raised on diets containing 66 % wheat; 62 % wheat; 62 % barley; 56 % barley; 44 % wheat/20 % barley; 40 % wheat/20 % barley with and without Avizyme 1110, 1210 and 1310 - liquid products for barley, wheat/barley and wheat based diets, respectively. Body weight gain and feed intake were measured from 7-21 days. Viscosity was measured at 21 days.

(c) Evaluation of Lipase in a Wheat-based Diet

Broilers (AAxH, male) were raised on a mash diet (60% wheat, 32 % soybean meal (48 %), 3.7 % canola oil; 5.5 % total fat) in the presence and absence of 0.1 % Avizyme TX - a feed enzyme product for wheat based diets - and a lipase (0.01 - 0.04%) for 0-21 days. Body weight gain, feed intake and intestinal viscosity were measured at 21 days.

(d) The Influence of Diet and Intestinal Viscosity on Growth Depression Due to Coccidiosis

Preliminary trials were undertaken to develop a model to test the effects of diet and enzyme supplementation on growth depression due to coccidial infection.

Trial 1: Female Lohmann Brown chicks were fed a maize (42 %)/wheat (25 %) diet for 0 - 12 days and then randomised into groups of 10 birds and fed diets containing maize (67 %) or wheat (67 %) (10 birds per treatment) with and without *T. longibrachiatum* xylanase supplementation. At day 14, birds were dosed orally with coccidial oocytes (a mixture of *Eimeria acervulina* (50,000 per bird) and *E. tenella* (5,000 per bird)). Birds were

individually weighed at days 14, 17 and 21. Lesion scoring was performed at day 21. The lesion scoring system was on a scale of 0 (low) to 5 (high).

Trial 2: Male Ross 1 broilers were raised on maize/wheat or wheat or wheat + enzyme diets as described in Trial 1. At day 12, the birds were randomised into control and challenge groups (20 per group). At day 14, the challenge group birds were orally dosed as in Trial 1. Body weight gain was measured at day 21 at which time 10 birds were removed for lesion scoring and intestinal viscosity measurement.

(e) Statistical Analysis

Data were subjected to ANOVA according to the GLM procedure of SAS.

III. RESULTS AND DISCUSSION

(a) Intestinal Viscosity - Wheat Variability

The relationship between variable wheat quality and intestinal viscosity has not been examined previously in any detail. A tentative analysis can be made using data from trials that have been made involving wheat-based diets and broilers. Using data from six trials in which diets contained 60 - 67 % wheat, it can be seen that the variation in viscosity ranges from 6.73 to 18.38 cps with a standard deviation of 4.2 (Table 1). Since a good correlation has been demonstrated between intestinal viscosity and performance parameters in wheat-based diets for broilers (Bedford and Classen, 1992), it can be concluded that viscosity is likely to be one of the main determinants of wheat quality.

One of the most important advances in animal nutrition in the last decade has been the development of feed enzymes and, in particular, products for wheat-based diets for poultry. In the example above, intestinal viscosity was reduced in all cases by the addition of Avizyme. In fact, the viscosity range was reduced to 2.66 - 6.85 cps with a standard deviation of 1.62 (Table 1). What is clear is that the addition of the enzyme can reduce viscosity of wheats with relatively high viscosity to below that of wheats with relatively low viscosity. Moreover, wheats exhibiting relatively low levels of viscosity also respond to enzyme addition in terms of viscosity reduction.

Table 1. Viscosity (cps) of different wheats, with and without enzyme, measured *in vivo* in broilers at 21 - 22 days.

Wheat No.	Control	+ Avizyme
1 (60 %)*	6.73	2.92
2 (60 %)	7.23	2.88
3 (62 %)	9.49	4.87
4 (60 %)	10.20	4.30
5 (60 %)	10.41	6.85
6 (67 %)	18.38	2.66
Mean	10.41	4.08
SD	4.20	1.62

* Wheat %

Annison (1991) found a highly significant negative correlation ($r = -0.91$, $P < 0.0001$) between the AME values of 13 different Australian wheats and the water-soluble NSP content, which consisted mainly of arabinoxylan. Since AME and intestinal viscosity values are both reliable indicators of performance, the evidence points to a relationship between all three of these factors, strongly suggesting that viscosity measured at the level of the GI tract is related to the soluble arabinoxylan content of wheat. In a recent study by Scott and Bedford (unpublished) the AME of 5 different wheats was measured with and without Avizyme supplementation. The wheats varied in AME as expected, and consistent with the hypothesis advanced, enzyme treatment increased AME in all cases to at least that of maize. Perhaps equally important was the observation that the standard deviation for the control wheats was 347 kJ/kg whereas that for the Avizyme supplemented wheats was reduced to 151 kJ/kg. In this study at least, the variability in wheat quality, as measured by the AME assay, was resolved by the addition of Avizyme. Consistent with this observation is the demonstration by Choct *et al.* (1994) that a low AME wheat (12.02 MJ/kg) had a relatively high intestinal viscosity (20.28 cps) compared to a normal AME wheat (14.52 MJ/kg; 9.65 cps) and that the increase in AME due to Avizyme was far greater for the low AME wheat (+ 24 %) than for the normal wheat (+ 2 %).

(b) Relationship between Intestinal Viscosity and Performance in a Diet with Relatively Low Starting Viscosity

In a recent trial involving the use of wheat, barley and wheat/barley diets with and without Avizyme, the maximum viscosity measured was < 10 cps. Although the correlation between liveweight gain and viscosity was not strong ($R^2 = 0.358$, $P = 0.089$), a very good correlation was observed between viscosity and FCR ($R^2 = 0.645$, $P = 0.009$), regardless of the basis of intestinal viscosity, mixed-linked β-glucans or arabinoxylans. This reinforces the importance of viscosity as a major constraint on digestibility even in diets where the relative viscosity is low.

(c) Evaluation of Lipase in a Wheat-based Diet

There is increasing evidence that the variable energy value for wheat is related to solubilisation of arabinoxylans from cell walls of the endosperm and the intestinal viscosity resulting from this, and that feed enzymes such as Avizyme can resolve this. However, viscosity per se is not considered to be a "growth depressing" factor, rather the consequences of viscosity at the level of the ileum. Viscosity is considered to be a major constraint on the process of digestion through interference with the diffusion of pancreatic enzymes, substrates and reaction products. This is exemplified by the effect of viscosity on the digestibility of added fat (Classen *et al.*, 1985). Theoretically, it should be feasible to overcome the constraints imposed by viscosity by increasing the concentration of pancreatic enzyme activities such as amylase, protease and lipase. In order to test this, and in view of the known sensitivity of fat digestibility to viscosity, the effects of supplemental lipase were evaluated in a wheat-based diet (Table 2).

Lipase improved performance, though not to the same extent as Avizyme and without a similar reduction in viscosity, supporting the hypothesis outlined above. The viscosity-reducing activity of the lipase preparation, though relatively poor, is most probably due to a small amount of β-glucanase present as a side activity. A further example is the effect of adding pepsin, which has been shown to give significant ($P < 0.05$) improvements in body

weight gain in a wheat-based diet (Bedford and Classen, unpublished). It can be concluded, therefore, that one of the main effects of viscosity in wheat-based diets is interference with the digestion of nutrients, especially fat, and the problems created by viscosity can, at least in part, be resolved by means other than viscosity reduction.

Table 2. The effects of lipase with and without Avizyme in broilers fed a wheat-based diet for 0 - 21 days.

Avizyme (%)	Lipase (%)	Body Wt Gain g	FCR g:g	Viscosity cps
0	0	421.13	1.619	61.22
0	0.01	514.43	1.441	47.82
0	0.02	535.80	1.450	49.00
0	0.04	533.46	1.452	28.32
0.1	0	529.39	1.415	10.14
0.1	0.01	551.28	1.412	13.10
0.1	0.02	542.56	1.412	11.00
0.1	0.04	509.28	1.454	13.71
Pooled SEM		9.00	0.015	4.220
P Value		0.0028	0.0015	0.0005

Significant effects ($P < 0.05$) due to Avizyme, Lipase and Avizyme * Lipase on body weight gain and FCR.

(d) The Influence of Diet and Intestinal Viscosity on Growth Depression Due to Coccidiosis

It has been shown previously that the pathogenicity of *Eimeria tenella* is lower in maize-fed chickens compared to those fed a wheat-based diet (Williams, 1992) and that this is the likely basis for the apparently greater potency of the ionophore monensin in maize- compared to wheat-based diets. Williams (1992) has suggested that these differences are due to the protective effects of vitamins A and E, which are present at higher concentrations in maize compared to wheat, and/or to the enhancement of pathogenicity by the higher levels of niacin and riboflavin in wheat compared to maize.

It is of interest to examine the effects of supplementing wheat diets with enzymes on the pathogenicity of *E. tenella* and *E. acervulina*, both major pathogens of poultry infecting mainly the caeca and the foregut, respectively. Two preliminary studies have been undertaken partly with a view to establishing a model for this type of work (Morgan, Bedford, Catchpole and Taylor - unpublished).

In the first experiment using pullets, the effect of diet was confirmed. Birds fed the maize diet resisted growth depression due to coccidial challenge to a greater degree than those fed the wheat-based diet (Table 3). Of particular interest was the effect of adding a xylanase from *Trichoderma longibrachiatum* to the wheat-based diet. There was a significant ($P < 0.005$) reduction in the level of growth depression due to enzyme addition. Lesion scoring logically followed this pattern but there were no statistically significant differences between challenged groups.

Table 3. The effects of diet and enzyme addition on body weight gain and lesion score in Lohmann Brown chickens with and without coccidial challenge.

Treatment	Challenge	Body Wt Gain, g 14 - 21 days	Body Wt Gain, g 14 - 17 days	Lesion Score
Maize	-	91.7^{a}	54.5^{a}	0^{a}
Maize	+	60.3^{b}	25.4^{b}	2.8^{b}
Wheat	-	89.2^{a}	51.8^{a}	0^{a}
Wheat	+	42.4^{c}	8.6^{c}	3.8^{b}
Wheat + Enzyme	-	91.4^{a}	54.7^{a}	0^{a}
Wheat + Enzyme	+	63.5^{b}	30.1^{b}	3^{b}
Pooled SEM		13.2	9.0	2.5

Values in a column with different superscripts are significantly different ($P<0.05$).

It was of interest to develop a broiler model to examine interactions of diet, enzyme, coccidial infection and growth depression where, additionally, intestinal viscosity could also be measured. In this preliminary study, the effects of coccidial challenge on body weight gain were clear at 7 days post-challenge (Table 4). Although there was no apparent effect of enzyme at this stage in terms of body weight gain and lesion score, there were pronounced effects on FCR, though statistical analysis of FCR was not feasible since it was possible to measure feed intake only on a group basis.

Intestinal viscosity measurements demonstrated a significant effect of enzyme addition. In addition, it was clearly evident that coccidial infection alone causes a reduction in intestinal viscosity.

Although these results are by no means conclusive, they do suggest that it is worthwhile investigating further the effects of wheat and enzyme supplementation on the pathogenicity of *Eimeria*. The tentative results presented here at least suggest that the differences between maize and wheat diets described by Williams (1992) and confirmed in the present study are possibly related to intestinal viscosity.

Table 4. The effects of diet and enzyme addition on body weight gain, FCR, lesion score and intestinal viscosity in broilers with and without coccidial challenge.

Treatment	Challenge	BWG, g 14 - 21 days	FCR 14 - 21 days	Lesion Score	Viscosity cps
Maize/Wheat	-	341.9^{a}	1.81	0^{a}	8.6^{a}
Maize/Wheat	+	264.4^{b}	2.23	4.1^{b}	4.7^{a}
Wheat	-	329.3^{a}	2.11	0^{a}	18.4^{b}
Wheat	+	241.9^{b}	2.52	3.7^{b}	6.1^{a}
Wheat + Enzyme	-	346.5^{a}	1.93	0^{a}	6.3^{a}
Wheat + Enzyme	+	243.7^{b}	2.05	3.9^{b}	2.7^{a}
Pooled SEM		50.6		0.8	4.4

Values in a column with different superscripts are significantly different ($P < 0.05$).

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INTRAPERITONEAL IMMUNISATION PRIMES FOR A MUCOSAL RESPONSE TO *SALMONELLA TYPHIMURIUM* IN CHICKENS

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Salmonellosis in chickens causes production losses and also poses a serious public health risk. Bacterial colonisation of the intestine can be reduced using immunisation strategies which stimulate local production of antigen specific immunoglobulin IgA (Husband, 1993). We have shown using non-replicating antigens and appropriate adjuvant formulations, that priming via the intraperitoneal (ip) route followed by an oral booster, provokes local secretion of IgA antibody in the chicken intestine (Muir *et al.*, 1994). The present study was designed to assess the ability of ip vaccination to protect chickens from infection with *S. typhimurium*.

Ten, male broiler chickens (19 days old and individually caged) were vaccinated ip with 10^{11} formalin-killed *S. typhimurium* emulsified in Auspharm adjuvant. Two weeks later an oral booster of the same dose of organisms was administered. After another week, vaccinated and control unvaccinated birds were challenged orally with 10^{12} live organisms homologous to the vaccine strain. An unvaccinated, unchallenged control group was also included in the study. Protection was assessed two weeks post challenge by identification of infected birds via isolation of organisms from cloacal swabs, caecal wall and contents, spleen and liver, and enumeration of organisms per g of excreta.

Vaccination provided partial protection from infection with *S. typhimurium*. A reduced percentage of infected, vaccinated birds had positive cloacal swabs (see Table) and shed lower numbers of organisms compared to infected, unvaccinated control birds. Nevertheless, *S. typhimurium* was isolated from all organs sampled in challenged birds regardless of vaccination. *S. typhimurium* was not isolated from unvaccinated, unchallenged controls.

Percentage of birds with positive cloacal swabs for *S. typhimurium* following challenge with *S. typhimurium*.

Vaccination	Challenge	Day[1]		
		2	6	13
-	-	0	0	0
-	+	100	90	60
+	+	56	56	0

[1] Days post challenge.

These results indicate the potential for ip vaccination using non-replicating antigens to reduce intestinal colonisation by pathogens. However, the *S. typhimurium* challenge dose used in this study was probably greater than challenge inocula experienced during field exposure and may have exceeded the capacity of the mucosal immune system to control colonisation.

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YOLK RECYCLING IN THE OVARY OF THE LAYING HEN: A CRITICAL FEATURE OF PAUSE

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Summary

During the early phase of regression of large yolky follicles in the ovary of the laying hen, the internal follicular wall develops defects which allow the yolk to pass from the follicle into the stroma of the ovary. This process ("bursting atresia") produces a large volume (as much as 70mL) of complex compounds which must be resorbed in an orderly manner if normal ovarian function is to be resumed at the beginning of the next laying cycle. The mechanism of resorption of this mass of yolk has been largely unexamined. We have used standard morphological and tissue culture techniques to study this process in layer and broiler breeds and have shown that the large spaces (lacunae) in the ovarian stroma, and the cells that line them, play a major role in the breakdown and resorption of yolk during regression of large yolky follicles.

I. INTRODUCTION

There is surprisingly little published research on ovarian regression in commercial breeds of chicken. This phenomenon is a normal event in natural populations of birds, and is the process whereby the ovary becomes inactive after a period of intense follicular activity (Smith *et al.*, 1957). During such active periods there are at any time several large yolky follicles being prepared for ovulation. Upon cessation of ovulation, the remaining large yolky follicles are redundant and their bulky, fragile nature may interfere with future ovarian function; moreover, their content represents a concentrated accumulation of nutrients which the bird might well use. To this end there seems to have evolved an effective method of rapid resorption of large yolky follicles, because it is known that these structures undergo rapid shrinkage following cessation of ovulation in all bird species so far studied. This process has recently been recognised as being the most common mode of atresia of large yolky follicles, and has been named "bursting" atresia (Gupta *et. al.*, 1988).

Diagnostic poultry pathologists frequently recognise abnormalities in the process of follicular atresia during routine diagnostic necropsy examination of layer and broiler breeding hens, but this type of ovarian disease is poorly documented because the process of normal bursting atresia is itself poorly understood.

II. MATERIALS AND METHODS

(a) Gross examination and light microscopy

Broiler breeder hens were culled from commercial flocks soon after exhibiting behavioural and other signs of going out of lay. A total of 53 birds of the same strain from 2 flocks were examined *post mortem*; they ranged from 38 to 54 weeks of age. A total of

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19 birds were found to be affected with systemic diseases; these included 6 cases of lymphoid leucosis and other myeloproliferative disorders, 2 cases of bacterial oophoritis, and 10 cases of wasting of unknown cause. These diseased birds were excluded from the study and the morphological studies were focused on 17 otherwise normal birds whose ovaries were in the process of undergoing spontaneous regression.

Laying "pause" was induced in 29 (14 Siro CB and 15 Tegel Tint) laying hens (59 weeks old) by substituting barley for their regular laying ration for 7 days.

Birds were killed by intravenous injection of barbiturate and the ovaries were compared with those from normal hens of the same age in full lay. Tissue samples were fixed in neutral buffered formalin, and haematoxylin and eosin-stained sections were prepared from them using routine histological procedures.

(b) Transmission and Scanning Electron microscopy (TEM and SEM)

Eleven Tegel Tint 52-70 week old laying hens from the University's Veterinary Science Farm were anaesthetised by intramuscular injection of ketamine (25mg/kg) and xylazine (20mg/kg). Fixation was achieved by a brief intra-aortic perfusion with normal saline followed by a gluteraldehyde-paraformaldehyde mixture (Karnovsky's solution), and blocks of ovary were prepared by standard methods for SEM and TEM.

(c) Monocyte studies

Mononuclear cells were isolated from the buffy coat of 10mL heparinised blood samples from laying hens. The buffy coat, obtained by centrifugation, was diluted in Hanks solution and monocytes were separated by centrifugation over Ficoll-Hypaque followed by a series of washing stages. Monolayer cultures of monocytes were achieved by culture of isolated cells in a controlled atmosphere (95% O_2, 5% CO_2) incubator for several days, with regular changes of culture medium (macrophage culture medium supplemented with 8% foetal calf serum and 2% chicken serum) to dispose of non-adherent and non-viable cells.

Monolayer cultures of monocytes were prepared for light and transmission and scanning electron microscopy after being grown on plastic coverslips in individual chambers. Fresh homologous yolk was prepared aseptically and diluted with the cell culture medium to a final concentration of 1%; then the supernatant from the cell culture chambers was replaced with 1.5 mL of yolk-containing medium. After 36h the coverslips were taken out and stained for light microscopic examination. Other coverslips were fixed in 2.5% gluteraldehyde/2.0% formaldehyde in 0.066 M cacodylate buffer containing 0.15 M sucrose (pH 7.2) for 30 min. The standard procedure for TEM was then followed. After osmication and partial dehydration, cover slips were cut in two; one was used for SEM and the other prepared for TEM.

After careful agitation, 200μL of the supernatant from control and treatment groups were transferred to 96 well cell culture chambers. The turbidity of the suspensions was then measured directly in a scanning densitometer using a 620 nm filter. The experiment was repeated 4 times with blood from different birds.

(d) Ovarian and embryonic fibroblast studies

One normal healthy laying hen was killed by barbiturate injection and slices of ovary were taken immediately and aseptically into sterile warm phosphate-buffered saline

(PBS) and the ovarian tissue fragments were minced into small pieces. The PBS was discarded and the fragments were trypsinized for 35 min at 37°C. The suspension was then passed through a gauze mesh filter and centrifuged and washed 3 times in PBS in order to remove the enzyme. Cells were counted (final cell concentration $6x10^5$/mL). The cells were cultured in 199 medium containing kanamycin 200U/mL; penicillin/streptomycin 100U/mL and amphotericin B 0.125 μg/mL.

Ten-day old embryonated eggs from healthy laying hens were used for isolation of embryonic fibroblasts by the same method as that used for the ovarian tissue culture.

Fibroblasts from both sources were incubated with 1% yolk for 36 h, when the supernatants were removed and their turbidity measured as described above. The cells on the coverslips were stained as before for light microscopy.

In all cell-culture preparations, cells were counted to ensure that a constant number had been added to the wells.

III. RESULTS

(a) Gross examination and light microscopy

No significant differences were seen between the morphological aspects of atresia of large yolky follicles in layer and in broiler breeder hens, so the process in both breeds is described below.

During bursting atresia the diameter of the follicle rapidly diminished and its outline became partially hidden by several loose, baggy, thin-walled, yolk-filled chambers between which the reduced, but still spherical, follicle appeared to sink. Meanwhile the stigma, which in a maturing follicle was identified merely as an avascular band around the free part of the follicular circumference, rapidly became thick and contracted, thus forming a barrier to the escape of follicular contents by this route. Mitotic activity was frequently noted in the fibroblasts which made up the bulk of the stigma in the regressing follicle.

Thus, the occurrence of bursting atresia was confirmed as being the escape of yolk from the follicle into voluminous thin-walled chambers within the stroma surrounding the follicles: these chambers filled with escaped yolk so rapidly and so uniformly that it was apparent that they were extensively interconnected.

In histological sections of the normal active ovary of the laying hen the ovarian stroma between the follicles and other cortical structures presented an extremely loose appearance due to the presence of large, irregular but sharply demarcated spaces. These spaces (lacunae) were lined by flattened cells which appeared identical to vascular endothelium in neighbouring blood vessels. The lacunae were therefore differentiated from veins primarily by their being consistently free of blood cells and by their extremely irregular outline. They were judged to be thicker-walled than lymphatics, but, apart from the extensive, irregular profiles of the lacunae and the absence of blood in their lumen, clear distinction between lacunae, veins and lymphatics in normal active ovaries was often difficult. The lacuna spaces were present in both the medulla and the cortex of the avian ovary, and the only part of the ovarian stroma in growing and regressing follicles which was free of lacuna spaces was the stigma.

In sections of regressing follicles in ovaries from "paused" birds, the lacunae could be seen to contain large amounts of yolk and, in some sections which included the site of rupture of the follicular wall, continuity could be seen between the yolk in the follicular lumen and the yolk in the lacunae. The lining cells of such lacunae bore little resemblance to the flat mesothelial-type cells of empty lacunae; instead, these cells were rounded,

macrophage-like, and their cytoplasm greatly distended by yolk particles in various stages of degradation. They also appeared to have greatly increased in number, since they were often present in layers many cells thick. Mitotic figures were sought but difficult to discern, because the nuclei of these cells was often obscured by the cytoplasmic content.

Free yolk was occasionally found in the abdominal cavity of birds which had recently ovulated normally (ovum present in oviduct), and in which the remaining yolky follicles were undergoing bursting atresia. In these cases the mesothelial cells in contact with the yolk were seen in histological sections to have undergone the same changes shown by lacuna-lining cells; ie, they had rounded up and taken the form of macrophages filled with yolk granules.

(b) Transmission and Scanning Electron microscopy (TEM and SEM)

In TEM preparations, cells lining the lacunae and the veins could not easily be distinguished from one another: both presented scattered microvillous projections from their luminal surfaces and both showed micropinocytic activity. The junctions between vascular endothelial cells appeared to be more dense than those between the lacuna-lining cells, but these differences were difficult to quantify.

The SEM preparations confirmed the extremely extensive configuration of the ovarian lacunae, and also confirmed that these spaces intercommunicated extensively.

(c) Monocyte studies

Monocytes growing on coverslips in yolk-free media were elongated and sometimes stellate, and resembled fibroblasts. Within 12 h of exposure to yolk in the culture medium, these cells showed the same features seen in lacuna-lining cells in regressing ovaries, ie, engorgement of cytoplasm by yolk particles, distortion of the nucleus, and a tendency to pile up and become loosened from the underlying surface.

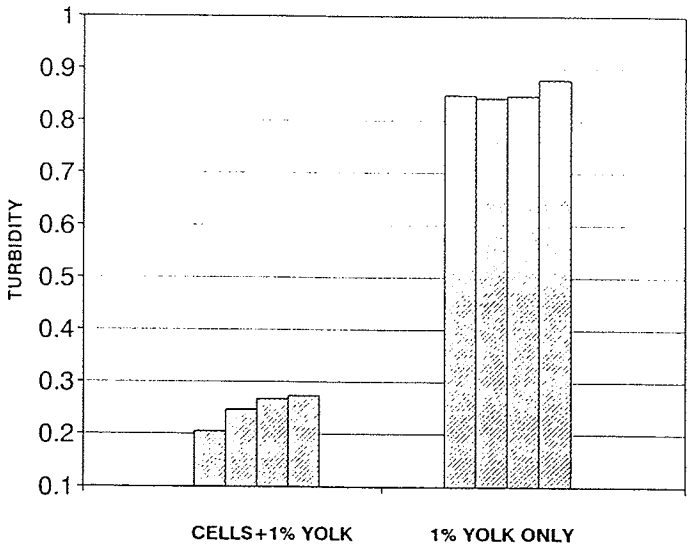

Figure 1. Change in turbidity of yolk suspension in cell culture medium after 36 h incubation in the presence of autologous monocytes (yolk and monocytes from the same bird), compared with cell-free medium.

The turbidity of the yolk-containing culture medium taken from the monocyte cultures showed significant reduction in turbidity after 36 h of exposure to the monocytes, compared to yolky culture medium added to chambers free of cells (Figure 1.). The differences were highly significant ($P < 0.0001$).

(d) Ovarian and embryonic fibroblast studies

Ovarian cells, after removal of debris and formation of monolayers on coverslips, also showed morphological features typical of fibroblasts. After exposure to yolk, they showed some morphological changes such as formation of variably-sized lipid droplets. These cells reacted to the presence of yolk but much less dramatically than did the monocytes: the ovarian cell nuclei remained visible in 50% of cells, and they remained attached to the coverslips and did not become suspended in the cell culture fluid.

Embryonic fibroblasts did not show morphological evidence of phagocytic activity and retained their fibroblastic conformation after exposure to yolk. Significant ($P > 0.0001$) clarification occurred in the yolk-containing culture fluid from both ovarian and embryonic fibroblasts (Figure 2.) but to a much lesser degree than that seen in the monocyte cultures.

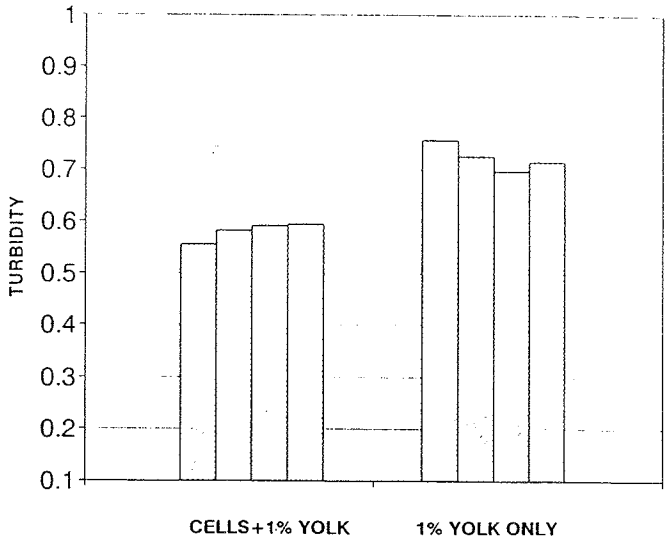

Figure 2. Change in turbidity of yolk suspension in cell culture medium after 36 h incubation in the presence of embryo fibroblasts, compared with cell-free medium.

IV. DISCUSSION

(a) The function of the ovarian lacunae

One of the most characteristic aspects of atresia of large, yolky follicles is resorption of follicular contents. However, there has been controversy for a long time regarding the mechanisms involved in the rapid resorption of this large volume of yolk (up to 70 mL in total) following ovarian regression. Some authors believe that the yolk is resorbed through the vascular system (Johnson 1986), although no convincing evidence has been presented for this. Other workers have stated the ovarian stroma to be the main site of yolk resorption (Davis, 1942; Gupta, 1986; Gupta, 1988), without specifying the exact site and route of such resorption.

The existence, structure and function of lacuna spaces in both the active and regressing avian ovary have received little attention in the past. Gilbert (1979) reviewed all the structural details of the avian ovary but did not mention the existence, function or morphology of this prominent structure. Callebaut (1979) demonstrated the existence of these spaces in the ovary of immature chickens and quails and, in fact, named them lacunae. He cited earlier work that suggested that, at the beginning of ovarian development, there was no communication between blood vessels and lacunae; however later, by erosion of the vessels in the vicinity of the lacunae, a communication is established between lacunae and the vascular system. We were unable to confirm this. Callebaut (1979) also demonstrated direct communication between lacunae and the peritoneal space in immature chickens, quail and chick embryos, and showed that experimental intraperitoneal injection of yolk or of Hela cells resulted in uptake of these substances into the lacunae. However, we did not observe communications between the peritoneal cavity and the lacuna system in our non-atretic, actively-laying hens, although some leakage may occur through the thin walls of the lacunae during bursting atresia. The main function of the system of lacunae therefore seems to be to aid in the disposal of yolk from large regressing follicles in laying hens, by providing voluminous spaces in which phagocytic cells can break down yolk to material which can be easily metabolised.

It seems that the hen has a "back-up" mechanism for disposing of yolk in the event of escape of follicular contents into the abdominal cavity. In the few cases where free yolk had escaped into the peritoneal cavity from regressing ovaries, we observed transformation of peritoneal-lining cells into macrophage-like cells in the same manner of the transformation of lacuna-lining cells when exposed to yolk. Sturkie (1955) has described the rapid disappearance of yolk from the peritoneal cavity after experimental surgical rupture of yolky follicles in the laying hen: our observations illustrate a mechanism whereby (in part at least) this phenomenon might be effected.

The extreme distensibility and very extensive disposition of these lacunae within the ovary may also allow for the extremely rapid expansion of maturing follicles that takes place shortly before ovulation. A typical follicle may expand from 8mm to 37mm in diameter over 7 days (Johnson, 1986) and, with several such follicles maturing at the same time, the lacunae may well be necessary to allow the stroma to accommodate this rapid increase in volume.

(b) The origin and function of the lacuna-lining cells

Upon demonstration of the activity of the lacuna-lining cells in regressing ovaries, the question arose as to their origin. In normal resting lacunae the flat lining cells are present as a single layer, while in the presence of the escaped yolk the lining cells appear to have increased in number as well as changed their morphology. Since the cells now clearly had phagocytic properties, it occurred to us that there may have been active recruitment of blood monocytes which may have migrated into the spaces from the adjacent blood vessels. This may in fact happen, but was not observed in our material. Our findings show that avian monocytes are able to respond morphologically and phagocytically to the presence of yolk in a relatively short period of time. Moreover, cells very similar to monocytic phagocytes accumulate in lacunae together with large volumes of extruded yolk. At this stage we cannot determine the relative contribution, if any, of blood monocytes to the phagocytic lacuna-lining cells in the regressing ovary. Further studies are planned to determine to what extent (if any) mitosis of lacuna-lining cells contributes to the population of lacuna macrophages. At this stage, the avian blood monocyte appears to react to the

presence of yolk in the same manner as do the lacuna-lining cells. The relatively poor capacity of ovarian and embryonic fibroblasts to phagocytose yolk suggests that structural and undifferentiated mesenchymal cells are not particularly well-differentiated with respect to this function.

(c) The role of the stigma in bursting atresia

Following rupture of the follicular wall (thecal layer) in bursting type atresia, most of the wall undergoes atrophy. The stigma, however, which in the developing follicle is extremely thin and represents the site through which normal ovulation takes place, in the regressing follicle rapidly becomes thickened and shrunken. Some of this may simply be the result of sudden reduction in follicular pressure and diameter, which allows the stigma to shrink. But the mitotic activity seen in the stigmal fibroblasts suggests that the stigma, on initiation of bursting regression, is stimulated to thicken and prevent the escape of yolk into the peritoneal cavity.

Having partially defined this aspect of ovarian function, we now plan to study ovarian morphology in laying birds of different strains which have been subjected to pause, in order to see if there are differences between strains in their ability to regain normal ovarian form and function following a "pause" cycle.

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STUDIES ON GROWTH AND PRODUCTION IN LAYING HENS HOUSED IN CONTROLLED ENVIRONMENT SHEDDING

G. B. PARKINSON* and A. ALMOND**

Summary

A problem of weight loss and slow growth between 24 and 30 weeks of age has been observed in all the controlled environment production systems in the Victorian egg industry. The growth problems in early egg production appear to be a precursor to a significant reduction in mature body size and an inability of flocks to express their full genetic protential for egg production The period of low growth is associated with daily feed intakes well below the levels conventionally expected from flocks as they approach peak egg production.

A model of the phenomenon was established in a controlled environment shed using an Australian brown egg laying strain to test the hypothesis that the weight loss and reduced production may be ameliorated by increases in dietary protein and energy concentrations provided between 20 and 35 weeks of age.

The provision of the additional dietary protein and energy failed to ameliorate the weight loss between 24 and 30 weeks of age although body size was larger at 35 weeks of age. The apparent inappetance and low growth in flocks at peak production remains an intractable problem which appears unresponsive to the traditional nutritional approach of increasing nutrient density to compensate for low appetite.

1. INTRODUCTION

Recent collaborative research undertaken by the Department of Agriculture, Victoria and the Victorian College of Agriculture and Horticulture has demonstrated substantial variations in flock growth rates between point of lay (18-20 weeks) and peak egg production, which have important ramifications for life time production of flocks. Many flocks have been observed with depressed growth rates and even significant weight loss between 24 and 30 weeks of age. These flocks appear incapable of compensatory growth after this period and, as a consequence, the flocks remain 20% smaller for life. Associated with the reduction in body size, average egg size has been observed to be depressed by 2-3 g, peak egg production is below genetic potential by approximately 5%-10% and there may be an accelerated deterioration in shell quality (Almond, 1993). In some of the flocks with the weight loss at 24 to 28 weeks of age, mortality has also been elevated, but the exact reasons for this are unknown.

Similar problems of low appetite at peak egg production, weight loss and a marked post-peak slump in egg production have also been described recently in North America (Leeson, 1990; Mutalib, 1993), and it, therefore, appears that the phenomenon is more widespread than originally hypothesised.

Earlier research undertaken in Australia by Balnave (1984) and Johnson *et al.* (1984) indicated that flocks approaching peak egg production experienced a plateau in growth for a period of a few weeks, followed by a marked acceleration in growth rate between 28 and 40 weeks of age. The final mature weights of the flocks appeared

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unaffected by the transitory growth depression occurring at or near peak egg production.

The plateau in growth described by Balnave (1984) and Johnson *et al.* (1984) appears similar to the phenomenon recently observed in Australia and North America, but these recent studies indicate that flocks are experiencing significant weight loss (5-10%), rather than a slowing of growth rate. Furthermore, the recent research indicates that there are long term consequences of this weight loss on mature body size (Almond, 1993) and probably on tissue reserves.

The decline in growth rate of flocks at 25 to 27 weeks of age observed by Balnave (1984) is believed to result from a decline in feed consumption at sexual maturity and has been observed by many other authors (Foster, 1968; Meyer *et al.*, 1970; Hurvitz *et al.*, 1971; Classen and Scott, 1982; Johnson *et al.*, 1984). Scott and Balnave (1989) have hypothesised that the decreased feed intake is likely to be in response to a behavioural or physiological/ endocrinological stimuli initiated by oviposition and or ovulation of the first egg. The response occurs in all hens and is manifested over a two to four week period in most flocks.

The close association between the growth problem and the demands of peak egg production has resulted in the hypothesis that the flocks subject to this phenomenon are experiencing a metabolic crisis between 25 and 30 weeks of age. Nutrient intake is probably unable to meet the dual demands for growth and production during this period (Summers, 1983). Furthermore, Summers (1983) indicated that pullets with minimum nutrient reserves at sexual maturity are unable to meet the demand for nutrients as they are subject to high levels of nutrient output with relatively low levels of nutrient intake. In other words, underweight flocks would not have the nutrient reserves to sustain production during this period of peak metabolic demand.

Mutalib (1993) has hypothesised that modern laying strains may have an altered metabolic state which can potentially accentuate the problems of low nutrient intakes at peak production. Mutalib (1993) suggested that the selection pressure for feed conversion has produced genotypes with a reduced appetite responsiveness. The modern genotypes may, therefore, be more susceptible to imbalances in the equilibrium of appetite, tissue reserves and productive output.

Research is required to thoroughly quantify the production consequences of the phenomenon, and to examine the role of nutrition as a means of ameliorating both the weight loss and the detrimental effects on production. A model of the weight loss phenomenon has been established in a controlled environment shed and the responsiveness of the phenomenon to dietary nutrient manipulation has been examined using an Australian brown egg laying strain.

II. METHODS

A total of 960, 18-week old (Australorp x New Hampshire) pullets were divided into four groups of approximately equal weight and uniformity. Each 240 bird flock was distributed into 5 replicates of 48 birds. The birds were housed in 4-bird cages in a controlled environment shed and were fed four different diets from 18 to 35 weeks of age. The four diets ranged in crude protein (CP) concentration from 165-200 g/kg and metabolizable energy (ME) from 11.5 to 12.14 MJ/kg and were prepared by a commercial stockfeed mill. Diet 1 was a conventional layer diet (165 g CP,11.5 MJ of ME/kg), Diet 2 was a high protein diet (200 g CP,11.5 MJ of ME/kg), Diet 3 was a high energy diet (165 g CP,12.14 MJ of ME/kg), and Diet 4 was a high energy and high protein diet (200 g CP,12.14 Mjof ME/kg). The high protein diets contained 4.4 g methionine and 11.0 g

total lysine/kg, whilst the low protein diets contained 3.4 g methionine and 8.1 g total lysine/kg. The dietary fat increased from 60-70 g/kg on the low energy diets to 80 g/kg on the high energy diets. Similar dietary mineral specifications were maintained on all four diets.

The body weight and egg production were monitored weekly between 20 and 36 weeks of age.

III. RESULTS

The flocks fed Diet 2 produced the greatest response in both growth and production, with rates of egg production exceeding the flock fed Diet 1 by 3-4% at ages of 25 and 35 weeks of age (Table 1). The flock fed Diet 2 was approximately 100 g heavier than the flock fed the conventional diet at 35 weeks of age (Figure 1).

The flocks fed Diets 3 and 4 also showed improvement in growth rates and production (Figure 1, Table 1), but the use of Diet 4 failed to produce any significant enhancement of production or growth. The experimental model was unable to clearly differentiate a protein from an energy affect, and the use of Diet 4 failed to produce any synergistic or additive affects on either growth or production.

The growth responses to additional energy and protein occurred on all diets between 20 and 24 weeks of age and the provision of a higher nutrient density failed to ameliorate the weight loss and low growth problems (Figure 1). At 35 weeks of age the hen-housed egg production on Diets 2 and 4 exceeded that of Diet 1 by 2-3 eggs, and the magnitude of the difference appeared to be increasing with time.

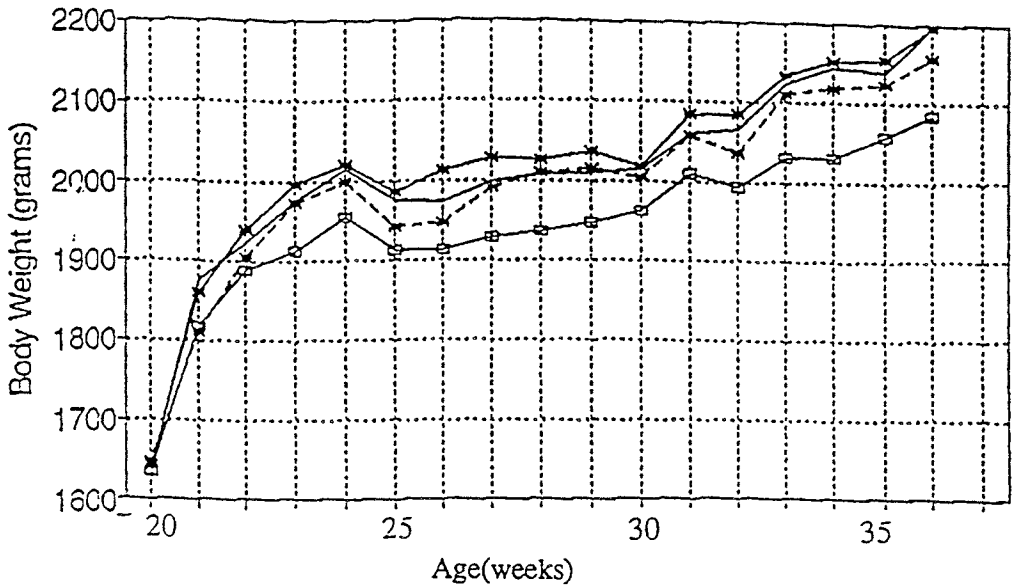

Figure 1. Average body weights of four groups of laying hens fed the experimental diets between 20 and 36 weeks of age.

Table 1. Percentage rates of lay at 5 weekly intervals in four flocks fed the four experimental diets.

Diet Age (wks)	1	2	3	4
20	6.0(1.2)[a]	5.0(1.2)[a]	4.5(0.9)[a]	4.3(1.1)[a]
25	81.4(1.7)[a]	87.7(1.8)[a]	84.0(3.0)[ab]	85.1(1.0)b
30	94.3(1.9)[a]	96.0(2.0)[a]	94.8(1.3)[a]	95.6(0.6)[a]
35	86.0(0.7)[a]	90.7(1.4)[a]	87.4(1.9)[a]	87.67(1.7)[ab]

Mean with (SE), n = 5 replicates of 48 hens
[ab] Different superscripts denote significant differences ($P < 0.05$) between treatments at each age.

IV. DISCUSSION

The brown egg laying strain used in this experiment appeared susceptible to both the weight loss at peak production and the inability of the flock to achieve the accepted mature body weight. The provision of the additional dietary energy and protein failed to significantly alter the pattern of the weight loss between 24 and 30 weeks of age. The response in growth and production to the additional energy and protein was primarily a consequence of the faster growth achieved between 20 and 24 weeks of age.

Calculations of daily energy requirements indicated that flocks subject to this phenomenon between 24 and 30 weeks of age are in negative energy balance, with ME deficits of between 0.12 and 0.16 MJ/day. Clearly the energy-regulating mechanisms within the bird are unable to maintain a balance under all circumstances, and the period between point of lay and peak production is a critical period where constraints to nutrient intake can produce long term ramifications on production.

The brown egg hens in this experiment had very low feed consumptions of 98 to 100 g/day in all groups during the period of peak egg production (30 weeks of age), The feed intakes recorded are difficult to reconcile with the normal appetite patterns manifested by this particular brown egg laying strain when housed in conventional shedding. Clearly the constraint to appetite is not related to the physical ability of the hens to consume feed.

The problem of low nutrient intake between point of lay and peak production appears at this stage to be a problem of appetite. Flocks subject to this problem have been recorded consistently with feed intakes of less than 100 g/day. The vexing question is why are the flocks unable to increase their feed consumption to take account of the increasing demands of both growth and production. Feed intake would only need to increase to 100-115 g/day and the flocks would begin to meet their energy requirements.

Scott and Balnave (1987) have suggested that the inappetance is a result of a physiological/endocrinological stimulus which is an inevitable consequence of the initiation of egg production. It is clear from these findings, however, that the reduced growth rate associated with the inappetance at peak egg production can differ in magnitude and severity and that it can be the precursor to a significant reduction in mature body size.

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PREDICTION OF BREAST MEAT YIELD IN JAPANESE QUAIL BY ULTRASOUND

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The high price of breast meat in chickens relative to other cuts provides an incentive to the chicken meat industry to maximise breast meat yield. A genetic approach is indicated but is hampered by the availability of accurate objective measures of yield in the live bird. Herein, the results are presented of a study of the prediction of breast meat yield in Japanese quail, as a model for meat chickens, using measures of muscle length and width and real time ultrasound to measure muscle depth. The study was undertaken to provide prediction equations for a now-commenced selection experiment for breast meat yield in Japanese quail.

One hundred and fifty Japanese quail were hatched and reared to 42 d on a broiler starter diet containing 12.5 MJ ME and 230 g CP/kg. At 42 d 74 males and 63 females were weighed (LWT) and measures made of breast muscle length, width and depth. Muscle width was measured with vernier callipers at the cranial point of the keel (WA) and also 20 mm cranial to the caudal termination of the keel (WB), and muscle length was measured as the length of the keel (L). Muscle depth was measured with a 5.0 Mhz prove attached to an Aloka SSD-500 real time ultrasound scanner with the probe placed parallel to and 6-7 mm away from the keel bone with depth measures taken adjacent to the cranial point of the keel (Da) and 40 mm caudal to this point (DB). The birds were killed the following day and the breast muscle excised and weighed. Bird and breast muscle weights were measured in g and muscle length, depth and width in mm. Prediction equations were determined for breast muscle weight using a stepwise multiple linear regression procedure where the model chosen in each step showed maximum R-square value. Optimal prediction equations for breast meat yield (BMY) in the two sexes were:

$$\text{BMY males} = -1.8 + 0.073\,\text{LWT} + 0.27\,\text{DA} + 0.42\,\text{DB} \qquad R^2 = 0.51$$
$$\text{BMY females} = -13.0 + 0.033\,\text{LWT} + 0.29\,\text{WA} + 0.24\,\text{L} + 0.83 \qquad R^2 = 0.69$$

In each case, the addition of further traits resulted in only very marginal improvement in prediction of breast muscle weight.

The above equations showed a significant improvement in the prediction of breast meat yield from liveweight alone, where R^2 values were 0.44 in both males and females, indicating substantial benefit from inclusion of certain breast muscle measurements. In the case of the females, the inclusion of DA in the 2- variable model increased the R^2 value from 0.44 (with LWT alone) to 0.60. Thus the ultrasound-derived thickness measures had the greatest influence of all the breast muscle measures on the improvement in prediction. The differential contribution of the different measures in the two sexes is presently under study with the aim of producing robust equations that can be used effectively in the different lines of the selection experiment to select for increased or decreased breast meat yield.

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NUTRITIONAL EVALUATION OF GRAIN AMARANTH FOR CHICKENS

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The growing interest in the possible use of grain amaranth in poultry nutrition arises from its potential nutritional value. Compared to common cereals, amaranth has a high content of protein and a better balance of essential amino acids (National Research Council, 1984). The present study was conducted to investigate the effects of incorporating graded levels of raw grain amaranth (*Amaranthus hypochondriacus*) in nutritionally balanced diets on broiler performance. The influence of autoclaving on the energy utilization values of amaranth was also determined. The grain amaranth sample used in the study was analyzed and found to contain (g/kg DM): crude protein, 168; crude fat, 58; crude fibre, 60; ash, 26; calcium, 2.2; total phosphorus, 5.6; lysine, 10.1 and methionine, 3.5.

In Trial 1, raw amaranth was incorporated into a maize-soyabean meal-meat meal diet at 0, 200, 400 and 600 g/kg levels. All diets were balanced to contain similar amounts of metabolizable energy, protein, lysine and sulphur-containing amino acids. Four replicates of six broiler chicks were offered each of the experimental diet from 7 to 16 days of age. Individual chick weights and pen feed intake were monitored. At the end of the trial, three chicks from each replicate were sacrified and organs (heart, liver, spleen and pancreas) were excised and weighed. Weight gains and feed intake were depressed ($P<0.01$) with increasing levels of raw amaranth in the diet. Feed/gain values were similar between birds fed on diets containing 0 and 200 g/kg amaranth, suggesting that the growth depression at 200 g/kg amaranth level was due largely to a reduction in feed intake. Beyond the 200 g/kg level, feed/gain was increased ($P<0.01$) by the inclusion of raw amaranth. Although not statistically significant, heart weight, when expressed as a proportion of liveweight, was lower and pancreas weight was higher in birds given the raw amaranth diets compared to those given the control diet.

In Trial 2, energy utilization of raw and autoclaved (130^{o} C for 1 hour) amaranth was evaluated in a classical apparent metabolizable energy (AME) assay (Mollah *et al.*, 1983) using individually caged 6-week-old broilers. A maize diet was included in the assay as a control. The AME of grain amaranth was improved by autoclaving. The AME (MJ/kg DM) values of raw and autoclaved amaranth were 11.85 ± 0.29 and 13.10 ± 0.26, respectively. The results indicate that *A. hypochondriacus* grain is a potentially useful energy supplement for poultry. However, the growth data suggest the presence of anti-nutritional factor(s) in raw amaranth and the need for some form of processing to maximize its nutritional value. Whether the growth depressing effects of amaranth can be completely overcome by autoclaving cannot be determined from the present data and needs to be evaluated in future studies.

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PHYTASES IN POULTRY NUTRITION - AN OVERVIEW

V. RAVINDRAN

Summary

Phytate, the hexaphosphate of *myo*inositol, is the primary storage form of phosphorus in plant seeds. For the phosphorus in phytate to be utilized by poultry, phytate must be hydrolysed to yield inorganic phosphorus. Phytase (EC.3.1.3.8), either of microbial origin or endogenous to certain ingredients, must be present in order to hydrolyze significant amounts of phytate. Endogenous phytase in wheat is effective in degrading phytate, but the degree of hydrolysis varies. The usefulness of commercial phytase preparations as a means of releasing the phosphorus from the phytate is highlighted. Recent results clearly demonstrate the efficacy of supplemental phytase in improving phytate-phosphorus availability and decreasing phosphorus excretion in the manure. In its native state, phytate is complexed with various cations or with proteins. In the foreseeable future, the effects of phytase supplementation on these biologically important nutrients will be studied and may open up exciting new avenues.

I. INTRODUCTION

Phytic acid, *myo*inositol hexakis dihydrogen phosphate, is an ubiquitous compound that is abundant in all seeds (cereals, grain legumes and oilseeds) serving as the chief storage form of phosphorus (Maga, 1982). Phytate-phosphorus accounts for 50 to 80% of the total phosphorus present in these plant materials. Oilseed meals and cereal brans contain high levels of phytate-phosphorus, whereas cereals and grain legumes contain moderate amounts (Table 1). Since a major portion of practical poultry diets consists of plant-derived ingredients, the phytate assumes considerable nutritional significance. Typical poultry diets contain 2.5 to 4.0 g phytate-phosphorus/kg. The ability of poultry to utilize phytate-phosphorus is generally assumed to be poor (Nelson, 1967), thus posing two problems for the producer: (1) the need to add inorganic phosphorus supplements to diets, and (2) the excretion of large quantities of phosphorus in the manure. The ability of phytate to complex with metal ions and proteins, and to make them biologically unavailable is another nutritional concern associated with phytate.

The availability of phytate phosphorus for poultry has been researched and speculated on for almost half a century. Available evidence indicates that phytate-phosphorus utilization by poultry ranges from zero to over 50%, depending on age of birds, ingredient type, and dietary levels of calcium, phosphorus and vitamin D (Ravindran *et al.*,1995). It is now becoming increasingly clear that older birds have a greater ability to utilize the phytate form of phosphorus. The purpose of this brief review is to focus on aspects of phytate hydrolysis and on the usefulness of supplemental phytase as a feed additive, and to consider these in relation to poultry nutrition. The influence of phytase on other phytate-bound nutrients and the possible "extra-phosphoric" effects associated with the enzyme will also be highlighted.

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Table 1. Phytate-phosphorus content and phytase activity of some common feed ingredients.

Ingredient	Phytate phosphorus, g/kg[1]	Phytate phosphorus, as % of total P[1]	Phytase activity units/kg[2]
Cereals and by-products			
Wheat	2.4 (1.9-2.9)[3]	68 (61-78)	1190
Maize	2.0 (1.6-2.6)	73 (61-85)	15
Sorghum	2.2 (1.9-2.9)	68 (61-76)	25
Barley	2.1 (1.9-2.4)	58 (55-62)	580
Oats	2.8 (1.6-3.5)	69 (48-78)	40
Wheat bran	8.8 (6.0-12.7)	76 (68-93)	2960
Grain Legumes			
Lupins	3.0 (2.9-3.0)	55 (54-55)	0
Peas	1.7 (1.3-2.1)	45 (36-53)	115
Chick peas	2.1 (2.0-2.3)	51 (49-53)	-
Oilseed meals			
Soyabean meal	3.7 (2.8-4.0)	57 (46-61)	40
Canola meal	6.5 (4.6-7.8)	58 (36-70)	15
Sunflower meal	4.4 (3.2-5.1)	44 (35-47)	60

1 Data adapted from the following sources: Eeckhout and De Paepe (1994); Kirby and Nelson (1988); Nelson *et al.*(1968a); Ravindran *et al.* (1994).

2 Data from Eeckhout and De Paepe (1994). One unit is defined as that amount of phytase which liberates inorganic phosphorus from a 0.0015M Na-phytate solution at a rate of 1 mol/min at pH 5.5 and 37°C.

3 Values within parantheses refer to ranges reported in the literature.

II. PHYTATE HYDROLYSIS IN POULTRY

In order for the phosphorus to be utilized by poultry, phytate must be hydrolyzed into inorganic phosphorus. The dephosphorylation of phytic acid is the result of phytase (*myo*inositol hexaphosphate phosphohydrolase; EC.3.1.3.8) activity. Phytases comprise a family of enzymes that catalyze the removal of inorganic orthophosphate from phytate in a stepwise manner producing five classes of intermediate products (*myo*inositol pentakis-, tetrakis-, tris-, bis- and monophophates).

The degradation of phytate within the digestive tract of poultry may be ascribed to the action of one or more phytases and there are three possible sources: (1) intestinal phytase in digestive secretions, (2) phytase activity originating from resident bacteria, or (3) endogenous phytase activity present in some feed ingredients. The available data on the presence of phytase activity in intestinal secretions (Bitar and Reinhold, 1972; Maddaiah *et al.*,1964; Davies and Flett, 1978) and intestinal bacteria (Warden and Schaible, 1962) are limited and controversial, and it may be concluded that such activity in poultry is negligible. However, the presence of native phytase in some feed ingredients is well known. Wheat, barley and wheat bran are rich in phytase activity, whereas maize, sorghum, oats and oilseed meals contain little or none of the enzyme (Table 1).

Several workers have demonstrated that phytate phosphorus utilization in poultry diets can be significantly improved, and the use of inorganic phosphate supplements can be

totally avoided, by incorporating feed ingredients with known phytase activity. It has been shown that phytate phosphorus in diets based on wheat and wheat by-products, without feedstuffs of animal origin and without inorganic phosphate supplements, is well utilized by young chickens (Temperton *et al.*, 1965a) and laying hens (Temperton *et al.*, 1965b; Salmon *et al.*, 1969) for bone formation and production purposes. However, it is noteworthy that the effectiveness of wheat phytase in hydrolysing phytate appears variable. Nelson (1976) found that laying hens hydrolyzed only 13% of the phytate when the diet contained wheat. It is possible that the amount of active phytase in feed ingredients can vary depending on cultivar, age of wheat and/or drying and storage conditions. Temperatures employed during ingredient processing or during pelleting of diets based on these ingredients can also influence the native phytase activity. Higher temperatures (> 70° C) have been reported to partially or totally inactivate phytases present in wheat and barley (Jongbloed and Kemme, 1990).

III. PHYTASE AS A FEED ADDITIVE

(a) Effects on phytate-phosphorus availability

Poultry can utilize phytate phosphorus provided a source of active phytase is present in the diet. In this context, the use of microbial phytase has attracted attention in recent years as a mean of releasing phosphorus from phytate and reducing the excess amounts of phosphorus excreted in the manure (Simons *et al.*, 1990). Almost three decades ago, Nelson *et al.* (1968b) were the first to show that phytase produced by *Aspergillus ficuum* can hydrolyze the phytate phosphorus in plant-derived ingredients. Although this was confirmed in subsequent studies (Rojas and Scott, 1969; Nelson *et al.*, 1971), the low supply and high cost of microbial phytase limited its commercial exploitation in the past. However, the recombinant DNA technology currently available to synthesize the enzyme, from genetically modified *aspergilli* strains, has generated new possibilities and this is evidenced by the upsurge of interest in using microbial phytase as a feed additive. Recent research on this topic has been reviewed by Swick and Ivey (1992). Reported improvements in phosphorus availability are generally in the range of 20 to 45%, the amount of phytate phosphorus released being dependent on the level of added phytase, the phytate content of the diet, source of phytate, and dietary levels of non-phytate phosphorus, calcium, calcium:total phosphorus ratio and vitamin D. In addition, phosphorus excretion may be decreased by up to 60% by supplemental phytase (Yi *et al.*, 1994b; Kornegay *et al.*, 1994) and immediate reductions of at least 30% appear possible under practical feeding situations.

(b) Effects on digestibility of cations and amino acids

The structure of phytic acid is indicative of tremendous chelating potential. It is a strong acid and forms a wide variety of insoluble salts with di- and tri-valent cations at neutral pH, potentially rendering these minerals biologically unavailable. It is now known that phytate lowers the bioavailability of several nutritionally important minerals, including Ca, Mg, Zn and Fe. Other minerals which are believed to be affected are Cu, Mn, Mo and Co (Maga, 1982). Evidence is accumulating to indicate that the apparent digestibility of Ca is improved by supplemental phytase (Schoner *et al.*, 1991; Huyghebaert *et al.*, 1992; Kornegay *et al.*, 1994; Yi *et al.*, 1994a). Studies on the influence of phytase on other phytate-bound minerals have been limted. Pallauf *et al.* (1992) found that

phytase addition increased the apparent absorption of Mg, Zn, Cu and Fe by up to 13, 13, 7 and 9%, respectively. Similar positive responses on the bioavailability of Zn have also been reported by Lei *et al.* (1993).

The nutritional significance of phytate is further complicated by its interaction with proteins and its inhibitory effects on proteolytic enzymes. These effects may be expected to adversely affect the digestibility of proteins and amino acids. Thus, theoretically phytase must be able to release the phytate-bound protein for utilization, but published data on this aspect is limited. Officer and Batterham (1992) observed improvements of 7-12% in the ileal digestibility of protein and essential amino acids in pigs with the use of supplemental phytase. Improved nitrogen digestibility has also been reported in several other studies (van der Klis and Versteegh, 1991; Mroz *et al.*, 1994; Yi *et al.*, 1994b). The possible effect of phytase on amino acid digestibility will be of immense practical interest and needs to be confirmed in future studies.

VI. CONCLUSIONS

Phytase, either of microbial origin or active within an ingredient, must be present in poultry diets in order to release significant amounts of phosphorus from phytate. An active phytase in wheat is effective in dephosphorylating phytate although the amount degraded may be variable. Recent studies have demonstrated consistently significant improvements in phytate phosphorus utilization through supplementation of poultry diets with dried preparations of microbial phytase. More research is needed to resolve the factors that may influence the efficacy of the enzyme in poultry diets and, these factors include *inter alia* Ca:P ratio and dietary levels of Ca and vitamin D. Limited studies suggest that vitamin D may function synergistically with phytase to enhance phytate hydrolysis (Edwards, 1993) and that high Ca levels may be detrimental to phytase activity (van der Klis and Verteegh, 1991). In the long term, however, the acceptability of phytase by the feed industry will depend not only on the magnitude of its efficacy, but also on the cost, product stability and ease of application (Swick and Ivey, 1992). Based on data from maize-soybean meal diets, and using current US prices, Yi *et al.* (1994c) estimated that the cost of adding phytase enzyme to broiler diets is 1.3 times the cost of an equivalent amount of an inorganic form of phosphorus; this cost, however, did not include the cost of phosphorus disposal or the possible "extra-phosphoric" effects of phytase. In the foreseeable future, one can speculate that much attention will be centred on the effects of phytase on the availability of nutrients other than phosphorus. In this context, the potential for wider application of phytase technology to enhance overall nutrient utilization in diets for monogastric animals is exciting.

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THE EFFECTS OF GROWTH FACTORS ON CHICKEN OVARIAN CELLS *IN VITRO*

R.D. ROBERTS and B.M. GORDON

Summary

The growth factors, IGF-1, IGF-2, EGF, TGFa, FGF-1 and FGF-2 all stimulated DNA synthesis in cultured granulosa and thecal cells in a dose-dependent manner. The potency of each growth factor was different, with TGFa being the most potent and EGF the least potent for each cell type. Western lig and blotting revealed the presence of IGF binding proteins (BP) in both granulosa and thecal cell-conditioned medium, the concentrations of which were highest in thecal cell-conditioned medium. Production of IGFBP was inducible in granulosa cells by IGF-1 treatment. Stimulation of DNA synthesis by treatment with IGF-1 or an analog, Long-[Arg^3]-IGF-1, was significantly different in thecal cell cultures at 0, 24 and 48 h after a cell culture medium change, but was significant in granulosa cells at only 48 h.

I INTRODUCTION

Chicken pre-ovulatory follicles are among the fastest growing structures found in higher vertebrates. Disruption of the well-ordered development of follicles as they approach ovulation is probably responsible for the industry problem of low reproductive efficiency in broiler breeder females. Understanding the mechanisms which control normal follicular development will help to identify which of these are dysfunctional in broiler breeders.

The involvement of insulin-like growth factor-1 (IGF-1) in the growth and differentiation of mammalian ovarian cells is now well established, the evidence being consistent with an autocrine/paracrine mode of action (Baranao and Hammond, 1984; Adashi *et al.*, 1991). Previous studies to determine the role of IGF-1 in the chicken ovary revealed that the gene is expressed and that receptors to IGF-1 are present in granulosa and thecal cells which form the follicular wall and which support and nurture the developing oocyte. Further, IGF-1 was found to be mitogenic for these cells *in vitro* (Roberts *et al.*, 1994), but comparative effects of IGF-1 and the related peptide, IGF-2 are unknown. The IGFs are associated with binding proteins (IGFBP), with six having been identified in mammals. They have various effects on the actions of IGFs in different tissues (Shimasaki and Ling, 1992) and could be potential regulators of IGF actions in the avian ovary.

There is little information on the role of other growth factors. Epidermal growth factor (EGF) receptors have been located on both ovarian cell types and the peptide has been found to be both steroidogenic and mitogenic for these cells (Onagbesan *et al.*, 1992, 1994; Peddie *et al.*, 1994). There are no reports on the actions of the EFG-related peptide, transforming growth factor alpha (TGFa) or the fibroblast growth factors (FGFs) in the avian ovary.

In addition to their roles in ovarian steroidogenesis, the pituitary gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), have also been tested for their growth effects on ovarian cells. Treatment with LH stimulates DNA synthesis in granulosa cells, in a similar manner to IGF-1 (Yoshimura and Tamura, 1988). When IGF-1 and LH treatments were combined, they were found to have a synergistic effect on DNA synthesis (Roberts and Goddard, 1992). The mechanism of the IGF-1/LH synergy remains unknown.

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A study was undertaken in an attempt to clarify the role of various growth factors on chicken ovarian cells. In addition, the existence and possible role of binding proteins for the insulin-like growth factors in the chicken ovary was investigated. To this end, Long-[Arg^3]-IGF-1, an analog of IGF-1, which differs from the wild type peptide in not binding to the IGFBPs while still binding to the IGF type 1 receptor (Francis *et al.*, 1992), was used.

II MATERIALS & METHODS

(a) Animals

Individually caged White Leghorn hens fed *ad libitum* were reared under commercial restricted lighting conditions as used for broiler breeder hens, the light period being stepped up to 15.25 h daily by the beginning of lay. Hens were killed by cervical dislocation between 30 and 50 weeks of age. Birds used in each study were of the same age and were laying daily.

(b) Reagents

Medium 199 (M199) containing Earl's salts and sodium bicarbonate (GIBCO) 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). Foetal calf serum (FCS) was obtained from ICN Biomedicals Pty Ltd. Recombinant chicken IGF-1, IGF-2 and long-[Arg^3]-IGF-1 were gifts from Dr. Zee Upton, CSIRO Division of Human Nutrition, Adelaide. Murine EGF was produced at CSIRO Division of Animal Production, Prospect. The TGFa, FGF-1, FGF-2 and all other reagents (unless stated) were obtained from Sigma Chemical Co.

(c) Tissue collection and cell culture

The granulosa and thecal layers were separated from F1-F4 yellow yolky follicles in sterile Dulbecco's phosphate-buffered saline, then transferred to 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 1-5 minutes at room temperature prior to centrifugation, while the thecal layers were digested for 45 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% FCS prior to plating on plastic tissue culture flasks or multi-well plates. Cells were cultured in a humidified incubator containing 5% $C0_2$ at 41°C. Granulosa and thecal cells were plated at a density of 50,000 and 250,000 viable cells per cm^2, respectively. Following an attachment period of 48 h, the medium was removed and replaced with medium containing 0.1% FCS. This was repeated after a further 24 h of culture and then the effects of various treatments were investigated. In each experiment the granulosa and thecal tissues were obtained from the same hen. All reported experiments were repeated twice.

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

For the final 16 h of treatment, 0.25 μCi [^{3}H]-thymidine label (Amersham Australia) was added to each well containing 1 mL of medium. The treatment was terminated by aspiration of the medium. The cells were then solubilised by the addition of 500 μL of

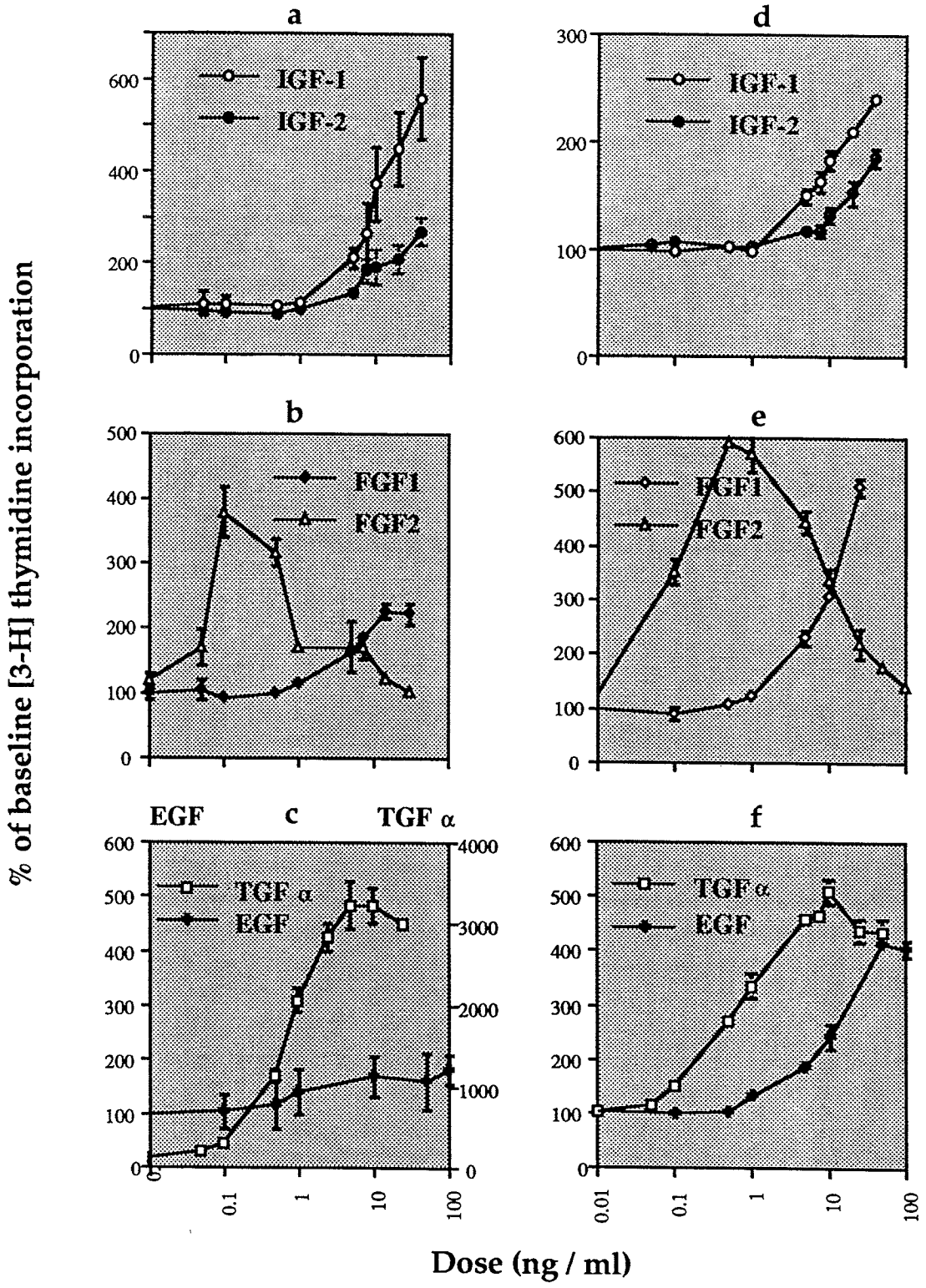

Figure 1. The effects of growth factor treatment on granulosa cell (a, b, c) and thecal cell (d, e, f) DNA synthesis *in vitro*, measured by incorporation of [3-H] thymidine as a percentage of the unstimulated control. Mean values are shown, bars represent the standard error.

sodium hydroxide (0.5 mol/L), and incubation at room temperature for 2 h. The radioactivity in the samples was then measured in a scintillation counter.

(e) Western Ligand Blotting

Samples of cell-conditioned medium were concentrated, then denatured, by boiling for 5 minutes in Laemlli buffer (Tris-HCl (50 mmol/L, pH 6.8), SDS (1% w/v), glycerol (10% (v/v) with bromophenol blue) prior to separation on a 12% SDS polyacrylamide gel. The running buffer was Tris base (0.3% w/v), glycine (1.44% w/v) and SDS (0.1% w/v). Gels were electroblotted to nitrocellulose filters, which were then placed in chambers containing TS buffer (Tris HCl (10 mmol/L, pH 7.4), sodium chloride (0.15 mol/L)) with sodium azide (0.5 mg/mL) and Nonidet P40 (3% v/v) and incubated for 30 minutes. The filters were then rinsed with TS buffer and incubated with TS buffer containing BSA (1% w/v) for 2 h. This was followed by incubation for 2 periods of 20 minutes with TS buffer containing Tween 20 (0.1% v/v), then an overnight incubation with TS buffer containing BSA (1% w/v), Tween 20 (0.1% v/v) and [^{125}I]-IGF-1. The filter was then washed in TS buffer containing Tween 20 and then in TS buffer. All incubations and washes were carried out at 4°C. The filter was then autoradiographed.

III RESULTS

(a) DNA synthesis

Treatment with IGF-1, IGF-2, EGF, TGFa, FGF-1 and FGF-2 in doses ranging from 0.01 ng/mL to 100 ng/mL stimulated uptake of [^{3}H]-thymidine in a dose-dependent manner by both granulosa and thecal cell cultures (Figure 1). The IGF-1 was a more potent stimulator than IGF-2 in both cells. The dose-response curves for FGF-1 and FGF-2 treatment indicated that at doses lower than 10 ng/mL FGF-2 was the more effective stimulator and at doses greater than 10 ng/mL FGF-1 was more effective for both cell types. The maximum stimulation by both FGFs was greater in thecal cells than granulosa cells. The TGFa was the most potent stimulator and EGF the least potent stimulator of [^{3}H]-thymidine uptake in granulosa cells. The maximum stimulation by these peptides was similar in thecal cells; however, at doses lower than 10 ng/mL TGFa was considerably more effective than EGF. All the cultures responded to the addition of FCS (positive control) with an increased [^{3}H]-thymidine incorporation compared with untreated cells.

(b) Binding protein analysis

Granulosa and thecal cells cultured in flasks were used to condition culture medium for 24 h in the presence or absence of IGF-1 (25ng/mL). Analysis of granulosa cell-conditioned medium showed that there were five proteins with IGF-1 binding capacity; these had molecular weights of 32.5, 31.5, 30.5, 29.5 and 24.5 kDa. Thecal cell-conditioned medium contained four of these proteins (32.5, 31.5, 29.5, and 24.5 kDa). The abundance of the proteins in untreated thecal cell-conditioned medium was greater than that in untreated granulosa cell-conditioned medium.

(c) IGF-1 and IGFBP interactions

Treatment with IGF-1 caused increased production of binding proteins in granulosa cell-conditioned medium. However, IGF-1 treatment did not alter the level of production in thecal cell-conditioned medium.

Thymidine incorporation was measured in granulosa and thecal cell cultures when treated with IGF-1 or the analog, Long-[Arg^3]-IGF-1 in doses ranging from 0.01 to 20 ng/mL (Figure 2). Cultures were treated at three separate times: T1, immediately following medium change; T2, 24 h following medium change; T3, 48 h following medium change. There were no significant differences between the responses of granulosa cells to IGF-1 and the analog at T1 or T2. There was a significant difference in cells treated at T3 with a dose of 0.5 ng/mL. The responses of thecal cells treated with IGF-1 were significantly different from those treated with the analog at most doses and at all three times. The shape of the dose-response curves suggest that IGFBPs are inhibitory for IGF-1 mitogenic actions only when the concentration of this growth factor is less than 10 ng/mL.

IV DISCUSSION

Insulin-like growth factor -1 has been clearly established as influencing the growth of both thecal and granulosa cells (Roberts *et al.*, 1994). Here it is shown that IGF-2 has a similar role. The difference in mitogenic activity of these two peptides may be due to the differences in affinity they both show for the type-1 IGF receptor.

The mitogenic effects of EGF and the presence of its receptors on both cell types has been shown previously (Onagbesan *et al.*, 1992, 1994; Peddie *et al.*, 1994) We confirmed the mitogenic role of EGF; however, it was the least mitogenic of the growth factors included in the study. The marked difference between the effects of TGFa and EGF on both thecal cells and granulosa cells may be due to the greater affinity which TGFa has for the chicken EGF receptor (Lax *et al.*, 1988). Thus, TGFα may be more important for growth in the chicken ovary than EGF, the existence of which in chickens has yet to be confirmed.

Fibroblast growth factor has been shown to have differentiative effects on chicken granulosa cells (Tilly and Johnson, 1990). The mitogenic effects of two members of this family of growth factors are shown here. The large differences in the potency of FGF-1 and FGF-2 on DNA synthesis in both granulosa and thecal cells may indicate separate roles for these factors in follicular growth.

Western blot analysis of granulosa- and thecal-cell conditioned medium showed that cells which develop adjacently differ in their IGFBP secretion profiles. Granulosa cells produced lower quantities of IGFBPs than thecal cells, but production to the level of thecal cells was inducible by treatment of granulosa cells with IGF-1.

There was no significant difference in the effects of IGF-1 or the analog on granulosa DNA synthesis until endogenous IGFBP had been secreted for 48 h. This contrasted with the situation in thecal cells in which the peptides had different dose-response curves at each stage of the experiment, suggesting that IGFBPs in thecal-conditioned medium have altered the capacity of exogenous wild type IGF-1 to bind to thecal cell IGF receptors. The comparative lack of IGFBP effects in the granulosa system may be explained by the much lower level of unstimulated production of these proteins by these cells.

This study provides evidence that IGFBP synthesis by granulosa cells is regulated by IGF-1 and that the growth-stimulating actions of IGF-1 on thecal and granulosa cells are attenuated by endogenous IGFBPs. Thus IGF-1 and IGFBPs appear to form a paracrine / autocrine growth regulating mechanism within the pre-ovulatory follicle.The precise role of

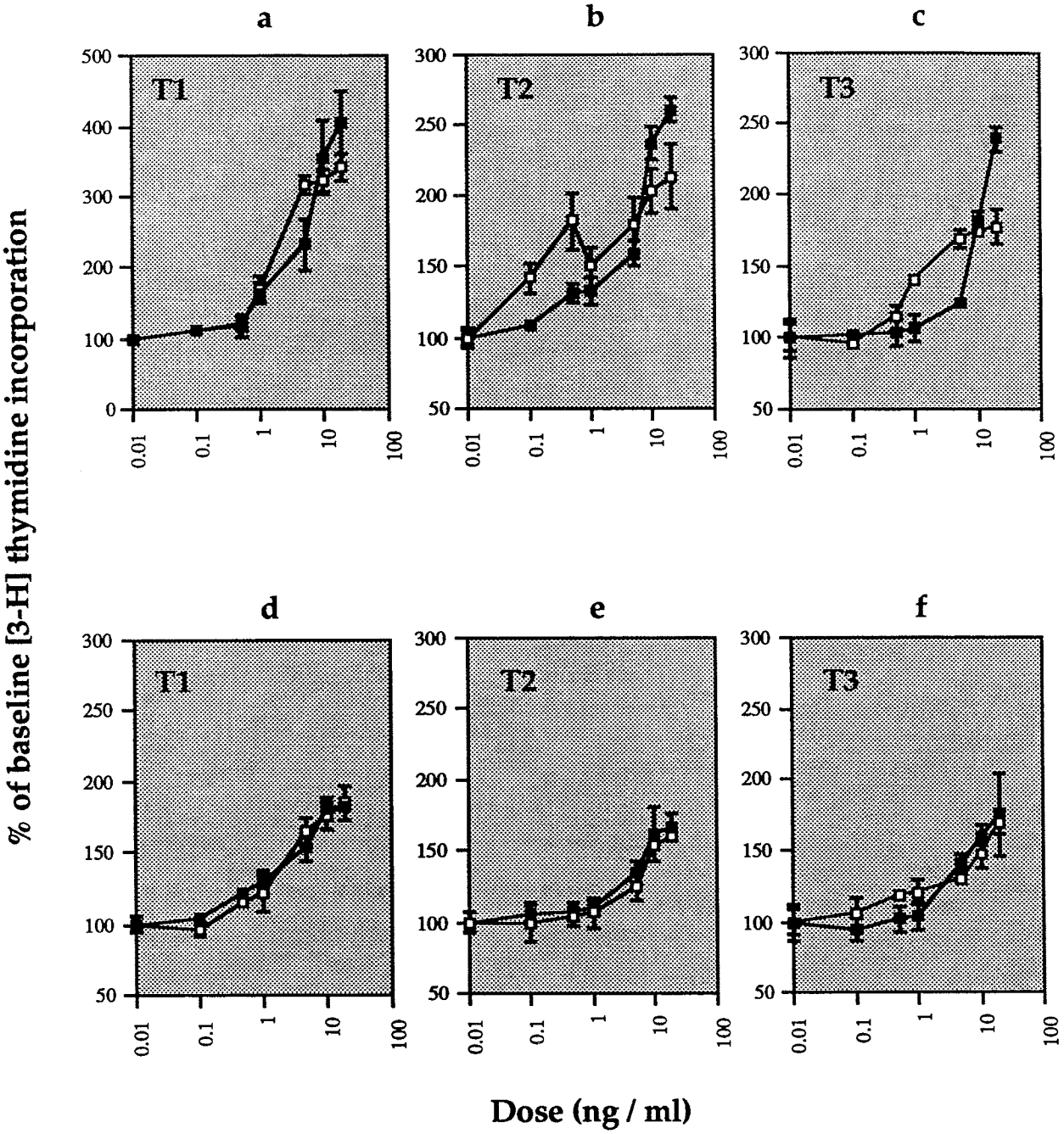

Figure 2. The effects of IGF-1 (■) or long [Arg^3] IGF-1 (□) on thecal cell (a, b, c) and granulosa cell (d, e, f) DNA synthesis *in vitro*, measured by incorporation of [3-H] thymidine as a percentage of the unstimulated control. Cells were treated at 0 hr (T1), 24 h (T2) or 48 h (T3) after replacing the culture medium. Mean values are shown, bars represent the standard error.

each different IGFBP in this system is, at present, unknown. Functional studies with purified IGFBPs may elucidate their roles in the regulation of follicular growth.

The growth of chicken ovarian cells appears to involve a wide range of factors in addition to the IGFs. Further study will be required to elucidate their relative importance, which may change as the cells age. The model used here is static relative to the development of the follicle. Therefore, a developmental model needs to be considered in order to determine more precisely the role of growth factors in the hierarchical development of follicles in the chicken ovary. Future studies will compare growth factor-mediated mechanisms in normal and growth-disrupted ovaries to determine the causes of inefficient ovarian function in broiler females.

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EGG SHELL QUALITY: A REVIEW OF METHODS OF ASSESSMENT

J.R. ROBERTS

Summary

Egg shell quality is of vital importance to the poultry industry. What constitutes good shell quality for a table egg may be different from the requirements of the eggs of breeders. However, most studies of egg shell quality have focused on the table egg. Egg shell breaking strength is the only direct measure of egg shell strength although it is not widely used because of time and cost considerations. Instead, specific gravity, which assesses the relative amount of shell present, is most commonly employed in the field situation. Other measurements of the amount of shell present include shell weight to egg weight ratios and shell thickness. Assessment of the ultrastructure of egg shells takes into account the quality of construction of the shells. The use of a range of methods provides the most reliable assessment although, commercially, it is necessary to utilise a cost-effective method.

I. INTRODUCTION

This paper will provide an overview of what is meant by egg shell quality, how it can be measured and the advantages and disadvantages of the various methods employed to assess egg shell quality.

The egg shell of the domestic hen has two important roles from a commercial perspective. In the case of the laying hen, the egg shell provides a package or container for the egg shell contents. Therefore, the shell must be strong enough to withstand the processes of being laid by the hen, collected, sorted, transported to the market place and handled by the consumer. If an egg shell is faulty it may crack or break during this series of processes. Another potential problem is that of bacterial contamination and such contamination may occur via egg shell defects that are invisible to the naked eye.

The fundamental biological function of an avian egg shell is to provide an incubation chamber in which a new individual can develop. The requirements of an egg shell, in this capacity, are somewhat different from those of a table egg. The egg shell of a developing chick must not only provide protection but it must allow for adequate movement of water vapour and respiratory gases (via the pores in the shell). In addition, the chick must be able to release itself from the egg shell at the end of the incubation period.

The "quality" of egg shells has been assessed mainly in terms of the table egg. Relatively few studies have investigated the relationship between shell quality and hatchability. Gross egg shell defects and abnormalities are detected during the process of "candling" of the eggs. Most shell quality measures have focused on the amount of egg shell present. These measures include egg specific gravity, shell thickness, and shell weight to egg weight ratio. However, the egg shell is like any mechanical structure in that its strength depends not only on its weight and thickness but also on the quality of its construction. The quality of construction of an egg shell is reflected in its ultrastructure, particularly of the mammillary layer which provides the "foundation" for the egg shell as a whole. Egg shell quality may be assessed in terms of the strength of the shell and

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measurements such as shell breaking strength may be employed. Hunton (1990) provides an overview of available methods.

II. EGG SHELL STRENGTH

The strength of egg shells has been assessed in a number of ways: by measuring the "snapping strength" of pieces of egg shell (Tyler and Thomas, 1967); by the extent of non-destructive shell deformation in response to a given force (Schoorl and Boersma, 1962) and by measuring shell breaking strength (Hunt and Voisey, 1966; Richards and Staley, 1967; Voisey and Hunt, 1967). The strength of the attachment between the shell and shell membranes has also been used as an indicator of shell strength (Orberg, 1990). Non-destructive deformation is essentially a measure of the elasticity of the shell whereas shell breaking strength is related more to the "stiffness" characteristics of the shell. Bain (1990) described egg shell failure as occurring in two stages: initial failure at the point of contact followed by propagation of these cracks. She introduced the term "fracture toughness" to describe the resistance of a shell to unstable crack growth and found that there was a link between this characteristic and the ultrastructure of the shell (see section IV below).

Egg shell breaking strength provides the only truly direct measure of egg shell strength. Breaking strength has been measured in a variety of ways from dropping ball bearings or similar objects onto eggs, to compression of eggs under controlled conditions. This latter method is known as "quasi-static compression" and is generally regarded as the best way of measuring shell breaking strength. The commercially-available Canadian Egg Shell Tester operates on this principle and a similar shell breaking strength machine has now been built in the author's laboratory.

III. THE AMOUNT OF SHELL PRESENT

Generally speaking, the strength of an egg shell is related to the amount of shell present, relative to the egg size. The shape of the egg is also important (Shape index = width x 100/length). The specific gravity (SG) of an egg gives a good indication of how much shell is present. Specific gravity may be measured either by the flotation method where eggs are compared with saline solutions of known S.G. or by Archimedes' method of weighing the egg in both air and water (Pym, 1969; Hempe *et al.*, 1988). Experimentally, eggs may be weighed, emptied of their contents and dried to allow determination of shell weight as a percentage of egg weight. In addition, shell thickness can be measured by use of a suitable micrometer or dial comparator gauge (Fagan *et al.*, 1988). Shell surface area and shell density can be calculated (Curtis *et al.*, 1985).

IV. THE QUALITY OF CONSTRUCTION

As mentioned earlier, the strength of any structure depends not only on its size and thickness, but also on the quality of its construction. Solomon and coworkers at the University of Glasgow reasoned that the mammillary layer of the egg shell is the foundation of the shell and that any weakness or abnormality in this layer will affect the performance of the shell as a whole (Solomon, 1991). A method was devised for removing the shell membranes without damaging the shell itself - the process of plasma ashing or etching (Reid, 1983). The Glasgow Poultry Research Group, over a period of approximately 10 years, described a number of abnormalities of the mammillary layer:

early fusion, late fusion, type A mammillae, type B mammillae, confluent mammillae, cuffing, alignment of mammillae, aragonite, cubics, pitting (depressions, erosion, holes) and changed membrane, and these are summarised by Solomon (1991).

Members of the Glasgow group recorded the incidence of these features in abnormal eggs, and observed the ultrastructure of egg shells at crack sites, following quasi-static compression. They concluded that some abnormalities of the mammillary layer are beneficial to shell strength (early fusion, cuffing, confluent mammillae, a low mammillary density) whereas others were associated with decreased shell strength (late fusion, type B mammillae, aragonite, pitting, alignment of mammillae, a high mammillary density) (Bain, 1992). Similarly, some ultrastructural features were associated with a low resistance to bacterial penetration (late fusion, type A and B mammillae, aragonite, pitting, alignment of mammillae, cubics, changed membrane, a low mammillary density), whereas some ultrastructural features increased the resistance of the shell to bacterial penetration (early fusion, good cap formation, cuffing, confluent mammillae, a high mammillary density) (Nascimento, 1992).

A collaborative project involving Dr. Solomon, the author, Ms. C. Brackpool and other Australian researchers (Associate Professor D. Balnave, Mr. R. Hughes, Dr. B. Sheldon, Associate Professor W. Bryden, Dr. P. Glatz, Dr. J. Barnett, Mr. R. Bishop) has resulted in egg shell ultrastructure assessments being conducted at the University of New England.

V. COMPARISON OF METHODS

Egg shell breakage results in the loss of up to 20% of all eggs laid (Hamilton, 1982; Balnave, 1988). Shell breakage is related to shell strength which is, in turn, related to a range of other indicators. Measurement of shell breaking strength (or impact, puncture or crushing strength) gives a direct indicator of shell quality but results in the destruction of the egg. It also requires specialized equipment. Therefore, indirect measurements are frequently used. These include specific gravity, shell weight, shell weight to egg weight ratios and shell thickness. A number of researchers have attempted to compare and correlate egg shell quality, as assessed by the different methods discussed. Most studies have shown that all the various measures are correlated to varying extents (Frank *et al.*, 1965; Richards and Staley, 1967; Abdallah *et al.*, 1993). Correlations between egg shell strength and egg shell ultrastructure have been observed by a number of workers (Simons, 1971; King and Robinson, 1972; Bunk and Balloun, 1978; Solomon, 1991).

Studies at the University of New England have highlighted the need to use a range of measures in order to fully assess egg shell quality. For example, Brackpool *et al.*, (1993) found that thinner egg shells laid by hens during heat stress were ultrastructurally superior in certain respects. The study reported at this symposium by Thomas and Roberts (1995) found that these thinner, but better constructed, egg shells had shell breaking strengths which were as high as those of eggs laid at moderate environmental temperatures.

VI. CONCLUSIONS

Studies of egg shell quality have focused mainly on the table egg and the need to ensure that the egg shells are strongly constructed. Egg shell breaking strength is the only direct measure of egg shell strength although it is not widely used commercially because it is time-consuming and requires relatively costly equipment. Instead, specific gravity,

which assesses the relative amount of shell present, is most commonly employed in the field situation. Other measurements of the amount of shell present, such as shell weight to egg weight ratios and shell thickness, have been used in experimental trials. An assessment of the ultrastructure of the egg shell allows the quality of construction of the shell to be taken into account. In an experimental situation, it is desirable to use a range of methods for assessing egg shell quality. However, in the commercial situation, time and cost considerations dictate the method of choice and a non-destructive method such as specific gravity tends to be used.

Studies conducted at the University of New England have highlighted the importance of assessing both the quantity of the egg shell present and the quality of its construction in order to fully appreciate egg shell quality.

VII. ACKNOWLEDGMENTS

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ECONOMICS OF MULTIPLE CYCLE MANAGEMENT STRATEGIES FOR TWO STRAINS OF LAYER

D. ROBINSON, P.C. TRAPPETT and K.M. BARRAM

Faced with higher operating costs and lower returns many poultry farmers have adopted the practice of putting their laying flocks through two or more production phases separated by "pauses", with the aim of reducing hen depreciation and exploiting the demand for higher priced grades of eggs. Initial investigations (Robinson *et al.*, 1992) indicated that multiple cycle strategies may have economic advantages even when hen replacement costs and prices received for large eggs are relatively low. In a further trial spanning a total production period of 102 weeks, hens of two hybrid strains (tinted egg, A, and brown egg, B) were subjected to fifteen laying cycle programmes which differed in respect to the age (40 or 60 weeks) at which the first pause (if any) was initiated, the number (1-5) and length (15-30 weeks) of the post-pause laying phases and the length of the pause inducement periods (4-12 days). Pauses were induced by replacing the normal diet by unground oats. For each strain the economic effects of the husbandry regimens were compared by computing annual margins (AM: egg and carcase revenue minus feed and pullet costs per hen housed, converted to an annual basis), using a range of price structures and a variable termination age at which it was assumed the hens would be replaced by 18-week-old pullets. A price modelling technique was employed to enable both mean egg weight data and grading data to be utilised. Median egg prices ranged through L, M and H levels, resulting in AMs in the region of $0, $5 and $10 respectively, and egg grade price differentials were zero (constant price/kg), negative (N: constant price/dozen), or positive (P: biased in a reciprocal sense to N).

Physical and economic performance differences between strains were generally greater than differences between strategies. While egg number, egg weight, feed intake and mortality under the single cycle regimen favoured strain A, the response of strain B to pausing was more pronounced than that of A. Multiple cycle strategies employing cycles of 20-30 weeks' duration usually resulted in higher egg numbers and achievable profit margins than the single cycle regimen or cycles of 15-16 weeks. With the longer cycles, longer (5-6% of cycle length) pause inducement periods tended to be superior to shorter periods.

With no constraint on replacement age the single cycle regimen was never the optimum strategy for either strain under any price structure, but became the preferred option for A when replacement age was set at <80 weeks and egg price at M or H. With L or M egg price, multiple cycle regimens terminating at 100-120 weeks of age yielded the highest AMs. With L egg price, replacement age had a greater effect on AM than regimen, while with H egg price the reverse was true. As egg price decreased from H to L optimum replacement age tended to increase from approximately 80 weeks to $>=120$ weeks. At M and H prices, N grade differential greatly reduced the differences between AMs of the different strategies, while the main effect of P was to increase AMs of all strategies. The optimum strategy overall (average AM $0.80 higher than peak AM of the single cycle regimen) employed pauses at 40, 67 and 94 weeks of age, with hen replacement at 120 weeks. However, AM was most consistently high with replacement at 100-105 weeks of age following three or four cycles (including the first).

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OYSTER SHELL VERSUS LIMESTONE AS SOURCES OF CALCIUM FOR LAYERS

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Laying hens usually have about 20 g calcium (Ca) in bone stores but deposit about 10% of this amount of Ca in every egg laid. Thus, they require a ready supply of dietary Ca. This Ca may be supplied as finely ground Ca in a pellet, crumble or loose mix, or as particulate Ca. In this study, a comparison was made of oyster shell and a much less expensive limestone, mined at Attunga, NSW, as sources of dietary Ca in a standard layer diet.

Forty-eight SIRO CB laying hens (32 weeks of age) housed two per cage in an open shed at the University of New England's poultry farm "Laureldale" were assigned to one of three treatments for each Ca source (4 cages per treatment). For Treatments 1-3, limestone was finely ground and mixed into a Ca-deficient diet to produce three diets containing 37, 43 and 50 g Ca/kg, respectively. Treatments 4-6 were similar to Treatments 1-3 except that oyster shell was substituted for limestone. Feed intake, egg production and egg characteristics were recorded for 12 weeks.

There were no mortalities over the 12-week period and production was excellent (>93%, hen housed basis) for both Ca sources, at all three levels of dietary Ca inclusion. Food conversion ratio did not differ between treatments. There were, however, statistical differences in egg characteristics between the Ca sources that slightly favoured the oyster shell.

Effect of calcium source and level of dietary inclusion on egg production and egg characteristics.

	Egg weight (g)	Egg mass production (g/b/d)	Egg specific gravity	Shell density (g/cm^3)	% Shell	Shell thickness (μm)
SOURCE						
Limestone	56.3	52.3	1.084	209	9.22	363
Oyster Shell	59.2	55.4	1.085	209	9.21	369
Significance	***	NS	NS	NS	NS	*
LEVEL						
37 g/kg	59.1	55.3	1.083	208	9.03	362
43 g/kg	57.5	55.4	1.083	209	9.18	363
50 g/kg	56.7	52.8	1.086	209	9.42	372
Significance	***	NS	***	NS	***	***

* $P<0.05$, *** $P<0.001$.

Higher levels of Ca in the diet were associated with lower egg weight, higher specific gravity, higher weight of shell relative to total egg weight, and thicker shells. However, shell density did not differ ($P>0.05$) between treatments.

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BIOLOGICAL EFFECTS OF LIPID PEROXIDES AND THEIR BY-PRODUCTS IN FEED

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Summary

This study was designed to provide information about the mechanism responsible for poor bird performance resulting from the addition of rancid feed ingredients in the diet. In the present study, gain, feed conversion and hematocrit were negatively affected by the feeding of oxidized fat. Changes in gastrointestinal (GI) structure, cell proliferation, and transient effects on the intestinal microflora were observed. These changes can be associated with alterations in GI function, including nutrient uptake, maintenance requirements and resistance to opportunistic pathogens. Capacity for energy uptake measured *in vitro* was found to increase, while that of other nutrients, such as methionine, was not affected. There also was evidence that the efficacy of the gut-associated immune system is impaired. Hepatic cell proliferation increased, possibly due to liver cell damage by secondary products of fat oxidation. These indicators of oxidative stress may be factors leading to reduced body weight gain and feed efficiency seen in the animals fed unstabilized diets containing oxidized ingredients.

I. INTRODUCTION

Antioxidants are used in feed ingredients and complete feeds to prevent oxidative losses of fat-soluble vitamins, pigments, and to prevent loss of metabolizable energy (ME) value in the fat component of the feed. Furthermore, oxidized fat contains a variety of poorly characterized by-products including lipid hydroperoxides and secondary autoxidation products. Although the toxicity of reactive oxygen and peroxides can readily be demonstrated, these are generally not fed to the animal directly. The biological costs associated with feeding unstabilized feeds are inadequately understood and poorly documented, particularly those associated with marginal degrees of oxidation.

Poor performance using diets containing oxidized fat and other unstabilized feed ingredients can be due to a variety of associated effects. Vitamins and polyunsaturated fatty acids deteriorate in the absence of antioxidants, leading to deficiencies. Lipoperoxides and other secondary products of autoxidation can exert deleterious effects directly on cells. Incorporation of oxidized fat into cell membranes can result in changes in membrane permeability, nutrient uptake, secretory activity and membrane-bound enzyme activity. The

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ramifications of these primary effects could lead to secondary systemic effects such as poor gain, reduced feed conversion, impaired immune function and other performance problems.

Poor performance as measured by decreases in gain and feed efficiency associated with feeding oxidized fat to animals have been reported in rats (Raced *et al.,* 1963) and broilers (Cabel *et al.,* 1988). It has been demonstrated on numerous occasions that animals require certain polyunsaturated fatty acids and that deficiencies can be associated with weight loss, fatty liver, kidney malfunction and poor reproduction (Balnave, 1970,1971; Nakmura *et al.,* 1973). Also, it has been demonstrated that dietary oxidized fats can become incorporated into liver cell membranes (Ashida *et al.,* 1988) and adipose tissue (Reddy and Tappal, 1974), and that feeding oxidized fat reduces the amount of tocopherol present in subcellular membranes (Asghar *et al.,* 1989). Severe vitamin E deficiency in chicks is associated with well known syndromes such as exudative diathesis and encephalomalacia. Several *in vitro* methods were used to determine if similar effects of feeding oxidized fats could be observed in the GI system and how such changes are reflected in performance.

II. MATERIALS AND METHODS

In these studies, systemic effects of feeding standard starter diets formulated to Maryland nutrition standards were measured with weight gain, feed conversion and hematocrits. Fat was added at 3.8% of the diet. Hubbard x Hubbard cockerels (8 birds/pen, 3 replicate pens/treatment) were used in the study. Treatments included control fat without and with ethoxyquin (Treatments 1 and 2) and oxidized fat without and with ethoxyquin (Treatments 3 and 4). Associated functional changes occurring in the GI system were measured using a variety of *in vitro* assays.

III. RESULTS AND DISCUSSION

(a) Bird Performance

Differences in cumulative feed conversion were detected at 14 ($P=0.05$) and 21 days ($P=0.07$) (Figure 1). For both time points, birds fed the control (non-oxidized) diet containing the antioxidant gave the best feed conversion. Although not significant, this trend could be detected after only seven days. Figure 2 shows the final body weight of the birds that remained on study at 21 days. The body weights of birds fed a diet containing the unstabilized, oxidized fat (4.2 meq peroxide/kg diet) were significantly lower than the body weights of birds fed the diet containing non-oxidized fat plus ethoxyquin ($P=0.01$). These results suggest that sufficient oxidation occurs in unstabilized feed, even with good quality fat, within the lifetime of normal feed to be detectable in reduced performance.

The same pattern was seen with hematocrits (Figure 2). Birds fed oxidized fat in the absence of ethoxyquin had the lowest hematocrit, with the highest seen in birds fed non-

oxidized fat in a diet stabilized with ethoxyquin. The incorporation of oxidized fat into the erythrocyte cell membrane caused changes in membrane viscosity and permeability, ultimately leading to a reduction in red cell lifespan (Girotti *et al.*, 1987; Claster *et al.*, 1989).

(b) Nutrient Uptake Effects

To examine functional aspects of the GI system, *in vitro* nutrient uptake experiments were performed using mid-small intestine from birds fed the test diets. Nutrient uptake is dependent upon the properties and integrity of the apical membrane of intestinal absorptive cells. Data have been published indicating that oxidized fats can become incorporated into cellular and subcellular membranes (Ashida *et al.*, 1988), and this phenomenon has been associated with changes in membrane permeability as measured by the escape of hemoglobin from erythrocytes (Girottii *et al.*, 1987). A non-metabolizable glucose analog, O-methyl-glucose, and an amino acid, L-methionine where used to study nutrient transport.

Figure 3 shows the effect of dietary oxidized fat and ethoxyquin on the *in-vitro* uptake capacity for O-methyl-glucose by everted slices of intestine (Dibner *et al.*, 1992). There are three pathways for glucose uptake; energy dependent uptake, energy independent uptake, and diffusion. These results demonstrate that birds fed a diet containing oxidized fat without ethoxyquin developed an increased capacity for energy independent, carrier specific glucose uptake ($P=0.05$). In addition, there was an increase in the capacity of the energy dependent uptake, significant at the $P=0.10$ level. The overall result of these changes was that total *in vitro* uptake was increased under conditions of O-methyl-glucose saturation. The uptake of other nutrients, represented by L-methionine, was not affected by diet. These results suggest that the increase in glucose transport due to oxidative stress represents a need for energy *per se* rather than a general breakdown in the transport of essential nutrients due to changes in membrane fatty acid composition in the presence of oxidized fat.

The increased glucose uptake may be due to the lower ME of oxidized fat or may indicate that the bird requires more energy under conditions of oxidative stress. Uptake of secondary oxidation products by the liver is associated with a reduction in metabolic energy resulting from a decrease in the activity of the citric acid cycle (Ashida *et al.*, 1987a) and a depletion of NADPH and other high energy metabolites (Ashida *et al.*, 1987b). Thus, the glucose uptake increase reported here may be a reaction to changes in hepatic carbohydrate metabolism. In addition, uptake results are similar to the increase in uptake capacity for glucose which was observed during heat stress (Dibner *et al.*, 1992). Higher circulating blood glucose during heat stress has been reported and may represent a general response to stress (Donkoh, 1989).

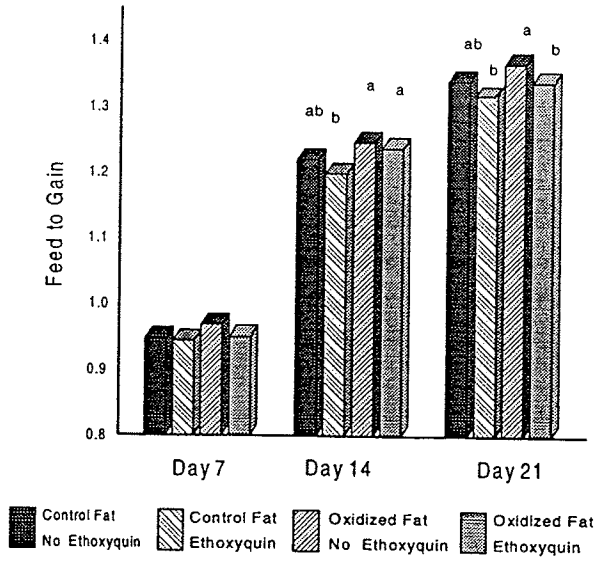

Fig. 1. Effect of Oxidized Fat and Ethoxyquin on Feed Conversion in Broilers.

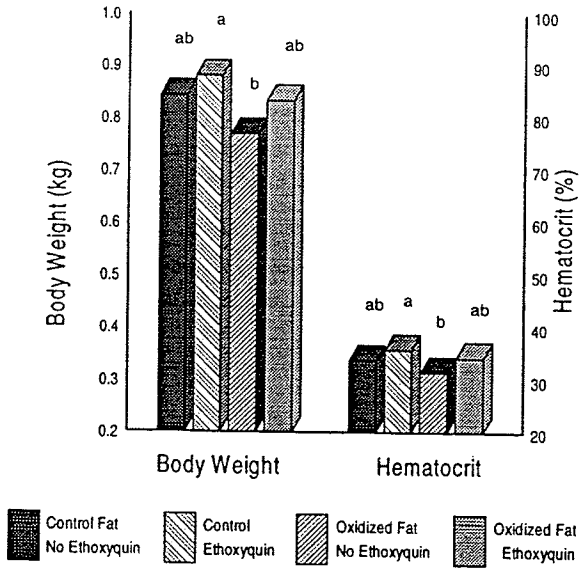

Fig. 2. Effect of Oxidized Fat and Ethoxyquin on 21-Day body Weight and Hematocrit.

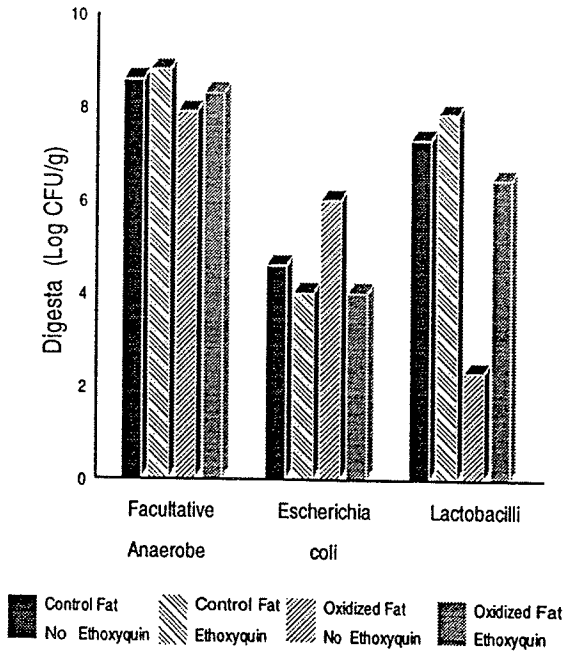

Fig. 3. Effect of Oxidized Fat and Ethoxyquin on Microflora of the Small Intestine at 11 Days.

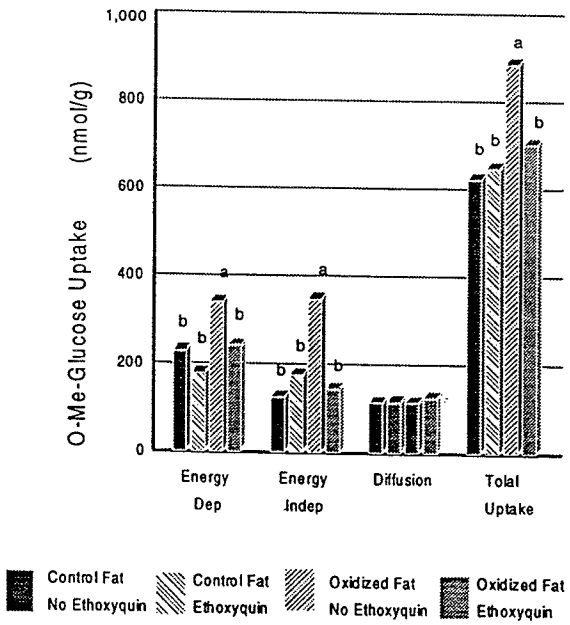

Fig. 4. Effect of Oxidized Fat and Ethoxyquin on Uptake of O-Methyl Glucose (20mM) by Intestine.

(c) Intestinal Microflora

When intestinal microflora were examined, a transient decrease in *Lactobacilli sp.* and a transient increase in *E. coli* were observed at day 11 in the animals fed the diet containing oxidized fat without ethoxyquin (Figure 4). A reduction in *Lactobacilli sp.* was also observed in the ceca of birds on this diet. By 18 days, however, the intestinal microflora of birds on the four diets did not differ. *Lactobacilli sp.* do not have a superoxide dismutase system to respond to oxidative stress, as do E. coli and most other bacteria (Fridovich, 1988). *Lactobacilli sp.* show oxygen tolerance, however, in that their multiplication is only temporarily inhibited by peroxides and they do recover (Fridovich, 1988) from contact exposure to peroxides. Clearly, however, a reduction in *Lactobacilli sp.*, especially accompanied by an increase in *E. coli*, may increase the chances for an opportunistic infection by *E. coli* or other pathogens.

(d) Cell Proliferation

When tissue sections from the gut and other target organs were stained with bromodeoxyuridine for cell proliferation differences in staining index were observed. Low levels of hepatocyte proliferation were seen in the control animals. In contrast, a marked increase of proliferation was observed when birds were fed oxidized fat. This was independent of the presence of antioxidant. Increased proliferative activity in the liver may reflect increased cell death due to secondary products of autoxidation such as aldehydes and ketones, (Fridovich, 1988) in the feed. This requires the bird to replace nonfunctional or nonviable liver cells. Any increase in cell proliferation which is not reflected in growth will increase maintenance requirements and ultimately reduce feed efficiency. An antioxidant does not protect the animal against oxidative stress due to secondary products of autoxidation because these molecules are not free radicals but are toxins formed from the decomposition of peroxides present in the oxidized fat sample. The only way to ensure against the presence of these secondary products is to add an antioxidant to the fat before it has a chance to oxidize, i.e. before the fat is rendered.

(e) Gut Associated Immune Function

Primary and secondary immune tissues are also targets for oxidant stress (Fritsche *et al.*, 1990, 1991). There is some indication that reduced disease resistance may be one of the effects of feeding oxidized fats. Further, there is strong evidence that dietary fat source affects immune function. For example, the dietary n-3 fatty acids found in some fish oils can raise the antibody response to cellular antigens (Fritsche *et al.*, 1990, 1991).

Immune protection against the microorgansims that normally live in the gut or that are carried into the gut on feed, is accomplished in part by the secretion of the

immunoglobulin IgA into the tissues and lumen of the lower gut. Plasma cells synthesize and secrete IgA which then diffuses throughout mucosal connective tissue below the gut epithelium. The IgA accumulates just below the apical membrane of the epithelial cells, particularly those in the glands deep within the mucosa and becomes incorporated in the apical membrane of the epithelial cells. When sections of large intestine from birds fed oxidized fat were stained for IgA, a change in the distribution of free IgA was observed. The plasma cells appeared to be synthesizing IgA, but it did not appear in the tissue. Therefore, the effect of oxidized fat is to either reduce secretion of IgA or to reduce the stability of the IgA in the epithelial cell membrane. The results of these changes are a reduction in the amount of IgA available to protect the animal against toxins produced by gut microorganisms and an increase in the possibility of opportunistic infection.

IV. CONCLUSIONS

This research was designed to gain a better understanding of the relationship of the GI system to the poor performance seen in animals fed unstabilized diets containing oxidized ingredients. The following effects were observed:

1. Feeding oxidized fat produced a decrease in gain, feed efficiency and hematocrits.
2. Feed mixing generates ideal conditions for fat oxidation, including increased surface area of the fat, exposure to pro-oxidant salts (iron and copper) and heat. The poorer cumulative feed conversion and lower body weights and hematocrits of birds fed the unstabilized diet containing control fat compared to birds fed the stabilized diet containing control fat observed in this study suggest that oxidation products can be generated during the normal lifetime of poultry feed. Addition of an antioxidant is advisable even when good quality fat is added to the feed.
3. Intestinal microflora were affected, with *Lactobacilli sp.* decreased and *E. coli* increased for a short period of time.
4. Changes were observed in nutrient uptake which may reflect an energy deficit associated with oxidative stress.
5. Increased cell proliferation in the gut and in the liver was observed which may be related to the cytotoxic effects of lipoperoxides, and likely decreases the efficiency of feed utilization.
6. There appear to be changes in the large intestine which result in reduced levels of IgA and a less effective function of the gut associated lymphoid tissue.

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GROWTH PERFORMANCE AND DEVELOPMENT OF DIGESTIVE TRACT AND ENZYMES OF FEED RESTRICTED CHICKENS

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Restriction of feed to 75% or 50% of *ad libitum* intake between 5 and 11 d of age results in subsequent compensatory growth and an increase in the relative weight of the digestive tract (Susbilla *et al.*, 1994). The effect of feed restriction on digestive tract development and activity of enzymes involved in protein digestion were studied.

Two feeding trials, one in floor pens and one in individual metabolism cages, were conducted with broilers fed commercial starter feed to 25 d and finisher feed thereafter. *Ad libitum* feeding (Control) and feed restriction of 60% (FR60) between 5 and 11 d of age were used. In the pen study, there were 4 pens of 10 birds per treatment and 2 birds per pen were killed on d 41 after an overnight fast. In the cage study, there were 24 birds per treatment and birds were killed on days 5, 12, 19 and 26 for enzyme measurements (Tarvid, 1992).

At 12 d of age the FR60 birds in the floor pens had a significantly ($P<0.01$) lower bodyweight (BW) (212 vs 362 g). On day 40, no significant differences were observed between the Controls and the FR60 birds in BW (2268 vs 2165 g), feed conversion (1.79 vs 1.72), dressed weight (73.9 vs 73.7 %BW) or in abdominal fat (8.7 vs 9.5 g/kg BW). The results for birds in cages are shown below:

Age	Day 12		Day 19		Day 26	
Treatments (n)	Control (6)	FR60 (6)	Control (5)	FR60 (5)	Control (6)	FR60 (6)
Body Wt (BW), g	273±6	168±5††	664±9	459±35††	1063±39	901±17††
Pancreas, g/kg BW	4.0±0.2	4.0±0.2	2.7±0.1	3.4±0.1††	2.5±0.1	2.7±0.1
SI, g/kg BW	30.2±1.4	35.3±2.6	22.4±1.3	22.3±1.7	18.5±0.8	18.1±0.7
Pancreatic enzymes						
GPA[a]	37.0± 8.1	3.2±1.0††	50.6±11.0	28.6±3.5	78.3±8.6	39.1±4.2††
CPA[b]	44.8±1.8	11.7±1.6††	60.2±5.6	48.0±2.7	53.3±1.6	53.5±3.6
Intestinal enzymes						
Aminopeptidase[b]	0.25±0.01	0.35±0.02††	0.19±0.02	0.25±0.01†	0.18±0.01	0.20±0.01
Dipeptidase[c]	7.67±0.49	11.87±1.20†	6.28±0.48	7.88±0.63	5.69±0.53	4.92±0.35

[a] GPA (General Proteolytic Activity) expressed in μM tyrosine/g tissue per min.

[b] CPA (Carboxypeptidase A) and aminopeptidase expressed in μM leucine/g tissue per min.

[c] Dipeptidase expressed in μM glycyl leucine/g tissue per min.

†P<0.05; ††P<0.01 compared with the Control group at the same age.

The relative growth of the pancreas and the small intestine (jejunum and ileum) during the period of feed restriction was maintained. At the end of the restriction period, the activity of the pancreatic enzymes was decreased and the activity of the intestinal enzymes was increased. The greater activity of the intestinal enzymes may have compensated for the lower pancreatic enzyme activity so that compensatory growth could occur.

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SOME CONSEQUENCES OF THE INTERMITTENT FEEDING OF PARTICULATE CALCIUM

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The determination of the calcium requirements of laying hens and methods of providing adequate calcium appear to be based around the perceived need to have calcium available daily. It appears that no work has been directed towards the responses of laying hens to the supply of a calcium source on an intermittent basis.

Commercial layers (SIRO CB), introduced to choice-feeding from 8 weeks of age, were caged in pairs in an uninsulated, slatted shed. Whole wheat (110g crude protein/kg) and a protein concentrate (10.72 MJ AME, 415 g crude protein and 40 g calcium/kg) in mash form were mixed in a feed trough across half the cage front. An identical trough contained 200 g of a mixed shell grit, sieved to >4 mm diameter, available *ad libitum*. At 37 weeks, 3 treatments were imposed: 1. Shell grit available *ad libitum*; 2. Shell grit available *ad libitum* every second day; 3. Shell grit available *ad libitum* every fourth day.

Egg production, feed and shell grit consumption were determined weekly. From the second week eggs were collected once weekly for 4 equal periods, between 0700 and 1200 h, and egg weight, egg specific gravity (SG), shell weight % (SW) and shell thickness (ST) were measured. Egg production and wheat intakes were not significantly different ($P>0.05$) across the treatments and feed consumption was similar for the 3 treatments. Grit consumption (g/hen/day) by the hens is shown in the Table.

	WEEK					
Treatment	1	2/3	4	5	6	
1.	7.6±0.43	8.2±0.31^a	7.7±0.43^a	7.4±0.41^a	7.3±0.48	n=17
2.	7.5±0.43	6.5±0.30^b	5.6±0.42^b	6.2±0.40^b	7.3±0.46	n=18
3.	8.1±0.45	6.2±0.31^b	7.6±0.43^a	8.1±0.41^a	7.2±0.48	n=17
		***	**	*		

^{ab} Values within columns with different superscripts are significantly different.

Egg weights were not significantly different across treatments and nor were SG, SW and ST, except for eggs from hens on Treatment 3 where SG, SW and ST were all significantly ($P<0.001$) lower on the third day after grit was withdrawn. It appears that the laying hen has the capacity to adjust rapidly its daily intake of particulate calcium when denied access for fixed periods. This adjustment occurred during the first week of the trial and in the case of Treatment 3 occurred on the first day of access to the shell grit. Overall, the mean daily intakes per bird were 7.5 g, 6.6 g and 7.4 g on Treatments 1, 2 and 3 respectively. These intakes of particulate calcium allowed the hens to produce at similar rates without sacrificing shell quality until the third day after grit was denied. The shell grit was apparently identified as the principal calcium source as the intake of the protein concentrate was not increased. The early training for choice feeding may be involved in preventing excess intake of protein. The laying hen fed *ad libitum* on a Ca-deficient diet plus particulate Ca appears to exhibit the capacity to adjust its intake of particulate Ca to maintain production and shell quality even when access is restricted.

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THE EFFECT OF HEAT STRESS ON EGG SHELL BREAKING STRENGTH IN AN IMPORTED LAYER STRAIN

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Heat stress is known to have a deleterious effect on egg shell quality (Say, 1987). Egg production may decrease and the shells tend to become thinner. However, the ultrastructure of these thinner shells is improved, as if the hen is attempting to utilise the available calcium in such a way as to maximise shell strength (Brackpool *et al.*, 1993; Roberts and Brackpool, 1994).

This study was designed to investigate the effect of heat stress on the breaking strength of egg shells to see if the improved ultrastructure was able to compensate for the weakening of the shells as the result of decreased shell thickness. Hens of an imported brown egg layer strain, at 50 weeks of age, were divided into two groups, each of 10 birds. Each group was housed in a separate constant temperature room in the University of New England Animal House. Birds were maintained at 20°C for two weeks prior to the commencement of the experiment. Both groups were then maintained at 20°C for one week during which all eggs laid were collected. The temperature of one room was then increased to 25°C for 2 days and then to 30°C. For the next 4 weeks, feed intake was monitored and eggs were collected daily.

All eggs were weighed, the length and width measured and specific gravity determined by Archimedes method (Pym, 1969). The eggs were allowed to dry prior to measurement of egg shell breaking strength by quasi-static compression. Quasi-static compression tests were conducted using a shell breaking strength machine custom-made by the workshop of the Department of Physiology, University of New England. Eggs were compressed at the equator at the rate of 20 $cm.min^{-1}$ until an audible crack was heard. The breaking force, in Newtons, was recorded via a T.W.L. force gauge. The deformation to breaking force was measured by a Mitutoyo electronic dial comparator gauge. The egg contents were emptied and the shells dried in an oven at 50°C prior to weighing. Shell thickness was measured by means of a Mitutoyo dial comparator gauge.

The heat stress resulted in significant decreases in egg weight, egg breadth, shell weight and shell thickness. However, there was no significant effect on egg shell breaking strength. Deformation to breaking point decreased in both groups of birds over time. These data suggest that the improved ultrastructure of eggshells laid by hens during heat stress is able to compensate for the thinning of the shells. This results in shells which are of equivalent breaking strength to the thicker shells laid at the lower temperature.

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HOW GOOD ARE EMPIRICAL EQUATIONS FOR ESTIMATING EGG PARAMETERS?

E.S. THOMSON, S. ROBINSON and J.V. NOLAN

Egg quality and value are dependent on characteristics such as egg weight, shell weight and thickness, and weight of contents (yolk and white). Shell characteristics and egg contents do not vary in direct proportion to egg weight: yolk weight, for example, tends to be relatively heavier in smaller eggs (Jaffe, 1964). Narushin (1994a) proposed the following equation for predicting eggshell weight (W_S) from egg weight (W_e) based on measurements of 320 eggs (45-70 g) from a single strain of White Leghorn hens.

$$W_S = 0.081316 W_e^{1.0865} \quad (1)$$

Similarly, specific gravity (SG) has been widely used for predicting shell thickness. Narushin (1994b) reported that mean shell thickness (cm) could be predicted as follows:

$$T = 0.000853\ V_e^{0.33}/(1.175-D_e) \quad (2)$$

where V_e is egg volume and D_e is egg density (equivalent to egg mass/unit volume or SG).

The present evaluations of the prediction equations of Narushin (1994) were based on characteristics of 475 eggs from 70 SIRO CB hens fed an Australian Layer diet at the 'Laureldale' Poultry Farm, University of New England. The eggs were collected over two days on each of three occasions at 3 week intervals starting when the birds were 32 weeks of age. Egg weight and SG were determined within 24h of the eggs being laid. The eggs were then broken and the shells and membranes were washed and dried, then weighed. Mean shell thickness was determined from measurements made with a micrometer gauge at three points on the equator of each egg.

Equations 1 and 2 were applied to the egg data, and the calculated values were compared with the measured values using linear regression analysis. The shell weight correlation was:

$$W_S\text{calc} = 3.06 + 0.691 W_S\text{measured} \qquad R^2=0.31$$

The shell thickness correlation was:

$$T\text{calc.} = 0.0136 + 0.597 T\text{measured} \qquad R^2=0.66$$

In both cases the intercepts are significantly greater than zero ($P<0.001$), and the slopes significantly less than 1 ($P<0.001$).

Applying the formulae to data from Hisex White hens reported by Kaminska et al. (1994) indicated similar disagreement between calculated and measured shell weight to that reported above (i.e. overestimated W_S by about 20%). Calculated shell thickness also differed to a similar extent (2.8%) from the measured value, but in the opposite direction from our data i.e. overestimated rather than underestimated shell thickness.

The development of equations for non-destructive estimation of various egg parameters has obvious attractions. However, extrapolating these from different strains, stages of lay, feeds and husbandry systems is not valid. The constant factors in such equations need to be verified with a sample taken from the relevant flock.

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EFFECTS OF BETAINE ON METHIONINE REQUIREMENT OF BROILERS UNDER VARIOUS ENVIRONMENTAL CONDITIONS

E.I.VIRTANEN and L.ROSI

Summary

Two trials were formulated to compare the effect of betaine and methionine supplementation in broilers fed a marginally methionine-deficient diet. In Trial I, broilers were grown in floor pens with built-up litter and natural coccidiosis challenge. They were fed a basal commercial-type diet adequate (NRC) in other nutrients except for methionine (75-80 % of commercial level), supplemented with graded levels of DL-methionine or betaine (0, 0.5, 1 or 1.5 g/kg). In Trial II, the birds were grown under minimized coccidiosis challenge or inoculated experimentally via drinking water. These birds were fed a similar basal diet, supplemented with 1.5 g DL-methionine or 0.75 g betaine/kg.

In Trial I, the broilers showed a linear growth and feed conversion response to supplementary methionine. Interestingly, the response to betaine was significantly greater, indicating a two-fold efficacy of betaine to promote growth and feed efficiency. Also, the carcass responses (percent fat pad and breast meat) were more pronounced with betaine than methionine. The second trial demonstrated that betaine decreases the severity of intestinal lesions under coccidiosis challenge, leading to significant effects in broiler performance. However, even in the conditions of minimized challenge, betaine exerted a clear methionine sparing effect.

I. INTRODUCTION

The only traditionally known metabolic function of betaine in animals is its ability to donate methyl groups for various methylation reactions. One of these is the methylation of homocysteine to methionine. Pesti *et al.* (1979) concluded that with a practical maize-soya diet for starter broilers, 3.7 g methionine or 7.4 g total sulphur amino acids (TSAA)/kg was enough to meet the specific requirement of methionine and TSAA, and above these levels there is just a need for methyl donors. However, their conclusion has not been shared with many researchers and industry nutritionists.

While most of the work dealing with interactions between methionine and methyl donors has been carried out using methionine and choline, little attention has been paid to the fact that choline may be inefficient as a methyl donor. Stekol *et al.*. (1952) reported that choline is relatively inefficient in methylation of homocysteine and creatine, compared to betaine and methionine. Moreover, ionophores present in broiler diets may inhibit choline oxidation to betaine (Tyler, 1977), being necessary to render the methyl group labile.

In this study the efficacy of betaine to promote growth and feed efficiency in broilers fed a methionine-deficient diet was examined. The basal diet was formulated to be similar to that used by Pesti *et al.* (1979), and supplemented with graded levels of betaine or methionine. Because it was concluded from the results of this experiment that coccidiosis challenge may play a major role in betaine responses, a second experiment was formulated to compare betaine and methionine responses under minimized coccidiosis challenge and experimentally induced subclinical coccidiosis.

Cultor Ltd., Finnsugar Bioproducts, P.O.Box 105, 00241 Helsinki, Finland

II. MATERIAL AND METHODS

Trial I.

The trial was conducted at PARC Institute, Maryland, USA. The experimental chicks (2240 males and 2240 females) were of commercial broiler strain (Peterson x Arbor Acres) and grown from 1 to 45 days of age. They were grown on built-up litter in floor pens of 4.76 m^2 , 80 birds per pen, 8 pens per treatment. Wet litter from the previous trial was removed and approximately one inch of new litter was placed in each pen.

Broilers from each sex were randomly distributed into pens. The birds were given the experimental diets commencing the first day of the trial, both feed and water being available *ad libitum*. Artificial lighting was provided continuously and the temperature of the pens was checked daily for the first two weeks and that of the experimental building throughout the trial. All birds were vaccinated against Marekcs Disease and Newcastle-Bronchitis at the hatchery. The weights and day of removal of all dead and culled birds were recorded.

Broiler feed in crumble form was fed from day 1 to day 21 (starter diet) and pellets from day 22 to day 45 (finisher diet). The basal diet consisted of yellow miaze, soybean meal (48%), soy oil, NaCl, L-lysine-HCl, limestone, defluorinated phosphate, DL-methionine, vitamin premix, trace mineral premix, salinomycin and Bacitracin MD. The calculated analyses were (/kg) crude protein 210/190 g, metabolizable energy 13.5/13.7 MJ/kg, lysine 12.0/10.0 g, methionine 3.7/.3.1 g, methionine+ cysteine 7.3/6.5 g, choline 1420/1440 mg, calcium 9.0/9.0 g and available phosphorous 4.5/4.0 g for starter/finisher diets, respectively. The other diets were produced by mixing graded levels (0.5, 1.0 or 1.5 g/kg) of either DL-methionine (99 % purity) or anhydrous betaine (97 % purity) into the basal feed mix.

Live weight based on average pen weight and feed efficiency were determined on days 21 and 45. Individual body weights were recorded on day 45. Ten males and 10 females per pen were processed on day 45 to determine the weight of abdominal fat pad and breast meat.

Trial II.

The trial was conducted at Colorado Quality Research, Inc, Colorado, USA. The experimental chicks (1320 males and 1320 females) were of commercial broiler strain (Ross x Arbor Acres) and grown from 1 to 47 days of age. They were grown in floor pens of 4.09 m^2, 55 birds per pen, 8 pens per treatment. Prior to bird placement, the research facilities were cleaned by remowing wet litter and top dressing with new wood shavings.

Broiler from each sex were randomly distributed into pens. The birds were given the experimental diets commencing the first day of the trial, both feed and water being available *ad libitum*. Artificial lighting was provided continuously. The temperature of the pens was checked twice a day. All birds were vaccinated against Marek's Disease and Newcastle-Bronchitis on day 5. The weights and day of removal of all dead and culled birds were recorded.

On day 14, birds in treatments 4-6 (separate facility, see Table 1.) were challenged via the feed with a mixed inoculum of coccidia oocysts (Eimeria tenella, E. acervulina and E. maxima).

Broiler starter feed was provided from day 1 to day 23, grower feed from day 24 to day 41 and finisher feed from day 42 onwards. The basal diet consisted of maize, soybean meal (46.5 %), meat and bone meal (48 %), blended fat, L-lysine-HCl, DL-methionine, NaCl, defluorinated phosphate, limestone, choline chloride-60%, salinomycin, Bactracin

MD-50, 3-nitro-20, trace mineral premix, vitamin premix and fine sand. The calculated analyses were (/kg): crude protein 220/200/180 g, metabolizable energy 13.0/13.3/13.5 MJ/kg, lysine 11.6/10.2/8.8 g, methionine 3.8/3.7/3.0 g, methionine+cysteine 7.1/6.7/5.8 g, choline 1650/1550/1320 mg, calcium 9.2/8.6/8.0 g and available phosphorous 4.8/4.5/4.0 g for starter/grower/finisher diets, respectively. The other diets were produced by replacing sand with either 1.5 g DL-methionine or 0.75 g anhydrous betaine/kg.

Six birds (three males and three females) per pen were randomly selected for lesion scoring (Johnson and Reid, 1970) on day 21. Live weight based on average pen weight and average weight of males and females, and feed efficiency were determined on day 47. Nine birds per pen were processed on day 47 and live weight, hot weight, chill weight and weight of breast fillet were determined, and the skin was scored for skin tears.

The data of both trials were analysed using a randomized block design in an ANOVA model, and Tukey¢s test for treatment means. Values with the same superscript do not differ significantly ($P>0.05$). The data are given in Tables 1 (Trial I) and 2 (Trial II).

III. RESULTS AND DISCUSSION

In Trial I, the broilers showed a linear growth and feed efficiency response to both methionine and betaine (Table 1). Interestingly, while Pesti *et al.* (1979) demonstrated that betaine could be as effective as methionine to promote growth and feed efficiency in starter broilers fed 7.4 g TSAA/kg, the present data, when analyzed by the slope ratio technique, indicated that betaine was two times more effective than methionine when added to a similar diet.

Table 1. Growth performance (1 to 45 days) and carcass composition of broilers fed a basal, methionine-deficient diet or the basal diet supplemented with three levels of DL-methionine or betaine (Trial I).

Treat-ment	Added methionine g/kg	Added betaine g/kg	Body weight kg	Feed: gain g:g	Mortality /100 birds	Fat pad g/kg	Breast meat g/kg
T1	0.0	0.0	2.100^{A}	1.862^{A}	8.9^{A}	27.8^{A}	129.7^{AB}
T2	0.5	0.0	2.124^{AB}	1.849^{AB}	7.5^{AB}	27.1^{AB}	126.6^{A}
T3	1.0	0.0	2.154^{BC}	1.842^{AB}	3.9^{BC}	26.8^{ABC}	130.0^{AB}
T4	1.5	0.0	2.169^{CD}	1.839^{AB}	6.7^{AB}	25.5^{BC}	134.8^{BC}
T5	0.0	0.5	2.155^{BC}	1.846^{AB}	4.1^{BC}	26.9^{ABC}	129.0^{A}
T6	0.00	1.0	2.193^{CD}	1.831^{BC}	5.2^{BC}	25.0^{C}	130.8^{AB}
T7	0.0	1.5	2.204^{D}	1.811^{C}	1.9^{C}	22.5^{D}	139.8^{C}

Since it was evident that betaine used at 1.5 g/kg affected the mortality of the birds under natural coccidiosis challenge, and the studies of Virtanen *et al.* (1993) and Rosi *et al.* (unpublished) demonstrated that betaine decreases the severity of gut lesions and improves

the performance of coccidia-challenged broilers, a second trial examined how coccidiosis challenge affected betaine and methionine responses with a methionine-deficient diet.

Table 2. Growth performance (1 to 47 days) , lesion score at day 21 and breast meat content of broilers fed a basal, methionine-deficient diet or the basal diet supplemented with 1.5 g DL-methionine or 0.75 g betaine/kg. The broilers were fed with a clean (Treatments 1-3) or a coccidia-inoculated diet (Treatments 4-6) (Trial II).

Coccidia challenge	Diet	Body weight kg	Feed:gain g:g	Mortality /100 birds	Lesion score	Breast meat g/kg
No	Basal	2.336^{ABC}	1.808^{AB}	4.1^{A}	0.13^{A}	172.6^{AB}
No	Basal+Met	2.380^{D}	1.792^{A}	4.1^{A}	0.85^{A}	181.9^{C}
No	Basal+Bet	2.372^{D}	1.788^{A}	3.2^{A}	0.19^{A}	180.0^{C}
Yes	Basal	2.276^{A}	1.875^{D}	2.3^{A}	4.44^{CD}	168.8^{A}
Yes	Basal+Met	2.325^{BC}	1.846^{C}	2.0^{A}	4.75^{D}	179.8^{C}
Yes	Basal+Bet	2.310^{ABC}	1.835^{BC}	4.3^{A}	2.27^{B}	178.0^{BC}

Based on the observation in Trial I that the response to 1.5 g methionine/kg could be achieved with half that amount of betaine, Trial II was designed to determine how much this was related to the effects of methionine and betaine on coccidiosis. Only betaine was effective in reducing lesion score under coccidiosis challenge, while 0.75 g betaine and 1.5 g methionine/kg produced similar effects on growth, feed efficiency and carcass composition. Although the mild coccidiosis challenge did not significantly increase mortality, the "clean" birds had a significantly better growth performance than the challenged ones. Surprisingly, the methionine response was very low in the "clean" brids, and did not differ from the response to betaine.

It is not yet known how betaine exerts its effects on gut health status in broilers under coccidiosis challenge. Similar effects are not produced by methionine or choline supplementation (Rosi *et al.*, unpublished). In addition to the methyl donor function, betaine has another metabolic role, the osmoprotectant function, which has been demonstrated in various organisms and tissues, including plants, microbes, fish and mammalian kidney (Yancey *et al.*, 1982; Bagnasco *et al.*, 1986; Clarke *et al.*, 1994). While coccidiosis and coccidiostats affect the ionic balance of the gut (Gwyther and Britton, 1989), betaine may counteract some of these changes which are potentially harmful to nutrient absorption. Accordingly, betaine improved the digestibility of protein, pigments, and some amino acids and minerals in coccidia-challenged broilers (Virtanen *et al.*, unpublished).

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THE COMMERCIAL PRODUCTION OF AMINO ACIDS BY FERMENTATION

J. C. WEIGEL

Summary

Amino acids are the basic building units of protein and are essential for all living organisms. Demand for amino acids have, therefore, spread over many uses, including medicines, food and feed use and fine chemicals. As a result, the production of industrially produced amino acids has grown and will continue to play an important role in the world biochemical industry.

This paper will identify and discuss the basic concepts involved in the production of lysine, threonine and tryptophan. The paper will discuss the four phases of production; fermentation/separation, recovery, purification, finished product.

I. INTRODUCTION

Lysine is one of the largest industrially produced amino acids, second only to methionine. Lysine, along with threonine and tryptophan, is produced by the microbial fermentation of certain organisms on a media consisting of key carbon sources. These carbon sources include sugar from beets or cane, dextrose from the corn wet milling industry and fibre carbohydrate sources. Nitrogen comes from soybean processing, alternative oilseed processing, or recycling of components from within the process.

II. FERMENTATION

The selection of new microbial strains has been very important for the establishment and improvement of amino acid production. At the early stage wild strains were employed for the accumulation of amino acids. Since the biosynthesis of amino acids is strictly controlled by the regulatory mechanisms of living cells, their overpopulation would not be expected. Genetic alteration of amino acid producers was done by mutagenesis and selection of mutants to give various auxotrophic and regulatory strains with improved characteristics. Now, the newly developed technology of genetic manipulation *in vivo* (such as transduction, cell fusion) and *in vitro* (such as DNA recombination) is also applied to the construction of new types of amino acid-producing organisms. Mutagenesis has provided new genetic characters such as drug resistance, and the techniques of transfusion and cell fusion have been used for the recombination of desired genetic characters, while the techniques of DNA recombination have been used for the amplification of gene copy numbers.

When selecting the organism for the production of the desired amino acid, microbiologists evaluate how well the organism will produce and yield the desired amino acid on the selected energy source. Much of this is done with lab scale 1-litre fermenters.

Once the organism has been selected, it is placed in the Vial Lot Laboratory. This is where the original media are prepared and where commercial fermentation commences.

The viable vial of the culture is introduced into the shake flask and the flask is prepared to inoculate the plant fermenters.

Archer Daniels Midland Company, Decatur, Illinois, USA

Most fermentation facilities use three phases of fermentation:

Propagation	-	Cell Division,
Seeding	-	Cell Division,
Main production	-	Amino Acid Production.

There are numerous types of fermentation vessels ranging from 1 litre to 650,000 litre vessels or reactors. The fermenters consist of pH probes and controls, oxygen probes and regulator temperature probes, pumps for acids/bases, antifoam addition pumps and nutrient addition pumps for continuous nutrient addition during fermentation.

Oxygen, a raw material in the aerobic conditions used in amino acid fermentation, is consumed in large amounts. Due to low solubility to oxygen in fermentation broth, it is essential to continuously supply large amounts of dissolved oxygen to the fermenters. Depletion of oxygen severely restricts amino acid production. Huge driving motors, in some cases up to 1000 HP are used in each reactor..

Three methods are used for the fermentation of amino acids. These methods include:

1. Batch,
2. Fed Batch,
3. Continuous.

Prior to their addition any raw materials are assayed for purity and safety, along with a complete sterilisation. Sterilisation is done not only for the medium, but also for all equipment.

The raw materials used in fermentation consist of products from beet and cane sugar processing, the corn wet milling industry, soy and alternative oilseed processing, certain non-protein nitrogen sources, plus numerous trace nutrients. The nutrient requirements for the amino acid producing organisms do not differ much from those of conventional farm animals. Biochemists are constantly re-evaluating the nutrient demands of the organism. These nutrients are supplied to the organism(s) by the medium. In the feed industry it is called the "feed".

The fermentation process is scientifically and computer driven. Some of the problems experienced by biochemical engineers are:

1. Maintenance of the pure culture,
2. Automatic control of pH, temperature, foam,
3. Agitation and aeration.

The success of the fermentation is based on the following:

1. Productivity (g amino acid/hour),
2. Concentration (g amino acid/litre),
3. Yield (g amino acid/ g sugar).

These values are very similar to what animal specialists look for in the evaluation of animal production.

III. SEPARATION

Once the fermentation has ended, the biomass is exposed to heat-to-kill conditions to ensure no live organism is left prior to recovery. The fermenter is then harvested. The broth is passed through a series of ultrafiltration membranes for the separation of the amino acid as the permeate, and the waste biomass as the retentate. The retentate can be assayed and recycled as part of the nutrient medium. The ultrafiltration/microfiltration system is an integral part of the process. Both polymeric and ceramic hollow fibre membranes can be used.

IV. RECOVERY

Prior to the movement to the ion-exchange columns, the high pH broth is acidified with sulfuric acid to allow the main acid to reach its iso-electric point. Once the low pH broth passes through the ion-exchange columns the free L-lysine and small amounts of ammonia are separated from the liquid. The residual liquid can be recycled or used as a feed or fertiliser, depending on contamination.

V. PURIFICATION

Depending on the amino acid, the product can be spray-dried, and a low purity product can be produced. In other cases the purity is improved via crystallisation. Lysine, because of its stability problems in the presence of a reducing sugar, has to be put into the salt form. It is blended as a 70/30 ratio of lysine liquor and hydrochloric acid (HCl) forming lysine HCl dihydrate.

VI. FINISHED PRODUCT

The product is then dried to ambient temperature, sized to market requirements and checked for purity, granulation and isomeric purity.

The above are the basic methods for production of lysine, threonine and tryptophan. The methods of production are dynamic in relation to changes in processes concerning new fermentations, membrane separation, and basic and applied research relative to new organisms.

THE INTERACTION OF FACTORS AFFECTING OVARIAN FORM AND FUNCTION IN BROILER BREEDER HENS

F. E. ROBINSON

Summary

Body weight control is very important for broiler breeders as over-feeding results in lower total and settable egg production as well as reduced fertility, hatchability and livability. A high degree of variability in a flock can mean that the flock will not show high peaks in productivity. Even though the flock average may be on target, when there is a large number of "light weight" hens and a large number of "high weight" hens it is very likely that some hens will not achieve their genetic potential. Broiler breeder hens are very sensitive to mis-management due to over-feeding. When these hens are fed more nutrients than they need, they appear to deposit surplus energy in the ovary. This is not a desirable situation as extra follicles do not mean extra eggs. To avoid subjecting the hens to being in a major "positive energy balance", treat increases in feed allocation with caution. Avoid major sudden changes in feed allowance so as to assist hens to maintain control of follicular recruitment. This report is a review of several experiments conducted at the University of Alberta to examine the negative relationship between body weight and reproductive fitness of female broiler breeders.

I. INTRODUCTION

Broiler breeder hens have more expected of them than other classes of commercial poultry. Leghorns are expected to lay at least 280 eggs per year. However, with their small size this expectation is usually met. Broiler chickens are expected to grow rapidly but they are not expected to reproduce. Broiler breeders need to be fed and managed in a manner that maximizes reproductive traits (semen production, egg production, fertility and hatchability), while at the same time carrying the genetic material to have their offspring exhibit fast and efficient rates of growth.

The ovary of a broiler breeder hen usually consists of an orderly hierarchy of seven to ten large follicles greater than 1 cm in diameter. The largest follicle (F1) is the nearest to ovulation. To ovulate, a follicle must be sufficiently "mature" to synthesize progesterone in response to a preovulatory surge of luteinizing hormone (LH). The small ovarian follicles are a major source of estrogens and androgens, and this steroid output is highly LH responsive. Eggs are laid in sequences of one or more eggs. Hens that are laying at high rates lay long sequences. A sequence is terminated when the time of oviposition approaches 6 to 8 h after "lights on". A pause day follows, and a new sequence commences.

The biggest challenge faced in managing a broiler breeder flock is determining the optimum feed allocation. Breeding companies suggest target weights; however, the birds change genetically over the years and the exact target growth curve to follow may be somewhat unknown. A second challenge is that of flock uniformity. There can be considerable variation in a flock in terms of body weight, and this fact is a major limitation to maximizing returns. There is a well-documented negative relationship between body

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weight and reproductive fitness in poultry (Siegel and Dunnington, 1985; Robinson *et al.*, 1993), that severely limits the ability of meat-type stocks to reproduce. Broiler breeder females are severely reproductively compromised when they are allowed to full-feed (Robinson *et al.*, 1991; Yu *et al.*, 1992a,b). Reproductive inefficiency is apparent in reduced egg production and shell quality, in addition to an increased incidence of multiple-yolked eggs, obesity-related mortality, infertility and embryonic loss. The critical time in broiler breeder body weight management is during the period from photostimulation (lighting) to peak production (Robinson *et al.*, 1993: Wilson *et al.*, 1993). This period is characterized by relatively fast weight gains and the changes brought about by the presence of a hormone-producing ovary. This paper will focus on a review of obesity in breeders and how variations in feed allocation during rearing, laying and while birds are attaining sexual maturity impacts on reproductive function.

II. THE IMPLICATIONS OF OBESITY ON REPRODUCTIVE FITNESS

The main approach used to study the consequences of obesity in broiler breeders has been to compare feed restricted hens with full-fed hens. While this approach may at first seem to be somewhat extreme, it represents a good place to start. It must be remembered that, due to within flock variation (poor flock uniformity), there often may be situations where some very large birds exist, so observations made with full-fed birds may be quite relevant.

In one study, (Robinson *et al.*, 1991) Indian River broiler breeder pullets were raised to 22 wk of age following the suggested body weight targets. The photoperiod used was 23 h of light and 1 h of darkness (23L:1D) from 0 to 1 wk, 8L:16D from 1 to 18 wk, and 14L:10D from 18 to 62 wk. All hens received the same standard laying ration containing 11.96 MJ metabolizable energy (ME) and 165 g crude protein (CP)/kg. At 22 wk of age, the birds were housed in cages with 30 hens being feed restricted and 30 hens being full-fed to 62 wk of age. The body weights of the full-fed hens were approximately 700 g heavier than those of the restricted hens throughout lay (Table 1). Full-feeding resulted in increased fat deposition as 68% of the body weight difference between the two groups of hens was composed of fat. Mean egg output was lower in the full-fed hens compared to the restricted hens. A similar number of laying sequences was observed in the two groups of hens. However, the length of the longest (prime) sequence was significantly greater in the restricted hens than in the full-fed hens. The full-fed hens had a greater number of pauses (consecutive non-laying days) of greater than 11 days duration, indicating that ovarian regression had occurred. There was a trend for higher mortality in the full-fed hens. Six full-fed hens died due to fatty liver syndrome, while only two restricted hens died from this problem.

Table 1. Carcass traits and reproductive performance of full-fed versus feed restricted broiler breeder hens.

Parameter	Full-fed 22-62 wk	Restricted 22-62 wk
Body weight, 62-wk (kg)	4.31[a]	3.66[b]
Carcass fat content at 62 wk (%)	34.4[a]	27.7[b]
Total (including unsettable) egg production (eggs/hen)	136.2[b]	176.6[a]
Length of longest laying sequence (days)	14.9[b]	24.2[a]

In a second, larger scale study, Yu *et al.* (1992a,b,c) examined the consequences of full-feeding from 4 to 20 and from 20 to 68, wk of age in a 2 X 2 factorial design (two periods X two levels of feeding). The treatments were denoted as follows: RR (restricted during rearing and laying), RF (restricted during rearing and full-fed during laying), FR (full-fed during rearing and restricted during lay) and FF (full-fed during rearing and lay). A total of 200 hens (50 per group) were studied. The photoperiod used was 23L:1D for the first 3 days, 8L:16D (day 4 to 18 wk), 12L:12D (18 to 20 wk) and 14L:10D thereafter. All birds received a starter diet (11.42 MJ ME and 191 g CP/kg) from 0 to 3 wk, a grower diet (11.42 MJ ME and 155 g CP/kg) from 3 to 22 wk and a layer diet (12.09 MJ ME and 146g CP/kg) from 22 to 62 wk. During rearing the full-fed pullets consumed, on average, 2096 kJ of ME and 28.4 g CP per day. The restricted birds consumed only 778 kJ of ME and 10.6 g CP per day. During the laying period, on average, the RR birds consumed 86% to 88% of the feed that was consumed by FF birds.

Feed restriction during rearing reduced body weight at 18 wk of age from 4.2 kg to 1.9 kg. There was a large difference in percentage carcass fat at 18 wk (full-fed = 27.4%; restricted = 7.3%). The reproductive performance of the hens was poorer the longer the duration of full-feeding (Table 2). Fertility and hatchability were reduced with full-feeding. The FF hens exhibited severe reproductive problems (soft-shelled eggs, multiple yolked eggs, and the laying of more than one egg per day). Erratic (night time) laying was highest in full-fed hens. One of the most obvious signs of reproductive dysfunction was an increase in the number of large follicles. During the 62-wk experiment, mortality was as follows: eight FF, three RF, one FR and zero RR hens.

Table 2. Reproductive characteristics of broiler breeder hens varying in feed allocation (full-fed versus feed restricted) during rearing and lay.

Parameter	FF	FR	RF	RR
Body weight, 62-wk (kg)	4.9^{a}	3.9^{c}	4.5^{b}	3.4^{d}
Total (including unsettable) egg production (eggs/hen)	122.2^{c}	162.9^{ab}	132.5^{c}	176.6^{b}
Total settable egg production	102.6^{c}	143.9^{ab}	118.1^{b}	172.4^{a}
Incidence of shell problems (%)	32.6^{a}	22.7^{b}	20.0^{b}	4.5^{c}
Incidence of multiple-yolked eggs (%)	18.1^{a}	13.5^{a}	12.6^{a}	2.3^{b}
Incidence of multiple-egg days (%)	7.5^{a}	6.6^{ab}	4.7^{b}	1.2^{c}
Incidence of night-time laying (%)	40.8^{a}	28.7^{b}	24.5^{b}	13.3^{c}
Fertility (%)	78.0^{b}	82.3^{b}	82.0^{b}	91.9^{a}
Hatchability (%)	65.0^{c}	75.4^{b}	68.5^{bc}	86.4^{a}
No. large follicles at first egg	12.2^{a}	11.1^{a}	10.7^{a}	7.8^{b}
No. large follicles at 62 wk	6.9^{a}	4.6^{a}	6.2^{ab}	4.6^{b}

$^{a-d}$Means in rows with different superscripts are significantly different ($P<0.05$).

III. CONSEQUENCES OF VARYING FEED ALLOCATION DURING PULLET REARING

In this study (Wilson *et al.*, 1993) 750 Indian River pullets were raised to 20 wk of age on three feeding programs based on the level of feed allocation from 1 to 24 wk of age. At 7 days of age the birds were subjected to skip-a-day feed restriction following one of

three body weight target curves. The body weight targets from 1 to 24 wk of age are illustrated in Figure 1 and as described below:

"Standard" body target recommended by the primary breeder (this group received relatively linear increases in feed intake).

"Early slow" provided with lower than usual feed allocation from 1-19 wk, followed by more feed than was allocated to the standard group (wk 20 to 26).

"Early fast" fed more feed than the standard group from 1 to 20 wk followed by similar amounts as the standard group after 20 wk.

At 20 wk of age 25 birds from each of the feed allocation groups were individually caged. The photoperiod and diets used were identical to those described above for the Yu *et al.* (1992a, b, c) study.

The observed body weight profiles of the three groups are presented in Figure 2. The total egg production for the early slow, standard and early fast groups were 170.2, 183.5 and 181.3 respectively (Table 3). The early fast and the standard treatment hens produced significantly more settable eggs than did the early slow hens (168.4, 167.4, and 153.3 eggs respectively). For eggs set from 28 to 58 wk of age there were no significant differences in fertility between treatments. Hatchability and hatchability of all fertile eggs set were highest in the early fast group, being significantly higher than for either of the other two groups by 2.6 to 3.1%. The incidence of mortality was very low and was not different among treatments.

Table 3. Influence of feed allocation from 1 to 24 wk of age on reproductive performance of broiler breeder hens to 58 wk of age.

Parameter	Early slow	Standard	Early fast
Age at first egg (d)	178.0	174.1	172.7
Body weight at first egg (kg)	2.77	2.71	2.77
Total (including unsettable) egg production (eggs/hen)	170.2	183.5	181.3
Settable egg production (eggs/hen)	153.3^{b}	167.4^{a}	168.4^{a}
Mean egg weight (g)	61.81	61.63	63.19
Fertility (%)	84.06	83.49	84.57
Hatchability (%)	75.68^{b}	75.68^{b}	78.79^{a}
Hatch of fertile (%)	90.02^{b}	90.06^{b}	92.68^{a}
Number of chicks per hen (28 to 58 wk)	107.8^{b}	116.0^{a}	116.9^{a}

$^{a\text{-}b}$Means in rows with different superscripts are significantly different ($P<0.05$).

IV. CONSEQUENCES OF VARYING FEED ALLOCATION DURING THE EARLY LAYING PERIOD

Robinson *et al.* (1993) studied a flock of 250 Indian River pullets raised to 20 wk according to the Standard target program indicated in the preceding study. At 20 wk of age 75 pullets (those closest to the 20 wk target weight) were housed in individual laying cages.

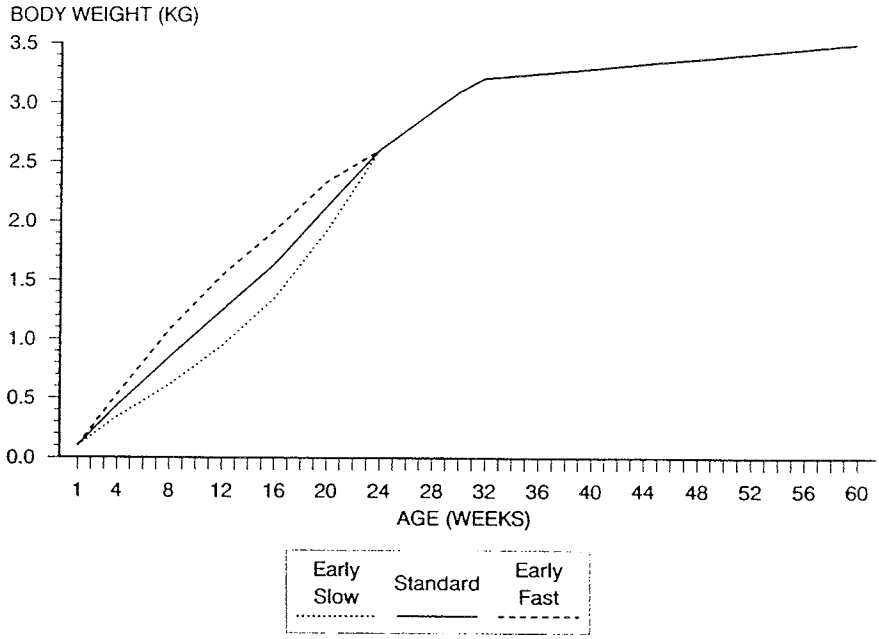

Figure 1. Target body weight of three treatment groups of feed restricted female broiler breeders varying in feed allocation from 1 to 20 wk of age.

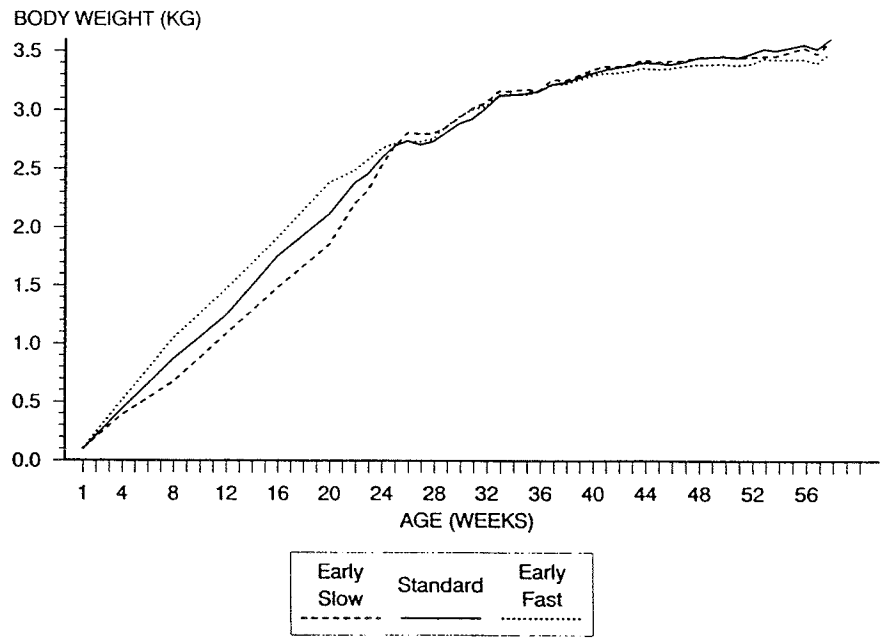

Figure 2. Actual body weight of three treatment groups of feed restricted female broiler breeders varying in feed allocation from 1 to 20 wk of age.

Three feed allocation treatments were fed from 24 to 32 wk of age (Figure 3):

"Standard" followed the body weight targets of the breeder (the most linear group with small weekly incremental changes in feed allocation).

"Early slow" lower feed intake during the period from 24 to 32 wk of age.

"Early fast" higher feed intake during the period from 24 to 32 wk of age.

The photoperiod and diets used were identical to those described above for the Yu *et al.* (1992a, b, c) study.

It is apparent from Figure 4 that it was very difficult to have the birds follow the body weight targets established and shown in Figure 3. Due to the more generous feed allocation to the early fast hens after 24 wk of age, they commenced lay at a significantly heavier weight than the other two groups. The early fast hens were 100 g heavier than the early slow birds and 140 g heavier than the standard birds at first egg. This increased body weight may have contributed to the poor egg production seen in this group. All groups of hens had a similar number of unsettable eggs, so that the early fast birds were still significantly poorer than the standard hens in terms of the number of settable eggs. There were treatment differences in hatchability and hatch of fertile eggs (Table 4). In both of these traits, the early slow treatment group exhibited superior performance to the early fast group. Hens of the early fast group produced significantly fewer chicks. These data also provide evidence that under the conditions imposed with the three different growth curves, problems were encountered when pullets and hens were provided with surplus energy around the time of puberty, through to early lay.

Table 4. Influence of feed allocation gain during the early lay period on reproductive performance of broiler breeder hens to 58 wk of age.

Parameter	Early slow	Standard	Early Fast
Age at first egg (d)	175.6	174.1	178.1
Body weight at first egg (kg)	2.75^{ab}	2.71^{b}	2.85^{a}
Total (including unsettable) egg production (eggs/hen)	174.1^{ab}	183.5^{a}	167.7^{b}
Settable egg production (eggs/hen)	155.8^{ab}	167.2^{a}	149.8^{b}
Mean egg weight (g)	61.89	61.71	61.84
Fertility (%)	82.42	83.49	81.47
Hatchability (%)	76.21^{a}	75.68^{ab}	72.99^{b}
Hatch of fertile (%)	92.05^{a}	90.06^{ab}	89.38^{b}
Number of chicks per hen	111.4^{a}	116.7^{a}	99.5^{b}

$^{a\text{-}b}$Means in rows with different superscripts are significantly different ($P<0.05$).

V. CONSEQUENCES OF VARIATION IN 20-WEEK BODY WEIGHT

The 75 hens used in the preceding experiment were sorted on the basis of 20 wk body weight into five groups as follows: 96%, 98%, 100%, 102%, 105% (Robinson *et al.*, 1993). The photoperiod and diets used were identical to those described above for the Yu *et al.* 1992a, b, c) study.

Significant differences in body weight at 20 wk of age persisted to 25 wk of age. All groups of birds commenced lay at a similar time (range of 5.9 days) and at the same body weight (range of 170 g) (Table 5). The total number of eggs laid and the number of settable eggs laid did not differ significantly between groups. There was a higher incidence

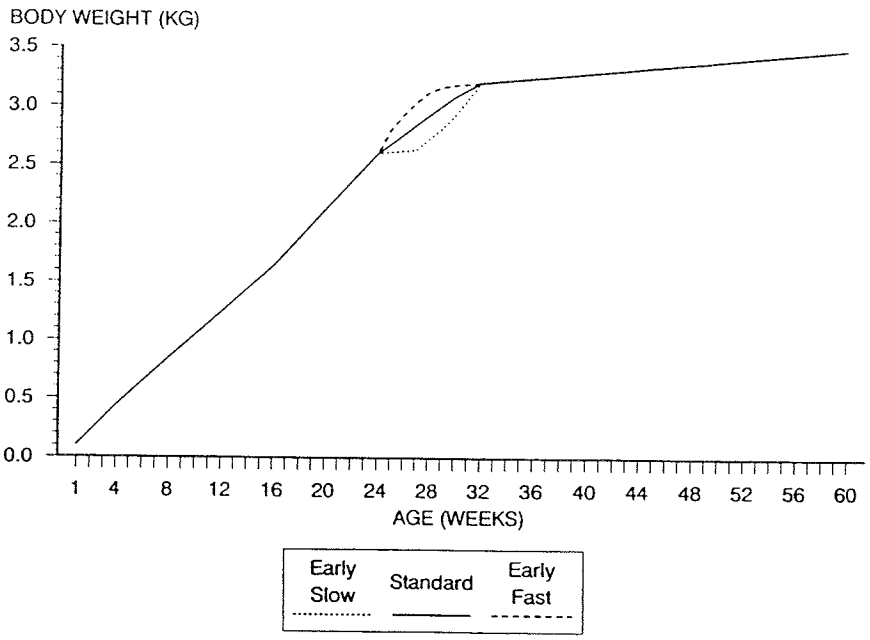

Figure 3. Target body weight of three treatment groups of feed restricted female broiler breeders varying in feed allocation from 24 to 32 wk of age.

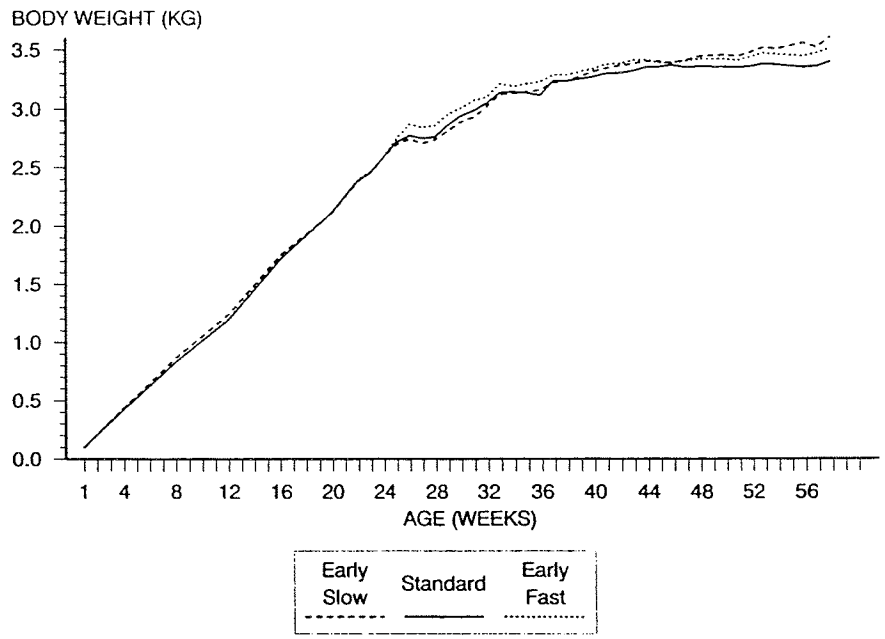

Figure 4. Actual body weight of three treatment groups of feed restricted female broiler breeders varying in feed allocation from 24 to 32 wk of age.

of broken and cracked eggs in the 96% group compared to all other groups. The greatest chick production was seen in the hens that were largest at 20 wk (105%). The 96% group exhibited the lowest rate of chick production (25.5 fewer chicks than from the 105% group). Some of the problem with the 96% group was with fertility, as this group had significantly poorer fertility than the 105% group (83.70%). While overall hatchability and hatchability of all fertile eggs were not significantly influenced by 20 wk weight, there were numerical differences observed that warrant further study with a larger sample size. It was apparent that the smaller hens were overfed and consequently showed signs of obesity. The smaller hens would have a lower maintenance requirement for energy and hence, when allocated the same amount of feed as the larger hens, would have been over-fed.

These trials were conducted in individual cages where there was no competition for feed. In commercial practice, where a larger bird may consume somewhat more that her share or, conversely, where a small bird may not get her entitlement, the situation described here may not be as severe. Nonetheless, whether hens are housed in cages or in floor pens, feed allocation should be done with great care to avoid predisposing the birds to excess energy.

Table 5. Influence of body weight at 20 wk of age on reproductive performance of broiler breeder hens to 58 wk of age.

Parameter	96%	98%	100%	102%	105%
No. of hens	13	15	14	14	13
Age at first egg (d)	175.1	175.7	179.5	173.6	176.8
Body weight at first egg (kg)	2.71	2.71	2.82	2.76	2.88
Total (including unsettable) egg production (eggs/hen)	170.5	174.6	175.5	175.0	180.0
Settable egg production (eggs/hen)	145.5	155.5	156.1	163.7	166.4
Broken or cracked eggs (%)	2.93^{a}	1.30^{b}	1.22^{b}	0.35^{b}	1.12^{b}
Double-yolked eggs (%)	1.35	0.94	0.76	0.97	0.51
Mean egg weight (g)	61.48	63.09	61.00	62.27	61.14
Fertility (%)	79.89^{b}	82.73^{ab}	83.04^{ab}	82.94^{ab}	83.70^{a}
Hatchability (%)	71.19	72.13	76.88	75.49	79.10
Hatch of fertile (%)	88.46	87.19	92.14	90.52	94.17
No. of chicks per hen	94.5^{d}	103.2^{c}	117.6^{ab}	110.8^{bc}	120.0^{a}

$^{a\text{-}d}$Means in rows with different superscripts are significantly different ($P<0.05$).

VI. DISCUSSION

These data support the hypothesis that broiler breeder pullets and hens are vulnerable to excessive follicular development in response to being in a positive energy balance. The effects seen with *ad libitum* feeding are extreme, as the number of large follicles at the time of sexual maturity can increase from 7.8 in feed restricted birds to 12.2 in chronically full-fed hens (Yu *et al.*, 1992b). It would seem that full-fed hens experience difficulty in having this increased number of follicles ovulate normally in sequences. A loss of control of the ovulatory cycle is seen in an increased incidence of double-yolked eggs, multiple eggs laid per day and night time laying. Further problems associated with a disruption of the normal transit time in the oviduct are seen in poor shell quality, which

presumably would contribute to excessive egg weight loss during incubation and, ultimately, in decreased hatchability. Further declines in hatchability may be related to variations in the time spent in the oviduct which may impact on the stage of development of the embryo at the time of lay. Eggs that pass quickly through the oviduct may be less well developed, and hence less capable of restarting the development process after egg storage-induced embryonic diapause. Fertility may be reduced in overweight birds due to mechanical problems in natural mating, or due to a reduced duration of fertility (sperm storage) in over weight females. It is also speculated that when hens are laying erratically the random passage of eggs through the oviduct may impede the transit of sperm from the uterovaginal sperm storage glands to the site of fertilization.

These results also provide evidence that a critical period in feed management in broiler breeders is the time from photostimulation to peak egg production. To maximize egg production, flocks should be subjected to small increases in feed allocation such that hens do not perceive "excesses" in feed allocation and, hence, "store" excesses in energy depots such as the ovary. Small, but frequent, feed increases should be used in the period from photostimulation to peak feeding so as to avoid excessive follicle recruitment. Further research is needed to determine the optimal degree of feed allocation after peak production. The rate at which feed allocation is reduced after peak production may influence the persistency of lay.

In summary, it cannot be overstated that body weight control of broiler breeders remains a powerful tool in improving reproductive efficiency. The importance of having a high degree of uniformity in body weight is great, as even small excesses in feed allocation can negatively impact on egg and chick production. With conditions of poor uniformity in body weight, allocating feed to the flock mean will imply that some birds are being over-fed and others under-fed.

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SIMPLE TECHNIQUES FOR EVALUATION OF PROTEIN QUALITY OF GRAIN LEGUMES FOR POULTRY

K.G. WIRYAWAN AND J.G. DINGLE

There is a need to be able to assess the protein quality of feedstuffs. In this study three short tests, Modified Limiting Amino Acid Score MLAAS (Dingle and Wiryawan, 1994), Net Weight Gain (NWG) and Net Protein Ratio NPR (Bender and Doell, 1957) were compared. Following 2 d adaptation to single cages and diets 7-d-old broiler chickens were given free access for 14 d to drinking water and to one of nine isoenergetic diets containing nominally 100 g crude protein/kg supplied by legume meals and one isoenergetic nitrogen-free diet. Each dietary treatment had eight replicates and chickens were caged in a temperature-controlled room at 31°C ± 0.5°C. Body weight gain and feed intake were measured between 7 and 21 d of age. Results are shown in the Table.

Legumes	MLAAS	Protein Intake (g)	Weigh Gain (g)	NPR	Order of Protein Quality MLAAS	NWG	NPR	Growth Test Category
Soybean meal[1]	55.2	21.52^{ab}	27.20^{a}	3.53^{a}	1	2	1	high
Chickpea cv. Desi	54.4	21.89^{ab}	27.62^{a}	3.49^{a}	2	2	1	high
Chickpea, cv.Kaniv	54.4	24.33^{a}	37.06^{a}	3.53^{a}	2	1	1	high
Green gram	47.0	19.34^{b}	6.61	2.86^{b}	4	5	4	medium
Pigeon pea	46.0	20.11^{b}	9.12	2.88^{b}	5	4	4	medium
Black gram	42.0	22.29^{ab}	1.22^{bc}	2.24^{c}	6	7	8	low
Faba bean	36.8	14.74^{c}	-7.54^{b}	2.80^{b}	7	8	7	low
Field peas	34.6	19.37^{c}	6.98	2.88^{b}	8	5	4	medium
Lupins	27.6	15.00^{c}	-15.54	2.22^{c}	9	9	9	low
SEM	-	1.43	5.06	0.18				

[1]Solvent extracted. The other legumes were unprocessed.

The values with common superscripts in the same column are not significantly different ($P > 0.05$).

The MLAAS, NWG and NPR methods distinguished legume proteins of high, medium and low feed values. MLAAS correlated well with NWG ($r = 0.90$; $P < 0.001$) and NPR ($r = 0.78$; $P < 0.01$) in these diets where processed soybean meal and unproccessed grain legumes were used as the sole protein concentrate in diets for meat chickens. However, it did not predict the exact order of NPR and NWG. Growth was also limited as dietary methionine, the first limiting amino acid, providing only 27 to 55% of the recommended proportion in the protein. Although the results should be interpreted cautiously since a small sample size was used, it is concluded that the MLAAS calculation can be used to estimate the relative protein quality of most grain legumes. However, NWG and NPR are better methods since they detect limiting factors in raw and processed legumes other than limiting amino acids.

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