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CONTENTS

INVITED PAPERS	PAGE
ECONOMIC EVALUATION OF OUTPUT TRAITS GENETICALLY ENGINEERED INTO CROPS USED IN ANIMAL FEED <i>P.E.V. Williams</i>	1
GENETICALLY MODIFIED PLANTS FOR POULTRY FEEDS <i>P.J. Sharp</i>	12
THE MANIPULATION OF INTERMEDIARY METABOLISM BY TRANSGENIC TECHNOLOGY TO ALTER NUTRIENT REQUIREMENTS IN ANIMALS <i>K.A. Ward</i>	18
VARIATION IN NUTRITIONAL VALUE OF CEREAL GRAINS ACROSS LIVESTOCK SPECIES <i>J.L. Black</i>	22
FACTORS INFLUENCING THE ENERGY VALUES OF AUSTRALIAN CEREAL GRAINS FED TO BROILERS <i>R.J. Hughes, M. Choct and R.J. van Barneveld</i>	30
THE METABOLISABLE ENERGY VALUE OF SORGHUM AND BARLEY FOR BROILERS AND LAYERS <i>M. Choct, R.J. Hughes, R. Perez-Maldonado and R.J. van Barneveld</i>	39
FROM JUNGLE FOWL TO FACTORY FLESH: CHANGING PERSPECTIVES ON THE CHICKEN IN CONTEMPORARY SOCIETY <i>G. Albrecht</i>	43
RECENT DEVELOPMENTS IN LAYER HOUSING AND WELFARE IN AUSTRALIA <i>G.D. Stewart</i>	51
ASSESSING THE WELFARE OF HENS AND BROILERS <i>D.M. Broom</i>	61
THE SCIENTIFIC ASSESSMENT OF ANIMAL WELFARE AND ITS APPLICATION TO SOME ISSUES IN THE EGG INDUSTRY <i>J.L. Barnett</i>	71
THE EUROPEAN UNION LAYING HEN DIRECTIVE AND OTHER EUROPEAN UNION DEVELOPMENTS <i>D.M. Broom</i>	79

THE EUROPEAN BAN ON THE PROPHYLACTIC USE OF ANTIBIOTICS AS GROWTH PROMOTERS IN ANIMAL NUTRITION: POLITICAL AND ECONOMIC ASPECTS <i>P.E.V. Williams</i>	83
EUROPEAN POULTRY PRODUCTION WITHOUT ANTIBIOTIC GROWTH PROMOTERS – ONE YEAR ON <i>J. Ratcliff</i>	94
THE APPLICATION OF YEAST AND YEAST DERIVATIVES IN THE POULTRY INDUSTRY <i>K.A. Dawson</i>	100
THE ANTIBIOTIC RESISTANCE REDUCTION EFFECT OF FLAVOPHOSPHOLIPOL (FLAVOMYCIN®) <i>K. Seitz and F. During</i>	106
INFLUENCE OF FEEDING PROGRAM ON BROILER BREEDER MALE MORTALITY <i>S.D. Peak and J. Brake</i>	113
RESEARCH PRIORITIES IN BROILER BREEDER NUTRITION <i>C.J. Weirrusz</i>	121
THE RELEVANCE OF THE ANTIOXIDANT SYSTEM TO THE HEALTH AND GROWTH OF THE DEVELOPING CHICK <i>P.F. Surai</i>	126
LONG PAPERS	
EFFECT OF ROUTE OF INFECTION AND VACCINATION STATUS ON DETECTION OF MAREK'S DISEASE VIRUS FROM BLOOD OR SPLEEN BY POLYMERASE CHAIN REACTION <i>A.F.M.F. Islam, S.W. Walkden-Brown and P.J. Groves</i>	135
EPIDEMIOLOGICAL STUDIES OF <i>CAMPYLOBACTER</i> COLONISATION OF BROILER FLOCKS IN SOUTH EAST QUEENSLAND <i>J.K. Miflin, J.M. Templeton and S.J. More</i>	140
INFLUENCE OF TIAMULIN AND ZINC BACITRACIN ON AVIAN INTESTINAL SPIROCHAETE INFECTION <i>D.J. Hampson, S.L. Oxberry and C.P. Stephens</i>	144
THE AUSTRALIAN OSTRICH INDUSTRY: CRITICAL ISSUES <i>D.J. Farrell</i>	148
PREDICTION OF FEED INTAKE IN MODERN BROILERS <i>P.C.H. Morel, J.A. Timmers, T.A.T.H. De Wit, G.R. Wood, R. Sheriff, B.J. Camden, D.V. Thomas and V. Ravindran</i>	152

UPPER LIMITS OF INCLUSION OF CANOLA MEAL AND COTTONSEED MEAL IN DIETS FORMULATED ON A DIGESTIBLE AMINO ACID BASIS FOR BROILER CHICKENS <i>R.A. Perez-Maldonado, G.W. Blight and J. Pos</i>	156
COMPARISON OF APPARENT ILEAL AMINO ACID DIGESTIBILITY IN MEAT AND BONE MEAL MEASURED WITH BROILERS AND RATS <i>V. Ravindran, W.H. Hendriks, C.A. Butts, D.V. Thomas, B.J. Camden and P.C.H. Morel</i>	160
COMPARISON OF THE INFLUENCE OF THE METHODOLOGY USED FOR DETERMINING THE METABOLISABLE ENERGY OF SOYABEAN MEAL <i>W.D. Cowan, G. Huyghebaert and R.J. Hughes</i>	164
HYDROGEN AND METHANE BREATH TESTS FOR ASSESSING METABOLIC ACTIVITY OF GUT MICROFLORA IN BROILER CHICKENS <i>R.J. Hughes, D.R. Tivey and R.N. Butler</i>	168
GENETIC IDENTITY AND DISTANCE AMONG LONG-TERM SELECTED AND CONTROL LINES OF WHITE LEGHORN CHICKENS <i>S. Kumar, K.G. Kumar and R. Singh</i>	172
SELECTION FOR THE COMPONENTS OF LEAN TISSUE GROWTH IN JAPANESE QUAIL (<i>COTURNIX COTURNIX JAPONICA</i>) <i>H. Sutedjo, L. Knott and R.A.E. Pym</i>	175
EVALUATION OF COMBINING ABILITY OF NATIVE BREEDS WITH SYNTHETIC BROILER LINES FOR LEAN MEAT USING A PARTIAL DIALLEL MATING SCHEME <i>R. Singh, L. Shaktivel and S. Kumar</i>	179
LYSINE REQUIREMENTS OF BROILER BREEDER HENS <i>C. Fisher, R. Gous and L. Goddard</i>	183
THE EFFECT OF WHOLE WHEAT, GROUND WHEAT AND DIETARY ENZYMES ON PERFORMANCE AND GASTRO-INTESTINAL MORPHOLOGY OF BROILERS <i>R.D. Taylor and G.P.D. Jones</i>	187
ILEAL STARCH DIGESTIBILITY OF NORWEGIAN LOW-AME WHEATS IS IMPROVED BY ENZYME SUPPLEMENTATION, FEED FORM AND DILUTION WITH CELLULOSE <i>B. Svihus and H. Hetland</i>	191
WHOLE GRAIN FEEDING AND ENZYMES: A PROMISING OPPORTUNITY! <i>P.A. Geraert, F. Rouffineau, F. Baron and B. Barrier-Guillot</i>	195
ENZYMATIC PRE-TREATMENT OF LUPINS FOR BROILER DIETS <i>A. Ali, I.H. Williams, G.B. Martin and D.J. Harris</i>	199

NON-STARCH POLYSACCHARIDE ENZYMES AND VISCOSITY: THE RELATIONSHIP REVISITED! <i>P.A. Geraert, F. Rouffineau and B. Barrier-Guillot</i>	208
UTILISATION OF DIETARY PROTEIN BY MODERN LAYING HENS <i>A.E. Widodo, E.S. Thomson and J.V. Nolan</i>	212
EFFECTS OF DIET COMPOSITION AND BEAK TRIMMING ON THE INCIDENCE OF CANNIBALISM IN LAYING HENS <i>S. Hartini, M. Choct, G. Hinch and J.V. Nolan</i>	216
OPTIMISING THE TIMING OF INCREASED CALCIUM IN THE DIET FOR LAYING HENS <i>N. Gongruttananun, J.R. Roberts and W. Ball</i>	220
EGGSHELL QUALITY OF CAGED LAYING HENS IN DIFFERENT AMBIENT TEMPERATURES GIVEN DRINKING WATER OF DIFFERENT TEMPERATURES <i>E.S. Tangkere, B. Bhandari and J.G. Dingle</i>	224
EGGSHELL QUALITY OF CAGED LAYING HENS GIVEN DRINKING WATER MAINTAINED AT 20-26°C IN NORMAL AND HIGH AMBIENT TEMPERATURES AND GIVEN FEED WITHOUT OR WITH BARNYARD GRIT® OR EGG SHELL-49® <i>E.S. Tangkere, B. Bhandari and J.G. Dingle</i>	228
SHORT PAPERS	
DEVELOPMENT OF A NON-INVASIVE TEST FOR STRESS IN LAYING HENS <i>J.A. Downing, D.R. Fraser and W.L. Bryden</i>	232
EGG ALBUMEN CORTICOSTERONE CONCENTRATION IN HENS EXPOSED TO HIGH TEMPERATURE <i>J.A. Downing and W.L. Bryden</i>	233
ARTHRITIS AND THE CHICKEN ANKLE JOINT <i>C.A. Lunam and M.J. Gentle</i>	234
CLAW ABRASIVES IN LAYER CAGES <i>P.C. Glatz</i>	235
DIETARY VITAMIN E MODULATES INTESTINAL IMMUNITY <i>W.I. Muir, A.J. Husband and W.L. Bryden</i>	236
OPTIMISING INFECTIOUS BRONCHITIS VACCINATION PROTOCOLS FOR LAYING HENS <i>A. Sulaiman, J.R. Roberts and W. Ball</i>	237

THE EFFECT OF SCALDING ON THE STRUCTURAL INTEGRITY OF EMU SKIN <i>K.A. Weir and C.A. Lunam</i>	238
PRACTICAL APPLICATION OF TOTAL AND DIGESTIBLE AMINO ACID DATA IN THE FORMULATION OF POULTRY FEEDS <i>M. Blair</i>	239
APPARENT METABOLISABLE ENERGY CONTENT OF NEW ZEALAND WHEATS <i>V. Ravindran, D.V. Thomas, B.J. Camden and S.H. Voon</i>	240
INFLUENCE OF WET AND DRY FEED ON BROILER CHICKEN PERFORMANCE UNDER HEAT STRESS <i>F. Shariatmadari</i>	241
COMPARISON OF PROTEIN DIGESTIBILITY DETERMINED WITH EITHER C ₃₆ N-ALKANE OR ACID INSOLUBLE ASH AS INDIGESTIBLE MARKERS <i>X. Li, H. Dove and W.L. Bryden</i>	242
EVALUATION OF DIGESTIBLE AMINO ACID CONTENT OF FEEDSTUFFS WITH EMPHASIS ON NIRS TECHNOLOGY <i>M. Blair</i>	243
ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS IN LONG-TERM SELECTED LINES OF WHITE LEGHORNS FOR PART-PERIOD EGG PRODUCTION <i>D. Sharma and D.B. Reddy</i>	244
EFFECT OF EGG STORAGE TEMPERATURE AND DURATION ON HATCHABILITY <i>J. Ruiz, C. Lunam, P.J. Groves and P.C. Glatz</i>	245
INDIVIDUAL AND COMBINED SUPPLEMENTATION OF WHEAT-BASED BROILER DIETS WITH PHYTASE AND XYLANASE <i>P.H. Selle, P.H. Pittolo and W.L. Bryden</i>	246
LOW GLUCOSINOLATE CANOLA MEALS FOR LAYING HENS <i>P.C. Trappett, K.M. Barram, M.K. Kemsley and R.A. Perez-Maldonado</i>	247
METHODS OF BEAK TRIMMING <i>P.C. Glatz</i>	248

ECONOMIC EVALUATION OF OUTPUT TRAITS GENETICALLY ENGINEERED INTO CROPS USED IN ANIMAL FEED

P.E.V.WILLIAMS

Summary

The use in animal feed of crops containing genetically modified output traits (OPT's) will be governed by the reduction in feed cost that can be achieved from using the novel crop. This will be the case until the new crop can offer some specific nutritional advantage over traditional dietary components. Least cost formulation is the driver in this instance. Input traits (IPT's)(herbicide and insecticide resistance) offer considerable benefits to growers. The economic value of nutritional OPT's is limited by the shadow price of standard raw materials. Economic evaluation suggests that the use of crops containing nutritional OPT's will be severely limited until the OPT can be stacked with existing IPT's and a number of OPT's can be stacked to achieve additional benefit.

I. INTRODUCTION

The debate surrounding genetic engineering of crops has taken an unexpected turn. It was probably never envisaged that a premium would be paid to guarantee that the crops used to produce compound feed had not been genetically manipulated or were not contaminated with genetically modified (GM) material. Bennett (2000) reported that feeding non-GM soyabeans and corn to UK livestock would cost producers £61m/ year if it became required by retailers. Hardest hit would be pig and broiler producers. The European Union (EU) calculated that keeping non-GM and GM crops separate from the farm gate to the consumer could raise raw material costs by as much as 6 - 17%, depending on the grains and separation systems involved (Farmers Weekly, 2000). The GM issue in all aspects is one of the most contentious recent scientific debates. The argument surrounding these issues is essentially risk versus benefit. The object of this manuscript is to examine one particular issue and that is the assessment of the economic value of the genetic traits. The genetic manipulation of crops is defined by the type of trait, agronomic traits related to the growth and protection of the plant (input traits (IPT's)) or quality factors related to the composition of plants (output traits (OPT's) or quality traits). It is the development of OPT's in crops with respect to animal production which is of greatest interest to the animal nutritionist. Below are listed six categories of OPT's adapted from those proposed by Barre and Aumaitre (1998) which would be of interest with respect to improvements in nutritional value:

- reduction or suppression of anti-nutritional and toxic factors in plants
- increase in the resistance of plants to cryptogamic diseases reduction in plant components having low digestibility (modify the fibre fraction)
- increase in nutrient density, protein and/or energy content
- increase biological value of protein by improving the profile of amino acids to achieve a composition closer to animal needs thus reducing nitrogen excretion
- improve mineral availability in the plant (e.g. incorporate phytase).

A wide range of potentially modifiable OPT's have already been identified. In a recent literature survey, 34 different traits were identified of which 20 were associated with the oil content of the seed. However, genetically modified plants will offer no interest to animal production unless the new traits will in some way significantly reduce the cost of feed

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formulation. Barre and Aumaitre (1998) stated that the cost benefit must be attainable in all the countries involved, to avoid delocalisation of animal production.

II. THE GENETIC MODIFICATION ISSUE

Before considering the issues raised in capturing the benefit of GM traits it is worthwhile reviewing the present situation and the problems caused by the unwillingness of European consumers to accept genetically modified crops. Fuelled by a lack of consumer confidence in modern biotechnology retailers have insisted that animal produce is derived from animals that have not been fed GM material or material containing GM DNA. The EU has defined a GM feedstuff (GM) as being "any biological entity capable of replication or of transferring genetic material in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural combination" (Directive 90/220; EC 1990). In order to comply with this directive work has been carried out to examine the viability of DNA from feedingstuffs that have been processed for animal feed (Forbes *et al.*, 1998). Forbes *et al.* (1998) reported that GM DNA in expeller linseed and in expeller and solvent extracted soyabean meals was degraded during processing. The interpretation of the Directive differs dramatically in different European countries. On the one hand, the Dutch consider that processing will effectively destroy GM DNA and, therefore, corn gluten derived from GM corn may be used in feedingstuffs, whilst the French will not permit the use of material containing any GM fragments. In France it is not permitted to use any non-registered GM crop or material derived from such plants. To offset the great difficulty in guaranteeing freedom from any GM derived DNA retailers have stated that they will accept a degree of inadvertent inclusion of GM material. This level of inclusion appears to range from 1 to 5%. However the precedent has been set in Europe that consumers have a right to choose meat and produce from animals that have been given feedingstuffs free of GM material and it is this which is driving the premium for non-GM crops. In response, identity-preserved supply routes are being set up but because of the world-wide acceptance of the benefits of GM input traits to the grain producers (see later), there is now additional cost to producing the non-GM alternative. Bennett (2000) stated that in future "GM feeds will cost less than non-GM's". US growers can achieve benefits in terms of yield, ease of management and reduced use of crop chemicals by growing GM crops. Bennett calculated that the present premium for non-GM crops is approximately 8% of the value of the crop. The reduction in gross margin obviously varies according to the use of different crops in the manufacture of feedingstuffs.

III. INPUT TRAITS AND THE MAJOR BENEFITS FOR GROWERS

There are three major criteria which farmers consider when deciding on a strategy for planting crops with new agronomic traits. The first is the opportunity to enhance profit on the farm. The second is the reduction in the cost of inputs. When genetically engineered herbicide resistance is incorporated into crops, management is made easier and less expensive for growers since the number of herbicide applications to the crop is reduced. The third is the ease of incorporation of the new technology into existing farm operations. The rapid adoption of IPT's is in no small way due to the very rapid and quantifiable capture of the value of the trait by the farmer, apart from the value gained by the seed producer who can effectively ring fence the use of branded insecticides and herbicides. Gene stacking will allow the combination of these two traits with subsequent major advantages for the grower.

Bt corn containing the insecticide protein derived from *Bacillus thuringiensis*, was introduced in 1996, and was presented to the market in 1997, whereupon it achieved high market penetration and high grower satisfaction. The introduction of this trait not only

addressed areas with known European corn borer (ECB) problems but also suggested that ECB was causing more wide spread yield losses than had previously been anticipated. All major seed companies marketed Bt corn in 1997, although the Bt trait came from different events and different sources. Approximately 5.5 million acres of Bt corn were planted in 1997 and it is estimated that supply could accommodate the planting of 20 million acres in 1998 with predictions that 60% of corn acreage will be planted to Bt corn in the year 2005.

All seed companies are pricing Bt hybrids at a premium over conventional hybrids. Bt hybrids were selling for a premium of approximately \$5-10 per acre or \$15-30 per unit. In 1997 farm performance of Bt corn indicated that yield increases were in the range of 10-30 bushels (bu) per acre over conventional non-Bt hybrids. The benefits provided for the grower are closely tied to the value of the corn and are greatly diminished in years of either low ECB infestation or low corn prices. Compared with control using insecticides, the net economic profit from the Bt trait will range from at least \$8 to \$15 per acre.

It is estimated that the increased yield achieved with Roundup Ready soyabeans is of the order of 1-2 bu/acre (Wheat 1998). This plus the savings on herbicides amount to an increase in income amounting to 30-50 cents / bu., or between \$11-18 per metric ton even allowing for the premium payments on the genetically modified seed (Wheat 1998). Roundup Ready seed is priced so that the farmer is likely to see several dollars in savings per acre. With time additional benefits are being realised. Hartnell and Fuchs (1999) reported that research from Iowa State University and the USDA showed 96%, 54% and 64% reduction in the severity of insect damaged ears with the Bt gene in studies in 1994, 1995, and 1996. There were associated reductions in visible mold and rot, and reductions in mycotoxigenic ear molds.

Thus, for seed producers and growers the success of IPT's is immediately evident. They offer a rapid, substantial economic return. The value of the technology can be calculated and to date there has been no recorded negative impact on yield. The technology is easily incorporated into existing practise and there has been high grower satisfaction. The above examples indicate the level of return which can be achieved and which are the base line for the value of any alternative trait that would either be included instead of, or stacked with, nutritional traits. The advantage to the agronomic traits described is that there is no alteration in the composition of the grain which could influence returns, for example, to the soyabean crusher, and there is no need for segregation and identity preservation.

IV. OUTPUT TRAITS AND THE CHALLENGE OF VALUE CAPTURE

The reasons why growers may adopt output/quality traits, are, somewhat different to those that drive the adoption of IPT's. Again, the first and foremost criteria is profit enhancement, Secondly, farmers are keen to be part of any emerging technology and, thirdly, is risk management with a desire not to be left behind. For most if not all of the quality trait crops, the need to identity to preserve them, coupled with the current tendency for these crops to experience some yield lag has resulted in premiums being paid to producers to contract-grow them. Generally, there are three types of costs associated with quality traits; 1) grower premiums / incentives, 2) identity preservation costs and 3) end user costs of adoption. To be sold in significant volume, a feed quality trait needs to be adopted by at least two end user segments, poultry, pig, beef, dairy or export. Without broad appeal a quality trait cannot become a major percentage of production. For traits to have broad appeal in the USA they must compete directly with the two major crops, standard corn, of which approximately 72% is used for feed production, and soyabeans, of which 80% is in some way used in feed production. Furthermore, corn and soyabean are the primary targets for development of output traits in feed crops.

Producers have become accustomed to being paid premiums for anything new, novel or under contract. Assuming that growers will act rationally, they will require a premium or some other form of compensation for the increased risk of growing a quality trait. Typically these premiums have been at the level of \$0.15/bu for waxy corn to a high of \$18.00/bu for organic clear hylum soyabeans. The majority of premiums fall in the \$0.20 to \$0.50/bu. Typical premiums for speciality grains without any yield lag are expected to be in the region of \$0.15/bu minimum. There are few speciality crops without some sort of yield lag that would need to be additionally recompensed.

In order to capture the value of an output/quality trait when used as a major component in animal feed, changes will be required in the infrastructure of the feed business. Capture of value from OPT's is more complex compared with IPT's since there are more links in the value chain. For IPT's there are typically three links in the chain, the gene-engineer, the seed producer and the farmer who grows the crop. For OPT's there may be as many as four additional participants in the chain (grain storage, grain treatment, compound feed producer, livestock producer). In order to achieve a situation in which the nutritional benefit imparted into the crop is transferred to the livestock producer it is essential that at each stage of the movement and processing, each of the participants who contribute in the process can gain economic benefit. It is even more important that none suffer any financial loss or are in any way compromised by handling the crop.

A recent study (Maltsbarger and Kalaitzandonakes, 2000) has paid particular attention to the hidden costs associated with identity preservation. They indicate that differences in local supply conditions and asset configurations at the farm, elevator and processor can produce substantial variation in segregation and identity preservation costs. Under-utilised capacity was a major concern for elevator managers considering IP. Three case studies of elevators of differing configuration were studied involving segregation of high oil corn (HOC). The additional costs of segregation were up to 36 cents/bu, which is considerable. However, the lowest cost of 16 cents/bu was achieved with an elevator which had large capacity but split between many storage bins. The reduction in IP preservation cost was achieved through greater flexibility in filling patterns to maximise storage utilisation within the batch-processing IP system. The most interesting scenario for the smallest elevator was achieved when the entire facility was dedicated to the processing of HOC but even then it did not achieve the lowest per bushel cost. At the other extreme the largest facility suffered greatly from under-utilised capacity.

Identity preservation starts with segregation of the crop on the farm where it is grown. New storage capacity or modification of current capacity may be needed. Changes include the way the quality trait is delivered from the farm to the end user and in the way end users make feed. Identity preservation places new responsibilities on the traditional commodity grain system. The costs associated with identity preservation include:

- Additional storage: reduced turnover, partially full bins, delivery over time vs. all at once. Separate harvesting together with additional separate storage space will be required on farm. On-farm storage with flexibility to handle smaller lots of speciality crops is usually limited and storage flexibility will severely limit the number of farms that can participate in the production of crops with specific agronomic traits.
- The logistics of handling speciality crops during the harvest poses an additional problem since this is a period of major pressure with the high volume of grain that must be processed in a limited time.
- Separate storage facilities will severely limit the adoption of transgenic crops. In the United States grain handling systems are set up to handle large volumes and the normal bin size in elevators is between 100,000 to 300,000 bushels.
- Risk management : quality traits cannot be hedged exactly ; contamination risks.

- Transportation: containment; transport identification; transportation is via trainloads where the unit is 75 + cars.
- Analysis: testing and identification of the improvement in the quality trait.
- Marketing: identification of specific buyers, contracting, co-ordination of delivery.
- Consumer specifications: the reluctance of consumers to adopt GM material in the feed chain will further add to identification costs.
- The hidden cost of blending. In the traditional system, high and low quality grain lots are often blended together in order to achieve an overall quality which just achieves the required standard.

Typical costs for identity preservation of corn and soyabean meal are shown in Table I. These costs compensate the grower, the distribution system and the processor. However, the technology developer and/or seed supplier and end user have not been rewarded and the end user has only paid for costs incurred. For corn this represents a minimum of an additional value of 10\$US/tonne in order to adequately compensate all the participants in the chain and for soyabean, 28\$US/tonne to compensate for the additional participants in the chain.

Soyabean production is a particular case where additional consideration must be given to the value chain. Oil and meal are two co-products from the harvesting of soyabean meal. The oil is used as a high value product in human nutrition with just 5% for industrial purposes and 97% of the meal is used for animal feed. However, the price of the oil and meal is significantly influenced by world markets of other commodities. The world price of food grade oil is in competition with palm oil and the meal competes with fishmeal and rapeseed. Soyabean crushing and the extraction of oil from the bean are crucial steps in processing and the profitability of the oil crushing process is critical.

Table 1. Costs to move quality traits through the system.

Description	Corn		Soyabeans	
	Low end (\$/bu)	High end (\$/bu)	Low end (\$/bu)	High end (\$/bu)
Grower premium	0.10	0.40	0.25	1.00
Identity preservation	0.10	0.25	0.10	1.80
Cost of adoption for end user	-	0.05	-	0.03
IP at processor	-	-	0.05	0.80
TOTAL	0.20	0.70	0.40	3.63

It is the oil content of the bean that drives the price received by the grower since they are penalised if the oil content falls below specific norms. The oil content of the bean, therefore, becomes a critical factor in defining the value of the crop and cannot be permitted to fall at the expenses of an added trait.

There is one caveat to the above when the challenges to infrastructure do not apply and that is when the feed quality trait is to be fed directly on-farm. The barriers in terms of infrastructure may also cease to apply in situations where an integrated end user can contract sufficient acres near a local feed mill, assuming that the product can be stored on the farm or at the mill.

One of the most important factors in limiting the numbers of growers who adopt either speciality or bio-engineered grains will be the changing value of the crop itself. A premium of \$0.3/bu for speciality grain when the commodity grain is valued at \$3.00/bu represents a 10% increase in value. However if the commodity crop is valued lower at \$1.75/bu the same premium represents a 17% increase in return, which is obviously more attractive.

V. HIGH OIL CORN, A MODEL FOR VALUE CAPTURE OF OUTPUT TRAITS

High oil corn (HOC) is a relatively recent development in crop production, which has been rapidly adopted by growers and feed compounders as a result of the benefits that can be realised in feed production. Although HOC was a trait achieved by traditional selection and not developed by genetic engineering, the capture of the additional economic value in the crop is an excellent example of the levels of value essential for adoption of the technology and the constraints which may be encountered. In the first instance the problem of yield lag, which is an immediate constraint and disincentive for the grower, was overcome by using the Top Cross technique

In HOC a double benefit is claimed, the oil concentration rises from 40 to 70 g/kg and there is an additional 10 g protein/kg (88.6 to 97.5 g/kg) with additional methionine and lysine. Although there is a reduction in starch content of the grain, the overall metabolizable energy content increases by .74 MJ/kg (equivalent to + 4.5%) for poultry. The higher energy and protein content increases the energy and nutrient density of the grain permitting an increase in overall nutrient density or alternatively the use of cheaper feed ingredients as a higher proportion of the ration. The improved protein quality with higher levels of lysine and methionine also allows a reduction in the supplementation with synthetic amino acids.

The primary determinants of the value of HOC as a feed ingredient are the price and nutritional value of normal corn (2-yellow dent corn) and the price of the alternative energy sources of which the most important is fat. Figure I shows the increase in value compared with 2-yellow dent corn when HOC is used in a typical broiler grower ration.

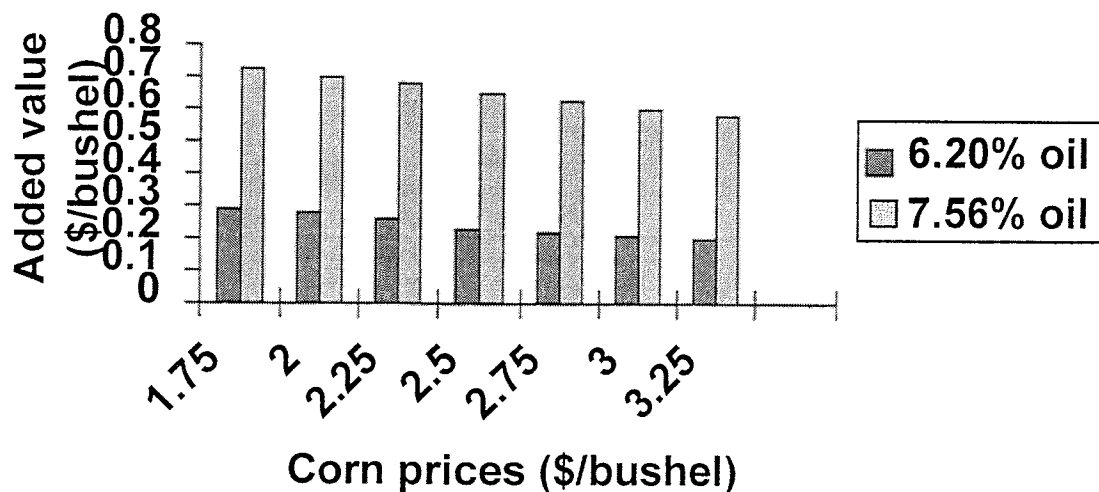


Figure 1. The influence of the price of corn on the added value of High Oil Corn in broiler feed.

There are two key points. It can be seen that there is a substantial increase in the value of the HOC but the increase in value is dependent on the price of the grain. When there was an 85% increase in the price of the grain the added value of the HOC grain fell by between 20-30%. This would at first appear illogical. However, as the price of the grain increases, it is the influence of the least cost formulation procedure that selects alternative energy sources and, hence, the value of the new trait is reduced. The situation is the reverse when the cost of alternative feed ingredients increases. The cost of supplementary fat has a major influence on

the value of HOC. Elevated prices for fat increase the value of the extra energy in HOC. Regional differences in the price of fat will also influence the potential markets for HOC.

The importance of these examples is to demonstrate that there are several factors that have significant influence on the value of the added trait. Feed formulations are derived by least cost formulation and both the cost of the basic grain and the cost of the potential alternative feed ingredients will significantly influence the value of the trait.

Finally, the value of HOC is also influenced by the effect that the oil content has on manufacturing feed costs. With HOC there is additionally, a potential 12% increase in grinding efficiency plus other factors such as dust reduction, mixing efficiency and pellet quality. All these factors need to be taken into consideration to determine the final value of the added trait. In order for HOC to become widely adopted it must be attractive to both integrated poultry and pig producers. Allied to this is the fact that the commodity fat market would be reluctant to allow significant penetration into these segments unless an alternative use is found for this commodity. Thus, with such a wide range of factors to take into consideration, as well as the dynamics of feed ingredient pricing, it is difficult to provide a precise value for HOC on which to base an investment plan. However, this type of evaluation is essential before the value of a new OPT can be precisely quantified in animal nutrition.

VI. DEVELOPMENT OF PROTEIN AND AMINO ACID TRAITS IN SOYABEANS

Research to genetically modify soyabeans has targeted four broad areas: a) improvement of protein concentration and/or essential amino acid composition, b) reduction and elimination of anti-nutritional factors, c) improvement in the profile and composition of soyabean oil, and d) development of disease resistance, herbicide resistance and insect tolerant lines. In least cost formulations soyabean meal has a high value due to its ability to supply lysine that is often a first limiting amino acid in formulations. Increasing the methionine content of soyabean meal would improve the biological value of the meal.

Three alternative approaches have been considered for increasing the methionine content of soyabeans using genetic engineering. The advantages and disadvantages of each approach cannot be evaluated until samples of the meal are obtained and tested *in vivo*. The three methods described are:

- Increase in the free methionine content.
- Insertion of a foreign protein with a high methionine content.
- Replacement of non-essential amino acids in an endogenous protein with methionine.

There is a limit to the level of methionine that can be inserted into the plant since the high sulphur content of the amino acid results in high levels of methionine being toxic to the plant. The type of insertion may also influence the availability of the amino acid.

Modification of the protein and amino acid composition of soyabean meal is important because the value of the meal represents approximately 65% of the value of the bean. Chung and Pettigrew (1998) considered that high-protein soyabean meal had the greatest potential for technical feasibility while the remaining alternatives still required significant research progress for their commercialisation. High-protein soyabean meal has the potential to reduce the proportion of meal used in diets for both pigs and poultry. This compares with the situation when individual amino acids are the target. High lysine, with a 1.3 % increase in lysine content in the meal, would result in a lower proportion of meal in the diet, a higher proportion of feed grains and no supplemental synthetic lysine. The meal would be of greatest benefit to pig producers and there would be little advantage to the poultry industry. Alternatively, for high-methionine soyabean meal with a 0.32% increase in methionine content, the primary interest would be from poultry producers who would be able to reduce reliance on supplemental synthetic methionine.

These examples of modification of amino acid content demonstrate that the market application of specific traits can be highly specific and that raw materials that presently have universal application can rapidly be restricted to specialised markets. Storage and identity preservation of such materials for feed formulation in mills that produce feed for either pigs or poultry then becomes an additional consideration.

Chung and Pettigrew (1998) evaluated imputed prices (level of specific nutrient x shadow price) of the new soyabeans in a number of formulations to determine the price premiums which could be expected for a range of soyabean meals with different OPT's. The estimated imputed prices are considered as the maximum amount of premium prices that livestock producers can pay for each alternative soyabean meal. If livestock producers can gain extra benefit, the estimated prices are the premium prices in the market. Table 2 is adapted from the data presented by Chung and Pettigrew (1998). Their analysis is based on industrial prices for supplementary amino acids and mean commodity prices in the period January 1990 to December 1992. The objective of presenting this data is to compare the magnitude of the different premium estimates, rather than to consider the actual values that alter considerably, based on the price of raw materials.

Table 2. Imputed prices for a range of different soyabean meals.

Animal	Stage	Base model feed cost (\$/ton)	Cost savings (\$/ton)		
			HPSM ¹	HLSM ²	HMSM ³
Turkeys	0-4 weeks	148.16	7.97	2.89	4.08
	8-12 weeks	132.32	5.50	5.63	2.83
	16-20 weeks	115.21	3.44	0.07	0.74
	Weighted average	124.99	4.72	2.11	1.89
Broilers	0-3 weeks	148.11	6.44	0.48	3.30
	6-8 weeks	122.21	4.11	0.48	1.75
	Weighted average	129.77	4.84	0.48	2.28
Layers	0-6 weeks	115.63	1.63	0	0.66
	14-20 weeks	99.97	0.46	0.48	0.20
	Laying	105.84	0.99	1.03	1.23
	Weighted average	105.77	0.97	0.89	1.04
Swine	10-20 kg	127.95	5.28	0	NT
	50-80 kg	109.62	1.65	2.42	NT
	Weighted average	111.19	2.05	2.19	NT

¹HPSM; high-protein soyabean meal: ²HLSM; high-lysine soyabean meal: ³HMSM; high-methionine soyabean meal.

There is a wide range in value that can be captured from the modified crop, which severely limits its market potential. It would appear therefore that these crops would need to be produced for particular species specific, niche markets. In each instance, the highest level of benefit is found in the diet requiring the highest concentration of protein and highest amino acid specification, thus in the early growth phase. However, since this is the lowest volume of feed produced, compared with the later growth and finisher phases, the overall value is strongly weighted to that value which can be achieved in the late growing animal.

Based on this analysis, the results indicate that the highest additional value that could be achieved for the HPSM diet was 11% above the conventional soyabean price. Thus, any premium paid by livestock producers would not be likely to exceed 11% of the price of commodity grain. Again it must be emphasised that these evaluations are based on current

market prices of synthetic amino acids. The key question is whether an 11% increase in the value of soyabean meal would be sufficient for all the players in the chain leading to the production of the meal to gain sufficient benefit to invest in the identity preservation of the meal. Faced with such a challenge there would undoubtedly be an adjustment in the price of synthetic amino acids, reducing the shadow price with a consequent decline in the imputed price. Changes in the availability of alternative protein sources such as meat and bone meal also resulted in relatively large effects. This is exactly the same situation that was described for HOC, where the price of alternative energy sources such as fat had a major influence on the additional value of the oil in the grain. Since these alternative sources of energy and protein are by-products of the rendering industry it must be expected that the price of such materials could and would be adjusted downwards in the face of a challenge from alternative sources arising in plants.

Estimates were made of the increased value of transgenic soyabean meal, based on a value of soya of 200 \$US/tonne, current market prices for lysine and methionine, and containing increased levels of both lysine and methionine targeted to markets for either pigs (+30% methionine and +40% lysine) or poultry (+100% methionine and +10% lysine). For pigs the increased amino acid content was worth approximately 24 \$US/tonne and for poultry 20 \$US/tonne. In neither case was the increase in value in excess of 28US\$/ton which was the minimum premium needed to compensate all the participants in the chain in order to identity preserve the meal.

VII. BETWEEN TRAIT ECONOMIC COMPARISONS

An economic study was made using least cost formulation comparing 6 different kinds of corn. The effects of the added traits on feed costs were determined for each of the modified traits for pigs and poultry (Baumel *et al.*, 1999). However, the analysis was performed assuming that the cost per bushel was the same for each of the corn varieties containing the added-value traits. The corn modifications were: increased protein +80 g/kg (from 87 to 167 g/kg dry matter basis); enlarged germ size (from 11.1 to 27.1 % of kernel size); +8% in starch digestibility in poultry diets; doubled methionine content (from 2.1 to 4.1 g/kg dry matter basis); doubled lysine content (from 3.0 to 6.0 g/kg dry matter basis), and doubled available phosphorus (from 0.7 to 1.2 g/kg dry matter basis) (Table 3). The additional cost of producing the speciality corns (yield lag, seed cost, handling) was estimated at \$0.35 per bushel and was subtracted from the added value of each corn to provide a net added value for the modified corn. The net value of each of the modifications is shown in Table 3 adapted from Baumel *et al.* (1999). A negative net value indicates that the additional costs (35 cents) exceeded the value of the modification of the feed, a point which has been raised earlier. None of the modifications had positive net values in formulations for pigs. The highest feed cost savings of \$11.75 per ton of feed was for formulations utilizing corn with increased protein content followed by modifications in corn starch digestibility, which reduced the cost of feed by \$7.68 per ton. The increased corn germ size lowered feed cost by \$7.06 per ton of feed. The methionine-enhanced corn saved \$1.57 per ton in poultry rations and the lysine-enhanced corn \$2.10 per ton for swine diets. The corn with the increased available phosphorus yielded the minimum saving of only \$ 0.12/ton. The poultry diets were the only diets to have positive net values for the six corn modifications. The high protein and enlarged-germ corn had relatively large positive net values in broiler and tom turkey rations. The diets containing the corn with increased starch digestibility gave an added value of \$.048 per bushel for broiler rations only.

Table 3. Net value of each of six genetic modifications of corn in pig and poultry rations in cents per bushel.

Modification	Pigs			Poultry	
	Piglets 4 – 6kg	Finishers	Broilers	Tom turkeys	Layers
High protein	-5.6	-19.5	22.3	10.0	-8.0
Enlarged germ	-35.0	-24.8	12.9	9.2	1.2
High starch digestibility	-	-	4.8	-1.6	-3.9
High methionine	-	-	-27.6	-31.0	-29.3
High lysine	-35.0	-29.8	-	-	-
High available phosphorus	-33.3	-33.4	-	-	-

It is important to note how the economic values of the traits differed according to the dietary use made of the crop. It is obvious that where a range of economic values is obtained for a single trait, in order to obtain maximum use of the crop it is the lowest value which will allow maximum usage. It is impossible to achieve differential costings for a single raw material. Furthermore, not only would the traits need to be identity preserved, some actually impart an apparent negative value when they are not appropriate for a particular species. Such segmentation of the market for a raw material such as corn would require dramatic and improbable alterations in the infrastructure of the cereal raw material markets.

IX. STACKING AND MULTIPLE TRAIT INSERTION

The analysis of agronomic IPT's at the start of this manuscript identified the major benefits for the grower. Therefore, until a combination of IPT's plus OPT's can be inserted and stacked into plants there will always be competition between the choice of the grower for immediate benefit brought about by IPT's, compared with the down stream benefits in nutritional value from OPT's which must be shared amongst the participants of the feed production chain. For growers, therefore, it is likely that IPT's will always be the traits of first choice.

Although techniques of gene insertion are now taken as a matter of course the simultaneous transfer of several genes is less common since technically it is more difficult. However, recent developments suggest that simultaneous insertion of agronomic plus OPT's may soon be possible. Using ballistic bombardment with plasmids each containing a transgene, Chen *et al.* (1998) succeeded in inserting 13 transgenes into a rice plant. Of the 125 plants grown from the tissues, 85% contained at least two new genes, and 17% contained more than nine insertions. The work demonstrated for the first time that in excess of 300 kb of foreign DNA could be inserted into a plant without affecting its morphology, growth or fertility. These results offer significant promise for the eventual introduction of combinations of traits. Nutritionally important OPT's, which could not compete alone in terms of value when the benefits from agronomic characteristics are an alternative, will become a more attractive choice when there is the option of stacking both OPT's and IPT's compared with making a choice between one or the other.

X. CONCLUSIONS

Least cost ration formulation is the corner stone on which feed formulation is based and this system attributes simple economic values to specific quantities of available nutrients. Many of the nutrients that are under consideration for transgenic insertion into plants are

presently supplied as commodities with defined prices and nutritional values. Prime examples are the synthetic amino acids, lysine and methionine. The presence of such readily available and defined commodities places severe limitations on the additional value that can be gained in plants from OPT's. Furthermore, for certain traits the value is highly sensitive to the price of the crop and to competing raw materials. The capture of the value is technologically and logistically challenging. Insufficient value within a trait, to share within the members of the feed production chain, may well limit the adoption of the technology. However, these problems apart, the present environment is such that consumers, particularly in Europe, have signalled that they are unwilling to accept this new technology. It is important to heed the recommendations made in a recent publication supported by a number of international academies of science which recommended that transgenic crop research should focus on plants that (i) improve production stability, (ii) give nutritional benefits to the consumer, (iii) reduce environmental impact of intensive and extensive agriculture, and (iv) increase the availability of pharmaceuticals and vaccines (The Royal Society, 2000). Unfortunately, OPT's for use as previously described in animal nutrition, do not figure highly in this list.

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GENETICALLY MODIFIED PLANTS FOR POULTRY FEEDS

P.J. SHARP

Summary

Plant breeding, both conventional and by the production of transgenic plants, has and is developing grains more suited as animal feed, including poultry feed. Past progress has focussed on easily determined feed quality traits. Present progress is using more sophisticated analysis methods. Future development will rely on clearer definitions of the requirements for animal feed and research on the constituents of grains that affect these requirements.

I. INTRODUCTION

The development of crop varieties with targeted qualities that make them more suitable for animal feeding purposes is a relatively recent phenomenon. It has occurred for longest in grain sorghum, where selection for lower tannin content has increased digestibility and palatability (Tsaftaris, 1997). It is much more recent in other grains where the animal feed classification is a lower value grade used for grain not fitting the demanding specifications for human food uses.

This paper reviews some of the history and current state of breeding crop plants for feed quality traits. Only work on grain traits is considered as these are of interest to the poultry industry. Research on forage quality traits and the manipulation of these in grasses and legumes is proceeding, with benefits for grazing animals.

II. CONVENTIONAL PLANT BREEDING

Conventional plant breeding for most major commodity grain crops has now proceeded in the West, with various levels of input, for between seventy to over a hundred years. Considerable increases in commercial yields and reliability of production resulted from the combination of both improved cultivars and agronomic practices, with feedback between the two factors (Duvick, 1984; Evans, 1993). This increased production, other technical and infrastructure advances, and economic pressures contributed to the development of intensive food animal rearing as a major industry.

Conventional plant breeding is also having an influence on feed grain quality by targeting components known or thought to modify grain feed value. The most obvious of these is the strong attachment of the relatively indigestible husk or hull to barley and oat grains. The simple genetic control of the presence versus absence of the husk or hull on the thrashed grain has enabled the development of "naked" cultivars of both barley and oats. Commercial naked barley and oat cultivars are available overseas in the UK, USA and Canada (Mannsell, 2000) as well as in Australia (van Barneveld *et al.*, 1998), and have increased digestible energy per unit volume. Naked oats, in particular, is highly suitable for broilers (Maurice *et al.*, 1994) but they have harvest problems with shedding of grain from the crop (Perry, 1995). In addition, cultivars with thin husks and with higher oil content are available in the UK (Mannsell, 2000).

Another specialty crop developed by conventional plant breeding is "waxy" corn or

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maize. This single gene trait results in the virtual absence of amylose (linear starch) from the endosperm starch, so that the starch is, therefore, composed entirely of the branched polymer, amylopectin (Ferguson, 1994). The difference can be assayed easily by iodine/potassium iodide staining of the grain endosperm. Though initially developed in the 1940s as a tapioca replacement, and largely used for the isolation of "waxy" starch for many food uses, it also finds a good market due to its higher (than non-waxy) feed efficiency for most animals, including poultry (Ferguson, 1994). Waxy barley is also available in commercial cultivars (Blatty, 1997) but these have higher than normal β -glucan levels and are, therefore, not favoured as feed, especially for poultry.

High lysine genotypes of maize and barley are available, based on the *opaque-2* and *hipoly* genes, respectively. In maize, breeding effort in the USA has overcome many of the deleterious effects of the original *opaque-2* sources and has resulted in the development of Quality Protein Maize (QPM). Quality protein maize is in quite extensive commercial use in a number of countries with more than a million tons produced in the USA mostly for on-farm animal, mainly pig, feeding (Vasal, 1994).

III. ACCELERATED PLANT BREEDING

Conventional plant breeding is undergoing something of a revolution that is enabling an acceleration of progress. This is coming about by the application of the growing body of knowledge of plant genomics and biochemistry and the availability of molecular tools for selection in plant breeding. In recent times this has resulted in improved "output traits" such as grain quality for animal feeding. It relies on two processes; germplasm screening by high throughput analytical tests for trait identification, and subsequent use of DNA-based molecular markers to incorporate the traits into commercial cultivars (Mazur *et al.*, 1999).

Mazur *et al.* (1999) have indicated success with this process in two cases, soyabean and maize. In soyabean, screening germplasm and mutated lines found lines with low levels of antinutritional oligosaccharides. Combining these sources led to even lower levels and the lower levels were transferred into commercial high-yielding plants with DNA marker assistance. Feed as well as other uses are envisaged (Mazur *et al.*, 1999).

The development of commercial crops of high-oil corn is a current commercial success in feed grains. This started with the now classic high-oil lines of corn developed by long-term recurrent selection at the University of Illinois (Alexander, 1988). The lines are very poor agronomically but their grain has very high feed value, including for poultry (Lambert *et al.*, 1994). Quantitative genetic studies of the high-oil lines indicated that the trait was controlled by more than 12 genes so that conventional breeding to produce well adapted and high-yielding cultivars would be extremely difficult. To overcome this the TopCross® crop method was developed (Mazur *et al.*, 1999). This involves commercial fields containing a low density of the (agronomically inferior) male-fertile high-oil line with a normal density of an elite F1 hybrid that is male-sterile. The cross-pollination of this later line by the inter-planted high-oil line results in high yield of grain with up to a doubling of grain energy content compared with normal hybrid corn. This system has now performed well in a wide area over a number of seasons and is a highly successful product for DuPont Agricultural Products in the USA.

These examples discussed so far partly illustrate an important point concerning plant breeding. Intimate knowledge of the phenotype desired and rapid ways to select for that phenotype, as early in the breeding cycle as possible, are necessary for progress. Other examples will result in products in the next decade.

Mutants of maize and barley with low phytic acid levels in the grain were isolated in the 1990s (Larson and Raboy, 1999). The genetic control of this trait is simple, by one or two *lpa* loci. This, coupled with the availability of rapid screening tests (Rasmussen and Hatzack, 1998) that rely on the presence of high inorganic phosphate levels in the *lpa* grains, means that the introgression of this trait into commercial cultivars will be relatively rapid. Low phytate grains will have nutritional value in animal feed, with higher phosphate uptake and higher bioavailability of minerals and proteins, and have environmental advantages as a result of the lower phosphate load in effluent from animals fed such grain (Ertl *et al.*, 1998; Mazur *et al.*, 1999).

The high lysine trait in grains is beginning to be understood better and rapid tests developed. In maize and sorghum the concentration of elongation factor 1A (eEF1A) in the grain is a very good predictor of grain lysine content (Sun *et al.*, 1997). Although eEF1A has a high content of lysine it represents only a small proportion of the grain lysine. ELISA tests of eEF1A levels will enable more rapid breeding progress than could be achieved by conventional lysine determinations as these take longer and are more expensive. Work in wheat suggests that the level of eEF1A is not closely related to grain lysine levels but that the concentration of another lysine-rich protein, aldolase, may be more strongly related to wheat grain lysine levels (Singh *et al.*, 2000).

As consideration moves to less well-defined chemical constituents, and to bioassays such as feeding trials, evidence of the direction to select (if any) becomes less certain (O'Brien, 1999). Two particular interrelated problems of wheat for poultry, soluble non-starch polysaccharides (NSP) and low apparent metabolisable energy (AME) grain, are becoming more clearly defined with the degree of branching of soluble NSP being positively correlated with AME (Austin *et al.*, 1999). Additionally, regions of the wheat genome controlling the viscosity of water extracts of wheat grain are becoming defined by molecular mapping studies (Martinant *et al.*, 1998). These studies suggest that progress in selecting for higher AME in wheat for poultry would be possible.

One way to overcome the limitations of cultivar and environment comparisons in determining grain components important in determining feed quality outlined by O'Brien (1999) is to use precise genetic stocks, such as isogenic lines that differ only at one gene. This approach is being used in wheat to examine poultry feed quality (Anonymous, 1998). These studies indicate that the widespread (especially overseas) wheat-rye translocation chromosome (1BL/1RS) lowers AME while hard grain gives higher starch digestibility than soft grain. Other isogenic line pairs are being investigated (Anonymous, 1998).

Other traits of potential importance for poultry feed quality are under development. Waxy (zero amylose) wheat cultivars are expected in the next few years (Graybosch, 1998; Zhao and Sharp, 1998). It is not yet known if these will have a similar increase in NSP content as waxy barley. Another aspect of wheat starch, the distribution between the large A-granules and the small B-granules is beginning to be investigated (Stoddard, 1999). Any association of this property with feed quality remains to be determined. Quick progress in changing this property can be expected as testing is relatively easy (Stoddard, 1999). Very recently, a mutant sorghum with very high protein digestibility was isolated and the biochemical nature of the trait is being elucidated – one feature is the presence of highly irregular shaped protein bodies, in contrast to the near spherical protein bodies of normal sorghum (Oria *et al.*, 2000). This mutant may be significant to sorghum breeding for feed quality. In addition, the research on the nature of the trait may open up the development of methods to screen for similar changes in other grain crops.

IV. TRANSGENIC PLANTS

The generation of transgenic or genetically modified (GM) grain crops is another possible technology with potential to alter grain feed qualities (Mazur *et al.*, 1999). An initial GM target of significance for animal feeding was increased grain methionine and lysine levels. Two approaches were taken to achieve this; introducing and high expression of a gene encoding a high methionine/lysine seed protein or inserting genes for biosynthetic enzymes of lysine/methionine with altered feed back inhibition.

A well-known example of the first approach is the use of a Brazil nut 2S storage protein. This protein contains 19% methionine (Altenbach and Simpson, 1990). The gene encoding this protein was cloned from Brazil nut (Altenbach *et al.*, 1992), and inserted into canola and soyabean (Altenbach *et al.*, 1992) under the control of seed-specific promoters. This led to significant benefits in nutritional value with increased methionine levels of over 30%. These products have not proceeded to commercialization. This is because the 2S protein is responsible for the sometimes life-threatening human allergenicity of Brazil nuts – a point not known when the work started (Day, 1996).

The incorporation of genes of the lysine biosynthetic pathway insensitive to lysine feedback inhibition has proceeded (Mazur *et al.*, 1999). This has used aspartokinase (AK) and dihydrodipicolinic acid synthase (AHDPS) genes from various bacterial species (Falco *et al.*, 1995) or a lysine-insensitive form of a plant AHDPS produced by site-directed mutagenesis (Mazur *et al.*, 1999). The results from the incorporation these genes have depended on the host plant species and the tissue-specificity of the promoter controlling the gene. In soyabean and canola lysine levels were more than doubled but the lysine catabolic products, saccharopine and α amino adipic acid, also accumulated in the seed. In contrast, in GM corn these genes gave no lysine accumulation under an endosperm-specific promoter while expression under an embryo-specific promoter gave a 50 to 100% increase in lysine levels with only little accumulation of the catabolism products (Mazur *et al.*, 1999). Commercial release of GM corn and soyabean lines with increased lysine and methionine is due in the next few years in the USA (Mazur *et al.*, 1999).

An early research success in modifying grains for feed use was GM tobacco seeds expressing an *Aspergillus niger* phytase. This resulted in seed that, when added to chicken feed at about 20g/kg, produced a similar growth response to the use of a commercial feed enzyme supplement (Pen *et al.*, 1993). Clearly, this is not commercially viable but recent work has developed GM wheat expressing the same *A. niger* phytase (Brinch-Pedersen *et al.*, 2000). Although not yet tested in feeding trials the level of phytase expressed in the wheat grain suggests that only limited amounts of the phytase GM wheat will be required in compound feed to give a beneficial effect. It may be that GM wheat expressing microbial xylanase genes as well would be of additional benefit (Zyla *et al.*, 1999).

V. ECONOMIC AND OTHER ASPECTS

Feed quality enhanced grains will have to bear the cost of “identity preservation” (Mazur *et al.*, 1999) down the production and distribution chains. This is because one is attempting to convert a bulk commodity into a specialized higher-value product (Kidd, 1993). DuPont have such a system organized for their high-oil corn in the USA. Another consideration is how the current controversies concerning GM food will play out. In particular, the public view of GM grains as animal feed and the products from animals fed such grains will be important in their final commercialization.

VI. CONCLUSIONS

Grain cultivars with improved feed quality are in agriculture. Many products are under development. Consequently, we can expect more high-feeding value grain cultivars to come on the market in the next ten years.

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THE MANIPULATION OF INTERMEDIARY METABOLISM BY TRANSGENIC TECHNOLOGY TO ALTER NUTRIENT REQUIREMENTS IN ANIMALS

K.A. WARD

Summary

This paper describes the potential for the manipulation of the intermediary metabolism of animals by the use of transgenic technology. The aim is to introduce into animals the genetic information required for the enzymes involved in the biosynthesis of organic nutrients that must at present be supplied by the diet. The general concept requires the isolation of functional genes from bacteria, modification for expression in animals and transfer to embryos to create transgenic animals. Examples given are the introduction of a cysteine biosynthesis pathway and a glyoxylate cycle to mammals. It is envisaged that the general principles may also be applicable to poultry.

I. INTRODUCTION

For intensive animal industries such as the poultry, pig, feedlot cattle and aquaculture industries, the supply of inexpensive feedstuffs is a major factor in overall production costs and thus becomes a critical factor in any producer's economic survival. Much of the expense of these feedstuffs derives from the need for supplementation with various organic nutrients that cannot be synthesised by the animal and are either completely absent or not present in sufficient quantity in normal inexpensive feed sources. Such nutrients must be supplied by the diet because animals have during evolution lost the genetic information necessary for the enzymes involved in the relevant biochemical pathways. There are two obvious ways to improve this situation for the producer. The first approach is to alter the genetic properties of the primary feedstuff source (predominantly plants) so that they contain appropriate levels of the missing nutrients. The other approach is to modify the genetic properties of the animal itself so that it becomes capable of synthesising the missing nutrients *de novo*, thus removing the dietary requirement. The latter approach requires modification of the intermediary metabolism of the animal.

The modification of intermediary metabolism by the introduction of a new biochemical pathway is a significant challenge because the physiological homeostasis of an animal is maintained by the complex interaction of many different biochemical and hormonal regulatory systems. It is reasonable to expect that the introduction of a new pathway to the existing biochemical repertoire could upset this delicate balance. Nevertheless, the potential improvement in productivity and reduction in feedstuff costs makes such an objective worthy of the attempt, provided that the techniques for such manipulations are available. Normal evolutionary processes are very unlikely to restore any of the lost genetic information to full functionality, so conventional selection approaches would be unlikely to succeed. However, the development of transgenic technology (Palmiter and Brinster, 1986) now makes this possible because it provides a mechanism for the transfer of genes between organisms that cannot breed by conventional methods. Utilising these new techniques it is now possible to introduce a missing biochemical pathway into an animal by identifying and isolating a functional equivalent of the missing genetic information from any organism in nature and then transferring it to the target organism. Two examples of such manipulations that have achieved some measure of success are summarised in the following text.

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II. INTRODUCTION OF A CYSTEINE BIOSYNTHETIC PATHWAY TO ANIMALS

Sheep require a large supply of the amino acid cysteine to maintain optimal wool growth because of the high content of this amino acid in the keratin proteins that contribute more than 90% of the mass of the wool fibres. The amino acid can only be synthesised in an animal from the sulphur amino acid methionine and the amount of methionine in most feeds is insufficient to provide an adequate cysteine supply. Cysteine cannot be synthesised from other substrates because animals lack two crucial enzymes, serine transacetylase and O-acetylserine sulphhydrylase. However, the genes for these enzymes are still present and fully functional in bacteria. In *Escherichia coli*, the *cysE* gene encodes the enzyme serine transacetylase and the *cysK* gene encodes O-acetylserine sulphhydrylase. The carbon pathway for cysteine biosynthesis is shown in Figure 1.

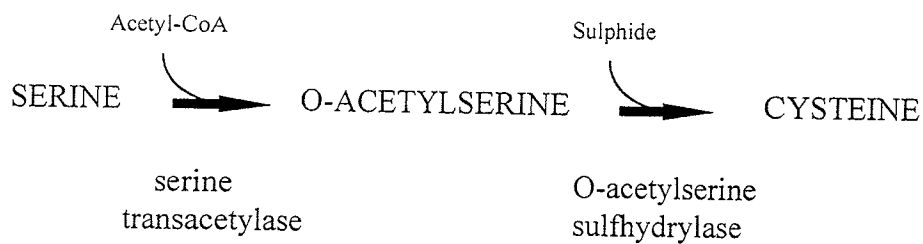


Figure 1. The carbon pathway for cysteine biosynthesis in *E. coli*.

The *cysE* and *cysK* genes have been isolated and modified for expression in mammals (for review see Ward, 1999). The DNA construct used is shown in Figure 2.

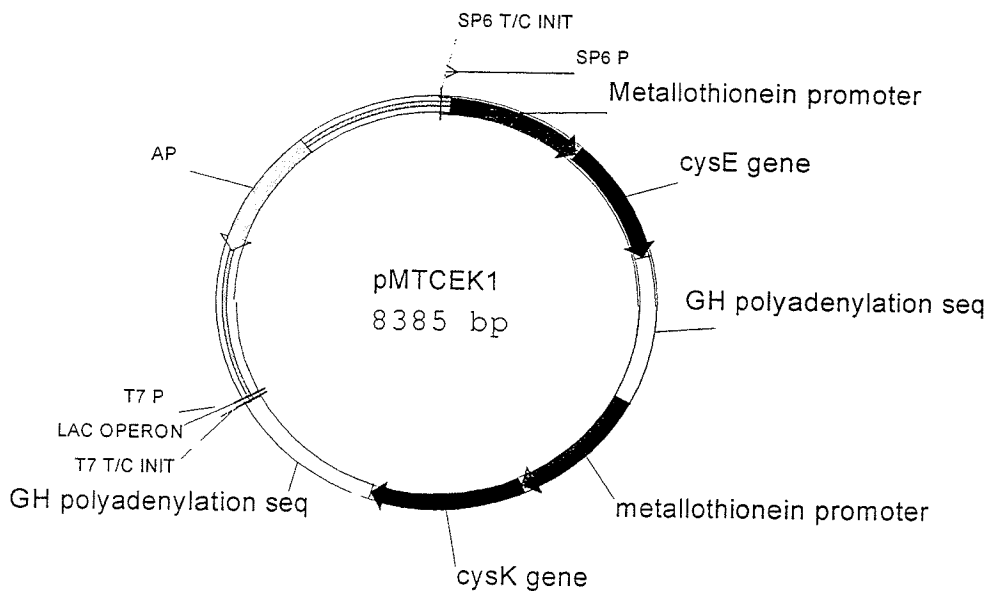


Figure 2. The plasmid MTCEK1 containing the genes from *E. coli* that encode serine transacetylase and O-acetylserine sulphhydrylase.

When inserted into transgenic mice the recombinant DNA provides the genetic information necessary for the production of the two enzymes required for cysteine biosynthesis. That the new biochemical pathway was fully functional was tested experimentally by placing transgenic and non-transgenic mice on a synthetic diet which was supplemented with sodium sulphide as a sulphur source but which contained no cysteine and only trace amounts of methionine. As expected, non-transgenic mice were unable to survive on such a diet for any extended period of time. On the other hand, the transgenic mice containing the introduced cysteine biosynthetic pathway were capable of synthesising their entire cysteine requirement for the full duration of the experiment (Ward *et al.*, 1994). This research demonstrates that, at least in some instances, it is possible to introduce new biochemical pathways into animals to remove a dietary requirement.

III. THE INTRODUCTION OF A GLYOXYLATE CYCLE TO ANIMALS

The second example of a modification to mammalian biochemistry is summarised to indicate the extent to which new pathways can be designed to interface with existing intermediary metabolism. This research aims to introduce to mammals a functional glyoxylate cycle which allows the biosynthesis of glucose from the volatile fatty acid acetate. While this is mainly relevant to ruminant animals that have very large concentrations of acetate circulating freely in their blood stream, it may have some application in non-ruminants in tissues that are highly dependent on glucose as an energy source and have access to reasonable quantities of acetate. The overall concept underlying this project is similar to that described above for the biosynthesis of cysteine in that the glyoxylate cycle is non-functional in animals because the genes encoding two critical enzymes, isocitrate lyase and malate synthase, are both missing. These genes are fully functional in *E. coli*, the *aceA* gene encoding isocitrate lyase and the *aceB* gene encoding malate synthase. These genes have been isolated and modified for expression in animals, after which they have been transferred to mice and expression analysed in a variety of tissues from the transgenic animals. Expression of both genes at the mRNA and protein level (Saini *et al.*, 1996) has been found in the intestinal epithelium of these animals, this being the most likely site for expression of the transgene because of the metallothionein gene promoter that was used to regulate its expression. While no dietary experiments have yet been carried out on these animals, the results to date indicate that it is possible to introduce genes that encode enzymes that interact directly with fundamental components of the animal's biochemistry which, in the case of the glyoxylate cycle, is the tricarboxylic acid cycle.

IV. THE APPLICATION TO POULTRY FEEDSTUFFS

Transgenic techniques are more difficult to carry out in poultry than in mammals but considerable progress has been made in the last 5 years or so in the development of suitable methods. Therefore, it is feasible to consider the application of the principles outlined above to poultry if problems relevant to the industry can be identified. It is clear that the biochemical pathways for the synthesis of various amino acids, such as lysine, could be introduced to poultry, thus removing the need for dietary supplementation of these components. While the pathway for lysine biosynthesis presented a significant challenge several years ago because of the number of enzymes required (at least 7), new developments in the past couple of years have made it possible to create fusion proteins that fold to contain several different enzymic activities in the one protein molecule. The use of fusion proteins can greatly reduce the size and complexity of the recombinant DNA needed for metabolic pathway construction. This concept has been under active research within the author's group,

for example, to create a single fusion protein containing both the serine transacetylase and O-acetylserine sulphydrylase activities needed for cysteine biosynthesis and progress in this work has been most encouraging. Therefore, it is not beyond realistic expectation that the biochemical pathways for some of the more complex molecules needed by poultry for adequate growth might be introduced by this transgenic approach.

A further possibility for this approach is to introduce a gene combination which allows the biochemical processing of a compound in feedstuffs that cannot at present be utilised by poultry. The introduction of cellulases to degrade the cell walls of plant material is a simple example of such an approach and attempts to achieve this in monogastric animals have already made interesting progress (Hall *et al.*, 1993). More complex enzyme combinations can be envisaged that would allow the processing of much of the plant material that at present is not usable as a nutrient source for poultry.

V. SOCIAL IMPLICATIONS OF TRANSGENESIS

At present society is skeptical of the value of transgenic organisms and remains reluctant to consume plants or animals that contain recombinant DNA. This situation will slowly alter as evidence accumulates that there are no inherent dangers in this sort of genetic manipulation. However, it is unlikely that animals with modified biochemical pathways will be readily accepted for some time yet. When introducing new enzymic pathways to animals it is clearly important to ensure that the new pathway is compatible with the existing biochemistry of the target organism, that no novel and potentially hazardous biochemical intermediates are likely to be produced and that the new enzymes that are synthesised in the animal's tissues present no dietary problems to consumers if eaten. It is critical for public acceptance of this technology that all experimental results be readily available for full scrutiny and that public opinion be sought at all stages of the work. Under such circumstances the benefits of this approach may be more fully appreciated and eventually accepted by most consumers.

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VARIATION IN NUTRITIONAL VALUE OF CEREAL GRAINS ACROSS LIVESTOCK SPECIES

J.L. BLACK

Summary

Variation in the available energy content (MJ/kg DM) of Australian cereal grains has been examined across sheep, cattle, pigs, broiler chickens and laying hens. There were only small differences in the available energy content of individual grains across animal types, except for the low energy content of sorghum for cattle. Much of the variation between grains could be explained by gross chemical composition. However, other factors likely to affect the energy available from grains for animals include endosperm cell wall characteristics, grain hardness, fatty acid content and composition, relative proportions of amylose and amylopectins in starch, chemical and physical nature of the protein-starch matrix and phenolic acid bonds with lignin, polysaccharides and proteins.

I. INTRODUCTION

Cereal grains provide the major source of energy for animals raised in intensive production systems. The energy available from cereal grains can vary widely between both grain and animal species. For example, the digestible energy (DE) content of wheat and barley for pigs has been reported to range from 13.3 to 17.0 and from 11.7 to 16.0 MJ/kg, respectively (van Barneveld, 1999). Similarly, Hughes and Choct (1999) reported ranges in apparent metabolisable energy (AME) for broiler chickens, from 10.4 to 15.9 MJ/kg for wheat, 10.4 to 13.5 MJ/kg for barley and 8.6 to 16.6 MJ/kg for triticale. There are also large differences between animal species in their capacity to digest cereal starch. The digestibility of sorghum starch across the whole digestive tract of poultry is 0.99 compared with 0.87 for cattle and 0.30 for horses (Rowe *et al.*, 1999). Significant variation exists also between grains in the digestibility of starch in the rumen of cattle, with reported values of 0.92, 0.65 and 0.62, respectively, for oats, maize and sorghum (Rowe *et al.*, 1999).

The variation in energy available to animals from cereal grains can have a substantial impact on the profitability of intensive animal enterprises. Kopinski (1997) predicted that a change of approximately 5 percent in the DE content of wheat grain (0.70 MJ/kg) could alter the annual profitability of a 200 sow piggery from \$7,500 to \$15,000 depending on grain price. Steam-flaking sorghum to improve its energy value for feedlot cattle costs about \$35-40 per tonne. In 1996, the Grains Research and Development Corporation, in collaboration with several of the animal Research and Development Corporations, established a new research program, "Premium Grains for Livestock", with the major aims of improving the quality and marketing opportunities of cereal grains for the livestock industries. A primary aim of the Program was to identify the extent of, and reasons for, variation in the nutritional value of cereal grains available within Australia. This paper provides an outline of some results obtained from the Program.

II. VARIATION IN THE ENERGY VALUE OF CEREAL GRAINS

(a) Outline of experimental procedures

Over 2000 grains with a wide range in chemical and physical characteristics thought to influence nutritional value have been collected. Many of the grains were obtained from plant breeders. Some were grown specifically and others were selected because of suspected wide variation in nutritional value due to severe drought, frost damage or pre-harvest germination. All grains were scanned with near infra-red spectrometry (NIR) and the extent and rate of digestion of components of selected grains examined within *in vitro* systems simulating rumen fermentation and intestinal digestion. A subset of approximately 100 grains selected on the basis of NIR scans and *in vitro* analyses have been fed to animals, including sheep, cattle, pigs, broiler chickens and laying hens. A relatively small number of grains were offered to all animal types. Measurements made during animal experiments included voluntary intake, ileal and whole tract digestibility of energy and amino acids for pigs and poultry. The digestibilities of grains when offered at maintenance intake to sheep and cattle were also determined.

Comprehensive chemical and physical analyses were conducted on all grains fed to animals. These analyses covered the range in grain characteristics that may influence nutritional value and included individual carbohydrate, fatty acid and amino acid components, α - and β -amylase and anti-nutritional factors such as lectins, tannins and phytic acid. Physical properties measured included grain weight, hydration capacity, seed colour, seed diameter, seed size distribution, seed hardness index and profile, and the viscosity of whole grain, starch extract and acid-soluble extract. Light microscopy has been used to examine the physical structure of some grains and scanning electron microscopy has commenced.

(b) Variation between grain and animal types

The available energy content of selected grains offered to pigs, broiler chickens and laying hens is shown in Figure 1 and compared in Table 1 with values for sheep and cattle where the same grains were offered across the animal species. There was considerable variation in available energy content of most grain species for each animal type. The range was relatively small for sorghum grain for all animals except cattle, whereas it was relatively large for triticale and barley in all animals. The range for oat grain was large, but this was due to a comparison between the naked cultivar, Numbat, and the normal cultivars, Yarran and Echidna. Frost damaged grains had low energy availability, whereas naked oats and sorghum grains had the highest values.

The comparison of individual grains across animal types (Figure 1 and Table 1) shows small differences in available energy content between animal types, except for the extremely low values for sorghum when fed to cattle. For all other comparisons, the available energy content of grains was similar for ruminants and non-ruminants with the largest differences occurring for the frosted grains (3828, 6805) with a high fibre content.

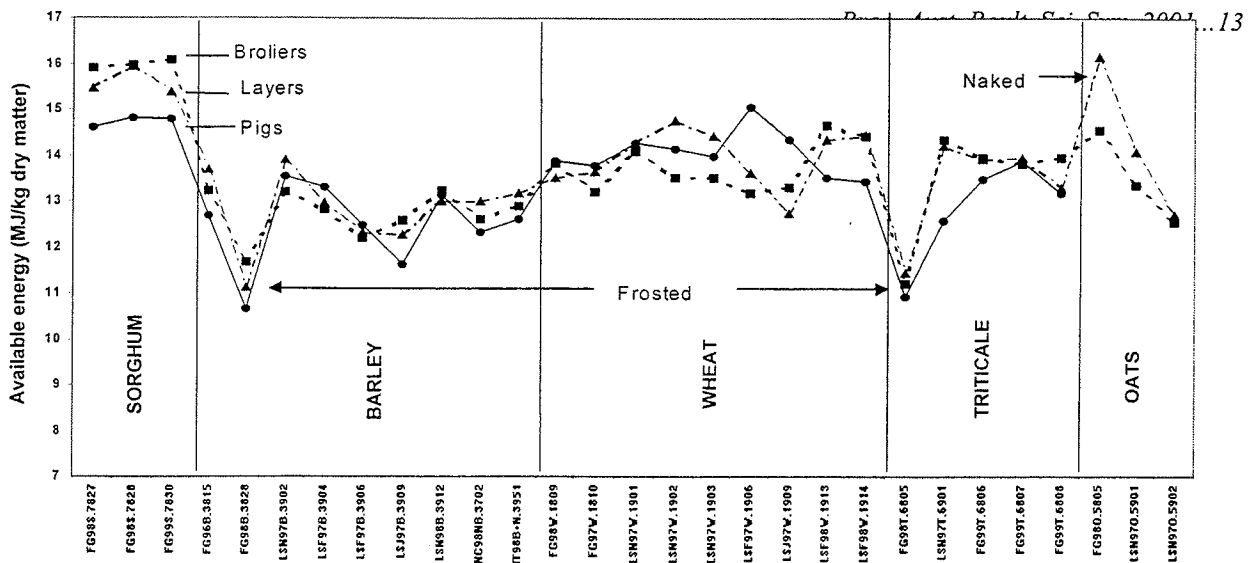


Figure 1. Available energy content of grains (MJ/kg DM) as apparent metabolisable energy for broiler chickens and laying hens and as digestible energy for pigs.

Table 1. Available energy content of grains (MJ/kg DM) fed across animal species as digestible energy for sheep, cattle and pigs and as apparent metabolisable energy for poultry.

Grain	Sheep	Cattle	Pigs	Broilers	Layers
Sorghum	5805	14.56	9.73	14.60	15.90
	5901	14.79	13.21	14.83	15.96
	5902	14.53	10.17	14.79	16.08
Barley	3828	11.51	11.91	10.65	11.68
	3829	13.59	13.51	13.55	13.20
	3906	12.86	-	12.47	12.19
Wheat	1809	13.86	13.84	13.88	13.84
	1810	14.28	-	13.78	13.27
	1901	14.31	14.23	14.28	14.22
Triticale	6805	12.26	12.44	10.91	11.21
	6901	13.66	13.74	12.58	14.36
Oats	5805	15.90	-	-	14.55
	5901	13.41	13.33	-	13.37
	5902	12.56	12.38	-	12.55

III. REASONS FOR DIFFERENCES BETWEEN GRAINS

(a) Gross chemical composition

The amount of energy available to an animal from a grain depends on the relative proportion of each chemical constituent, its energy content and the extent of digestion. The chemical composition of all grains fed to animals has been determined and the gross energy content of these constituents is known. The extent of digestion of each component depends on the availability of appropriate enzymes, concentration of the enzymes relative to amount of substrate, the accessibility of enzymes to the substrate and the time the enzymes are in contact with the substrate. It is possible to predict the potential energy available to an animal

from knowledge of the gross chemical composition of a grain and the extent of digestion of each component, which will vary between animal types. Other factors, discussed below, influence the extent of digestion and reduce the actual energy available below the potential.

The AME content of grains fed to broiler chickens has been predicted from the proportion of the following chemical components and, shown in parentheses respectively, the gross energy (MJ/kg) and assumed digestibility (fraction) of each component; ash (0,0), lignin (15.0, 0), cellulose (16.0, 0), insoluble arabinoxylans (16.0, 0.05), soluble arabinoxylans (16.0, 0.25), β -glucans (16.0, 0.25), other polysaccharides (16.0, 0.25), oligosaccharides (16.0, 0.10), glucose (15.7, 1.0), starch (17.4, 0.98), crude protein (23.2, 0.90), lipid (39.3, 0.90), phytic acid (18.0, 0.10) and tannins (18.0, 0.10). The digestibility of protein and lipid was reduced below 1.0 to allow for endogenous gut losses. The gross composition was adjusted to sum to unity and predicted AME was corrected for grain water content and compared with observed values in Figure 2.

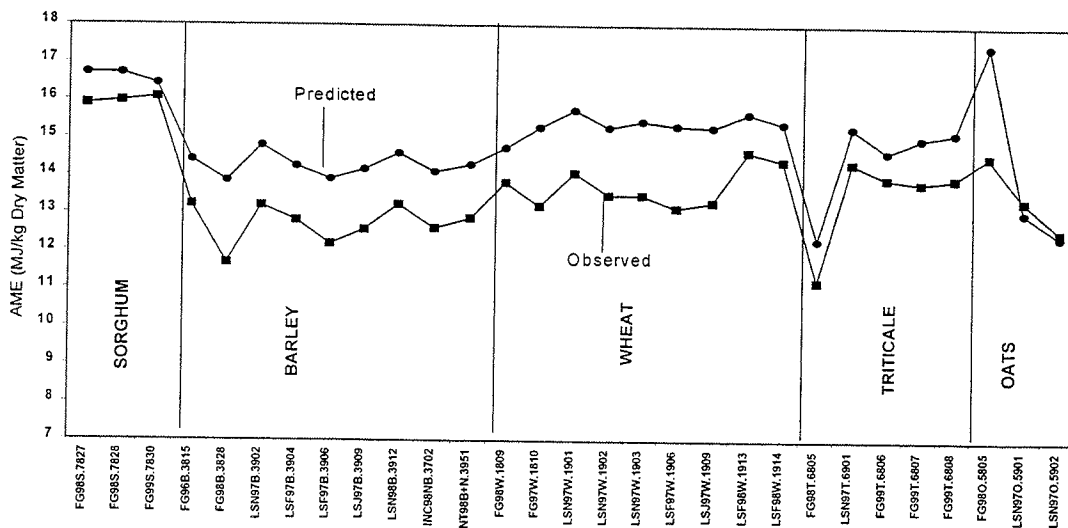


Figure 2. Predicted compared with observed Apparent Metabolizable Energy (AME) for broiler chickens.

The pattern of changes in predicted AME follows closely the observed pattern indicating that much of the variation in available energy between grains can be explained by gross chemical composition. The most accurate predictions are for normal oat grain. The predicted values were substantially higher than the observed values for barley and wheat. Although the accuracy of the assumed endogenous energy losses can be questioned, it is probable that other characteristics of the grain affect the digestion of nutrients.

(b) Cell wall composition and grain hardness

Endosperm cell walls are composed of a cellulose skeleton filled with soluble and insoluble arabinoxylans, xylans and β -glucans (non-starch polysaccharides, NSP). There is strong evidence that the availability of energy from cereal grains in poultry is inversely related to the content of soluble NSP. Choct and Annison (1990) observed a linear decline in broiler AME from 17.5 MJ/kg for rice to 11 MJ/kg for rye with increasing soluble NSP content of grain. Soluble NSP compounds are thought to increase the viscosity of digesta, reduce the diffusion of digestive enzymes and reduce the rate of substrate digestion. Choct and Annison (1992) demonstrated that the chain length of soluble NSP polymers was more important for reducing the AME of wheat for broilers than was the total soluble NSP content,

because of the greater increase in digesta viscosity which reduced the digestion of starch, amino acids and saturated fatty acids.

The difference between predicted AME based on gross chemical composition and observed AME for broiler chickens is related in Figure 3 to measured ileal digesta viscosity, the content of various grain NSP compounds and grain hardness. Although the log

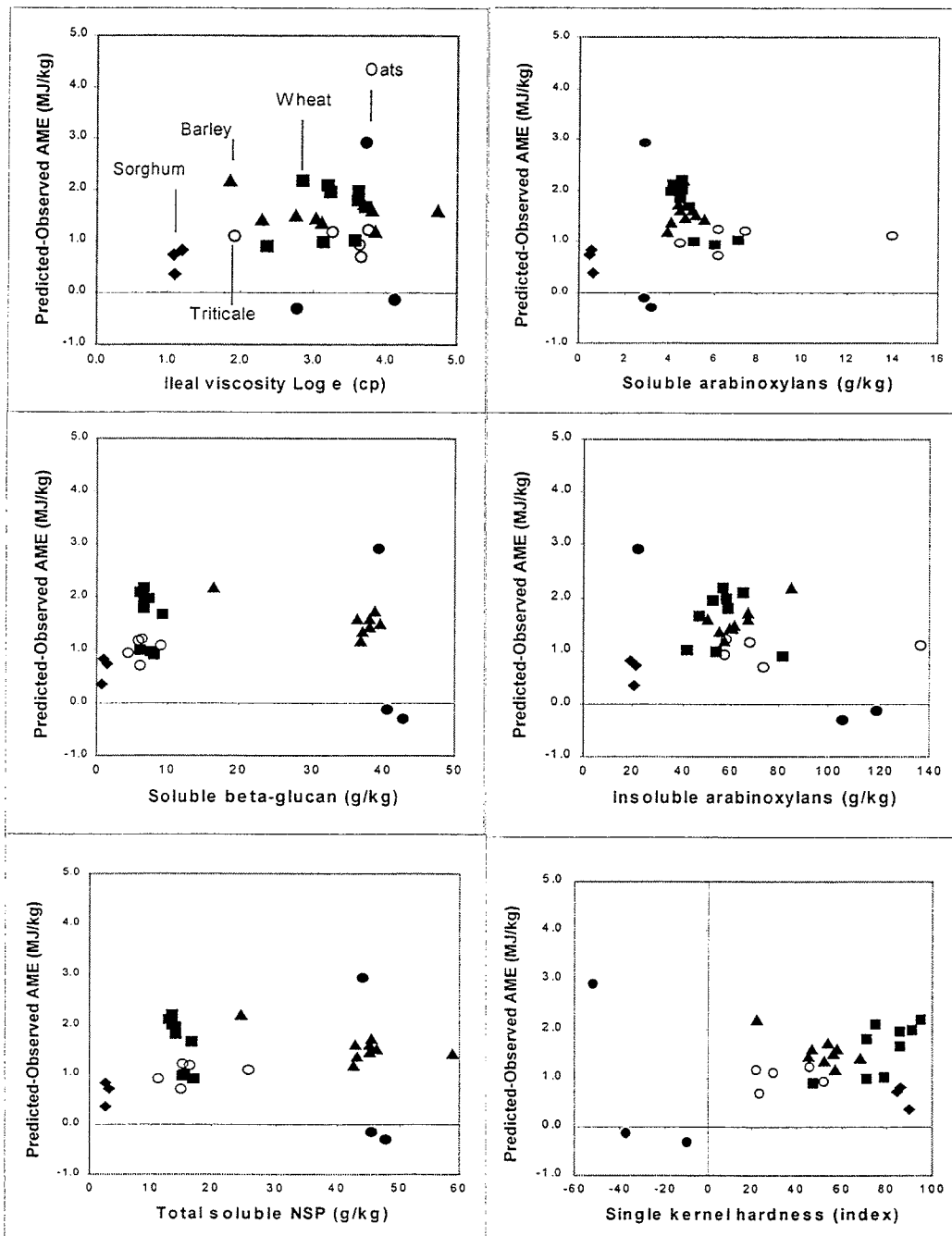


Figure 3. Relationship between grain characteristics and predicted – observed apparent metabolisable energy (AME) for broiler chickens. A key to symbols is given in the top left sub-figure.

transformed ileal viscosity ranged from approximately 1 to 5, it was related poorly to the difference between predicted and observed AME. Thus, digesta viscosity appeared unlikely to be the major reason for the reduction in observed AME from predicted values for comparisons across grain species. Similarly, there were poor relationships between the difference in predicted and observed AME and soluble arabinoxylans, soluble β -glucans and insoluble arabinoxylans. The relationship with total soluble NSP was slightly stronger but the differences between predicted and observed AME were similar for barley and wheat, whereas the total soluble NSP content of wheat grain was less than half that found in barley. Except for the sorghum grains and naked oats, the difference between predicted and observed AME was most strongly related to grain hardness. Wheat grain was harder than barley. Hard grains are known to take up moisture more slowly than soft grains (Kent and Evers, 1994) and, thus, reduce the time available for digestive enzymes to interact with the grain as it passes rapidly through the digestive tract of chickens.

(c) Fatty acid type and content

The digestion of fatty acids by pigs and poultry decreases as the chain length and the degree of saturation of the fatty acids increase (Freeman *et al.*, 1968; Choct and Annison, 1992). The digestibility of the C18 fatty acids in poultry has been observed to increase from 0.71 to 0.97 as the degree of unsaturation increased from 0 to 3 (Choct and Annison, 1992). Lipid molecules are frequently located within the amylose helix of cereal starches. The hydrophobic nature of lipid is believed to inhibit amylase accessibility to the starch molecule and to reduce starch digestibility (Asp *et al.*, 1996). The lipid content, fatty acid chain length and degree of saturation are known to vary widely between cereal grains. The lipid content of the grains in Figure 2 range from 20-40 g/kg for sorghum, 10-20 g/kg for barley, wheat and triticale, 98 g/kg for naked oats and almost 60 g/kg for normal oat cultivars. Oat lipid contains a relatively high proportion of the saturated C16:0 fatty acid and the large discrepancy in predicted AME for naked oats compared with the observed value indicates that digestibility may be reduced by the high lipid content.

(d) Starch composition

Starch is composed of amylose and amylopectin. Amylose consists of long chains of α -(1-4)-linked glucose that form tight helical structures and are relatively inaccessible to amylases, whereas amylopectin contains some α -(1-6) linkages that produce branches in the molecule and provide an open structure more readily attacked by digestive enzymes. High amylose starches are poorly digested compared with starches containing mainly amylopectin. The rate of *in vitro* enzyme digestion of starch from sorghum and maize grains declines substantially as the amylose content increases (Table 3). The same sorghum samples (waxy 7828, non-waxy 7827 and normal 7830) were offered to sheep, cattle, pigs and poultry (Table 1). For all animal types except broiler chickens and pigs, digestion of waxy sorghum was greater than for the non-waxy isolate. The difference for cattle was particularly large at over 3 MJ/kg. Pettersson and Lindberg (1997) observed a significantly higher digestibility of starch in the small intestines of pigs when amylopectin-rich barley (9:91, amylose:amylopectin) was compared with normal barley (30:70, amylose:amylopectin). Granfeldt *et al.* (1993) found that digestion in the small intestines of rats of starch from low-amylose maize was 0.96 compared with 0.68 for high-amylose maize.

Table 3. *In vitro* digestion of starch from sorghum and maize genotypes varying in the ratio of amylose:amylopectin.

Grain	Starch content (g/kg)	Amylose in starch (g/kg)	Starch enzyme digestion (g/kg)
Sorghum			
Waxy isoline	630	240	560
Non-waxy isoline	640	350	330
Conventional	660	460	300
Maize			
Cultivar 1	638	0	550
Cultivar 2	663	300	350
Cultivar 3	586	570	210

(e) Protein matrix

Starch granules are imbedded to varying degrees in a protein matrix and, in some grains like sorghum, protein bodies can form a contiguous layer around the edge of the endosperm and individual starch granules. These proteins must be degraded to expose fully the starch to amylases. The degree of granule encapsulation, nature of proteases and the presence of anti-nutritional factors such as tannins and trypsin inhibitors will affect starch digestion. The protein matrix surrounding the starch granules in sorghum grain contains a high concentration of γ -kafirins with many disulphide bonds which are resistant to some enzymes (Rooney and Pflugfelder, 1986). The marked difference in digestion of sorghum starch between cattle and horses compared with pigs and poultry could be due to differences in the capacity of proteases to degrade the protein matrix.

(f) Lignin bound to phenols and proteins

Cell-wall compounds, particularly arabinoxylans and protein, can bind covalently through phenolic acids to lignin, thereby reducing digestibility. The whole tract digestibility of dry matter in sheep has been examined for four cultivars of oat grain grown at the same site. Grain digestibility varied from 0.62 to 0.76 and was associated negatively with lignin content. However, digestibility was not related strongly to either the hull content of the grains or to the lignin content of hulls. Most oat grain hulls with a lignin content greater than 60 g/kg had low digestibility (0.3-0.5), whereas those with lignin content less than 60 g/kg could have either low (0.35) or high (0.75) digestibility. A possible reason for differences in digestibility between oat hulls with low lignin content is the presence of covalent bridges between phenolic acids, polysaccharides and lignin (Iiyama *et al.*, 1994). Phenolic acids, mainly ferulic acid and *p*-coumaric acid, are linked to carbohydrates through ester linkages and to lignin through either ester or ether linkages. There are also phenolic acid-protein-polysaccharide ester cross-links directly through tyrosine and cysteine. The ester linkages are more easily broken than the ether links and the number of these linkages could alter cell wall digestibility.

IV. FACTORS AFFECTING ENERGY AVAILABILITY FOR POULTRY

The gross chemical composition, particularly the relative contents of fibre and starch, has a major influence on the energy available to poultry from cereal grains. Much of the variation observed in AME between grains can be predicted from knowledge of the gross

energy content of each grain component and its assumed potential digestibility. However, other grain characteristics are particularly important in reducing the energy availability of wheat and barley below their calculated potential. The ranges observed in the AME values (MJ/kg) for non-frosted grains presented in this paper for broilers and laying hens were 13.17-14.66 and 12.73-14.66, respectively, for wheat and 12.19-13.24 and 12.27-13.91, respectively, for barley. The variation in the grains examined was not related closely to either the viscosity of ileal digesta or to the content of soluble arabinoxylans or β -glucans. Although there is ample published evidence demonstrating the importance of soluble NSP and digesta viscosity in reducing AME in poultry, the results presented suggest that grain hardness index was most strongly related to the difference between predicted and observed AME. The analyses suggested also that laying hens digest lipid well up to a lipid content of at least 100 mg/kg in oat grain, but digestibility appears to decline for broiler chickens when lipid exceeds 60 g/kg.

V. ACKNOWLEDGMENTS

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FACTORS INFLUENCING THE ENERGY VALUES OF AUSTRALIAN CEREAL GRAINS FED TO BROILERS

R.J. HUGHES¹, M. CHOCT² and R.J. VAN BARNEVELD³

Summary

The apparent metabolisable energy (AME) values for selected samples of wheat, barley, triticale, oats and sorghum grown in Australia were determined in energy balance experiments with young broilers of both sexes. Ileal digestible energy values were measured for most of these samples. Inherent characteristics of grains induced different responses in male and female chickens. Results indicated that the metabolic activity of gut microflora influenced the energy values in a sex-dependent manner. This has very important implications for the nutrition and husbandry of commercial broiler flocks. It may become economically worthwhile to feed and manage broilers in single sex flocks rather than jointly as is the current situation. In conclusion, sex-related differences may be important in the uptake and utilisation of energy and other nutrients, in responses to anti-nutritional factors (including non-starch polysaccharides; NSP), feed enzymes, prebiotics, probiotics, and other feed additives, and in vaccinations against gut pathogens.

I. INTRODUCTION

Feed is the largest single cost factor (60%) in the production of chicken meat with cost of energy being a major consideration given that birds eat to satisfy energy requirements. The Australian chicken meat industry is highly dependent on the supply of energy from cereals such as wheat, triticale and barley which are known to vary widely in AME. In contrast, sorghum is generally assumed to be a relatively consistent source of energy. Cereal grains, combined with legumes and oilseed meals, provide not only the bulk of the energy and other essential nutrients for commercial poultry production, but are also the prime source of anti-nutritive components which are likely to have a significant bearing on how effectively all dietary components are utilised by poultry. Of the known causes of variation in energy value of grains, soluble NSP stand out as a major determinant of the availability of energy and other nutrients for poultry (Hughes and Choct, 1999).

Glycanase enzyme products are able to depolymerise NSP thereby reducing the viscosity of digesta and, hence, the opportunity for proliferation of enteric bacteria (Choct *et al.*, 1996). Feed enzyme technology has proven to be a very effective tool for not only increasing the energy values of grains for poultry but also for improving the uniformity of growth and feed efficiency of broiler flocks. Despite the huge success of feed enzymes, questions remain about the specific modes of action of enzymes (Smits and Annison, 1996; Williams, 1997), and why enzymes can reduce, but do not eliminate, variation in energy values for grains (Bedford 1996; Kocher *et al.*, 1997). Remaining causes of variation require elucidation.

This paper examines reasons for, and the magnitude of, differences in energy values between grains commonly used in Australia, and discusses how inherent grain characteristics can influence the responses of individual chickens. This paper draws together results from

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recent experiments conducted at the Pig and Poultry Production Institute (PPPI) as part of the Premium Grains for Livestock (PGLS) program described by Black (1999) and an RIRDC Chicken Meat project on energy metabolism.

II. EXTENT OF VARIATION IN ENERGY VALUES FOR CEREAL GRAINS

(a) Differences between birds fed the same grain

The AME surveys by Mollah *et al.* (1983) and Rogel *et al.* (1987) indicated a range of 10-16 MJ/kg in wheat. More recently, Hughes and Choct (1997) demonstrated extremes of AME in a single experiment involving only one sample of wheat containing a high level of soluble arabinoxylan given to broilers hatched and reared under identical conditions (Figure 1). They concluded that the "low-ME" wheat phenomenon was not entirely dependent on the physico-chemical nature of wheat but it was a multi-faceted problem closely linked with the individuality of digestive physiology of broiler chickens.

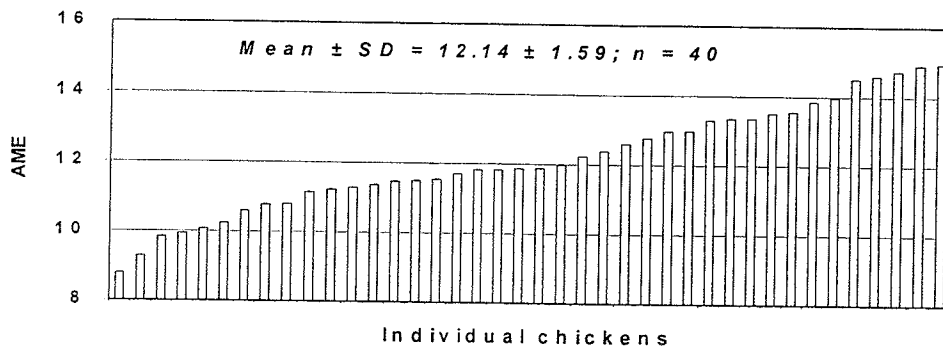


Figure 1. AME (MJ/kg dry matter) values for a single sample of wheat fed to 40 broiler chickens housed individually in metabolism cages from 27 to 34 days of age.

(b) Differences between birds fed different grains

The first PGLS experiment (conducted in 1998/99) with broilers examined the AME value for a control sample of barley used in all feeding experiments with other animal species. The barley control diet was fed to 96 single-sex groups each with six chickens of 17-24 d of age housed in metabolism cages (48 cages of males and 48 cages of females). Then, AME values for 24 test diets based on 12 samples of barley (including the PGLS control barley) and 12 samples of sorghum were examined in the following seven days (24-31 d of age). Each test diet was fed to two cages of males and two cages of females. Sorghum and barley were chosen to represent low and high NSP grains, respectively. The grains were of different varieties grown in different localities. Results are summarised in Figure 2.

Despite wide variation in AME values for the control barley in week 1 (12.9 ± 0.26 MJ/kg dry matter), there was no indication that AME values for weeks 1 and 2 were correlated. Nor were there any indications that other measurements in week 1 such as feed intake, growth rate or feed conversion were linked with AME of test grains or performance of chickens in week 2. This tends to suggest that any de-stabilising influences in week 1 such as proliferation of intestinal microflora (Choct *et al.*, 1996) or re-modelling of the villus-crypt axis in the intestinal mucosa (Tivey and Butler, 1999) did not extend into week 2.

The AME values for 12 samples of barley ranged from 11.2 to 13.3 MJ/kg dry matter and mean values for 12 sorghum samples ranged from 15.4 to 15.8 (96 individual data points are shown in Figure 2). The AME and ileal digestible energy (DE) values for the 12 sorghum

samples were measured in a follow-up experiment with chickens 22-29 d of age.

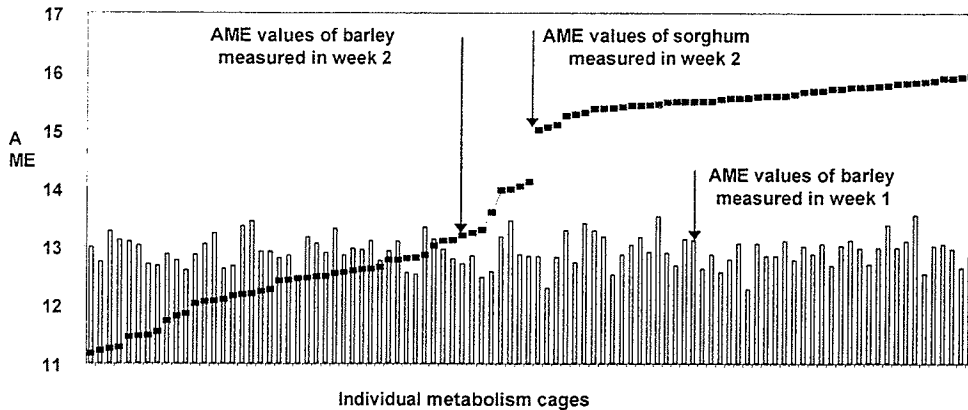


Figure 2. Comparison of AME (MJ/kg dry matter) of barley in week 1 with AME of test cereal in week 2 (AME data in week 2 are sorted in ascending order).

These results are summarised in Figure 3. In this experiment, mean AME values for the 12 sorghum samples ranged from 14.4 to 15.1 MJ/kg (Figure 3), and 14.8 to 15.4 MJ/kg DM for ileal digestible energy (DE). Larger variation has been observed in Australian sorghum samples. Connor *et al.* (1976) observed AMEn (AME corrected for nitrogen) values for sorghum ranging from 13.5 to 17.7 MJ/kg DM (mean value 15.8, $n = 39$).

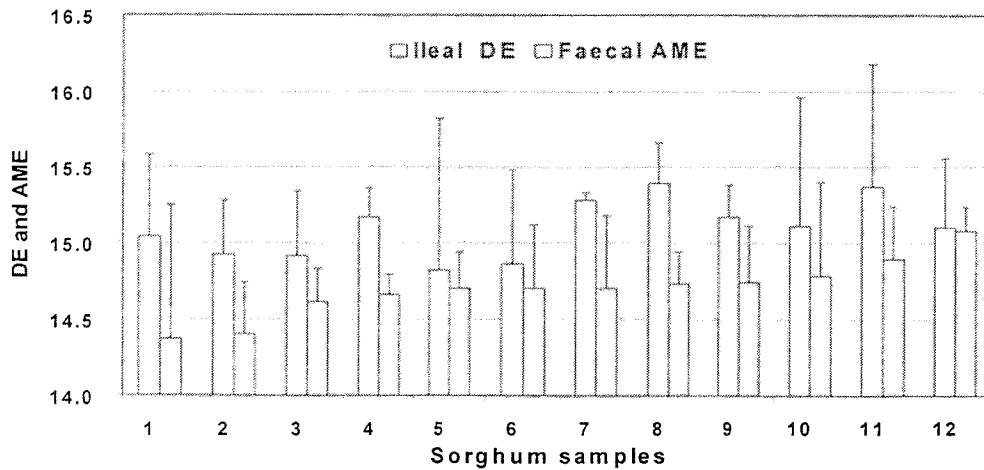


Figure 3. Ileal DE (MJ/kg dry matter) and faecal AME (MJ/kg dry matter) values for various sorghum samples fed to broiler chickens (22-29 days of age) housed in groups of five in metabolism cages (means \pm SD).

An interesting aspect of the results shown in Figure 3 is that faecal AME was less than ileal DE for all of the 12 sorghum samples. In addition, AME values for sorghum shown in Figure 3 are somewhat lower than the corresponding values obtained previously (data shown in Figure 2). This indicates that the two batches of chickens responded differently to the same grains. The underlying reasons for this could be associated with factors such as age and health of the donor flocks, and the feeding and rearing conditions prior to experimentation.

(c) Energy values of grains with widely differing physical and chemical characteristics

The AME and ileal DE values for a selection of samples of barley, oats, sorghum, triticale and wheat were measured in a series of five experiments in 1999/2000 for PGLS. Samples within each grain type were chosen on the basis of widely differing physical and chemical characteristics likely to influence the nutritive value to a range of livestock species. The PGLS control barley and a PPPI control sorghum were included in each of these experiments. Samples of grains of each type were measured in the same experiment with the exception of one sample of wheat. The results are summarised in Figure 4.

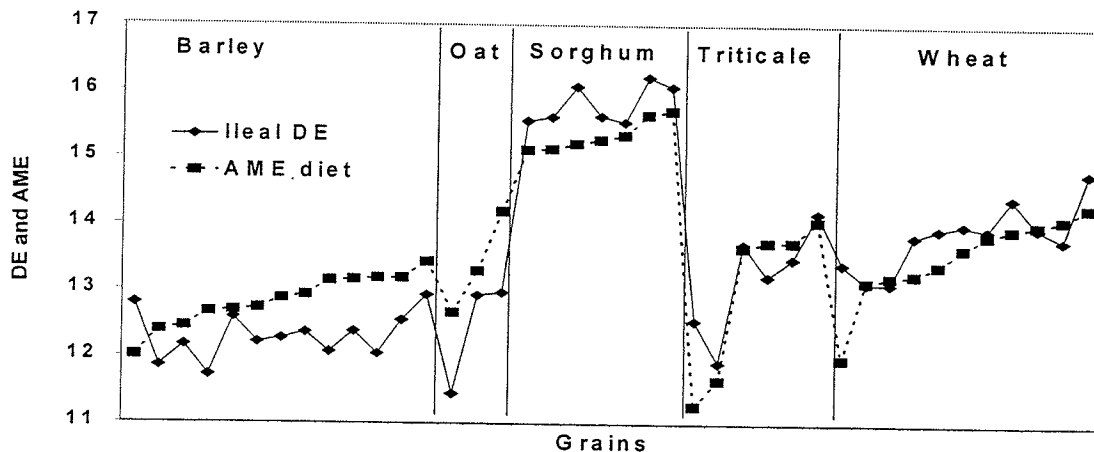


Figure 4. Ileal DE (MJ/kg dry matter) and faecal AME (MJ/kg dry matter) values for various cereal grains fed to broiler chickens (22-29 days of age).

The results shown in Figure 4 indicate a pattern for barley and oat samples in which AME exceeded ileal DE by about 0.4 MJ/kg, whereas for sorghum samples, ileal DE was approximately 0.3 MJ/kg higher than AME. Furthermore, responses differed between males and females (results not shown). In particular, AME and DE values for barley and oats were lower for males than females, whereas for sorghum, only the AME values were lower. These differences resulted in a widening of the gaps between AME and DE for males. There were no obvious patterns for wheat or triticale. The conclusion was that for barley and oats microbial proliferation in the hindgut utilised energy in the form of non-digestible carbohydrates which reduced the gross energy content of the excreta when volatile fermentation products were lost. In the case of sorghum, there was little loss of energy through microbial proliferation in the hindgut, and the difference between DE and AME represented endogenous energy losses.

Two key hypotheses were developed on the basis of these results and conclusions, one relating to the effect of sex and the second to the effect of gut microflora. These are discussed in the following sections.

III. SEX INFLUENCES ENERGY VALUES FOR CEREAL GRAINS

The relative importance of sex and breed was examined in a 7 d metabolism study with chickens 21 or 22 d of age at the start. AME results are summarised in Figure 5.

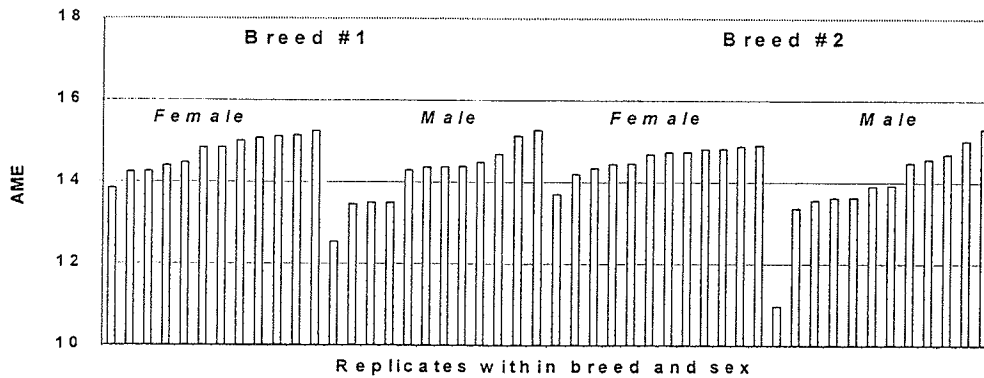


Figure 5. Variability in AME (MJ/kg dry matter) values for a wheat-based diet given to male and female chickens of two commercial breeds. Individual data points for 12 males and 12 females within each breed.

The breed effect (14.4 vs 14.2 MJ/kg dry matter) was not significant, whereas females had a significantly higher mean AME than males (14.6 vs 14.0 MJ/kg dry matter). Similar differences due to sex have been observed in several other experiments at PPPI. The plot of individual data points shown in Figure 5 suggests a higher degree of variability in males than in females, irrespective of breed, with a relatively large proportion of males showing a poor capacity for uptake of energy. These observations are consistent with the widening of the gap between DE and AME values for barley and oats as discussed in relation to Figure 4 above. Further aspects of the importance of sex on energy values of grains are examined in Section V below.

IV. GUT MICROFLORA INFLUENCE ENERGY VALUES FOR CEREAL GRAINS

It is likely that dietary factors which lead to increased activity of gut microflora will depress AME (Choct *et al.*, 1996), apparent protein digestibility (Smits *et al.*, 1997), and availability of amino acids (Steenfeldt *et al.*, 1995).

If avoidance of microbial overgrowth of viscous digesta in the small intestine is a key factor in reducing variation in energy metabolism, then it was reasoned that the use of antibiotics at therapeutic levels in the feed should have a similar effect through direct action on the microflora. This hypothesis was tested in a 7 d energy balance experiment with single-sex groups of six chickens of 22-29 d of age in 48 metabolism cages. Hydrogen and methane contents of the breath from one chicken per cage were measured on days 0 and 6 to gauge the metabolic activity of the gut microflora. The results are summarised in Figures 6 to 8.

The inclusion of antibiotics in the feed did not significantly affect AME or ileal DE values, nor AME and DE expressed as proportions of the gross energy (GE) of the diets (Figure 6). However, antibiotic treatment improved weight gain and feed conversion for each cereal type, except triticale (results not shown). Antibiotic treatment reduced the coefficients of variation in AME:GE for male and female chickens given the wheat diet, and for male chickens given barley or triticale diets (Figure 7). Coefficients of variation for other combinations of grain and sex were relatively low with little scope for change due to antibiotic treatment.

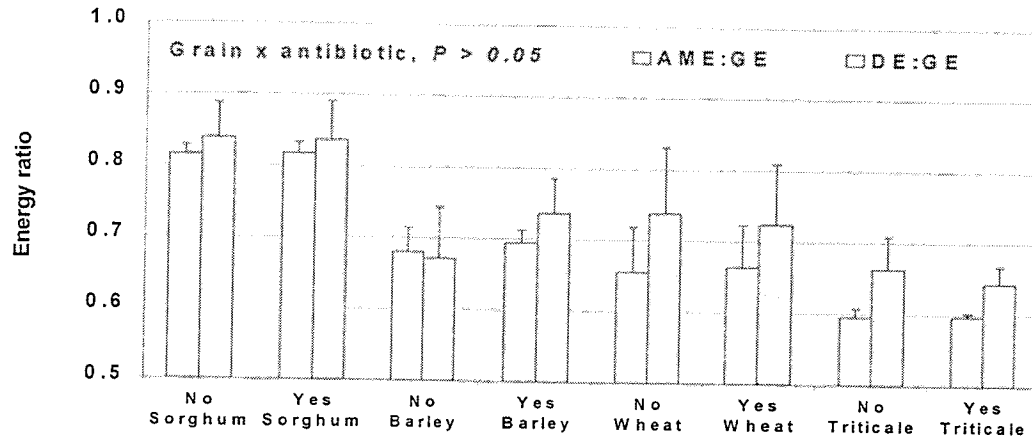


Figure 6. Effects of type of grain and dietary antibiotic treatment on ileal DE to gross energy ratio (DE:GE) and AME to gross energy ratio (AME:GE) (means \pm SD).

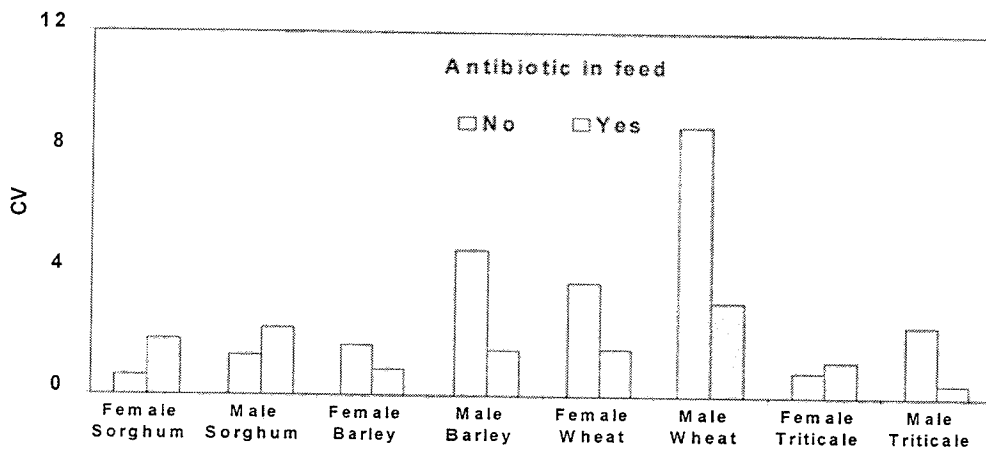


Figure 7. Coefficients of variation (CV) for DE:GE and AME:GE for each combination of grain type, sex and dietary antibiotic treatment.

The effects of antibiotic treatment on metabolic activity of the microflora differed across grain types according to breath hydrogen content (Figure 8). Antibiotic treatment resulted in an increase in hydrogen production in chickens given sorghum but a decrease in chickens given barley (Figure 8) compared with the respective control diets. It is evident from these results that antibiotics did not reduce the gap between AME and DE values, and that antibiotics altered, but did not eliminate, the gut microflora.

Recently, Choct and Kocher (2000) concluded that between-bird variation in AME was associated with the ability of gut microflora to produce xylanase which degraded NSPs and lowered viscosity of excreta. These results led to the ideas that expression of genes for xylanase and β -glucanase are increased in caecal microflora by the presence of high concentrations of arabinoxylans and β -glucans, respectively, in caecal digesta, and that excreta viscosity was an indicator of microbial enzyme activity. To test these hypotheses, practical diets based on wheat or barley were fed to single-sex groups of five chickens of 22-29 d of age housed in 48 metabolism cages. Viscosity of ileal and caecal digesta was measured at the end of the 7 d experiment along with short-chain fatty acids in freshly collected excreta.

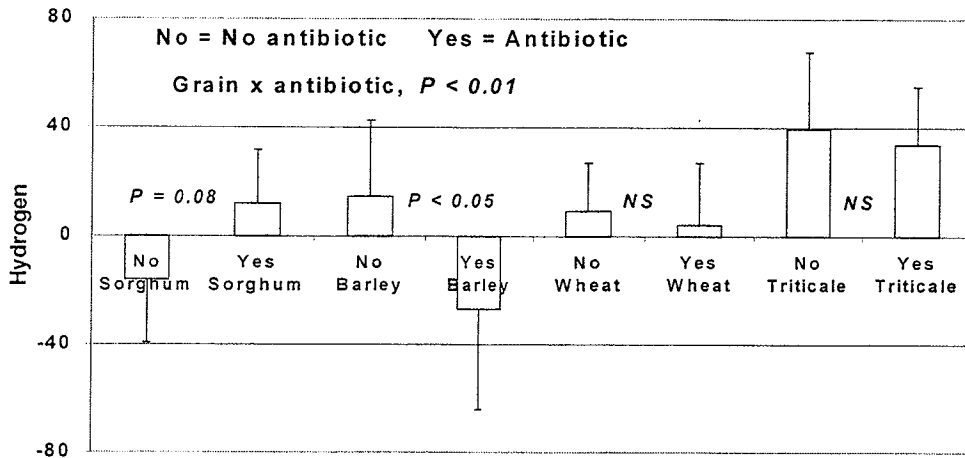


Figure 8. Effects of grain type and dietary antibiotic treatment on the change in breath hydrogen concentration in chickens from day 0 to day 6 (means \pm SD).

A decrease in viscosity of excreta relative to viscosity of ileal digesta (Figure 9) is indicative of microbial production of xylanases capable of cleaving insoluble NSP in wheat but not barley. Clearly, this response was not evident in some chickens given the wheat diet. Also, production of acetic and butyric acids differed widely between male and female chickens and between wheat and barley diets (data not shown). If these observations are indicative of changes in the profiles of bacterial populations in these chickens then it follows that variation in production of microbial enzymes could contribute to the variability in energy uptake by birds through mechanisms associated with the effects of digesta viscosity on digestion and absorption of nutrients (Smits *et al.*, 1997; Williams, 1995), use of nutrients from digesta to support microbial proliferation (Hughes *et al.*, 2001), and effects on gut motility and rate of passage of digesta through the gut (Tivey and Butler, 1999).

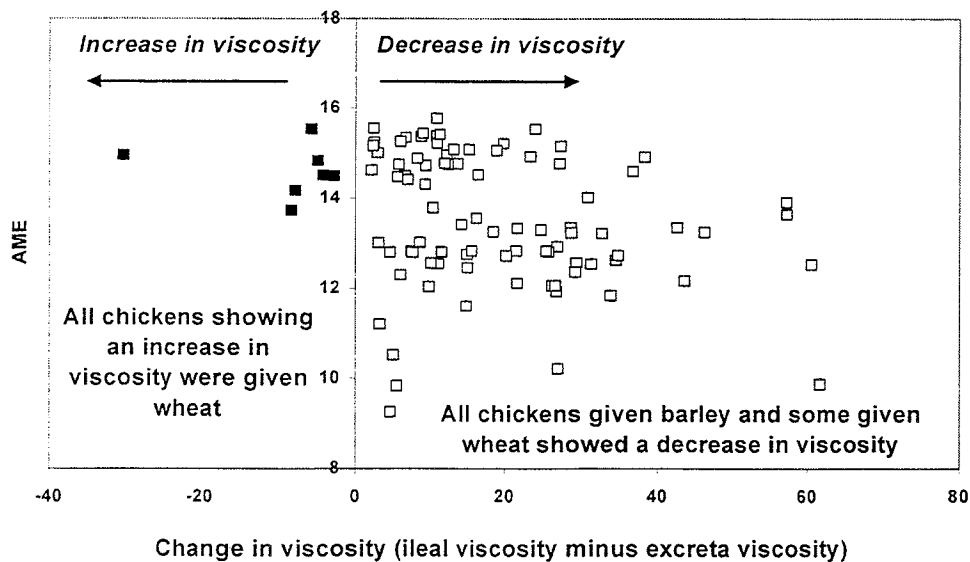


Figure 9. Association between AME (MJ/kg dry matter) and change in viscosity (mPa.s) as digesta pass through the caeca (Hughes, Choct and Kocher, unpublished data).

V. ASSOCIATION BETWEEN SEX AND MICROFLORA

In the PGLS experiment with antibiotics discussed in the previous section, ileal DE values for wheat tended to be lower for males than females. The lack of a significant difference (at the 5% level) in the DE:GE ratio between males and females on barley and wheat diets (Figure 10) implies that digestive and absorptive processes in the small intestine were unaffected by the sex of the chicken. On the other hand, male chickens had significantly lower AME values than females (Figure 10) when given barley ($P < 0.01$) or wheat ($P < 0.001$). These differing effects of sex on DE and AME values of these grains strongly imply that processes and events in the hindgut were affected by sex of the chicken.

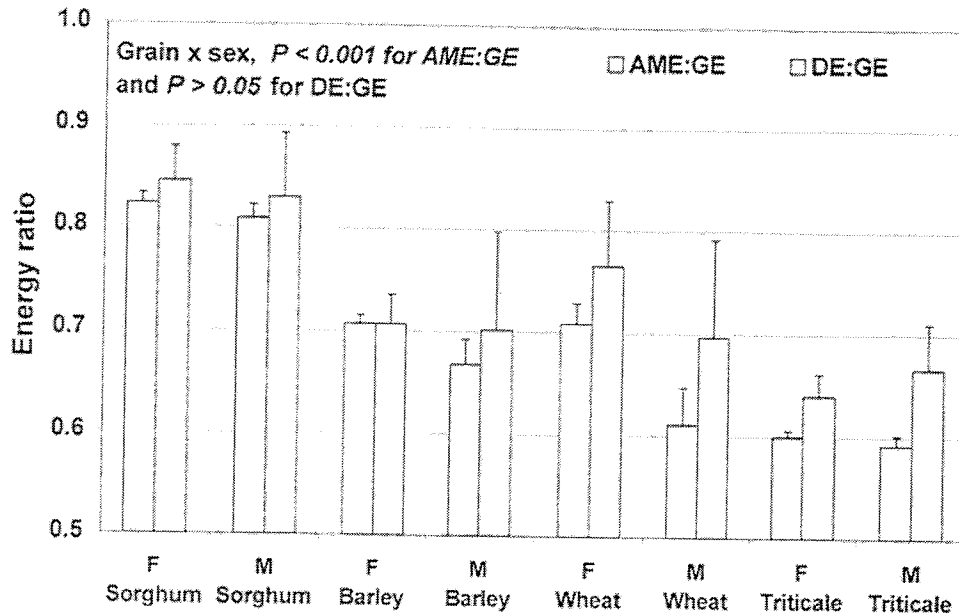


Figure 10. Effects of grain type and sex on AME:GE and DE:GE (means \pm SD).

VI. CONCLUSIONS

The effect of gut microflora on the nutritive value of different cereal grains is at least partially dependent on the sex of the chicken. That is, there is circumstantial evidence of "communication" between the host and gut microflora which has a differential effect on metabolic activity of the bacteria, and possibly the host tissue, also. Alteration of the balance between the host and resident microflora (by feeding different grains, enzymes, antibiotics, prebiotics, probiotics and other feed additives) is likely to result in outcomes which are difficult to predict given the complicated inter-relationships between various key factors contributing to the digestive capacity of the chicken.

VII. ACKNOWLEDGMENTS

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THE METABOLISABLE ENERGY VALUE OF SORGHUM AND BARLEY FOR BROILERS AND LAYERS

M. CHOCT¹, R.J. HUGHES², R. PEREZ-MALDONADO³ and R.J. van BARNEVELD⁴

Summary

A series of experiments was conducted to determine the apparent metabolisable energy (AME) values of 11 sorghum and 11 barley samples using broilers and layers. In addition the AME values of three selected samples were compared using laying hens and adult cockerels. The mean AME value (MJ/kg DM) of sorghum was 15.0 (range: 14.9-15.2) in broilers and 15.1 (range: 14.8-15.5) in layers, and the mean AME of barley was 12.5 (range: 11.6 to 13.8) in broilers and 13.0 (range: 12.5-13.5) in layers. Digesta viscosities (mPa.s) in birds fed sorghum diets averaged 2.7 and 2.6 in broilers and layers, respectively, with little variation between samples. Digesta viscosities in birds fed barley diets differed widely ($P < 0.01$) between samples with the mean value in broilers being 25.5 (range: 8.4-70.7) and in layers 11.8 (range: 3.4-20.5) mPa.s. Dietary ME values obtained using broilers and layers were highly correlated ($R^2 = 0.947$). In addition, in two of the three samples examined AME values determined with adult cockerels and hens were similar.

I. INTRODUCTION

Despite the persistent debate on energy systems for evaluation of dietary energy for poultry (Farrell *et al.*, 1991), perhaps the question about the suitability of using AME data generated in broilers, layers at various egg laying stages, and adult cockerels interchangeably is more pressing (Härtel, 1986; Bourdillon *et al.*, 1990). This is due to the age effect on the ME of grains, especially that of viscous grains. For instance, Rogel *et al.* (1987) reported that the ME value of low-ME wheats was significantly higher when the assay was done with 6-week old rather than 3-week old broilers. It has been suggested that the age-related difference in the ME of feedstuffs for poultry is related to carbohydrate and lipid digestion (Carré *et al.*, 1995). The current study examined the AME values of sorghum and barley samples for both broilers and layers. Gut viscosities were also measured.

II. MATERIALS AND METHODS

Eleven samples of both sorghum and barley were assayed for AME using broilers (Pig and Poultry Production Institute, SARDI) and layers (the Queensland Poultry Research and Development Centre; QPRDC). Measurements of AME were also made with groups of adult cockerels using three of the diets in pelleted form so that comparisons could be made with the results obtained with laying hens (QPRDC). Data were analysed using ANOVA and regression analysis (Statgraphics, Manugistics, Inc., Maryland, USA).

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(a) Broiler bioassay

Ross broiler chicks were reared to 24 d and were then transferred in mixed-sex groups of four replicates of six birds (three male and three female) per diet to metabolism cages located in controlled-temperature rooms kept at 20-25°C. A classic ME trial was conducted over a 7-d period using the Australian ME Standard diet described by Mollah *et al.* (1983). At the end of the experimental period, two chickens per cage were sacrificed to collect ileal digesta (pooled) for gut viscosity determination.

(b) Layer bioassay

Four replicates of six ISA Brown hens which had been in lay for 35-40 weeks were placed in individual wire-mesh, sloping floor cages (23 cm wide x 45 cm long x 48 cm high) with individual feeders and drinking cups. Plastic trays were placed beneath the cages for excreta collection. Experimental diets (g/kg): test cereal, 940.05; dicalcium phosphate, 30.00; sodium chloride, 2.75; minerals, 0.70; vitamins, 0.50; lysine, 5.00, choline chloride, 1.00, and celite (as a source of acid insoluble ash marker) 20.00 were offered 4 d prior to excreta collection. Starting from d 5 excreta were collected daily for 7 d. Coarse shell grit was offered in a separate container to prevent egg shell deterioration and reduced egg production. At the end of the trial the birds were killed by cervical dislocation and the contents of the upper part of the small intestine were squeezed into plastic tubes, centrifuged and the viscosity of the supernatant measured.

(c) Adult cockerel bioassay

White Leghorn adult cockerels were placed in individual, specially constructed cages (36cm wide x 45 cm long x 48 cm high) with feeders that minimised spillage. Galvanised metal trays were placed beneath the cages. Weighed plastic sheets were placed in the trays for excreta collection. The birds were trained to consume their daily pelleted feed allowance in one hour. When this was achieved, the six birds per diet were starved for approximately 32 h before receiving their feed allowance of 100 g. Excreta were collected for the next 42 h and dried at 70°C in a forced draft oven for 48 h.

III. RESULTS

(a) Apparent metabolisable energy and digesta viscosity

The mean AME value (MJ/kg DM) of the 11 sorghum samples was 15.0 (range: 14.9-15.2) and 15.1 (range: 14.8-15.5) in broilers and layers, respectively, whereas corresponding values for the 11 barley samples were 12.5 (range: 11.6 to 13.8) and 13.0 (range: 12.5-13.5). The digesta viscosity value (mPa.s) for the sorghum diets averaged 2.7 and 2.6 in broilers and layers, respectively, with little variation between samples, whereas the corresponding values in birds fed the barley diets were 25.5 (range: 8.4-70.7) and 11.8 (range: 3.4-20.5), respectively (Table 1).

(b) Correlations

Dietary AME values for broilers and layers were significantly ($P<0.01$) correlated ($R^2=0.947$). There were significant ($P<0.01$) negative correlations between ileal viscosity and

dietary AME with broilers ($R^2=0.494$) and layers ($R^2=0.652$). Ileal viscosity values obtained with broilers and layers were also significantly ($P<0.01$) correlated ($R^2=0.508$).

Table 1. Apparent metabolisable energy (AME) (MJ/kg DM) and digesta viscosity values (mPa.s) of 11 sorghum and 11 barley samples fed to broilers and layers.

Parameter	Sorghum		Barley	
	Broiler	Layer	Broiler	Layer
Mean AME	15.0 (0.03) ¹	15.1 (0.07)	12.5 (0.14)	13.0 (0.10)
AME range	14.9-15.2	14.8-15.5	11.6-13.8	12.5-13.5
Mean viscosity	2.7 (0.07)	2.6 (0.10)	25.5 (5.69)	11.8 (2.03)
Viscosity range	2.3-3.0	1.9-3.0	8.4-70.7	3.4-20.5

¹ Values in parenthesis are standard errors.

(c) Comparison between laying hens and cockerels

In two out of three samples examined there were no differences between AME values determined with adult cockerels and laying hens (Table 2).

Table 2. Comparison of apparent metabolisable energy (AMEn) values determined with laying hens and adult cockerels.

Grain	N	AMEn (MJ/kg DM)		
		Cockerels	Layers	SEM
Sorghum, composite control	6	14.47	14.84	0.182
Barley, Grimmatt PBI Narrabri	6	13.60	13.64	0.092
Sorghum, Thunder Biluela	6	14.40	15.50 ¹	0.090

¹ Significantly different to cockerels ($P<0.05$).

IV. DISCUSSION

There was no significant difference between the AME of sorghum measured using laying hens and broiler chickens but the AME of barley was on average 0.5 MJ/kg higher in layers. Carré *et al.* (1995) demonstrated that laying hens could obtain 0.8 MJ/kg more ME through effective use of lipids and carbohydrates compared to young broilers. The inference of adaptation of the gut microflora of the chicken to high non-starch polysaccharide (NSP) diets is suggested by the viscosity data in the current study where the mean gut viscosity in birds fed barley diets was 25.5 mPa.s in broilers and 11.8 mPa.s in layers. The ranges of viscosity values were extremely wide (8.4-70.7 mPa.s for broilers and 3.4-20.5 mPa.s for layers). Choct and Kocher (2000) reported that the caecal microflora of the chicken can produce xylanase and β -glucanase. It is possible that the enzyme levels produced by the gut microflora vary from bird to bird or perhaps the extreme variation in viscosity value reflects different times of caecal emptying and/or refluxing of caecal contents into the small intestine. If the latter is true, the enzymes produced by the caecal microflora could be refluxed into the small intestine at different rates and times, thus alleviating the anti-nutritive effect of the soluble NSP to a variable extent. Since older birds utilise NSP better, the higher the NSP level in the grain then the larger the difference in the AME between broilers and layers.

The negative effect of NSP in poultry diets is related to the viscous nature of the polymers and their ability to increase gut viscosity (Antoniou *et al.*, 1981). Consequently, attempts have been made to use gut viscosity as a predictor of the AME value of some grains for broilers with mixed success (Bedford and Classen, 1992, 1993; Choct and Kocher, 2000). In the current study gut viscosity was negatively ($P < 0.01$) correlated with AME with R^2 values of 0.494 in broilers and 0.652 in layers.

It has been argued that the use of ME values obtained in adult birds may not be applicable to young chickens (Härtel, 1986; Bourdillon *et al.*, 1990). This appears to be true for viscous grains such as barley but not for sorghum. The AME values obtained using broilers and layers were closely correlated ($R^2 = 0.947$). However, it is not known whether variations in broiler and layer AME values for a particular grain remain the same under different conditions. Traditionally, adult cockerels are used for the measurement of dietary ME of feedstuffs for layers because the availability of laying hens at appropriate ages, the high dietary mineral concentration and stress to the hens during excreta collection make ME determination in laying hens difficult. In this study the AME values were similar in two of the three diets selected for comparison of adult cockerels and laying hens. More systematic studies are required for such a comparison.

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FROM JUNGLE FOWL TO FACTORY FLESH: CHANGING PERSPECTIVES ON THE CHICKEN IN CONTEMPORARY SOCIETY

G. ALBRECHT

Summary

This paper explores the history of the use of the chicken in contemporary society. It compares the chicken in its natural setting with factory farming. The origins of attitudes that humans have towards animals in general, and chickens in particular, are discussed. The ethical issues raised by factory farming are evaluated from the perspectives of individual and ecological ethics. It is argued that the poultry industry faces major ethical problems on a number of different fronts. The tensions created by the manipulation of a natural species to fit into the artificial environment of a factory system of production will continue to generate intense ethical debate into the future.

I. INTRODUCTION

We cannot be certain about the exact origins of the domestic fowl. However, it appears in China c6000 BC and India c2000 BC. Charles Darwin concluded that the domestic fowl was descended from *Gallus gallus* or the Indian Red Jungle Fowl. However, other species of *Gallus*, the Ceylon Jungle Fowl, the Grey Jungle Fowl and the Java Jungle Fowl are all possible progenitors of the modern fowl. Stevens (1991), after examining a wide range of morphological, protein and genetic evidence, concluded that, "on balance the evidence supports the idea that *G. gallus* is the species most closely related to the domestic fowl" (1991:11). Some authors refer to the Southeast Asian Red Jungle Fowl (*Gallus bankiva*) as the origin of all domestic fowls.

The Red Jungle Fowl is described as chromatic with a comb on its head and wattles beneath its down-curved beak. The female has more sombre plumage and much reduced comb and wattle size. It flies on blunt wings for short distances, followed by a slow glide. It has sturdy, naked legs on which it runs well. Its normal habit is to actively free-roam and scratch the ground with its well-developed claws on the three front toes and peck for seeds, fruits, invertebrates and insects. The jungle fowl lives in small groups of about 4-6 and the hens have a strong nesting instinct that sees them 'hide' their nests in dense undergrowth. The rooster defends territory and his group of hens and he has a hind spur that is used for fighting. Like most bird species, the wild fowl regularly preens its feathers and dust bathes to maintain plumage health and to remove parasites. The species is mainly ground-dwelling but will take off quickly and spiral up vertically through the canopy if threatened by predators. They roost high in trees in close knit groups at night. The Red Jungle Fowl is a bird well adapted to the hot, tropical jungles of SE Asia.

II. DOMESTICATION

As indicated above, it is thought that the domestication of the fowl occurred in both

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China and India. Cock fighting was one of the reasons for such domestication, followed later by egg consumption and the table qualities of the flesh. The movement of the fowl from the east to the west is thought to have taken place at about the time of Roman occupation of Britain. As indicated by Edlin:

... jungle fowl were domesticated in the East from very early times, and had spread to the West by the Roman era; remains of fowls have been found at Silchester, a Roman station in Hampshire, and no doubt these birds have been with us ever since (Edlin 1952:90).

New varieties of chicken have been produced via selective breeding, mutation and cross breeding after migration. Until the early twentieth century, the fowl was bred as a multi-purpose bird for human needs. It satisfied the need for eggs and meat as well as providing 'permaculture' functions such as pest control. Roosters were also the alarm clocks of the farm and village. The number of breeds in the USA increased from 12 to 34 between 1814 and 1864 (Stevens 1991:12). The standard way of keeping chickens for the market c1900 was as a small farm flock that produced a modest seasonal surplus of both eggs and meat. Hen-hatched chicks were the source for replacement birds in the farm flock.

By the 1930s, about 50% of chickens in the USA were raised by hatcheries specifically for sale to growers and egg producers and, in 1934, 34 million table birds, or broilers, were produced for chicken meat. These birds were mainly males surplus to the requirements of the egg industry. The idea of specifically producing chickens more efficiently came as an offshoot of the application of the scientific management of time and motion produced by Frederick W. Taylor. Taylor was able to show that by breaking the production process down into optimal and efficient units, greater efficiency could be achieved. In 1913, Henry Ford used scientific management to produce the assembly line production system that was able to reduce the unit cost of a car by 50% within a six-year time span.

The transition to full factory production methods for the production of eggs and chicken meat took place after the second World War. Despite the ability to increase production by the 1950s chicken meat was a comparative luxury, seasonal and "Sunday only". Today, in the USA, over 8 billion birds are slaughtered per year and Americans eat chicken virtually when they wish. Chicken meat production has grown about 200% in the last 60 years and per capita chicken meat consumption continues to rise. Americans now eat 34.5 kg of chicken meat per person per annum. In 1940 the average number of eggs produced per annum by a laying hen was 134, and by the 1990s the figure approached 250 (HSUS www).

In Australia, about 600 thousand tonnes of chicken meat is produced annually. This represents the slaughter of about 395 million birds per annum. The consumption of chicken meat is increasing at a rate of about 4% per annum and "chicken meat is now Australian consumers' second most popular meat" (RIRDC www). Australians currently consume approximately 23.7 kg of chicken meat per person per annum (Animals Australia www).

The key factors that enabled the creation of full factory production included:

1. Scientific principles were applied to the breeding of chickens to increase their size and rate of growth.
2. Poultry processing and egg production plants embraced full Taylorism.
3. Intensive rearing was made possible with vaccines for common diseases and antibiotics for optimal flock health.

4. Nutrition science was able to produce feed formulations that maximised growth and minimised costs.
5. Producers became more 'vertically integrated' with greater control over all aspects and stages of production; a small number of producers dominate the market.

III. THE ETHICAL IMPLICATIONS OF THE FACTORY SYSTEM OF PRODUCTION

While achieving exponential increases in the production of meat and eggs, the poultry industry in all its forms has attracted criticism from ethical perspectives. These ethical perspectives range from a focus on the individual bird and the impact of the industry on the environment to the role of humans in the production and consumption process. The key issues that contain ethical implications include:

- The suffering that is inflicted on birds from hatching to death.
- The industry is a major polluter of the environment.
- The industry is implicated in causing human ill health.
- Human epidemics of food poisoning linked to overuse of antibiotics in the industry.
- Use of growth hormones linked to sexual and reproductive problems in humans.
- The industry is implicated in serious human occupational health and safety issues.
- The industry is implicated in the exploitation of workers in both the production and fast food industries.

It is the suffering of birds in the system of production that has attracted the most publicity in the last four decades. Since the animal liberation movement of the early 1970s public attention has been focussed on the plight of battery- and meat-production hens. The raising of ethical awareness about the suffering of chickens in the production of meat and eggs has occurred after a long history of humans not seeing all types of animals as candidates for ethical concern. This history will be presented in three very much abbreviated parts.

IV. HISTORICAL PERSPECTIVES ON CHICKENS

(a) Biblical

There is a long history of humans believing that they are superior to animals and have the power and the moral right to do with them anything they choose. Such views have their origins in biblical and other ancient sources that suggest that animals are here for our use and that it is our lot in the scheme of things to rule over them. In *Genesis* it is made clear:

And God said, let us make man in our own image, after our likeness: and let them have dominion over the fish of the sea, and the fowl of the air, over the cattle, and over all the earth, and over every creeping thing that creepeth upon the earth. (*Genesis* 2, 26)

Noah is told:

And the fear of you and the dread of you shall be upon every beast of the earth, and upon every fowl of the air, upon all that moveth upon the earth and upon all the fishes

of the sea: into your hands they are delivered. (Genesis 9, 2)

It is not difficult to construct an anthropocentric view of biblical accounts of the relationship between humans and nature. This view sets in train a perspective that sees the fowl as the legitimate object of human manipulation and domination.

(b) Cartesianism and chickens

The French philosopher Rene Descartes (1596-1650) put the case in the first half of the 17th century that animals should be thought of as examples of machines. Their cries and sounds, even those that are uttered when they are tortured or injured, are but the sounds of a machine; like the groan of the rusty hinges of an old gate as it swings in the wind. As suggested by Singer, Descartes believed that animals:

... experience neither pleasure nor pain, nor anything else. Although they may squeal when cut with a knife, or writhe in their efforts to escape contact with a hot iron, this does not mean that they feel pain in these situations. They are governed by the same principles as a clock (Singer 1975:218).

Such a perspective extends the biblical view in that the religious view of the treatment of animals is supported by science. In the context of this paper, with this view chickens can be perceived as objects that humans can manipulate with impunity.

(c) Capitalism and chickens

Increasingly there has been a tendency to see nature and animals as nothing over and above a set of 'natural resources' that can be commercialised and made profitable within the capitalist mode of production and consumption. Private ownership of animals then leads to profit-making in the capture, trade, domestication, manipulation and consumption of animals and/or their parts. This perspective treats animals as objects to be owned and disposed of just like non-living objects in commercial trade. With an animal such as a chicken, this mentality suggests that the domestication of the chicken and its transformation through selective breeding and genetic engineering into a product which maximises flesh production, egg production and hence profits, is a normal part of the capitalist mode of production. The maximisation of flesh, eggs and profit within the Capitalist system is supported by both Biblical and Cartesian traditions.

(d) Overview

In all three traditions, the common thread is that ethical considerations apply to humans, not animals. The chicken is viewed as an object that is legitimately under the control and ownership of humans who do not need to consider the welfare implications of their actions because chickens are on this earth for our use and they cannot feel and think as we do. Evidence that all three traditions are alive and well can be found in the modern factory farm and the way the chicken and its genome are being altered to suit commercial imperatives. The transition from jungle fowl to factory flesh is completed with little or no regard to the well-being of the bird, either at an individual or a species level.

V. THE EVOLUTION OF ETHICS FOR CHICKENS

It is worth noting that there are ancient Christian traditions that encourage an attitude of respect for animals. St Francis of Assisi (early 13th century) showed compassion to all types of animals. However, his beliefs were well outside Christian norms for human-animal relationships.

At the beginning of the Enlightenment, the early anatomists and physiologists found a belief in Cartesianism very convenient. It enabled them to vivisect animals (without anesthetic) to expose the 'workings of the machine' without any consideration for the 'feelings' of the animal in question. However, their discoveries revealed that animals are remarkably similar to humans in their 'inner workings'. As Voltaire (1694-1778) argued:

There are barbarians who seize this dog, who so greatly passes man in fidelity and friendship, and nail him down to a table and dissect him alive, to show you the mesaraic veins! You discover in him *all the same organs of feeling as in yourself*. Answer me, mechanist, has Nature arranged all the springs of feeling in this animal *to the end that he might not feel?*
(Singer 1975:220).

The most obvious reason why we should have ethical concerns about human interaction with animals is because they are very much like us! In particular, as has been argued since Bentham, their capacity to suffer, to experience pain, just like humans, is sufficient to make them candidates for our ethical attention. Bentham argued in 1780 that:

The day may come when the rest of the animal creation may acquire those rights which never could have been withholden from them but by the hand of tyranny ... It may one day be recognized that the number of legs, the villosity of the skin, or the termination of the *os sacrum* are reasons equally insufficient for abandoning a sensitive being to the same fate. What else is it that should trace the insuperable line? Is it the faculty of reason, or perhaps the faculty of discourse? But a full-grown horse or dog is beyond comparison a more rational, as well as more conversable animal, than an infant of a day or a week or even a month old. But suppose they were otherwise, what would it avail? The question is not, Can they *reason?* nor Can they *talk?* but, *Can they suffer?*

(Bentham, J. *Introduction to the Principles of Morals and Legislation* 1780, as quoted in Singer 1975:8)

Hence, via the science of comparative anatomy, questions about parity of ethical treatment based on the capacity to feel pleasure or pain have entered the ethical arena. The supporters of the Animal Liberation movement, under the intellectual leadership of philosopher, Peter Singer, have become champions of the chicken and many other species that are subjected to what they consider 'speciesism', that is, the unequal treatment of sentient species for no justifiable reason. As argued by Singer:

Once we place nonhuman animals outside of our sphere of equal consideration and treat them as things we use to satisfy our own desires, the outcome is predictable.
(Singer 1975:99)

From Bentham's argument that an animal has a capacity to suffer, Singer has helped to create an international movement that opposes the use of animals as "things" to be controlled and manipulated by humans. Chicken ethics at this level is focussed on the fact that chickens can feel pain and can suffer. Those who oppose the chicken industry in all its forms do so because they believe that the industry systematically inflicts pain and suffering on sentient creatures. The response to this argument ranges from complete avoidance of all meat (vegetarianism) to attempts to minimise the suffering involved (reform). The focus for the ethical concern that the chicken industry inflicts pain and suffering lies in the following 'management' practices:

- Overcrowding where both broiler and egg producers maximise production at the expense of bird well-being (restriction of movement, feet and claw damage).
- Battery cages that confine hens so that they cannot perform their normal behaviours (cannot properly stand, roost, stretch wings, preen feathers, dust bathe, lay eggs in nests and mix with other birds).
- Debeaking with its associated immediate pain and long-term distress.
- Removal of basic health and behavioural requirements such as sunlight.
- Breeding that produces birds incapable of normal function and in chronic pain.
- Handling that causes premature mortality and injury (broken bones).
- Starvation prior to slaughter and during forced molting.
- Suffering and pain during the slaughter process.

Given the scope and magnitude of these problems in the industry it is not surprising that critical attention has been paid to them. The Animal Liberation Movement and the RSPCA have highlighted these problems in very public ways. Very few are prepared to ignore the weight of this social movement and its expression in the form of legislation designed to prevent cruelty to animals.

VI. CHICKENS AND ECOLOGICAL ETHICS

While Singer sees a divide between sentient and non-sentient animals, proponents of ecocentrism see all such divisions as united within a unified whole. From an ecocentric perspective, humans exist in complex ecosystems only by virtue of the role played by thousands, perhaps millions, of other organisms that exist in interaction with each other. It is these interactions that constitute food chains, build new components of landscape, assimilate waste products and produce foundational essentials of life such as fresh water, clean air (oxygen) and fertile soils. Without the combined interaction of all this biodiversity, life in general, and human life in particular, would be impossible. Therefore, a life respecting ethic is also a human respecting ethic.

The American environmental philosopher, Aldo Leopold created his now famous 'Land Ethic' as a response to the newly emerging science of ecology and its implications. He said of this ethic that "A thing is right when it tends to preserve the integrity, stability, and beauty of the biotic community. It is wrong when it tends otherwise" (1949:224-225) and, that "the land ethic simply enlarges the boundaries of the community to include soils, waters, plants, and animals, or collectively: the land" (1949:204).

The role of the chicken in the biotic community is its contribution to the quality of the land. As Red Jungle Fowl, wild birds satisfy the ethical imperative of preserving the integrity, stability and beauty of the biotic community within a tropical rainforest setting. In the traditional family free-range farm or a modern permaculture setting, strong arguments could be given that the presence of domesticated chickens contributes to the ideal of the land ethic. However, in the context of a factory farm, the judgement might be that all these ideals are compromised or lost. It is certainly the case that the intensive chicken industry is a massive polluter of the environment once it gets as large as the industry in the USA. The EPA in the USA have identified manure and corpses from the chicken industry as "the third most primary pollutant from agricultural runoff" (HSUS www). Very few would argue that a modern intensive shed, full of chickens, is a thing of beauty.

Other ecological problems associated with the intensive form of the industry are linked to the potential of the routine use of antibiotics to render useless an array of previously efficacious drugs. In addition, new theories suggest that after wiping out many different types of salmonella bacteria with antibiotics, a highly resistant form (*S. enteritidis*) is left in the food chain. The consequences of having an avian ecosystem populated by virulent strains of bacteria capable of crossing species barriers is a serious concern for all life. In addition, there has been a recent report that Penguins have become infected with a poultry virus in the Antarctic. Infectious Bursal Disease Virus (IBDV) might be the first of many micro-organisms in poultry to cross species barriers and create major problems for wild avian species.

Some advocates of ecologically derived ethics argue that domestication of animals such as the fowl reduces the need for ethical consideration. Callicott, for example, has argued that domesticated animals such as the chicken "... have been bred to docility, tractability, stupidity and dependency" (Callicott 1980:330). As a consequence, chickens do not have the same moral claim on us as have wild animals and do not need 'liberation' by animal activists. Davis has counter argued that despite our best efforts to denature the chicken of its wild instincts and behaviours, we have failed to achieve such a result and that chickens "... retain their ancestral repertoire of behaviors" (Davis 1995:www), including virtual dust baths. Tensions between feminist and environmentalist (masculinist?) perspectives on the plight of the modern chicken remain.

VII. CONCLUSION

The evolution of the chicken from Jungle Fowl to factory flesh is a story that highlights the extent of control that humans now have over the things that 'moveth and creepeth' on this planet. Concern about the impact of this control over individual animals has been expressed by the animal liberation movement and other pressure groups within society. The focus of ethical concern has been to reject all forms of speciesism and to apply the principle of equal consideration of interests. The interest of avoidance of pain by all sentient creatures is the foundation of this ethical response.

While not immune to the plight of individual animals, ecologically inspired ethics focuses on the total environment within which individual animals live. The foundation of ethical concern here is the maintenance of complexity and diversity within natural systems. Such complexity and diversity fosters self-organised resilience and ultimately ecosystem health. By contrast, monocultures produce the need to constantly add inputs in the form of antibiotics, technologies, supplements and fossil fuel-based energy. In addition, the size of

the industry now means that it is a major producer of pollution that has detrimental impacts on ecosystem health.

To the combined insights of individual animal and ecological ethics can be added a number of important issues in human ethics. The industry faces criticism for its poor performance with respect to conditions of work and pay. Occupational health issues are significant with the rate of illness and injury in the industry in the USA “more than twice the national average” (Poultry Org: www).

The modern intensive chicken industry is under intensive ethical attack. The pursuit of the ‘perfect chicken’ has come at a cost and the industry must expect continued scrutiny of its activities. This scrutiny will expose the worst excesses of the industry and challenge it to raise the ethical bar. However, deeper ethical concerns relate to the rationale and structure of the whole industry. Raising the bar means more research, more interventions, more selective changes to the chicken to accommodate its shift from the jungle to the factory. Such increased inputs might prove to be too difficult for the bird and its consumers to handle. The degree of change we have inflicted on a species that had millions of years of evolutionary fine-tuning to be perfectly adapted to its jungle environment is enormous. From the perspective of the chicken we have gone too far already and to think that we can go further is perhaps to embrace old despotic traditions dressed in new clothes.

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RECENT DEVELOPMENTS IN LAYER HOUSING AND WELFARE IN AUSTRALIA

G.D. STEWART

Summary

This paper reviews some of the recent advances in the understanding of poultry welfare and looks at how Australian producers, society and Governments are developing Codes of Practice and legislation to enhance welfare positive outcomes in poultry production. As the result of the recent Agricultural Resource Management Council of Australia and New Zealand (ARMCANZ) 'Review of Layer Hen Housing and Egg Carton Labelling', specific recommendations have been made regarding stocking density and cage design. Australia has a vast range of climatic and management conditions under which poultry are produced and, if production diversity is to remain throughout the country, there is unlikely to ever be a 'one housing / management system suits all' scenario which will deliver the highest standards of welfare, production, egg quality, ecological sustainability and viability all year round.

I. INTRODUCTION

Historically, poultry have developed in a symbiotic manner with society. Jungle fowl display several characteristics which predisposed them to domestication: (1) they naturally live in small groups of one to two males and two to five females and their young, (2) their hierarchical structure reduces the risks from fighting, (3) they have promiscuous sexual behaviour which lends itself to managed selection and breeding, (4) they have flexible dietary requirements (omnivores) and (5) they are adaptable to a wide range of environments (Wood-Gush, 1959; Yamada, 1988; Appleby *et al.*, 1992). The determination of what facilities promote the 'best positive welfare outcome' under commercial conditions and over all seasons is extremely complex and no one system of husbandry / management provides a complete answer.

Brambell (1965) defined welfare as 'a wide term that embraces both the physical and mental well-being of the animal and that the evaluation of welfare should take into account scientific evidence available concerning the feelings of animals that can be derived not only from their structure and function but also from their behaviour'. The word 'welfare' like 'stress' lacks adequate definition, and however it is measured it is usually a continuous scale. A decision has to be made where to draw the line between the acceptable and non-acceptable. Physiological measures fit easily into a continuum scale whereas behavioural measurements are often more discrete.

Good welfare underlies 'biological fitness' (i.e. longevity and reproductive success) (Broom, 1991). It often seems that definitions of welfare are constructed of opposing views between the intensity of suffering and biological functioning but, as measurement techniques improve, there is more evidence to suggest a close integration of behaviour, physiology and immunology.

Animal welfare is concerned wholly or partly with suffering that the animal consciously (physically and mentally) experiences (Duncan and Dawkins, 1983; Dawkins, 1990; Duncan, 1993). One needs to distinguish between 'needs' which are essential for survival and reproduction and 'wants' which are the animal's cognitive representations of its needs. Duncan (1993) argued that welfare is primarily associated with 'wants' not needs.

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The inability to satisfy 'wants' will lead to frustration and consequently welfare will be compromised. Therefore, welfare is about the animal's perception of its environments and not just with the environments themselves.

In the broader context of welfare in agricultural production systems Tauson (1991) states that it is not only important to consider the 'well-being' of the animal but also of the people who work within the production system. At a practical level the development and assessment of alternative systems of production (other than intensive cage systems) which were thought to be more 'welfare positive' has not yet achieved the expected 'welfare positive' results. As a benchmark on which to compare various systems of egg production, the Agricultural Committee of the Swedish Parliament defined the following four criteria (Sorensen, 1994) :

- (1) Animal health should not be worse.
- (2) The use of medications/chemicals should not increase.
- (3) The working environment should not be impaired.
- (4) Beak trimming should not be necessary.

It should be noted that in the above Swedish model no weight was given to the cost of production because the top priority in assessing and comparing systems was clearly welfare. This author suggests that two more criteria be added to the above list:

- (5) That the natural environment be enhanced or protected.
- (6) That product quality be maintained or enhanced.

The conditions which lead to better welfare in one geographic location may have a negative effect in another. Barnett and Newman (1997) argued that it is important that welfare recommendations from overseas research and developments are validated under Australian conditions (e.g. ventilation problems in summer with the use of solid sided modified cages) in order to prevent compromising bird welfare with vastly different climatic conditions, different shedding and management systems, and different genetic stock.

In addressing the broad issues regarding the interrelationship of society, farmers and agricultural animals, Hurnik (1991) suggested the following basic 'self interests' of each group.

Table 1. Examples of interests specific to society, farmers and farm animals

Society	- healthy and affordable food - variety of available food - ecological sustainability of food production
Farmers	- occupational opportunity and fair income - satisfactory working conditions - good social reputation
Farm animals	- satisfaction of life-sustaining needs - satisfaction of health-sustaining needs - satisfaction of comfort-sustaining needs

Although the above table is possibly over simplified (e.g. society has a growing interest in the manner in which its food is produced, farmers want predictability of production outcome, etc.), it does set a basis on which to consider farm animal production in context.

The progression of 'welfare standards' within society relies on three major obligations (Varner, 1991):

- (1) regulations must be enforced to prevent objectionable practices, so that producers employing more humane techniques are competing on a level playing field with 'traditional' or 'factory' practices.
- (2) consumers must be willing to pay higher prices for products produced using more humane methods.
- (3) producers must be willing to accept slimmer profit margins in order to produce animals/animal products using more humane methods.

II. IMPROVING WELFARE 'ON FARM'

Suffering is a natural part of life in animals and man, and most people would agree that the aim of a caring society must be to minimise suffering where it can command the resources to do so. The ethical question is where is the line drawn in terms of benefit to mankind over animals and how much 'cost' is society prepared to pay to minimise suffering in the animals it uses for its benefit. Dawkins (1980) described 'suffering' in broad terms as '*a wide range of unpleasant emotional states including fear, frustration and pain*'. It leads to both aversion and deprivation behaviours. Suffering implies a long lasting and intense cumulative state of negative stimuli (Duncan and Mench, 1993).

When does behavioural deprivation become suffering? Dawkins (1988) stated that the mere existence of differences in behaviour between two groups of similar animals does not necessarily imply deprivation or suffering because (1) the animal may have acclimatised or adapted to its environment, (2) there may be genetic differences which lead to different responses to similar stimuli (e.g. intensive animals and wild counterparts) and (3) behaviour strongly dependent on external stimuli may not be shown because of the absence of the appropriate eliciting stimuli (e.g. anti-predator behaviour in intensive housing).

Jensen and Toates (1993) questioned the need to perform particular behaviours when the physiological needs of the animal are taken care of. They argued that in assessing the needs of animals a holistic approach is required to consider the motivational control of behaviour. Species specific needs are a complex interaction of obtaining a goal and performing the motor patterns and this is dependent on the environment and the stimuli/resources the animal finds itself in. Therefore, a behaviour may be called a need in a particular, but not necessarily in every, situation.

Behavioural stereotypies performed by an individual indicate imperfect adaptation, but such stereotypies may be the mechanism by which the individual copes with its imperfect adaptation. Beilharz (1982) warned that it is difficult to be certain of any criteria other than poor reproduction, injury or mortality as reliable indicators of disturbed welfare. The use of breeding stock which have performed well in a particular environment has been of significant importance in production in intensive management systems. Domestication of, and selective breeding of, animal species has increased the rate of genetic change which might have occurred naturally in the wild. This has been further skewed by parent selection in developing intensive husbandry and housing environments. Beilharz (1982) argued that, from the point of view of evolutionary adaptation, it does not make sense for instinctive behaviour to have to occur in the absence of the appropriate stimuli.

In today's society, most consumers do not want 'hands on' experience with animal production but, when forced to think about it, would like an assurance that the animals which produce the food they eat are cared for in an ethical and compassionate way (Poultry World, 1997).

Hill (1986), in assessing the welfare aspects of conventional and alternative systems of housing, argued that caution should be observed by opponents of cages who state that

cages should be banned because they cause a restrictive repertoire of behaviour. She stated three reasons for this:-

- (1) Welfare was multifaceted and if the focus was just on a single aspect of the hen's life, then one set of welfare problems would just be replaced by another.
- (2) There was little evidence to support a concept that a restricted behaviour repertoire was indicative of suffering.
- (3) Human judgements (anthropomorphism) about what was important to the hen.

III. ARMCANZ REVIEW OF LAYER HEN HOUSING IN AUSTRALIA

The most important recent Australian developments in poultry welfare are the decisions taken by the Agricultural Resource Management Council of Australia and New Zealand) in August 2000. These decisions related particularly to the cage housing of layers. In essence the (ARMCANZ) decisions proscribed that :

- *All new cage systems commissioned from 1 January 2001 must provide a floor space of 550 cm² per bird (including the baffle).*
- *All cage systems that do not meet the 1995 standards are to be scrapped on or before 1 January 2008 unless they are modified by then to meet contemporary standards at that time.*
- *All cage systems that comply with the 1995 standards, or if constructed after 1995 the standards at the time of construction, are to have an economic life of 20 calendar years from the established date of purchase, but must comply with the contemporary standards after that time. Cages which cannot be adapted to meet these new standards must be scrapped.*

ARMCANZ indicated some key areas for further research into housing systems :

- *Field evaluation of commercial laying systems, including alternative systems (with particular emphasis on barn and modified cages). This will identify welfare positive combinations of housing systems/management/genotype/and environment.*
- *Field identification of layer strains which may be best suited to individual alternative laying systems (i.e. barn and range).*

From a general community perspective, animal welfare fits in with a range of other issues such as wholesome nutrition, chemical, pesticide and antibiotic residues, and the environment. The ability to make considered decisions about ethical issues regarding animals depends on the interpretation and reliability of measurements regarding the 'well-being' of the animals in question. There is always the pitfall that human interpretation of such measures is flawed by our subjectivity in addressing such issues (anthropomorphism).

Welfare issues cannot be seen in isolation from the economic and political environments in which they function. The Global Agreement on Tariffs and Trade (GATT) will have a continuing affect on the economic returns in various countries. Countries which have low costs of labour can force competing countries to cut standards in order to survive as barriers to international trade are dismantled. Australian legislators need to ensure that welfare standards are set with the provisions of GATT in mind or else they just may export the responsibility for the welfare of laying hens to overseas countries with lesser standards. Poor agricultural returns are almost always associated with reduced husbandry, nutrition and welfare (Swarbrick, 1995).

The role of legislators is to reflect majority accepted standards and 'draw the line' based on wide spread consensus. Of course, attitudes will change as welfare is further

defined and scientific evidence regarding welfare issues increases and the 'line' will be continually re-drawn. Enactment of legislation falls into three broad categories:

- (1) Legislation prohibiting deliberate, unjustifiable acts of unnecessary cruelty has been the cornerstone of most animal protection acts throughout the world.
- (2) Legislation promoting quality of life. This often refers to 'Codes of Practice' which are usually recommendations for minimum standards with no regulatory penalties. Producers who can demonstrate they meet the 'Code' requirements can use this as a defence against accusations of cruelty or mistreatment. The most common regulation now enacted is that of minimum space which, once defined, can be objectively measured. The development of 'ethics committees' to oversee and approve/not approve the scientific use of animals in research and education is a positive step in actively promoting the 'quality of life' of animals used for the benefit of man.
- (3) Legislation which controls and/or prohibits specific management and invasive procedures (e.g. mutilations, slaughter methods, etc.).

Legislation regarding systems of production must give a period of certainty to producers if they are to be encouraged to invest in better forms of housing. The Australian Council of Egg Producers (1994) in its submission to the Standing Committee of Agriculture's 'Working Group on Layer Hen Housing' noted the following points:-

- (1) In Australia 90% of all egg producers in the industry in 1970 had left the industry by 1993.
- (2) New cages have an economic life of 20 to 25 years.
- (3) Unless intended legislation regarding systems of production contains minimum periods of certainty for acceptance of approved systems once purchased, then producers will not want to risk investment in improved welfare systems and, consequently, advances in welfare will be limited.
- (4) If legislation reflects the wish of society to increase minimum standards of welfare then tax incentives should be provided to help farmers upgrade to new standards.

In relation to the possibility of Governments legislating what systems of production may be acceptable, Sorensen (1994) from Sweden's Kronagg argues: *'If consumers are going to be motivated to make the right choice by buying eggs from welfare enhanced birds, why not simply let the consumers decide through their purchase decisions instead of making laws prohibiting certain systems. Market mechanisms will then dictate production systems.'*

In many cases in agriculture animals are now reared in countries and environments in which they would never have evolved naturally. In Australia, most layers are housed in open sided, naturally ventilated sheds with hens in single deck layer cages or in offset tiers to facilitate better ventilation in Australia's extremes of temperature (Stewart, 1993). This open housing for summer comfort may present welfare problems in winter in Australia due to the lack of body heat which can be accessed as a 'heat tank' due to the relatively low house stocking density compared to European sheds with multi deck battery cage systems.

IV. WELFARE POSITIVE SYSTEMS - HUMAN / ANIMAL / ENVIRONMENT INTERACTION

Domestication has been a symbiotic evolutionary process between humans and animals. The human / animal interaction has affected many behaviours as humans act as a 'buffer' between the animal and its domesticated environment (Price, 1997).

Good stockmanship can overcome many of the deficiencies in an outdated husbandry system but no system, however upgraded, will overcome the negative welfare outcomes created by a poorly trained or non-caring stock person. This point should not be overlooked by legislators as new standards are prescribed in 'Codes of Practice'.

If systems of management which lead to reduced standards of working conditions for farmers are promoted then true welfare is at risk as workers will spend the least time possible with the animals for which they are supposed to be caring. Already there are a number of workplace health and safety issues emerging in some of the alternative systems of management (particularly multi-storied aviaries). Although the hens in these systems may be able to perform most behaviours, workers are subject to health problems such as dust inhalation, physical injury as hens fly down on top of them from high perches as they collect floor eggs or do inspections, or have the unpleasant experience of having hens defecate on them from high perches. From the hens' perspective, high levels of dust may lead to increased respiratory problems and, if workers do not carry out adequate 'in pen' inspections, sick or injured hens may not be detected and treated or removed from further injury.

V. STOCKMANSHIP AND HUSBANDRY TRAINING

The attitude and behaviour of stockpersons towards their animals can influence their productivity and welfare by affecting their fear levels and stress responses (Hemsworth *et al.*, 1989; Barnett *et al.*, 1992). The importance of attitudinal training courses for stockpersons cannot be overstressed as a means of improving the welfare of animals in any system. In Denmark, a potential poultry farm buyer must have certain educational qualifications (Anderson, 1985). The European Commission (1998) has adopted a regulation (with effect 1 July 1998) which requires all people who have the ultimate responsibility for farm animals on any farm to pass an appropriate examination demonstrating their knowledge of the welfare requirements for their classes of stock (United Egg Producers, 1998). Failure to pass the examination after three attempts leads to disqualification from holding a farm licence. Therefore, education is being seen as an essential component to improving animal welfare.

VI. THE DEVELOPMENT AND ACCEPTANCE OF CODES OF PRACTICE

The development of 'Codes of Acceptable Practice' by broad cross-sectional society committees has done much to provide a realistic reference for the general population to acquaint themselves with accurate factual information about production methods and problems. The 'Codes', as well as providing minimum guideline standards for producers, provide consumers with a reassurance that their food is being produced with an ethical regard for the welfare of the animals in the production chain. For this reason, both producers and consumers should see the development of 'Codes' as a positive action within the community. Random 'third person' audits of farms by truly independent bodies can act as a community safeguard that 'Codes' are being adhered to.

VII. DEVELOPMENTS IN MODIFIED LAYING CAGES

Nicol and Dawkins (1990) have voiced their concern at increased aggression and fearfulness which is associated with increasing group size, as required in some alternative systems. They noted that hens preferred a small group size of about four if they were able to select a favoured size. They suggested that from a welfare point of view, a modified cage transformed with suitable nests, perches, operant feeders and enriched surroundings (e.g.

carpet patches, pecking wires, etc.) might be as suitable as a modified perchery – without the disadvantages.

Blockhuis and Metz (1992) noted that one of the practical concerns with modified cages was the spillage of abrasive dust bath or nest litter material into automatic feeding and collection equipment. Another problem was the difficulty of inspection of all hens because sick or injured hens hide under or behind equipment. Equipment layout may interfere with catching. Glatz and Barnett (1995) studied the addition of perches to cages. They reported that perches in cages caused significantly more cracked eggs (particularly line cracks caused by eggs being laid from hens on the perches) and line cracks were more than double similar types of cracks in cages without perches. They reported manure build up under the perches, particularly at the sides of the cage, and this led to a significantly higher percentage of dirty eggs. Much more research needs to be focused on the type, number and placement of resources in furnished cages to deliver positive welfare outcomes (Stewart, 1996a).

VIII. STRAIN ADAPTABILITY TO HOUSING SYSTEM

Craig (1982) proposed genetic selection techniques to develop ‘cage adapted’ family lines. Beilharz (1982) supported the concepts of genetic adaptability of strains to certain systems and of selection for particular systems. This approach would require the dual development of strains and systems. This approach was further developed by Craig and Adams (1984). They acknowledged that the social environment can be manipulated readily by changes in group size, beak trimming and claw trimming. Breeding companies need to do more research in this area.

IX. APPROPRIATE REARING FOR SUBSEQUENT LAYER HOUSING

Wegner (1984) stressed that hens to be housed in cages during lay should be grown in cages rather than on litter. This procedure resulted in higher egg production, lower feed consumption and lower mortality compared to hens reared on litter and subsequently housed in cages. Elson (1986) stressed the need for rearing systems to mirror the layer housing system, particularly where multiple level nest boxes and feeding and watering arrangements are in place in alternative laying housing systems.

X. CONCLUSIONS

The ideal outcome for welfare enhancement ‘on farm’ is a voluntary aspiration on the part of operators to adapt scientifically proven welfare advances in housing and management to their own farming situation on a continuous incremental basis, and a willingness for consumers to pay for products derived from welfare positive production systems.

There is always going to be a need for a ‘Code’ which contains minimum standards which must be met by poultry producers and which is enforced through independent ‘third party’ audit.

An interdisciplinary approach to welfare which takes into account the widest possible range of parameters will provide the best opportunity for improving our domestic animal environments. The ‘incremental approach’ to welfare improvement in every sphere of the science of animal production is likely to have the greatest and quickest practical outcome for farmed animals, as there is not one ‘big fix’ solution and no one production system which provides all the answers.

There is still much research needed to elucidate the ‘essential behavioural needs’ of various classes of stock under a variety of management and housing / climatic conditions.

Laying hens both react to, and adapt to, the environment in which they are placed. If the ultimate assessment of welfare is 'biological fitness' then welfare is much more than just the ability to perform certain behaviours. Such an approach is too simplistic and fails to fully acknowledge the complex interaction between a hen and its total environment over the whole period of its life.

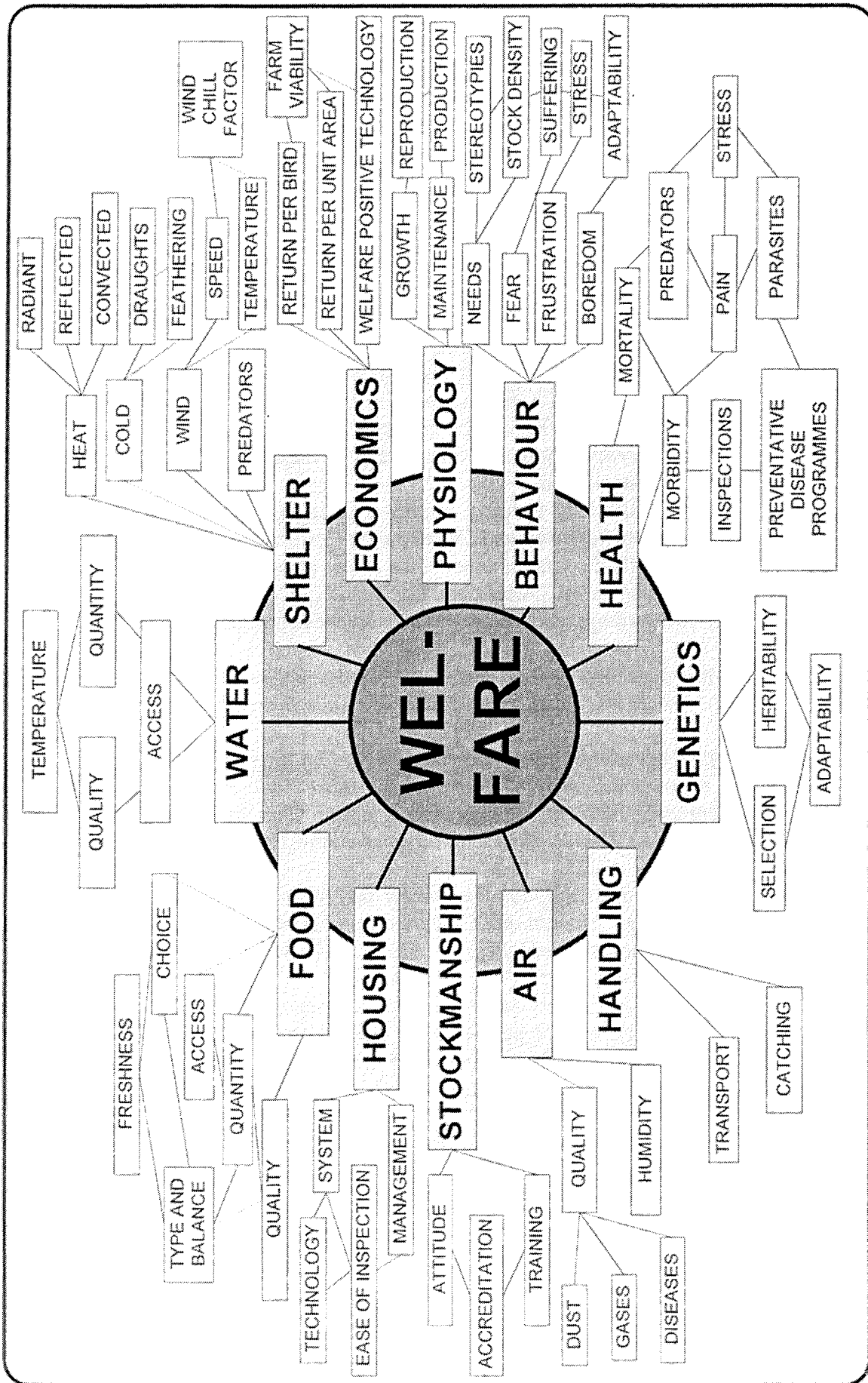
Welfare is not about the benefits of a particular system of housing and management on the best days of the year – rather, it is the combined benefits which an interactive housing / management system may offer the birds over 365 days of the year which is important. That is why striving for an incremental approach in every area is likely to achieve the most welfare positive gains in both the short and the long term.

The following model is designed to encapsulate the complexity of the relationship between a hen and its environment. Biological fitness depends on the outcome of the interaction of 'the sum of all the parts'. Any improvement to any 'part' will enhance the ultimate welfare outcome and will generally provide immediate and tangible welfare benefits.

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ASSESSING THE WELFARE OF HENS AND BROILERS

D.M. BROOM

1. INTRODUCTION

Welfare is a term which is restricted to animals, including man. It is regarded as particularly important by many people but requires strict definition if it is to be used effectively and consistently. A clearly defined concept of welfare is needed for use in precise scientific measurements, in legal documents and in public statements or discussion. If animal welfare is to be compared in different situations or evaluated in a specific situation, it must be assessed in an objective way. The assessment of welfare should be quite separate from any ethical judgement but, once an assessment is completed, it should provide information which can be used to take decisions about the ethics of a situation.

An essential criterion for a useful definition of animal welfare is that it must refer to a characteristic of the individual animal rather than something given to the animal by man. The welfare of an individual may well improve as a result of something given to it but the thing given is not itself welfare. The loose use of welfare with reference to payments to poor people is irrelevant to the scientific or legal meaning. However, it is accurate to refer to changes in the welfare of an initially hungry person who uses a payment to obtain food and then eats the food. The word welfare can be used in relation to a person, as above, or to an animal which is wild or is captive on a farm, in a zoo, in a laboratory or in a human home. Effects on welfare which can be described include those of disease, injury, starvation, beneficial stimulation, social interactions, housing conditions, deliberate ill treatment, human handling, transport, laboratory procedures, various mutilations, veterinary treatment or genetic change by conventional breeding or genetic engineering.

Welfare has to be defined in such a way that it can be readily related to other concepts such as needs, freedoms, happiness, coping, control, predictability, feelings, suffering, pain, anxiety, fear, boredom, stress and health.

(a) Welfare definition

If, at some particular time, an individual has no problems to deal with, that individual is likely to be in a good state including good feelings as indicated by body physiology, brain state and behaviour. Another individual may face problems in life which are such that the individual is unable to cope with them. Coping implies having control of mental and bodily stability and prolonged failure to cope results in failure to grow, failure to reproduce or death. A third individual might face problems but using an array of coping mechanisms, be able to cope but only with difficulty. The second and third individuals are likely to show some direct signs of their potential failure to cope or difficulty in coping and they are also likely to have had bad feelings associated with their situations. *The welfare of an individual is its state as regards its attempts to cope with its environment* (Broom, 1986). This definition refers to a characteristic of the individual at the time. The origin of the concept is how well the individual is faring or travelling through life and the definition refers to state at a particular time (for further discussion, see Broom, 1991a,b, 1993, 1996; Broom and Johnson, 1993). A crucial aspect of the concept is that welfare refers to how much an individual has to do when trying to cope, as well as the extent of any failure to cope and the extent of positive correlates of successful coping. The concept refers to the state of the individual on a scale from very good to very poor. This is a measurable state and any measurement should be independent of ethical considerations. When considering how to assess the welfare of an individual it is necessary to start with knowledge of the biology of the animal. The state may be good or poor. However, in either case, in addition to direct measures of the state, attempts should be made to measure those feelings which are a part of the state of the individual.

Welfare can be measured in a scientific way that is independent of moral considerations. Welfare measurements should be based on a knowledge of the biology of the

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species and, in particular, on what is known of the methods used by animals to try to cope with difficulties and of signs that coping attempts are failing. The measurement and its interpretation should be objective. Once the welfare has been described moral decisions can be taken.

(b) Welfare and needs

When attempting to determine what is an appropriate environment for an animal, most scientists involved in welfare research would agree with Appleby (1997) that a range of components of that environment, each of which is to some extent variable, should be considered. The environment is appropriate if it allows the animal to satisfy its needs. Animals have a range of functional systems controlling body temperature, nutritional state, social interactions, etc. (Broom, 1981). Together, these functional systems allow the individual to control its interactions with its environment and, hence, to keep each aspect of its state within a tolerable range. The allocation of time and resources to different physiological or behavioural activities, either within a functional system or between systems, is controlled by motivational mechanisms. When an animal is actually or potentially homeostatically maladjusted, or when it must carry out an action because of some environmental situation, it is said that it has a need. A need can, therefore, be defined as *a requirement, which is part of the basic biology of an animal, to obtain a particular resource or respond to a particular environmental or bodily stimulus*. As pointed out by Broom (1997c), these include needs for particular resources and needs to carry out actions whose function is to obtain an objective (Toates and Jensen, 1991; Broom, 1996). Needs can be identified by studies of motivation and by assessing the welfare of individuals whose needs are not satisfied (Hughes and Duncan, 1988a,b; Dawkins, 1990; Broom and Johnson, 1993). Unsatisfied needs are often, but not always, associated with bad feelings whilst satisfied needs may be associated with good feelings. When needs are not satisfied, welfare will be poorer than when they are satisfied.

Hens need to (Broom, 1992):

1. obtain adequate nutrients and water,
2. grow and maintain themselves in such a way that their bodies can function properly.
3. avoid damaging environmental conditions, injury or disease, and
4. be able to minimise the occurrence of pain, fear and frustration.

In order to achieve these ends, hens carry out a variety of activities, respond to certain stimuli and maintain certain physiological states.

Hence, they have other needs such as to:

5. show certain foraging and investigatory movements,
6. have sufficient exercise,
7. show preening and dust-bathing behaviour,
8. explore and respond to signs of potential danger,
9. interact socially with other hens,
10. search for, or create by building, a suitable nest-site.

(c) Welfare and feelings

The subjective feelings of an animal are an extremely important part of its welfare (Broom, 1991b). Suffering is a negative, unpleasant, subjective feeling which should be recognised and prevented wherever possible. However, whilst there are many measures which give information about injury, disease and both behavioural and physiological attempts to cope with the environment, fewer studies tell about the feelings of the animal. Information can be obtained about feelings using preference studies and other information giving indirect information about feelings can be obtained from studies of physiological and behavioural responses of animals.

Feelings are aspects of an individual's biology which must have evolved to help in survival (Broom, 1998) just as aspects of anatomy, physiology and behaviour have evolved. They are used in order to maximise fitness, often by helping to cope with the environment. It is also possible, as with any other aspect of the biology of an individual, that some feelings do

not confer any advantage on the animal but are epiphenomena of neural activity (Broom and Johnson, 1993). The coping systems used by animals operate on different time scales. Some must operate during a few seconds in order to be effectual, others take hours or months. Optimal decision-making depends not only on an evaluation of energetic costs and benefits but on the urgency of action. In other words the costs associated with injury, death or failure to find a mate (Broom, 1981). In the fastest acting, urgent coping responses, such as avoidance of predator attack or risk of immediate injury, fear and pain play an important role. In longer time-scale coping procedures, where various risks to the fitness of the individual are involved, feelings rather than just intellectual calculations are amongst the causal factors affecting what decisions are taken. In attempts to deal with very long-term problems which may harm the individual, aspects of suffering contribute significantly to how the individual tries to cope. In the organisation of behaviour so as to achieve important objectives, pleasurable feelings and the expectation that these will occur have a substantial influence. The general hypothesis advanced is that whenever a situation exists where decisions are taken which have a major effect on the survival or potential reproductive output of the individual, it is likely that feelings will be involved. This argument applies to all animals with complex nervous systems, such as vertebrates and cephalopods, and not just to humans. Feelings have not just a minor influence on coping systems, they are a very important part of them.

(d) Welfare and stress

The word stress should be used for that part of poor welfare which involves failure to cope. If the control systems regulating body state and response to danger are not able to prevent displacement of state outside the tolerable range, a situation of different biological importance is reached. The use of the term stress should be restricted to the common public use of the word to refer to a deleterious effect on an individual (Broom and Johnson, 1993). A definition of stress as just a stimulation or an event which elicits adrenal cortex activity is of no scientific or practical value. A precise criterion for what is adverse for an animal is difficult to find but one indicator is whether there is, or is likely to be, an effect on biological fitness. *Stress is an environmental effect on an individual which over-taxes its control systems and reduces its fitness or seems likely to do so* (Broom and Johnson, 1993, see also Fraser and Broom, 1990). Using this definition, the relationship between stress and welfare is very clear. Firstly, whilst welfare refers to a range in the state of the animal from very good to very poor, whenever there is stress, welfare is poor. Secondly, stress refers only to situations where there is failure to cope but poor welfare refers to the state of the animal both when there is failure to cope and when the individual is having difficulty in coping. For instance, if a person is severely depressed or if an individual has a debilitating disease but there is complete recovery with no long term effects on fitness then it would still be appropriate to say that the welfare of the individual was poor at the time of the depression or disease.

(e) Welfare and health

The word "health", like "welfare", can be qualified by "good" or "poor" and health varies over a range. However, health refers to the state of body systems, including those in the brain, which combat pathogens, tissue damage or physiological disorder. Welfare is a broader term covering all aspects of coping with the environment and taking account of a wider range of feelings and other coping mechanisms than those which affect health, especially at the positive end of the scale. Although people regularly refer to poor health they sometimes use the word to mean absence of illness or injury in the same way that people refer to welfare when they mean good welfare. However, the precise and scientific use of health and welfare must refer to states varying from very good to very poor. "Health" is encompassed within the term "welfare" and indeed is a very important part of welfare.

Health is a part of welfare and, hence, disease always has some adverse effect on welfare. There can also be effects in the other direction because specific aspects of health may be made worse when welfare in general is poor (Broom, 1988b). These relationships are summarised as follows.

Disease effects: poor welfare.

Difficult conditions: poor welfare, immunosuppression, increased disease.

Overall: poor welfare, disease.

The sequence could start with infectious disease which then causes poor welfare. Alternatively, inadequate housing conditions could lead to poor welfare and hence to increased disease susceptibility. If animals became diseased as a consequence, this would result in worse welfare than that caused directly by the housing conditions.

The general conclusions about the inter-relationships between welfare improvement attempts and disease are firstly, that disease is an aspect of poor welfare and many actions will be of benefit in both respects. Secondly, that the possible trade off between reduced immunosuppression and increased disease transmission risk should be carefully considered in all attempts to improve welfare. Thirdly, that there are differences between production- or system-related diseases and dangerous infectious diseases. While there is quite a lot of information about the former, the latter should also be borne in mind when new systems are being developed for housing and managing animals. The overall aim should be to improve welfare in total, including consideration of the effects on individuals of any disease which they might contract (Broom, 1992).

(f) Welfare assessment

The general methods for assessing welfare are summarised in Table 1 and a list of measures of welfare is presented in Table 2. Most indicators will help to pinpoint the state of the animal wherever it is on the scale from very good to very poor. Some measures are most relevant to short-term problems, such as those associated with human handling or a brief period of adverse physical conditions, whereas others are more appropriate to long-term problems. (For a detailed discussion of measures of welfare, see Broom 1988a; Fraser and Broom, 1990; Broom and Johnson, 1993).

Table 1. Summary of welfare assessment (modified after Broom, 1990a).

General Methods	Assessment
Direct indicators of poor welfare.	How poor?
Tests of (a) avoidance and (b) positive preference.	(a) Extent to which animals have to live with avoided situations or stimuli. (b) Extent to which that which is strongly preferred is available.
Measures of ability to carry out normal behaviour and other biological functions.	How much important normal behaviour or physiological or anatomical development cannot occur?
Other direct indicators of good welfare.	How good?

The net welfare at any moment will be the extent of good welfare indicated less the extent of poor welfare. If the extent of a problem is being evaluated and there are indications that net welfare is poor, the longer this state persists the worse is the evaluation. If the severity of the effect on the individual is measured and is plotted against time the total amount of poor welfare is indicated by the area under the curve produced (Broom, 1999b). A further factor to consider is the number of individuals involved. When a judgement is made about the overall magnitude of a problem the total amount of poor welfare in each individual whose welfare is poor should be summed. Most people would not consider it acceptable to allow compensation among individuals between good and poor welfare. If there are five thousand

contented people and two hundred desperately unhappy people in a town this does not add up to no problem. The large numbers of individual animals in the poultry industry mean that poor welfare in a small proportion of individuals is still a serious matter. If a high proportion of individuals are affected, the problem is greater.

Table 2. Measures of welfare.

Physiological indicators of pleasure.
Behavioural indicators of pleasure.
Extent to which strongly preferred behaviours can be shown.
Variety of normal behaviours shown or suppressed.
Extent to which normal physiological processes and anatomical development are possible.
Extent of behavioural aversion shown.
Physiological attempts to cope.
Immunosuppression.
Disease prevalence.
Behavioural attempts to cope.
Behaviour pathology.
Brain changes, e.g. those indicating self narcotisation.
Body damage prevalence.
Reduced ability to grow or breed.
Reduced life expectancy.

II. KEY DATA CONCERNING THE WELFARE OF HENS AND BROILERS

(a) Space for movements

If hens need to carry out a range of normal movements how much space is required for these? Measurements of the space occupied by a hen when carrying out such movements have been made (Dawkins and Hardie, 1989) (Table 3).

Table 3. Area required by hens for different behaviour patterns (cm²).

Standing	428 - 592
Turning	771 - 1377
Preening	818 - 1270
Ground-scratching	540 - 1005
Wing-stretching	653 - 1118
Wing-flapping	860 - 1980

If there are five hens in a cage these will not show all the different movements simultaneously and some hens might be relatively inactive whilst one bird uses more space. Some possible combinations of movement are considered in Table 4. It is clear from the calculations presented in Table 4 that a cage for five hens allowed 450 cm² each, and hence occupying 2250 cm², severely inhibits normal movements. Wing-flapping is not possible with commonly used cage heights of 50 cm or less.

If hens are allowed more space than 450 cm² per bird the amount of disturbed behaviour shown is decreased (Hughes and Black, 1974; Hansen, 1976; Zayan and Doyen, 1985; Cunningham *et al.*, 1987; Nicol, 1987). Hens will work for a larger space allowance of up to 1125 cm² per bird, and they continue to space themselves out in cages of 1410 cm² per bird, but in much larger space allowances of 5630 cm² per hen, they cluster. The effects of space allowance on the extent of injurious behaviour are not a linear relationship in battery

cages (Polley *et al.*, 1974; Al Rawi and Craig, 1975) but depend upon the complexity of the environment. In order to provide opportunities to escape and to hide from birds which tend to feather-peck or cause tissue damage by pecking, more space allowance than that normally provided in a battery cage is needed. Such escape possibilities are important in order to minimise injuries caused by other birds. As long as they are available injurious behaviour can be low at various space allowances.

Table 4. Space required for hens in a cage holding five birds.

	cm ² used	space per bird
4 hens crowded together plus 1 wing flapping	2720	544
4 hens crowded together plus 1 wing stretching	2185	437
4 hens crowded together plus 1 preening	2342	468
3 hens crowded, 1 turning, 1 wing flapping	3469	694
2 crowded, 2 turning, 1 wing flapping	4218	844
4 hens standing, preening	3074	615
4 hens standing, 1 wing flapping	3460	692
2 hens standing, 2 turning, 1 wing stretching	4050	810
2 hens standing, 2 turning, 1 wing flapping	4584	917

The space requirements of broilers in normal housing conditions, are those which allow normal movements, exploration and social interactions. At least enough space to exercise, maintain leg condition and have access to resources is needed. The problem arises in the latter stages of growth when birds are crowded together. Increasing stocking density above 25 kg/m² increases mortality, reduces locomotion, reduces litter quality, increases leg disorders and dermatitis, reduces calm behaviour and nesting and makes the finding of sick and injured birds more difficult.

(b) Bone fragility

The diet of hens is adequate for bone development, with calcium and vitamin D being key factors, but the bones of hens from battery cages break easily. In a series of studies, 25-40% of end-of-lay hens from battery cages were found to have at least one broken bone following handling prior to stunning and 98% of carcasses had a broken bone (Gregory and Wilkins, 1989; Gregory *et al.*, 1990, 1991). The numbers of broken bones from percheries and aviaries were much lower although hens from poorly designed or over-crowded percheries sometimes broke bones in the living conditions. The strength of the bones in wings and legs were reduced if there was insufficient opportunity for exercise. Birds which lived in cages in which they could not flap their wings had wing bones which were only half as strong as those of birds in a perchery which could and did flap (Knowles and Broom, 1990; Nørgaard-Nielsen, 1990).

(c) Investigatory pecking and dust bathing

Chickens strongly prefer litter floors to wire floors (Dawkins, 1982; Appleby *et al.*, 1988). The opportunity to peck at objects on the floor, scratch on the floor and dust bathe in a suitable substratum reduces the likelihood that injurious behaviour will be shown by hens and broiler breeders (Blokhus, 1986; Vestergaard *et al.*, 1990). Studies of the developmental and motivational basis of feather-pecking behaviour indicates links with deprivation of ground-pecking and dust-bathing opportunities.

(d) Nest boxes and egg laying

An appropriate nest box is used by almost all hens if it is readily accessible (Wood-Gush, 1975) and behaviour is clearly disturbed if none is available. The abnormal behaviour

most frequently observed when no suitable nest site is present is stereotyped pacing (Wood-Gush and Gilbert, 1969; Fölsch, 1981; Heil *et al.*, 1990). This stereotypy is a sign of long-term, intense frustration.

(e) Perching

Perches are preferred resting places for all but the youngest chickens. The design should be right and early experience of perches facilitates effective use (Appleby *et al.*, 1993). The presence of perches can increase leg strength (Hughes and Appleby, 1989). Where cloaca-pecking is a possibility, the perch should not be sited at such a height that the heads of some birds are level with the vents of others. This has been an important reason for the failure of some "getaway" cages because of injurious pecking (Moinard *et al.*, 1998). Young broilers use platforms and straw bales more than perches but appropriate perching facilities could improve welfare in general and leg strength in particular.

(f) Lighting

If hens, broilers or broiler breeders are kept in low light levels they are not able to show normal exploratory behaviour. At the lowest levels eye development is impaired. A review by Manser (1994) indicated clear welfare problems at light levels lower than 20 lux.

(g) Beak-trimming

Mutilations which involve tissue damage are painful at the time of the operation and can sometimes cause neuromas which result in lasting pain. Beak-trimming also seriously impairs sensory input and pecking behaviour. The effect on welfare of beak-trimming is substantial but is much greater if neuromas are present.

(h) Leg problems and ascites

The major causes of poor welfare in modern strains of broilers are leg disorders and ascites. The clinical conditions which impair walking include femoral head necrosis (Thorp *et al.*, 1993), dyschondroplasia (Lynch *et al.*, 1992), valgus-varus deformity (Lynch *et al.*, 1992), rickets and, in older birds, degenerative disorders (Hocking *et al.*, 1996). These conditions have become much commoner as growth rates have increased. The conditions must be painful as walking ability of birds with moderate lameness was improved after administration of the analgesic and anti-inflammatory drug carprofen (McGovern *et al.*, 1999).

Ascites is another pathological condition associated with fast growth in broiler chickens. It is also known as pulmonary hypertension syndrome and results in fluid from the blood leaking into abdominal cavities. It affects 5% of young broilers and 15-20% of the larger birds and whilst it can kill, it certainly weakens the birds and results in carcass condemnation. The main cause of ascites is failure of heart function associated with lack of oxygen supply to tissues. It is extremely rare in old strains of broilers and results from failure of the cardiovascular and pulmonary systems to grow fast enough to keep pace with the demands from the muscles and gut.

(i) Genetic selection

Farm animals have been selected for breeding on the basis of a range of criteria but by far the most important has been efficiency of production per animal. Fast growth, good feed conversion efficiency and high egg production have been selected for and this has had various consequences.

Broiler chickens have been selected, and their nutrition and management have been designed, so that they grow to market weight quickly and convert food to muscle efficiently. Thirty years ago chickens reached market weight at 12 weeks of age. Now the weight is often reached in 35 days and the age has been reduced by one day per year for the last five years. The change in the bird has been that muscle and gut grow very fast but problems can arise

because the bones and cardiovascular system do not grow as fast. As a consequence, the birds may suffer from leg problems even when the diet is ideal. Some broiler chickens have leg damage and leg pain especially in the last week before slaughter, one consequence being that their ability to walk is impaired. Kestin *et al.* (1994) reported that 90% of broiler chickens had some walking ability impairment in the last week before slaughter and 26% had a severe impairment. Sanotra (1999), studying a broiler strain used in many countries, found that 30% of birds on commercial farms had severe walking difficulties by market age. It is widely known that birds with weak legs sit on litter and when the litter quality is not good many chickens, as a consequence, have contact dermatitis visible on carcasses as breast or hock burn. A comparison of 1957 and 1991 strains of broilers showed that growth rates and, hence, leg problems have an origin which is much more a consequence of genetics than of food quality (Havenstein *et al.*, 1994).

The poor welfare which occurs in broiler chickens as they near the age of slaughter affects a very large number of individuals and may well be the most serious animal welfare problem in the world today. However, the problems are solvable. Birds can be bred for stronger legs but some slowing of growth, by genetic selection or management, and this is essential for a real solution. Leg problems can be reduced if food intake is limited for a period during growth (Classen, 1992). Some problems are exacerbated by high stocking density so this should be limited to a maximum of 25 kg/m².

For broiler breeders, the major welfare problem is probably hunger. Selection for fast growth means that the breeding birds would eat too much if fed *ad libitum*. However, the level of feeding normally used means that broiler breeders are hungry for most of their lives.

The selection of hens has taken insufficient account of the need to minimise injurious pecking and other welfare problems. Successful genetic lines in future will have to be those for which good welfare, as well as egg-production, has been a major factor in selection procedures.

III. CONCLUSIONS

There are very serious problems for hens in battery cages which result in poor welfare. In order to solve these, the basic needs of hens, including the need to show certain behaviours must be met. This is not possible unless the space available and design allows the provision of a nesting place, a perch, possibilities for dust-bathing and investigatory pecking, and room for walking and wing-flapping. No small cage can provide this. Design of hen accommodation and genetic selection of birds should be such that injurious pecking is minimised.

Broiler chickens must be genetically selected for stronger legs and slower growth. Stocking density must be limited and methods of enriching the environment in adequately illuminated conditions should be used.

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THE SCIENTIFIC ASSESSMENT OF ANIMAL WELFARE AND ITS APPLICATION TO SOME ISSUES IN THE EGG INDUSTRY

J. L. BARNETT

Summary

A recent government review of the layer hen industry in Australia has resulted in a number of resolutions by the State Agriculture Ministers that have implications for poultry housing. These include increased space in cages, scrapping of some older cages and research on furnished cages and non-cage systems. To help put these recommendations in some perspective, this paper examines the literature on some of the resolutions within the broader framework of the scientific assessment of welfare. It focuses on space and items of furniture such as perches, nest boxes, dust baths and abrasive strips. The homeostasis approach to the assessment of welfare is used in which welfare is evaluated on both how much has to be done by the animal in order to cope and the extent to which the coping attempts are succeeding. Using this approach there is evidence for increasing space in cages, based on reduced mortalities and increased production, and incorporating perches, based on a reduction in injuries at depopulation. Similar evidence for the inclusion of dust baths and nest boxes is lacking. The data on abrasive strips are equivocal with recommendations from overseas for their inclusion but local data indicating an increase in mortalities.

I. INTRODUCTION

Over at least the last 12 months there has been discussion by the Agriculture and Resource Management Council of Australia and New Zealand on options for a national approach to layer hen housing systems in Australia. At the last meeting of State Agriculture Ministers, in August, there were a number of resolutions that have implications for housing of poultry. These included that all new cage systems must provide a floor space of 550 cm²/hen, including the baffle, by 1 January 2001; that all cage systems that do not meet the 1995 standards are to be scrapped by 1 January 2008; research and development, based on furnished cages that include perches, nests, litter and abrasive strips, and non-cage alternatives such as barn and free-range be conducted in Australia by 2005, with the expectation that if the research is successful that industry will implement such system(s). The 1995 standards for cages include a space allowance of 450 cm²/bird, a slope on the floor of less than 8 degrees, a minimum of 40 cm height over 65 % of the cage floor area and more than 35 cm at all points, and cage fronts that are full height and width. In addition to resolutions on cage dimensions, the Code of Practice is being revised and this may result in additional changes. To help put some of these resolutions in perspective this paper examines some of the literature on space and cage modifications within the broader framework of the scientific assessment of welfare.

II. THE ASSESSMENT OF WELFARE

In making a decision on whether or not an animal's welfare is seriously compromised, individuals integrate moral views with biological facts. Thus, science has the important role of establishing the facts on how animals biologically respond to the practices under question.

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However, the assessment of welfare is a controversial subject. Within scientific disciplines, variations in definitions of animal welfare exist and combined with variations in methods and, in turn, interpretation, lead to disagreement (Hemsworth and Coleman, 1998). The following definition of Broom (1986) is favoured: "The welfare of an individual is its state as regards its attempts to cope". In this definition, the "state as regards its attempts to cope" refers to both how much has to be done by the animal in order to cope with the environment and the extent to which the animal's coping attempts are succeeding. Attempts to cope include the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses. Therefore, using such a definition, the risks to the welfare of an animal by an environmental challenge can be assessed at two levels: firstly, the magnitude of the behavioural and physiological responses and, secondly, the biological cost of these responses (Barnett and Hutson, 1987; Broom and Johnson, 1993; Hemsworth and Coleman, 1998). These behavioural and physiological responses include the stress response while the biological cost includes adverse effects on the animal's ability to grow, reproduce and remain healthy. This definition has been broadened to incorporate animal emotions (Broom, 1998) and there is no reason not to incorporate animal emotions into the homeostatic approach as they would have evolved on the basis of their survival values and contribution to biological fitness.

The disagreement over what is important for the welfare of animals led to attempts to study and conceptualise animal welfare in more scientific ways. It is generally accepted that there are three broad approaches used by scientists in studying animal welfare: the "feelings-based", the "nature of the species" and the "functioning-based" approaches (Duncan and Fraser, 1997). A more descriptive title for the last approach, which will be used here is the "homeostasis" approach. A fourth approach, the "animal preferences" approach, is sometimes included in the feelings approach but does not necessarily provide direct information on feelings or emotions. This approach involves studying the animal's choice for resources.

My colleagues and I favour the homeostasis approach, as outlined above, in assessing animal welfare. There is no reason why animal emotions cannot be incorporated into the homeostatic approach as they would have evolved on the basis of their survival values and contribution to biological fitness. This concept of biological fitness generally applies to natural populations and refers to "fitter" animals making a greater genetic contribution to subsequent generations (Pianka, 1974). This is based on their abilities to successfully survive, grow and reproduce. While the last attribute may not always apply to individual farm animals since reproduction is either controlled or absent for many farm animals, the ability to grow, survive and reproduce could be considered measurements of "fitness" within the limits of the management system. Most production systems in agriculture have breeding and growing components and these can generate considerable data on reproductive success of individuals. For example, conception rates and mortality, morbidity and growth of offspring can be used as a measure of "fitness". Similarly, Beilharz and Zeeb (1981) and Beilharz (1982) have linked reproductive performance of domestic species with welfare.

An attribute of the "homeostasis" approach that provides it with credibility within scientific circles is that it contains some widely accepted criteria of poor welfare such as health, immunology, injuries, growth rate and nitrogen balance. Furthermore, there are some excellent examples of the value of this "homeostasis" approach in assessing animal welfare (Hemsworth and Coleman, 1998). For example, handling studies on pigs have shown that fearful pigs have a sustained elevation of plasma free corticosteroid concentrations (Hemsworth *et al.*, 1981, 1986; Hemsworth and Barnett, 1991). The consequences of this chronic stress response in these fearful animals included depressions in growth and reproductive performance (Hemsworth *et al.*, 1981, 1986; Hemsworth and Barnett, 1991).

A counter argument is that our current knowledge may not allow detection of some of

the more subtle or less serious risks to welfare. Nevertheless, less serious challenges should be reflected in biological changes, admittedly of lower magnitude, with consequent effects on fitness variables such as growth, reproduction, injury and health. Short-term challenges can also be studied with this approach. Lay *et al.* (1992) studied the behavioural and physiological responses of cattle to two branding procedures to assess the relative aversiveness of the procedures and Hemsworth *et al.* (1996) utilised behavioural and physiological responses together with growth performance to assess the welfare implications of a husbandry procedure regularly imposed (daily injections) on pigs.

With current knowledge the "homeostasis" approach appears to offer science the best assessment of the welfare of animals. As a research tool, this approach involves comparing housing or husbandry systems and risks to welfare are assessed on the basis of relative changes in biological (behavioural and physiological) responses and corresponding decreases in fitness. This is the approach that is utilised in this review.

III. IMPLICATIONS FOR POULTRY

There has been considerable research in recent years on developing furnished cages as a replacement for conventional cages (Appleby and Hughes, 1990; Abrahamsson *et al.*, 1996; Abrahamsson and Tauson, 1997; Appleby, 1998; Tauson, 2000). Of particular interest has been the incorporation of perches (to improve bone strength), solid sides (to improve feather condition), abrasive strips (to reduce claw length and subsequent injuries), nest boxes (to provide for nesting behaviour) and sand-baths (to provide for dust-bathing behaviour).

(a) Space allowance

The literature on the effects of space allowance indicates that in general, within a range of 300 to 650 cm² per caged laying hen, increasing the area per bird increased egg production, food consumption and weight gain and decreased mortality (see Hill, 1977; Hughes, 1983; Adams and Craig, 1985). As identified by Hughes (1983), an obviously important explanation is the reduced feeding space that is generally associated with an area reduction in cages of generally constant depth. Another explanation is that crowding may lead to elevated corticosterone concentrations which, in turn, may adversely affect both production efficiency and health. Koelkebeck *et al.* (1987) reported an 11% increase in plasma corticosterone concentrations in caged hens when space allowance was decreased from 460 to 350 cm² per bird. However, Faure (1991) showed that birds would not consistently work for additional space. While these data, particularly those on production and mortality, would suggest that additional space is of benefit to birds, the precise area is difficult to define.

(b) Perches

A major problem that arises from keeping hens in cages is the problem of broken bones that occurs as a result of handling and transport (Gregory and Wilkins, 1989; Broom, 1990). Some of these problems occur as a result of cage design and certainly improved door design (eg S-shaped full width doors as described by Tauson (1985) and Elson (1990)) should improve access and reduce the risk of bone breakages when removing birds from cages. However, it would be preferable if bone strength was improved so that the risk of broken bones was reduced. While the provision of perches in cages improves bone strength (Hughes and Appleby, 1989; Norgaard-Nielsen, 1990; Gregory *et al.*, 1991), there can be detrimental consequences on production. In cages with perches there were increased numbers of cracked

eggs (Ruszler and Quisenberry, 1970) and reduced egg mass output (Tauson, 1984). However, this can be influenced by both perch design and the age of pullets when exposed to perches (Appleby *et al.*, 1998). While there appears to be clear advantages to the strength of some bones by the provision of perches, attention is required on the position, size and shape of the perch (see Tauson, 1984; Appleby and Hughes, 1990). Also, the effects of perches on non-load bearing bones is unclear and these bones may be adversely affected by, or derive no benefit from, perches; Appleby (1993) reported deformation of the sternum due to perches. Access to perches during rearing decreased cannibalism during the laying period (Gunnarsson *et al.*, 1999).

From a scientific perspective of welfare there is good argument, on the basis of the potentially improved bone strength and its positive consequences for health, for the inclusion of perches within cages. However, depending on the shape and location of the perch there can be a production cost in terms of increased numbers of cracked or dirty eggs i.e. an economic cost (see Glatz and Barnett, 1996). Integrating these two pieces of information is a political decision not a scientific one.

(c) Dust baths

In addition to providing a substrate for birds to dust bathe another reason for the inclusion of dust baths is to reduce the use of the nest box as a dust bath. Nevertheless, the welfare benefits of dust baths are far from clear. Studies have shown that hens do not make any great effort to obtain access to litter or sand (Faure, 1991; Faure and Lagadic, 1994), although they prefer litter to wire mesh (Lagadic, 1992). Petherick *et al.* (1993) suggested birds are not highly motivated to dust bathe and Widowski and Duncan (2000) have shown that the bird's motivation to gain access to litter is highly variable. Nevertheless, van Lier (1992) suggests that dust baths are essential to maintain feather integrity and for welfare. In terms of fitness variables, experiments with young chickens indicate a risk of pathological feather pecking when straw or wood-shavings are used as a substrate (Sanotra *et al.*, 1995) although Norgaard-Nielsen *et al.* (1993) showed that rearing with access to sand or peat reduced subsequent feather pecking and that access to straw, as an environmental enrichment, during the layer phase also reduced feather pecking. Rudkin (1997) has also shown positive effects of hay, both during rearing and the laying period, in reducing feather pecking. Nevertheless, the implications of these rearing experiments for the provision of dust baths in cages to improve welfare is unclear.

(d) Nest boxes

Duncan (1992) considered the lack of a nest site in conventional cages was the biggest welfare problem in this system of housing. The importance of the nest box is based on evidence of preference tests and evidence of frustration in the absence of a nest box (see review by Ekstrand and Keeling, 1994) and the strong motivation of hens to use a nest (Hughes *et al.*, 1989; Smith *et al.*, 1990). Cooper and Appleby (1995) have considered the controversy as to whether animals can be frustrated or experience a sense of deprivation by not having certain resources they have never experienced. For nesting, they found no differences between birds previously experienced or inexperienced with a nest in their motivation to use a nest, although it is not known if this leads to chronic frustration. However, Hughes *et al.* (1995) showed that naive birds did not recognise a visual stimulus with some features of a nest, although it must be recognised that the birds in this study were unable to physically interact with the 'nest'. A study by Webster and Hurnik (1994) suggests that birds may synchronise their behaviours within cages and this may have welfare

implications if nest sites are limited. Cooper and Appleby (1997) have found considerable variation in choice of nest-site but not nesting motivation.

Several aspects of nest design have been examined and current commercial nests incorporate some of these features, such as the use of artificial floors to increase attractiveness, sloped (roll-away) floors to improve egg quality, nest excluders to improve nest hygiene (Reed, 1994) and enclosed nests to reduce 'stress-associated' egg abnormalities (Walker and Hughes, 1998). Notwithstanding that developmental problems with the use of nest boxes, including roosting in the nest boxes, laying eggs outside of nest boxes, a higher incidence of cracked eggs and using the nesting material for a dust bath, can or have been solved, there is no evidence that nests affect fitness.

(e) Abrasive strips

One of the criticisms of keeping birds in cages is the excessive length that claws can reach by the end of the laying period as hens in conventional cages are not able to wear down their claws as effectively as birds kept in non-cage systems. Floor layers spend time scratching litter or soil and this behaviour wears the claws and keeps them blunt. However, in cages, claw length of the middle toe can reach over 40 mm (Hill, 1975; Tauson, 1977; Fickenwirth *et al.*, 1985) and in some strains the claws can become long, twisted and cracked and have a pronounced curl. A method by which claws can be kept short and blunt is to fit 8 mm wide strips of abrasive tape onto the egg guard; birds' claws scrape against this tape while they are feeding. Tauson (1986) showed that in cages with the strips, birds had significantly shorter claws than control hens throughout the laying period, the length of the claws of the middle digits did not exceed the length of those in pullets or birds kept on litter floors, and there were fewer broken or twisted claws. Rauch (1992) reported that the middle claw length of 42 week-old medium hybrid layers was 17.4 mm versus 7.2 mm for birds using the tape while for light hybrid layers at 61 weeks of age the measurements were 29.4 and 13.9 mm, respectively. Tauson (1996) considered an abrasive paint may improve the durability of the abrasive.

In Australia there have been two experiments with abrasive strips in layer cages. Both Murphy (unpublished data) and Stewart and Dingle (1997) indicated that abrasive strips were effective in reducing claw length. In the latter study the authors found that the angle and size of the egg baffle in different manufacturers' cages affected the reduction in claw length, although the abrasive strip was considered of benefit in all cage types examined. Glatz (2000) compared the effect of abrasive strips and abrasive paint in layer cages on claw length, claw sharpness, foot condition, feather cover, body scratches and mortality of hens. Abrasive paint was found to be more effective as a claw shortener than abrasive strips, based on length of claws and sharpness. However, hen mortality from prolapse and cannibalism combined was significantly higher in cages fitted with abrasives (6.3% for paint, 5.9% for strips and 1.6% for control. Glatz (2000) speculated that when birds were frightened or competed for a position at the feeder they might abrade their vent region on the paint or strip and this may encourage vent pecking. This finding raises concerns on whether claw shorteners should be installed in cages under Australian conditions. The increased light levels in the Australian study may account for the increase in cannibalism and prolapse.

IV. CONCLUSIONS

This review shows that there are welfare benefits from more space, on the basis of reduced mortalities, and from incorporating perches into conventional cages, based on a 'fitness' measure of the likely fewer 'injuries' due to improved bone strength. However, there

is no similar evidence for the incorporation of nest boxes or dust baths. Studies on solid sides and abrasives also indicate that overseas research may not be directly applicable to Australian conditions and that environmental factors (e.g. light or temperature) may affect behaviour, physiology or mortality. Therefore, it is important that these housing features are evaluated under local conditions prior to making any recommendations. Furthermore, the interactions between the items of furniture that together make up furnished cages also warrant examination as the findings from a furnished cage may be different from the reported literature on individual items of furniture. The issue of poultry housing is likely to remain a controversial topic until some fundamental issues, such as achieving an agreement on methodologies used to assess welfare, are addressed.

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THE EUROPEAN UNION LAYING HEN DIRECTIVE AND OTHER EUROPEAN UNION DEVELOPMENTS

D.M. BROOM

I. INTRODUCTION

The European Union Council Directive 1999/74/EC "laying down minimum standards for the protection of laying hens" was passed on 19 July 1999 and hence is being made law in each E.U. member state. Some key aspects of the legislation will be discussed here and then prospects for legislation on broiler chickens will be considered.

II. THE LAYING HEN DIRECTIVE

(a) Unenriched battery cages

The use of battery cages like those in use in much of the world will be prohibited from 1st January 2012. In the meantime, from 1st January 2003, hens in such cages must be provided with 550 square cm per bird, feed troughs must be 10cm per hen, cages must be 40 cm tall and cages must have claw-shortening devices.

(b) Enriched cages

Laying hens in enriched cages must have 750 square cm per bird, a nest, litter for pecking and scratching, perches allowing at least 15 cm per hen, 12 cm per bird of feed trough and claw-shortening devices.

(c) Alternative systems

This refers principally to aviary systems which must provide space on the ground and on tiers for nine laying hens per square m. There must be 10 cm of feeder, 15 cm of perch and 250 square cm of littered area per hen and one nest for each seven hens.

In all systems, beak-trimming of birds less than ten days old is permitted.

III. SOME PRACTICALITIES OF THE LAYING HEN DIRECTIVE

An important criticism of the battery cage system has always been that much space in the building is wasted. This space could be used by the birds with considerable beneficial effect on their welfare (Broom, 1992). Table 1 shows the volume of building needed for different systems. It is clear that aviaries can house as many birds in a building as battery cages.

Table 1. Space occupied by hens in different systems (E.U. Scientific Veterinary Committee Report, 1996).

Housing system	Floor area (Birds per m ²)	Volume of building (Birds per m ³)
Battery cages: 3, 4, 6, 8 tiers	20-22.2	10 - 11.9
Multifloor, Naturel or Natura aviary	8.3 - 9.6	11.4
25 birds per m ²		
20 birds per m ²	8.7 - 9.8	9.1
Dutch Tiered Wire Floor aviary	10.9	8.3
Deep litter	7.0	2.9

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Injurious pecking is a key problem in all laying hen systems. Mortality can be high when it occurs in large groups. However, in well designed systems its incidence can be low. In discussions about whether enriched cages or aviary systems are the future for egg production, questions about injurious behaviour and other aspects of welfare in relation to group size are important. Some evidence concerning this question is presented in Table 2.

Table 2. Number of birds per group : welfare.

In cages, when more than four birds are present, increased group size leads to more fearfulness, aggression, feather-pecking, cannibalism and adrenal weight and poorer egg production per bird (11 papers).

Feather pecking increases in a linear way with group size in cages.

In pens with a solid floor the effect of group size depends on design.

Commercial Tiered Wire Floor units in Holland with about 7,000 - 10,000 birds per unit have levels of feather pecking and cannibalism less than or similar to those in cages.

Several studies have compared costs and mortality in different systems. Badly designed or managed systems can always give bad results but the data in Tables 3,4 and 5 are from good, large scale, commercial systems.

Table 3. Comparison of battery cage and free range (Sanders, 1996).

	Battery cage	Free range
Mortality (%)	5.2	6.4
Eggs per hen	282	253
Feed per hen per egg (g)	6.0	6.3

Table 4. Comparison of battery cage and aviary (van Horne, 1996).

	Battery cage	Aviary (beak-trimmed mostly in Tiered-Wire-Floor)
Mortality (%)	9.2	6.7 *
Eggs per hen	331	325
g feed per g egg	2.27	2.20 *

* Significantly different at $P < 0.05$.

Table 5. Comparison of battery cage, enriched cage and get-away cage (Abrahamsson *et al.*, 1995)

	Battery cage	Enriched cage	Get-away cage
Mortality (%) Expt 1	8.0	7.8	13.3
Expt 2	5.8	2.6	8.6 *
Egg mass per hen Expt 1	51.7	51.0	50.0 *
Expt 2	53.4	53.2	50.3 *
Feed per hen per day (g.)	123.2	116.1	117.4
	129.0	127.0	120.6

* Significantly different at $P < 0.05$.

Careful comparisons of the costs of egg production in battery cages, somewhat enlarged battery cages, aviaries, enlarged/enriched cages, percheries, deep-litter and free range are summarised in Table 6. The data for enriched cages are not good because they are only just becoming available commercially. Some practical problems have not yet been solved in enriched cages, in particular the provision of dust-bathing facilities which do not result in rapid spreading of sand, etc., in the machinery for food provision and egg removal. Important problems in relation to welfare are cage height being too low for wing-flapping and insufficient possibilities for investigative pecking because of a non-preferred wire-mesh floor.

Table 6. Percentage difference from battery cage 450 cm²/bird in total cost per kg egg.

	Battery cage 560cm ² /bird	Aviary 20 birds/ m ² of floor	Cage 833cm ² /bird	Perchery	Deep litter	Free range
Elson (1985)	+ 5					
Haartsen and Elson (1989)		+ 4.6			+16.5	
Tucker (1989)				+ 11.8		+ 51.6
van Horne (1996)		+ 8.3				
Elson (1995)			+ 23	+ 26		+ 45
(estimates: 500cm ² /bird)						
EU Scientific Veterinary Committee (1996) estimate			enriched cage + 10-20			

IV. CONCLUSIONS CONCERNING HEN HOUSING

At present, the viable alternatives to the battery cage are the best of the aviary systems. These sometimes require beak-trimming with current designs and genetic strains. It seems unlikely that enriched cages will compete economically if they provide for the needs of the hens. New genetic strains of hens which show less injurious pecking are needed.

V. THE EUROPEAN UNION BROILER REPORT

On 21st March 2000 The E.U. Scientific Committee on Animal Health and Animal Welfare adopted a Report (SANCO.B.3/AH/R15/2000) entitled "The Welfare of Chickens Kept for Meat Production (Broilers)". The two principle recommendations of this report were as follows. Firstly, that genetic selection of broilers should change so that serious welfare problems associated with leg disorders and ascites are substantially reduced. It was considered that this would necessitate selection for slower growth. Secondly, that stocking density should be limited to 30 kg per square m in well-controlled environments and to lower stocking densities in less well-controlled environments. Environmental enrichment and measures to stimulate locomotion should be encouraged.

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THE EUROPEAN BAN OF THE PROPHYLACTIC USE OF ANTIBIOTICS AS GROWTH PROMOTERS IN ANIMAL NUTRITION: POLITICAL AND ECONOMIC ASPECTS

P.E.V. WILLIAMS

Summary

On 17 December 1998 the European Commission effectively banned the use of four antibiotic substances when used as additives in feed for the promotion of growth. The reason for the ban was stated as the need to protect consumers from the development of antibiotic resistance in pathogenic bacteria resulting from the widespread use of antibiotics in animal production. In the absence of scientific evidence to support the fact that the use of antibiotic growth promoters has resulted in the development of antibiotic resistance it can be questioned whether the action was politically motivated.

I. INTRODUCTION

Over the past fifteen years the European consumer has been faced with many food scares. In the autumn of 1988 the U.K. egg business was decimated following a statement that eggs in Great Britain were contaminated with Salmonella, made by Mrs Edwina Curry, who at the time was a senior member of parliament in the Conservative Government. Since then we have witnessed in 1996 an episode of food poisoning in which twenty one elderly people died in Lanarkshire in Scotland as a result of food purchased from a local butcher, which was contaminated with E.coli 0157. The last food associated crisis, and perhaps the most dramatic, was the Bovine Spongiform Encephalopathy issue which began in 1988 and continues even today. In television reports given by senior scientists, deaths on epidemic proportions were predicted. To date, thankfully, these have not materialised. Export of beef from the U.K. was banned, the sale of beef on the bone was banned and there was a catastrophic fall in the consumption of beef and devastation of the British beef industry. The market for beef in the U.K. has begun to return to levels approaching those before the crisis and at the beginning of August 1999 the ban on export was lifted. In the context of this present paper it is interesting to note that although the ban was lifted by the European parliament following a vote by all member states, Germany and France initially refused the importation of British beef on the grounds that it was still not safe. It could be said that they were merely following the example of the British government which at the time had not seen fit to remove the ban on the sale of beef on the bone, which tends to suggest that they feared that a risk still existed! It is against this background that the ban by the European parliament on the use of a number of antibiotics, when used sub-therapeutically as growth promotants, must be considered. It is difficult to identify another region of the developed world where consumers can demonstrate a choice with respect to the food which they purchase and eat, where food is not a price sensitive issue, and which has experienced so many, and such dramatic, crises with respect to the quality of their food.

In setting the scene for the events which led to the ban of the sale of certain antibiotics for growth promotion, there is one final point to consider and this is a philosophical issue which surrounds the concept that "we are what we eat". Consumers, and European consumers in particular, are able to make a choice with respect to the food that they buy. Food

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is no longer a price elastic commodity. If the price falls the European consumer no longer buys more. The overriding issue is quality and, in the absence of precise indicators of quality, it is often perceived quality which drives the purchasing choice. Since "we are what we eat" the consumer is constantly searching for the best perceived quality. What is identified as being good quality is difficult to define. However, perceived poor quality is more easily identified and the adulteration of food with additives is one of those perceived negative factors. The European consumer has, therefore, become highly suspicious concerning food quality and there have been instances when the media and governments have played on this suspicion for their own gains.

On 17 December 1998 the European Commission, by way of Council Directive (EC)2821/98 which amended an earlier directive, effectively banned the use of four antibiotic substances (virginiamycin, zinc bacitracin, spiramycin and tylosin phosphate) when used as additives in feed for the promotion of growth. These additives were in the main used in feeds for poultry and pigs, with benefits in terms of improved health, growth and feed conversion efficiency. The principal behind the ban was that of a precautionary measure. Analysis of risk and precautionary measures are concepts which are totally new and will be explained in greater detail at a later point. However, the merits of the precautionary measures and the factors which were brought into play with respect to this European ban on antibiotic growth promoters are the basis of this manuscript. Risks to the health of the population in general, economic policy and political gain were all factors which came into play.

II. MICROBIAL ANTIBIOTIC RESISTANCE IN RELATION TO FOOD SAFETY

It must be stated at the outset that the European Union (EU) law requires that animal feeds do not present any danger to the health of man or animals. The authorisation of veterinary medicines and zootechnical products (e.g. growth promoting additives) requires the submission of a detailed dossier demonstrating purity, efficacy and safety to animals, man and the environment. The four antibiotics which were banned had all previously passed successfully through this process and been authorised as safe.

In 1969 the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine (the Swann Committee) published its report (Swann Committee, 1969). One of the recommendations was that medicines used for the control of human disease should not be used as growth promoters in animal nutrition and where possible, different medicines should be used for human and disease control. Since then, and particularly in recent years, various bodies have begun to challenge the safety of the use of antibiotics in animal production on the basis of the development of potential antibiotic resistance. It has been suggested that resistance may build up in bacteria in animals, these bacteria then enter the human population by contact with the treated animals or food products derived from the animal. It is claimed that these bacteria may either cause disease in man or transfer resistance to other bacteria which are pathogenic to man.

In a report made by the House of Lords Select Committee, 23 April 1998 (House of Lords, 1998), it was stated that "Resistance to antibiotics and other anti-infective agents constitutes a major threat to public health, and ought to be recognised as such more widely than it is at present". The committee pointed out that on the evidence available, any microbial agent must be expected to encounter resistance sooner or later and that, as things stand, diseases such as tuberculosis, malaria, meningitis and the so called hospital "superbug" (methicillin-resistant *Staphylococcus aureus*, against which only vancomycin remains effective), are already showing signs of becoming untreatable. They considered that resistance

would take longer to emerge if antimicrobial use was controlled and prudent from the start. Whilst not suggesting that the problem of antibiotic resistance to pathogenic bacteria in man is not a serious problem in relation to our ability, in future generations, to control disease in humans, one must question the extent to which proper risk assessments have been carried out in relation to the use of antibiotic growth promoters in animal feeds.

Threlfall *et al.* (1996) reported concern about the increasing incidence of multi-resistant strains of *Salmonella*, particularly *S. typhimurium*, in humans and food animals (Table I).

Table 1. Drug resistance in *S. typhimurium* from humans and bovine animals in England and Wales, 1981 – 1995

	Humans			Bovines		
	Total	%DR ¹	%MR ²	Total	%DR	%MR
1981	3992	36	6	1157	71	15
1990	5451	54	18	1178	79	66
1994	5603	78	62			
1995	5663	83	60			

¹DR drug resistance (to one or more antimicrobials)

²MR multiple-resistance (to 4 or more antimicrobials)

During the 1980's the dominant phage types were DT204 and DT193 which demonstrated resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulphonamides, tetracyclines and trimethoprine. However from 1991 onwards the dominant phage type was DT104, resistant to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline. Although *Salmonella enteritidis* phage type PT4 is almost entirely associated with poultry and poultry products, *S. typhimurium* DT 104 is associated with a range of food animals such as the bovine, sheep, pigs, chickens and turkeys. Threlfall *et al.* (1996) linked the DT 204 problem of the late 1970's and 1980's to the prophylactic use of antibiotics in animals but no explanation was given for the marked reduction in this phage type by the 1990's. However, one of the most interesting points to emerge from the study of Threlfall *et al.* (1996) is that none of the antibiotics to which resistance had been found were used for growth promoting purposes in the UK since the Swann Committee report of 1969. This would surely cast into doubt the association between the development of antibiotic resistance and the prophylactic use of antibiotics as growth promotants in animal husbandry.

A report recently prepared by the HAN Foundation (Stichting Heidelberg Appeal Nederland)(HAN, 1999), commissioned by the Federation Europeenne des Fabricants d'Adjuvants pour la Nutrition Animale (the Federation which represents the feed additives industry in Europe) came to two key conclusions:

- 1) The assessment of risk as to whether prophylactic use of antibiotics as growth promoters (AGP's) in animals will result in a rise of resistant bacteria revolves around the question as to what extent, if at all, the use of AGP's in animal rearing *contributes* to bacterial antibiotic resistance *already present in humans*.
- 2) It is clear the reproducible and documented data concerning antibiotic resistance transfer from animals to humans is lacking.

The workings behind the HAN report and additional conclusions will be referred to later.

III. THE SCANDINAVIAN EXPERIENCE

In 1986 Sweden which at the time was not a member of the EU banned the use of antibiotics for growth promotion and allowed the use of antimicrobials in animal production on veterinary prescription only. The response by Swedish farmers was mixed (Viaene, 1997) and initially veterinarians were forced to prescribe increased levels of antibiotics for therapeutic use due to increases in diarrhoea and post weaning mortality as well as reductions in performance. Between 1986 and 1991 the Swedish animal production policy was quite protective. Import taxes and support for the internal market price levels were in force. This artificial support compensated for the reduced levels in performance enabling farmers to receive adequate margins. For pork production, the attitude was to control *Salmonella*, leave the AGP's out and so aim for pig meat with more sales appeal. This flag was soon taken up by other Scandinavian countries, namely Denmark and Finland (Anon; Pig International, 1996). Finland went a stage further by not only demanding freedom from *Salmonella* and the exclusion of AGP's, particularly during the finishing period, but also set aside the meat from any pig that required individual medication during its life. When Sweden and Finland joined the EU in 1995 there was, therefore, a dichotomy between livestock production systems in the northern European countries and the production systems in the rest of the community where AGP's were not banned. When it joined the EU, Finland was given a derogation from EU law allowing it to retain a ban on in-feed antibiotics until the end of 1997. In 1997 Finland submitted scientific evidence to support its demand to prolong the ban after the end of the year and successfully extended the period to the end of 1998.

Far from being willing to accept the production systems of the remainder of the EU, the Scandinavians were keen to impose their system on the rest of Europe. In November 1997 the Swedish Ministry of Agriculture organised a conference in Brussels entitled "Today we defeat bacteria. What about tomorrow?" Whilst evidence was presented which clearly demonstrated the problem of antibiotic resistant bacteria in human diseases, very little evidence was presented that supported the link between resistance and the use of AGP's in livestock. The clear objective of this conference was to persuade the rest of Europe and, in particular, the EU Commission that all should follow the Swedish experience and ban AGP's as they had done in 1986. This would have raised EU production costs to those experienced in the Scandinavian countries and, thus, levelled the playing field, albeit at the higher rather than the lower level. It would also be an effective protective measure prohibiting the import of livestock produce into the EU from regions and countries where AGP's were still permitted. It must be taken into account that at the time animal production in Sweden accounted for only approximately 2% of total European production and was also characterised by many small scale units in direct contrast with the UK, France and Holland.

An independent analysis of the Scandinavian system was required and in 1997 Dr J. Viaene from the University of Ghent, Department of Agricultural Economics examined the premise that the Swedish animal production model could be extended to the total EU, to the benefit of farmers, allied trades and consumers. The Viaene (1997) study concluded that the Swedish ban on anti-microbials for in-feed use and associated legislation had lowered production efficiency and increased costs. Consumption of in-feed antibacterials had remained relatively constant at 30-35 tonnes per year but scientists were not convinced that antibiotic resistance issues had been resolved by the ban. Viaene stated that the economic burden for consumers and farmers had been heavy, through increased feed use, loss of production and increased use of therapeutic levels of antibiotics. Increased feed use had increased the environmental impact of animal production and high use of zinc oxide as an alternative to

AGP's had increase zinc levels in the soil. The Swedish animal production system had come under increasing pressure from exporting countries and disadvantages to trade at the EU level were arising. He indicated that the EU was facing increasing trade competition, particularly from the USA. He concluded by stating that with open world markets European farmers needed access to all technologies which would allow them to hold costs at a minimum and to remain competitive, tantamount to arguing that European farmers needed access to AGP's.

The Swedish Veterinary Association and national animal health service were quick to refute the statements of the Viaene study. Given that antibiotic use, via veterinary prescription, increased it would have been the Swedish veterinarians who had most to lose had the tables been turned. In an interview with Animal Pharmaceuticals (9 May 1997) a representative of the Swedish Animal Health Service stated that there was no evidence that the national livestock industry was at a serious disadvantage. However, it must be emphasised that the Swedish system was not one of major industrialised agriculture.

Throughout 1997 and 1998 Sweden and Finland continued to lobby the EU to adopt their system and introduce a European ban on AGP's. An ally was found in Denmark who in 1996 had requested a review of the authorisation of Avoparcin as a growth promoter. The situation was reaching a point when the European Commission needed to make a final decision. The period of the derogation permitting Sweden to operate a system prohibiting feed additives which were allowed in the rest of Europe was reaching a deadline and such a two tier system was totally contrary to the policy of the EU.

An important point to note was that the pressure from the Scandinavians had kept the issue in the public gaze. Furthermore, although the feed additive industry was supported by the Viaene study there was little concrete evidence to refute the stance of the Swedes to ban AGP's, as much as they had no hard evidence to support the association between use of AGP's and antibiotic resistance.

IV. ACTIONS OF THE EUROPEAN COMMISSION WITH RESPECT TO ANTIBIOTIC GROWTH PROMOTERS

In the past the European Commission has relied on the SCAN Committee (Scientific Committee on Animal Nutrition) to advise and make recommendations on the use of additives in animal feed. It is the SCAN Committee that is responsible for assisting the Commission, at the latter's request, on all scientific questions relating to the use of additives in animal nutrition. It was established in 1976 and is composed of independent experts which are appointed for their scientific excellence. Their assessment is based on objective scientific grounds of safety, quality and efficacy. The Committee reviews all dossiers for submission of new feed additives and gives impartial advice to the European Commissioners on matters related to animal production. The SCAN Committee has in the past reviewed all dossiers on AGP's that are used in animal production and following the recommendation of the committee the products have been released for sale.

Since the dossiers for the four AGP's in question had earlier been reviewed by the SCAN committee, the question arises as to whether it was either a misinterpretation of the original data or the emergence of new data that precipitated the introduction of the ban by the EU commission. In fact, with respect to the ban on AGP's, the opinion of SCAN was not sought. In July 1998 the SCAN committee issued an opinion on the immediate and long-term use of virginiamycin as an animal growth promoter on antibiotic use in human medicine. SCAN concluded that "the use of virginiamycin as a growth promoter does not constitute an immediate risk to public health (in Denmark)" and that "a full risk assessment cannot be made

until quantitative evidence of the extent of transfer of antimicrobial resistance from livestock sources is obtained". Following the Commission's proposal to instigate a ban, a member of SCAN was cited to have stated that the Commission's "proposal to ban is contrary to scientific evidence" and that "there is not yet any evidence which proves that these substances have caused adverse effects to humans and therefore the proposal for an immediate ban is disproportionate. And yet despite this scientific opinion from the SCAN committee the Community decided to ban four antibiotics from being used as growth promotants in animal production.

This action was a fundamental move from the principles which had been adopted and used for over the past twenty years since SCAN was first instigated. It was a move which shocked the feed additive industries who have grown used to the scientific presentation of data on safety, quality and efficacy to support their products. However, the European Commission has strong principles and it is certain that these principles would not have been compromised in reaching their final decision.

The question arises as on what basis the decision was made and under what legal framework. As has been indicated earlier, the Scandinavian situation had raised the profile of the antibiotic resistance issue. The media used the opportunity to sensationalise the issue and drum up public support. Above all the European Commission was seeking transparency with respect to the food production policy of the community and after the scares of the past was not willing to risk the health of the community by failing to supply safe food in a safe manner. The risk existed that development of antibiotic resistance might be associated with feeding antibiotics to animals and it was felt that the risk was too great to go unchallenged. For the first time the European Commission adopted a policy of risk assessment and precaution.

The Precautionary Principle (EU 1998) was adopted and it was this principle which heralded a major change in decision making with respect to food and feed products. This was the legal basis on which AGP use was banned, in spite of the contrary SCAN opinion.

V. THE PRECAUTIONARY PRINCIPLE

In their preamble to the guidelines on the application of the Precautionary Principle the European Commission makes several enlightened comments with respect to present day principles and the industrialised production of consumer goods. They state that consumers have become increasingly sensitive to the risks associated with the industrial production of consumer goods and that the mass media has fuelled this new sensitivity. A second key issue is that public opinion urges political decision makers to accommodate consumer perceptions and fears and to adopt preventative measures. On the other hand, consumers often confound the notion of hazard and risk. Risk is a function of the probability of an adverse health effect and the severity of this effect resulting from the presence of a hazard associated with a product. When a hazard is clearly identified and perceived as such by consumers, the risk assessment has to be based on a body of scientific and statistical data. The precise link between the science that produces the risk assessment and the precautionary principle, which comes under the head of risk management, has yet to be defined. The precautionary principle represents a choice made by decision makers responsible for the welfare and safety of their fellow citizens. It is an eminently political decision exercised in conditions of scientific uncertainty. They state "The Commission will be guided in its risk analysis by the precautionary principle, in cases where the scientific basis is insufficient or some uncertainty exists".

The definition of the precautionary principle is “an approach to risk management that is applied in circumstances of scientific uncertainty reflecting the need to take action in the face of a potentially serious risk without awaiting the results of scientific research”. The precautionary principle takes into account the sustainability of agriculture since it begins to consider the principle of equality between generations and that decisions may need to be taken today to protect future generations. When effects do not materialise until long after exposure it is far harder in the absence of scientific certainty to convince decision makers and stake holders to adopt preventative measures which will have important and immediate repercussions on industry or agriculture.

The precautionary principle is a risk management approach that is exercised in a situation of scientific uncertainty, reflecting a need for action in the case of a potentially serious risk without awaiting the results of scientific research. It was perhaps the later point which so incensed scientists since the principle has transferred the decision making process out of the hands of scientists into the hands of politicians. Six guidelines have been written upon which the principle is based:

1. Implementation of an approach based on the precautionary principle should start with an objective assessment, identifying at each stage the degree of scientific uncertainty.
2. All stake holders should be involved in the decision to study the various management options that may be envisaged once the results of the risk assessment are available and the procedure should be as transparent as possible.
3. Measures based on the precautionary principle must be proportionate to the risk which is to be limited or eliminated.
4. Measures based on the precautionary principle must include a cost/benefit assessment (advantages/disadvantages) with an eye to reducing to a level that is acceptable to all the stake holders.
5. Measures based on the precautionary principle must be able to establish responsibility as to who must furnish the scientific proof needed for a full risk assessment.
6. Measures based on the precautionary principle must always be of a provisional nature, pending the results of scientific research performed to furnish the missing data and perform a more objective risk assessment.

It is a fact that the precautionary principle has introduced a new concept into the evaluation of issues affecting animal production. Without a doubt a subjective assessment has partly replaced the totally objective scientific assessment of the past and this is a situation which will lead to controversy and debate. Transparency in the decision-making process will be paramount.

Responding to the guidelines enumerated in the precautionary principle the Commission requested the Scientific Steering Committee (SSC) to scientifically evaluate the current position regarding the prevalence and development of antimicrobial resistance and examine the implications for human and animal health, particularly with regard to the development and management of infections. The SSC's evaluation (EU 1999) revealed that action needed to be taken promptly to reduce the overall use of antimicrobials in a balanced way in all areas: human medicine, veterinary medicine, animal production and plant protection. They recommended EU-wide co-operation and agreement as a matter of urgency to prioritise actions. They called for tighter controls on sale, supply and distribution of antimicrobials and, in addition to considering the use of antimicrobials for humans and in

plants, made specific recommendations with respect to their use as growth promotants in animals.

They specifically recommended that the use of agents from classes which are or may be used in human or veterinary medicine (i.e. where there is a risk of selecting for cross resistance to drugs used to treat bacterial infections) should be phased out as soon as possible and ultimately abolished. They also recommended that efforts should be made to replace those antimicrobials promoting growth with no known risk of influencing intestinal bacterial infections by non-antimicrobial alternatives. Thus they effectively recommended that the use of all antimicrobial growth promoters be banned. They supported their remarks by suggesting that husbandry practises with health control programs should be devised and implemented in order to reduce the need and demand for routine addition of antimicrobials in animal production. The SSC conceded that information was inadequate to determine which facets of antimicrobial use and which areas of use were the major contributors to the development of antibiotic resistance but concluded that the evidence was sufficiently compelling to merit immediate action.

On a world-wide basis the final recommendation of the SSC was perhaps the most pertinent. They stated that development of resistance is a global problem and the intervention by the EU would not be successful in isolation. They suggested that regulatory action may be needed in order to control access of animals, meat or foods from non-EU countries should there be significant threat perceived or detected for importation of resistant bacteria. This can only be interpreted as a suggestion to ban the imports of animals and animal products from non-EU countries which had not adopted the equivalent control of the use of antimicrobial materials. It can be questioned whether this was either a suggestion to protect European producers from the higher production prices that they would experience without the aid of AGP's or whether indeed it was an effort to control a global problem. Without doubt, however, the major components were in place to support a European ban on AGP's based on the Precautionary Principle and supported by a strong recommendation for immediate action from the Scientific Steering Committee.

VI. THE INDUSTRY ACTIVITY AND RESPONSE TO THE BAN

For many years the feed additives industry has worked closely with the EU to supply information and draft legislation to control the registration of new products. This registration procedure has protected the health of the consumer and *yet allowed* the development of efficacious, safe, high quality products which have been of benefit to the farming industry and profitable for the additives business. Many of these products have been exported world wide and they are the basis of a multi-billion dollar industry. They have also contributed to the success of European agriculture at a level roughly competitive with world agriculture. The registration of new products has in the past always been on the basis of objective scientific grounds relating to the efficacy, safety and quality of the products to the exclusion of political or other considerations. It was obvious that in the first instance the industry would revert to the course of action that in the past had been its cornerstone on which all evidence was based, that is, fundamental scientific principles.

When the industry became aware of the antibiotic resistance issue it commissioned a number of studies both in collaboration with the EU (Multidisciplinary Scientific Steering Committee on Antibiotic Resistance) and also by individual companies (Surveillance Programme) to evaluate the risk of resistance transfer and also the evidence for transfer of resistance. The protocols for these programs were elaborated on the request of the

Commission experts and received approval from the member states. The studies were based on an analysis of the resistance to microbes focusing on seven antibiotics, including those which were eventually subject to the ban. Samples were to be taken in six Member states one year apart. The second sample was scheduled for the end of 1999 which was a time point past the introduction of the ban. Introduction of the ban effectively withdrew from circulation, midway through the trial, four of the antibiotics which were part of the Surveillance Program, to which the EU had been part to the original planning. In order to carry out the Surveillance Program it is estimated that the industry made available in excess of US\$1 million. Obviously with over half of the data from the trial missing, the evaluation was severely jeopardised.

The feed additives industry was also not slow to commission an independent report on the likelihood of the transfer of resistance. The HAN (1999) report has already been alluded to and was prepared by the Heidelberg Appeal Nederland Foundation, an independent scientific supervisory committee. The conclusions of the HAN report were quite clear and the eight summary points had two clear messages: a) there was insufficient data to fully assess the contribution of antibiotic use in animals to human antibiotic resistance, more data was required, and b) there is presently no evidence to show that antibiotic use in animals has either compromised the health of humans or has resulted in the spread of antimicrobial resistant Gram-positive bacteria from livestock to humans. They stated that a comprehensive multidisciplinary research effort was needed to properly assess all aspects of the use of AGP's in animal husbandry. This final conclusion was pre-empted by the establishment of the Surveillance Program which, as indicated, was unfortunately invalidated by the introduction of the ban.

Based on all previous experience the industry had played by the rules but at a stroke, the rule book had been altered. The industry was duly highly offended and aggrieved by the decisions of the EU. The decisions would not only destroy highly profitable businesses on which many jobs depended but would also add to the pollution burden in Europe, reduce profitability of the European farmer and reduce the health status of pigs and poultry in Europe. It was also cited that in Sweden the ban on antibiotic use had resulted in increased use of zinc oxide as an alternative bacterial control measure and this, in turn, had resulted in high levels of zinc pollution when manure was applied to the soil. As a final resort, Pfizer Animal Health, the producer of virginiamycin, with the support of two European Federations which represent the animal health and feed additives industries at the European level (FEDESA and FEFANA respectively) took their case to the Court of First Instance of the European Communities in an attempt to either overturn or delay the ban. The decision of the court is pending.

VII. THE LESSONS TO BE LEARNED

With respect to the issue of the European ban on AGP's, it is very difficult to separate the political issues from the decisions based on scientific evaluation, irrespective of whether there was sufficient scientific evidence or not, to enable a fully reasoned decision to be taken. It is obvious that a new era has started when scientific evidence is no longer the sole criterion and in the future will be an insufficient basis on which to make decisions on the suitability of feed additives in animal production. As indicated above, for many in the population food is no longer price elastic, a reduction in price not leading to an increase in consumption. Indeed with a price premium nearly double for organically produced food, and the steady growth in the market for organic produce, it is obvious that efficiency of production may not be the key issue. The perception of quality will be an over-riding issue and it is very difficult in each

instance to define the factors relating to good quality. Furthermore, it is obvious that it is the perception of the consumer that will be a key factor driving political decision making.

Development of medicines whether for humans or animals is a long and expensive process. Particularly for animals, where the returns are not as profitable as in human medicine, it is essential that the industry has a consistent and predictable environment in which to operate. The actions of the EU have resulted in a major lack of trust between the industry and politicians. Under such circumstances it is very difficult to foresee major investment in the development of new feed additives. However, this is a short-sighted view since it is very difficult to predict the food needs of future generations. Whilst it will be the animal producers who will lose in the short term the ultimate loser will be the consumer. It is only those consumers who can afford increased prices who will have the real choice in terms of food quality. There is still a major proportion of the population, even in Europe who look first at the price before having the luxury of choice. Unfortunately, this proportion of the population is not as vociferous as their wealthier counterparts.

The Precautionary Principle is very difficult to contest. However, to function effectively it must be totally transparent. The real test will be when an action taken under the precautionary principle is revoked. However, the chance of an industry re-establishing itself after an action taken as a precaution must be very slim indeed.

Without doubt the EU ban on AGP's will have a dramatic adverse economic effect on pig and poultry production in Europe. Additionally, there will probably be adverse effects on the health and welfare of animals as the industry readjusts to the new requirements. There are presently no alternatives which can effectively take the place of the banned AGP's although the industry is making a major effort to develop alternative strategies.

The food production industry is an industry which requires stability in order to attract investment. Only with investment in the industry can the needs of the future be guaranteed. The actions of the EU, whilst taken on behalf of the consumer, have destroyed the confidence of industry in the legislative process. A worst case scenario is that the major pharmaceutical and fine chemical industries will turn their back on animal production in Europe and the industry will decline into a non-competitive shadow of its former self. Before taking such radical steps such as the imposition of the ban, the European Commission must have meaningful dialogue with the industry and precipitous action must be avoided. The two sides must plan together for the good of the consumer, the food producing industry and the feed additives business. Unfortunately, in Europe there does not presently seem to be the means nor the will for this dialogue.

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EUROPEAN POULTRY PRODUCTION WITHOUT ANTIBIOTIC GROWTH PROMOTERS – ONE YEAR ON

J. RATCLIFF

Summary

The use of growth-promoting antibiotics is being placed under increased pressure due to consumer concerns in both Europe and Asia Pacific about associated resistance against pathogenic bacteria in humans. The majority of the broiler production in the UK is now without antibiotic growth promoters and much of the past twelve months has been spent evaluating the effects of their removal and the various feed and management factors that influence performance and disease, particularly necrotic enteritis (NE).

I. INTRODUCTION

Just over one year ago a significant proportion of the European broiler industry removed in-feed antibiotic growth promoters (AGPs) following pressure from the major supermarkets due to concerns regarding possible antibiotic resistance in humans (Ratcliff, 2000). At the same time, tight monitoring procedures were implemented to ensure that the practice of prophylactic treatment was stopped and therapeutic antibiotic treatment did not increase, as was the case in the preceding twelve months following the removal of AGPs in Sweden (Best, 1996).

Following the European Union (EU) ban on avoparcin, the most commonly used growth promoters in Europe were zinc bacitracin and virginiamycin. These growth promoters were selected not just for their improved production performance but because they also provided effective protection against NE, even in the absence of an ionophore coccidiostat. Experience from Denmark and Sweden has confirmed that one of the key problems for countries reliant on wheat-based formulations is the control of NE once antibiotic growth promoters have been removed (Inbarr, 2000).

In 1999 the extended growth promoter ban within the EU left the broiler industry with only avilamycin or bambarmycin for use as in-feed AGPs. Neither was considered to be as effective as zinc bacitracin or virginiamycin in the control of NE and inevitably NE breakdowns were occurring even before the move to remove all AGPs came at the end of 1999.

As a consequence, current European broiler programmes without AGPs have tended to concentrate on limiting the increase in health problems, especially NE, associated with the removal of growth promoters.

II. NECROTIC ENTERITIS

Necrotic enteritis is caused by the intestinal proliferation and toxin production of *Clostridium perfringens* (CP) types A and C (Ficken *et al.*, 1997). This bacterial species is so prolific and environmentally resistant that it represents one of the major threats to intensive poultry production. Intestinal proliferation of CP has been associated with acute clinical disease and increased mortality (Kaldhusdal *et al.*, 1992) whilst the sub-clinical disease is associated with impaired performance and liver lesions (cholangiohepatitis) resulting in

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increased carcass condemnation at slaughter (Randall *et al.*, 1996).

The damage caused by *CP* depends largely on those factors that favour colonisation of the gut. It is thought that the slowing down of the gut flow during the process of digestion allows the *clostridium* bacteria to proliferate. Factors that increase the viscosity of the gut contents further encourage proliferation. Outside of the bird in the litter and the feed, *clostridium* bacteria will form spores that are highly resistant to desiccation, chemicals and heat. These spores present the risk of infection and re-infection.

III. STRATEGIES TO CONTROL NECROTIC ENTERITIS IN THE ABSENCE OF ANTIBIOTIC GROWTH PROMOTERS

Strategies to control NE in the absence of AGPs, without resorting to prophylactic or therapeutic treatment, have centred upon dietary and management practice (Table 1). The influence of feed and feed characteristics is evident throughout this list and highlights the importance of feed production in the control strategy.

Table 1. Factors associated with the occurrence of necrotic enteritis.

Cocciostat programme	Feed toxins
Processed feed characteristics	Gut conditioners
Raw material characteristics	Nutrition
Whole wheat addition	Alternative additives
Protein digestibility	Management practice

(a) Cocciostat programme

Coccidial stress has been shown to sensitise the broiler chicken to NE (Al-Sheikhly *et al.*, 1980). Control of coccidiosis is, therefore, an essential element in the strategy for the control of NE. The inclusion of the polyether ionophore group of coccidiostats in feed is known to have a beneficial effect in the control of NE (Williams *et al.*, 1999). Experience with commercial coccidial vaccines for broilers may lead to an increase in the incidence of NE as may the use of chemical in-feed coccidiostats. The inclusion of an ionophore coccidiostat would be the recommended option in the absence of an AGP.

(b) Processed feed characteristics

Normal gut motility is regulated by the gizzard (Duke, 1994). In birds, gut refluxes (reverse peristalsis) are normal and are an adaptation to compensate for a short intestine. The gut refluxes serve to re-expose intestinal ingesta to gastric secretions, to vigorously mix digesta with enzymes, to enhance nutrient absorption over a short segment of the gut and to discourage pathogenic proliferation that may cause disease or compete for nutrients (Ferket, 1995). It is observed that highly processed feeds can lead to atrophy and malfunction of the gizzard which then acts more like a transit organ rather than a grinding organ (Cumming, 1994). Normal gastric reflex does not occur when birds consume highly processed feed and as a consequence more undigested proteins end up in the hindgut where they are subject to microbial fermentation. One of the consequences is the potential for finely ground material to trigger the proliferation of *clostridia* and hence NE. As a consequence particle size is now seen as an important precursor for NE and efforts have been made to maximise the particle

size without compromising pellet quality. Grinding raw materials over a 4mm sieve has been found to be beneficial.

(c) Raw material characteristics

The viscosity characteristics of wheat are well documented and, even in the presence of an enzyme, wheat is known to be associated with a higher incidence of NE compared with maize (Kaldhusdal, 1996). As a result, in some countries, such as Denmark, they have replaced up to 30% of the wheat in the starter and grower ration with maize although in many poultry producing areas such a strategy is not practical. However, recent DNA profile methods of analysing microbial communities in the gastrointestinal tract have shown significant shifts in the microbial population which may favour the development of NE when changing from maize to wheat or rye during the broiler cycle (Apajalahti *et al.*, 2000).

(d) Whole wheat addition

Many broiler companies in Europe have been diluting the broiler feed by adding up to 30% whole wheat over the top. The economic benefits of this practice are well documented (Peterson, 1997). However, the feeding of whole wheat to turkeys and broilers is now considered beneficial, not only because of the commercial returns, but also because of the stimulation to the function of the gizzard and the resulting benefits on gut motility. This, in turn, is felt to be beneficial in helping to inhibit the proliferation of clostridia.

(e) Protein digestibility

Reports on the influence of protein sources on the frequency of NE are conflicting. There has been a suggestion that protein ingredients of animal origin (meat meal and fish meal) predispose to NE (Teglof *et al.*, 1992) although more recent studies have not confirmed this. The issue is one of nutrient density and protein quality rather than protein source. High levels of low quality protein sources are poorly digested in the foregut and, thus, pass to the hind gut where they are degraded by proteolytic bacteria such as *clostridia* resulting in the production of alpha toxin. Consequently protein levels should be kept to a minimum with the emphasis on protein quality and constraining to available amino acid levels rather than total protein.

(f) Feed toxins

Spoiled meat and poor quality fish by-products are a source of biogenic amines, histamine and tyramine, which can cause or aggravate an enteritis problem if they exceed 100 mg/kg in the finished feed. The purchase of high quality fish and animal by-products is, therefore, recommended. Personal experience with a feed company in the UK traced a NE problem last year back to the biogenic amine levels in the source of fishmeal. Other toxin problems may result from mycotoxins or oxidised fats. Again, the emphasis must be on quality control.

Kaldhustal (2000) has speculated that the possibility of contamination of poultry feed with *CP* should not be excluded. Heat treatment and the application of anti-microbial acids would not be sufficient to destroy the *CP* spores. By effectively reducing other pathogens in the feed such treatment may actually encourage the proliferation of *CP* within the bird.

(g) Gut conditioners

It is well documented that addition of carbohydrase enzymes to barley-, rye- or wheat-based diets can reduce or eliminate the anti-nutritive properties of viscous polysaccharides in broiler chicks. Lipase, phytase and protease preparations are also now widely used in poultry rations as a means of improving digestion and nutrient absorption although their effect on NE occurrence is not documented.

The accepted use of betaine as a methyl donor in poultry formulations can also provide beneficial protection to the structure of the epithelial cells in the gut, improving nutrient absorption and fluid retention, particularly under stress conditions (Ferket, 1995).

(h) Nutrition

While a tremendous amount of research is devoted to amino acid and energy requirements of poultry the importance of trace minerals is frequently ignored. Dietary inclusions of many trace minerals have remained constant despite the significant improvement in food conversion and the incredibly rapid growth rate of modern broiler breeds. Greater understanding of the co-factor relationship between many trace minerals, vitamins and key enzymes has been accompanied by a greater awareness of the significant difference in bioavailability between organic and inorganic forms of mineral supplementation and the effect this can have on performance, the immune defence and antioxidant protection systems (Surai *et al.*, 2000).

(i) Alternative Additives

A summary of the main categories of alternative products available in Europe has already been documented (Ratcliff, 2000). Currently the three most widely used alternatives in the UK are organic acids, essential oils and mannanoligosaccharides (MOS), either individually or in combination.

The first alternative programme with which the author became involved in the UK was the evaluation of MOS compared with avilamycin in broilers under commercial conditions, both in combination with monensin. The results, repeated over twelve months, showed comparative performance between the two treatments. It was observed on a number of farms however that both treatments showed an increase in the incidence of cholangiohepatitis resulting in an increased level of condemnations at the processing plant.

Since experience with organic acids had suggested a potential benefit in terms of NE control it was decided to apply an organic acid preparation in the drinking water for the first five days in combination with MOS in the feed. Since progressing to this programme little or no incidence of NE or cholangiohepatitis has been observed. The addition of organic acids to feed is widely practiced as a means of controlling microbial growth but nutraceutical benefits have also been discussed (Adams, 2000). Any pH effect in the bird is likely to be very limited and restricted to the crop and the proventriculus. Beyond the gizzard the free organic acids are rapidly metabolised and, therefore, there is unlikely to be any effect on the pH in the hind gut unless the acid in the feed is in some way protected through to the small intestine and caecum (Hyden, 2000). The bacteriostatic action of the acids in the intestine will depend not only on their ability to reduce the pH value but more importantly their ability to penetrate and destroy the bacterial cell (Cerchiari *et al.*, 2000). A range of products are now available that exploit this technology in an attempt to influence the growth of pathogenic bacteria in the hind gut.

The comparison of alternative products with avilamycin became academic towards the end of 1999 because the UK moved rapidly towards the complete removal of AGPs such that currently the majority of broilers reared in the UK are now AGP free. A number of companies decided that initially they would try to run without any alternative product. Companies that were using a chemical coccidiostat soon ran into problems with NE and were having to resort to treatment with antibiotics. Where an ionophore coccidiostat was used, in many cases the first two or three crops showed little setback in performance in terms of liveweight, feed conversion and mortality. The parameter that did deteriorate was liveweight uniformity. After three successive crops the performance did, however, start to suffer both in terms of feed conversion (up to four points), liveweight and mortality resulting in an increase in therapeutic treatment. With pressure being applied by the supermarkets to reduce therapeutic treatments the option of no alternative was not considered a long-term viable alternative.

The verdict on essential oils and herb extracts is still not conclusive. Personal observations indicate a lack of consistency over a sustained period of time such that in many cases it is difficult to achieve an economic benefit against negative controls. A problem also arises from the lack of active ingredient studies and dose response data.

Finally, competitive exclusion products may provide effective means of controlling NE in the absence of AGPs. Further evaluation is, however, required.

(j) Management practice

It is clear from published data that there is an important association between dietary ingredients and nutrition and NE in poultry, albeit that some of these data are conflicting due to the complex multifactorial nature of the disease. Diet is an important predictor of disease risk but many other factors are involved. The brooding stage (0-10 days) remains of key importance. With increasing growth rate the bird spends proportionately more time in this stage than in the past and with increasing genetic potential for growth, inputs during the brooding process become more important (Ross Breeders Technical Bulletin, 1998). Correct temperature, humidity and feed presentation are essential during this phase of development.

Other factors that can influence NE include, drinkers, stocking density, litter material, clean out and turn-around time. Immunity and concurrent diseases (particularly coccidiosis) will also be a major influence.

IV. CONCLUSIONS

The removal of antibiotic growth promoters from broiler and turkey feeds in Europe has led to a complete re-assessment of management practice and, in particular, the influence of feed and its components on necrotic enteritis. The use of an ionophore coccidiostat is the preferred option in conjunction with a careful evaluation of individual feed ingredients and nutrient levels and their physical presentation within the pelleted feed. Gut conditioners, particularly enzymes, are considered an essential and effective tool for counteracting the anti-nutrient factors in feed ingredients that may predispose birds to NE. The use of no alternative would not appear to be a viable long-term option for broilers due to a deterioration in performance and the corresponding reliance on therapeutic medication. A number of alternative treatments have been shown to be effective compared with AGPs either individually or in combination, e.g. MOS and organic acid. Further work is required to evaluate the efficacy of competitive exclusion products against NE. Finally, any strategy involving feed can only be considered in conjunction with other management factors such as environment, hygiene and disease control.

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THE APPLICATION OF YEAST AND YEAST DERIVATIVES IN THE POULTRY INDUSTRY

K.A. DAWSON

Summary

Yeast and yeast-derived preparations can provide inexpensive feed supplements that can have major impacts when used in poultry management systems. Research has shown that these preparations can be used to control the composition of the microbial population in the gastrointestinal tract, prevent colonization with pathogens, bind toxins, and modulate the immune system. These activities can directly or indirectly influence animal performance and can be used as tools for improving the efficiency of poultry production systems. Many of these activities provide economic benefits that are comparable to commonly used antimicrobial growth promotants.

I. INTRODUCTION

Yeasts and yeast fermentations have been intimately associated with human history for centuries. The diverse biochemical capabilities of the active yeast cell have been used to process foods and beverages, provide fuels and serve as a rich source of nutrients. Studies of yeast have also provided the base for our understanding of many biochemical principles and have provided tools for understanding basic molecular biology and genetics. In the last two decades, there has been increased interest in using yeast and specific components of yeast cells as feed supplements. These applications have been based on both empirical observations and on new scientific evidence that suggest a significant strategic role for yeast-derived products in modern animal production systems. This paper will examine some of the products derived from yeast and attempt to define their role in poultry management systems.

II. YEAST CULTURES AS FEED SUPPLEMENTS FOR LIVESTOCK

Yeast cultures are microbial supplements that, by definition, contain dried yeast cells that maintain some of their fermentative activities. These materials have been successfully used to enhance animal performance for many years. Current understanding of these supplements suggest that they can have a significant impact on the microbial populations in the gastrointestinal tract and can indirectly influence animal performance by enhancing the beneficial activities associated with these microorganisms (Dawson, 2000). The information obtained in studies of yeast cultures in ruminant animals has been extended to promote the use of these materials in other livestock and poultry. However, it is clear from studies in poultry that the effects of yeast culture on microbial activities in the gastrointestinal tract could not explain many of the effects of yeast cultures.

In recent years, various components of yeast culture preparations have been examined to determine their potential effects on animal performance and health. The yeast cell wall is a complex matrix containing mixtures of carbohydrates and proteins that can provide specific adsorptive capacity. Studies and applications of yeast cell wall preparations in poultry feeds have shown that materials in the cell wall can be used to influence livestock production and address specific production problems. Through processing these materials can be attenuated to increase their ability to bind to specific proteins and toxins. It is now possible to take

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advantage of some of these activities and use yeast cell wall material as a base for management of animal health and performance.

III. BINDING MYCOTOXINS WITH THE YEAST CELL WALL

Mycotoxins are secondary products of fungal metabolism that may be produced in contaminated feeds during production and storage. It has been estimated that at least 300 fungal metabolites are potentially toxic for man and animals, and that as much as 25% of the world's cereal grains are contaminated with measurable levels of mycotoxins (Devegowda *et al.*, 1998). Many of these compounds are known to have significant effects on animal health and may have a major impact on animal production. The ability of mycotoxins to negatively influence the growth and health of poultry makes them a major problem in many production systems.

There has been a great deal of interest in using biological products to reduce the bioavailability of mycotoxins in poultry. Some of these organic agents overcome the inherent drawbacks associated with use of large quantities of inorganic adsorbents that have been traditionally used to address intoxication problems. One available strategy for attenuating the effects of some groups of mycotoxins uses the unique adsorptive capacity of the yeast cell wall. The potential usefulness of these types of materials was first demonstrated in poultry in the early 90's. Initially used as a nutritional aid and growth promoter, a commercially available viable yeast culture product based on *Saccharomyces cerevisiae* strain 1026 (Yea-Sacc) was found to improve hatchability (McDaniel, 1991) and broiler body weights (Stanley *et al.*, 1993). In controlled studies, viable yeast cultures added to broiler diets containing aflatoxin resulted in significant improvement in weight gain and enhanced immune response (Devegowda *et al.*, 1995). *In vitro* studies clearly established the binding of aflatoxin to yeast cells in a dose-dependent response up to 90% (Devegowda *et al.*, 1994). Recent modification in manufacturing techniques have allowed for the production of specific modified yeast cell wall preparations that appear to have enhanced abilities to bind a range of mycotoxins (Table 1). The yeast cell wall-derived glucomannan product, Mycosorb, has also been shown to reduce the toxic effects of mycotoxin-contaminated grains in broiler chickens (Figure 1). Data from this and similar studies suggest that organic adsorbents prepared from yeast cell wall preparations may have a critical role in strategies for controlling the toxicity of mycotoxins in poultry feeds.

Table 1. Efficiency of adsorption¹ of various mycotoxins by Mycosorb.*

Mycotoxin	Strong binding (%)
Total aflatoxins (B1 + B2 + G1 + G2)	85.23
Zearalenone	66.66
DON	12.58
Ochratoxin	12.49
Citrinin	18.41
T-2 toxin	33.39
DAS	12.72
Nivalenol	8.16
Fusariotoxin X	7.87
Fumonisin	67.00

¹(minus values of control group).

*Adapted from Devegowda *et al.* (1998).

IV. CONTROL OF PATHOGENIC MICROORGANISMS AND IMMUNE RESPONSES WITH YEAST CELL WALL PREPARATIONS

The outer surface of the yeast cell wall is composed of a complex mixture of carbohydrates that contain mannose as a main constituent. The ability of mannan oligosaccharides in yeast cell walls to decrease the prevalence and prevent colonization of pathogens in the gastrointestinal tract has been well documented (Spring *et al.*, 2000). Many enteric pathogens use type-1-fimbriae to attach to the intestinal lining. These fimbriae specifically recognize mannan-based sugar residues and often define the ability of the organism to initially colonize the intestinal tract. In a recent screening study looking at bacterial attachment mechanisms, 66% of the strains of *Escherichia coli* tested expressed mannose-specific fimbriae (Finucane *et al.*, 1999a). The percentages of *Salmonella typhimurium* and *Salmonella enteritidis* strains that attached to mannose receptors were 80% and 67%, respectively. Mannose-type sugars and yeast-derived mannan oligosaccharides in the diet can block bacterial attachment by adhering to specific proteins of the bacterial cell surface and, therefore, reduce pathogen colonization. The effects of dietary mannan oligosaccharide on the gastrointestinal microflora have been investigated in a series of broiler and turkey studies, and significant reductions in both salmonella and pathogenic *E. coli* have been reported (Spring *et al.*, 2000).

Mannan oligosaccharides from yeast cell walls have also been shown to have indirect effects on bacterial populations and colonization by pathogenic bacteria. In a recent turkey trial, a reduction in *Clostridium perfringens*, the causative agent of necrotic enteritis, was reported in response to dietary mannans (Finucane *et al.*, 1999b). In this study, the turkeys fed oligosaccharides tended to have greater cecal concentrations of beneficial anaerobic bacteria. Clostridia are not known to express type-1-fimbriae. However, changes in clostridial concentration may be brought about through indirect effects exerted by oligosaccharides on the gut flora. Such shifts in the growth of beneficial anaerobes could competitively inhibit the growth and activities of the clostridia. These types of shifts in microbial populations are consistent with the reported effects observed with antimicrobial supplementation and could account for some of the growth-promoting effects associated with the use of mannan oligosaccharides in field trials.

One of the most interesting effects of the mannan oligosaccharides may be their ability to alter the immune function of animals. These activities can be used strategically to enhance the resistance of the animals to disease processes. Several lines of evidence indicate that the addition of yeast cell wall preparations (Bio-Mos) containing mannan oligosaccharides to production diets can enhance immune function. An early study conducted at Oregon State University reported increased concentrations of secretory IgA of about 25% with the addition of Bio-Mos to the diets of chickens (Savage *et al.*, 1996). In recent studies of swelling responses, Cotter (2000) demonstrated that Bio-Mos could modulate cell-mediated immune function resulting in less swelling upon repeated exposure to a mitogen. Such studies suggest directed effects of the oligosaccharide on immune function. However, it is currently not clear how the oligosaccharide acts to modify the immune systems. The wide-range of immune effects associated with this material indicate that there is a generalized mobilization of both B- and T-cell mediated immune systems when they are first exposed to an antigen. One explanation for these effects would be that the enhanced immune response simply reflects an immune response to the mannan complex itself. However, the ability of oligosaccharides to enhance responses to disease challenges in both conventional and germ-free animals suggests that the oligosaccharides can prime the immune system and allow it to respond more rapidly to disease challenge (Spring and Pirvulescu, 1998).

V. COMPARISONS OF MANNAN OLIGOSACCHARIDES (MOS) WITH ANTIMICROBIAL GROWTH PROMOTANTS

Several groups have recently compared the effects of yeast-derived mannan oligosaccharide preparations (Bio-Mos) with those of specific growth-promoting antimicrobial supplements in poultry (Table 2). These studies focus on the production advantages of yeast cell wall preparations and their potential roles as alternatives to large amounts of antimicrobials used in poultry diets.

Table 2. Comparison of relative performance of poultry fed antimicrobial growth promotants or yeast-derived mannan oligosaccharide (Bio-Mos)¹

Antimicrobial growth promotant	Type of bird and period	Parameter	% Improvement with:		Investigator/ Site
			Antimicrobial	Bio-Mos	
Avilamycin	Tom turkeys, 8 weeks	Weight gain	6.3	7.8	Valancony <i>et al.</i> , France, 2000
		FCR	0	0	
Bacitracin MD	Tom turkeys, 18 weeks	Weight gain	4.9	5.7	Sims and Sefton, Virginia, 1998
		FCR	6.4	7.4	
Flavomycin	Commercial broilers, 42 d	Weight gain	5.8	5.6	Roch, Canada, 1999
		FCR	1.6	3.8	
Zinc bacitracin	Commercial broilers, 21 d	Weight gain	2.3	6.5	Mateo, Philippines, 1999
		FCR	0.1	0.2	
Virginiamycin	Commercial broilers, 42 d	Weight gain	2.2	1.6	Mathis, Georgia, 1999
		FCR	-0.4	0.5	
Bacitracin MD	Commercial broilers, 29 d	Weight gain	7.8	4.8	Sims, Virginia, 1998
		FCR	9.8	9.1	

¹Data from unpublished reports on demonstration trials carried out during 1998 and 1999.

Sims and Sefton (1999) reared tom turkeys to 18 weeks of age on used turkey litter and fed diets with bacitracin methylene disalicylate (BMD), a yeast cell wall preparation (Bio-Mos), or in combination of these supplements, along with a control diet. There were no significant differences in the body weights of the birds at 6 or 12 weeks of age (Table 3). However, at both 15 and 18 weeks of age, turkeys fed BMD plus Bio-Mos were heavier ($P < 0.05$) than birds fed the control diet, while the birds fed either of the feed additives alone were intermediate in body weight. At 18 weeks of age, birds fed Bio-Mos or BMD alone were heavier than control birds but were not as heavy as those fed Bio-Mos and BMD in combination ($P < 0.05$). Changes in body weight were reflected to some extent in feed conversion, since it was also improved ($P < 0.05$) at 18 weeks of age for those birds fed Bio-Mos plus BMD compared to control fed birds, while those fed either Bio-Mos or BMD alone were intermediate in their feed efficiency.

Table 3. Effects of Bio-Mos and BMD on average liveweight (kg) of turkeys at 6, 12, 15 and 18 weeks (adapted from Sims and Sefton, 1999)

Treatment	Week 6	Week 12	Week 15	Week 18
Control	1.993 ^a	7.281 ^a	9.348 ^b	11.868 ^c
Bio-Mos	2.031 ^a	7.518 ^a	9.698 ^{ab}	12.563 ^b
BMD	2.127 ^a	7.545 ^a	9.716 ^{ab}	12.455 ^b
Bio-Mos+ BMD	2.116 ^a	7.535 ^a	9.804 ^a	12.787 ^a

^{a,b} Means in a column without a similar superscript differ significantly ($P < 0.05$).

The comparative effects of Bio-Mos and Flavomycin on the growth of turkey poults have also been examined (Fairchild *et al.*, 1999). In birds that had been challenged with *E.*

coli, both Bio-Mos and Flavomycin improved ($P < 0.05$) poult growth during the first week. Cumulative three-week body weight gains for unchallenged poult were improved by both Bio-Mos and Flavomycin ($P < 0.05$). These studies suggested that dietary Bio-Mos and Flavomycin were most effective in poult faced with an *E. coli* challenge during the first few weeks of life.

In general, these production studies indicate that the yeast-derived mannan oligosaccharide preparations (Bio-Mos) can provide many of the same production advantages that have been long associated with the use of antimicrobial growth promotants in poultry, and that these materials may serve as useful alternatives to antimicrobial supplements in many production systems (Table 2). However, since the responses to the combination of yeast cell wall preparations and antimicrobials are often greater than those associated with either supplement alone, it appears that the mechanisms that explain the overall effects of yeast preparations may differ from those used to describe the growth-promoting activities of antibiotics. In many cases, beneficial production responses to mannan oligosaccharides can be obtained both in the presence and absence of antimicrobial growth promotants.

VI. APPLICATION OF YEAST EXTRACTS

Autolysis of yeast cells using the enzymes present in the cell or hydrolysis of the yeast cell wall using exogenous enzymes or acids releases yeast extract. This material has long been known as a rich source of nutrients and contains vitamins, minerals, peptides, and amino acids that can contribute to a nutritionally balanced diet. Typically, the free amino acid content of yeast extract is around 35-40%, but there also are substantial amounts of small molecular weight (<600 Daltons) peptides and water-soluble vitamins present. The predominant amino acids are glutamic and aspartic acids, both of which contribute to the use of yeast extract as flavoring agents. Yeast extracts are used primarily in the fermentation industry as growth substrates or in the food industry as flavor enhancers. They are valued for their ability to enhance flavors and to mask sour and bitter tastes and are used in a wide variety of familiar applications, including the flavor base of food products such as soups, gravies, and sauces, as well as microbial growth medium in microbiology. In recent years, other components in yeast extract have been of interest from the point of view of animal health and production. Increasing interest in the nucleotide content of yeast extracts has resulted in a number of lines of investigation that have examined the value of these materials as important nutritional factors. Beneficial effects of nucleotides on nitrogen metabolism, immune function, intestinal development and overall growth have been documented, but not clearly defined (Carver, 1994; Pickering *et al.*, 1998).

Table 4. Effects of yeast derived biopeptides on the feed conversion (g feed:g gain) of male broilers¹

Dietary treatment	Period	
	0-7 days of age	0-17 days of age
Control	1.66	1.71
2% Biopeptides	1.47	1.70
4% Biopeptides	1.52	1.84
6% Biopeptides	1.45	1.88
SEM	0.05	0.03

¹Adapted from a preliminary report from Dozer and Moran (2000).

Until recently, use of yeast extracts as sources for biopeptides for animal feeds was cost-prohibitive. However, today the increased demand for yeast cell wall-based products has increased the availability of yeast extracts and will result in decreased costs. Preliminary studies with broiler chicks have shown that yeast-based biopeptides improved the efficiency during the first week of age, but that supplementation over a longer period did not provide any long-term advantages (Table 4). Such studies suggest a strategic role for yeast extract-derived biopeptides during the starter phase of chicken development.

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THE ANTIBIOTIC RESISTANCE REDUCTION EFFECT OF FLAVOPHOSPHOLIPOL (FLAVOMYCIN®)

K. SEITZ and F. DÜRING

Summary

Various antibiotics have been used as feed additives for livestock and poultry for the purpose of growth promotion. Consequently, drug resistant bacteria have considerably increased among the enteric bacteria of these animals and a majority of these bacteria have been found resistant to multiple drugs due to the presence of R factors (transferable drug resistance factors). The potential of Flavomycin to overcome or control plasmid-mediated antimicrobial resistance was determined in a series of *in vivo* and *in vitro* experiments. A possible mode of action is the selective killing or inhibition of bacteria carrying R plasmids. Investigations have indicated that the effect of Flavomycin is correlated directly with the presence of sex pili on the cell's surface which may have served as a pathway for the drug allowing it to enter the cell and then destroy it.

I. INTRODUCTION

The subject of antibiotic drug resistance has long been the object of much study and debate within the scientific community. Low inclusion levels of antimicrobial drugs are fed to livestock and poultry in order to increase the production of animal protein. Since the discovery and widespread use of antibiotics in the 1940's, bacterial pathogens of man and animals have engaged in a battle for survival. For 50 years new generations of antibiotics have been discovered, or chemically modified, as researchers identify better weapons against the surviving pathogens. In recent years, antibiotic treatment of infectious diseases has become more difficult because certain bacteria have grown resistant to them. The issue of resistance has led to worldwide investigations on the interaction of modern antibiotic use practices on bacterial resistance and human disease.

By definition, bacteria are selected for resistance when exposed to an antibiotic. This natural biological process, which results in the survival of the most resistant strains, was observed in bacteria that were collected before the discovery of penicillin, highlighting the fact that resistance is a natural phenomenon that has always existed.

Overuse or inappropriate application of antibiotics in human therapy quickly became apparent as the major contributor to the emergence of antibiotic resistance. Veterinary medicine and animal farming, however, also received attention, when a laboratory study from The Netherlands suggested that the use of an antibiotic in animal farming may co-select for resistance to an antibiotic from the same class used in human medicine, and that resistance may be transferred from harmless bacteria in animals to potentially pathogenic bacteria of a different species in man.

Co-selection for resistance to antibiotics from the same class and the potential transfer of resistance from animal to man established the major alert to the resistance issue. However, extensive studies, conducted over a period of several decades, have failed to document convincing evidence for this transmission, thus, not revealing any practical threat to human health. In addition, recent investigations initiated by the European Commission have shown that significant differences exist between the various types of compounds used.

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II. DIFFERENT TYPES OF ANTIBIOTIC RESISTANCE

There are two types of antibiotic resistance, chromosomal resistance, which all members of a species share and which is inherited, and acquired resistance, transferable via extrachromosomal DNA located on transposons, integrons or plasmids to other bacteria in the environment. The point of concern in the debate regarding animal growth promotants is the acquired transferable resistance by the conjugation form:

- conjugation -- DNA transfer by cell-to-cell contact (extrachromosomal resistance)

Conjugation is one of the most important mechanisms for spreading antimicrobial resistance for two reasons: it can occur in a broad range of bacteria species and DNA may be transferred that encodes for resistance to multiple drugs.

Extrachromosomal antibiotic resistance is caused by plasmids (genetic material not attached to the cell nucleus) carrying a resistance factor (R-factor). Unlike chromosomal resistance, R-Factor occurs frequently and can render a bacterial population resistant to a given antibiotic at a greatly accelerated rate.

III. R-FACTOR

In its simplest form R-factor resistance can be described as the transfer of genetic resistance information located on e.g. a plasmid (mobile genetic material) from one mature bacterium to another. The mechanism of this information exchange is not completely understood but is believed to follow a rather basic procedure.

A resistant and a sensitive bacterium meet and conjugate, forming a pilus that acts like a bridge between the two cells. After conjugation and transfer both donor and recipient will harbor a copy of the plasmid. The plasmid information then becomes part of the genetic structure of the sensitive cell, allowing it to become resistant.

The transfer of plasmid DNA via sex-pili occurs more frequently in gram negative than gram positive bacteria, but is also reported to happen between bacteria species. When the transfer to antibiotic-sensitive bacteria is fulfilled these recipients immediately become resistant and they try to spread the R-plasmid to still sensitive cells. These actions combined with normal cell reproduction allow the resistance to spread even more rapidly.

As a result, the antibiotic treatment of human bacterial diseases is becoming more and more difficult due to increasing cross-resistance among certain bacteria. Also antibiotics once useful in controlling disease outbreaks in animals can be made completely ineffective, limiting the already restricted number of usable compounds available for livestock producers. Inadequate exposure of an originally sensitive population of bacteria to an antibiotic will lead to a selection of bacteria that harbor resistance against the antibiotic. If insufficient selective pressure pertains the resistant bacteria will continuously pass resistance capabilities on to successive generations, eventually resulting in increasingly higher levels of and, finally, total resistance within the entire population.

R-factor resistance becomes even more significant when considering the types of bacteria that it affects. Bacterial pathogens including *E.coli* and *Salmonella* have been proven to harbor and pass on R-factors. Considering the health problems presently associated with *E.coli* and the public controversy surrounding *Salmonella* control, the ability to reduce R-factor resistance in just these two species would be tremendously beneficial.

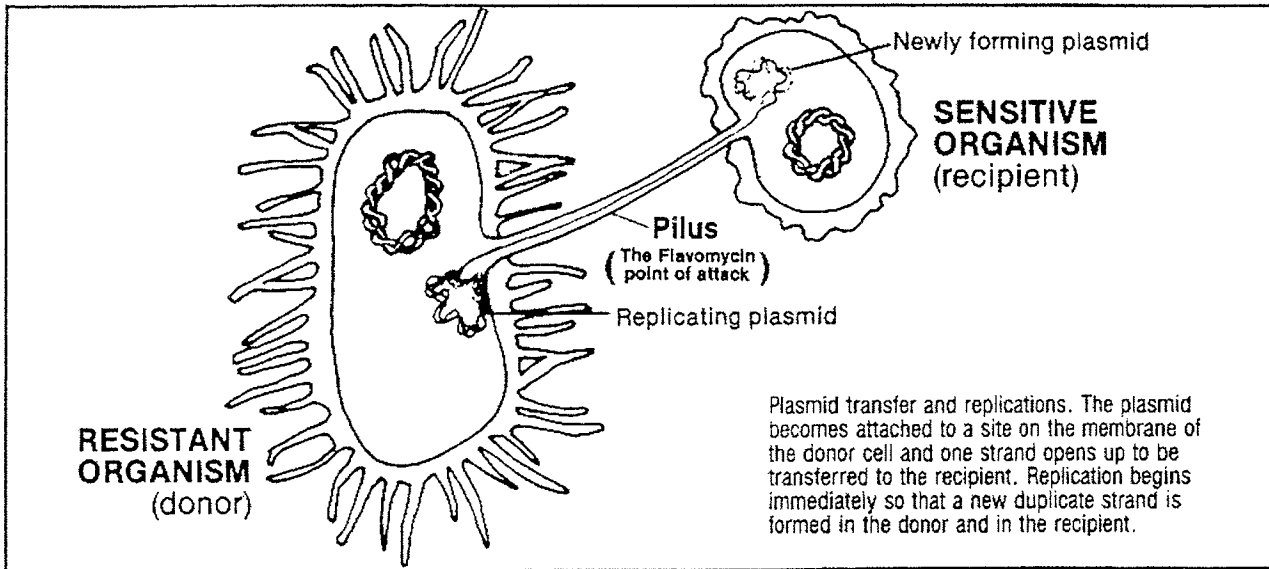


Figure 1. Plasmid transfer and replication.

IV. FLAVOMYCIN'S ABILITY TO REDUCE R-FACTOR TRANSFER

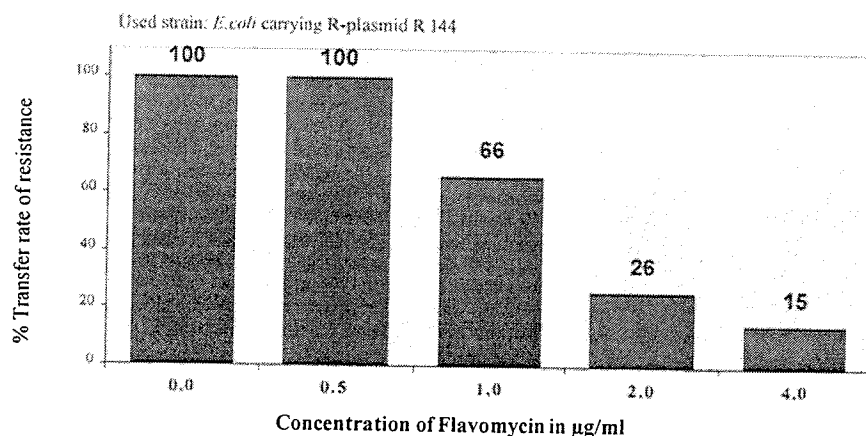
Flavomycin was found repeatedly to reduce the transfer of antibiotic resistance between bacteria and, consequently, to decrease resistance levels in bacterial populations, while in itself not making any contribution to the occurrence of resistance in pathogenic bacteria to antibiotics used in human medicine. Flavomycin's ability in reducing transferable drug resistance has been documented in a number of *in vivo* and *in vitro* trials. Flavomycin administered via feed decreased the incidence of transferable sulfonamide, oxytetracycline and streptomycin resistance among strains of *E.coli* and *Salmonella* populations known to be resistant to ampicillin, streptomycin, sulfonamides and oxytetracyclines. Flavomycin prevents the transfer of resistance to sensitive microorganisms and, therefore, lowers the spread of resistance against therapeutic drugs.

Flavomycin's R-factor control comes from two basic areas:

- elimination of r-plasmid-containing bacteria,
- prevention of r-plasmid transfer between cells.

Looking at the Minimum Inhibition Concentration (MIC) value, normal *E.coli* and *Salmonella*, which are not carrying plasmids, are not sensitive at all to Flavomycin under *in vitro* conditions. Earlier studies from the 70's and 80's conducted with *S. typhimurium* and *E.coli* harboring R-factors showed a reduction of recipient and donor cells of both species compared to a non-reduction of cells free of R-factors (Watanabe, 1971; Sokol, 1972; Dealy, 1976; Fagerberg, 1982, 1984) The reduction of transfer of resistance is correlated to the concentration of Flavomycin in the population as can be seen in Figure 2 based on an *in vitro* investigation done by George and Fagerberg (1984).

Flavomycin reduces the transfer rate and presence of resistance against antibiotics



Ref.: Beverly A. George and Diane J. Fagerberg, Am J Vet Res 45, No 11

Figure 2: Flavomycin reduces the transfer rate of resistance against antibiotics.

Another *in vitro* trial (Schady, 1980) clearly indicates that Flavomycin at a concentration of 2 ppm completely inhibited the transfer of resistance plasmids from *E.coli*, being resistant against Amikacin, Kanamycin, Neomycin and Tetracycline ("donors") to sensitive *E.coli* bacteria. At the same time, the number of donor *E.coli* was reduced by 76 % (Table 1).

Branà *et al.* (1973) indicated that the efficacy of Flavomycin in that respect is directly correlated to the presence of the sex-specific pili on the cell surface, which may have served as a pathway for the drug, allowing it to enter the cell and then destroy it.

In vivo trials in pigs and calves have confirmed the same effect (Figure 3).

With the onset of the scientific and public debate on the emergence of antibiotic resistance Flavomycin's feature of reducing antibiotic resistance has gained major attention.

Transfer of Resistance Determinants

FLAVOMYCIN inhibits the transfer of resistance plasmids and diminishes the number of bacteria harbouring an R-plasmid ("donors").

Concentration of FLAVOMYCIN (mcg/ml)	Transfer of resistance determinants (%) from <i>E. coli</i> (R+) RP 1 to <i>E. coli</i> (R-) 8632				No. of Donors
	Amikacin	Kanamycin	Neomycin	Tetracycline	
0 control	33	32	32	33	3,7 x 10 ⁷
2	0	0	0	0	9,0 x 10 ⁷
5	0	0	0	0	1,8 x 10 ⁸
10	0	0	0	0	9,0 x 10 ⁷
15	0	0	0	0	no donors
20	0	0	0	0	no donors

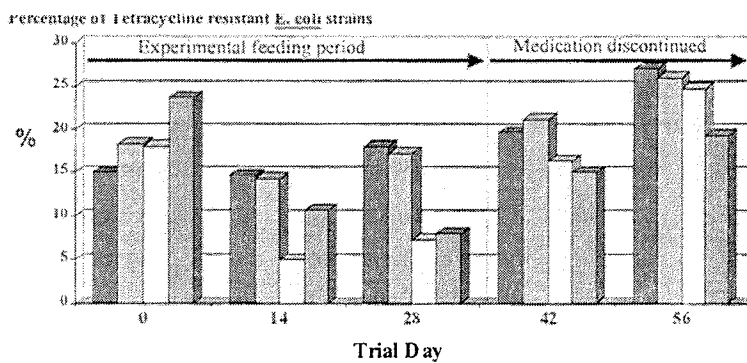
Ref: K.M. Schady: An Investigation of the selective action of FLAVOMYCIN on R+ *E. coli*. New Brunswick, 1980, p. 81

Table 1: Transfer of Resistance Determinants

Flavomycin Effect on Tetracycline Resistance

E. coli strains were collected from rectal swabs of weaned piglets, 60 piglets per trial group, initial weight 15 to 17 kg. Five *E. coli* isolates were selected from the culture of each pig sample. Flavomycin feeding began on Trial Day 0, and was discontinued after Day 28.

■ zero control □ Chlortetracycline (28 ppm) ◻ Flavomycin + Chlortetracycline (5+28 ppm) ▣ Flavomycin (5 ppm)



Ref: A. Sokol et al., Symp. in Czechoslovakia, 1972

Figure 3: Flavomycin effect on Tetracycline resistance.

Recent studies under controlled laboratory condition (*in vitro*) have shown that Flavomycin reduces the transfer rates of genetic elements carrying antibiotic resistance against Vancomycin (University Würzburg, Germany, 1999; accepted for publication 2000), and reduces the stability of certain types of resistance plasmids, or even causes resistance plasmid loss, from a variety of gram positive and gram negative bacteria (University Hohenheim, Germany, 1999).

An *in vivo* study performed in pigs to confirm the relevance of laboratory data under farm conditions shows that Flavomycin reduces the level and the prevalence of antibiotic resistance in *E. coli* microorganisms not sensitive to Flavomycin (University Maastricht, NL, 2000; prepared for publication 2000).

Flavomycin reduced antibiotic resistance in a number of studies

Year	Investigator	in vitro		in vivo		
		Inhibits or kills bacteria containing plasmids	Reduces transfer or eliminates plasmids	animal species	Resistant bacteria inhibited, and/or plasmids reduced	Reduced number of animals shedding resistant bacteria
1971	Watanabe	X				
1972	Lebeck		X			
1972	Sokol, et al.			Pigs		X
1973	Fedenc & Sokol			Pigs	X	
1976	Dcaly & Moeller			Pigs	X	X
1977a	Dcaly & Moeller			Calves	X	X
1977b	Dcaly & Moeller			Calves		X
1980	Schady	X				
1984	George & Fagerberg	X	X			
1999	University of Würzburg/D		X			
1999	University of Hohenheim/D		X			
2000	University of Maastricht, NL			Pigs	X	

Table 2: Summary of *in vivo* and *in vitro* studies, showing the antibiotic reducing effect of Flavomycin

Another unique point in the discussion with Flavomycin and Food Safety is the reduction of Salmonella shedding. A recent investigation at the Institute of Animal Science and Health in Lelystad / Netherlands (Bolder, 1999) confirmed the former results of Humbert (1991) and Dorn (1991), that Flavomycin, without having a direct MIC against Salmonella, had significantly reduced *S. enteritidis* shedding ($P < 0.05$) and reduced the number of Salmonella positive market age broilers.

V. CONCLUSIONS

Current concerns are related to:

- a) the increase of resistance against antibiotics essential in human medicine,
- b) the use in animals of antibiotics related to those in human medicines causing cross resistance to human antibiotics, and
- c) the increase of *salmonella* shedding as a food borne pathogen.

In this regard Flavomycin

- a) is not used in therapy nor are any relatives of its class. Therefore, there is no threat to human health and therapy,
- b) can be of significant benefit in reducing transferable antibiotic resistance, and
- c) reduces significantly *Salmonella* shedding in broilers and helps, therefore, to support a food safety program.

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INFLUENCE OF FEEDING PROGRAM ON BROILER BREEDER MALE MORTALITY

S.D. PEAK and J. BRAKE

Summary

Broiler breeder male mortality has become a persistent problem. However, recent evidence suggests that the feed allocation pattern during the rearing period can have a tremendous impact on overall male livability. Two experiments were conducted to determine the impact of feeding program during the grower period on subsequent male mortality. Results indicate that feeding males in a "concave" manner with larger feed increases late in the growing period is more advantageous than feeding males in a "linear" manner that allocates constant feed increases. In addition, all males, regardless of feeding program, had similar body weights, which further indicates that management of feeding program is more critical than management for proper body weight.

I. INTRODUCTION

Broiler breeder male mortality during the laying period has become a constant and costly problem to the poultry industry. Maintaining adequate male numbers is critical for good flock performance and fertility. Figure 1 demonstrates the average U.S. male mortality during the laying period for a typical male meat-type breeder crossed with three different female lines. From this figure, it is evident that the average male mortality from 22 to 64 weeks during the years 1995 to 1999 was approximately 43% (Figure 1). There appeared to be only slight fluctuations in the mortality from year to year or among female strains. The cause of the majority of this mortality is unknown. However, recent research by the present authors suggests that it may be possible to prevent some male mortality by adjusting the feed allocation program during the growing period.

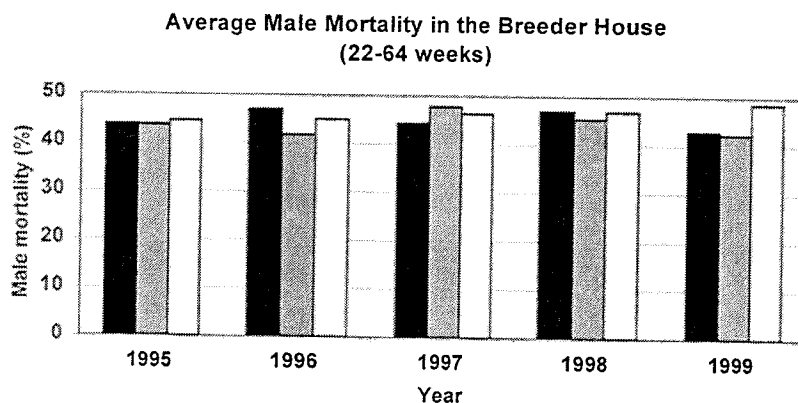


Figure 1. Average percentage male mortality in the breeder house from 1995 to 1999 for a meat-type breeder male crossed with 3 different female lines. Data furnished by Agri Stats Inc., 6510 Mutual Drive, Fort Wayne IN 46825, USA.

II. PRELIMINARY RESEARCH

Three broiler breeder flocks were examined retrospectively to determine the impact of

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feeding program on male mortality. These flocks had been grown consecutively in the same facilities under similar management. The first of the flocks, BB11, was placed in November 1995, followed by BB12 placed in July 1996, and BB13 placed in April 1997. All of the flocks utilized Ross males crossed with Ross 308 females. In all cases, males were grown separate from females using a diet containing 170 g crude protein (CP)/kg (see Table 1). Examination of the flock feeding programs indicated that BB11 males had been grown on a "linear" feed allocation program while BB12 and BB13 were fed in a more "concave" manner.

Figure 2 shows the feeding programs for each of these flocks. The linear feed allocation program consisted of 3 g feed increases per male/week from 8 to 21 weeks. The concave program had weekly increases of 2 g feed per male from 5 to 11 weeks, 3 g feed increases from 13 to 17 weeks, 5 g feed increases at 18 and 19 weeks and 7 g feed increases at 20 and 21 weeks. From Figure 2 it is evident that BB11 males received slightly more feed during the grower period. All flocks were moved to the laying house and photostimulated at the end of 21 weeks. From 22 to 64 weeks, all males received the same feed allocation.

Table 1. Metabolisable energy (ME), crude protein (CP) and feeding age for the starter, grower and breeder diets used to feed females, males grown separate from females, and males grown intermingled with females.

Diet	ME (MJ/kg)	CP (g/kg)	Females	Separate grown males	Mixed grown males
			Feeding age (weeks)		
Starter	12.22	170	1-2	1-21	1-4
Grower	12.22	150	3-25	22-25	5-25
Breeder	12.22	160	26-64	26-64	26-64

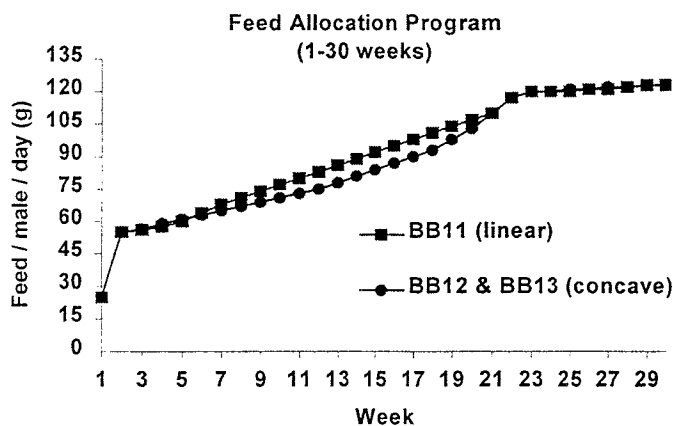


Figure 2. Feed per male (g/d) versus weeks of age (1-30 weeks) for BB11, BB12, and BB13 flocks.

Table 2 displays the body weights for each of the three flocks. The body weights during the grower period were slightly higher for males on the linear program due to the extra feed allocated. However, these differences diminished by 28 weeks. Table 3 shows the percentage male mortality for each of the flocks divided into four time periods and overall.

The highest overall percentage male mortality was observed in BB11, which had an overall male mortality of 50.8%. The BB12 and BB13 flocks had similar mortality patterns resulting in overall mortalities of 38.4% and 37.2%, respectively.

Table 2. Male body weight (kg) for each flock at 12, 20, 24, 28, 40, and 52 weeks of age.

Flock	Male body weight (kg)					
	12 weeks	20 weeks	24 weeks	28 weeks	40 weeks	52 weeks
BB11	2.1	3.2	4.0	4.2	4.4	4.6
BB12	1.8	3.0	3.7	4.0	4.9	5.0
BB13	1.9	2.9	3.9	4.1	4.5	5.0

Table 3. Percentage male mortality for five time periods: 22-29 weeks, 30-44 weeks, 45-64 weeks, 30-64 weeks and overall (22-64 weeks).

Flock	Male mortality (%)				
	22-29 weeks	30-44 weeks	45-64 weeks	30-64 weeks	22-64 weeks
BB11	7.5	25.8	17.5	43.3	50.8
BB12	9.6	12.8	16.0	28.8	38.4
BB13	8.4	12.0	16.8	28.8	37.2

It appeared that the males on the "linear" feed allocation program exhibited higher mortality than males on the "concave" feed allocation programs with the majority of the mortality differences occurring between 30 and 44 weeks. Mortality from 45 to 64 weeks was similar between flocks. Based on these preliminary findings, two experiments were conducted to determine the impact of male feeding program on subsequent male mortality.

III. MATERIALS AND METHODS

In Experiment 1, 2400 Ross 308 females and 600 Ross males were placed in a black-out growing house. The house consisted of 16 pens. Four pens held 75 males each and were used to raise males separate from females on either a "linear" or a "concave" feed allocation program. The remaining 12 pens contained 200 females and 25 males each. Birds were grown on a daily 8 h light and 16 h dark and both feed and water were controlled. At the end of 21 weeks the birds were moved to a curtain-sided laying house and photostimulated. The females from each pen were moved to a corresponding pen in the laying house. Each pen was equipped with 4 bell waterers, 2 separate male feeders, 4 nest boxes, and 12 female feeders equipped with male exclusion grills to ensure males would not eat female feed. Four pens from the growing house were left intact and both the females and males from these pens were moved together to their corresponding pen in the laying house. The males in the other eight pens were removed and replaced with males grown separate from females resulting in four pens with males grown on a "linear" program and four pens with males grown on a "concave" program. Therefore, there were 3 male treatments and 4 replicate pens per treatment.

Figure 3 shows the feed allocations for each treatment from 1 to 30 weeks. "Linear" grown males received constant feed increases of 2.4 g per male/week from 4 to 28 weeks. After 28 weeks, males received a constant feed amount of 117 g per bird. "Concave" grown males were fed the same as BB12 and BB13 through to the end of 21 weeks, after which time the feed amount was held constant at 117 g per bird. The diets utilised for the females and males of each treatment are displayed in Table 1. All separate grown males received the same amount of cumulative feed through 21 weeks that resulted in a cumulative CP intake of 1600 g and a cumulative ME intake of 133.9 MJ per male at photostimulation.

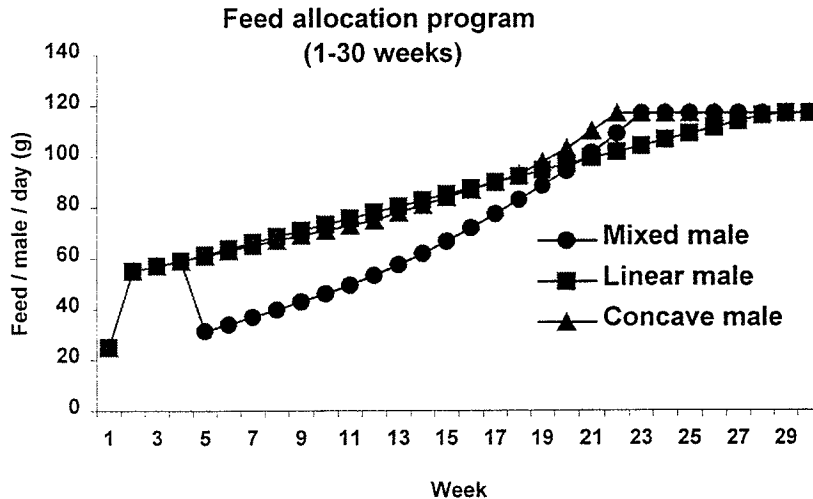


Figure 3. Feed per male (g/d) versus weeks of age (1-30 weeks) for males grown intermingled with females (Mixed), and males grown separate from females on either a "linear" feed allocation program (Linear) or a "concave" feed allocation program (Concave).

In Experiment 2, 2700 Ross 308 females and 600 Ross males were placed in the same black-out growing house as used in Experiment 1. There were 225 females and 25 males placed into each of the 12 pens used to raise females and males together. The 4 pens previously used to raise males separately from females were modified into twelve 0.91m x 1.93 m pens that contained 25 males each. These pens were used to raise males on either the "concave" feed allocation program utilized in Experiment 1 or on a "sigmoid" feed allocation program. All birds were grown on a daily 8 h light and 16 h dark, and both water and feed were restricted. At the end of 23 weeks, birds were moved to the same laying house with the same equipment as utilised in Experiment 1 and photostimulated. In this case, all males were removed from the intermingled pens and replaced with males grown separately. There were 200 females from each pen and 20 separately grown males placed into each of the 12 experimental pens in the laying house. Therefore, there were 2 male treatments and 6 replicate pens per treatment.

Figure 4 shows the feed allocation program from 1 to 30 weeks for each treatment. The "sigmoid" program provided relatively more feed early in the growing period and less feed late in the growing period. This program consisted of weekly feed increases of 5 g from 3 to 4 weeks, a 4 g increase at 5 weeks, a 3 g increase at 6 weeks, a 2 g increase at 7 weeks, a 1.5 g increase at 8 weeks, 1 g increases from 9 to 17 weeks, 2 g increases from 18 to 22 weeks and 3 g increases from 23 to 27 weeks. At 28 weeks birds were fed 110 g feed/bird/d through to the end of the experiment at 64 weeks. The "concave" program was the same as that utilised previously in Experiment 1, BB12 and BB13 except there was only one 7 g feed increase at 20 weeks resulting in feed allocation of 110 g feed/bird/d. The birds were fed this

constant amount of feed until the termination of the experiment. Diets were the same as for previous experiments (Table 1). Again, this experiment was designed to yield the same cumulative nutrition through 21 weeks, approximately 1600 g of CP and 133.9 MJ of ME, irrespective of feeding program.

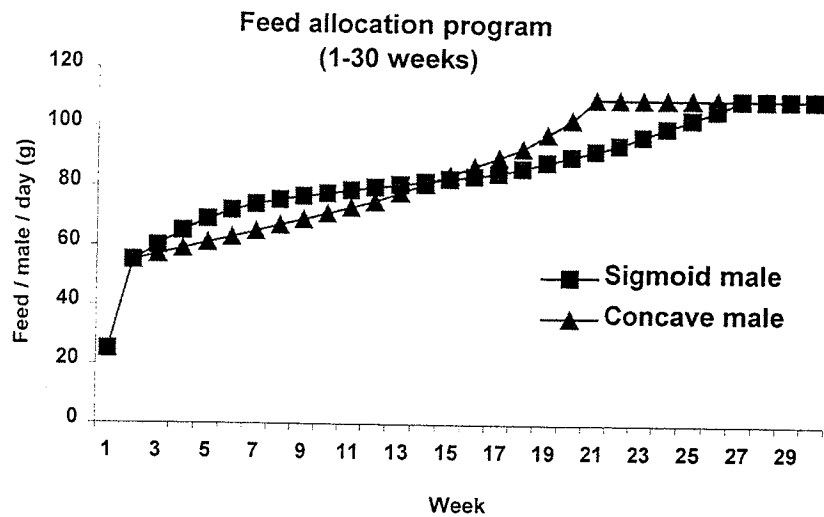


Figure 4. Feed per male (g/d) versus weeks of age (1-30 weeks) for males grown separate from females on either a "sigmoid" feed allocation program (Sigmoid) or a "concave" feed allocation program (Concave).

Data were collected for body weight, fertility, and mortality in both experiments. All males were weighed every 4 weeks from 4 to 60 weeks and mortality was recorded daily. The largest difference between the two experiments was the age at photostimulation and the feed amounts during the laying period. Experiment 1 was photostimulated at the end of 21 weeks and received a constant feed amount of 117 g per bird per d. This was fed to "concave" males from 22 to 64 weeks and to "linear" males from 28 to 64 weeks. Experiment 2 was photostimulated at the end of 23 weeks and received a constant feed amount of 110 g per bird per d. "Concave" males received this amount from 21 to 64 weeks and "sigmoid" males received this amount from 28 to 64 weeks. These differences are important for interpretation of the results.

IV. RESULTS

(a) Body weight

Table 4 displays the male body weights for Experiment 1. Males grown intermingled with females had significantly lower body weights at 12 and 16 weeks when compared to the separately grown males. Separately grown males on the "linear" program had significantly higher body weights than separately grown males on the "concave" program. However, there were no differences in body weight due to treatment after photostimulation (22 weeks). All males had similar body weights at 22, 26, 28, 40, and 52 weeks of age.

Male body weights for Experiment 2 are shown in Table 5. Males grown separately on the "sigmoid" program had significantly higher body weights than males grown separately on the "concave" program at 12 and 16 weeks. Again, these differences diminished over time and there was no difference in body weight due to treatment at 22, 26, 28, 40, or 52 weeks of age.

Table 4. Male body weights (kg) at 12, 16, 22, 26, 28, 40 and 52 weeks of age for Experiment 1. Males were grown "mixed" with females or grown separate from females on either a "linear" or "concave" feed allocation program.

Male treatment	Male body weight (kg)						
	12 weeks	16 weeks	22 weeks	26 weeks	28 weeks	40 weeks	52 weeks
Mixed	1.4 ^c	2.0 ^c	3.2	3.8	3.9	4.3	4.6
Linear	1.9 ^a	2.3 ^a	3.1	3.8	4.0	4.3	4.7
Concave	1.8 ^b	2.2 ^b	3.1	3.7	4.0	4.3	4.6

^{a-c} Means with different superscripts within columns differ significantly $P < 0.05$.

Table 5. Male body weights (kg) at 12, 16, 22, 26, 28, 40 and 52 weeks of age for Experiment 2. Males were grown separate from females on either a "sigmoid" or "concave" feed allocation program.

Male treatment	Male body weight (kg)						
	12 weeks	16 weeks	22 weeks	26 weeks	28 weeks	40 weeks	52 weeks
Sigmoid	2.1 ^a	2.6 ^a	3.1	3.8	4.0	4.1	4.4
Concave	1.9 ^b	2.5 ^b	3.2	3.9	4.0	4.0	4.2

^{a,b} Means with different superscripts within columns differ significantly ($P < 0.05$).

Body weights in the two experiments were similar at 12 weeks except for the "mixed" grown males, which were lighter than the separately grown males in Experiment 1. At 16 weeks it appeared that males in Experiment 2 were heavier than males in Experiment 1 despite the fact that "concave" males received the same amounts of feed in both experiments. However, by 22 weeks these differences were not evident and all males in both experiments weighed within 0.1 kg of each other at 22, 26, and 28 weeks. The experimental body weights were also similar to the body weights observed for the three previous flocks (Table 2), except for the body weights at 40 and 52 weeks in Experiment 2. Late in the breeder period, males from Experiment 2 were slightly lower in body weight due to the fact that they received less amounts of feed during this time.

(b) Mortality

Table 6 displays the percentage male mortality for Experiment 1. During the early breeder period (22-29 weeks), mortality in the two groups of separately grown males was similar. It appeared that the "mixed" grown males had less mortality during this period although these differences were not statistically significant. Males grown separately on the "linear" program or "mixed" with females had significantly higher mortality from 30 to 44 weeks when compared to males grown separately on the "concave" program. From 45 to 64 weeks, "linear" males numerically had the highest mortality with "mixed" and "concave" males having similar mortality. When mortality was compared from 30 to 64 weeks, "concave" males had significantly lower mortality when compared with the "linear" males. "Mixed" males were intermediate. This same trend was observed overall (22-64 weeks).

Table 7 displays the percentage male mortality for Experiment 2. The initial mortality (22-29 weeks) was significantly higher for the "concave" males when compared to the "sigmoid" males. This trend appeared to be reversed from 30 to 44 weeks and from 45 to 64 weeks. "Sigmoid" males had significantly higher mortality between 30 to 64 weeks. Overall, mortality appeared to be similar.

Table 6. Percentage male mortality from 22-29 weeks, 30-44 weeks, 45-64 weeks, 30-64 weeks and overall (22-64 weeks) for Experiment 1. Males were grown "mixed" with females or grown separate from females on either a "linear" or "concave" feed allocation program.

Male treatment	Male mortality (%)				
	22-29 weeks	30-44 weeks	45-64 weeks	30-64 weeks	22-64 weeks
Mixed	3.8	17.5 ^a	8.7	26.2 ^{ab}	30.0 ^{AB}
Linear	8.7	17.5 ^a	13.8	31.3 ^a	40.0 ^A
Concave	7.5	10.0 ^b	7.5	17.5 ^b	25.0 ^B

^{a,b} Means within columns without a similar superscript differ significantly ($P < 0.05$).

^{A,B} Means within columns without a similar superscript differ significantly ($P < 0.10$).

Table 7. Percentage male mortality from 22-29 weeks, 30-44 weeks, 45-64 weeks, 30-64 weeks, and overall (22-64 weeks) for Experiment 2. Males were grown separate from females on either a "sigmoid" or "concave" feed allocation program.

Male treatment	Male mortality (%)				
	22-29 weeks	30-44 weeks	45-64 weeks	30-64 weeks	22-64 weeks
Sigmoid	1.7 ^b	18.6	23.7	42.3 ^a	44.0
Concave	16.9 ^a	8.4	11.9	20.3 ^b	37.2

^{a,b} Means with different superscripts within columns differ significantly ($P < 0.05$).

Comparison of the two experiments indicated that the "concave" males in Experiment 2 had higher mortality than "concave" males in Experiment 1 despite the fact that males were fed similarly from 1 to 20 weeks. The majority of the mortality differences were observed from 22 to 29 weeks with "concave" males from Experiment 2 having 9.4% higher mortality during this time. It should be noted that Experiment 2 "concave" males were allocated a constant amount of feed of 110 g per male per day starting at 20 weeks and photostimulated at the end of 23 weeks. However, Experiment 1 "concave" males were allocated a constant amount of feed of 117 g per male per day at 21 weeks and photostimulated at the end of 21 weeks. Despite these feeding and photostimulation differences mortality from 30 to 64 weeks was similar in the two experiments.

The overall male mortality of "linear" males in Experiment 1 (40.0%) was similar to the overall male mortality of "sigmoid" males in Experiment 2 (44.0%). However, mortality patterns or ages when the males died appeared to be different in the two groups. "Linear" males had 7.0% higher mortality than "sigmoid" males from 22 to 29 weeks, while "sigmoid"

males had 9.9% higher mortality than "linear" males from 45 to 64 weeks. Mortality from 30 to 44 weeks was similar for the two feed allocation programs.

V. DISCUSSION

It is possible to alter male body weight during the grower period by changing the feed allocation program. Smaller body weights are achieved by feeding less feed early in the growing period such as when males are grown intermingled with females or separate from females using a "concave" feed allocation program. Allocating males more feed early in the grower period using either a "linear" or "sigmoid" shaped feed allocation program resulted in larger body weights from 12 to 16 weeks for the separately grown males. However, it appears that photostimulation is the equalizer due to the fact that no body weight differences were observed in either experiment after this time. This was even true for the males grown intermingled with females that obviously received less feed than the males grown separate from females.

It appeared that 117 g of feed per male per d (Experiment 1) was adequate to keep males slowly gaining weight from 28 to 60 weeks of age under current management that utilizes strict male and female exclusion grills. Feeding 110 g per male per d (Experiment 2) did not supply enough nutrition to ensure body weight gain. This was evident by the fact that these males did not appear to gain weight from 28 to 40 weeks and had smaller body weights at 52 weeks when compared to the males that received 117 g per male per d in Experiment 1.

Results from Experiment 1 supported the results from the preliminary research. All data indicated that males grown on a "linear" feed allocation program exhibited higher mortality than males grown on a "concave" feed allocation program. It appears that the majority of the mortality due to "linear" feeding can be expected to occur between 30 to 44 weeks of age. The results of Experiment 2 supported these conclusions. The "sigmoid" shaped feed program also employed a feed allocation program that was relatively linear late in the growing period and increased male mortality was observed from 30 to 44 weeks.

The mortality differences between treatments in Experiment 2 were not more pronounced due to the excessive male mortality observed in the "concave" males from 22 to 29 weeks. This high initial mortality was probably due to the fact that feed was held constant at 110 g per male per d starting at 20 weeks. The males were not photostimulated until the end of 23 weeks which meant there were no feed increases for four weeks prior to photostimulation. The "sigmoid" males received constant feed increases during this time period and high initial mortality was not observed in this group. In addition, male mortality rates from 22 to 29 weeks for the four other experiments (Tables 3 and 6) were not excessive and in all cases males received feed increases until photostimulation.

The lowest initial mortality was observed with the "sigmoid" grown males in Experiment 2. This was probably due to the fact that photostimulation was delayed to the end of 23 weeks. It is thought that initial male mortality in the breeding period is mainly due to aggression from other males, which is primarily influenced by male maturity. When males are photostimulated at a later time they are allowed more time to mature and, thus, there is less early male aggression and mortality.

Therefore, it appears that the feed allocation program used during the growing period in association with the time of photostimulation can influence broiler breeder male mortality. It appears that this occurs irrespective of body weight. The various groups of males employed in these experiments exhibited average body weights that were not remarkably different. Thus, one can conclude that management of feeding programs should take precedence over body weight management.

RESEARCH PRIORITIES IN BROILER BREEDER NUTRITION

C.J. WIERNUSZ

Summary

Today's commercial broiler is the fastest growing and most efficient bird ever produced. It represents the combined efforts of genetics and management. However, with this tremendous improvement in broiler performance comes a greater challenge in the ability to manage broiler breeders properly. Much like the broiler, the broiler breeder is also changing rapidly and, although geneticists are also selecting for increased egg production, the customers demand that the emphasis be placed on broiler traits. This does not create broiler breeders which are any more difficult to manage than in the past but, as the bird changes, innovative nutritional and management approaches must also be developed to match these needs.

I. INTRODUCTION

Consumer demand for lean poultry products necessitates that product leanness and uniformity be improved. As a result, technologies resulting in greater protein production, not overall bird mass, will be emphasized. The shift of focus to the profit center of lean tissue mass necessitates that nutritional advances occur which enable muscle growth at optimal rates while minimizing fat accretion. Though this direction may slow bird growth rate research directed at reducing the growth-depressing consequences of stress may help offset this dilemma. Nonetheless, technological developments must occur within the bounds of increasing environmental restrictions, which are becoming more intense. Knowledge related to bird energetics, stress management and waste production are evolving and new management approaches are being employed. A more thorough understanding of energy metabolism is fundamental to improving profitability of production enterprises.

Future poultry expertise will undoubtedly be similar to today's highly technical industry practices on numerous fronts including nutrition, ventilation, hatchery, health and management. Research related to broiler breeder energy and amino acid requirements throughout their life cycle will become a priority. For example, research has shown that the way pullets are grown may have a dramatic effect on subsequent hen performance. Recommendations for pullet rearing programs are and will continue to focus not only on body weight but also on body composition. Breeder nutrient requirement research will be conducted to optimize pullet body composition and change the growing curve to improve egg production and, more importantly, chick production.

There are large differences between various countries in relation to chick production. Broiler breeder hen performance statistics show that countries such as the United States lag significantly behind performance figures for the same breed in other areas of the world. These differences are especially evident with the heavier, fast growing, high yielding broiler breeds. Many companies focus only on pullet costs and often neglect what impact changes in pullet rearing may have on subsequent hen performance. Controlling costs are essential for a company to survive but there must be a balance between cost reduction and performance. There are many opinions as to why certain countries have better flock performance. Clearly, feed formulations, feed management, body weights and body compositions differ. However, what these differences mean for bird health and productivity remains uncertain.

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There is a great wealth of information available on nutrition and management of broiler breeders but the industry often lacks basic knowledge of pullet and breeder hen metabolism and the nutrient requirements for optimal productivity. Though many companies offer feed and weight guidelines for rearing pullets it seems that much of the industry practices and rearing management guidelines are based more on circumstantial opinions and trial-by-error than on scientific evidence and documented solutions. Therefore, the intent of this paper is to describe some of the trends that may take place in broiler breeder research and to add fundamental knowledge describing relationships between nutrition, growing curves, bird body composition, hen performance and mortality.

II. ENERGY REQUIREMENT SCHEMES

Broiler breeder research directed at optimizing body composition of pullets and hens will be essential in understanding what the flock requires for good chick performance. The degree of carcass fatness is well documented to be impacted by both non-nutritional and nutritional factors. The effects of age (Edwards, 1971; Kubena *et al.*, 1972), sex (Summers *et al.*, 1965) and ambient temperature (Swain and Farrell, 1975) are known among the non-nutritional factors. Fraps (1943) described the nutritional effects of varying dietary ingredients on carcass fat. Since then many studies have established that as the dietary energy:crude protein ratio is widened carcass lipids increase (Donaldson *et al.*, 1956; Summers *et al.*, 1965; Kubena *et al.*, 1972). The effect appears to be independent of energy source because dietary fat substitution for carbohydrate at a constant dietary energy:crude protein ratio has little effect on carcass fat (Barton, 1979; Lauren *et al.*, 1985). Without exception such studies have utilized the metabolizable energy (ME) system as a basis for varying ingredient concentrations and ratios.

An understanding of energy and protein metabolism for all poultry classes is fundamental to diet formulation and profitable production. The nitrogen corrected metabolisable energy (ME_n) system currently is accepted as the standard for ration formulation. However, by definition ME_n does not quantitatively predict energy deposition by birds. Any difference in heat increment alters energy retention and, thereby, may affect cellular energy:nutrient ratios. Other factors affecting ME_n utilization include stress factors such as disease agents (bacteria, viruses and protozoa), social stress created by other animals and man, malnutrition, toxicities and the thermal environment. Diets based on ME_n do not necessarily correlate with bird energy retention and may have energy:nutrient ratios varying independently of ME_n .

Efficiency of ME_n use for tissue gain depends on numerous variables. Efficiency varies with substrate source, for lipogenesis being approximately 75, 84, and 61% for carbohydrates, fats and proteins, respectively (De Groot, 1969; Chudy and Schiemann, 1971; Hoffmann and Schiemann, 1971). The high availability of fat ME_n for tissue gain, however, requires that fat is used for lipogenesis (Bossard and Combs, 1961). Utilization of protein for tissue energy gain depends upon the biological value of the protein source and should not be constant (De Groot, 1973). Indeed, one could summarize that the bird's energetic efficiency for use of protein or any substrate is the net result of partitioning consumed substrate energy into maintenance needs versus accretion of protein and fat.

Dietary protein recommendations for optimum rates of lean tissue accretion range from high (Kubena *et al.*, 1972) to low levels complemented with specific amino acids (Waldroup *et al.*, 1976). Whether the carcass leanness associated with feeding high protein diets is attributable to substrate limitations (amino acids), or due to greater heat production per unit of ME_n for dietary amino acids, carbohydrates and fat is subject to debate. Research conducted at Oklahoma State University by Mittelstaedt (1990) examined the true

metabolizable energy (TME) utilization of carbohydrate, protein and fat sources for energy, protein and fat gain. Despite similar TME consumption among the energy-supplemented groups carcass energy was impacted significantly. Total carcass energy gain was 17, 27, and 30% greater for gelatin-, starch- and corn-oil supplemented groups, than for birds fed the basal diet. Estimated energy gain from the basal ration was similar among the energy supplemented groups due to nearly identical feed consumptions. However, total energy gained differed ($P < 0.05$) across experimental groups with the highest value of 1.82 MJ/bird observed for the birds fed corn oil verses only 0.70 MJ/bird for birds fed gelatin. As a result, energetic efficiency varied among the energy supplemented groups. Efficiency of ingredient TME usage for carcass energy deposition averaged 50.0, 39.1, and 19.9%, for supplemental corn oil, starch and gelatin, respectively.

An additional consequence of low protein ME_n utilization efficiency is that the birds heat load is increased. Elevated heat load has little consequence when birds are housed at or below thermoneutral temperatures. However, if the bird's heat load is elevated by high ambient temperature stress, without a concomitant increase in heat dissipation, elevated heat load can be devastating (Wiernusz and Teeter, 1993). Belay and Teeter (1992) fed birds various protein levels and energy:protein ratios. Increasing dietary energy and/or narrowing energy:protein ratios by relaxing restrictions on amino acid balance (which necessitated increased dietary protein) significantly impacted bird carcass composition. Improving amino acid balance and lowering dietary crude protein concentration increased survival both in the thermoneutral environment (4.4%) and in the heat stressed environment (10.8%). Lowering crude protein (at adequate amino acid balance) for birds subjected to heat stress can prove beneficial. Research is needed to identify which amino acid excesses cause the greatest risk.

Diets formulated on the ME_n system do not necessarily correlate with bird energy retention since the energy:nutrient ratios of depot tissue can vary independent of ME_n . In order for the broiler breeder to achieve optimum carcass composition with maximum energetic efficiency an energy-requirement scheme must account for the variation in substrate-mediated heat production.

III. AMINO ACID REQUIREMENT SCHEMES

Rearing broiler breeders for optimum chick output is a complex and multifaceted undertaking. Pullet nutrition and feeding, as one portion of that undertaking, is constantly in flux as genetic packages and management styles change. Nutrient modulation during the replacement phase can and does influence a number of important factors involved in the laying period. Unfortunately, follow-up can be difficult as final egg/chick numbers are so far removed in time from nutritional changes that may be made during the early rearing period. A number of research programs have looked at pullet nutrition and correlated changes in diet with subsequent reproductive performance.

Protein and amino acid inclusion levels of the pullet starter, developer, pre-breeder and breeder feeds have been investigated in a number of laboratories. Results become difficult to interpret because feed intake varies and this results in differing protein intakes even when similar dietary crude protein levels are fed. Reproductive performance enhancements have been noted when increased protein levels were fed throughout the rearing period in quail (Lilburn *et al.*, 1992) or during the prelay period in broiler breeders (Brake *et al.*, 1985, Cave, 1984, Lilburn and Myers-Miller, 1990). Trials by Walsh and Brake (1997) indicated that breeder female fertility was increased if protein intakes to 20 weeks of age were increased.

Recent broiler breeder research at Auburn University has explored the influence of early protein nutrition on body composition and reproductive performance. Pullets were fed isoenergetic starter diets with 120, 160 and 200 g crude protein/kg to six weeks of age. Amino acid densities were scaled to the protein levels. Protein intakes to six weeks were 177g, 231 g and 278 g for the 120, 160 and 200g crude protein/kg treatments, respectively. Breast meat growth was enhanced by dietary crude protein intake while carcass fat was reduced. This relationship was supported by internal research conducted at Oklahoma State University which indicated higher early crude protein intake increases lean tissue accretion, skeletal mass and flock uniformity. Egg production increased with increases in starter protein levels such that cumulative and settable egg production to 31 weeks was greatest in the 200 g crude protein/kg group. Promoting optimal growth during the first few weeks of a chick's life appeared to positively influence hen performance. This may relate to the concept of nutritional programming discussed recently by a number of groups (Knight and Dibner, 1998; Giesen, 1998).

In a second study conducted at Auburn University, early crude protein intake benefits were assessed by measuring egg production through 65 weeks of age. Similar body composition trends were noted as in the first experiment. The study is still ongoing but pullets fed higher crude protein levels early came into production slightly earlier and maintained a higher level of egg production through 45 weeks. Although final results have not been analyzed it appears that hens fed 180 g of crude protein to four weeks of age were substantially ahead of those fed 140 g during the same period. A relatively small increase in total protein intake appears to have a positive influence on egg production through peak lay and beyond.

IV. CARCASS COMPOSITION

Future research will also be directed not only towards early protein intake but also to establishing relationships between optimal body composition, body weight programs and subsequent breeder performance. University studies have been conducted and corroborated by field data which indicate that there are critical periods during pullet rearing where growth rate needs to be enhanced for better hen performance. In effect these observations indicated that the shape of the pullet body weight curve has an effect on hen performance. Rearing recommendations will be modified in the future once a greater understanding of the bird's needs are reached.

A broiler breeder study conducted at Oklahoma State University was designed to examine feeding curve (linear, sigmoid), starter composition (protein level) and overall program effects on hen live weight, live weight variability, change in body composition (lean, fat, bone mass), and egg production. Further, the relationships between body composition, egg production, egg weight and bird mortality were also extensively modeled. Chicks consuming higher amounts of crude protein had increased muscle and skeletal tissue accretion and better flock uniformity. In this study a number of correlations were also evident. Bird lean gain from 12 to 16 weeks of age was positively correlated with egg production to 32 week of age. The correlation of lean gain from 28 to 32 weeks of age was negatively correlated with egg production at 32 weeks of age. After week 20 it appears that hen performance is negatively affected when birds are both gaining weight and producing eggs. This supports recommendations that once light stimulation is initiated the flock must be at the correct body weight and have the ideal body composition.

V. CONCLUSION

Rearing broiler breeders for optimum chick production and quality can be a complex enterprise. As broiler breeder nutrition and feeding guidelines are established they must be constantly updated as the genetic packages and management styles change. Nutrient intake during the replacement phase can and does influence a number of important factors involved in lay. However, final chick numbers are so far removed from the rearing program that correlations between them are difficult. Research must be conducted in such a way that bird requirements are elucidated and modeled. Proper body composition will require more thought and understanding of the bird's development (fleshing) from feeding programs and nutrient consumption. Nutrient consumption will likely be fundamental to addressing the issue of proper pullet growth and optimum carcass composition.

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THE RELEVANCE OF THE ANTIOXIDANT SYSTEM TO THE HEALTH AND GROWTH OF THE DEVELOPING CHICK

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It is generally accepted that living organisms pay a price for living in the oxygen atmosphere. Although oxygen is absolutely necessary to maintain energy production in living organisms, when in excess it is toxic. Information is accumulating to indicate that free radicals and other reactive oxygen species are produced in the metabolic pathways of aerobic cells and affect a number of biological processes (Halliwell, 1994). Furthermore, the electron transport chain of the mitochondria is considered to be the main source of free radicals (mainly as superoxide radical) in biological systems. Other sources of free radicals include immune system cells (macrophages) and enzymes of xenobiotic metabolism. Free radicals and toxic products of their metabolism have been postulated to play a role in aging and are implicated in a number of degenerative diseases (Hogg, 1998). In fact, it is believed that most human diseases at different stages of their progress are associated with free-radical-mediated processes. Furthermore free radicals are considered to play a role as subcellular messengers in gene regulatory and signal transducing pathways (Sen, 2000). Redox regulation of gene expression by superoxide and other related oxidants and antioxidants is beginning to unfold as a vital mechanism in health and disease (McCord, 2000). Unfortunately, this subject is much less studied in relation to animals but information is also accumulating which shows the role of free radicals in animal production. In particular, in the poultry industry such diseases as Pulmonary Hypertension Syndrome (PHS) (Bottje and Wideman, 1995), nutritional muscular dystrophy, encephalomalacia, exudative diathesis (Combs, 1994) and some others are associated with overproduction of free radicals. Therefore, antioxidants could play an important role in their prevention.

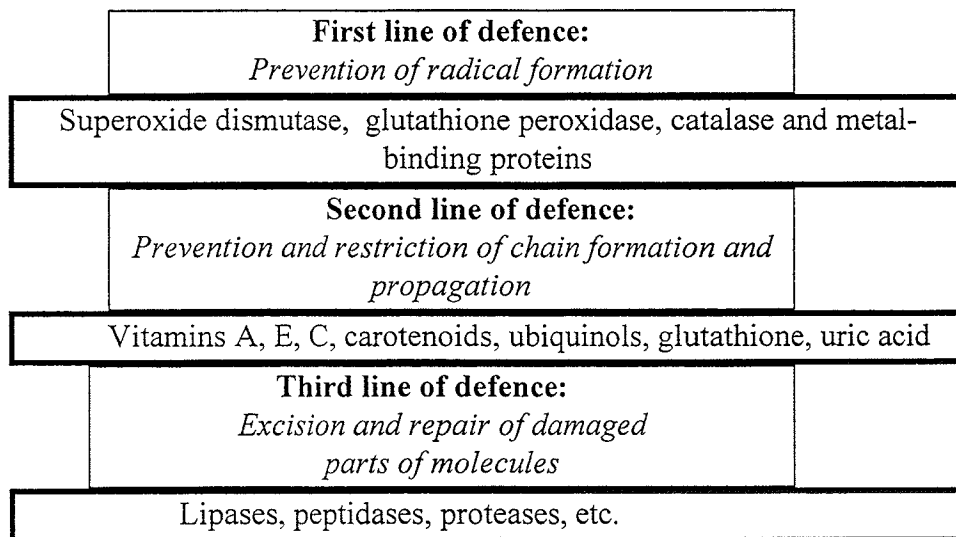


Figure 1. Three lines of defence in animal cells.

During evolution living organisms have developed specific antioxidant protective mechanisms to deal with the free radicals which are constantly produced in the cells. These mechanisms are described by the general term "antioxidant systems". They are diverse and are responsible for the protection of cells from the actions of free radicals. They include natural fat-soluble antioxidants (vitamins A, E, carotenoids and ubiquinones), water-soluble antioxidants (ascorbic acid, glutathione and uric acid) and antioxidant enzymes: glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) 1999; Figure1). The protective antioxidant compounds are located in organelles, subcellular compartments or the extracellular space enabling maximum cellular protection to occur. Therefore, all the antioxidants in the cell operate in association with each other forming integrated antioxidant systems. The cooperative interaction between antioxidants in the cell is vital for maximum protection from the deleterious effects of free radicals. It has been suggested that the antioxidant-prooxidant balance is a major determinant of successful chick embryo development and of early postnatal development (Surai, 1999).

This assumption is based on the following characteristics of the newly hatched chick.

(a) High levels of polyunsaturated fatty acids (PUFA)

The newly hatched chick is considered to be an intermediate stage between prenatal and postnatal development. In this respect avian embryo development is associated with the accumulation of highly polyunsaturated lipids within the tissues (Noble and Speake, 1997). Also, the rate of oxidative metabolism increases dramatically over the hatching period (Freeman and Vince, 1974). The hatching process itself can be considered as a high stress condition which the newly hatched chicken has to accommodate. Thus, effective antioxidant protection may be vital for posthatch viability and subsequent productive and reproductive performances. In such conditions, oxidative stress may be a problem during the last days of prenatal and the first days of postnatal chick life. These necessitate the development of effective antioxidant capacities in the tissues to prevent lipid peroxidation.

The tissues of the newly hatched chick show distinctive features in antioxidant profile and susceptibility to lipid peroxidation. The susceptibility of tissues to peroxidation depends on a number of factors including primarily the content of PUFA, levels of natural antioxidants (vitamins A, E, C and carotenoids), activities of antioxidant enzymes (SOD, GSH-Px and CAT), their cofactors (Se, Zn and Mn) and content and availability of prooxidant cations (Fe and Cu).

The brain clearly displays the greatest susceptibility to spontaneous and Fe-stimulated lipid peroxidation. High levels of lipid unsaturation and comparatively low antioxidant protection make the brain vulnerable to free radical attack. This is of particular importance in the chick in respect to the development of encephalomalacia which is associated with an antioxidant system compromised by a deficiency of vitamin E (Fuhrmann and Sallmann, 1995). In such conditions the cerebellum was shown to display particular oxidative stress. Packer and Landvik (1989) suggested that in biological systems vitamin E can be recycled from its oxidised form, ascorbic acid being one of the possible antioxidants involved in such a recycling (Packer, 1992). In the embryonic brain it has been suggested that antioxidant defence is afforded by high levels of ascorbic acid which is able to effectively recycle the low concentrations of vitamin E and maintain membrane protection against free radical attack (Surai *et al.*, 1996).

The liver is the main site of natural antioxidant accumulation and metabolism. Vitamin E and carotenoid accumulation in the liver reached a maximum at hatching (Surai *et al.*, 1996)

and is accompanied by high activities of GSH-Px and CAT (Table 1). Thus, the susceptibility of the liver lipids to peroxidation in the newly hatched chick immediately following hatching is low. The high levels of endogenous antioxidants within the liver can clearly serve as a major adaptive mechanism for the protection of the tissue during the oxidative stress experienced at hatching. Carotenoids can also be considered as a group of natural antioxidants of some importance for avian embryo development (Surai and Speake, 1998). Lutein and zeaxanthin are characterised by high antioxidant activity (Rice-Evans *et al.*, 1997) and are the main carotenoids accumulated in high concentration in the liver, but in much lower concentrations in other tissues. In general, carotenoid accumulation in the chicken embryo occurs in a similar manner to that of vitamin E, reaching a maximum level at hatching (Surai and Speake, 1998). Within the liver of the newly hatched chick it has been shown that the massive accumulation of lipids occurs through the formation of droplets within the cytosol (Noble and Cocchi, 1990) thus providing in turn a suitable intracellular milieu for the storage of large amounts of lipid-soluble vitamins.

Table 1. Antioxidant and fatty acid composition of tissues of a newly hatched chick (Adapted from Surai *et al.*, 1999b).

	Liver	Brain	Kidney	Heart	Lung	Muscle
Vitamin E, µg/g	678.2	6.6	19.5	24.3	21.0	14.8
Vitamin A, µg/g	10.3	0.1	1.3	0.4	-	0.1
Carotenoids, µg/g	30.8	-	2.3	3.3	2.7	2.0
Ascorbic acid, µg/g	151.0	839.1	130.6	59.0	124.6	57.9
Glutathione, µg/g	43.3	40.2	54.7	32.9	23.6	21.3
Mn-SOD, U/mg protein	3.8	3.7	3.0	5.8	0.1	1.1
Cu-Zn-SOD, U/mg protein	1.5	6.9	3.1	2.7	5.8	6.1
Se-GSH-Px, U/mg protein	177.0	29.8	159.8	99.0	99.8	45.8
Non-Se-GSH-Px, U/mg protein	114.6	6.2	58.6	11.6	53.0	12.6
Catalase, U/mg protein	35.8	1.9	29.5	5.8	6.0	3.2
18:2n-6, % ¹	12.3	1.5	11.8	9.8	8.5	15.0
20:4n-6, % ¹	21.5	9.5	19.9	21.8	10.1	15.3
22:6n-3, % ¹	10.1	16.2	6.3	3.3	3.3	8.1

¹ PUFAs in the phospholipid fraction.

The importance of the heart-vascular system for posthatch development determines the strategy of antioxidant defence. Data (Surai *et al.*, 1999b) has shown that the heart is characterised by moderate levels of both fat- and water-soluble antioxidants. Most significantly, the activity of Mn-SOD in the heart was much higher than that in the other tissues. With the considerable increase in the activity of the heart mitochondria associated with the hatching process leakage of electrons from the electron-transport chain has been suggested to be the main source of superoxide radicals in this tissue (Turrens, 1997). The SOD forms the first line of defence against free radical damage and, thus, the high activity of the mitochondrial SOD is possibly of vital importance to the heart at the critical period of hatching.

The requirement in antioxidant/prooxidant balance in the lung is exemplified by its involvement in the development of PHS in the chicken (Bottje and Wideman, 1995).

However, the lung also displayed a very high concentration of Fe and the enhanced susceptibility of the lung to spontaneous lipid peroxidation compared to other tissues may be associated with such high concentration of Fe. Nevertheless, it is clear that the lung possesses considerable protection against peroxidation through its content of Cu,Zn-SOD, Se-GSH-Px and Non-Se-GSH-Px.

As in the case of the brain the high levels of PUFAs in the muscle made the tissue vulnerable to lipid peroxidation, especially in the presence of catalytic amounts of Fe. With only low levels of fat-soluble antioxidants, comparatively low levels of Se, reduced glutathione and ascorbic acid, the muscle presumably relies upon its high levels of Cu,Zn-SOD for antioxidant protection. In the case of the chick embryo there is evidence that in conditions under which vitamin E concentrations are compromised, the levels of Cu,Zn-SOD are unable to afford adequate protection with the result that at hatching exudative diathesis with muscular degeneration can be observed (Hassan *et al.*, 1990).

A highly significant correlation between the Se level and the activity of Se-GSH-Px was found in the majority of the tissues (Surai *et al.*, 1999b). A carry over effect of Se from the maternal diet via the chicks has been shown (Hassan *et al.*, 1990; Surai, 2000) with an associated increase in the activity of GSH-Px and reduction in exudative diathesis. It has been suggested that the effect of Se on the activity of GSH-Px is achieved through pretranslational mechanisms including Se-GSH-Px gene expression and cytosolic mRNA stabilisation (Christinsen and Burgener, 1992).

Thus, tissue-specific distinctive features associated with the level of PUFA, antioxidant enzyme activity, natural antioxidant accumulation and the susceptibility to lipid peroxidation have been shown (Surai *et al.*, 1999b). In another investigation (Surai, 1999a) it was shown that different tissues of the chick embryo displayed distinct developmental strategies with regard to the acquisition of antioxidant capacity and it was suggested that during embryogenesis natural antioxidants (vitamins A, E, C and carotenoids) play a crucial role in antioxidant defence of the embryo tissues against lipid peroxidation. However, it may also be concluded that in postnatal development, when oxygen concentrations in the tissues are higher, metabolic activity and superoxide radical production are increased and tocopherol and carotenoid concentrations are decreased, the required protection is afforded through the major antioxidant enzymes SOD, GSH-Px and CAT. In fact, recent observations (Surai, 2000) indicate that GSH-Px activity in the chicken liver significantly increased between hatch and 10 days of postnatal development suggesting the different strategy in antioxidant system regulation. Indeed, during embryonic development, vitamin E played a key role in antioxidant defence, but after hatching its level in the liver decreased quickly (more than 10 times during the first 10 d) while simultaneously the activity of GSH-Px was increasing. It seems that the antioxidant systems of the growing chick rely more on GSH-Px as a major defence. Therefore, dietary provision of physiological levels of selenium is an essential step in maintaining antioxidant defence.

(b) Immune system development

In the newly hatched chick the immune system is immature and is not completely functional. This system is actively developing in postnatal life and this process involves accumulation of PUFAs and increased susceptibility to lipid peroxidation. Furthermore, immune cells use free radicals as an effective weapon to kill pathogens (Kettle and Winterbourn, 1997) and, as a result, the surrounding tissues could be damaged if antioxidant protection is not appropriate (Schwarz, 1996). In stress conditions the requirement for

antioxidant defence substantially increased. For example, under commercial conditions, chicks may be delayed access to feed for a considerable time after hatch and this increases the likelihood of ketosis and dehydration (Vieira and Moran, 1999). The delays between emergence and access to food and water resulted from the time spent in the hatchery and transportation to the farm and could be considered as substantial stress conditions. The asynchrony of chick emergence (eggs from older breeders and smaller eggs tend to hatch earlier) from the egg means that chicks hatching early may be held 36 h longer than those hatching late (Vieira and Moran, 1999). This could also result in dehydration and a shortage of available energy and lead to subsequent reduction in the rate of nutrient absorption and growth rate and increased early mortality. Immune system development is also compromised due to this stress (Wyatt *et al.*, 1986; Casteel *et al.*, 1994). Therefore, during these extreme stress conditions the antioxidant system could be the crucial factor in maintaining chicken health. It is necessary to underline the fact that any birds with restricted nutrition immediately after hatching are not able to recover completely and do not reach the same weight gain as those that are fed early (Vieira and Moran, 1999). In the absence of growth promoters in the chicken feed, the role of natural antioxidants in immune system modulation is difficult to overestimate.

(c) Digestive system development

The contents of the yolk sac are the main source of nutrients during the first 2-3 d after hatching and after this time the main energy source for the chick changes from yolk-based lipid to dietary carbohydrate (Vieira and Moran, 1999). The digestive and absorptive activities of the intestine actively develop during the first 2 weeks posthatch (Vieira and Moran, 1999) and this development is also associated with PUFA metabolism and possible lipid peroxidation. At the same time the ability to utilise carbohydrates is developing (Siddons, 1969) and by 4 d after hatch the ability to digest starch can reach 85% (Noy and Sklan, 1995). In contrast, pancreatic lipase activity increases until 16 d after hatching (Vieira and Moran, 1999). The intestine attains maximum growth between 3 and 7 d posthatch (Murakami *et al.*, 1992). In addition, early access to food stimulates the growth of the intestines and their absorptive capacity (Moran, 1985). On the other hand, a delay in access to food and absence of stimulation from food intake causes shortening and thinning of the villi (Michael and Hodges, 1973; Vieira and Moran, 1999) which results in decreasing absorptive efficiency of the intestine.

It is generally accepted that the ability to absorb dietary lipids is not well developed in the newly hatched chick but improves with age and it is recommended to avoid long chain saturated fatty acids and to use unsaturated fat during at least the first week posthatch (Vieira and Moran, 1999; Noy and Sklan, 1995). It is obvious that vitamin E is poorly assimilated from the diet during this period (at least during first 5 d posthatch) when it is extremely important as a natural antioxidant and as a result the chicken actively uses tocopherol reserves accumulated in the liver during embryonic development.

For example, during early postnatal life the level of tocopherols in the turkey liver have been observed to decrease sharply (Surai *et al.*, 1998; Soto-Salanova and Sell, 1995). Therefore the reserve of vitamin E is used during the first 2 weeks posthatch. During this period vitamin E concentration in the liver decreased by 10 times in chickens (Surai and Ionov, 1994), goslings and ducklings (Surai *et al.*, 1993) and more than 50 times in turkeys (Soto-Salanova *et al.*, 1993). On the other hand, during this period of development, tissues with incompletely developed antioxidant regulation required an effective protection against

lipid peroxidation. These data also confirmed the biological importance of a very high vitamin E concentration in the embryonic liver at hatching time.

A variety of approaches aimed at improving the vitamin E status of turkey poults has been investigated, including dietary supplementation of the poults with high levels of vitamin E (Applegate and Sell, 1996), bile salts (Soto-Salanova *et al.*, 1993) and fat (Soto-Salanova and Sell, 1995) as well as vitamin E injection (Soto-Salanova and Sell, 1996) and alterations in dietary fatty acid profile (Applegate and Sell, 1996). However, none of these attempts were able to reverse the process and only slowed down the vitamin E depletion. Nevertheless, observations with chickens indicate that increased vitamin E supplementation, as well as inclusion of organic selenium in the maternal diet, could substantially improve the situation (Surai, 2000). Therefore, maternal diet plays a crucial role in maintaining physiological levels of vitamin E in chicken tissues during the first 10 d of postnatal development.

(d) Antioxidant system regulation in embryonic and postnatal development of the chicken

Antioxidant transfer from the maternal diet to the egg yolk and further to the developing chick could be considered as a valuable means of regulating the antioxidant system of the newly hatched chick. Vitamin E transfer from the yolk to the developing tissues of the chick takes place during the last week of incubation, reaching a maximum in the newly hatched chick (Surai *et al.*, 1996). There also are species, specific differences in vitamin E and carotenoid transfer from the feed to the egg yolk and further to the developing embryonic tissues and chickens seem to have a better ability to accumulate these nutrients compared to turkeys, ducks or geese (Surai *et al.*, 1998). There is also discrimination between tocopherols and tocotrienols during embryonic development and alpha-tocopherol is the major vitamin E form in chicken tissues (Surai and Speake, 1998a).

Vitamin E transfer from the feed to the egg yolk is a fast process (Surai *et al.*, 1999) but there are no real reserves of vitamin E in the body of the laying hen and, therefore, with a single egg more vitamin E is released than the total amount present in the liver (Surai *et al.*, 1998a). Therefore, the vitamin E level in the diet is extremely important in maintaining physiological levels of this vitamin in the yolk. Increased vitamin E supplementation of the maternal diet significantly increased the vitamin E level in the egg yolk and embryonic tissues (Surai *et al.*, 1999a) and, as a result, the liver of newly hatched chicks became more resistant to lipid peroxidation. Similarly, carotenoid supplementation of the maternal diet was also associated with increased carotenoid accumulation in the egg yolk and the developing tissues (Surai and Speake, 1998). The carotenoid-enriched egg yolk, yolk sac membrane and liver of the newly hatched chick was characterised by a decreased susceptibility to lipid peroxidation.

Inclusion of organic selenium in the maternal diet is an effective means of increasing the selenium concentration in the egg yolk and egg white. A combination of organic selenium with increased vitamin E supplementation did not change selenium accumulation in the egg. Increased selenium level in the egg yolk and egg white resulted in an increased selenium concentration in the liver of the newly hatched chick. As a result the activity of GSH-Px significantly increased (Table 2).

Organic selenium had also a positive effect on vitamin E accumulation in the egg yolk and embryonic liver as well as increased accumulation of reduce glutathione in the liver. The increased antioxidant levels in the chick liver as a result of organic selenium supplementation translated into increased tissue resistance to lipid peroxidation. The most striking feature of the effect of selenium in the maternal diet on the antioxidant systems of the developing chick is that it was still obvious 5 d posthatch and, in the case of the combination of vitamin E and

selenium in the maternal diet, the beneficial effect was still seen at 10 d of postnatal development: vitamin E level increased and lipid peroxidation declined.

Table 2. Effect of organic selenium on the antioxidant status of the egg and liver of a newly hatched chick¹ (Adapted from Surai, 2000).

Parameter	Diet		
	Commercial (C)	C+ 0.2 mg/kg Se	C+ 0.4 mg/kg Se
Se in egg yolk, ng/g	298.3	605.3	854.0
Se in albumin, ng/g	50.7	193.7	403.7
Vit. E in egg yolk, µg/g	19.6	32.2	45.5
Vit. A in egg yolk, µg/g	6.3	6.0	6.1
Vit. E in liver, µg/g	119.6	144.2	166.1
Vit. E in plasma, µg/g	8.2	9.9	10.2
Vit. E in brain, µg/g	1.5	1.9	1.9
Glutathione in liver, µg/g	482.9	667.6	696.5
Se-GSH-Px, U/g fresh tissue	15.8	24.5	27.1
MDA in liver, µg/g	22.7	16.4	14.2

¹ The level of selenium and vitamin E in the commercial diet was 171 µg/kg and 10.1 mg/kg respectively. Selenium was supplemented in the form of Sel-Plex.

The important point is that effects of dietary antioxidants on the chicken are most pronounced when any stress conditions appear. Indeed, in ideal physiological conditions, when hatchability is higher than 85% and mortality for the first 10 d posthatch is negligible, there is not much scope to improve the situation. However, when stress conditions occur and free radical production exceeds antioxidant protection dietary antioxidant supplementation is especially helpful. This was shown in the case of ascites (Roch *et al.*, 2000) when antioxidant (vitamin E and selenium) supplementation substantially decreased mortality. Similar results were obtained when chickens experienced anaemia and organic selenium supplementation of the maternal diet significantly (more than 3 times) decreased mortality for the first two weeks posthatch (Lanning *et al.*, 2000). Another confirmation of the positive effect of antioxidants for poultry under stress conditions came from studies where very high vitamin E supplementation prevented the decline in egg production due to high temperature (Bollengier-Lee *et al.*, 1998, 1999).

In conclusion, information is actively accumulating to indicate that antioxidant systems are among the major regulators of many physiological processes, and antioxidant/prooxidant balance in the chicken body is responsible for maintaining their health, growth and future productive and reproductive performances. Clearly more work is needed to address this question.

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EFFECT OF ROUTE OF INFECTION AND VACCINATION STATUS ON DETECTION OF MAREK'S DISEASE VIRUS FROM BLOOD OR SPLEEN BY POLYMERASE CHAIN REACTION

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Summary

Polymerase chain reaction (PCR) has recently emerged as an additional tool for the diagnosis of Marek's disease (MD). The effect of route of infection and vaccination status on detection of Marek's disease virus (MDV) in blood or spleen of infected commercial broiler chickens by serotype-specific PCR was investigated. Commercial vaccinated and unvaccinated broilers were placed in isolators and challenged or not challenged with virulent MDV at day 3 of age either by intra-peritoneal injection or by inhalation of feather dust. On days 7, 14, 21, 28 and 35 post infection (PI), 50 birds were weighed, blood sampled and euthanased for various measurements including MDV serotype 1 (MDV-1) PCR of spleen tissue and peripheral blood lymphocytes (PBL). Spleen and PBL were found to provide similar sensitivity of detection of MDV with a slight advantage in favour of spleen. Infection of birds by inhalation of infective feather dust resulted in a slightly later but more complete appearance of MDV in challenged birds. Vaccination delayed the detection of MDV by approximately 2 weeks and it did not protect against the lower live weight observed at day 35 PI in infected birds.

I. INTRODUCTION

Diagnosis of clinical Marek's disease (MD) is based on gross and microscopic lesions plus isolation and identification of MD virus (MDV) in the infected tissues. Virus isolation is usually by virus propagation in cell culture and identification/quantification by cytopathic changes (plaque formation) or identification of infected cells by immuno-staining (De Laney *et al.*, 1998). Polymerase chain reaction has emerged in recent years as an additional diagnostic tool offering the advantages of serotype specificity (Davidson *et al.*, 1995; Walkden-Brown *et al.*, 2000) and the ability to differentiate between vaccinal and field strains of MDV serotype-1 (MDV-1) (Silva, 1992). It is a potentially useful tool for routine monitoring of vaccinated or unvaccinated commercial broilers for early exposure to MDV. However, before this potential is realised a number of issues need to be resolved. Firstly, the timing of appearance of MDV in peripheral blood lymphocytes (PBL) and other lymphoid organs after natural challenge is not known. Most experiments investigating this issue have used birds infected by the intra-peritoneal (IP) route rather than by the inhalation route (INHA) considered to be the natural route of infection (Venugopal and Payne, 1995). The extent to which the route of infection influences the appearance of the virus in various tissues is, therefore, of considerable interest. Secondly, the effect of vaccination on the appearance of virus in lymphoid tissues is of interest as most studies have been carried out with unvaccinated specific pathogen free birds. Thirdly, the tissue sampled is of practical importance as the separation of PBL from blood is expensive and tedious, and should be completed within 24 h of collection, whereas spleen may be stored at -20°C without further processing.

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This experiment was designed to determine:

- whether route of infection with MDV (IP or INHA) influences the timing of MDV detection in PBL and spleen.
- whether MDV will be detected earlier in spleen than PBL.
- whether MDV detection will be ablated or delayed by prior vaccination of birds with HVT.

II. MATERIALS AND METHODS

(a) Vaccine and challenge viruses

The vaccine used was cell-associated herpes virus of turkey (caHVT), strain FC-126 (The Marek's Company, Australia). The challenge virus was an Australian pathogenic MDV strain MPF-57 (Delaney *et al.*, 1998).

(b) Experimental design

The experiment utilised a 2x3 factorial design with two doses of vaccine (0 or 8000 pfu of caHVT) administered subcutaneously at hatch, and 3 types of challenge with virulent MDV (IP, INHA and no challenge). Female 1-d-old Cobb broiler chickens (320), derived from a parent flock vaccinated against MD with serotype 1 MDV were used in this experiment. Half of them (160) were vaccinated at 1-d-old. Equal numbers of vaccinated and unvaccinated chickens were placed in each of 10 positive pressure isolation units (n=32/isolator). Chickens in 4 isolators were challenged IP 3 d after vaccination with 50 pfu of MPF-57. Chickens in another 4 isolators were challenged via the respiratory route by circulating 12-15g of infective chicken dust collected from a previous challenge experiment using the same virus. Chickens in the remaining 2 isolators were not challenged (Control). Each week up to day 35 PI 50 chickens were removed (5 from each isolator) weighed, blood sampled (in K3EDTA tubes) and euthanased, followed by dissection to determine the weights of bursa and spleen.

(c) Sample preparation for polymerase chain reaction

Peripheral blood lymphocytes were separated from fresh blood by density gradient centrifugation with Ficoll-paque, washed with saline phosphate buffer, placed in M199 growth medium containing 10% DMSO and 20% calf serum and stored at -70°C until used. Thawed samples were digested using proteinase K and used as DNA template. Spleen was stored at -20°C. The DNA was extracted from spleen tissues using QIAamp DNA Mini Kit (QIAGEN Genomics Inc.) following the manufacturer's protocol.

(c) Polymerase chain reaction assay

Polymerase chain reaction amplification was carried out using the MDV-1 specific PCR described by Walkden-Brown *et al.* (2000).

(e) Statistical analysis

Live weight and organ weight data were analysed by ANOVA using Statview (SAS Institute Inc). Effects of route of infection and vaccination on PCR detection were analysed by ANOVA using the generalised linear model for binomial data of S-plus 2000 (Mathsoft Inc. Cambridge, MA, USA).

III. RESULTS AND DISCUSSION

(a) Detection by polymerase chain reaction of MDV-1

No MDV was identified from spleen or PBL of control chickens at day 35 PI. As these chickens were reared in two isolators located amongst the infected isolators, the absence of infection in these chickens confirms the efficacy of the isolators.

Detection from spleen versus PBL: Challenge virus was recovered from spleen earlier than from PBL throughout the experiment but the differences were not statistically significant in any given week (Figure 1). However, by pooling data from all birds the difference in isolations from spleen (85/200) and PBL (67/198) approached significance ($P < 0.08$). For field diagnosis of MD, spleen might be the preferred sample because of this advantage in sensitivity and also because collection and storage of spleen is easier. Analysis of variance of PCR data from both spleen and PBL of challenged birds produced almost identical results. In both cases there was no main effect of route of infection ($P > 0.7$), but significant effects of vaccination status ($P < 0.001$) and day PI ($P < 0.001$), and a significant interaction between the route of infection and day PI ($P = 0.05$) (Figure 1).

Effect of vaccination: Vaccination reduced the overall proportion of MDV positives in challenged chickens (60/100 in unvaccinated vs 25/100 in vaccinated chickens, $P < 0.001$) and also delayed the time of detection of virus in both spleen and PBL. The percentage of MDV positives exceeded 50% by day 21 PI in unvaccinated birds, but not until day 35 PI in vaccinated birds (Figure 1). Although the protective mechanism of MDV vaccines is not clear, suppression of MDV replication in tissues by vaccine has been reported (Lee *et al.*, 1999) and is consistent with our findings. These data raise the prospect of identifying MDV-1 infection in vaccinated flocks. However, some issues remain to be resolved. Whether all of the vaccinated challenged chickens would eventually show the presence of MDV or to what extent MDV-positive chickens will exhibit clinical disease are not known and might be fruitful areas for future work.

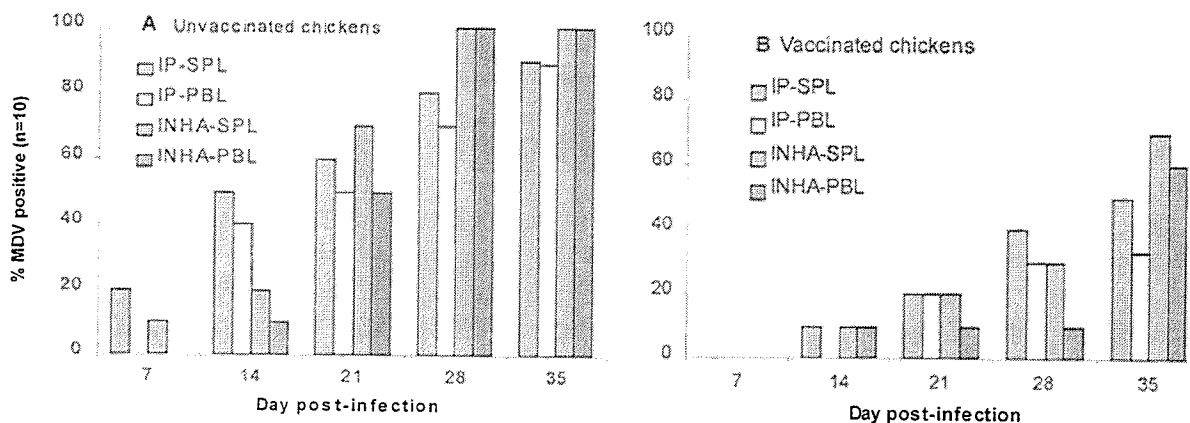


Figure 1. MDV positive chickens in unvaccinated (A) and vaccinated chickens (B) challenged by either intra-peritoneal (IP) injection or inhalation (INHA) at 3 days of age. Diagnosis was by serotype-specific PCR of spleen (SPL) tissues or peripheral blood lymphocytes (PBL).

Effect of route of infection: While the main effect of route of infection was not significant there was a significant interaction between route of infection and day post challenge ($P = 0.05$). This interaction was manifested in higher levels of detection of MDV in birds infected IP up to day 14 PI, but higher levels in birds infected INHA after that time (Figure 1A). Furthermore, MDV detection level reached 100% in unvaccinated birds infected by INHA

from day 28 onwards, whereas in birds infected IP, this level of detection was not achieved, suggesting a higher rate of failure of infection in the latter. These data suggest that while experimental IP challenge with MDV broadly mimics natural challenge through the respiratory route, there are small differences in the timing and magnitude of the response.

(b) Live weight

Live weights (LW) of chickens did not differ between treatments up to day 28 PI. At day 35 PI, LW of challenged birds (1689 ± 57 g) was significantly lower than control (1909 ± 27 g) birds ($P < 0.01$). Vaccination had no effect on LW in the challenged chickens ($P > 0.67$) and did not protect against reduced weight gain at day 35 (1704 ± 44 g vs 1674 ± 56 g for unvaccinated and vaccinated birds, respectively). The cause of reduced growth in infected birds is unknown but may represent a combination of reduced feed intake and/or reduced efficiency of conversion. The failure of HVT vaccine to protect against reduced growth late in the experiment is worthy of note and suggests that vaccination may not be enough to protect against significant production loss with broilers due to subclinical MDV infection with very virulent strains.

(c) Lymphoid organ weights

Bursa and spleen weights were expressed relative to body weight. There was no effect of challenge, route of infection or vaccination status on relative bursal weights at any stage of the experiment. However, spleen weight was affected by both vaccination and challenge but these effects were influenced by day PI. Splenic mass was higher in vaccinated than unvaccinated chickens at day 10 post-vaccination (day 7 PI) irrespective of whether the chickens were challenged or not ($P < 0.001$). This is consistent with the elevated spleen weight at day 10 after vaccination with HVT observed in an earlier experiment (Islam *et al.*, unpublished data). At day 28 PI, spleen weight in challenged chickens was higher than in control chickens ($P < 0.05$) and it was also higher in unvaccinated than vaccinated chickens ($P < 0.05$). Splenic enlargement from day 14 PI is typical for MDV infection. These data suggest that vaccination may reduce this consequence of MDV infection.

IV. CONCLUSIONS

Regarding the first objective, this experiment demonstrated that, in general, the pattern and timing of MDV detection from spleen and PBL were similar following infection by the intra-peritoneal or respiratory route, although the former appears to produce slightly earlier detection and the latter a slightly more complete infection rate. Regarding the second objective, the data indicate that MDV detection in spleen and PBL was similar but there was a consistent trend towards earlier detection from spleen. Regarding the third objective, it was found that vaccination with HVT delayed the detection of MDV following challenge by approximately 2 weeks. Vaccination did not protect infected chickens from the reduced live weight observed at day 35. Overall, these findings extend the potential usefulness of PCR as a diagnostic aid in MD by demonstrating its ability to detect challenge MDV in different tissues in both vaccinated and unvaccinated commercial broilers. They also confirm that virulent Australian strains of MDV are able to induce infection and production loss in birds vaccinated with HVT.

V. ACKNOWLEDGEMENTS

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EPIDEMIOLOGICAL STUDIES OF *CAMPYLOBACTER* COLONISATION OF BROILER FLOCKS IN SOUTH EAST QUEENSLAND

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Summary

Detailed epidemiological studies on selected broiler farms were conducted in an attempt to elucidate mechanisms of transmission of *Campylobacter* spp. in poultry. *Campylobacter* colonisation of flocks was not detected prior to 28 d of age. Results to date indicate that drinking water and darkling beetles were not the primary source of introduction to the flock but did play an important role in horizontal transmission. Transport crates used at partial depopulation can introduce *Campylobacter* spp. into a flock. However, with stringent biosecurity on flocks housed in modern tunnel sheds, *C. jejuni* colonisation can be prevented to final slaughter at 47 d of age.

I. INTRODUCTION

Campylobacter enteritis is the most common notifiable infection in humans in Australia. The causative bacteria, *C. jejuni* and *C. coli*, cause a spectrum of illness in which diarrhoea is the most common clinical sign. Since *Campylobacter* spp. live as commensals in the intestinal tract of a wide range of birds and mammals there are a number of pathways of human infection. The vast majority of *Campylobacter* cases occur as sporadic infections rather than as large outbreaks. Handling or consumption of raw or undercooked poultry is considered to be the most important risk factor and errors in food handling are also often involved in sporadic cases. The infectious dose in humans is 500-800 bacterial cells.

The optimum growth temperature for *C. jejuni* and *C. coli* is around 42°C. The primary site of colonisation in the chicken is the lower gastrointestinal tract, especially the caeca. During evisceration, campylobacters spill over onto the carcass, and further cross-contamination occurs during the spin-chilling process. Prevalence at the retail level can be very high with overseas studies recording up to 98% of samples positive (Jacobs-Reitsma, 2000).

The low infectious dose means that reducing the number of flocks that carry *Campylobacter* spp. will have a greater impact on public health than reducing the number of these bacteria on carcasses. It is generally accepted that control strategies should, therefore, be applied at the farm level to prevent colonisation of flocks.

II. METHODS

In order to study the source of *Campylobacter* and also aspects of transmission, a longitudinal study design was adopted. Sampling began at placement and continued at frequent intervals to determine at what age colonisation was first detected and how rapidly the organism spread within the flock. Systematic random sampling was used during faecal sample collection. Faecal samples were plated directly onto Karmali agar and incubated at

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42°C in an atmosphere of 5% O₂, 10% CO₂ and 85% N₂. Sample size (100 faecal samples per shed) was sufficient to detect a single positive sample with 95% confidence when at least 3% of the birds were colonised. Samples of drinkers, litter and darkling beetles and larvae were enriched in Preston broth overnight and then plated onto Karmali agar.

Molecular methods were used to elucidate the epidemiology of *Campylobacter* in broiler flocks. The DNA typing technique adopted was the *flaA* PCR (Nachamkin *et al.* 1993), which is based on one of the genes encoding the protein in the bacterial flagellum. The method involves PCR amplification of the *flaA* gene followed by restriction enzyme digestion of the amplified product to generate a series of DNA fragments of different sizes. These fragments are sorted according to size on an agarose gel, and the resulting pattern characterises a particular isolate (see Figure 1).

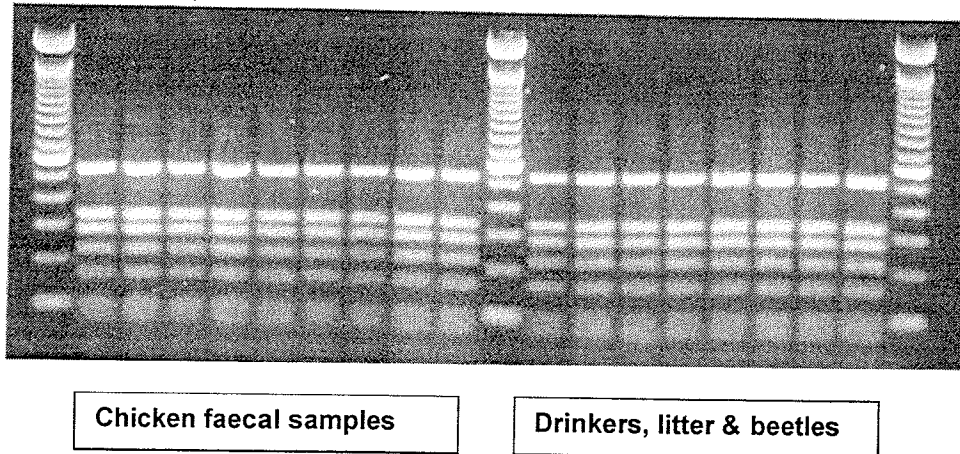


Figure 1 A single *flaA* type present in different samples in one shed

III. RESULTS

All flocks tested to date have been *Campylobacter*-free to 28 d of age. When *Campylobacter* spp. were detected before partial depopulation, colonisation occurred in a window between 28 and 35 d of age, irrespective of clean-out procedures, breed of bird, or age of donor flock. Once the first positive sample was detected the flock became 100% positive within 4 to 6 d. Table 1 gives an example of how rapidly the organism spreads within a flock. Transmission within the flock can be directly via the faecal-oral route or via contaminated drinkers.

Table 1 *Campylobacter* isolation results for one shed on a study farm

Sample	Day 0-28	Day 31	Day 34	Day 37	Day 42	Day 49
Faeces	negative	2/100 +ve	85/100 +ve	100/100 +ve	30/30 +ve	29/30 +ve
Drinkers	negative	negative	10/12 +ve	10/12 +ve	12/12 +ve	12/12 +ve
Litter	negative	negative	1/3 +ve	1/3 +ve	negative	negative
Beetles/larvae	negative	negative	2/4 +ve	negative	1/6 +ve	2/8 +ve

(a) Drinking water

As shown in Table 1, at Day 31, 2/100 faecal samples were positive while all other samples including drinkers were negative. *Campylobacter* spp. were not detected in drinkers

prior to the chickens becoming positive, suggesting that drinkers were not the source of introduction to the flock.

(b) Darkling beetles and larvae

Litter beetles found at placement were invariably *Campylobacter*-negative. Furthermore, *C. jejuni* was only detected in the beetles or larvae after the chickens became colonised. On the basis of results obtained to date, litter beetles did not serve as a reservoir of *Campylobacter* as they do not maintain the organism between cycles.

(c) Litter/shed environment

To date, *C. jejuni* has not been isolated from litter prior to colonisation of the chickens. On one of the farms studied, litter was re-used from one batch to the next. In one of the first sheds colonised in the second production cycle on this farm, the *flaA* type observed was the same type as was present in that shed in the previous batch. This could suggest carryover in the litter or within the shed environment in this shed. The results could also be interpreted to illustrate the existence of a common reservoir that infected this shed in both cycles. A new, different *flaA* type appeared simultaneously in one of the other study sheds which makes interpretation more complex. Future work will investigate whether wet floors might be involved in this situation.

(d) Farm animals

Campylobacter isolation was attempted from 70 separate samples of cattle faeces from one poultry farm over three production cycles. From these samples, one *C. jejuni* isolate was obtained from each of two consecutive production cycles. Typing of these two isolates demonstrated that they were identical to each other. However, this was a different *flaA* type from any of the types seen in the chickens on the farm in any of the three cycles. In this particular instance, there was no evidence of movement of *C. jejuni* from cattle to poultry, or vice versa.

(e) Vertical transmission

In an experiment to examine whether or not vertical transmission was occurring, 1-d-old chicks from a 56-week old parent breeder flock were placed on two different farms. The breeder flock was unusual in that it was colonised with only one *flaA* type. On one farm the progeny broilers remained negative throughout the sampling period until final slaughter. On the other farm, the flock was fully colonised by 35 d of age with a distinctly different *flaA* type from that found in the breeder flock. These results indicate that vertical transmission did not occur in this experiment.

(f) Crates

On one study farm, *flaA* typing has demonstrated that unwashed transport crates used at partial depopulation introduced *Campylobacter* spp. into a flock.

(g) Risk reducing measures

The longitudinal studies conducted to date have included one farm with very high standards of biosecurity. The farm has modern tunnel sheds with stabilised concrete floors. Each shed has an entrance room where outside shoes are changed for shed-dedicated footwear. Birds in shed 3, the shed initially selected for detailed study, were negative throughout the growing period, but were slaughtered early at 38 d of age. Detailed monitoring (100 faecal samples per visit) was continued in Shed 1. This shed was thinned at 38 and 42 d of age. All samples were negative till final slaughter at 47 d of age. The results suggest that with stringent biosecurity, *Campylobacter* spp. can be kept out of poultry flocks.

IV. DISCUSSION

At the commencement of this study, there was little published information on *Campylobacter* spp. in Australian broiler flocks, apart from two small-scale surveys in the early 1980s (Smeltzer, 1981; Shanker *et al.*, 1982). The results reported above are by no means complete, but are starting to contribute to a local picture that may be used in comparisons with overseas studies. For example, the studies to date have indicated that drinking water is unlikely to be responsible for introduction of *Campylobacter* to poultry sheds. However, one study in the UK clearly identified drinking water as the source (Pearson *et al.*, 1993) despite the fact that the organism could never be cultured. Furthermore, non-disinfected surface water was shown to be strongly associated with *Campylobacter* colonisation in Norway (Kapperud *et al.*, 1993).

Despite two decades of concentrated research overseas the sources of infection of poultry flocks are still debatable (Newell and Wagenaar, 2000). Most of the evidence points to horizontal transmission from the environment and the primary strategy employed overseas has been to enhance biosecurity to prevent the entry of the organism into the broiler house. However, it is important to be able to target biosecurity measures to the demonstrated source or sources. Over the next twelve months further studies will be conducted to clarify the source or sources of the organism and to determine the most appropriate on-farm control measures.

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INFLUENCE OF TIAMULIN AND ZINC BACITRACIN ON AVIAN INTESTINAL SPIROCHAETE INFECTION

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Summary

Infection with the intestinal spirochaete *Brachyspira (Serpulina) intermedia* has been shown to cause increased faecal water content and reduced production in layers. This study aimed to examine the efficacy of the antibiotic tiamulin in controlling infection. The possible role of the antibiotic growth promotant, zinc bacitracin, was also examined. While tiamulin was initially effective in reducing infection, reinfection occurred relatively quickly following the cessation of medication. Evidence was obtained that zinc bacitracin may suppress the growth of intestinal spirochaetes when fed a ration containing this antibiotic.

I. INTRODUCTION

Intestinal spirochaetes of the genus *Brachyspira (Serpulina)* are anaerobic, spiral-shaped bacteria that colonise the large intestine and can cause enteric disease in a range of animal species (Hampson and Stanton, 1997). Colonisation with intestinal spirochaetes has been recognised as a reason for previously unexplained production losses and/or diarrhoea in layers and meat breeders in Europe, the United States and Western Australia (Davelaar *et al.*, 1986; Griffiths *et al.*, 1987; Dwars *et al.*, 1989; Swayne *et al.*, 1992, 1995; Trampel *et al.*, 1994; McLaren *et al.*, 1996).

In a recent study in Western Australia (WA), intestinal spirochaetes were isolated from birds in 64% of flocks with signs of intestinal disease, compared with 28% of clinically normal flocks (McLaren *et al.*, 1996). Of the WA isolates identified, 41% were identified as *B. intermedia*, 56.4% as belonging to a previously undescribed and unnamed group of uncertain pathogenicity (provisionally designated as "*Brachyspira pulli*"), and the rest as *Brachyspira innocens*, a non-pathogenic species known to colonise pigs (McLaren *et al.*, 1997). No isolates of *B. pilosicoli* or *B. alvinipulli* were recovered in WA. An isolate of *B. intermedia* from a WA layer was subsequently used to experimentally infect layer hens, causing increased faecal water content and reduced egg production and thus confirming its pathogenic potential (Hampson and McLaren, 1999).

In 1998 the present authors conducted a survey in which faecal samples from 69 meat breeder, layer or meat chicken flocks in the eastern states of Australia were cultured for intestinal spirochaetes. Overall, birds in 42.9% of meat breeder and 68.2% of layer flocks were colonised with spirochaetes but no birds in meat chicken flocks were infected. There was a significant association between colonisation with spirochaetes and the occurrence of wet litter and/or reduced production (Stephens and Hampson, 1999). A subset of 57 spirochaete isolates from birds in 16 flocks were identified to the species level. Isolates from nine (56%) of these flocks were spirochaetes that are known to be pathogens of poultry: *Brachyspira pilosicoli* was isolated from birds from five flocks, birds from two flocks were infected with *B. intermedia* and in two other flocks both species were identified. Isolates

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from the other seven flocks belonged to other *Brachyspira* species of unknown pathogenicity.

A recent trial sought to evaluate the efficacy of antimicrobial control of intestinal spirochaete infection. This paper outlines some preliminary results of this trial.

II. METHODS

Fifty 18-week old layer hens were obtained from a commercial producer. The birds were placed in an airconditioned facility in individual cages with mesh floors. They were fed a commercial, vegetable-based layer diet *ad libitum* and had constant access to water. Individual faecal samples from each bird were cultured once a week for spirochaetes throughout the trial.

Thirty birds were orally inoculated with two mL of an actively growing culture of *Brachyspira (Serpulina) intermedia*, strain HB60, for 3 d per week for a period of three weeks. The broth contained approximately 10^8 bacterial cells per mL. The remaining 20 birds were placed in another room as control birds and were not inoculated. Ten weeks later, ten infected birds were orally dosed by crop tube with tiamulin in water at a rate of 25 mg per kg bodyweight per d for 5 d.

Initially the diet fed to the birds contained zinc bacitracin at 100 ppm. This was removed after nine weeks, all birds then being given the same diet free of bacitracin. After four weeks, bacitracin was re-introduced into the diet of half (10) of the control birds and half (10) of the infected but untreated birds.

Individual faecal samples were collected from each bird weekly, cultured for spirochaetes and the percentage faecal moisture determined. Egg production and egg weight of each bird were recorded daily and accumulated to provide weekly egg mass output.

III. RESULTS

(a) Faecal moisture and culture

There was no statistically significant difference in the faecal moisture content of the birds in each of the groups. All faecal cultures carried out prior to inoculation of the birds were negative and all faecal cultures of the twenty control birds were negative throughout the trial. In the first six weeks following inoculation, up to five of the 30 inoculated birds were positive on culture. In the week following removal of the zinc bacitracin, as shown in Figure 1 below, this figure rose to 18 positive birds.

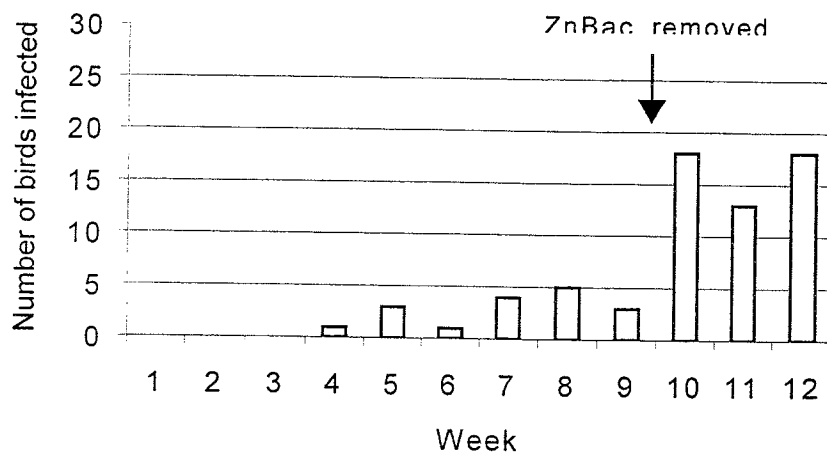


Figure 1. Level of infection before and after removal of zinc bacitracin from the diet.

One week after treatment with tiamulin all ten birds were negative when cultured for spirochaetes. This figure rose slowly over the next month, until seven out of the ten treated birds were positive. At postmortem one week later, that is, six weeks following treatment, nine out of ten birds were positive. In contrast, as shown in Figure 2, in the infected but untreated group between five and seven of the birds remained positive throughout.

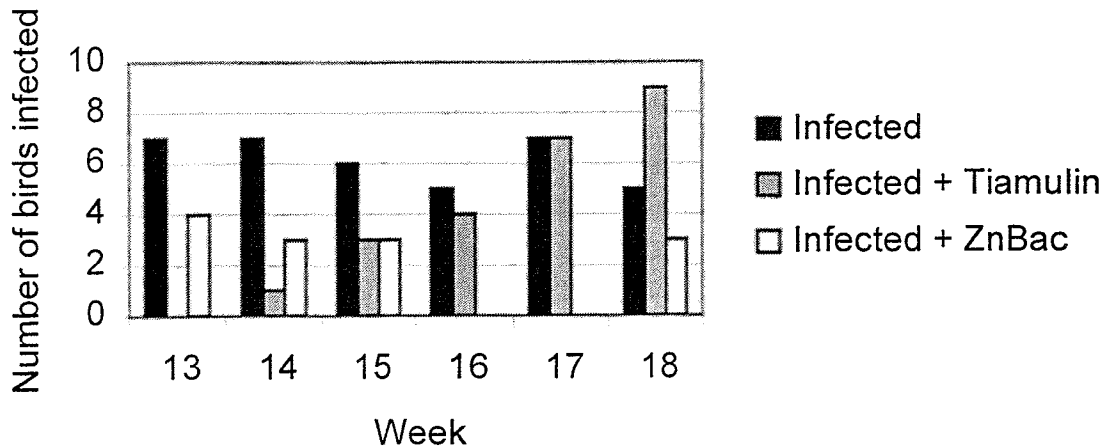


Figure 2. Influence of tiamulin and zinc bacitracin on infection level.

Of the ten infected birds put back onto the diet containing zinc bacitracin, four birds were positive the following week, with this figure dropping slightly to three birds for the following two weeks. Four weeks following reintroduction of the zinc bacitracin, no birds were culture positive in this group, although three birds were found to be positive on postmortem.

(b) Egg production

Egg mass output was calculated by multiplying the number of eggs produced by the mean weight of the eggs in g. This was calculated daily for each bird and accumulated to provide weekly figures for each group.

Egg output from the control birds was significantly better than that from the infected birds. For example, in week 15, total output by the control group was 423.2 g whereas that of the infected group was 340.3 g ($P < 0.0182$). Treatment with tiamulin significantly improved egg output in infected birds. In week 17, egg output in the treated group was 466.5 g, compared with 400.2 g in the control group and 364.7 g in the infected group.

IV. CONCLUSIONS

This trial has only just been completed and the results are yet to be fully analysed. However, the results confirm the potential that infection with the intestinal spirochaete *B. intermedia* has to depress egg output. Treatment with the antimicrobial tiamulin appeared to be effective in removing the bacteria for a limited time but reinfection occurred. This is not altogether surprising given that the birds were housed in the same room as other infected, but untreated, birds. It would be useful in future studies to determine whether treatment of all birds in a room, together with appropriate disinfection of the environment, would completely eradicate the infection. The fact that the birds were reinfected suggests that infection does not

stimulate a strong protective immunity. Infection levels post treatment were actually higher than those prior to treatment, possibly due to the removal of competing intestinal bacteria.

The results of the trial showed that zinc bacitracin at an inclusion rate in feed of 100 mg/kg suppressed the growth of *B. intermedia* over an extended period following experimental infection. Subsequent removal of the zinc bacitracin allowed proliferation of the spirochaetes whilst, when reinstated in the diet, it once again reduced growth of the organisms. These preliminary results suggest that removal of zinc bacitracin as a growth promotant may result in increased problems associated with intestinal spirochaete infections in the chicken industries.

V. ACKNOWLEDGEMENTS

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THE AUSTRALIAN OSTRICH INDUSTRY: CRITICAL ISSUES

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Summary

The ostrich industry in Australia has undergone significant change from rapid expansion to a commercial phase with a huge reduction in producer numbers, especially small producers. Poor reproduction, hatchability and chick survival have had a severe negative impact. Feed costs are normally 50% of growing costs and there is a need for these to be reduced. The digestive system of the ostrich is unique with significant fermentation occurring in the hindgut. The initial aim was to produce ostriches for meat. However, export markets are highly competitive and the local costs of red meat are so low that ostrich meat cannot compete. Focus has changed to leather and currently hides are being sent to South Africa to be tanned. A good income can result but this depends on producing high-quality leather, which is difficult. Cost estimates of producing birds, by either purchasing chicks or having a breeding flock, are made. In both cases profitability, if it is to occur, not only relies on product price but on the number of birds farmed.

I. INTRODUCTION

The ostrich industry in Australia has undergone a series of crises such that producer numbers have diminished greatly. Many made fortunes during the expansion phase of the industry and many more went broke subsequently.

Ostrich meat in Australia is expensive compared to meat from conventional livestock and a viable export market has not yet been established. Although ostrich meat is reputed to be low in saturated fat, cholesterol and salt, these characteristics no longer attract much interest in a world faced with a vast amount of health information and health products on the market. There is now a change of emphasis from ostrich meat to leather with Japan seen as a key market outlet.

In Zimbabwe and South Africa, where there are established ostrich industries, leather commands much more of the income (70%) than meat and feathers. The change in focus from meat to leather means that feeding and management strategies will also change, and growth rate will not be the major target. Instead of ostriches reaching a slaughter liveweight of 95 kg in nine months, they will take at least 12 months to reach this weight and will probably be killed at about 100 kg. The emphasis will be, therefore, on low-cost diets and on managing ostriches extensively and on pasture. There is not much good information on dietary needs of ostriches for meat and considerably less for leather production, but feed costs are about 55% of total costs for producing a meat bird to slaughter.

II. OSTRICH CHICKS

The most critical stages of ostrich production and reproduction are incubation and chick rearing. It is very difficult to obtain reliable data on either of these. In an intensive system, in which a hen will lay 44 eggs/year, only about 40% of these eggs will hatch and the chicks survive to 10 weeks of age (Cooper, 2000). In Australia, a survey was undertaken in early 1997 of producers in Queensland and Northern New South Wales (Farrell *et al.*, 2000a).

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From that information, it is unlikely that farmers achieve anything like 17-19 chicks per hen each year. In part, this is due to inferior genotypes. Until now little attempt has been made to practise selection for favourable traits, such as egg production, fertility and growth rate. Thirty eight percent of producers surveyed had a hatchability of less than 40% of eggs set.

The rearing of ostrich chicks to about 12 weeks of age requires excellent management skills. Disease is a major problem and dietary requirements are not well established, and are often taken from those for poultry. These specifications are normally generous, resulting in expensive diets. However, relative to lifetime production costs, for this phase of growth the feed component is comparatively small. In Zimbabwe Cooper (2000) estimated total costs of rearing a chick to 13 weeks of age to be US\$36 (A\$61). An optimistic loss of only 15% from hatch to 13 weeks was used by Cooper.

III. DIGESTION AND NUTRITION

The ostrich is unique as an avian herbivore with a prodigious hindgut. Each of the two caeca are about 130 cm long in a 45 kg bird, and the length of the colon is 760 cm (Swart *et al.*, 1993b). In these studies Swart *et al.* (1993a) found that young ostriches (5-10 kg) were able to digest the fibre component (neutral detergent fibre) of the diet to a greater extent than older (42-50 kg) birds (0.52 vs 0.46). *In vitro* fermentation studies by this group indicated that energy from the volatile fatty acids (VFA), the end products of hindgut microbial fermentation, could contribute 76% to daily apparent metabolisable energy (AME) needs (Swart *et al.*, 1993b). However, the efficiency of utilisation of AME from a diet containing 50% lucerne meal was only 0.32 (Swart *et al.*, 1993c). When emus, cockerels and young ostriches were fed four diets containing different sources of fibrous feeds, dry matter digestibility and AME were consistently and significantly higher in the ostriches but values for emus and cockerels were the same (Farrell *et al.*, 2000a). Studies by Angel (1993) showed that the AME of a diet when fed to chickens was 8.3 MJ/kg; it yielded 7.2 MJ AME in ostriches at three weeks of age but this increased to 11.4 MJ at 17 weeks and remained constant thereafter. It is not surprising to find in the literature generally substantially higher AME values for most feedstuffs when fed to ostriches compared to poultry, particularly those high in fibre (Cilliers *et al.*, 1994; Cilliers *et al.*, 1997). The dilemma is whether ostriches should be fed in a similar fashion to horses or chickens. The problem is that sources of roughage (fibre) are expensive in Australia, particularly lucerne meal. It is uncertain whether fibre is a dietetic requirement for gut function, or a dietary requirement, or neither. If the latter, this suggests that although lucerne and other fibrous sources may yield twice as much AME in ostriches as in poultry, the net availability of that energy is so low that these feeds become much more expensive than grains when expressed as \$/MJ net energy and should be used sparingly.

Grazing can provide a significant contribution to daily feed needs. The area required depends on the quality of the pasture. Van Niekerk (1995) reported that 5 to 10 hectares of unimproved pasture are needed to support one mature bird. He also pointed out that intensively-grazed, high quality, pasture can be stocked at 6.5 birds/hectares but much of the forage may be trampled and wasted. He suggested zero grazing in these circumstances, with the mechanically-harvested forage fed to ostriches in a feedlot situation. Very preliminary studies suggest that high-quality pasture may contribute up to 25% of the food needs of fast-growing meat birds (Farrell *et al.*, 2000b). If leather production is the target then pasture contribution may be much higher. The difficulty is to maintain a constant supply of good quality pasture. If ostriches are being grown mainly for meat they should be fed like chickens but if leather is the main production perhaps they should be fed more like a horse.

The production of leather as opposed to meat requires two different husbandry and feeding strategies. In the latter case the birds are grown rapidly. On high-quality diets it has been demonstrated that ostriches gain almost 0.5 kg/day between 24 and 65 kg liveweight (Farrell *et al.*, 2000b). A major constraint to the economical production of ostriches for meat is that the current final slaughter weight (90-100 kg) is close to their mature liveweight (115-125 kg). Consequently, feed conversion ratio increases from about 2:1 at 20 kg to 11:1 at 95 kg even when birds are fed a high-quality turkey starter diet (Degen *et al.*, 1991). Rapid growth may give a carcass that has excess fat resulting in a low yield of favoured meat cuts.

The nutrient requirements of growing ostriches are uncertain. Du Preez (1991) has estimated requirements for energy, protein and amino acids on the basis of egg and body composition, rate of gain and maintenance energy costs. Requirements for vitamins and minerals are usually those for growing chickens and breeder hens. However, Van Niekerk (1997) has identified vitamin E as being critical for growth along with additional amounts of copper and selenium. Since male and female breeding birds are given the same diet, it is sometimes recommended that calcium be omitted from the diet and given free choice.

On the basis of the research by Swart (1988) on the low efficiency of utilisation of the VFA from dietary fibre sources, the worth of these fibrous feeds will be much lower than indicated by their AME. Cereal grains are superior in terms of cost per MJ of AME. However, for leather production, when ostriches may be grown slowly, there is opportunity to reduce nutrient needs thereby allowing formulation of low-cost diets. The concept of "no frills feeding" (Farrell *et al.*, 2000b) is an area that has not been well researched and requires urgent attention. This is based on the feeding of whole grain with appropriate supplements.

IV. PROFITABILITY

In August 2000, Harrierville Trading Pty Ltd (Atkins, 2000) anticipated net returns on an average grade 2 hide of A\$319. This was based on hides varying from US\$21 (grade 1) to US\$9 (grade 5) per square foot. Fillet steak into the European Union was fetching A\$7.50/kg. The recent decline in the Australian dollar will likely increase these amounts.

Tuckwell (1997) presented an analysis of costs of rearing an ostrich to slaughter weight in Australia to be \$366 assuming a realistic annual production of 10 yearlings per breeding hen. If yearling numbers reach 15 production costs would be reduced to \$335; of this 40% was required for feed. Tuckwell (1997) estimated income per bird from meat to be \$135 assuming a wholesale price of A\$10.90/kg. Leather returns of A\$303 gave an estimated farm gate value of a 95kg yearling of A\$438, or a profit of over A\$100/bird. These figures have changed significantly since Tuckwell (1997) made these calculations.

Tuckwell (1999) calculated gross margins for two production systems. The estimate for a producer purchasing 0-2 month old chicks showed profitability to be related to two critical factors. One was the purchase price; as it decreased profitability increased. The second was flock size. A profit was not realised until over 1000 chicks were purchased at A\$45/chick. The enterprise gross margin increased from A\$14,729 for a flock of 2000 birds, to \$76,573 for a flock of 5000 birds when chicks were purchased at A\$45 each. These calculations included a generous survival rate of 86%. Net carcass weight was assigned A\$6/kg, again an optimistic valuation. However, a hide value of only A\$14.33/sq foot was used compared to A\$20.60 recently forecast by Atkins. Currently, fillet steak is fetching from A\$5 to 7.50/kg with one company exporting to Europe meat from 400 birds/week with an immediate expected increase to 1200 (De Groot International, pers. Comm., 2000).

Using the same costs and income, Tuckwell (1999) calculated a gross margin for an established ostrich breeding flock of 100 hens with 50 cocks. His fertility, hatchability and survival rate gave an optimistic 16 growers slaughtered per hen each year. This is well above

the industry norm and a realistic figure of 12 would be more acceptable. When total income from the 1584 growers was deducted from expenses, gross margin was A\$12,425.

V. CONCLUSIONS

The future of the Australian ostrich industry will rely on establishing reliable markets, particularly for leather. Recently, birds have been sent to South Africa to be tanned although there is the possibility of facilities being established in Australia. However, it is difficult for producers to meet the stringent requirements for grades 1 and 2 hides. The sales of ostrich meat overseas are still unreliable and the price volatile. Opportunity of obtaining a reasonable price for ostrich meat in Australia is bleak as long as the price of traditional meats remains low. Fundamental to the future of the industry may be farming birds in an extensive system, growing the meat birds at a slower rate with lower feed costs, and hens with improved reproductive performance. One thing seems certain, there is little room for the faint hearted and, at this stage, little room for the smaller producer.

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PREDICTION OF FEED INTAKE IN MODERN BROILERS

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Summary

The influence of varying dietary nutrient concentrations on feed intake was examined in male broiler chickens from 1 to 38 d of age. Individual body weights and pen feed intakes were recorded daily. A stepwise regression analysis was carried out and the following equation was derived for the prediction of feed intake in Ross male broiler chickens.

$$FI = 50.25 - 0.159 LW + 1.715 LW^{0.75} + 0.15 ADG - 3.37 AME + 1.67 LYS - 0.191 CP$$

$$(R^2 = 0.96 \text{ and } RSD = 13.3)$$

where FI = daily feed intake (g), LW = live weight (g), $LW^{0.75}$ = metabolic live weight, ADG = change in live weight (g/day) on the previous day, AME = apparent metabolisable energy content of the diet (MJ/kg), LYS = lysine content of the diet (g/kg) and CP = crude protein content of the diet. This equation was valid over a range of live weight from 50 g to 2400 g.

I. INTRODUCTION

Feed intake is economically important to the broiler industry because feed is the major cost item. Furthermore, to determine optimum nutrient concentrations in broiler diets, it is necessary to be able to accurately predict feed intake of broilers varying in body weight. Although a number of equations are available in the literature for the prediction of feed intake by broilers (Zoons *et al.*, 1991), none are based on the fast-growing broilers currently available. Feed intake in poultry is affected by a number of factors (Forbes, 1986) that are related to the feed (e.g. energy and protein levels), bird (e.g. gut capacity, age and sex), environment (e.g. temperature) and husbandry (e.g. stocking density). Because of the complexity of biological processes involved, mathematical modeling can be useful in integrating the various factors that control feed intake. The objective of the present study was to derive equations for the prediction of daily feed intake of male broiler chickens as a function of live weight, growth rate and dietary nutrient concentrations.

II METHODS

Four diets (1 to 4) were initially formulated to contain varying concentrations of energy and protein (Table 1). Low feed intake and problems with behaviour were observed during week 4 in birds fed Diets 2 and 4. To overcome this, two more diets (Diets 5 and 6) were formulated with higher concentrations of protein and amino acids. The feeding schedule employed during the 38-d trial is presented in Table 2.

Day-old male broiler (Ross) chicks were obtained from a commercial hatchery, wing-tagged and randomly assigned to 36 pens (4 chicks/pen) in electrically heated, raised wire-floored starting batteries in an environmentally controlled room. The six dietary treatments

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Table 1. Composition (g/kg) of diets.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Wheat	650.0	720.0	510.0	590.0	655.2	548.7
Broll ¹	-	-	150.0	150.0	-	139.5
Soyabean meal	250.0	200.0	250.0	200.0	263.0	249.0
Fish meal	30.0	-	30.0	-	9.0	7.0
Soyabean oil	30.0	30.0	10.0	10.0	27.3	9.3
Limestone	20.0	20.0	20.0	20.0	18.2	18.6
Dicalcium phosphate	11.0	21.0	21.0	21.0	19.1	19.5
DL methionine	3.0	3.0	3.0	3.0	2.7	2.8
Salt	3.5	3.5	3.5	3.5	3.2	3.3
Premix	2.5	2.5	2.5	2.5	2.3	2.3
AME (MJ/kg)	13.7	13.7	12.2	12.3	13.5	12.3
Crude protein	203	171	209	179	195	197
Lysine	11.5	8.6	11.9	9.1	10.8	10.8
Methionine + cystine	9.7	8.8	9.9	8.9	9.2	9.3

¹ Wheat milling by-product.

Table 2. Treatments and feeding schedule used in the study.

Treatment	Day 1 to 24	Day 25 to 38
A	Diet 1	Diet 3
B	Diet 2	Diet 5
C	Diet 3	Diet 3
D	Diet 4	Diet 6
E	Diet 1	Diet 1
F	Diet 1	Diet 6

(Table 2) were each randomly assigned to six pens in a completely randomised design. The birds were transferred to colony cages in an environmentally controlled room at 14 d of age. Room temperature was maintained at 30 ± 1 °C during the first week and gradually decreased to 22 ± 2 °C by the end of the third week. The diets were fed from 1 to 38 d of age. Feed and water were available *ad libitum* and uniform light was provided on a 24-hour basis. Individual body weights and pen feed intakes were recorded daily.

Live weight and feed intake data were subjected to analysis of variance to calculate least-square means, standard errors and statistical significance between dietary treatments using the General Linear Models procedure (SAS, 1997). The STEPWISE option in PROC REG was used and the probability level to include variables in the prediction equation was set at 5%. The predictors in the multiple regression equations are therefore all significant at $P < 0.05$. The factors investigated included live weight (LW), metabolic live weight ($LW^{0.75}$), daily growth rate on the previous day (ADG) and diet composition measured in terms of apparent metabolisable energy (AME), crude protein (CP), lysine (LYS), and methionine plus cystine (MC).

III. RESULTS AND DISCUSSION

Dietary treatments significantly ($P < 0.05$) influenced live weight and feed intake (Table 3). The effects on growth rate and feed efficiency will not be discussed herein. Birds on treatments A and F had the highest feed intake and live weights, and those on treatment B ate less and were the lightest. Intake of birds on treatment C, D and E were intermediate.

Table 3. Least-square means of average live weight (LW) and feed intake (FI) of broilers at different ages as influenced by dietary treatments.

Age (day)	TREATMENT						Pooled SE
	A	B	C	D	E	F	
LW (g)							
1	43	43	43	43	43	43	0.1
7	131 ^b	106 ^a	146 ^c	114 ^a	132 ^b	140 ^{bc}	3.6
14	349 ^c	216 ^a	396 ^d	259 ^b	372 ^{cd}	379 ^{cd}	13.5
21	758 ^c	446 ^a	855 ^d	588 ^b	801 ^c	822 ^c	26.1
28	1377 ^c	853 ^a	1431 ^c	1063 ^b	1368 ^c	1405 ^c	39.5
38	2365 ^b	1794 ^a	2325 ^b	1937 ^a	2368 ^b	2370 ^b	58.5
FI (g/day)							
1-7	21 ^{ab}	18 ^a	23 ^b	18 ^a	22 ^{ab}	23 ^b	1.4
8-14	54 ^c	39 ^a	62 ^d	47 ^b	59 ^c	60 ^{cd}	2.3
15-21	103 ^c	75 ^a	116 ^d	94 ^b	107 ^c	109 ^c	2.0
22-28	156 ^{cd}	123 ^a	151 ^c	138 ^b	143 ^{bc}	161 ^d	3.2
29-38	205 ^c	181 ^a	196 ^b	188 ^{ab}	182 ^a	207 ^c	2.9

^{a-d} Means in the same row without a similar superscript differ significantly ($P < 0.05$).

The results of the stepwise regression analysis are shown in Table 4. During all stages of growth, most of the variation in feed intake was explained by variation in live weight (partial R^2 ranging between 75.2 and 93.8 % for $LW^{0.75}$ and between 0.5 and 3.1 % for LW). Interestingly, differences in diet composition (AME, CP, LYS, MC) contributed to less than 1 % of the total variation in feed intake and this effect was seen only after two weeks of age. Average daily gain contributed to only 0.1 % of the total variation. The daily feed intake (g/day) of broiler chickens between 1 and 38 d of age was predicted by the following multiple regression equation.

$$FI = 50.25 - 0.159 LW + 1.715 LW^{0.75} + 0.15 ADG - 3.37 AME + 1.67 LYS - 0.191 CP$$

This equation was used to predict daily feed intake over the 38-d trial period and the observed and predicted daily intakes for treatment groups B, C and F are shown in Figure 1. Group B presents a measure of the effects of early feeding of a diet with low nutrient concentrations followed by a return to a more adequate diet, whereas group F presents the reverse situation. It can be seen that the equation accurately predicted the increase in feed intake observed after change of diets in groups B and F on day 25.

Table 4. Partial coefficient of determination (pR^2) for different predictors, coefficient of determination (R^2) and residual standard deviation (RSD) of daily feed intake from 1 to 38 days of age.

Age	LW	LW ^{0.75}	AME	CP	LYS	MC	ADG	R ²	RSD
1-7	0.005	0.752	NS	NS	NS	0.006	NS	0.763	4.5
1-14	0.004	0.893	NS	NS	NS	NS	NS	0.893	6.8
1-21	0.020	0.923	0.001	NS	NS	NS	NS	0.923	8.7
1-28	0.018	0.938	0.001	NS	0.004	0.001	0.001	0.964	9.6
1-38	0.031	0.922	0.001	0.001	0.005	NS	0.001	0.961	13.3

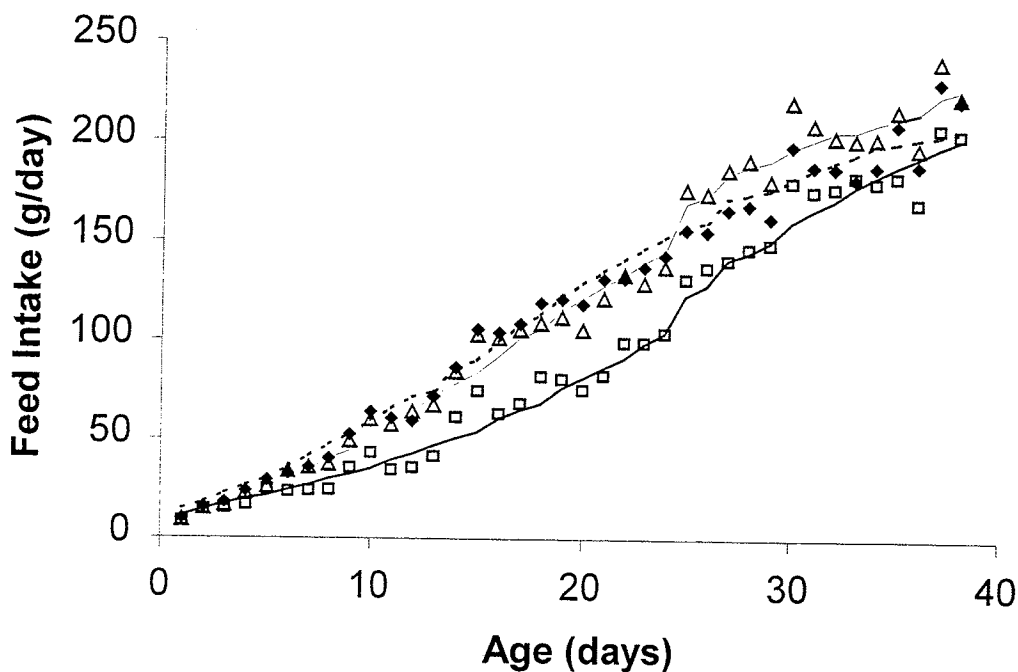


Figure 1. Observed feed intake for groups B (\square), C (\blacklozenge) and F (\triangle) compared to predicted intake for groups B (—), C (---) and F (· · ·).

The proposed equation is not intended to provide a comprehensive description of intake patterns in broilers. However, despite the limitations of this study and the small data set used, the multiple regression equation generated enabled the prediction of daily feed intake of Ross male broiler chickens over a 38-d growing period under the conditions provided in this experiment. Live weight was found to be the main factor affecting feed intake and adjustment in intake for differences in diet composition occurred only after two weeks of age.

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UPPER LIMITS OF INCLUSION OF CANOLA MEAL AND COTTONSEED MEAL IN DIETS FORMULATED ON A DIGESTIBLE AMINO ACID BASIS FOR BROILER CHICKENS

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Summary

Two broiler experiments evaluated graded levels (100, 200, 300, and 400 g/kg) of four Australian canola meals (CM) and one cottonseed meal (CSM) in diets formulated on a digestible amino acid (AA) basis. The results showed that bird performance was not significantly ($P>0.05$) affected by the level of CSM in the diet and feed intake was reduced ($P<0.05$) only at inclusions of 200 and 400 g CSM/kg. This excellent broiler performance was obtained by formulating the diets on a digestible AA basis with an adjusted lysine coefficient value. The results also showed that feed intake and weight gain were significantly ($P<0.05$) affected by the level and the source of CM in the diet. The overall feed efficiency was significantly ($P<0.05$) improved with 3 of the 4 CMs. This experiment demonstrated that substantial amounts of CM can be used in broiler diets formulated on a digestible AA basis.

I. INTRODUCTION

In Australia, the widespread use of canola meal (CM) and cottonseed meal (CSM) in poultry diets has been seriously restricted due to antinutritive factors (ANF), mainly glucosinolates (GSL), erucic fatty acid (EFA) and sinapine in CM and cyclopropene fatty acids (CPFA), gossypol and high fibre in CSM (Fernandez *et al.*, 1995). In the past these ANF were responsible for liver damage, increased thyroid weight, decreased energy utilisation, reduced feed intake (FI) and weight gain, and caused leg problems in broilers fed high levels of rapeseed meals (Leeson *et al.*, 1986). Gossypol binds to iron molecules in the diet and in blood cells and this may lead to anaemia and laboured breathing. Gossypol may also bind with lysine during heat processing, thus reducing CSM digestibility and availability (Fernandez *et al.*, 1995). Variation in the nutritional value and ANF of the meals would be expected due to location, environmental factors, cultivars, and industry processing conditions. It is well known that in Australia CM is produced from "double zero" varieties low in ANF. Also, CSM is derived from cultivars containing little gossypol and any residual gossypol can be inactivated by adding soluble iron compounds (Watkins *et al.*, 1994). In addition, solvent-extracted CSM contains less oil, thus reducing the negative effects of CPFA. This study evaluated the upper limits of inclusion of CM and CSM in diets formulated on a digestible amino acid basis in order to determine their utilisation in broiler diets.

II. MATERIALS AND METHODS

Commercial CM was obtained from four representative Australian processors located in Newcastle (NSW), Melbourne (Victoria), Numurkah (Victoria) and Pinjarra (West Australia). Solvent extraction was used to obtain all CM, except for the Pinjarra processor who used expeller extraction. Solvent-extracted CSM was obtained from a single supplier

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located in Narrabri (NSW). Prior to the trial, CM and CSM samples were analysed for proximate analysis, AA, gross energy (GE), GSL, gossypol, total condensed tannins (TCT), CPFA, AME and ileal digestible AA. The AME of the meals was determined using the classical total collection method with four replicates each of six male broiler chickens (21 d old). Meal samples were included in a basal diet (g/kg) as follows: CM or CSM 300, sorghum 667 plus minerals, vitamins and AA. Samples of food and dried excreta were analysed for GE and nitrogen. Ileal AA digestibility for each meal was determined with three replicate groups of four (37-42 d old) broilers (Ravindran *et al.*, 1999). Feed and digesta AA analysis were performed using a standard HPLC method (Farrell *et al.*, 1999).

Crumbled starter and pelleted finisher diets, in the growth experiments, contained graded levels of 100, 200, 300, and 400 g/kg CM or CSM and were formulated on a digestible AA basis as described by Farrell *et al.* (1999). The CM trial used a control diet based on wheat-soyabean meal but sorghum-soyabean meal comprised the control diet for CSM. Determined digestibility coefficient values were used for both meals for all AA except for lysine in CSM where a digestibility coefficient of 0.6 was used. Ferrous sulfate provided a 2:1 iron to gossypol ratio in each CSM diet.

Starter and finisher diets were fed from 4 to 25 d of age and from 25 to 41 d of age, respectively, to male broiler chicks (Cobb) grown in wire cages in an insulated, air conditioned house. Food and water were offered *ad libitum*; light and temperature followed industry practice. At 41 d two birds on diets with 200 and 400 g/kg of CM and CSM, were killed, weighed, blood sampled and their liver and pancreas weighed. In total, there were two experiments (CM and CSM) in the one design and layout. In the CM experiment, there were 17 treatments comprising a control diet plus all combinations of four levels x four sources of meal in a factorial design. In the CSM experiment there were five treatments comprising a control diet plus four levels of CSM. In each experiment, a cage of eight birds was the experimental unit. The layout was randomised blocks, with four replicates in blocks of 22 cages. Data were analysed separately for the CM and CSM experiments. In the CM study, the 17 treatments were compared in an initial randomised blocks ANOVA, and then in a follow-up ANOVA in which the full error term (48 degrees of freedom) from the initial ANOVA was used, the main effects and interaction of the embedded four x four factorial were tested.

III. RESULTS AND DISCUSSION

The AME values obtained with broilers (14-21 d) were much higher than those reported in the literature and AME values obtained with laying hens were used instead. The AME values of 11.2 and 10.9 MJ/kg DM for the Numurkah and Pinjarra CMs, respectively, were higher than for the Newcastle and Melbourne CMs (10.4 and 9.8 MJ/kg DM, respectively). Since all the canola varieties currently grown in Australia have been selected for low ANF, the AME differences among CMs may be attributed to differences in location and in processing conditions. The proximate analysis showed that the crude protein (CP) of the Pinjarra (335 g/kg) CM was lower than the for other three CMs (mean of 416 g/kg). Calcium, phosphorus and the essential AA content of all CMs were generally similar to expected values. The low GSL levels (3-7 $\mu\text{mol/g}$) in the CMs were one third those reported for Canadian "double zero" varieties. The TCT content of Newcastle CM (71 g/kg DM) was 50 per cent higher than the other CMs. This was attributed to differences in processing and this relatively high TCT level may cause a reduction in CP digestibility. The low gossypol CSM on the other hand was high in CP (519 g/kg DM) but low in NDF (182 g/kg), gossypol (0.05 g/kg) and TCT values which are typical of Narrabri where these ANFs are mostly removed during processing.

The AA digestibility coefficients of lysine, threonine, methionine and tryptophan in the CSM were 0.74, 0.78, 0.73 and 0.64 respectively. The average values of the CMs for the

same AA were 0.84, 0.83, 0.78 and 0.71, respectively. The responses of graded levels of CSM and CM, compared against the control diet, on growth performance and other parameters are shown in Tables 1 and 2, respectively.

Table 1. Feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) and organ weights (g/100 g live weight) means for broiler chickens (41 d) fed graded levels of cotton seed meal.

Diet	FI (g)	LWG (g)	F C R	Liver	Pancreas
Control	4429 ^a	2493	1.77	2.22	0.212
CSM (100 g/kg)	4369 ^{ab}	2508	1.75		
CSM (200 g/kg)	4199 ^{bc}	2379	1.78	2.31	0.192
CSM (300 g/kg)	4280 ^{abc}	2419	1.77		
CSM (400 g/kg)	4198 ^c	2395	1.73	2.30	0.197
LSD (P=0.05)	153	124	0.04	0.23	0.049

Means within a column with no common superscript are significantly different (P<0.05).

Table 2. Feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) and organ weights (g/100 g live weight) for broiler chickens (41 d) fed graded levels of canola meal from various sources.

Diet	FI (g)	LWG (g)	F C R	Liver	Pancreas
Control	4555 ^a	2519 ^a	1.820 ^a	2.026 ^a	0.172 ^a
Newcastle (100 g/kg)	4412 ^a	2505 ^{ab}	1.773 ^{ab}		
Newcastle (200 g/kg)	4351 ^b	2465 ^{ab}	1.767 ^b	1.927 ^a	0.179 ^a
Newcastle (300 g/kg)	4229 ^b	2401 ^b	1.791 ^{ab}		
Newcastle (400 g/kg)	3986 ^c	2215 ^c	1.809 ^a	2.393 ^b	0.245 ^b
Melbourne (100 g/kg)	4489 ^a	2600 ^a	1.734 ^b		
Melbourne (200 g/kg)	4166 ^b	2390 ^b	1.744 ^b	1.926 ^a	0.185 ^a
Melbourne (300 g/kg)	4032 ^b	2358 ^b	1.710 ^b		
Melbourne (400 g/kg)	4110 ^b	2361 ^b	1.744 ^b	2.074 ^a	0.226 ^b
Numurkah (100 g/kg)	4405 ^{ab}	2527 ^a	1.744 ^b		
Numurkah (200 g/kg)	4404 ^{ab}	2502 ^a	1.762 ^b	1.880 ^a	0.170 ^a
Numurkah (300 g/kg)	4256 ^{bc}	2494 ^{ab}	1.741 ^b		
Numurkah (400 g/kg)	4155 ^c	2390 ^b	1.734 ^b	2.341 ^b	0.232 ^b
Pinjarra (100 g/kg)	4203 ^b	2509 ^a	1.687 ^b		
Pinjarra (200 g/kg)	4145 ^{bc}	2483 ^{ab}	1.670 ^b	2.269 ^a	0.198 ^a
Pinjarra (300 g/kg)	4266 ^b	2546 ^a	1.672 ^b		
Pinjarra (400 g/kg)	3967 ^c	2373 ^b	1.674 ^b	2.186 ^a	0.235 ^b
LSD (P=0.05)	193.8	110.1	0.0468	0.2693	0.0321

Means for each canola meal source within a column with no common superscript are significantly different (P<0.05).

Table 1 shows that LWG and FCR were not affected (P>0.05) by the level of CSM in the diet. However, FI was significantly reduced but only at inclusions of 200 and 400 g

CSM/kg. Watkins *et al.* (1993, 1994) also found that CSM gave no depression in LWG when used at 300 g/kg in broiler diets formulated on a digestible AA basis. Contrary to our findings, these workers reported increased FI and higher FCR but in the present study FCR was not affected ($P>0.05$) by the level of CSM in the diet. In a quest to improve their previous results Watkins and Waldroup (1995) adjusted their diets for digestible AA content and reduced previously assigned AME values. However they still observed a depression in FI and LWG even at an inclusion of 300 g CSM/kg. Other workers similarly reported depressed chick performance on diets at 200 g CSM/kg (Ravindran and Bryden, 1999) and at 300 and 400 g CSM/kg (Fernandez *et al.*, 1995) even when formulated on a digestible AA basis and supplemented with free AA. In the current study, diets were formulated on a digestible AA basis using determined digestibility coefficients for all CSM AA except lysine where a coefficient of 0.60 was used to account for low utilisation of lysine availability in over heat-processed meals.

Liver and pancreas were not affected at any level of CSM in the diets so good broiler performance can be obtained with inclusions of up to 400 g/kg of pre-press solvent-extracted CSM.

The results in Table 2 showed that after 41 d, except for the Newcastle source, the FCR was significantly ($P<0.05$) improved with each CM source and level. However, Newcastle and Melbourne sources significantly ($P<0.05$) reduced LWG when fed at 300 and 200 g CM/kg, respectively. This positive effect on FCR was largely due to the significantly ($P<0.05$) reduced FI and good LWG, particularly with the Numurkah and Pinjarra CM sources where no significant adverse effect ($P>0.05$) was found when birds were fed up to 300 g CM/kg. Ravindran *et al.* (1998) also reported that CM gave no depression in LWG, FI, and FCR when used at up to 200 g/kg in broiler diets formulated on a digestible AA basis. The improved FCR in our experiment may be partly attributed to an underestimated AME value used in formulation since in this experiment, AME values for CM obtained with broilers were not used and AME values obtained with laying hens were used instead. Since low GSL levels were found in all CMs, it is likely that the differences in the nutritional value of the CMs were influenced by other factors such as differences in oil processing conditions. The high levels of TCT (71.1 g/kg) found in the Newcastle CM may be responsible for the impaired bird performance. The enlargement of the pancreas with all CM sources at 400 g/kg may also indicate that a trypsin inhibitor is present in these meals.

These results have demonstrated that substantial amounts of CM can be used in broiler diets formulated on a digestible AA basis but more detailed studies are required.

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COMPARISON OF APPARENT ILEAL AMINO ACID DIGESTIBILITY IN MEAT AND BONE MEAL MEASURED WITH BROILERS AND RATS

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Summary

The apparent ileal amino acid digestibilities in 15 meat and bone meal samples were determined using 28-d old broiler chickens and growing laboratory rats. The apparent digestibility for ten of the 16 amino acids determined were similar ($P > 0.10$) for the two species, but those for arginine, leucine, lysine, phenylalanine and cystine were higher ($P < 0.01$ to 0.05) in the rat. Differences approaching statistical significance ($P < 0.06$ to 0.10) in favour of the rat were observed for isoleucine and tyrosine. The data suggest that the ileal digestibility values generated with the growing rat for some amino acids in meat and bone meal cannot be directly applied to the broiler chicken. However, the significant relationship between digestible amino acid concentrations determined with the broiler and rat models indicates that it is possible to develop regression equations to predict digestible amino acid concentrations in meat and bone meal for the broiler from values generated with rat assays.

I. INTRODUCTION

Analysis of ileal digesta contents rather than excreta is a reliable method for assessing amino acid digestibility in poultry (Ravindran *et al.*, 1999) and pigs (Low, 1980). With pigs, the laboratory rat has been shown to be a suitable and relatively inexpensive model for the determination of ileal amino acid digestibility in a range of feed ingredients (Pearson *et al.*, 1999). Published data on the correlation between the digestibility estimates for the rat and the broiler chicken, are however, scanty (Johns *et al.*, 1985). In the present study, 15 meat and bone meal samples were assayed to determine the ileal digestibility of amino acids in broiler chickens and rats. The aim was to examine whether the rat can be used as a generalised model animal for simple-stomached species in amino acid digestibility assays.

Also, a new technology based on near infrared reflectance analysis will soon be available for the rapid determination of digestible amino acids in New Zealand meat and bone meals. The rapid assay has been developed as part of the on-going Public Good Science Funding programme that uses the rat as a model animal and will allow discrimination of meat and bone meal samples based on amino acid digestibility (Hendriks, 1999). A comparison of rat and broiler amino acid digestibility values is necessary for the transfer of this technology to the poultry industry.

II METHODS

Fifteen meat and bone meal samples, selected from the 74 samples assayed using the rat model in the Public Good Science Fund Project, were utilised in this study. The selected samples were representative of the digestibility ranges determined in the rat model.

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Each sample was ground to pass through 1-mm mesh and was included as the sole source of protein in assay diets. Assay diets were based on maize starch and meat and bone meal, and the proportions of maize starch and the ingredient were varied to obtain a dietary crude protein level of 10 and 16%, respectively, in rat and broiler assays. All diets contained 3 g chromic oxide/kg as an indigestible marker.

Each diet was fed *ad libitum* to six growing rats (males; average body weight, 160 g) housed in stainless steel metabolism cages, or to six 28-d old broiler chickens (males; average weight, 1420 g) housed in colony cages. Water was available at all times. After eight (for rats) or three (for broilers) days on the test diet, the animals were euthanased and digesta from the terminal ileum was obtained, pooled within diets, lyophilised and analysed for amino acids and chromium. Amino acids were detected on a Waters ion exchange HPLC system, and the chromatograms were integrated using dedicated software (Maxima 820, Waters, Millipore, Milford, MA) with the amino acids identified and quantified using a standard amino acid solution (Pierce, Rockford, IL). Cysteine and methionine were analysed as cysteic acid and methionine sulphone by oxidation with performic acid for 16 h at 0°C and neutralisation with hydrobromic acid prior to hydrolysis. Tryptophan was not determined. The chromium content was measured on an Instrumentation Laboratory atomic absorption spectrophotometer following the method of Costigan and Ellis (1987).

Apparent ileal amino acid digestibility coefficients were calculated, using chromium as the indigestible marker, as shown below.

$$\text{Apparent digestibility} = \frac{(\text{AA} / \text{Cr})_d - (\text{AA} / \text{Cr})_i}{(\text{AA} / \text{Cr})_d}$$

where, $(\text{AA} / \text{Cr})_d$ = ratio of nitrogen/ amino acid to chromium in diet and
 $(\text{AA} / \text{Cr})_i$ = ratio of nitrogen/ amino acid to chromium in ileal digesta.

The digestibility values for each amino acid were statistically analysed using the General Linear Models procedure of SAS (1997) for the effects of species and sample.

III. RESULTS AND DISCUSSION

Wide variations were observed in the gross content of amino acids, but the gross amino acid data will not be discussed here. The average ileal amino acid digestibilities of the 15 meat and bone meal samples for the broiler and the rat are presented in Table 1. The apparent ileal digestibility of lysine in the 15 meat and bone meal samples for broilers and rats ranged from 43 to 82% and from 48 to 85%, respectively. The corresponding ranges for methionine, cystine, threonine and amino acid N were: 55 to 84% and 53 to 87%; 28 to 56% and 39 to 73%; 49 to 73% and 47 to 83%; 43 to 76% and 52 to 84%, respectively. This was an expected result since the meals were obtained from different rendering plants that used widely differing raw materials and processing procedures (Hendriks *et al.*, 2000).

Comparison of digestibility data shows that for 10 of the 16 amino acids determined, apparent ileal amino acid digestibility was similar ($P > 0.10$) for the 28-d old broiler and the growing rat. However significant ($P < 0.01$ to 0.05) differences were observed between the estimates in the rat and the chicken for arginine, leucine, lysine, phenylalanine and cystine. Differences approaching statistical significance ($P < 0.06$ to 0.10) were observed for isoleucine and tyrosine. For these amino acids, the values obtained for broilers were consistently lower than those in the rats. The species difference was marked for cystine, with

digestibility in broilers being 17 percentage units lower than in the rat. The significance of this observation is unclear.

Table 1. Apparent ileal digestibility of amino acids from meat and bone meal for broiler chickens and rats¹.

Amino acid	Broiler	Rat	Pooled SE	Probability, P=
Indispensable amino acids				
Arginine	69.4	76.7	1.49	0.01
Histidine	61.6	60.1	3.30	0.76
Isoleucine	69.4	73.8	1.49	0.06
Leucine	70.1	74.7	1.38	0.04
Lysine	69.2	75.0	1.28	0.01
Methionine	74.0	76.9	1.31	0.13
Phenylalanine	72.4	77.3	1.17	0.02
Threonine	61.8	60.7	2.32	0.74
Valine	68.3	72.0	1.68	0.14
Dispensable amino acids				
Alanine	68.1	70.7	1.67	0.29
Aspartic acid	47.2	46.8	3.50	0.94
Cystine	38.9	56.1	2.91	0.01
Glycine	63.6	62.7	2.02	0.75
Glutamic acid	66.6	67.3	1.75	0.77
Proline	60.6	60.9	2.00	0.81
Serine	58.2	56.1	2.49	0.56
Tyrosine	69.6	73.3	1.50	0.10
Amino acid N	64.9	67.2	1.81	0.39

¹ Each mean represents the average value from 15 samples.

These results indicate that the rat assay cannot be used as a direct model for the determination of digestibility of some amino acids in meat and bone meal for broilers. For the amino acids that differed between the species (arginine, isoleucine, leucine, lysine, phenylalanine, cystine and tyrosine), linear regression analysis was carried out (SAS, 1997) to develop equations to predict broiler amino acid digestibility from digestibility estimates in the rat. When amino acid digestibility values were considered, only low to moderate relationships ($r^2 = 0.25$ to 0.41) were observed between the two estimates. However, when digestible amino acid concentrations were considered, the rat model accounted for most of the variation in the broiler values for arginine ($r^2 = 0.75$; $P < 0.001$), isoleucine ($r^2 = 0.81$; $P < 0.001$), leucine ($r^2 = 0.80$; $P < 0.001$), lysine ($r^2 = 0.84$; $P < 0.001$), cystine ($r^2 = 0.45$; $P < 0.01$) and tyrosine ($r^2 = 0.65$; $P < 0.001$).

In conclusion, these findings suggest that the digestibility values generated with the growing rat for some amino acids in meat and bone meal are not directly transferable to the broiler chicken. However, the significant relationships between digestible amino acid concentrations estimated with the broiler and rat models indicate that it is possible to develop

linear regression equations to predict digestible amino acid concentrations in meat and bone meal for the broiler from values generated with rat assays.

IV. ACKNOWLEDGEMENTS

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COMPARISON OF THE INFLUENCE OF THE METHODOLOGY USED FOR DETERMINING THE METABOLISABLE ENERGY OF SOYABEAN MEAL

W.D. COWAN¹, G. HUYGHEBAERT² and R.J. HUGHES³

Summary

The metabolisable energy corrected for nitrogen retention (MEn) for soyabean meal was estimated by a 'substitution' method using three standard test diets, each containing different cereal bases, (wheat, wheat + xylanase or sorghum). Diet formulation had a marked effect on feed intake, weight gain, feed efficiency, moisture output, N-retention and dietary MEn values. Dietary supplementation with xylanase did not generally increase MEn values of the wheat-based diets. A higher MEn value for soyabean meal in combination with wheat-based diets was observed. The range of estimated MEn values for soyabean meal varied widely (8.1-12.7 MJ/kg) casting doubt on the principle of additivity used in diet formulation and the tabulated values for soyabean meal MEn.

I. INTRODUCTION

Nitrogen corrected ME values for feedstuffs are a key component in diet formulation and different methodologies are used to determine them. Least-cost diet formulation is based on the additivity of nutrient values but interactions between fat and wheat have been observed (Cowan, 1997). Possible effects of diet composition, namely type of cereal, have never been investigated or taken into account with soyabean meal. It would be useful to determine if there was an interaction between the type of cereal (sorghum or wheat as the main cereal in the MEn determination), MEn methodology and the MEn value of soyabean meal derived from this determination.

Xylanase enzymes are widely used to improve nutrient utilisation from wheat-containing diets. Nutritional improvement with exogenous enzymes depends mainly on the target substrate (its dietary concentration, its NSP-content and composition, and the structural arrangement of the polymers) and the enzyme preparation. The influence of different enzyme activity profiles has been reported previously (Huyghebaert, 1995). One technique used to establish the effect of xylanases is to examine their effect on the MEn value of wheat and it has until now been assumed that this effect was independent of the method chosen.

The objective of the present balance trial with broiler chickens was to establish the effect of different approaches to diet formulation for estimation of the MEn of soyabean meal by the substitution method, and to examine the influence of a xylanase in the respective wheat-based diets.

II. METHODS

All experimental diets, including the addition of all enzyme preparations were prepared in the feed mill of the CLO-DVV. Nutrient values for feed raw materials were taken from the CVB tables (1999). There were 3 types of diet formulation, based on the Australian Standard Diet (ASD), the European Standard Diet (ESD), and modification to the European

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Standard Diet (MESD), respectively (Hughes *et al.*, 2000). The other factors were type of cereal (sorghum vs. wheat), the level of soyabean meal and the supplementation of the wheat-based diet with xylanase (from *Thermomyces lanuginosus* active against soluble and insoluble wheat arabinoxylans). The compositions of all diets are given in Table 1.

Five hundred Ross broiler chicks were obtained from a local commercial hatchery. From 1 to 14 d of age, the birds were housed on deep litter under conventional conditions for lighting, heating and ventilation. The birds were fed *ad libitum* a normal mash starter diet. On d 14 the birds were weighed individually and birds having excessively high or low body weights were discarded. The balance trial was carried out according to the EU-reference method (Bourdillon *et al.*, 1990) consisting of a 7 d period of adaptation to the respective experimental diets (14-21 d of age) and a 4 d main balance period (21-25 d of age) with restricted feeding and total excreta collection. Groups of 4 birds were assigned randomly to each of the 75 pens housed in balance cages. Five replicates were used for each of the 15 treatments. Samples of wet excreta were freeze-dried. Samples of feeds and freeze-dried excreta were analysed for gross energy (GE), nitrogen (macro-Kjeldahl: protein = N x 6.25). The MEN contents of the experimental diets were calculated from their respective freeze-dried excreta/feed (E/F)-ratios as well as their corresponding GE-contents, followed by a correction for N-retention to zero by using an energy equivalent of 34.36 kJ/g N retained. The MEN of soyabean meal was determined by ascribing all changes in MEN to the test ingredient.

The main balance results of the dietary treatments were analysed statistically by 1-way and 2-way ANOVA and the LSD-multiple range test (Statgraphics version 6.2, 1992).

Table 1. Composition of diets used in the ME determinations (g/kg).

Ingredient (g/kg)	ASD					ESD					MESD					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Diet Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Sorghum	800	-	-	548	-	572	-	-	429	-	600	-	-	450.00	-	
Wheat	-	800	800	-	548	-	572	572	-	429	-	600	600	-	450.00	
Soyabean meal (solvent ex.)	-	-	-	300	300	320	320	320	490	490	303	303	303	477.25	477.25	
Sunflower oil	-	-	-	-	-	60	60	60	45	45	49	49	49	36.75	36.75	
Casein	152	152	152	104	104	-	-	-	-	-	-	-	-	-	-	
Di-Calcium phosphate	20	20	20	20	20	20	20	20	15	15	20	20	20	15.00	15.00	
Limestone	11	11	11	11	11	11	11	11	8.25	8.25	11	11	11	8.25	8.25	
DL-methionine	7	7	7	7	7	7	7	7	5.25	5.25	7	7	7	5.25	5.25	
Vit./ min. premix	5	5	5	5	5	5	5	5	3.75	3.75	5	5	5	3.75	3.75	
Salt	3	3	3	3	3	3	3	3	2.25	2.25	3	3	3	2.25	2.25	
Choline chloride (60%)	2	2	2	2	2	2	2	2	1.50	1.50	2	2	2	1.50	1.50	
Xylanase	-	-	0.2	-	-	-	-	-	0.2	-	-	-	-	0.2	-	-

Australian Standard Diet (ASD), Diets 1-5, European Standard Diet (ESD), Diets 6-10, Modified European Standard Diet (MESD), Diets 11-15. Xylanase from *Thermomyces lanuginosus*, 1,000 FXU/g.

III. RESULTS

The energy related balance results are shown in Table 2. The calculated values for soyabean MEN are shown in Tables 3 and 4. For all method and cereal combinations the addition of soyabean meal depressed dietary MEN. Dietary MEN values for sorghum-containing diets were higher with the ASD and MESD methods but not with the ESD method.

Soyabean meal MEn was lower in all methods with the sorghum-based diet compared to the wheat-based diet and varied considerably from method to method (9.88 ± 2.0 MJ/kg). Xylanase supplementation did not significantly increase MEn of all wheat-based diets.

Table 2. Metabolisable energy (ME) and nitrogen retention (N ret) values for the various diets determined using the three methods of assessment.

Diet	Method	ME, MJ/kg	N ret., kJ/kg	MEn, MJ/kg
1	ASD-S	12.50 d	486 f	12.01 de
2	ASD-W	11.92 ef	493 f	11.42 fg
3	ASD-W+	12.07 e	506 f	11.56 f
4	ASD-S+Soya	11.87 ef	645 cd	11.22 gh
5	ASD-W+Soya	11.73 f	664 cd	11.06 h
6	ESD-S	13.05 bc	663 cd	12.38 bc
7	ESD-W	13.13 ab	659 cd	12.47 ab
8	ESD-W+	13.30 a	666 c	12.64 a
9	ESD-S+Soya	11.84 ef	719 b	11.12 h
10	ESD-W+Soya	12.02 e	666 c	11.36 fg
11	MESD-S	12.87 c	637 cde	12.23 cd
12	MESD-W	12.54 d	598 e	11.94 e
13	MESD-W+	13.08 abc	620 de	12.46 abc
14	MESD-S+Soya	11.71 f	648 cd	11.06 h
15	MESD-W+Soya	11.73 f	656 cd	11.08 h
Probability		<0.001	<0.001	<0.001
LSD (P=0.05)		0.24	45	0.23

+ indicates a diet supplemented with xylanase at 200 FXU/kg. W = wheat, S = sorghum, Soya = additional soyabean meal.

Means in a column without a common letter are significantly different ($P < 0.05$).

IV. DISCUSSION

There were some marked differences in feed intake among treatments. Feed intake was lower for Treatments 1-3, i.e. those diets where the casein level was at least 15%. These differences in feed intake for the ASD method might be an important input factor for gastrointestinal physiology with implications for MEn determinations and subsequent calculations. The findings of Bourdillon *et al.* (1990) showed that a difference in feed intake of 0.45 vs. 0.90 of *ad libitum* resulted in a MEn change of 0.6 to 1.8% because of the variable contribution of endogenous secretions or metabolic losses. However, in the current experiment it was not possible to determine the effect of reduced feed intake on the final MEn result.

The MEn values were slightly higher with the sorghum diets with the ASD and MESD methods. Xylanase only gave a significant response with the MESD diet and the effect of xylanase was reduced with all three methods after the addition of soyabean meal. There was a clear effect of diet on MEn and this has significant implications for feed formulation. Feedstuff MEn values are considered to be independent of the method used to derive them and these results indicate that this is not correct. Both experimental design and cereal type

affected the end result and suggested that current methods of feed formulation based on additivity are not correct. The wide range of observed MEn values for soyabean meal (Table 3) indicates that nutrient requirements generated using one system may not be transferable to diets formulated using a different feedstuff nutrient base.

Table 3. The calculated nitrogen corrected metabolisable energy (MEn) value of soyabean meal.

MEn method and cereal	MEn (MJ/kg)
ASD – Sorghum	11.86 a
ASD – Wheat	12.65 a
ESD – Sorghum	7.08 c
ESD – Wheat	8.12 bc
MESD – Sorghum	8.8 bc
MESD – Wheat	10.53 b

Means without a common letter are significantly different ($P < 0.05$).

Addition of xylanase in this experiment did not generally affect dietary MEn. This might be due to the unsaturated nature of the lipid fraction. The other cereal related observation could be due to the fact that the adverse effect is more dose related for wheat (because of the anti-nutritional effects of the NSP-fraction) than for sorghum.

Table 4. Two factor ANOVA for soyabean meal MEn

ANOVA	P-value
Type of cereal	0.0019
Type of methodology	<0.001
Interaction	0.92
LSD m.r. test	MEn, MJ/kg
Type of cereal	
Sorghum	8.28 b
Wheat	9.09 a
LSD ($P=0.05$)	0.48
Type of methodology	
ASD	10.39 a
ESD	7.67 b
SSD	8.01 b
LSD ($P=0.05$)	0.59

Means within each factor with different letters are significantly different at $P < 0.05$.

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HYDROGEN AND METHANE BREATH TESTS FOR ASSESSING METABOLIC ACTIVITY OF GUT MICROFLORA IN BROILER CHICKENS

R.J. HUGHES¹, D.R. TIVEY² and R.N. BUTLER³

Summary

Breath hydrogen measurement is used as an indicator of carbohydrate malabsorption in humans and for estimating the rate of passage of digesta through the small intestine. Both tests rely on bacterial fermentation of undigested carbohydrate in the large bowel. The development of similar breath tests for chickens for non-invasive measurement of gastro-intestinal functions will provide more insight than the snap-shot view obtained in conventional nutrient balance studies involving the slaughter of birds to obtain digesta. Using breath tests, it should be possible to pre-select individual chickens with known physiological characteristics, subject them to dietary or other treatments, and follow the resulting changes in gastro-intestinal functions such as digesta transit time and microbial proliferation in the small intestine. Preliminary breath testing studies with broiler chickens indicated that gut microflora competed for energy and other nutrients thus slowing the rate of growth and reducing feed efficiency.

I. INTRODUCTION

Analysis of expired breath is a non-invasive method for diagnosing gastro-intestinal function in humans (Butler, 1996). Hughes *et al.* (2000b) demonstrated that ¹³CO₂ breath tests could be developed as non-invasive tools for studying gut physiology in broiler chickens. Other breath tests used routinely in medical practice are based on release of hydrogen and methane following microbial fermentation of carbohydrates such as lactulose which is a disaccharide not absorbed in the small intestine (Wutzke *et al.*, 1997). Studies on humans and other species indicate that samples of breath can be taken with simple, inexpensive equipment and remain stable for long periods, enabling these tests to be used in the field. Tivey and Butler (1999) concluded that breath tests should prove to be powerful analytical tools for nutrition research and veterinary diagnostics. The breath tests likely to be of most benefit for broiler nutrition studies include those which examine digesta transit time and microbial growth in the small intestine in order to address problems such as variation in energy metabolism in broilers (Hughes *et al.*, 2000a).

This paper describes (1) an initial experiment to determine whether gut microflora of broiler chickens produce hydrogen and methane, and (2) application of a hydrogen breath test to assess metabolic activity of gut microflora in chickens given a wheat-based diet.

II. MATERIALS AND METHODS

Helmets of different dimensions (40 or 50 mm internal diameter and 95 or 100 mm length, respectively) were constructed from capped PVC pipe to suit chickens of different ages and hence size. The helmet was placed over the head and neck of the chicken and held firmly against the shoulders and breast to minimise loss of expired H₂ and CH₄. After 30 sec, a sample of breath was drawn through the cap via Luer lock fittings into a 10 mL evacuated tube. Preliminary work indicated that 30 sec provided a suitable breath sample without distressing the

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chicken. Hydrogen and methane concentrations were analysed by gas chromatography.

In Experiment 1, sixteen 23-d-old chickens fed a commercial diet *ad libitum* were breath tested. Two days later, after an overnight fast, chickens were weighed and breath tested commencing at 0830 h to establish a base-line. Each chicken was given 5 mL of diluted lactulose solution via a disposable syringe fitted with a plastic tube which was inserted 4 cm into the oesophagus. A total of 12 chickens were given approximately 130 mg lactulose, two other chickens were given double the dose (260 mg lactulose) and a further two were given quadruple the dose (520 mg lactulose). The lower dose rate (1 g carbohydrate per 10 kg body weight) was equivalent to that given to human subjects to assess carbohydrate malabsorption. The higher dose rates (2 g per 10 kg and 4 g per 10 kg) were given to obtain an indication of whether carbohydrate loading needed to be greater to achieve measurable levels of hydrogen or methane in expired breath for subsequent experiments with chickens. Each chicken was breath tested 3 h post-feeding of lactulose. Chickens were denied access to feed in this 3 h period.

In Experiment 2, a total of 48 chickens of 21-22 d of age were housed individually in metabolism cages and given a practical diet comprising (per kg): wheat 700 g, meat and bone meal 76 g, soybean meal 170 g, sunflower oil 40 g, salt 2.5 g, lysine HCl 2.5 g, methionine 3 g, and vitamins and minerals 6g. Cold-pressed pellets were fed for seven d. The first 3 d enabled the chickens to adapt to the cages and feeds. During the following 4 d, all excreta were collected and dried. Feed intake was measured during the adaptation and collection phases of the study. Birds were weighed at the start and end of the 7 d period. Dry matter contents of samples of pelleted and milled feed were measured. Gross energy values of dried excreta and milled feed were measured with a Parr isoperibol bomb calorimeter for calculation of apparent metabolisable energy (AME) of the diet. Chickens had free access to feed and water throughout the 7 d experimental period. On days 0 and 6 each chicken was breath tested commencing at 0830 h.

III. RESULTS AND DISCUSSION

There was large variability in hydrogen concentration (in ppm) in breath samples from non-fasted chickens given a commercial diet, and in fasted chickens both before and after dosing with lactulose in Experiment 1 (Figure 1). In 9 out of 12 chickens studied, there was an increase in hydrogen concentration in the 3 h period following dosing with lactulose. There was no change in one chicken and the other two chickens showed a small decline.

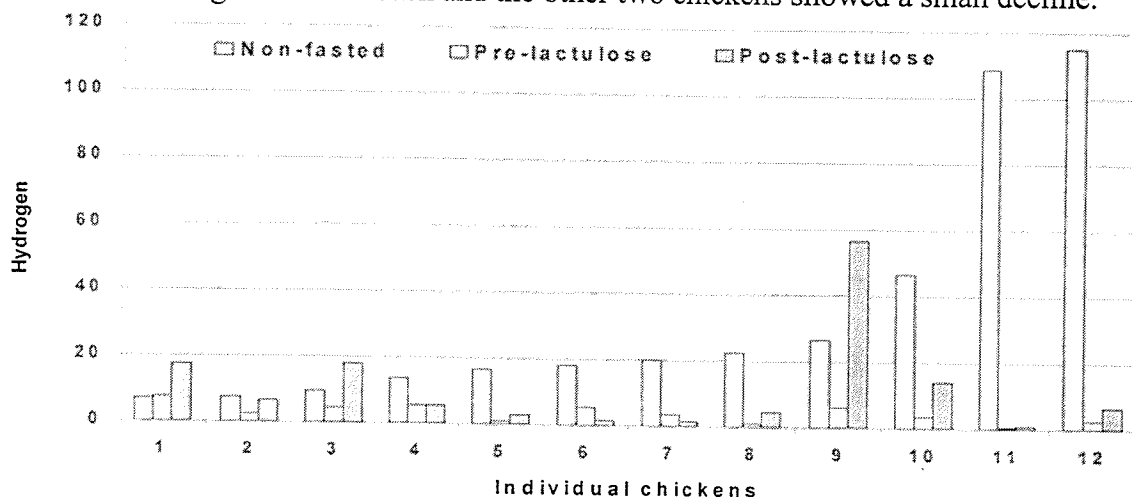


Figure 1. Breath hydrogen concentration (in ppm) in chickens fed a commercial diet *ad libitum*, and then two days later from the same chickens (fasted overnight) immediately before and 3 h after dosing with lactulose (130 mg in 5mL water).

All chickens hosted microflora (presumably in the caeca) which were capable of fermenting carbohydrate to hydrogen. In addition, methane (up to 13 ppm) was detected in the breath of all 16 chickens at some stage during this experiment, either before or after dosing with lactulose (data not shown). These results tend to suggest that other factors such as the rate of passage of digesta, proliferation of facultative anaerobes (Choct *et al.*, 1996) in the small intestine, and combinations of these contributed significantly to the large variation in breath hydrogen and methane. It is also possible that the dose rate of non-absorbable carbohydrate was too low.

In Experiment 2, change in hydrogen concentration in breath during the 7 d study was highly variable, as were the concentrations at the start and end of the study (Figure 2).

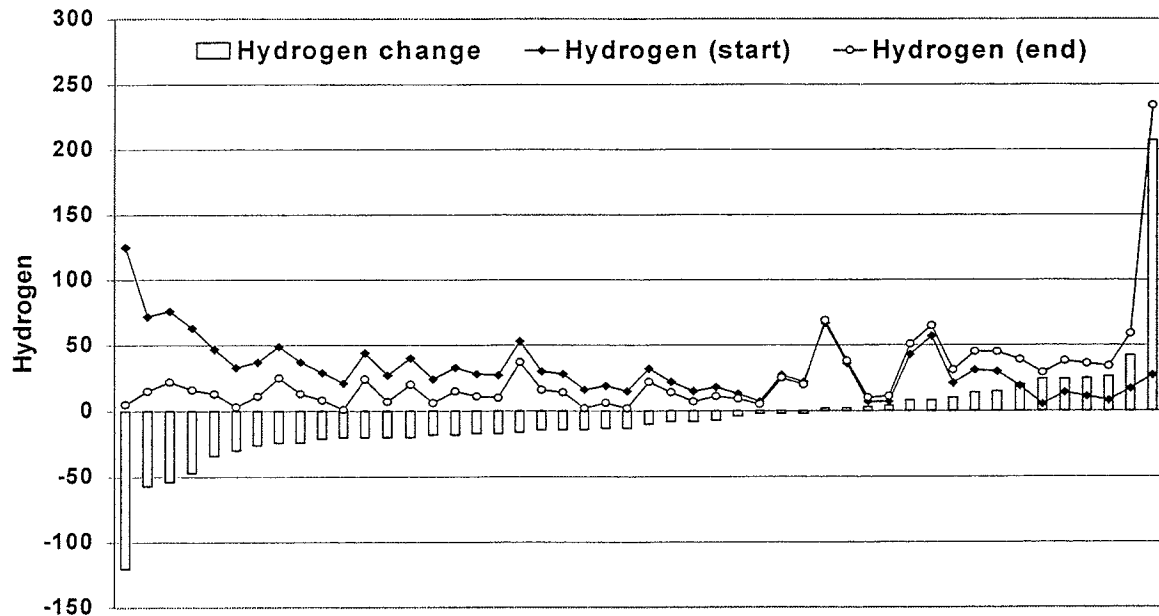


Figure 2. Variation in expired concentrations of breath hydrogen (in ppm) from chickens given a wheat-based diet (30 g crude fibre/kg). Data from individual chickens are sorted by increasing change from start to end of the 7 d metabolism experiment.

There were no associations ($P > 0.05$) between AME and breath hydrogen or between feed intake and breath hydrogen. However, growth rate of chickens was negatively correlated ($P < 0.01$) with breath hydrogen samples taken at the end of the study and feed conversion ratio was positively correlated ($P < 0.001$) with breath hydrogen (Figure 3). These preliminary results demonstrate the potential usefulness of non-invasive breath tests for detecting malabsorption of carbohydrate in commercial broiler flocks and for measurement of digesta transit time in nutrient balance experiments.

In addition, it was clearly evident that gut microflora competed for energy and other nutrients thus slowing the rate of growth and reducing feed efficiency. The possibility that undigested carbohydrate leaving the small intestine was fermented to volatile products such as short-chain fatty acids also in a variable manner, like hydrogen and methane production, needs further study. Bacterial overgrowth of the gut is likely to have detrimental effects in addition to significant losses of nutrients. Microbial proliferation could ultimately lead to health problems through general inflammation of the gut and invasion of tissue by organisms pathogenic to the bird or to humans consuming contaminated carcasses.

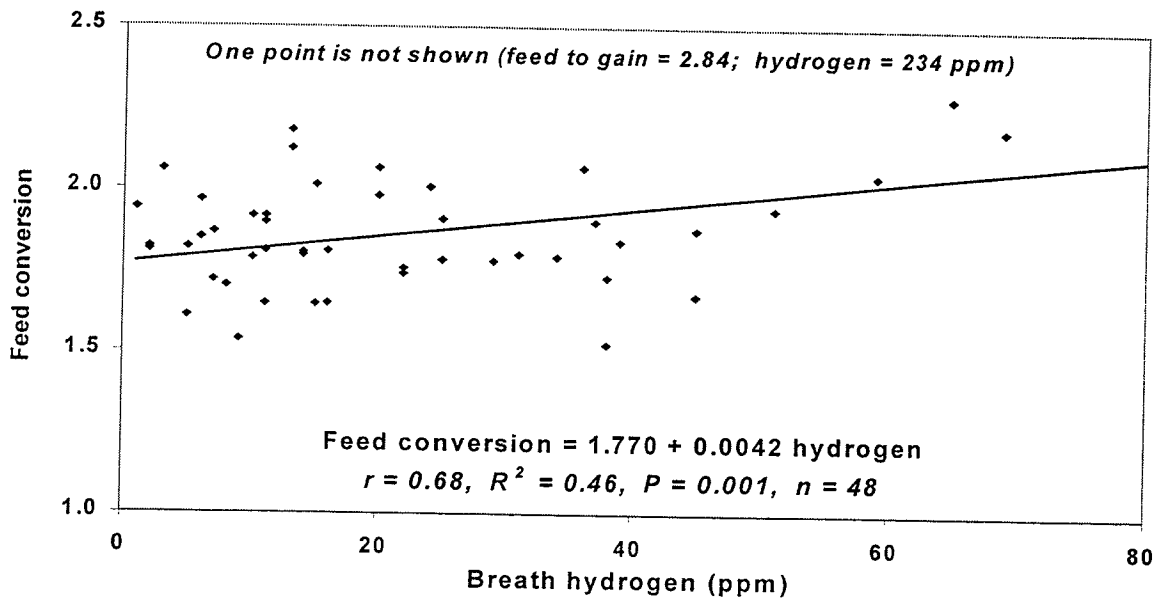


Figure 3. Relationship between feed conversion and hydrogen concentration in breath samples taken at the end of a 7 d metabolism experiment with chickens given a wheat-based diet (30 g crude fibre/kg).

IV. CONCLUSIONS

Changes in hydrogen and methane concentrations in breath during two metabolism studies were highly variable. However, observed variation between individual birds in AME was not directly associated with breath hydrogen concentration. Elevated levels of hydrogen in breath were indicative of significant reductions in growth rate and feed efficiency brought about by malabsorption, coupled with losses of energy and other nutrients through proliferation of gut bacteria.

V. ACKNOWLEDGMENTS

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GENETIC IDENTITY AND DISTANCE AMONG LONG-TERM SELECTED AND CONTROL LINES OF WHITE LEGHORN CHICKENS

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Summary

The randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique, with 11 polymorphic markers, was employed to evaluate the genetic similarities and distance among four selected lines, viz. IWH, IWG, IWJ and IWI, and a random bred control (IWC) line of White Leghorn chickens. The band sharing frequency (BSF) analysis (pooled over primers) revealed that the control line had the highest within line BSF. The highest between line BSF was observed between control and one selected (IWJ) line. The estimates of genetic distance calculated on the basis of BSF were 0.119 ± 0.033 , 0.06 ± 0.026 , 0.091 ± 0.027 , 0.128 ± 0.048 , 0.086 ± 0.021 , 0.114 ± 0.032 , 0.090 ± 0.030 , 0.063 ± 0.027 , 0.133 ± 0.042 and 0.144 ± 0.031 between C-H, C-G, C-J, C-I, H-G, H-J, H-I, G-J, G-I AND J-I, respectively.

I. INTRODUCTION

The application of recent molecular techniques has revolutionized the genetic analysis of poultry. The advent of polymerase chain reaction (PCR) has added a new dimension in the field of molecular biology (Mullis, 1990). It is characterized by high selectivity, sensitivity and fast speed. It is non-radioactive, easy to execute and require few simple reagents. There are many offshoots of PCR and one of the powerful techniques based on PCR is randomly amplified polymorphic DNA (RAPD) analysis (Williams *et al.*, 1990), which utilizes 8-10 mer synthetic oligonucleotides of random sequence as primers that can detect the amplification of several unrelated regions of the genome. The RAPD-PCR analysis has been used in genetic analysis of relatedness and diversity in chickens (Smith *et al.*, 1996) and for genetic characterization of inbred chicken lines (Plotsky *et al.*, 1995; Wei *et al.*, 1997). DNA Fingerprinting (DFP), another technique developed by Jeffreys *et al.* (1985), that involves southern blot hybridization with microsatellite DNA probes and yields specific fingerprint patterns, was first employed in poultry by Burke and Bruford (1987). It allows the evaluation of polymorphic DNA specific to individuals. Although DFP is powerful it involves high cost, is technically demanding and often requires use of radioisotopes which are hazardous to health. White Leghorn stock, brought to CARI, Izatnagar in 1972 from Israel and the USA, has been developed into 4 selected lines. A random bred control population has also been maintained since then. The selected lines have undergone 23 generations of selection for egg production. Presently, the age at sexual maturity (ASM) is 156.10 ± 0.25 , 135.25 ± 0.18 , 134.24 ± 0.43 , 133.63 ± 0.25 and 143.95 ± 0.23 in IWC, IWH, IWG, IWJ and IWI, respectively. The corresponding mean values for egg production up to 64 weeks of age are 152.5, 230.3, 215.4, 195.8 and 223.6, respectively (CARI Report, 2000).

However, these birds, which are amongst the best layers in the country, have not been analyzed by RAPD-PCR or DFP, the modern biotechnological tools, for evaluation of genetic biodiversity. Therefore, the present study was carried out to evaluate the genetic variations within and between lines for future exploitation in breeding programs.

II. METHODS

The White Leghorn stock of CARI, Izatnagar was brought from Israel and the USA in 1972. Five populations viz. 4 selected lines (IWG, IWH, IWI, IWJ), which have undergone selection for part-period egg production for 23 generations and one random bred control (IWC) were used in this study. The dams were selected on the basis of egg production up to 64 weeks of age and they were mated with males in the ratio of 1:5. In every generation 50 males and 250 dams were used as parents. The isolation of genomic DNA was done by the phenol extraction method (Kagami *et al.*, 1990). Purity and concentration were checked by spectrophotometry. The RAPD-PCR reaction was carried out using 11 primers that revealed polymorphic patterns. The products were then analyzed on 1.4% gel to obtain fingerprints. The 20 μ l reaction mix had 75 ng genomic DNA; 100 μ M each of dATP, dCTP, dTTP, dGTP; 1 μ M tetramethyl ammonium chloride (TMAC); 0.5 U Taq DNA polymerase; 2 μ l 10 X Taq polymerase buffer (500 mM KCl, 100mM Tris-HCl pH 8.8, 15 mM Mg Cl₂ 1% Triton X - 100). Only distinct and prominent bands were scored. The presence or absence of a band within the RAPD pattern was scored as one or zero, respectively. The methods of Nei *et al.* (1979), Lynch (1991) and Smith *et al.* (1996) were used for the determination of BSF and the estimation of genetic distance.

III. RESULTS

The PCR conditions were optimized and all the primers were observed to yield scorable amplified fragments in the individuals of control and selected lines. The molecular sizes of scorable amplified fragments ranged from 300 to 3100 bp, and products beyond this range were not considered in the analysis owing to their poor resolution.

(a) Band sharing frequency

The BSF, which is an indicator of the genetic similarities/relatedness among different lines (Plotskey *et al.*, 1995; Smith *et al.*, 1996), was calculated by considering only resolvable amplified products. The overall average within line BSF (pooled over primers) in each of the five lines along with between line BSF estimates are presented in Table 1. The BSF estimates were found statistically different from zero and one. The overall average within line BSF estimates ranged from 0.69 \pm 0.06 (IWI) to 0.67 \pm 0.05 (IWG). The average BSF estimates between lines ranged from 0.67 \pm 0.05 (IWH-IWI) to 0.68 \pm 0.05 (IWC-IWG).

Table 1. Average band sharing frequency estimates (pooled over primers) within lines (at diagonal) and between lines (above diagonal) in different WLH lines.

Lines	IWC	IWH	IWG	IWJ	IWI
IWC	0.79 \pm 0.05	0.67 \pm 0.045	0.68 \pm 0.05	0.71 \pm 0.04	0.66 \pm 0.05
IWH	-	0.74 \pm 0.06	0.65 \pm 0.05	0.68 \pm 0.04	0.67 \pm 0.05
IWG	-	-	0.67 \pm 0.05	0.69 \pm 0.05	0.60 \pm 0.05
IWJ	-	-	-	0.79 \pm 0.04	0.64 \pm 0.04
IWI	-	-	-	-	0.69 \pm 0.06

(b) Genetic distance

The within and between line BSF estimates were used to determine the genetic distance among lines (Lynch, 1991). The average genetic distance (pooled over primers) estimates among WLH lines are presented in Table 2.

Table 2. Average genetic distance between lines (pooled over primers).

Lines	IWC	IWH	IWG	IWJ	IWI
IWC	-	0.12±0.03	0.07±0.03	0.09±0.03	0.13±0.05
IWH	-	-	0.09±0.02	0.11±0.03	0.09±0.03
IWG	-	-	-	0.06±0.03	0.13±0.04
IWJ	-	-	-	-	0.14±0.03

IV. DISCUSSION

The size of the scorable bands ranged from 300 and 3100 bp, and the products beyond this range were not considered due to their poor resolution. Smith *et al.* (1996) also considered amplified products in the range 250-2500 bp. The medium to high estimates of between lines BSF represented the closer genetic relatedness among the lines (Plotsky *et al.*, 1995) taken in this study. It has been reported that the intra-inter population differences varied with the types of random primers used in the RAPD analysis (Smith *et al.*, 1996). The D estimates ranged from 0.06 ± 0.03 to 0.14 ± 0.03 . The IWI line appeared to be the genetically farthest from rest of the lines and, hence, can be utilized successfully to exploit the heterosis.

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SELECTION FOR THE COMPONENTS OF LEAN TISSUE GROWTH IN JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*).

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Summary

Seven lines of Japanese quail was selected to 30 d of age for two generations for either high growth rate (line HW), low feed conversion ratio (FCR) (line FE), high or low body fat (lines HF and LF), high or low breast yield (lines HB and LB), or at random (line C). Direct and correlated responses in the lines were as follows: HW line – an increase in growth rate and proportion of breast meat; FE line – a decrease in growth rate, FCR and body fat; HF line – an increase in FCR and body fat and a reduction in breast yield; LF line – an increase in FCR, but no effect on the other traits; HB line – a decrease in growth rate and an increase in FCR; LB line – an increase in growth rate, FCR and body fat and a decrease in breast yield.

I. INTRODUCTION

The efficiency with which lean tissue is deposited is an exceedingly important determinant of profitability in broiler production, but it is also a complex composite trait regulated by a large number of interacting physiological factors. Improvement in lean tissue deposition efficiency in commercial broiler breeding programs is typically addressed through within and between line selection for growth rate, feed utilisation efficiency for growth, and body composition in terms of a reduction in fatness and an increase in meat yield.

The purpose of the study described here was to determine the direct and correlated responses to selection for growth rate, feed utilisation efficiency, carcass fatness and breast meat yield in Japanese quail to facilitate the assessment of appropriate selection strategies for improvement in the efficiency of lean tissue growth rate.

II. THE SELECTION EXPERIMENT

Birds used in the base population from which the lines were selected, were derived from the unselected control line of an earlier selection experiment (Pym *et al.*, 1998). The base population was constituted from matings between 50 males each mated to three females. From each of the four hatches birds were selected as parents of the selection lines shown in table 1, with equal representation from the half- and full-sib families as potential parents for the lines.

Lines were constituted each generation from matings between 10 males each with three females. There were four weekly hatches each generation, the first was used to determine body composition for sib selection for high and low fatness, and for correlated responses in the other lines. The other three hatches were used to produce birds from which parents of the subsequent generation were selected. For the individual selection lines (HW, FE, HB and LB), approximately 120 birds of each sex were available in each line for selection each generation (the *i* values in males and females were approximately 1.8 and 1.3 respectively).

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Table 1. Selection lines used in the study.

Line	Selected for
HW	Increased liveweight at 35d of age
HF	Increased 35d abdominal fatness – by sib selection
LF	Decreased 35d abdominal fatness – by sib selection
FE	Decreased 14-30d FCR – in individual cages
HB	Increased breast meat yield (proportion) – by ultrasound
LB	Decreased breast meat yield (proportion) – by ultrasound
C	Unselected control

All birds were given a crumbled broiler starter diet containing 220 g CP and 12.5 MJ ME/kg from hatch to 35 d of age. Selection in the HW line was based on liveweight at 35 d of age. The birds were reared to 14 d in brooder cages and then transferred to deep litter pens where they were reared intermingled with the high and low breast yield groups. The birds were given supplementary artificial lighting to provide 16h of light per day between 04.00 and 20.00h. Following the 35 d weighing, the breast yield lines were measured for breast depth and area using a 5.0 MHz probe attached to an Aloka SSD-500 real-time ultrasound scanner. After freezing the image, the area of the breast muscle was circumscribed by the cursor and the area enclosed calculated. Data from both sides were combined and the total area was divided by the liveweight of the bird to provide a proportional measure.

Following group cage rearing to 14 d of age, as for the other lines, feed efficiency in the FE line was determined in individual cages from 14 to 30 d of age. Birds in hatch 1 of the high and low fat lines were reared to 35 d as for the HW line birds and, after weighing, two birds per sex per dam family were killed and the abdominal fat pad excised and weighed. For each bird this was expressed as a proportion of liveweight and the dam family averages within each line were ranked on this criterion. Birds in the subsequent two hatches from the selected dam families were retained for breeding.

To reduce the deleterious effects of inbreeding, for the high- and low-fat lines, a restriction was placed on the number of males (2) and females (3) from the same full sib family that could be selected as parents within each line. Apart from that birds were selected from the highest (line HF) or lowest (line LF) full sib families. For the other lines, to reduce inbreeding, no more than two males were selected from any one sire family. There was no restriction on females, but in all lines there were no full- or half-sib matings. All birds were pedigree wingbanded at hatch.

III. SELECTION RESPONSES

Responses to two generations of selection were determined in a hatch of about 290 birds reared in group cages to 14 d of age and then transferred to the single-bird cages and reared there to 30 d of age. Birds were weighed at 14 and 30 d of age and individual feed intake over the 14 to 30 d interval was measured. The birds were then killed by neck dislocation and abdominal fat and breast meat were excised and weighed. Line mean values for 30 d liveweight, 14-30 d FCR and 30 d abdominal fat proportion and breast proportion, are shown in Table 2.

IV. RESULTS AND DISCUSSION

Liveweight was significantly increased in the HW and LB lines but decreased in the FE and HB lines. The liveweight increase in the HW line is in keeping with expectations, but the reasons for the increase in the LB line and for the decrease in the FE and HB lines requires explanation. The divergent correlated response in liveweight in the HB and LB lines is likely a reflection of the use of liveweight as the denominator in the calculation of the selection index for breast yield, where the cross-sectional area of the breast muscle determined by ultrasound was the numerator. Since weight is essentially a three dimensional trait associated with volume, the weight correction of the measure appears to have been excessive and the consequence was that selection for high breast proportion favoured individuals with moderate breast muscle area but reduced body weight, whilst selection for low breast proportion favoured heavy individuals with low breast meat yield. The reduced 30 d liveweight in the FE line was probably associated with a correlated reduction in initial (14 d) liveweight at commencement of the feed intake measure which would have led to reduced maintenance requirements during the measurement period. The HF line was significantly heavier than the LF line suggesting a strong positive genetic correlation between liveweight and fatness, something not observed in other studies with quail (Pym *et al.*, 1998) or in chickens (Leclercq *et al.*, 1980; Whitehead and Griffin, 1984; Pym, 1987, 1990). The factors responsible for the response in these lines have not been elucidated.

Table 2. Line means (\pm SE) for 30 d liveweight (g), 14 to 30 d feed conversion ratio (FCR) and abdominal fat and breast muscle proportion (g/kg) at 30 d in the 7 lines after two generations of selection.

Line	n	Liveweight	FCR	Abdominal fat	Breast muscle
HW	44	197 (2.4) ^a	3.15 (0.078) ^b	9.35 (0.57) ^b	21.77 (0.24) ^a
FE	47	162 (1.9) ^e	2.80 (0.043) ^a	5.24 (0.41) ^a	21.29 (0.18) ^{ab}
HF	43	178 (2.1) ^c	3.62 (0.083) ^{cd}	13.66 (0.86) ^c	19.70 (0.30) ^c
LF	46	171 (2.3) ^d	3.44 (0.084) ^c	8.72 (0.61) ^b	21.39 (0.26) ^{ab}
HB	40	162 (2.5) ^e	3.39 (0.074) ^c	9.59 (0.81) ^b	20.95 (0.25) ^b
LB	46	186 (1.9) ^b	3.82 (0.069) ^d	12.54 (0.84) ^c	19.64 (0.23) ^c
C	45	176 (2.6) ^{cd}	2.97 (0.045) ^b	8.81 (0.57) ^b	21.02 (0.22) ^b

Means in the same column without a similar superscript differ significantly ($P < 0.05$).

The FCR was substantially reduced as a direct response in this trait to selection in the FE line but not reduced, as might be expected, in the HW line. The LF line birds were marginally, but not significantly, more efficient than their HF counterparts. Most other studies (e.g. Whitehead and Griffin, 1984; Leclercq, 1988; Cahaner *et al.*, 1986; Pym, 1987) have shown the fat line birds to be less efficient than their lean line counterparts. The reasons for this discrepancy are likely related to the poor growth rate in the LF line in the present study.

It is interesting to note that the FE line birds were actually leaner than the LF line birds. This may relate in part to the relative efficiency of individual versus sib-selection but supports the studies in chickens by Pym (1990), Leenstra (1988) and Chambers (1987) which show considerable reduction in fatness in birds selected for improved feed efficiency and reflects the high energetic cost of fat deposition and the low moisture content of fatty tissue. Overall, fatness was increased in the HF and LB lines, and reduced in the FE line. The increased fatness in the low breast proportion line is in agreement with the correlated

responses obtained in an earlier selection experiment with Japanese quail (Pym *et al.*, 1998) although the underlying reasons have not been investigated.

There was a significant increase in breast yield only in the HW line but a significant decrease in the HF and LB lines. The lack of a positive response in the HB line suggests that the proportional liveweight adjustment applied to the ultrasound measure in deriving the selection criteria for high and low breast yield was inappropriate and that selection, in the upwards direction at least, was ineffective. The increase in breast yield in the HW line was not in agreement with an earlier report (Pym *et al.*, 1998), where such selection had no effect on breast proportion. The reason for the discrepancy between the two reports is not immediately apparent. The reduction in breast proportion in the HF line suggests a negative genetic association between fatness and breast yield in this line which is supported by the correlated responses in fatness in the high and low-breast yield lines.

Inferences that can be drawn from the results are limited by the relatively few generations of selection undertaken thus far. Fortunately, using Japanese quail it is possible to measure up to four generations per year, and results to six generations of selection will be reported at the next symposium.

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EVALUATION OF COMBINING ABILITY OF NATIVE BREEDS WITH SYNTHETIC BROILER LINES FOR LEAN MEAT USING A PARTIAL DIALLEL MATING SCHEME

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Summary

The present study was aimed at evaluating various crosses under a partial diallel cross for a number of blood lipid and cholesterol parameters to develop a suitable cross for production of lean broilers in tropical environments. Three native (set I males) and 3 exotic (Set II females) populations were used. Least square analysis of variance revealed a significant effect of genetic groups on all the parameters studied, whereas sex and its interaction with genetic groups were non-significant. Analyses of variances (two models) to determine combining abilities revealed significant general combining ability for set I and non-significant specific combining ability in both sets for total blood cholesterol and very low density lipoproteins (VLDL).

I. INTRODUCTION

In India the broiler industry is transforming at an incredible pace from an age-old backyard vocation to a dynamic mechanized industry with an annual production of 540 million birds in 1998 compared with 4 million in 1971. The positive growth of the broiler industry has been largely due to the availability of improved germplasm, balanced feed, optimum management and sound health. The rapid growth has introduced two major negative consequences; greater susceptibility to environmental stress and increased fat deposition. India, being a tropical country, has great seasonal fluctuations of temperature and humidity and needs to produce and develop stock that are best suited to a tropical environment. Crossing of native breeds with fast growing exotic populations has shown promising results in lowering the plasma cholesterol level and improving tropical adaptability (Singh *et al.*, 1998). In a crossbreeding program identification of superior crosses is of utmost importance (Goto and Nordskog, 1959).

The partial diallel cross mating system is suitable for estimation of combining abilities among crosses (Kempthorne and Curnow, 1961). In the present study a partial diallel cross using 6 purebred populations was carried out with the objective of evaluating the blood lipid and cholesterol levels in crossbred and purebred progenies. Six purebred populations, namely P1: Aseel (AS), P2: Kadakanath (KN) and P3: Frizzle (FZ) as set I (males) and P4: Coloured Synthetic Female Line (CSFL), P5: Naked Neck Line (NNL) and P6: Synthetic Dam Line (SDL) as set II (females) maintained at Central Avian Research Institute, Izatnagar, India, were utilized in the partial diallel cross experiment.

II. METHODS

The partial diallel cross was planned with nine crossbred genetic groups viz. P1xP4, P1xP5, P1xP6, P2xP5, P2xP6, P3xP4, P3xP5, P3xP6. The cross combination were referred as AS x CSFL-1x 4, AS x NNL-1x5 and so on. The SDL purebred (6x6) was maintained as control. Twenty-one sires from each male line and 120 dams from each female line were chosen and utilized in the ratio of 7 sires to 40 dams for each cross. Standard broiler managemental practices were followed.

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The blood samples were analyzed from 14 birds (7 of either sex) from each genetic group at 42 d of age for total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, low density lipoproteins (LD) and VLDL. The total cholesterol and HDL cholesterol were analyzed by the CHOD/POD method using a diagnostic kit (Beacan Diagnostics Pvt. Ltd., India). The triglycerides were analyzed by the GPO-PAP method using a kit supplied by Human Gesellschaft für Biochemical and Diagnostica mbH, Germany. The VLDL cholesterol and LDL cholesterol were analyzed using Friedwald's method (Friedwald, 1972). The least square analysis of variance was carried out using the following model:

$$Y_{ijk} = \mu + G_i + C_j + (G \times C)_{ij} + e_{ijk}$$

Where, Y_{ijk} = observation on the k^{th} individual of the j^{th} sex in the i^{th} genetic group.

μ = Overall population mean, G_i = the effect of i^{th} genetic group ($i=1..9$), C_j = the effect of j^{th} sex ($j=1,2$), $(G \times C)_{ij}$ = the interaction between i^{th} genetic group and j^{th} sex and e_{ijk} = random error with NID ($0, \sigma_e^2$). Analyses of variance for combining ability were performed using two models: A (Kempthorn and Curnow, 1961) and B (Wolf *et al.*, 1991-combining ability model type 4).

III. RESULTS

Least squares analyses of variance revealed that genetic groups had highly significant ($P < 0.01$) effects on all the blood lipid and cholesterol parameters, whereas sex and its interaction with genetic groups were found to be non-significant for all the traits (Table 1). The least squares means for the blood lipid and cholesterol parameters are given in Table 2.

Table 1. Least-squares analysis of variance for blood lipid and cholesterol parameters.

Source of variation	d.f.	Mean sum of squares				
		Total cholesterol	HDL-cholesterol	Triglycerides	VLDL	LDL
Genetic gps (G)	8	4102.31**	859.83**	4113.82**	164.57**	2413.76**
Sex	1	241.36	734.14	1513.41	60.56	347.80
G x Sex	8	979.57	222.47	712.82	28.50	1218.33
Error	108	860.69	215.65	471.28	18.85	856.03

** $P < 0.01$.

Table 2. Least squares estimates (mean \pm se) for blood lipid and cholesterol parameters (mg/100 mL) in the different genetic groups at 42 days of age.

Genetic group	Total Cholesterol	HDL Cholesterol	Triglycerides	VLDL	LDL
1x4	143.71 \pm 7.84 ^a	72.31 \pm 3.92 ^a	62.50 \pm 5.80 ^{abc}	12.50 \pm 1.16 ^{abc}	59.46 \pm 7.82 ^{abc}
2x4	188.47 \pm 7.84 ^c	91.53 \pm 3.92 ^{bc}	95.27 \pm 5.80 ^d	19.05 \pm 1.06 ^d	77.88 \pm 7.82 ^{bc}
3x4	151.93 \pm 7.84 ^a	78.31 \pm 3.92 ^{ab}	82.46 \pm 5.80 ^{cd}	16.49 \pm 1.16 ^{cd}	57.13 \pm 7.82 ^{abc}
1x5	144.54 \pm 7.84 ^a	82.70 \pm 3.92 ^{abc}	45.42 \pm 5.80 ^a	9.08 \pm 1.16 ^a	52.76 \pm 7.82 ^{ab}
2x5	181.73 \pm 7.84 ^{bc}	80.35 \pm 3.92 ^{ab}	94.08 \pm 5.80 ^d	18.82 \pm 1.16 ^d	82.57 \pm 7.80 ^c
3x5	171.27 \pm 7.84 ^{abc}	86.46 \pm 3.92 ^{abc}	57.31 \pm 5.80 ^{ab}	11.46 \pm 1.16 ^{ab}	73.35 \pm 7.82 ^{bc}
1x6	145.05 \pm 7.84 ^a	74.79 \pm 3.92 ^a	62.72 \pm 5.80 ^{abc}	12.54 \pm 1.16 ^{abc}	57.72 \pm 7.82 ^{abc}
2x6	171.05 \pm 7.84 ^{abc}	80.55 \pm 3.92 ^{ab}	82.25 \pm 5.80 ^{cd}	16.45 \pm 1.16 ^{cd}	74.05 \pm 7.82 ^{bc}
3x6	154.09 \pm 7.84 ^{ab}	96.81 \pm 3.92 ^c	68.89 \pm 5.80 ^{bc}	13.78 \pm 1.16 ^{bc}	43.50 \pm 7.82 ^a
SE	7.84	3.92	5.80	1.16	7.82
μ	161.32 \pm 2.61 ^x	82.64 \pm 1.31 ^x	72.32 \pm 1.93 ^x	14.46 \pm 0.39 ^x	64.27 \pm 2.61 ^x
6x6	226.18 \pm 7.84 ^y	99.72 \pm 3.92 ^y	108.95 \pm 5.80 ^y	21.79 \pm 1.16 ^y	104.67 \pm 7.82 ^y

Means in the same column without a similar superscript differ significantly ($P < 0.05$).

Applying Model A, analysis of variance for combining abilities of total blood cholesterol and VLDL (Table 3) revealed highly significant ($P < 0.01$) variation due to general combining ability (GCA). Applying Model B, the variation due to GCA was significant ($P < 0.01$) only for set I (Table 4). This indicated that the variation due to additive gene effects among the synthetic broiler genetic groups of set II was low and non-significant. The analysis also revealed a non-significant specific combining ability (SCA) for both total blood cholesterol and VLDL using both Models A and B, which suggests that additive is more important than non-additive genetic variance in the inheritance of blood cholesterol and VLDL parameters.

Table 3. Analysis of variance for total blood cholesterol (mg/100mL) and VLD (mg/100mL) using partial diallel design Model A (Kempthorne and Curnow, 1961).

Source of variation	d.f	Mean sum of squares	
		Total blood cholesterol	VLDL
GCA	5	450.74**	19.11**
SCA	3	76.82	2.17
Pooled error	108	61.48	1.35

** $P < 0.01$.

Table 4. Analysis of variance for total blood cholesterol (mg/100mL) and VLDL (mg/100mL) using partial diallel design Model B (Wolf *et al.*, 1991).

Source of variation	d.f	Mean sum of squares	
		Total blood cholesterol	VLDL
GCAI	2	737.21**	25.99**
GCAII	2	46.79	1.81
SCA	9	28.36	1.34
Pooled error	108	61.48	1.35

** $P < 0.01$.

Table 5. Estimates (mg/100 mL) and standard errors of different crossbreeding parameters using partial diallel Model A (Kempthorne and Curnow, 1961).

Parameter	Sign	Estimates \pm SE	
		Total cholesterol	VLDL
Overall mean	μ	161.32 \pm 4.65	15.02 \pm 0.69
GCA	g1	-13.49 \pm 12.48	-2.52 \pm 2.66
	g2	26.99 \pm 12.48	5.05 \pm 2.66
	g3	-3.33 \pm 12.48	-0.83 \pm 2.66
	g4	-3.33 \pm 12.48	0.98 \pm 2.66
	g5	-3.36 \pm 12.48	-2.75 \pm 2.66
	g6	-3.48 \pm 12.48	0.07 \pm 2.66
SCA	s14	-0.77 \pm 3.92	-0.43 \pm 0.91
	s24	3.50 \pm 3.92	-1.44 \pm 0.91
	s34	-2.72 \pm 3.92	1.87 \pm 0.91
	s15	0.07 \pm 3.92	-0.11 \pm 0.91
	s25	-3.21 \pm 3.92	2.05 \pm 0.91
	s35	16.64 \pm 3.92	0.58 \pm 0.91
	s16	0.70 \pm 3.92	0.53 \pm 0.91
	s26	-13.78 \pm 3.92	-3.13 \pm 0.91
	s36	-0.42 \pm 3.92	0.07 \pm 0.91

Table 6. Estimates (mg/100 mL) and standard errors of different crossbreeding parameters using partial diallel Model B (Wolf *et al.*, 1991).

Parameter	Sign	Least squares estimates \pm SE	
		Total Cholesterol	VLDL
Overall mean	μ	161.32 \pm 4.65	15.02 \pm 0.69
GCA(Set I)	g1	-16.88 \pm 5.00 ^a	-3.65 \pm 0.74 ^a
	g2	19.10 \pm 5.00 ^b	3.09 \pm 0.74 ^b
	g3	-2.22 \pm 5.00 ^a	0.56 \pm 0.74 ^b
GCA(Set II)	g4	0.05 \pm 5.00	0.99 \pm 0.74
	g5	4.53 \pm 5.00	-0.23 \pm 0.74
	g6	-4.59 \pm 5.00	-0.76 \pm 0.74
SCA	s14	-0.78 \pm 6.13	0.13 \pm 0.91
	s24	7.80 \pm 6.13	-0.05 \pm 0.91
	s34	-7.22 \pm 6.16	-0.08 \pm 0.91
	s15	-4.42 \pm 6.13	-2.06 \pm 0.91
	s25	-3.22 \pm 6.13	0.95 \pm 0.91
	s35	7.64 \pm 6.13	1.12 \pm 0.91
	s16	5.20 \pm 6.13	1.94 \pm 0.91
	s26	-4.78 \pm 6.13	-0.90 \pm 0.91
	s36	-0.42 \pm 6.13	-1.04 \pm 0.91

Means within a parameter with a similar superscript do not differ significantly ($P < 0.05$).

IV. DISCUSSION

The KN population had the highest estimate of GCA from both the Models for total cholesterol and VLDL. The AS population had the lowest estimate of GCA with both Models for total cholesterol while the NNL population in Model A and the AS population in Model B had the lowest estimate of GCA for VLDL. Using Model A, the crosses 3x5 and 2x5 had the highest estimates of SCA for total cholesterol and VLDL, respectively, and cross 2x6 had the lowest estimate of SCA for both the traits. Using Model B, the crosses 2x4 and 1x6 had the highest estimates of SCA for total cholesterol and VLDL, respectively, whereas crosses 3x4 and 1x5 had the lowest estimates of SCA for total cholesterol and VLDL, respectively.

The present mean values were found to be comparable with the findings of Darshan *et al.* (1987) and Settar *et al.* (1998). Overall, the AS x NNL population was identified as the best crossbred genetic group for lean meat production with better tropical adaptability.

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LYSINE REQUIREMENTS OF BROILER BREEDER HENS

C. FISHER¹, R. GOUS² and L. GODDARD²

Summary

Lysine dose-response experiments were done with caged broiler breeder hens at 26, 37, 48 and 60 weeks of age. Requirements, estimated from fitted asymptotic response curves for egg output, were 864, 859, 763 and 687 mg true faecal digestible lysine at respective ages. Extrapolation of response curves indicated a maintenance lysine requirement close to zero. Measured and calculated estimates of the efficiency with which dietary lysine was utilised for egg production were much lower than comparable data from egg laying hens. The results are discussed in relation to the calculation of amino acid requirements for broiler breeders.

I. INTRODUCTION

Fisher (1998) discussed the calculation of amino acid requirements of broiler breeder hens from specified levels of production. The model used considered egg mass, growth, maintenance and variation amongst individual hens as determinants of requirements. Amino acid utilisation coefficients for egg production were considered to be related to rate of lay and, thus, declined with the age of the flock.

An important conclusion was that requirements (as percent of the feed) for a flock being fed and producing as specified in a commercial manual (Ross Breeders Limited, 1995) reached a maximum requirement at 55 weeks of age and not at peak production as commonly supposed. This result arises from the combined effects of changes in egg output, efficiency of amino acid utilisation and rate of feeding.

Fisher (1998) concluded that questions about amino acid utilisation and about the lysine requirement for maintenance required further investigation. The current paper reports an experiment carried out by one of us (LG) to measure the responses of broiler breeder hens of different ages to dietary lysine. This experiment was not designed specifically to address these questions but the results are discussed in that context.

II. METHODS

Groups of broiler breeder hens (Ross) were housed in cages for four 9-week assays starting at 26, 37, 48 and 60 weeks of age (ages 1 to 4, respectively). In each assay 10 feeds were given, comprising 5 lysine levels x 2 energy levels. Supplementation of one feed in the first assay tested whether lysine was the first limiting amino acid. Each feed was made by mixing a summit diet (lysine 1.2 times, other amino acids 1.5 times, the calculated requirement) with a virtual protein-free dilution mixture. High (HE:13.1MJ/kg) and low (LE:10.8MJ/kg) energy (AMEn, calculated) summit and dilution diets were used (see Table 1). Lysine levels were 1.2, 1.0, 0.8, 0.6 and 0.4 times the calculated requirement in the first 2 assays. In the later assays levels ranged from 1.0 to 0.2 times requirement. Analysis of feeds for energy, digestible amino acids (both determined in adult cockerels by tube feeding as described by McNab and Fisher (1984)), protein, calcium and phosphorus confirmed the calculated levels except for ME. These ranged from 12.8 to 11.1 MJ/kg in the LE series and from 14.4 to 11.7 MJ/kg in the HE series. Data below are presented as true faecal digestible

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(TFD) lysine from the determined figures. The experiment was carried out in a light-tight, fan-ventilated house with a daily 16L:8D light regime throughout. Feed intakes at the start of the respective assays were 185, 178, 175 and 155 g/bird/day. All experimental feeds were fed at 160 g/bird/day with residues being accumulated, weighed back and discarded weekly.

III. RESULTS

Full results of this experiment are available in Goddard (1997). During all trials a total of 4.3% birds died, mostly in the final assay and mostly on the HE feeds. Adding lysine to the 0.4 times requirement level assay 1 increased egg output from 26.7 to 30.5 g/day ($P_t < 0.01$), thus confirming that lysine was the first limiting amino acid. In what follows all results refer to the last 4 weeks of each 9-week assay.

Table 1. Composition of high energy (HE) and low energy (LE) of summit and dilution diets (g/kg).

Ingredient	LE summit	HE Summit	LE Dilution	HE Dilution
Maize	436.77	436.77	-	-
Maize Gluten ml 60	100.00	100.00	-	-
Sunflower ml 37	210.67	210.67	-	-
Soybean ml 48	69.08	69.08	-	-
Sand	43.30	10.89	132.31	94.89
Oil	59.65	92.06	60.00	60.00
Sugar	-	-	295.13	332.55
Starch	-	-	295.13	332.55
Sunflower husks	-	-	132.31	94.89
Vits and Mins	80.53	80.53	85.13	85.13
Analysis (calculated)				
CP (Nx6.25), g/kg	220.50	220.50	8.00	5.60
AMEn, MJ/kg	11.88	13.10	11.88	13.10
Total lysine, g/kg	6.7	6.7	0	0

Table 2. Feed (g/bird/day) and energy (ME, kJ/bird/day) intakes.

Energy	Lys.	Age 1		Age 2		Age 3		Age 4	
		Feed	ME	Feed	ME	Feed	ME	Feed	ME
Low	7.06	154	1970	158	2020	-	-	-	-
Low	6.69	158	1960	159	1980	160	1990	141	1750
Low	5.04	160	1940	159	1920	157	1900	151	1830
Low	3.91	156	1840	154	1820	152	1790	153	1800
Low	2.52	140	1600	140	1600	140	1600	132	1510
Low	1.74	-	-	-	-	103	1140	97	1080
High	7.12	148	2130	159	2280	-	-	-	-
High	6.20	157	2170	150	2080	158	2190	148	2050
High	5.13	158	2110	158	2100	158	2100	148	1970
High	3.69	154	1960	152	1930	153	1900	142	1810
High	2.58	117	1430	121	1490	127	1560	115	1410
High	1.50	-	-	-	-	87	1020	46	1000

Energy determinations showed that the ME contents of the dilution mixtures were lower than the summit feeds leading to a range of energy values as indicated above. These variations in dietary energy are inevitably correlated with lysine. The combination of dietary ME, fixed feeding levels and weekly refusals yielded the mean feed and energy intakes shown in Table 2. The extent to which energy intake may have limited output or modified the response to lysine cannot be determined but must be borne in mind when interpreting the results of this experiment.

Figure 1 shows the response of egg output to lysine intake at each age. The 4 fitted asymptotic curves (one per age, pooled for energy level) were fitted by the model described by Curnow (1973) – the so-called Reading Model.

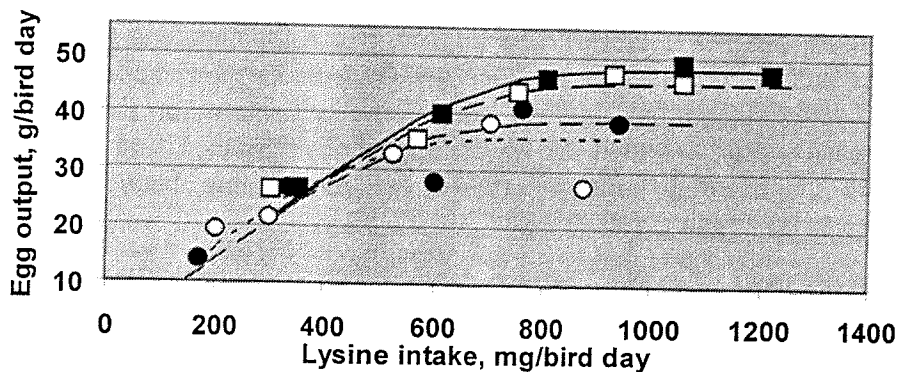


Figure 1. Response of egg output to lysine intake at four ages. Data points are shown only for age 1 (■ - LE; □ - HE) and age 4 (● - LE; ○ - HE). The asymptotic values for the 4 curves decline in order from age 1 to age 4.

The asymptotic values of the curves (48.1, 45.6, 38.9 and 36.9 g/bird/day for ages 1-4 respectively) are slightly higher than commercial standards (Ross Breeders Limited, 1995). Extrapolation of the curves to zero egg output indicates a 'maintenance' requirement for lysine close to zero ('b' coefficients of the Reading Model = 0.00, 0.02, 0.01, 0.01 mg/kg bodyweight). The 'a' coefficient of the Reading Model, which determines the slope of the line above maintenance and is expressed as mg lysine per g egg, was 14.04, 14.25, 14.23 and 12.19 mg for ages 1 to 4 respectively. Assuming egg contains 8.3 mg lysine per gram these estimates of the 'a' coefficient indicate efficiencies of utilisation of 0.59, 0.58, 0.58 and 0.68 respectively. These are very much lower than the values estimated for laying hens; 0.79 for total lysine (MacDonald and Morris, 1985) or about 0.83 for 'available' lysine (Fisher, 1994). For broiler breeders, Bowmaker and Gous (1991) estimated efficiency for 'available' lysine to be 0.47, similar to the present study.

IV. DISCUSSION

It is difficult to ascribe any precise biological meaning to the concept of amino acid requirements for maintenance when these are estimated by extrapolation of response curves. However, the data from the present experiment lead consistently to a very low estimated maintenance requirement. This is in agreement with some early concepts and with the experiments of Leveille and Fisher (1959). Similar estimates for laying hens are in striking contrast; MacDonald and Morris (1985) reported a pooled value of 79.5 mg/kg. For broiler breeders, Bowmaker and Gous (1991) reported a value of 11.2 mg/kg. Direct studies of lysine maintenance requirement in broilers by Edwards *et al.* (1999) showed a 13-fold difference in

the estimate for zero protein as opposed to zero lysine accretion. These authors' preferred estimate was 89.1 mg/d/kg^{0.75} based on lysine accretion, a figure which yields 68 mg/d/kg for a 3 kg broiler breeder. This is in the range indicated in laying hen studies. Fisher (1998) related maintenance lysine to body protein. The assumptions used give requirements per kg bodyweight of about 85 and 90 mg/d/kg. These now seem considerably too high for broiler breeders although it remains uncertain as to what is the most defensible estimate.

In calculating amino acid requirements Fisher (1998) related efficiency of amino acid utilisation to rate of lay. Using the asymptotic rates of lay observed at each age in this experiment the equation used in the predictions gives efficiencies of 0.82, 0.76, 0.59 and 0.57 for ages 1-4 respectively. These values are higher than those derived from the response curves (see above) in the early stages of lay but about the same in the older birds.

Amino acid utilisation was measured directly in each bird from the equation: utilisation = (lysine deposited in egg)/(lysine intake - lysine for maintenance). Lysine in egg was corrected for measured yolk and albumen weight but constant chemical compositions were assumed. Lysine deposition in shell and lysine utilisation for tissue growth were ignored. Maintenance was assumed to be 2mg/kg bodyweight. Using the broken-stick regression model suggested by Fisher (1994), the relationship between efficiency of utilisation and rate of lay (eggs per day) was:

$$\text{Efficiency} = 0.0938 + 0.759 (\text{rate of lay} < 0.5) = 0.512 (\text{rate of lay} > 0.5) (R^2 = 0.45)$$

The inflection point at rate of lay = 0.5 agreed with the suggestion of Fisher (1994) for laying hens. However the asymptotic efficiency of 0.512 is much lower than the value of 0.8-0.83 indicated by similar calculations in laying hens. In these data there was no indication that efficiency increased as rate of lay varied between 0.5 and 1.0 eggs per day. This suggests that the difference between layers and breeders is not simply a reflection of the difference in intensity of lay. The mean efficiencies for birds laying more than 0.5 eggs per day were 0.45, 0.45, 0.45 and 0.49 for ages 1-4 respectively. The corresponding figures for all birds, irrespective of rate of lay, were 0.42, 0.40, 0.36 and 0.33.

The 'requirements' of these birds, calculated from the fitted response curves in Figure 1 at 0.99 times the asymptotic output value, were 864, 859, 763 and 687 mg/day. Fisher (1998) suggested a requirement of 1037 mg/day at 31 weeks, roughly equivalent in age and output to the birds at age 1 in the present experiment. It is clear that, compared to the assumptions used by Fisher (1998) the experiment suggests a maintenance requirement that is very much smaller and an efficiency that is also lower. The former tends to reduce the calculated requirement, the latter to increase it.

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THE EFFECT OF WHOLE WHEAT, GROUND WHEAT AND DIETARY ENZYMES ON PERFORMANCE AND GASTRO-INTESTINAL MORPHOLOGY OF BROILERS

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Summary

Broiler chickens were offered commercially formulated diets containing 200 g/kg whole wheat with or without enzyme supplementation. Bird performance and proportional gastro-intestinal weights were recorded as well as bird mortality and the presence or absence of proventricular dilatation. Birds offered whole wheat had larger ($P < 0.05$) gizzards and higher ($P < 0.001$) levels of proventricular dilatation than birds offered ground wheat. No differences in bodyweight were apparent at 42 d of age. Feed conversion ratio was not affected by whole wheat inclusion and was improved in one experiment by exogenous enzyme supplementation.

I. INTRODUCTION

It is now recognised (Croom *et al.*, 1999) that absorption of nutrients is the rate limiting factor in optimising bodyweight gain and feed conversion ratio (FCR) of broilers. This is confounded by broilers having an immature (Croom *et al.*, 1999) and shorter (Cherry *et al.*, 1987) gastro-intestinal tract with a limited digestive capability (Nir *et al.*, 1978).

Currently, exogenous enzymes are commonly added to broiler diets to overcome gastro-intestinal immaturity and dietary ingredient deficiencies, although responses may not be consistent (Bedford, 1997) and may be related to differences in digestive tract development (Peterson *et al.*, 1999) or to differences in diet form or particle size.

Proventricular hypertrophy and poor gizzard development have been linked to the use of diets containing finely ground (Jones and Taylor, 2000), rather than coarse, grain. Coarse fibrous particles may lead to the enhanced development of the gastro-intestinal system allowing improved nutrient absorption and digesta motility (Williams *et al.*, 1997). Similarly, there is increasing evidence to suggest that the use of whole grain is beneficial to the bird's health (Belyavin, 1993).

The experiments reported here examined the effect of whole wheat inclusion in commercially formulated sorghum- or wheat- based diets on bird performance, gastro-intestinal development and mortality.

II. METHODS

Wheat (a commercial weather damaged durum:bread wheat (60:40) mix) was incorporated (200 g/kg) into sorghum/soybean/meatmeal-based starter and grower mixes (Experiment 1) or into wheat/soybean/meatmeal-based starter and grower mixes (Experiment 2), described in Table 1. The experiments were of a 2x2 factorial, randomised block design, each with 8 replicates per treatment. The wheat was added as either whole or as finely hammermilled grain and an exogenous feed enzyme (Avizyme 1302; 0.5 g/kg, Experiment 1 or Allzyme PT; 1.0 g/kg, Experiment 2) was either added to, or omitted from, each diet. After

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mixing, the diets were cold pelleted through a 4 mm die and were offered *ad libitum* to replicates of eight male broilers from 5 to 42 d of age housed in wire-mesh cages in continuously illuminated, environmentally-controlled rooms, initially maintained at approximately 33°C and reduced to 22°C by 28 d of age. The birds were weighed at the commencement of each experiment and at 21 and 42 d of age. At 42 d of age, three broilers, randomly selected from within each replicate group, were euthanased and dissected. Proventriculus, gizzard and intestinal segment weights were determined to elicit each organ's response to the diets offered. Mortalities during the course of each experiment and the number of birds dissected which showed dilatation of the proventriculus were also recorded.

Table 1. Composition (g/kg) of experimental diets.

Ingredient	Experiment 1		Experiment 2	
	Starter	Grower	Starter	Grower
Sorghum	453.9	435.2	--	--
Soybean meal	250.0	250.0	187.2	208.8
Wheat (ground)	--	--	457.5	465.1
Wheat (whole)	200.0	200.0	200.0	200.0
Meat meal	64.2	64.6	69.7	65.7
Tallow	12.1	30.0	30.8	36.7
Millrun	--	--	11.4	--
Vitamin/mineral premix	5.0	5.0	5.0	5.0
DL-methionine	3.4	3.4	2.9	2.2
Vegetable oil	2.5	4.3	5.0	5.5
Sodium bicarbonate	2.3	1.7	3.5	2.3
Potassium carbonate	--	--	3.5	2.8
Limestone	2.1	1.9	0.9	1.9
Salt	1.9	2.3	0.8	1.3
L-lysine HCl	1.9	0.8	3.2	1.3
Choline chloride	0.7	0.7	0.3	0.5
L-threonine	--	--	0.5	--
<i>Calculated analysis</i>				
Metabolisable energy (MJ/kg)	12.14	12.50	12.10	12.26
Crude protein (g/kg)	222	220	214	217

The production and organ weight data were treated by analysis of variance and mortality data by Cox's Proportional Hazards Regression (Cox, 1972). The proventricular dilatation scores (binary data) were tested by comparing the change in deviance due to each treatment contrast with the critical region of the X^2_1 distribution.

III. RESULTS

Bird weight was not affected by the inclusion of whole wheat in the bird's diet in either experiment (Tables 2 and 3). Feed conversion efficiency was not affected by the form

of the grain presented in either experiment but was influenced by the inclusion of exogenous enzymes in Experiment 2.

Table 2. Performance (bodyweight and feed conversion ratio (FCR)) and organ weights of 42-d old broilers offered ground or whole wheat in a sorghum basal diet with or without added Avizyme 1302 (Experiment 1).

Wheat	Enzyme	Weight (g)	FCR (g:g)	Prov ¹ (g/kg)	Gizzard (g/kg)	Duodenum (g/kg)	Jejunum (g/kg)	Ileum (g/kg)
Ground	--	2339	1.815	4.4	12.6	7.0	11.1	7.9
	+	2356	1.778	4.3	12.6	6.9	11.3	8.3
Whole	--	2309	1.844	4.1	14.1	7.0	11.3	8.8
	+	2405	1.812	3.8	13.8	7.0	10.5	8.0
Probability								
	Form (F)	NS	NS	NS	***	NS	NS	NS
	Enzyme (E)	NS	NS	NS	NS	NS	NS	NS
	F x E	NS	NS	NS	NS	NS	NS	**

¹ Proventriculus; ** P<0.01; *** P<0.001; NS not significant.

Table 3. Performance (body weight and feed conversion ratio (FCR)) and organ weights of 42-d old broilers offered ground or whole wheat in a wheat basal diet with or without added Allzyme PT (Experiment 2).

Wheat	Enzyme	Weight (g)	FCR (g:g)	Prov ¹ (g/kg)	Gizzard (g/kg)	Duodenum (g/kg)	Jejunum (g/kg)	Ileum (g/kg)
Ground	--	2603	1.742	5.7	11.3	8.0	10.3	9.0
	+	2635	1.708	4.7	11.8	7.7	10.1	8.4
Whole	--	2606	1.739	4.2	12.2	7.7	9.7	8.1
	+	2550	1.724	4.0	12.7	7.7	10.0	8.3
Probability								
	Form (F)	NS	NS	**	*	NS	NS	NS
	Enzyme (E)	NS	**	NS	NS	NS	NS	NS
	F x E	NS	NS	NS	NS	NS	NS	NS

¹ Proventriculus; * P<0.05; ** P<0.01; NS not significant.

Table 4. Total mortalities and the number of broilers showing proventricular dilatation at 42 d of age in Experiments 1 and 2.

Wheat	Enzyme	Experiment 1		Experiment 2	
		Mortalities	Dilatation	Mortalities	Dilatation
Ground	--	10	10	3	18
	+	5	11	5	17
Whole	--	4	3	4	8
	+	6	3	2	7
Probability					
	Form (F)	NS	***	NS	***
	Enzyme (E)	NS	NS	NS	NS
	F x E	NS	NS	NS	NS

*** P<0.001

The relative size of the gizzard (g/kg bodyweight) was increased in both trials by the inclusion of whole wheat. The other organs measured showed inconsistent responses to the whole wheat inclusion, typified by the response of the proventriculus which decreased in proportion to bodyweight in the second trial only (Table 3). There was no effect of enzyme inclusion on the size of the organs measured although a feed x enzyme interaction on ileal weight was observed in Experiment 1. There were no significant differences between treatments in bird mortality in either trial (Table 4). However, the numbers of birds exhibiting proventricular dilatation was greater ($P < 0.001$) in the ground wheat treatments.

IV. DISCUSSION

The gastro-intestinal weights and bodyweight data reflect those from previous work (Jones and Taylor, 2000) with whole triticale grain. However, FCR was not improved by the use of whole wheat in the experiments reported here and may be ascribed to the use of a blend of weather damaged, durum and bread wheats. Preston *et al.* (2000) showed an improvement in FCR when feeding whole wheat. They ascribed this to an improvement in AME, indicating that the technique of feeding part of the grain component of the diet as whole, rather than ground, grain is effective in offsetting the problems of 'low AME' wheat. The improvement seen is due to the enhanced development of the gastro-intestinal tract and better absorption of nutrients through increased digesta motility (Williams *et al.*, 1997). The higher level of proventricular dilatation in birds fed the ground diet, and the consequent lack of digesta movement, may indicate higher levels of fermentation of feed prior to acid digestion, which may lead to decreases in performance, particularly with 'problem' wheats.

V. ACKNOWLEDGEMENTS

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ILEAL STARCH DIGESTIBILITY OF NORWEGIAN LOW-AME WHEATS IS IMPROVED BY ENZYME SUPPLEMENTATION, FEED FORM AND DILUTION WITH CELLULOSE

B. SVIHUS and H. HETLAND

Summary

In a series of experiments with 5 varieties of wheat harvested on two locations in two years, a consistent low AME-value was observed when cold-pelleted diets containing 770 g/kg wheat was fed to young broiler chickens. Analysis of ileal contents revealed that all diets gave starch digestibility coefficients lower than 0.78. Enzyme supplementation raised starch digestibility to an average value of 0.92. In a follow-up experiment, ileal starch digestibility of a wheat-based diet increased significantly from 0.79 to 0.95, 0.93 and 0.91, respectively, when the diet was crushed and fed in a mash form, was diluted with cellulose, or when part of the wheat was fed as whole grain. These results indicate that an overload of wheat starch in the digestive tract may be the cause of poor availability of the starch for broilers. Grinding of the wheat may also influence starch digestibility.

I. INTRODUCTION

Wheat is a major ingredient in broiler diets in many countries and, due to its high protein and starch contents, it is considered to be a good source of nutrients for broilers. A negative effect of soluble non-starch polysaccharides (NSP) has been documented, but this problem can be alleviated by addition of fibre-degrading enzymes. Digestibility studies with wheat usually reveal a starch digestibility between 0.93 and 0.98 (Choct *et al.*, 1999; Steinfeldt *et al.*, 1998; Edney *et al.*, 1989; Yutse *et al.*, 1991; Annison, 1990). However, starch digestibilities lower than 0.82 have been reported in some Australian studies (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Choct *et al.*, 1995). These low starch digestibilities have not been observed elsewhere. It has been suggested that the low starch digestibility observed in some Australian wheats is related to antinutritive effects of NSP (Choct *et al.*, 1995, 1999), since addition of fibre-degrading enzymes overcomes this problem. In the present work, ileal starch digestibility of Norwegian wheats, and factors affecting starch digestibility, were studied.

II. METHODS

(a) Study of starch digestibility in Norwegian wheats

Five varieties of wheat were grown on experimental plots at two different locations in Eastern Norway (latitude 59° 19' and 60° 44') in 1998 and 1999. For use in diets, the grain was milled on a Skiold vertical hammer mill (Skiold Sæby A/S, Sæby, Denmark) through a 3 mm sieve. For each batch of grain, diets both with and without enzymes were made. The dry matter diet composition (/kg) was 770 g wheat, 100 g soybean isolate, 38.3 g fish meal, 30 g animal fat, 10 g soybean oil, 14 g mono CaP, 18.5 g ground limestone, 2.5 g salt, 0.9 g vitamin/mineral premix, 5 g titanium dioxide, 4 g DL-methionine, 3 g L-lysine, 1 g L-threonine, 1.5 g enzyme or maize starch, and 1.3 g choline chloride. The enzyme contained

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2500 U/g of xylanase and 800 U/g of protease (Finnfeeds Int. Ltd., Marlborough, UK). Each diet was pelleted without prior heating to pellets with a diameter of 3 mm. Temperature of the feed post-pelleting varied between 51 and 62°C.

Four experiments were carried out in late 1998 and 1999, after 2 to 3 months post-harvest storage of the grain. Day-old male broiler chicks (Ross breed) were reared in deep litter cages till 7 d of age. The same environmental conditions and the same starter diet, stored in a freezer between use, were used for all experiments. At the 7 d of age, 5 randomly selected birds were placed in each of 48 cages (50 cm x 35 cm x 20 cm, mesh floor) in a room with a constant temperature (28°C from 7 to 14 d of age and 26 °C from 14 to 21 d of age) and with 23 h light. Each bird was weighed, and only birds that deviated less than approximately 15 % from the average weight were used. Each diet was fed *ad libitum* to birds in 4 cages. At 21 d of age, individual birds were weighed, and the median three birds in each cage were killed by a cranial blow followed by cervical dislocation. To synchronize the feeding pattern of the birds, light was switched off for 4 h, followed by at least 3 h light before the chickens were sacrificed. The pooled contents from Meckel's diverticulum to the ileo-caeco-colonic junction from the three birds in the same cage were collected and frozen at -18 °C.

(b) Study of factors affecting starch digestibility

Diets with the same composition as described for the previous study, but without enzymes, were made with wheat variety 5. All diets were cold-pelleted. One diet served as a control, another diet was diluted with cellulose powder in the ratio 1/10 before pelleting and a third diet was crushed gently by the use of a lawn roller after pelleting to produce a mash feed. While all the wheat used in Diets 1 to 3 was hammer-milled through a 3 mm sieve, in Diet 4 half of the wheat (38.5 % of the ration) was fed as whole wheat together with pelleted other feed ingredients.

Diets were fed to broiler chickens from 10 to 21 d of age as described for the previous study, but with each diet fed to 3 birds in 8 cages. In addition, ileal contents from each of the 24 birds per treatment were collected for marker and starch analysis. The birds were starved for 2 h followed by at least 1 h access to feed before being sacrificed.

(c) Chemical and statistical analyses

Freeze-dried ileal samples were ground in an electrical household-type coffee mill, while feed was ground in a Retsch centrifugal mill (Model ZM 100, F. Kurt Retsch GmbH & Co., Haan, Germany) through a 0.5 mm sieve. Titanium dioxide content in feed and ileal contents were determined as described by Short *et al.* (1996), and starch content was determined enzymatically applying commercial kits (Megazyme Pty Ltd., Wicklow, Ireland).

Analysis of variance was performed using SAS software (SAS Institute Inc., Cary, USA).

III. RESULTS

(a) Study of starch digestibility in Norwegian wheats

All the 20 wheat-based diets tested had AME-values lower than 12.8 MJ/kg DM when enzymes were not used (data not shown). Starch digestibility was low for all varieties tested when enzymes were not used, but no significant differences occurred between varieties. (Table 1). Enzyme supplementation significantly ($P < 0.05$) increased starch digestibility with all varieties, and reduced the variation in starch digestibility between groups of birds.

Table 1. Ileal starch digestibility values for 21 d-old broiler chickens given pelleted diets containing 770 g wheat/kg DM.

	No. of replicates	Mean	Min.	Max.	Std. dev.
Variety 1	16	0.78	0.53	0.94	0.123
-with enzymes	8	0.93	0.75	0.99	0.081
Variety 2	16	0.76	0.56	0.94	0.112
-with enzymes	8	0.93	0.85	0.98	0.046
Variety 3	16	0.76	0.51	0.91	0.103
-with enzymes	8	0.91	0.87	0.96	0.032
Variety 4	8	0.72	0.57	0.94	0.140
-with enzymes	4	0.91	0.89	0.95	0.024
Variety 5	16	0.77	0.53	0.93	0.121
-with enzymes	8	0.89	0.78	0.98	0.074
Variety 6	8	0.70	0.54	0.84	0.129
-with enzymes	4	0.86	0.79	0.92	0.055

(b) Study of factors affecting starch digestibility

Crushing of the pellets resulted in a significantly ($P < 0.05$) reduced feed intake and weight gain (Table 2). This diet also gave the highest feed/gain ratio. All dietary treatments improved ($P < 0.05$) ileal starch digestibility compared to the control diet.

Table 2. Performance and ileal starch digestibility values for broilers fed pelleted diets containing 770g wheat/kg DM.

	Control	Dilution with cellulose	Crushed to produce mash diet	Wheat fed as whole grain
Weight gain	448 ^a	461 ^a	323 ^b	471 ^a
Feed intake	724 ^a	763 ^a	549 ^b	728 ^a
Feed/gain	1.62 ^{ab}	1.66 ^{ab}	1.71 ^a	1.57 ^b
Starch digestibility	0.79 ^b	0.93 ^a	0.95 ^a	0.91 ^a

^{ab}Means in a row without a common superscript are significantly different ($P < 0.05$).

IV. DISCUSSION

The results reported herein suggest a similar low starch digestibility, and thus a similar low nutritional value, for the wheats studied here as for the low-AME wheats reported by Mollah *et al.* (1983) and Rogel *et al.* (1987). In addition, there was a large variation in starch digestibility between replicates. This may indicate that individual birds have different abilities to digest starch. Alternatively, starch digestibility varies for a given bird with time due to other factors, for example feed load in the gut.

It has been suggested that the cause of the low energy value of some Australian wheats is related to antinutritional effects of NSP (Choct *et al.*, 1995, 1999). In the current study, a large improvement in starch digestibility was also observed when wheat diets were supplemented with enzymes. However, even with enzyme supplementation, starch digestibility was lower than normal. This indicates that factors other than NSP are also interfering with starch digestion. Further support for such this hypothesis may be found by taking into consideration the fact that fat and protein digestibilities were high in the wheat diets (data not shown). The NSP are reported to reduce both protein and, particularly, fat

digestibility. Thus, if the cause of low starch digestibility was related to antinutritional effects of NSP, it would be expected that fat and protein digestibility would also be low.

A review of the literature on starch digestibility reveals that a high starch digestibility usually coincides with mash feeding, whereas a low starch digestibility, such as that observed in this study, coincides with feeding cold-pelleted diets. In studies using pelleted diets, feed intake and weight gain are high and comparable to results obtained under commercial situations, but in those using mash diets feed intake and weight gain are usually considerably lower (Choct *et al.*, 1999; Steenfeldt *et al.*, 1998; Edney *et al.*, 1989; Yutse *et al.*, 1991). This observation leads to a hypothesis that a low starch digestibility of diets containing high levels of wheat is only observed in situations with a high feed intake. This is strongly supported by the current study where a significant increase in starch digestibility was observed when the diet was fed in mash form and feed intake was much lower. It is unlikely that the pelleting process itself caused a low starch digestibility through degradation of endogenous enzymes or through resistant starch formation. This is because the temperature increase was relatively small after pelleting. In addition, starch digestibility was high for the pelleted diet containing cellulose. The significant increase in starch digestibility when the wheat diet was diluted with cellulose powder indicates that feed intake itself is not limiting starch digestibility. These data rather suggest that the amount of wheat starch consumed is negatively correlated to starch digestibility.

The significant increase in starch digestibility when wheat was fed unground indicates that gut function, for example through changes in gizzard activity, interacts with the ability of the bird to digest starch.

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WHOLE GRAIN FEEDING AND ENZYMES : A PROMISING OPPORTUNITY !

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Summary

Experiments (digestibility and growth trials) were performed to study the potential of non-starch polysaccharide (NSP) enzymes in wheat-based diets for broilers with wheat incorporated either as ground or as whole grains. The effect of wheat genotype was also investigated using four wheat varieties : Soissons, Isengrain, Rialto and Baroudeur characterized by different *in vitro* viscosities and nutritional values. The incorporation of 300 g/kg of whole wheat grains prior to pelleting increased the apparent metabolisable energy (AME) value of the diet by about 0.42 MJ/kg, depending on the wheat cultivar. The addition of NSP-enzymes enhanced the nutritional value of the wheat, depending on the genotype, and reduced the difference between wheat cultivars.

I. INTRODUCTION

Wheat is known to contain anti-nutritional soluble pentosans which are particularly important when wheat is ground prior to pelleting. The NSP-enzymes such as xylanases have been designed to optimize ground wheat-based diet digestibility and utilization. This improvement with enzymes was also related to the wheat genetics (Dusel *et al.*, 1998). However, in spite of the numerous studies performed to establish a relationship between *in vitro* parameters and *in vivo* efficacy of NSP-enzymes in broilers, no clear relationship has been established between any chemical or physical parameters and nutritional value in response to enzyme addition. The most often addressed parameter, the *in vitro* wheat viscosity either relative, specific or after proteolytic treatment (Huyghebaert and Mombaerts, 2000) cannot explain more than 60 % of the total variability in wheat nutritional value.

Recent years have seen the development of the use of whole wheat in broiler feeding, especially in Northern Europe. Different modes of feeding have been tested : choice-feeding (complete feed and whole grains presented at the same time in different feeders), mixing complete feed and whole grains, or alternative feeding of complete feed and whole grains. Whole grain feeding proposed as choice-feeding has been demonstrated to enhance economic profitability by 3 to 5 % in broiler production (Hadorn and Wiedmer, 2000). The alternative technique has also been demonstrated to be beneficial with even further benefits under heat stress conditions (Noirot *et al.*, 1999). The effect of wheat genotype, when used as whole grains, on digestibility and bird performance (Yasar and Bedford, 2000) might be worthwhile considering with respect to the effect of whole wheat on gizzard development or digestive tract functioning. Indeed, recent results have demonstrated that whole-wheat feeding significantly enhanced gizzard weight whereas it decreased the length of the proventriculus (Demir *et al.*, 2000). To avoid variation in the nutritional value of a complete diet and, thus, to guarantee easy monitoring of animal performance, it has been proposed that whole grain should be included in pellets without prior grinding of the grains.

Whereas NSP-enzymes have been developed using ground wheat applications they appeared to work also with whole-wheat based diets. However, the interaction of the enzyme

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and the cultivar has not been investigated using this type of whole-wheat incorporation. The objectives of the present experiments were thus to examine the effect of the wheat cultivar on the response to whole grain feeding and to determine the enzyme response in relation to cultivar and wheat form, ground or whole grain.

II. MATERIALS AND METHODS

Four wheat cultivars were used : Soissons, Isengrain, Rialto and Baroudeur with specific viscosity ranging from 1.3 to 4.7 mL/g DM (Table 1).

Table 1. Measured wheat characteristics (g per 100 g DM)

	Soissons	Isengrain	Rialto	Baroudeur
Crude protein (Nx 6.25)	11.9	11.0	11.0	12.7
WICW ¹	10.4	10.2	12.0	11.4
Soluble pentosans	0.66	0.82	1.24	1.16
Relative viscosity	1.28	1.43	2.02	1.92
Specific viscosity ²	1.30	2.00	4.70	4.30

¹Water Insoluble Cell Walls (Carré *et al.*, 1997).

²In mL per g DM (Grosjean *et al.*, 1999).

Experiment 1 : Birds received either a basal diet containing corn, soyabean meal, gluten meal and soyabean oil and a premix (amino acids, minerals and vitamins) or different ground wheat-based diets containing the basal mixture, the premix and each of the four wheat cultivars at 50 % of the diet. Half the diets were supplemented with a NSP-enzyme (Rovabio™ Excel AP, containing xylanase and β -glucanase activities, at 50 g/t). Diets were in pellet form. Digestibility balances were carried out on ISA male chickens according to the modified European Reference Method with *ad libitum* feeding and total excreta collection for 3 d (Bourdillon *et al.*, 1990). The AME was measured between 22 - 25 d and between 34 - 37 d of age. On days 28 and 39 birds were killed for viscosity determination of the jejunal chyme supernatant using a cone-plate Brookfield digital viscometer. Intact adult cockerels also received the same feeds *ad libitum* in order to evaluate the AMEn by the same method.

Experiment 2 : Birds received a grower pelleted diet containing (/kg): 510 g wheat, 340 g soyabean meal and 50 g animal fat. Wheat was either included as ground (510 g/kg) or whole (300 g/kg) and ground (210 g/kg). The whole grains were included prior to pelleting. The enzyme, Rovabio™ Excel LC, was sprayed onto the pellets at 0.2 L/t. The AME was determined according to the European Reference Method with *ad libitum* feeding and total excreta collection using 3-week old Ross male chickens.

Experiment 3 : Using the same diets as in Experiment 1, short growth trials (2 wk periods) were performed in collective battery cages from 5 to 28 d of age using Ross male broilers. Weight gain, feed intake and feed conversion ratios were measured.

III. RESULTS AND DISCUSSION

In ground wheat-based diets, the difference between AME values measured at 22 – 25 d of age using the four wheat cultivars reached up to 0.85 MJ/kg DM (Table 2). Rialto and

Baroudeur exhibited lower energy values than Soissons and Isengrain. In adult cockerels, the difference was reduced. The enzyme supplementation decreased the cultivar effect. At 22 – 25 d of age the improvement with enzyme reached 0.49 MJ/kg DM i.e. + 2.8 % without any reduction in jejunal viscosity (data not shown).

Table 2. Wheat AME_n (MJ/kg) measured with wheat-fed broilers at 4 and 6 weeks of age and with adult cockerels : effect of wheat cultivar and enzyme addition.

	Soissons	Isengrain	Rialto	Baroudeur
22-25 days				
- enzyme	14.35	14.34	13.50	13.77
+ enzyme ^{1,2}	14.75	14.67	13.99	14.17
34-37 days				
- enzyme	14.47	14.38	13.34	14.33
+ enzyme ^{1,2}	14.17	14.72	14.21	14.36
Adult cockerel				
- enzyme	14.50	14.18	14.11	13.93

¹Enzyme : Rovabio™ Excel AP at 50 g/t.

²Statistical significance : Enzyme effect : P < 0.001; Wheat effect : P < 0.001.

RSD : 0.55 MJ/kg DM, Interaction : NS

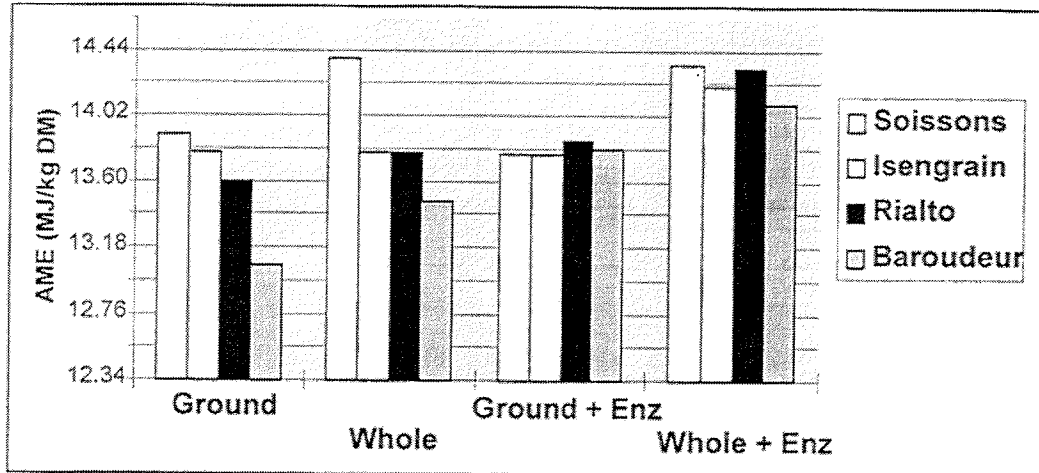
The effect of incorporation of whole wheat prior to pelleting is shown in Figure 1. The dietary energy value of the 510 g/kg ground wheat diets ranged from 13.9 to 13.1 MJ/kg DM. The enzyme addition reduced the cultivar effect, the average AME_n value of the ground wheat diets being 13.81 MJ/kg DM. The greatest response to enzyme addition was obtained with the Baroudeur wheat. Incorporating half the wheat as whole grains prior to pelleting, enhanced the energy value by up to 0.42 MJ/kg DM with Soissons and Baroudeur. The effect of the NSP-enzyme supplementation appeared greater with the whole grain-containing diets than with the ground wheat-containing diets. As observed with the ground wheat-based diets the variability between wheat cultivars was decreased with enzyme addition.

The growth trials (data not shown) confirmed the observations on digestibility. The whole grain feeding gave better growth (improved weight gain and feed efficiency from 2 to 4 %) with the Soissons and Baroudeur cultivars. With enzyme supplementation the feed conversion of birds fed the diet containing Baroudeur whole wheat was even better than for birds fed the Soissons wheat.

IV. CONCLUSIONS

The incorporation of whole grains prior to pelleting enhanced nutritional value, depending on the wheat cultivar. Moreover, even a high quality wheat (Soissons) responded to whole grain feeding by up to 0.45 MJ of ME/kg DM, suggesting potential for improvement.

Figure 1 : Effect of adding whole wheat grains on AME of 3-wk-old wheat fed broilers.



It also appeared that NSP-enzymes designed for use with ground wheat-based diets were equally or more efficient in improving the energy value of whole wheat-based diets. With enzyme supplementation the average improvement in AME reached 0.42 MJ/kg DM between whole wheat and ground wheat feeding. The enzyme addition was also efficient in decreasing the variability in wheat energy values, either ground or as whole grains.

The partial additivity of the effects of enzyme addition and whole grain feeding suggests that underlying mechanisms might be different and could be further developed.

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ENZYMATIC PRE-TREATMENT OF LUPINS FOR BROILER DIETS

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Summary

Dehulled lupins were treated for 1, 12 or 24 h with pectinase (endo-polygalacturonase, PG) in acid media in an attempt to hydrolyse the cell wall content and potentially improve their nutritive value in broiler diets. We found that addition of PG reduced solution viscosity after incubation for 1h but, at 12 and 24h, the viscosity of the treated samples returned towards untreated values. Filtration rate of supernatant was significantly increased by PG after all three incubation periods. PG substantially reduced the content of cell walls and pectic substances, but not water-soluble polysaccharides. The molecular weight of pectic substances was reduced by incubation in the absence of PG, and was improved by PG. PG failed to significantly shorten the polygalacturonic acid chains. Incubation with PG for 1h appears to be the most efficient pre-treatment of dehulled lupins that will be used in broiler diets.

I. INTRODUCTION

Despite the success of enzymes in the breakdown of indigestible polysaccharides, a major problem in adding enzymes to diets is their susceptibility to denaturation and loss of activity due to pelleting. Few enzymes produced today are resistant to high temperatures despite continuous attempts to produce enzymes that are thermally resistant. Nevertheless, enzymes that break down indigestible polysaccharides remain potentially useful in poultry diets. One way to overcome the risk of denaturation is to incubate a feedstuff with enzyme before pelleting. This approach avoids the risk of heat destroying the enzyme and may also give superior hydrolysis to enzyme action in the gastro-intestinal tract. For example, extending the time of incubation may allow greater hydrolysis than would occur with the more limited time of enzyme action in the digestive tract of poultry. Many reports have shown that increasing the reaction time can be beneficial. Increasing the time of incubation of cell walls or pectic substances with pectinase from 1 to 24 h leads to a further 5 to 20% hydrolysis of polygalacturonic acid. Increased hydrolysis lowers the viscosity, decreases the water-holding capacity, increases the filtration rate of the supernatant, and decreases the molecular size of the pectic substances (Kertesz, 1951; Endo, 1964; Thibault and Mercier, 1978; Lui and Luh, 1980; Kollar, 1998).

Thus, improving hydrolysis by pre-treating feed with enzymes before it is consumed might improve the value of some feedstuffs such as dehulled lupins. Amongst the improvements might be lowered viscosity of digesta, better digestion of nutrients, higher metabolisable energy and better weight gain. This hypothesis was tested by incubating dehulled lupins with polygalacturonase (PG) for periods of 1 to 24 h and studying the degradation of the cell wall components.

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II. MATERIALS AND METHODS

Dehulled lupins (*L. angustifolius* cv Gungurru) were incubated with pectinase (endo-polygalacturonase, PG, EC 3.2.1.15) of activity 3500 units/g) for 1, 12 or 24 h. Dehulled lupin flour (50 g) was incubated with (+PG) or without (-PG) 0.1 g PG. The incubation mixture was prepared by dissolving dehulled lupins in 70ml deionised water and 10ml McIlvane buffer according to the procedure described by the enzyme supplier. Nine replicates of each reaction mixture were incubated in an incubator-shaker at 150 RPM at 38°C for 1, 12 or 24 h, independently. The samples were centrifuged at 15000g for 10 min at 20°C and the filtration rate ($\mu\text{l}/\text{sec}$) of the supernatants was measured. Viscosity of the supernatants was also measured with a viscotester (HAAKE, PK 100) using cone plate PK5, shear rate 4802/s and speed rate 800/min at 22°C. Water-holding capacity was measured according to the method of Robertson and Eastwood (1981). Carbohydrate components of dehulled lupins were quantified by the method of Carre *et al.* (1985). Polygalacturonic acid concentration was measured by spectrometry absorption according to the method of El-Rayah and Labavitch (1977). The molecular weight of pectic substances and the degree of polymerisation of polygalacturonic acid polymer were measured by the methods of Smit and Bryant (1967) and Barash and Eyal (1970). Data were analysed using a 2×3 complete factorial model for the two enzymes (\pm PG) and three incubation periods (1, 12 and 24 h) and their interactions, using Genstat 5 software. If ANOVA of any measured trait was significant statistically, LSD ($P < 0.05$) was used to compare the differences among their means.

III. RESULTS

There were significant differences ($P < 0.01$) in filtration rate, viscosity and water-holding capacity due to the PG treatment at 1 and 12 h of incubation (Table 1).

Table 1. Effects of enzyme (PG) and incubation time on filtration rate, viscosity and water-holding capacity of dehulled lupins (mean \pm sem).

	1 h ¹	12 h ¹	24 h ¹	Enzyme ²
Filtration rate ($\mu\text{l}/\text{sec}$)				
-PG	61.2 \pm 2.1	70.7 \pm 3.6	70.4 \pm 3.1	67.4 \pm 5.4
+PG	73.2 \pm 3.4	75.2 \pm 2.9	74.8 \pm 2.8	74.4 \pm 1.1
Mean ³	67.2 \pm 8.5	72.9 \pm 3.2	72.6 \pm 3.2	
Viscosity (mPas/sec)				
-PG	1.61 \pm 0.02	1.64 \pm 0.02	1.63 \pm 0.03	1.62 \pm 0.02
+PG	1.45 \pm 0.02	1.58 \pm 0.01	1.58 \pm 0.02	1.53 \pm 0.02
Mean ³	1.53 \pm 0.02	1.61 \pm 0.02	1.61 \pm 0.02	
Water-holding capacity (g:g)				
-PG	4.28 \pm 0.11	4.05 \pm 0.33	4.05 \pm 0.39	4.12 \pm 0.13
+PG	4.21 \pm 0.16	3.89 \pm 0.36	3.99 \pm 0.38	4.03 \pm 0.16
Mean ³	4.24 \pm 0.05	3.97 \pm 0.11	4.02 \pm 0.04	

^{1,2,3} LSD for effects of enzyme, time and enzyme x time interaction. For filtration rate: 1.66 (enzyme), 2.04 (time), 2.88 (interaction). For viscosity: 0.03 (enzyme), 0.04 (time), 0.06 (interaction). For water-holding capacity: 0.21 (time).

Cell wall material, water-soluble polysaccharides, pectic substances and polygalacturonic acid values all decreased with time and the addition of enzyme (Table 2). In contrast to the above, the maximum degradation was found at 24h of incubation. The reverse held for the concentration of polygalacturonic acid, which decreased linearly with time of incubation. The last measurements, molecular weight of pectic substances and degree of

Table 2. Effect of enzyme (\pm PG) and incubation time on the cell wall components of dehulled lupins (mean \pm sem).

	1 h ¹	12 h ¹	24 h ¹	Enzyme ²
Cell wall materials (%)				
-PG	22.5 \pm 0.7	20.7 \pm 0.7	19.7 \pm 0.8	21.0 \pm 1.5
+PG	18.8 \pm 0.7	15.2 \pm 0.4	14.1 \pm 0.3	16.0 \pm 2.5
Mean ³	20.7 \pm 2.6	18.0 \pm 3.9	16.9 \pm 4.0	
Water-soluble polysaccharides (%)				
-PG	5.29 \pm 0.38	5.25 \pm 0.25	5.21 \pm 0.21	5.25 \pm 0.04
+PG	4.88 \pm 0.34	4.66 \pm 0.35	4.52 \pm 0.38	4.69 \pm 0.18
Mean ³	5.09 \pm 0.29	4.95 \pm 0.42	4.87 \pm 0.49	
Pectic substances (%)				
-PG	5.15 \pm 0.12	4.57 \pm 0.22	4.24 \pm 0.14	4.66 \pm 0.46
+PG	4.24 \pm 0.19	4.19 \pm 0.13	3.77 \pm 0.14	4.07 \pm 0.25
Mean ³	4.69 \pm 0.65	4.38 \pm 0.27	4.01 \pm 0.33	

^{1,2,3} LSD values for effect of enzyme, time and enzyme x time interaction. For cell wall materials: 0.65 (enzyme), 0.79 (time), 1.12 (interaction). For water-soluble polysaccharides: 0.33 (enzyme). For pectic substances: 0.17 (enzyme), 0.20 (time), 0.29 (interaction).

Table 3. Effect of enzyme (\pm PG) and incubation time on the pectic molecules of dehulled lupins (mean \pm sem).

	1 h ¹	12 h ¹	24 h ¹	Enzyme ²
Polygalacturonic acid (g/kg)				
-PG	66.6 \pm 4.5	64.3 \pm 3.7	58.7 \pm 3.4	63.2 \pm 4.1
+PG	64.4 \pm 4.1	63.3 \pm 2.6	58.6 \pm 3.5	62.1 \pm 3.1
Mean ³	65.5 \pm 1.6	63.8 \pm 0.7	58.6 \pm 0.2	
Molecular weight of pectic substances(kda)				
-PG	97.8 \pm 8.3	86.3 \pm 7.6	88.0 \pm 6.1	90.7 \pm 6.2
+PG	68.5 \pm 4.2	75.9 \pm 4.9	60.4 \pm 4.2	68.3 \pm 7.8
Mean ³	83.2 \pm 21	81.1 \pm 7.3	74.2 \pm 20	
Degree of polymerisation (no. galacturonic acid/chain)				
-PG	50.5 \pm 2.3	51.2 \pm 1.9	50.9 \pm 1.9	50.9 \pm 0.4
+PG	46.4 \pm 2.0	49.7 \pm 1.2	49.9 \pm 2.0	48.7 \pm 2.0
Mean ³	48.5 \pm 2.9	50.5 \pm 1.1	50.4 \pm 0.7	

^{1,2,3} LSD values for effect of enzyme, time and enzyme x time interaction. For polygalacturonic acid: 3.06 (time). For molecular weight: 4.15 (enzyme), 5.09 (time), 7.19 (interaction). For degree of polymerisation: 1.30 (enzyme), 1.59 (time).

polymerisation (number of units of galacturonic acid per chain, followed different patterns to the others. Molecular weight decreased with time and the degree of polymerisation decreased during the first hour of incubation but then remained unchanged up to 24h (Table 3).

IV. DISCUSSION

Polygalacturonase changed several physico-chemical properties of dehulled lupins, partially supporting the proposed hypothesis. It increased filtration rate and decreased viscosity, but did not reduce water-holding capacity or degree of polymerisation. The inability of PG to reduce water-holding capacity efficiently may be attributed to the presence of the other known carbohydrate fractions of the cell wall material, mainly cellulose and hemicellulose, that are also able to absorb and retain large quantities of water. The expected change in the degree of polymerisation was not observed and this may be due to the presence of methyl ester groups adjacent to the carboxyl group along the pectic chain. These groups would block PG from hydrolysing glycosidic bonds (Kertesz, 1951; Thibault and Mercier, 1978; Rombouts and Thibault, 1986).

Time of incubation also affected most factors because, as time increased, degradation was more complete, as indicated by the breakdown of cell wall material, the decrease in water-soluble polysaccharide and pectic substances, and the decrease in their molecular weight. The first hour of incubation produced the largest changes, contrary to expectation. One reason for this might have been reduced activity of PG with time because of changes in pH. The incubation was started at pH 3.5, the optimal pH for this enzyme, but as the incubation proceeded, the pH drifted up and reached 5.2 by 24 h. At this pH, PG would have been completely inactivated (Endo, 1964; Thibault and Mercier, 1978; Chun and Huber, 1988; Benen *et al.*, 1999). A small loss of water from the incubation flask might have caused the small rise in viscosity that, in turn, would be expected to decrease the filtration rate. Another possibility might be that incubation for 1 h is sufficient to achieve maximum hydrolysis of pectic substances. Other investigators agree that PG-enzymic hydrolysis for 15–60 min is sufficient for maximum effect on pectin viscosity, hydrolysis of polygalacturonic acid polymer, and molecular weight of pectin (Kertesz, 1951; Endo, 1964; Thibault and Mercier, 1978; Liu and Luh, 1980; Kollar, 1998).

In conclusion, incubation of dehulled lupins with PG prior to feeding to broilers looks promising as a method of obtaining better nutrient utilisation and, consequently, better performance of lupin-based diets. Results from this study may also aid feed manufacturers by enabling them to overcome the negative effects of pectic substances. Finally, this approach is not just restricted to lupins but may also be applied to other legumes and cereals that possess similar complex polysaccharides.

V. ACKNOWLEDGMENTS

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DO FEED ENZYMES IMPROVE THE NUTRITIVE VALUE OF SOYABEAN MEAL IN BROILER DIETS?

A. KOCHER and M. CHOCT

Summary

Two feed enzymes (Enzyme A, a multi-activity glycanase and Enzyme B, an experimental product containing mainly galactanase) were added at the recommended level and five times above this level to broiler diets containing soyabean meal (SBM) as the sole protein source. Enzyme B at both inclusion levels and Enzyme A at the high dosage improved ($P<0.05$) the apparent metabolisable energy (AME) of the diet but not the growth rate or the feed conversion ratio (FCR) of the birds. Both enzymes increased the amount of free sugars in the ileum. However, the increase was only significant ($P<0.05$) when enzymes were added at the high dosage. The addition of both enzymes reduced ($P<0.05$) the concentration of insoluble non-starch polysaccharides (NSP) in the ileum, whereas the concentration of soluble NSP was only reduced ($P<0.05$) when Enzyme B was added.

I. INTRODUCTION

The introduction of a total ban on the use of animal protein sources in feedstuffs in some countries has placed great emphasis on the nutritive value of vegetable proteins in poultry broiler diets. Soyabean meal is the most commonly used vegetable protein source in poultry diets, despite a range of anti-nutritive factors. Protease inhibitors and lectins can be successfully inactivated by heat treatment. However, the nutritive value of SBM is also influenced by the amount of non-starch polysaccharides (NSP). Soyabean meal contains approximately 180-210 g/kg NSP of which 25-30g/kg are soluble (Bach Knudsen, 1997). Endogenous enzymes of broilers are unable to digest NSP. However, Carré *et al.* (1995) showed that volatile fatty acids (VFA), the end product of bacterial degradation of soluble NSP, can be extensive in broilers. In order to improve the nutritive value of SBM several studies have investigated the effect of exogenous enzyme supplementation to SBM-based diets. Marsman *et al.* (1997) showed that the addition of a multi-activity enzyme preparation improved NSP digestibility by solubilising parts of the insoluble NSP fraction. However, the improved NSP digestibility did not transfer to improved animal performance. In contrast, Irish and Balnave (1993) found that the addition of a similar enzyme product resulted in significantly poorer growth compared to the control diet because of the partial breakdown of NSP. The presence of large amounts of low molecular weight NSP can result in increased fluid retention in the small intestine and can adversely affect nutrient absorption (Wiggins, 1984).

This paper reports the result of a study investigating the effect of two glycanase products on the composition of NSP in the intestine of broilers and the subsequent effects on broiler performance and energy utilisation of broilers.

II. MATERIALS AND METHODS

(a) Experimental diets, bird management and AME trial

The effects of two feed enzymes (Enzyme A with glucanase, hemicellulase and pectinase, Enzyme B with β -galactanase) included at the recommended level and five times above this level on SBM were examined. Five experimental diets were formulated (Table 1). Day-old male broiler chickens (Cobb) were raised in brooders on enzyme-free commercial starter crumbles. At 4 d of age, chickens were weighed in groups of 10. The experimental diets were fed for 32 d. Feed intake, mortality and body weight gain was measured over this period. At 16 d of age the birds were transferred to cages. The first 3 d enabled the chickens to adapt to their new environment. During the next 4 d feed intake was measured and all excreta collected. Gross energies of excreta and feed were determined by bomb calorimetry and the AME of each diet was calculated.

Table 1. Composition of experimental diets.

Ingredient g/kg	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize	570.3	570.3	570.3	570.3	570.3
Soyabean meal	365	365	365	365	365
Vitamins+Minerals	44.7	44.7	44.7	44.7	44.7
Celite (Marker)	20	20	20	20	20
Enzyme A g/kg ¹	---	0.4	---	0.94	---
Enzyme B g/kg ¹	---	---	2	---	4.7

¹ Enzyme A (Ronozyme VP), Enzyme B (Novozyme); supplied by F. Hoffmann-La Roche, Basel, Switzerland.

(b) Nitrogen, non-starch polysaccharide and volatile fatty acid determinations

At the end of the 4 d collection period, five birds from each group were killed by cervical dislocation. The contents of the ileum (from Meckel's diverticulum to 4 cm above the ileo-caecal junction) and caeca were collected. Samples from each replicate were pooled and stored on ice prior to centrifugation (12,000g, 10min). Supernatant and pellet were frozen and kept at -20°C for analysis. Ileal digesta were analysed for free sugars and soluble and insoluble NSP by gas liquid chromatography following the methods described by Annison *et al.* (1996). The levels of VFA in ileal and caecal digesta were determined by gas chromatography after distillation in Thunberg tubes. The nitrogen contents of the diets and ileal digesta were determined using a LECO FP-2000 automatic analyser. Protein content was calculated using a multiplications factor of 6.25.

III. RESULTS

The effects of adding enzymes to diets containing SBM as the sole protein source are shown in Tables 2, 3 and 4.

Table 2. Effect of enzyme type and dosage level on weight gain, feed conversion ratio (FCR) and apparent metabolisable energy (AME_N) of broilers fed diets containing soyabean meal.

Diet	Weight gain (g/bird)	FCR (g feed:g gain)	AME _N (MJ/kg DM)
Control	1976.5 ± 77.5	1.721 ± 0.044	12.84 ± 0.12 ^b
Enzyme A (normal)	1952.0 ± 70.1	1.700 ± 0.034	12.89 ± 0.06 ^b
Enzyme A (5x)	1981.8 ± 96.9	1.703 ± 0.025	13.04 ± 0.07 ^a
Enzyme B (normal)	1950.5 ± 110.5	1.703 ± 0.034	13.05 ± 0.07 ^a
Enzyme B (5x)	1917.2 ± 39.3	1.693 ± 0.038	13.07 ± 0.07 ^a

Values within columns without a similar superscript are significantly different (P<0.05).

Table 3. Effect of enzyme type and dosage level on ileal protein digestibility and volatile fatty acid (VFA) concentration in the ileum and caeca.

Diet	Ileal protein digestibility %	Ileal VFA production mMol/bird	Caecal VFA production mMol/bird
Control	81.97 ± 0.60 ^b	26.44 ± 8.8	568.1 ± 109.1 ^b
Enzyme A (normal)	80.19 ± 1.73 ^{bc}	24.60 ± 6.7	522.1 ± 113.8 ^b
Enzyme A (5x)	85.56 ± 0.98 ^a	25.88 ± 7.1	575.2 ± 116.6 ^b
Enzyme B (normal)	80.79 ± 0.87 ^{bc}	26.64 ± 8.4	753.5 ± 103.4 ^a
Enzyme B (5x)	80.63 ± 1.09 ^c	28.14 ± 10.8	744.3 ± 144.3 ^a

Values within columns without a similar superscript are significantly different (P<0.05).

Table 4. Effect of enzyme type and dosage level on the concentration of non-starch polysaccharides (NSP) in ileal digesta (g/kg acid insoluble ash).

Diet	Free sugars	Insoluble NSP	Soluble NSP
Control	939.4 ± 159.6 ^b	3980.2 ± 285.3 ^a	297.5 ± 40.0 ^a
Enzyme A (normal)	1072.6 ± 203.3 ^{ab}	3627.3 ± 281.6 ^b	279.3 ± 12.2 ^a
Enzyme A (5x)	1204.6 ± 337.9 ^a	3601.4 ± 421.3 ^b	272.0 ± 32.8 ^{ab}
Enzyme B (normal)	1152.3 ± 167.5 ^{ab}	3369.2 ± 263.1 ^b	221.2 ± 37.6 ^c
Enzyme B (5x)	1299.5 ± 155.5 ^a	3321.5 ± 169.0 ^b	244.4 ± 9.6 ^{bc}

Values within columns without a similar superscript are significantly different (P<0.05).

When included at five times the recommended dosage both enzymes significantly (P<0.05) improved AME_N of the diet, but not the growth rate or the FCR of the birds (measured over a 5 week growth period) (Table 2). At the recommended level only Enzyme B had an effect (P<0.05) on AME_N. Ileal protein digestibility was significantly (P<0.05) affected by the addition of enzymes (Table 3). Enzyme A at the high dosage improved protein digestibility, whereas Enzyme B at the high dosage decreased it. However, the addition of both enzymes at their recommended level had no effect on protein digestibility. The addition of Enzyme B, but not Enzyme A, at both dosages significantly (P<0.05) reduced

the concentration of soluble NSP in the ileum (Table 4). Both enzymes significantly reduced the concentration of insoluble NSP in the ileum (Table 4). The inclusion of Enzyme B, but not Enzyme A, significantly ($P < 0.05$) increased the concentration of VFA in the caeca (Table 3). However, ileal VFA concentration was not affected by enzyme supplementation.

IV. DISCUSSION

This trial was designed to investigate the effects of feed enzymes on the nutritive value of SBM. Enzyme B clearly had an effect on the AME_N of SBM-containing diets. This improvement was the result of the significant reduction of soluble and insoluble NSP into smaller fragments and the subsequent increase in production of VFA in the caeca. Enzyme B effectively depolymerised NSP of SBM in the ileum, making an increased amount of smaller molecules available to the caecal microflora (Choct *et al.*, 1996). Although the energy contribution via caecal fermentation is less effective than the absorption of glucose in the upper intestine, VFA as the end product of bacterial fermentation can provide some energy to the bird (Carré *et al.*, 1995). This is clearly evident in the increased AME_N when Enzyme B was added. There were no apparent differences between the two inclusion levels of Enzyme B, indicating the potential use of this experimental galactanase in practical diets.

Unlike Enzyme B, the addition of Enzyme A at its recommended level was clearly below the maximum response level. The addition of Enzyme A at the high dosage led to a significant improvement in protein and energy utilisation, whereas the same enzyme at the recommended level had no beneficial effects. Analysis of the NSP content in ileal digesta showed a marked increase in the amount of free sugars and a significant reduction in insoluble NSP at the high dosage level. This trend was also evident at the lower dosage level. However, when Enzyme A was added at the higher dosage the effects were more profound. Improvement in protein digestibility can be a direct result of the enzymatic breakdown of cell walls in vegetable proteins and the release of entrapped nutrients (Pettersson and Åman, 1989). The disruption of the cell wall matrix and the easy access of endogenous proteolytic enzymes as well as small amounts of exogenous proteases in Enzyme A (Marsman *et al.*, 1997) may explain the overall improved protein digestibility at the high dosage level.

This study demonstrated that the use of appropriate enzymes at suitable dosages can lead to increased energy release from SBM although an effect on bird performance was not demonstrated.

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NON-STARCH POLYSACCHARIDE ENZYMES AND VISCOSITY: THE RELATIONSHIP REVISITED !

P.A. GERAERT¹, F. ROUFFINEAU¹ and B. BARRIER-GUILLOT²

Summary

Controversy exists regarding the relationship between wheat nutritional value, mainly AME, and its *in vitro* viscosity. Different methodologies are used leading to a wide range of values for viscosity. This review proposes to revisit the viscosity – AME relationship as well as its consequences on non-starch polysaccharides (NSP) enzyme response. The relationship between AME and growth performance is also questioned. In order to benefit from enzyme utilization AME has long been preferred to feed conversion ratio for reformulating diets.

I. INTRODUCTION

Measuring the nutritional value, i.e. the energy value and the protein digestibility of feedstuffs and complete feeds, appears to be critical to allow the poultry industry to build valuable dietary formulations. Many chemical analyses have been developed and used in order to estimate the nutritional value of feedstuffs. However, in order to be efficient and useful in practice simple and reliable parameters have to be found.

Wheat is an important cereal for broiler diets in Europe, Canada and Australia. However, it has long been considered to be a highly variable feedstuff, especially for young chickens. Water-soluble NSP, mainly arabinoxylans, can affect wheat energy value through an increase in intestinal viscosity but recent publications have reported conflicting results regarding the relationship between soluble NSP content, *in vitro* viscosity, AMEn and growth performance (Dusel *et al.*, 1998 ; McCracken *et al.*, 1998 ; Francesch *et al.*, 2000).

The NSP-enzymes have been developed to overcome intestinal problems. However, enzyme users would benefit if they could predict the potential value of using enzymes with a particular wheat, or even knowing the magnitude of the energy improvement that could be obtained for reformulation purposes.

This short review proposes to summarize the different methodologies used to measure viscosity either *in vitro* or *in vivo* and to investigate the recent data dealing with the relationships between viscosity measurements and nutritional value, energy digestibility and growth performance. The effect of NSP-enzymes will be reviewed according to the range of wheat genotypes and their *in vitro* viscosities.

II. VISCOSITY : METHODOLOGIES AND VALUES

For enzyme users (e.g. formulators, nutritionists), it is often difficult to get a clear view of viscosity values. Indeed, various methodologies are used by different laboratories and this leads to a wide range of viscosities : from 1.0-2.5 to 5-4 to 100 cPs. In order to better understand published results it is valuable to summarize the different ways in which *in vitro* viscosities of raw materials and complete diets are measured as well as the values for *in vivo* intestinal viscosities usually measured at the jejunum or ileum.

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In vitro viscosities :

The wheat water extract viscosity measurement proposed by Grosjean *et al.* (1999) follow these main steps : grinding the grains at 0.5 mm, soaking with desionised water, centrifugation and denaturation of the endogenous activities by boiling at 100°C. The viscosity is then determined with a capillary-based viscometer. The specific viscosity (SV) is expressed in mL/g DM and is derived from the relative viscosity (RV) ($SV = (RV-1)/\text{sample weight}$).

Real and potential viscosities, relative to acetate buffer pH4.5, are measured using a rotative viscometer (Carré *et al.*, 1994). Denaturation of endogenous xylanase activities is obtained with boiling ethanol.

Finally, Huyghebaert and Mombaerts (2000) reported that a proteolytic treatment improves the correlation between measured viscosity and wheat nutritional value.

In vivo viscosities :

Usually the chyme is collected after slaughtering the animals between 16 and 35 d of age. The intestinal contents from the duodenum, jejunum or ileum, proximal or distal, are collected through gentle manual or mechanical pressure on the intestines. The samples are homogenized, centrifuged and the viscosity of the supernatant is then determined (Scott *et al.*, 1998 ; Barrier-Guillot *et al.*, 1998).

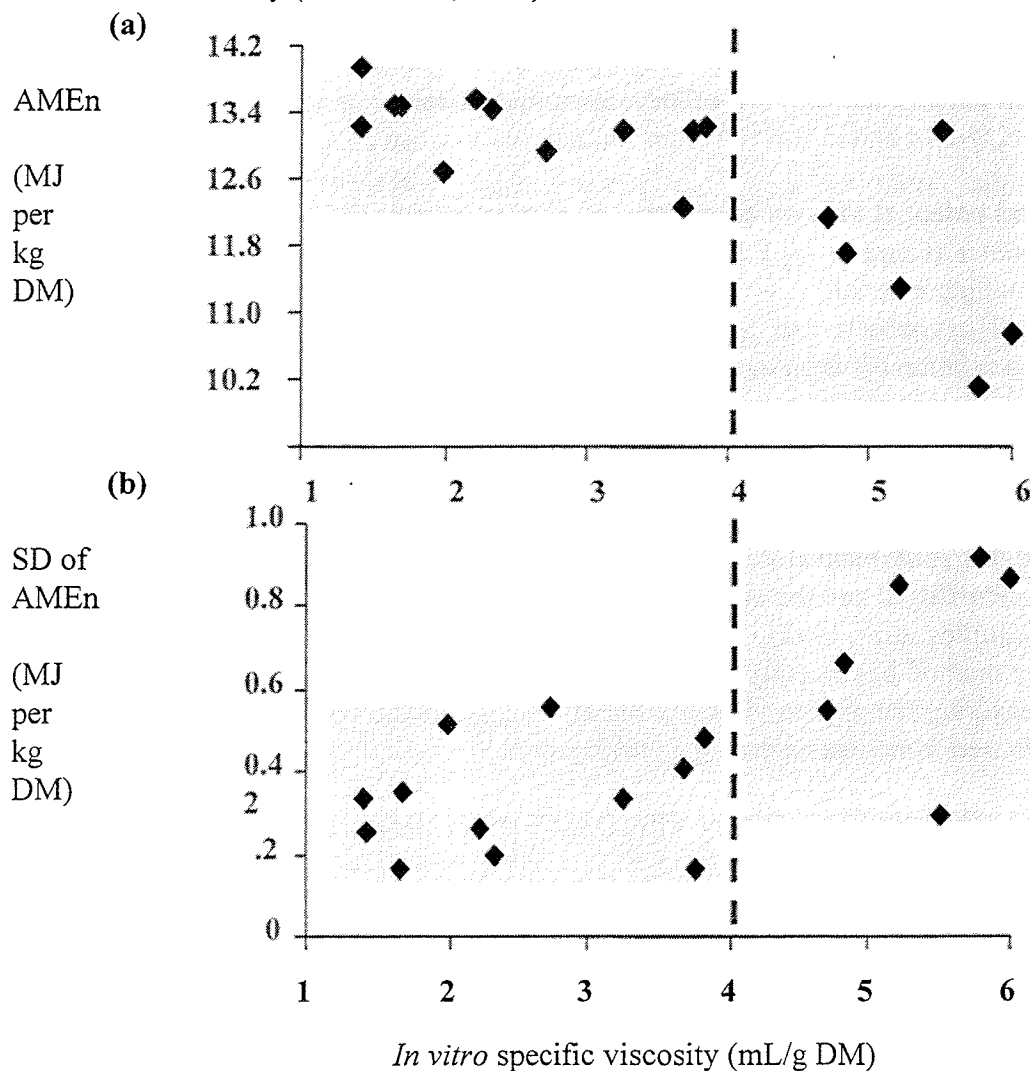
Another method has recently been proposed by Francesch *et al.* (2000) which consists not only of measuring the digesta viscosity but also the quantity, volume or dry matter of the supernatant released from digesta during centrifugation.

III. GENETIC VARIATION IN VISCOSITY AND NUTRITIONAL VALUE

Barrier-Guillot *et al.* (1998) considered 19 wheat samples from France and Great Britain, chosen to cover a wide range of *in vitro* viscosities (SV from 1.4 to 7.3 mL/g DM). The wheat AMEn values ranged from 9.96 to 14.06 MJ/kg DM. Whereas water-extract viscosity was highly correlated with jejunal viscosity ($r > 0.95$), the correlation between *in vitro* viscosity and AMEn only reached 0.65 which means that less than 50 % of the variation in AMEn could be attributed to viscosity. However, the main conclusion was that low viscosity varieties seem to have high AMEn whereas wheat varieties with the highest viscosities can lead to variable AMEn values. In order to complete this study Skiba *et al.* (1999) used 21 wheat varieties and also demonstrated that the correlations between AMEn and either SV or jejunal viscosity were weak : -0.58 and -0.57 respectively. Such results mean that viscosity, measured either *in vitro* or *in vivo*, was not the only factor to explain variability in wheat energy values. However, from these studies it was possible to define a threshold in viscosity (SV of 4.0 mL/g DM) below which wheat AMEn is less variable and relatively high while above it, the AMEn was usually lower and highly variable (Figures 1a and 1b).

Scott *et al.* (1998) studying 9 wheat cultivars grown in replicate in three locations and two crop years demonstrated a significant effect of location on feeding value and subsequent growth performance in broilers. Recently, McCracken and Bedford (2000) demonstrated the importance of diet composition in studying both the wheat AME value and its response to enzyme supplementation. Indeed, the enzyme response was lower when commercial-based diets were used compared with a cereal-casein-based diet. Also, animal fats rich in saturated fatty acids have long been reported to enhance the detrimental effect of wheat viscosity.

Figure 1 : Relationship between wheat AMEn (a) or standard variation of wheat AMEn (b) and *in vitro* viscosity (Skiba *et al.*, 1999)



IV. VISCOSITY AND RESPONSE TO ENZYME

The NSP-enzymes or hemicellulolytic and cellulolytic enzymes cover a wide range of enzymes. Voragen (2000) stressed the range of β -glucanases required to ensure complete breakdown of β -glucans. The same applies to arabinoxylans and even to more complexed cell wall components. Thus, trying to generalize the effect of NSP-enzymes through the action of one particular product is rather unrealistic. It would be more useful to relate the exact enzyme composition to the effect on different components. The presence of endogenous xylanase activities in wheat grains, and the recently discovered xylanase inhibitors, might be critical in explaining the somewhat weak effect of NSP-enzymes on high quality wheats (McLauchlan *et al.*, 1999).

The NSP-enzymes have often been used to reduce intestinal viscosity which should, thus, improve wheat nutritional value. However, whereas the reduction in viscosity is greater with high- than with low-viscous wheats, the improvement in feed to gain ratio may be similar (Dusel *et al.*, 1998). These authors also observed that the enzyme effect was greater between 29 and 35 d of age than between 1 and 28 d of age. Francesch *et al.* (2000) demonstrated that

the quantity of supernatant released from digesta by the action of enzymes was better correlated to feed conversion than was the supernatant digesta viscosity.

V. CONCLUSIONS

New criteria need to be developed or a more global approach used. An example is the NIRS technique which has been developed successfully to predict amino acid digestibility. Recently, a new measurement has been proposed by Chesson (2000) which appears to be better linked to assessing enzyme potential: the *in vitro* release of arabinoxylan oligomers. However, not only improvements in AME have to be considered. Reduction in the variability of bird performance and the effect on amino acid availability, often resulting in improved carcass quality, should be considered when evaluating the potential benefits of enzymes.

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UTILISATION OF DIETARY PROTEIN BY MODERN LAYING HENS

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Summary

A ^{15}N tracer technique was used to investigate dietary protein digestion and utilization in modern laying hens. A standard layer diet containing ^{15}N -labelled duckweed was offered to hens for one day and the subsequent appearance of ^{15}N in excreta and egg components was monitored. Apparent digestibility of protein was 86%. About 60% of the ingested ^{15}N appeared in eggs or excreta after 7 d while 40% was apparently retained, indicating that there was also extensive protein deposition and catabolism in tissues. In hens ingesting 19 g protein/d and depositing about 6.7 g protein/d in eggs, protein turnover rate was 35 g/d. Deposition of protein in egg white occurred mainly during the 24 h before eggs were laid, whereas protein deposition into developing egg yolks extended for more than 1 week.

I. INTRODUCTION

Protein is an expensive ingredient in poultry diets. The hen deposits about 6-7 g protein in each egg but needs to ingest 17-21 g protein/d. The additional protein is used to maintain body tissues and to allow for inefficiencies associated with digestion, tissue protein turnover and obligatory losses in the excreta. Hennig and Gruhn (1988) investigated dietary protein utilisation in laying hens at about 70% hen-day production by feeding ^{15}N -labelled wheat and then determining labelling patterns in egg proteins. The ability to label the aquatic plant duckweed by growing it on solutions containing ^{15}N enabled the undertaking of further investigations of the digestion and utilization of dietary protein in hens in peak production. The ^{15}N -labelled duckweed was included in the diet of hens for 1 d and the appearance of labelled N in excreta, egg yolk and egg white was monitored during the next 7 d to enable the utilisation of dietary protein for egg production to be described quantitatively.

II. MATERIALS AND METHODS

Commercial layers (ISA Brown) were obtained as 1-d-old chicks from the Baiada hatchery, Tamworth and reared at the University of New England on a commercial grower diet until 13 weeks of age. They were then moved into a temperature-controlled room (18-20°C) and held singly in California style wire-mesh layer cages (45 x 40 x 35 cm). Photoperiod was advanced by 30 min/week until the birds were 20 weeks of age after which a daily regime of 16 h light and 8 h dark was maintained. They were given commercial layer pellets *ad libitum* from 13-18 weeks, and a pre-layer diet from 18-20 weeks, of age. From week 20 the birds were given a standard layer diet containing 150 g protein/kg (as fed) and a calculated 11.3 MJ of ME/kg. The diet included (g/kg): wheat 280, sorghum 270, millrun 100, limestone 85, dicalcium phosphate, synthetic lysine and methionine, and trace minerals and vitamins. The protein concentrates used were (g/kg): sun-dried duckweed (*Spirodella* spp. containing 300 g protein/kg) 150, soybean meal 50 and meat and bone meal 50. From 20-27 weeks, all birds were weighed weekly and their feed intake and egg production were recorded daily.

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In week 26, 5 birds were offered, for 24 h only, a diet that was identical to the layer diet described above except that the duckweed portion was replaced with similar material that had been grown on water containing ^{15}N -ammonium sulphate. Excreta were collected daily for 1 d before and 7 d after the ^{15}N -duckweed was first offered and stored at -20°C , and oviposition time was recorded to within 5 min. Upon collection, eggs were hard-boiled and stored at 4°C . Later, shells were removed and the eggs were cut into halves along the long axis. Each half was weighed and one half was used to obtain representative samples (200 mg approx.) of yolk and white for N analysis. Excreta samples were thawed and, along with feed samples, dried to constant weight at 80°C to determine dry matter content. Uric acid in 150 mg excreta was extracted into 15 ml of 0.068 M borate buffer (Adeola and Rogler, 1994) and its concentration determined from the absorbance of the diluted filtrate at 293 nm. Total N content of egg components, feed and excreta was determined by semi-micro Kjeldahl digestion, steam distillation and titration of the ammonia with HCl. The resulting ammonium chloride was oxidized at 1000°C in O_2 and the resulting nitrogen oxides reduced to N_2 in a nitrogen analyzer (Carlo Erba Instruments, NA 1500) interfaced with a mass spectrometer (TRACERMAS, Europa Scientific) and the ^{15}N abundance was determined. Enrichment was calculated as the difference between the sample abundance of ^{15}N and the abundance in the corresponding material collected the day before labelled duckweed was offered.

III. RESULTS

Mean feed intake did not differ between the pre-experimental and the experimental weeks and was $128 \pm \text{SE } 4.5$ g/day but, on the day the ^{15}N -labelled feed was offered, intake was 11 g/d higher ($P < 0.01$) than the two-week average. There was a small decrease ($P < 0.05$) in mean live weight of the hens during the experimental week (mean start and end weights were 2070 and 2018 g, respectively). On the day the ^{15}N -labelled feed was first offered, and for the next 7 d, the hens maintained their high hen-day egg production ($>97\%$). Their egg mass production did not differ between the pre-experimental and experimental weeks and was $58.5 \pm \text{SE } 1.5$ g/d. Feed conversion ratio (g feed/g egg mass) was $2.18 \pm \text{SE } 0.02$.

During the experimental week, mean N intake of the birds was $3.08 \pm \text{SE } 0.11$ g/d. Output of N in excreta was $1.71 \pm \text{SE } 0.10$ g/d of which 58% was in uric acid, $0.32 \pm \text{SE } 0.01$ g/d was deposited in egg yolk and $0.76 \pm \text{SE } 0.03$ g/d in egg white, leaving a balance of $0.30 \pm \text{SE } 0.02$ g/d that probably included a small loss of volatile N during collection of excreta and other N in egg shell, feathers and dander. The recovery of ^{15}N in these components is shown in Figure 1.

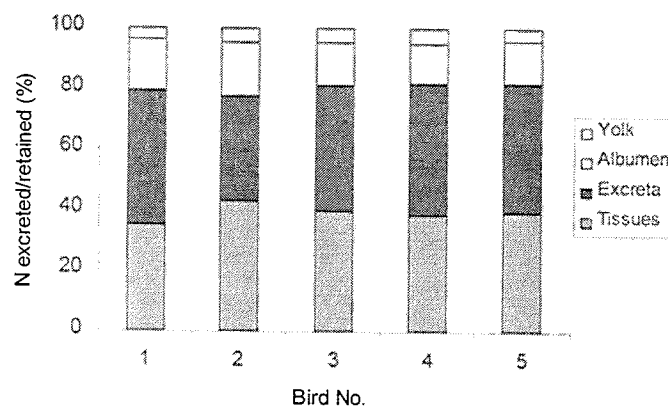


Figure 1. Recovery (%) over one week of ingested ^{15}N in excreta, eggs and miscellaneous components and (by difference) the retention (%) in tissues of 5 laying hens after they had ingested ^{15}N -labelled feed for 1 day.

The recovery of ^{15}N over 7 d in egg yolk and egg white (but excluding that in shell) averaged $19.6 \pm \text{SE } 0.8 \%$ of ^{15}N ingested, and the loss in excreta averaged $41.6 \pm \text{SE } 1.8 \%$.

All hens showed a similar pattern of ^{15}N enrichment in excreta in the period after they ingested ^{15}N -labelled feed. Enrichment was highest in excreta produced on the day that hens ingested labelled feed (about half the enrichment of the labelled feed) and then declined (Figure 2).

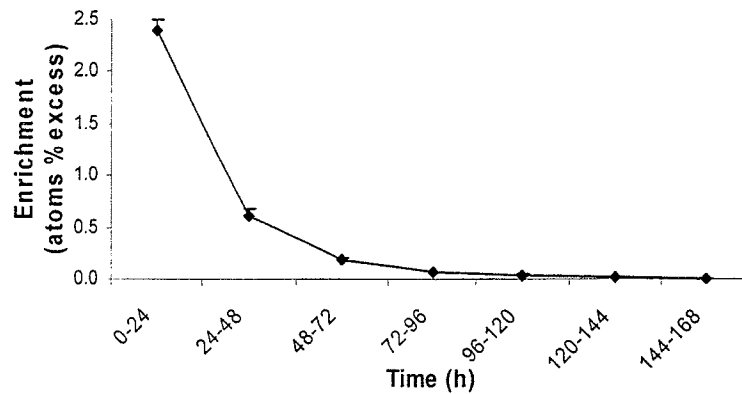


Figure 2. Enrichment (atoms % excess) in excreta after hens received ^{15}N -labelled feed from 0-24 h (Values are the means \pm SE for 5 hens, adjusted to 10 mmol ^{15}N ingested).

There was a low level of labelling in the white of the egg produced during the day the hens ingested ^{15}N -labelled feed. Labelling was highest in the egg produced on d 3 and then declined but remained higher than the excreta from d 4 to 7 (Figure 3A).

The pattern of enrichment in egg yolk was quite different from that in the egg white, increasing steadily after ^{15}N was ingested towards a maximum on d 6 and 7 (Figure 3B).

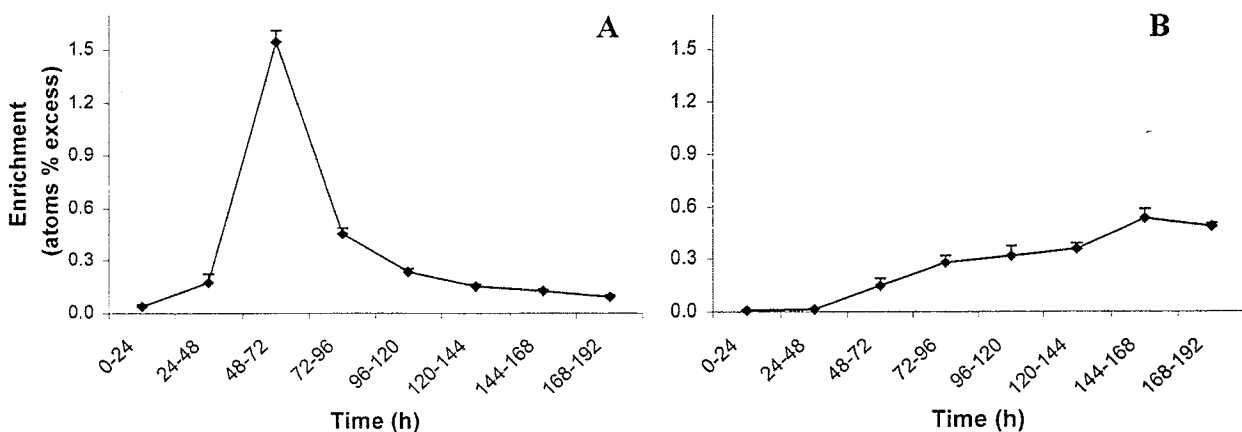


Figure 3. Enrichment (atoms % excess) in white (A) or yolk (B) of eggs formed after hens received ^{15}N -labelled feed from 0-24 h (Values are the means \pm SE for 5 hens, adjusted to 10 mmol ^{15}N ingested).

IV. DISCUSSION

Analyses indicated that most of the N of *Spirodella* spp was present in true protein. It has been assumed that when ^{15}N is incorporated into duckweed protein, it will be found in essential and non-essential amino acids, and that the duckweed protein (30% of the total dietary protein) and the protein in other dietary ingredients will be digested and used in a similar manner.

The hens appeared to have two feeding periods in the 24 h that they had access to labelled feed – one between 0900 and 2100 h on the first day and another between 0500 and 0900 the following day – so the highest enrichment of blood amino acids probably occurred in the morning on d 2. The enrichment in egg white probably reflected the enrichment of blood amino acids while the egg white was being deposited, mainly on the day before the egg was laid. This suggestion is consistent with the occurrence of the highest enrichment in egg white on d 3 (Figure 2). The pattern of appearance of ^{15}N was markedly different for egg white and yolk. Yolk protein enrichments (Figure 3) indicated that protein deposition continued in developing yolks for at least 6 d before ovulation.

The apparent digestibility coefficient of dietary protein was estimated to be $0.865 \pm \text{SE } 0.0397$ by assuming, based on data in Hennig *et al.* (1987), that uric acid N output in excreta ($1.02 \pm \text{SE } 0.22$ g N/d) was 78% of total urinary N excretion. Faecal N output was obtained by subtracting urinary N excretion (uric acid N/0.78) from total N output in excreta. The apparent retention of dietary protein in eggs and tissues, estimated from the difference between feed protein intake (19.3 g/day) and excreta protein output (10.7 g/day), was 45%. The apparent retention of ^{15}N in tissues and eggs was higher than that of unlabelled N, being 61% of that ingested during the 48 h after the labelled feed was first provided. This higher removal of labelled N from the 'metabolic pool' is indicative of a relatively high rate of tissue protein synthesis from intermediates in the blood. The ^{15}N incorporated into tissues was presumably widely distributed at low concentration in a relatively large mass of (mainly) proteins with relatively slow rates of turnover. These tissue proteins could thus be expected to release small amounts of ^{15}N over a period of several weeks and, in this connection, some ^{15}N was still finding its way into egg white on d 7 (Figure 3A).

Whole-body protein turnover was estimated from the results of this experiment using the end-product method (Golden and Jackson, 1984), by assuming that all N in excreta was derived directly from the 'metabolic pool' or indirectly via endogenous excretion into the gut, *i.e.* that true digestibility of dietary protein was 100%. Accordingly, an estimate of whole-body protein flux through the 'metabolic pool' of 35 g/day was made by dividing the excreta protein output from this pool during the same period ($10.7 \pm \text{SE } 0.64$) by the cumulative ^{15}N recovery in excreta on the day labelled feed was offered ($30.8 \pm \text{SE } 2.1\%$). This estimate of whole-body flux was almost twice the rate of estimated protein-N absorption from the gut and five times the rate of deposition of protein in egg mass. As live weight remained almost constant, there must have been a nearly similar rate of protein catabolism in tissues.

Despite the efficient use of dietary protein for egg protein production by the modern layer, it seems clear from these results that there are potential inefficiencies associated with tissue protein turnover and relatively high uric acid excretion. It may be possible to further increase efficiency of dietary protein utilization by hens when the factors regulating protein turnover are more fully understood.

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EFFECTS OF DIET COMPOSITION AND BEAK TRIMMING ON THE INCIDENCE OF CANNIBALISM IN LAYING HENS

S. HARTNI, M. CHOCT, G. HINCH and J.V. NOLAN

Summary

A total of 2880 ISA Brown hens were used to investigate the effect of diet composition on the incidence of cannibalism in a 2 (dim and bright rearing) x 2 (with or without beak-trimming) x 4 (commercial diet, high-insoluble fibre diet, high-soluble fibre diet, high-soluble fibre diet plus enzyme) factorial experiment. Beak trimming had a profound effect ($P < 0.001$) on cannibalism with mortality occurring predominantly in untrimmed birds. Total mortality for the trimmed birds was 0.14% and 0.77% for the pre-lay and early lay periods whereas it was 13.4% and 37.7% respectively for the untrimmed birds. Type of diet significantly affected cannibalism ($P < 0.01$) with high-fibre diets appearing to be preventative against cannibalism. The highest mortality occurred in birds on the commercial diet, i.e. 13% and 29% for the pre-lay and early lay periods. Light intensity during rearing did not influence the incidence of cannibalism. The beak-trimmed birds had lower feed intakes than the non-trimmed birds ($P < 0.05$). Diet also affected the feed intake ($P < 0.05$), being lower ($P < 0.05$) on the commercial diet than on the higher fibre diets. Egg production did not differ significantly between diets.

I. INTRODUCTION

Cannibalism is now widely considered to be the major cause of mortality in commercial layers in Australia. Management factors such as excessive light, crowding, restricted feeder space and poor rearing management are considered by some workers to be the factors that initiate this behaviour (Cain *et al.*, 1984; Johnsen *et al.*, 1998; Kjaer and Vestergaard, 1999). Others have related increased feather pecking, which is often correlated with cannibalism, to frustration associated with the lack of opportunity for dust bathing and ground pecking (Blokhuys and van der Haar, 1989) but the links between feather pecking and severe cannibalism are equivocal. Nutrient deficiencies have also been postulated as a cause of this behaviour with Curtis and Marsh (1992) linking protein deficiency with cannibalism in pheasants and Esmail (1997) relating cannibalism to dietary fibre deficiency. In addition, Cumming *et al.* (1995) observed increased cannibalism in layers fed a diet marginally deficient in available phosphorus.

Beak trimming is widely used in the industry to control pecking and cannibalism but has welfare implications as trimming is known to result in chronic pain for several weeks after completion (Duncan *et al.*, 1989). Consequently alternative methods of controlling cannibalism are being sought.

The present study examined the effect of light intensity during rearing, beak trimming and diet composition on the incidence of cannibalism in cage-housed hens.

II. MATERIALS AND METHODS

ISA Brown birds (2880) were used in a 2 x 2 x 4 factorial design with 2 replicates of 90 birds per treatment. The factors were: light level during rearing (L), beak trimming (B)

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and diet (D). Initially the chicks were housed in two large floor pens, one half being reared in dim light (5 lux) and the other half in bright light (60-80 lux). Half of each of the rearing groups were beak-trimmed (upper beak 1 cm, lower beak 0.5 cm) at 10 weeks of age and all birds were transferred to 5-bird cages in a layer shed at 15 weeks of age where they were held under a natural light regime supplemented by artificial light (>80 lux) for a total of 16 h/day. At 17 weeks the birds were fed one of four pelleted diets: a commercial diet (Diet 1), a high-insoluble fibre (millrun) diet (Diet 2), a barley-based diet high in soluble fibre (Diet 3) and a similar diet plus enzyme (Diet 4). All diets were formulated to be isoenergetic and isonitrogenous according to commercial specifications and produced at a commercial mill in Tamworth.

There were two recording periods, *viz.* pre-lay, 17 to 20 weeks and early lay, 21-24 weeks. In the pre-lay period, weekly feed intake, excreta moisture levels and daily mortalities were recorded. To prevent large imbalances in the number of birds per cage due to treatment differences in mortality, the dietary treatments were crossed over at the end of week 20. Diet 1 was swapped with Diet 2 and Diet 3 was swapped with Diet 4. In the early-lay period, feed intake, excreta moisture and egg production were recorded weekly and mortality daily. For welfare reasons, birds subjected to severe cannibalism were removed to another shed and recorded as 'dead'. At the end of week 24, the experiment was discontinued when cannibalism became unacceptably severe.

Data from periods 1 and 2 were analysed separately using least squares analysis of variance procedures. Mortality data were not normally distributed and were log transformed before analysis.

III. RESULTS

(a) Pre-lay period: weeks 17 to 20

Beak trimming (B) and diet (D) both affected mortality in the pre-lay period ($P < 0.001$, $P < 0.01$, respectively) (Table 1). Trimmed birds had lower mortality than untrimmed birds and birds fed Diets 2, 3 and 4 had lower mortality than those fed Diet 1. There was also a significant ($P < 0.01$) D x B interaction with the untrimmed birds given Diet 1 having the highest mortality. Diet, beak trimming and rearing condition (R) all significantly influenced feed intake ($P < 0.05$) with a significant D x R interaction ($P < 0.01$). Birds from the group reared in dim light and fed Diet 2 had a higher feed intake ($P < 0.05$) than the other groups. There were no significant treatment effects on excreta moisture content.

(b) Early lay period: weeks 21 to 24

The effect of diet on mortality was maintained after the crossover of diets at the end of week 20. The mortality levels were higher with birds on Diet 1 ($P < 0.01$, Table 1) but mortality occurred primarily with the untrimmed birds, with a significant D x B interaction ($P < 0.01$). Feed intake was different for all main effects ($P < 0.05$, Table 1) with intakes being as follows: untrimmed birds > trimmed birds; dim-light-reared birds > bright-light-reared birds; the high soluble-fibre diets (Diets 3 and 4) > Diets 1 and 2. Excreta moisture content was significantly ($P < 0.05$) higher with birds fed the high insoluble-fibre diet than with the other groups. Egg production was greater in the untrimmed birds ($P < 0.01$) and also in the birds reared in dim-light ($P < 0.001$). Egg production differences were reflected in the feed:egg (g:g) ratio (2.95 and 4.39 for dim and bright lighting, respectively) which was also influenced by beak-trimming ($P < 0.05$), with poorer conversion (3.94) in the trimmed birds

than the untrimmed birds (3.39). The birds reared in dim light (2.95) were more efficient ($P<0.001$) than those reared in bright light (4.39). A significant D x B interaction ($P<0.01$) indicated that birds reared in dim light on Diet 2 had the most efficient feed to egg mass conversion (2.81) in the early-lay period.

Table 1. Mean values for mortality, feed intake, and egg production of ISA Brown layers in response to beak trimming, diet and light intensity during rearing.

Treatments	Pre-lay period (17-20 weeks)		Early-lay period (21-24 weeks)		
	% Total mortality	Feed intake (g/d)	% Total mortality	Feed intake (g/d)	% Egg production
Beak trimming					
Beak-trimmed	0.14 ^a	88.2 ^a	0.77 ^a	102.0	58.9 ^a
Untrimmed	13.4 ^b	92.6 ^b	37.7 ^b	107.3	66.6 ^b
Diet					
Commercial diet	13.2 ^b	84.5 ^a	28.9 ^b	100.6 ^a	61.2
Insoluble fibre	3.9 ^a	99.3 ^b	14.3 ^a	101.4 ^a	64.1
Soluble fibre	5.8 ^a	88.5 ^b	15.9 ^a	108.9 ^b	63.2
Sol.fibre+enzyme	4.1 ^a	89.4 ^b	17.8 ^a	107.8 ^b	62.4
Rearing					
Dim	8.1	93.3	17.5	107.1 ^b	70.7 ^b
Bright	5.4	87.5	20.9	102.3 ^a	54.8 ^a

Within column groups, means with different superscripts are significantly different ($P<0.05$).

IV. DISCUSSION

Kjaer and Vestergaard (1999) observed that floor rearing of pullets in dim light (3 vs 30 lux) reduced the incidence of cannibalism. This finding was not confirmed in the present study with birds reared in dim light having no less mortality due to cannibalism. However, they did have higher feed intakes and superior egg production throughout the experiment.

This experiment, using young birds during the early stages of lay, showed that hens fed a high insoluble-fibre diet (Diet 2) or high soluble-fibre diets (Diets 3 and 4) had much lower levels of mortality due to cannibalism than hens fed a control 'commercial' diet. This finding, to a degree, confirms the report of Esmail (1997) that a low fibre diet will induce increased levels of pecking leading to cannibalism. Fibres with different physicochemical properties induce different physiological changes in the gastrointestinal tract of animals (Southgate, 1995). For instance, insoluble fibre acts as a bulking agent causing digesta to pass rapidly through the gastrointestinal tract of birds (Roberfroid, 1993), whereas soluble fibre is known to increase digesta viscosity and reduce the rate of feed passage (Salih *et al.*, 1990). In addition, Hughes and Black (1977) observed that the longer the birds spent in feeding, the lower their mortality due to cannibalism. Thus, the hypotheses examined in this work were that (a) diets high in insoluble fibre will require birds to spend a longer time eating in order to obtain the energy intake needed for production, and (b) diets high in soluble fibre will exacerbate cannibalism problems and degradation of the fibre with enzymes would, therefore, alleviate them. The current results seem to support the first hypothesis although cannibalism was still low during the early laying period when feed intake was lower. This suggests that aspects of fibre other than its bulking properties may be important in altering behaviour. The second hypothesis, however, was not supported by the results. In fact mortality due to cannibalism remained low in birds fed the high soluble fibre diets with or without enzyme supplementation.

It was postulated that increased gut viscosity and reduced nutrient absorption may lead to a situation where birds need to spend time increasing their feed intake to compensate for the reduction in nutrient absorption. However, enzyme supplementation was supposed to overcome the viscosity problem, thereby increasing nutrient digestion. The activity of the enzyme after pelleting and exposure to the viscosity of the gut contents in birds fed both soluble-fibre diets has yet to be determined. A lower mortality from cannibalism in the beak-trimmed birds highlights the difficult welfare dilemma where, on the one hand, beak-trimming causes pain and discomfort to the bird and, on the other, it is highly effective in reducing cannibalism.

This experiment provides evidence that, under commercial conditions, the use of diets high in fibre, especially insoluble fibre, may reduce the incidence of cannibalism. The impact of the high-fibre diets is large for non-beak-trimmed birds but further study is needed to confirm positive long-term effects in beak-trimmed hens. The mechanism whereby high fibre diets prevent cannibalism is not known at this stage.

V. ACKNOWLEDGEMENTS

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OPTIMISING THE TIMING OF INCREASED CALCIUM IN THE DIET FOR LAYING HENS

N. GONGRUTTANANUN, J.R. ROBERTS and W. BALL

Summary

ISA Brown birds were provided with a pre-layer diet at either 16 weeks (Control) or 13 weeks (Treatment) of age. The treatment group had lower feed intake and egg weight throughout the laying life of the flock and significantly lower levels of sodium, potassium and ionised calcium in blood plasma at 60 weeks. There were no differences between the control and treatment groups in egg production, egg shell quality and faecal moisture levels. The two groups of birds responded similarly to water deprivation. There were negative effects of re-vaccination for infectious bronchitis late in lay (76 weeks of age) including an increased incidence of defective egg shells which appeared to be worse in the treatment group, and tendencies to higher faecal moisture and lower Haugh Units. Presentation of a pre-layer diet at too early an age may result in minor kidney damage that has later negative consequences if the birds are subjected to a challenge that affects the kidneys.

I. INTRODUCTION

Adequate calcium in the diet of laying hens is essential for birds to produce strong egg shells at the same time as maintaining adequate stores of calcium in their bones. The complex mechanisms of calcium metabolism in laying hens are still not completely understood (Hurwitz 1987, 1989; Etches, 1987). Medullary bone provides the most immediate source of stored calcium and the amount of medullary bone is influenced by calcium levels in the diet (Clunies et al., 1992). Mobilisation of calcium from the bones into the blood is an essential part of the process of egg shell formation. However, the overall regulation of the calcium levels in the body of the hen also involves the kidneys (Wideman, 1987).

The quantity of calcium in the grower diet is typically 10 g/kg whereas a level of 36 g/kg is normally included in a layer diet. Some producers place birds onto a prelayer (22 g calcium/kg) from several weeks prior to the arrival of the first egg until the birds have reached about 5% production. Over the past few years some Australian producers have used a combination of imported layer strains (which come into lay at 16-18 weeks of age) and Australian-bred strains which usually come into lay several weeks later. Where both types of strains occur on the same farm, sometimes all birds have been placed onto prelayer or layer diets at the same time. This has, on occasion, resulted in some birds receiving the higher level of dietary calcium up to 4-5 weeks prior to the onset of lay. Observations with broiler breeder hens have shown that providing diets with high calcium concentrations before the birds are able to utilise these larger amounts of calcium for egg production can have disastrous effects on the kidneys, resulting in kidney damage and even death. Previous studies (Leeson and Summers, 1997) have suggested that giving a prelayer ration to pullets too early prior to the onset of lay may result in subsequent persistent problems with wet droppings. It was hypothesised that these wet droppings may be due to damage to the kidneys of the birds as the result of the high levels of calcium in the diet at a time when the birds were not forming egg shells.

The present study investigated the effect of early presentation of a prelayer ration to ISA Brown laying hens on later egg production, egg shell quality, faecal moisture, and water

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and electrolyte balance. It also assessed the responses of the control and treatment groups of birds to two situations of stress: water deprivation and revaccination for infectious bronchitis late in lay.

II. MATERIALS AND METHODS

Two hundred ISA Brown female chickens were received at 1-d old in May 1998. Birds were placed in brooders in an isolation laboratory and provided with commercial chick starter diet. At 6 weeks of age the birds were transferred to rearing cages in the University of New England Animal House. Birds were fed a commercial grower diet (10 g calcium/kg) from 6 to 13 weeks of age. At 13 weeks of age birds were transferred to a commercial-style layer shed and housed, individually, in California-style layer cages (used to house 3 birds in commercial situations). When birds were 13 weeks of age, the control group (100 birds) continued to receive the commercial grower diet. However, the treatment group (100 birds) received a prelayer diet containing 22 g calcium/kg. At 16 weeks of age the control group was also placed on the prelayer diet and at 19 weeks of age, when birds had reached 5% production, both groups were placed on a layer diet containing 36 g calcium/kg. At 17 weeks of age birds were exposed to a photoperiod of 12 h light and 12 h dark. Starting one week later, when birds were 18 weeks of age, the light period was increased by 30 min per week until the birds were receiving 16 h of light and 8 h of dark daily at 25 weeks of age. Birds were vaccinated at the hatchery for Marek's disease and infectious bronchitis (IB). Vaccination for IB was repeated at 4 weeks and 12 weeks of age and vaccination for avian encephalomyelitis (AE) was given at 12 weeks of age. Beak trimming was conducted at 4 weeks of age.

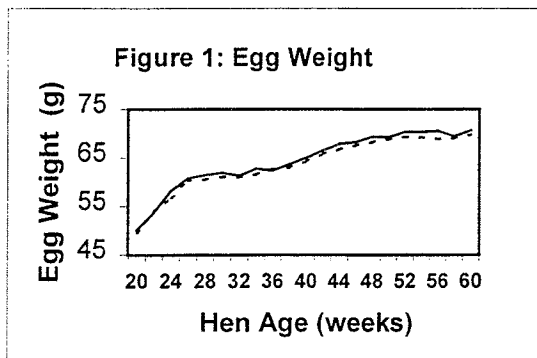
Birds were housed individually and, throughout the experiments, replicates were individual birds. Egg production was monitored continuously and hen-day egg production calculated weekly. Feed intake was measured weekly and feed efficiency calculated. In addition, manure moisture was recorded for 48 birds of each group on one day each week. A sample of 48 birds from each group was weighed at 4, 6 and 12 weeks of age and then every 4 weeks for the duration of the experiment. Detailed measurements of egg and egg shell quality were made every two weeks throughout the experiment. Measurements made on the eggs were: egg weight, shell reflectivity (an indication of the colour lightness of the egg shells), egg shell breaking strength, shell weight, shell thickness, albumen height and Haugh Units, yolk colour and yolk weight. At 60 weeks of age, blood samples were collected from a sample of 20 birds from each treatment group. Haematocrit was determined and plasma analysed for osmolality and the concentrations of sodium, potassium and ionised calcium. Bone breaking strengths were measured on the humerus bones from five birds from each group at the end of the experiment using a Lloyd LRX Materials Testing Machine.

When birds were 80 weeks of age, a sample of birds from each of the groups was subjected to the challenge of water deprivation. Thirty-six birds from each of the control and treatment groups were maintained on drinking water *ad libitum*. An additional 36 birds from each group were deprived of water for a 48 h period. Egg production, egg shell quality and faecal moisture content were measured before, during and following the period of water deprivation. Plasma electrolytes were measured before and after the water deprivation.

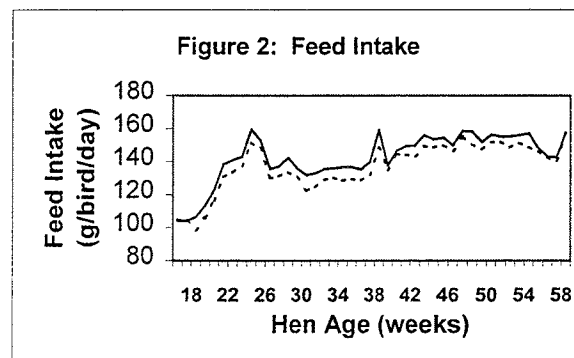
Thirty-six ISA Brown laying hens of 76 weeks of age were taken from each of the control and treatment groups. Birds were then allocated to one of six groups, each of 12 birds: Group 1 (control group + sham vaccination); Group 2 (control + Steggles Strain IB No. 1 vaccine); Group 3 (control + Websters Vic S strain vaccine); Group 4 (treatment group + sham vaccination); Group 5 (treatment + Steggles vaccine); Group 6 (treatment + Vic S vaccine). Vaccines and sham (distilled water) were administered by eye drop.

III. RESULTS

Egg production over the laying life of the flock was similar for both groups. However, both feed intake and egg weight were consistently higher for the control group of birds (Figures 1 and 2). Interestingly, the higher egg weight of the control group in the present study resulted largely from a greater volume of albumen as neither yolk weight nor egg shell weight was higher in the control group. Body weight and faecal moisture content were the same for both groups throughout the laying life of the flock. Also, there were no differences in egg shell quality. For the blood samples taken at 60 weeks of age, the osmolality and concentrations of sodium, potassium and ionised calcium were all significantly higher in the control group (Table 1). Bone breaking strengths measured on the humerus bones resulted in a higher mean result for the treatment birds, although this was not statistically significant ($P=0.08$).



Control Group: Solid Line
Treatment Group: Dotted Line



Control Group: Solid Line
Treatment Group: Dotted Line

Table 1. Plasma osmolality and electrolyte concentrations at 60 weeks of age.

Group	Plasma osmolality (mOsm/kg)	Plasma sodium (mmol/L)	Plasma potassium (mmol/L)	Plasma ionised calcium (mmol/L)
Control Group	322.6 ± 5.0 ^a	149.3 ± 0.7 ^a	5.44 ± 0.08 ^a	1.59 ± 0.03 ^a
Treatment Group	301.5 ± 3.0 ^b	136.7 ± 1.8 ^b	4.90 ± 0.08 ^b	1.29 ± 0.04 ^b

^{a-b}Means within columns with different superscripts differ significantly ($P<0.05$).

Birds that were subjected to water deprivation had lower feed intake, egg production and faecal moisture levels during the period of water deprivation. Egg shell quality (percentage shell, shell thickness and breaking strength) was reduced and Haugh Units and yolk colour were increased. Haematocrit and plasma osmolality, sodium and chloride were elevated. However, these changes were similar for both control and treatment groups of birds.

Revaccination for IB resulted in more defective egg shells (cracked, wrinkled and soft-shelled) than were found in the birds which were not revaccinated and the incidence was greater for the treatment group, particularly with the Steggles vaccine (Table 2). There was also a tendency for lower Haugh Units and increased faecal moisture in the revaccinated birds.

Table 2. Percentage good and defective egg shells following re-vaccination for infectious bronchitis.

Group	Percentage of Shell %			
	Good	Cracked	Soft Shelled	Wrinkled
Control + sham	96.2±1.5 ^a	3.8±1.5	0	0
Control + Steggles	83.3±4.8 ^b	4.0±1.7	3.6±2.9	9.1±3.4 ^b
Control + Vic S	78.7±6.7 ^b	6.6±2.0	5.1±3.4	9.6±6.7 ^b
Treat + sham	94.8±1.4 ^a	2.7±1.2	1.0±0.7	1.4±0.6 ^c
Treat + Steggles	69.4±6.9 ^c	7.7±3.4	4.6±3.2	18.4±5.4 ^a
Treat + Vic S	81.6±3.2 ^b	7.5±1.4	4.5±1.9	6.3±3.5 ^b

^{a-c}Means within columns with different superscripts differ significantly (P<0.05).

IV. DISCUSSION

The results of the present study indicate that early presentation of a pre-layer diet had some long lasting effects on the performance of the flock, including consistently lower feed intake and egg weight. A similar effect on egg size was recorded by Leeson *et al.* (1986). In addition, there were significantly lower concentrations of sodium, potassium, ionised calcium and osmolality in the blood of the birds at 60 weeks of age.

However, egg production, egg shell quality and faecal moisture were not significantly affected. The water deprivation challenge tested the hypothesis that early presentation of pre-layer diet results in minor kidney damage which leaves the birds more susceptible to later challenges that involve the kidneys. However, the response to water deprivation was similar for both groups. The revaccination of birds that had not been vaccinated since they were 12 weeks of age increased the incidence of defective egg shells in both groups but to a greater extent in the treatment group, and had some negative effects on Haugh Units and faecal moisture. These negative effects support the observation that, if the titre levels of birds are allowed to fall, revaccination can negatively affect the kidneys and oviducts of birds. In conclusion, the provision of additional calcium to laying hens prior to the onset of lay needs to take into consideration the potential negative effects of providing additional calcium too early.

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EGGSHELL QUALITY OF CAGED LAYING HENS IN DIFFERENT AMBIENT TEMPERATURES GIVEN DRINKING WATER OF DIFFERENT TEMPERATURES

E.S. TANGKERE¹, B. BHANDARI² and J.G. DINGLE¹

Summary

ISA Brown hens, 22 weeks of age, in 36 two-bird cages were allocated to one of two water temperature regimes, either (i) 5°C or 20°C in the day time or (ii) 35°C or 20°C in the day time, in a controlled environment room held constant at 22°C for 5 weeks, then at a cyclic room temperature that increased to 35°C from 07.00 to 19.00 h for 4 weeks, and then to a constant room temperature 22°C for 2 weeks. Eggshell breaking strength was significantly higher for hens receiving cold water at 5°C rather than at 35°C or 20°C when ambient temperature was 22°C or 35°C, respectively. Egg specific gravity was significantly better and eggshell reflectivity was significantly less (darker colour) when water was 5°C at a room temperature of 35°C. Overall, cooling the drinking water to 5°C during the day time gave better eggshell quality than cooling the water to 20°C or heating the water to 35°C at both normal and high ambient temperatures.

I. INTRODUCTION

Heat accumulation from heat waves occur in poultry sheds during summer (Bola, 1991). As ambient temperature rises, birds pant (hyperventilation), reduce feed intake and increase water consumption to maintain normal body temperature. However, the high temperatures usually heat the drinking water (Stewart and Dingle, 1996). Cooling the water helps hens to resist the effects of hot weather. Hens dissipated metabolic heat when they stood in cold water at 0°C (Van Kampen, 1988). Drinking water with a temperature less than 25°C stimulated hens to consume more food (Stewart and Dingle, 1996). Hens given drinking water maintained at 5°C and 10°C in an ambient temperature at or above 30°C consumed more food and produced more eggs with thicker, heavier shells (Brackpool and Roberts, 1995; Glatz, 1996; Glatz, 1997). In contrast, Damron (1991) found that drinking water at 10°C in an ambient temperature of 32°C did not improve egg production or feed intake and Degen *et al.* (1992) found that a drinking water temperature of 18.5°C in a maximum ambient temperature of 33°C did not improve egg production of broiler breeder hens. It has also been found that hens given drinking water at 17°C or 15°C in ambient temperatures of 30-35°C produced poorer eggshell quality (Brackpool and Roberts, 1995; Tangkere and Dingle, 1999). The current study compared the effects that cooling the drinking water to 5°C, maintaining it at 20°C or heating it to 35°C, in normal and high ambient temperature environments, had on eggshell quality.

II. MATERIALS AND METHODS

Seventy-two ISA Brown laying hens, 22 weeks of age, were used in this experiment. They were kept in 36 two-bird cages (experimental units) and allocated to one of two water temperature regimes. The first of these allowed for water temperatures of either 20°C or 5°C (cool group) and the second allowed for water temperature of either 20°C or 35°C (warm

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group). All cages were maintained in the same controlled temperature environment with room temperature set constant at 22°C for 5 weeks, then set to cycle daily from 22°C at 19.00-07.00 h to 35°C from 07.00-19.00 h for 4 weeks, and then back to a constant 22°C for 2 weeks. The sequences of ambient temperatures and water temperatures are shown in Table 1. Water was circulated by pump from a water bath maintained at the required water temperature using a water heater or water cooler and ice. Feed was given in separate troughs, one per cage. All birds were fed the same diet containing (/kg):11.4 MJ of ME, 168 g crude protein, 35 g calcium and 6.6 g phosphorus.

Table1. General conditions of the experiment.

Stages	Weeks	Ambient	Water Temperature (°C)	
		Temperature (°C) 07.00-19.00 h	Cool group (07.00- 19.00 h)	Warm group (07.00- 19.00 h)
1	1	22°C	20°C	20°C
1	2	22°C	20°C	20°C
2	3	22°C	5°C	20°C
3	4	22°C	20°C	35°C
4	5	22°C	5°C	35°C
5	6	35°C	20°C	20°C
6	7	35°C	20°C	35°C
7	8	35°C	5°C	20°C
8	9	35°C	5°C	35°C
9	10	22°C	20°C	20°C
9	11	22°C	20°C	20°C

Eggs used for examining external quality were taken each day during the period when the hens were subjected to a constant ambient temperature of 22°C and four days each week when the hens were subjected to a day time maximum ambient temperature of 35°C. The eggshell quality measurements taken were egg shape index (% width:length), egg specific gravity, eggshell colour reflectivity (Minolta CR100 Chromameter), egg breaking strength (using a texture analyser machine, Model TA-XT2), shell weight, percentage shell and shell thickness (using electronic digital callipers 0-150 mm/6"). This trial was an incomplete crossover design. Within each week unpaired t-tests were used to compare the two water temperature treatments on eggshell characteristics (SAS, 1990).

III. RESULTS AND DISCUSSION

There were no significant differences between the egg weight, shell thickness and percentage shell of the two groups of hens at any stage of the trial (Table 2). Egg specific gravity was significantly higher ($P < 0.05$) for the 5°C water group compared to the 35°C water group in stage 8 when ambient temperature was 35°C. The specific gravity of the eggs of hens that received the 35°C water decreased at this stage while the specific gravity of the eggs of hens that received the 5°C water did not decrease. It appeared that not as much eggshell was deposited when drinking water was kept at 20°C as when water was 5°C. Egg shape index was significantly higher in the hens which received the 5°C water in stage 8 when room temperature was 35°C. Eggshell reflectivity or lightness increased during the heat period and for the two weeks after. In stage 8 (the fourth week of heat period) shell reflectivity of hens which received warm water was significantly greater (i.e. shells were a

lighter colour) than hens that received cold water. The lighter colour of eggs may be caused by less pigment being deposited or from extra calcium layers over the pigment and it may be a reflection of a stress response (Leary *et al.*, 1997; Roberts and Ball, 1998). Numerically, the shell breaking strength of eggs from hens that received cold drinking water was slightly higher throughout the experiment, but was significantly better when drinking water was 5°C at both normal and high ambient temperatures (stages 4 and 7). The shell weight of hens which received cold water at 5°C was significantly greater than for hens which received the 20°C drinking water at an ambient temperature of 35°C.

Table 2. Eggshell characteristics of caged laying hens 22 to 33 weeks of age given different water temperature at different ambient temperatures (Means \pm SEM).

Stages		1	2	3	4	5	6	7	8	9
Room °C		22	22	22	22	35	35	35	35	22
Water °C		20-20	5-20	20-35	5-35	20-20	20-35	5-20	5-35	20-20
EW (g)	C	62.48 ± 0.42	62.17 ± 0.57	63.64 ± 0.58	62.50 ± 0.51	61.11 ± 0.54	61.24 ± 0.65	61.53 ± 0.81	58.77 ± 0.69	60.51 ± 0.73
	W	61.84 ± 0.55	62.88 ± 0.44	63.49 ± 0.55	63.18 ± 0.53	61.17 ± 0.55	60.62 ± 0.83	59.92 ± 0.88	59.20 ± 0.77	59.48 ± 0.98
SG	C	1.076 ± 0.0007	1.084 ± 0.0008	1.083 ± 0.0007	1.091 ± 0.0010	1.078 ± 0.0007	1.080 ± 0.0011	1.077 ± 0.0010	1.078* ± 0.0010	1.082 ± 0.0013
	W	1.077 ± 0.0005	1.084 ± 0.0005	1.085 ± 0.0008	1.089 ± 0.0009	1.080 ± 0.0010	1.078 ± 0.0009	1.074 ± 0.0011	1.073 ± 0.0013	1.081 ± 0.0012
SI (%)	C	77.78 ± 0.25	77.65 ± 0.38	76.61 ± 0.26	76.49 ± 0.38	76.40 ± 0.30	76.04 ± 0.47	76.15 ± 0.41	76.15* ± 0.28	76.11* ± 0.35
	W	77.28 ± 0.43	76.81 ± 0.41	76.70 ± 0.43	75.98 ± 0.35	75.80 ± 0.50	75.48 ± 0.43	74.98 ± 0.50	75.11 ± 0.42	74.77 ± 0.52
SR	C	60.54 ± 0.52	61.62 ± 0.50	62.13 ± 0.58	61.81 ± 0.68	65.70 ± 0.54	65.45 ± 0.58	64.86 ± 0.84	64.97* ± 0.58	65.73 ± 0.77
	W	60.77 ± 0.65	61.88 ± 0.81	62.22 ± 0.83	62.19 ± 0.91	65.37 ± 0.84	65.45 ± 0.82	67.47 ± 1.17	67.71 ± 1.06	66.44 ± 1.01
EBS (N)	C	43.48 ± 0.99	44.27 ± 1.11	43.27 ± 0.88	43.71* ± 0.97	41.92 ± 0.71	46.72 ± 1.01	47.19* ± 1.10	47.69 ± 1.31	46.74 ± 1.43
	W	43.68 ± 0.63	42.60 ± 0.70	42.76 ± 0.75	40.97 ± 0.83	40.70 ± 0.82	44.82 ± 1.07	43.16 ± 1.05	45.65 ± 1.44	46.21 ± 1.93
SW (g)	C	6.15 ± 0.06	6.07 ± 0.06	6.25 ± 0.08	6.30 ± 0.06	5.82 ± 0.09	6.09 ± 0.07	6.16* ± 0.10	5.89 ± 0.11	6.22 ± 0.12
	W	6.20 ± 0.08	6.19 ± 0.07	6.39 ± 0.08	6.33 ± 0.08	5.99 ± 0.08	5.97 ± 0.10	5.83 ± 0.12	5.86 ± 0.14	6.14 ± 0.14
ST (mm)	C	0.368 ± 0.004	0.370 ± 0.005	0.370 ± 0.004	0.363 ± 0.004	0.347 ± 0.003	0.356 ± 0.004	0.360 ± 0.004	0.362 ± 0.005	0.364 ± 0.007
	W	0.370 ± 0.003	0.369 ± 0.002	0.377 ± 0.004	0.365 ± 0.004	0.352 ± 0.004	0.354 ± 0.005	0.354 ± 0.006	0.359 ± 0.0063	0.363 ± 0.007
PS (%)	C	9.85 ± 0.11	9.78 ± 0.10	9.84 ± 0.11	10.10 ± 0.12	9.53 ± 0.09	9.97 ± 0.14	10.04 ± 0.12	9.93 ± 0.20	10.30 ± 0.19
	W	10.04 ± 0.11	9.86 ± 0.09	10.07 ± 0.12	10.03 ± 0.10	9.80 ± 0.11	9.86 ± 0.11	9.75 ± 0.14	9.91 ± 0.19	10.32 ± 0.18

* Means within a cell are significantly different from one another ($P < 0.05$);

C = cold; W = warm; EW = egg weight; SG = specific gravity; SI = shape index; SR = shell reflectivity; EBS (N) = eggshell breaking strength in Newtons; SW = shell weight; ST = shell thickness; PS = percent shell.

This study indicated that cooling the drinking water to 5°C gave better performance in all eggshell characteristics. This finding is in line with those of Brackpool and Roberts (1995) and Glatz (1996, 1997) who found that hens given drinking water maintained at 5 or 10°C had equal or higher egg production and eggshell quality at high ambient temperatures than hens with warmer drinking water. The better shell breaking strength of the eggs produced by hens from the cold water group may be due to the birds being able to cope better with high ambient temperature because cold drinking water may help dissipate body heat

(Van Kampen, 1988). Hence, the synthesis of the membranes and the shell of eggs from these birds may have been better (Parsons, 1982; Brackpool and Roberts 1995; Roberts, 1995).

IV. CONCLUSIONS

Overall there were not many differences in eggshell quality characteristics between hens which received water at either 5°C, 20°C or 35°C in normal or high daily temperature environments. However, cooling the drinking water to 5°C when ambient temperature was 35°C for 12 h during the day enabled hens to produce eggs which maintained their dark shell colour and had higher specific gravity, shell weight and eggshell breaking strength.

V. ACKNOWLEDGMENTS

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EGGSHELL QUALITY OF CAGED LAYING HENS GIVEN DRINKING WATER MAINTAINED AT 20 - 26°C IN NORMAL AND HIGH AMBIENT TEMPERATURES AND GIVEN FEED WITHOUT OR WITH BARNYARD GRIT OR EGGSHELL-49

E.S. TANGKERE¹, B. BHANDARI² and J.G. DINGLE¹

Summary

Twenty four 37-week old Lohmann Brown hens in eight replicate three-bird cages were allocated to each of three dietary treatments: Control (C), C + 5% Barnyard Grit and C + 0.1% Eggshell-49. Repeated measurements were made when ambient temperature was kept constant at 22°C (for 5 weeks), when daily ambient temperature was changed cyclically from 22°C (19.00-07.00 h) to 35°C (07.00-19.00 h) (for 4 weeks) and when changed back to a constant 22°C (for 2 weeks). Drinking water temperature was maintained between 20° and 26°C. There were no effects of the dietary additives and no significant interactions between the dietary groups and room temperature in terms of eggshell characteristics when the daily ambient temperature increased from 22°C to 35°C. However, egg weight, egg specific gravity, egg shape index and shell colour decreased, but eggshell breaking strength increased, when ambient daily temperature increased to 35°C but drinking water was kept between 20° and 26°C.

I. INTRODUCTION

One of the main causes of poor eggshell quality is high ambient temperature (Glatz, 1997; Gay, 1998; Roberts and Ball, 1998). Cool drinking water has prevented the deterioration of eggshell quality in hot weather (Glatz, 1996; Roberts and Brackpool, 1995). Some dietary additives have been reported to also improve eggshell quality. These include Barnyard Grit[®], which is produced in Australia and in preliminary studies increased egg production and minimised egg breakage (Perez-Maldonado, 1999) and Eggshell-49[®]. The latter is a combination of proteinated trace minerals such as manganese and zinc which are important in shell membrane synthesis and eggshell formation because they are cofactors of enzymes responsible for mucopolysaccharide synthesis and carbonate formation (Sefton, 1997; Gomez-Basauri, 1999). The effects of Barnyard grit and Eggshell 49 on the eggshell quality of laying hens given cool drinking water when caged at normal and high ambient temperatures were measured in this trial.

II. MATERIALS AND METHODS

Three dietary treatments, (Control diet (C), C + 50 g Barnyard grit/kg, C + 1 g Eggshell 49/kg) were each randomly allocated to eight replicate three bird cages containing 37 week old Lohmann Brown hens (72 total) in a controlled environment chamber. The composition and analysis of the control diet are shown in Table 1. Repeated measurements were made as the ambient temperature was kept at a constant 22°C for 5 weeks (period 1), changed to a daily cyclic 22°C from 19.00 to 07.00 h and 35°C from 07.00 to 19.00 h for 4 weeks (period 2), and then to a constant 22°C for 2 weeks (period 3). Feed was given in separate troughs, one per cage, and refusals weighed each day. Birds had access to drinking water via nipple lines and the water was circulated by pump from a water bath maintained at 20 to 26°C using ice. Eggs were collected and weighed daily. Eggs used for examining

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external and internal quality were taken each day in period 1 and four days each week in periods 2 and 3. The egg quality measurements made were egg shape index, egg specific gravity, eggshell colour reflectivity (Minolta CR100 Chromameter), egg shell breaking strength (using a texture analyser machine, Model TA-XT2), shell weight, percentage shell and shell thickness (using electronic digital callipers 0-150 mm/6"). Repeated measures ANOVA were made using the SAS GLM procedure (SAS, 1990).

Table 1. Composition and calculated analysis of control diet.

Ingredient	(CP g/kg)	g/kg	Analysis	g/kg
Wheat cracked	(125)	248.0	Crude protein	168.0
Sorghum cracked	(100)	402.6	ME (MJ/kg)	11.38
Mung beans	(240)	40.0	Calcium	35.0
Meat meal	(500)	72.3	Phosphorus	6.6
Soybean meal (U.S)	(480)	62.6	Av. phosphorus	3.7
Full fat soyabean	(380)	46.6	Sodium	1.40
Millrun	(160)	14.0	Potassium	5.6
Limestone (Fine)		68.6	Chlorine	1.7
Salt		1.0	Lysine	7.8
Sodium bicarbonate		0.6	Methionine	3.1
Bentonite		40.0	Threonine	5.7
DL-Methionine		0.8	Leucine	14.1
L-Lysine HCl		0.4	Isoleucine	6.5
Layer premix		2.0	Tryptophan	1.7
		999.9		

III. RESULTS AND DISCUSSION

There were no significant differences between the dietary treatments and there were no significant interactions between the dietary treatments and room temperatures in terms of feed consumption and eggshell characteristics. However, there were significant differences ($P < 0.01$) in feed consumption and eggshell characteristics at different ambient temperatures (Table 2). Feed consumption of the laying hens significantly decreased when the room temperature rose to 35°C. Egg weight, egg shape index and egg specific gravity significantly decreased when the day temperature increased from 22°C to 35°C and did not fully recover during the next two weeks when day temperature was again 22°C. Eggshell reflectivity was significantly greater (lighter colour) during and after the heat period. The lighter colour of eggs during and after the heat period may have resulted from a reduction in the amount of pigment deposited or from extra calcium layers over the pigment and it may be a reflection of a stress response (Leary *et al.*, 1997; Roberts and Ball, 1998b). Interestingly, eggshell breaking strength was significantly increased during the heat period and it was even greater after the room temperature was reduced. Numerically, eggshell breaking strength of the hens fed Barnyard Grit tended to be higher compared with those fed Eggshell 49 and the Control group but statistically there were no differences between these three dietary groups. There were no significant differences in shell weight and percentage shell of eggs which were taken before and during the heat period. However, the shell weight and percentage shell of eggs taken after the heat period were significantly higher than those of eggs before and during the heat period. The National Research Council (1981) and Siegel and Jordan (1997) have stated that many factors, including acclimation of the birds, influence the reaction of poultry to temperature changes. Therefore, the ideal temperature range for different classes and ages of poultry cannot be generalized. Birds with better genetic adaptation to hot climates can

maintain health and egg production under severe conditions. The current results are interpreted to mean that although the egg weight decreased, the egg quality of Lohmann Brown hens did not deteriorate when the maximum daily temperature increased to 35°C as long as the drinking water was maintained at 20° to 26°C. In addition, shell weight, shell strength and percent shell increased significantly when daily ambient temperature fell from 35°C to 22°C.

Table 2. The effect of Barnyard Grit, Eggshell-49 and ambient temperature on feed intake and eggshell quality of caged laying hens, 37-48 weeks of age (Mean \pm SEM).

	Nutrients group (NG)			Room temperature (RT)			Significant	
	Control	Barnyard	Eggshell49	Before	Heat	After	NG	RT
FI (g)	105.78 \pm 5.60	102.64 \pm 5.60	103.59 \pm 5.60	111.02 ^a \pm 0.82	96.02 ^c \pm 0.90	104.97 ^b \pm 1.34	NS	**
EW (g)	59.06 \pm 1.09	60.03 \pm 1.10	61.12 \pm 1.14	60.53 ^a \pm 0.15	59.49 ^b \pm 0.24	60.18 ^{ab} \pm 0.48	NS	**
SI (%)	75.16 \pm 0.56	75.52 \pm 0.57	74.88 \pm 0.59	76.02 ^a \pm 0.13	74.90 ^{bc} \pm 0.19	74.64 ^c \pm 0.40	NS	**
SG	1.077 \pm 0.0009	1.078 \pm 0.0009	1.077 \pm 0.0010	1.080 ^a \pm 0.0002	1.077 ^b \pm 0.0003	1.077 ^b \pm 0.0007	NS	**
SR	67.77 \pm 0.93	66.75 \pm 0.95	66.46 \pm 0.98	65.83 ^b \pm 0.13	67.38 ^a \pm 0.19	67.77 ^a \pm 0.39	NS	**
EBS (N)	44.41 \pm 1.10	46.05 \pm 1.12	44.27 \pm 1.16	42.80 ^c \pm 0.25	44.97 ^b \pm 0.38	46.95 ^a \pm 0.77	NS	**
SW (g)	5.76 \pm 0.12	5.92 \pm 0.13	5.89 \pm 0.13	5.81 ^{bc} \pm 0.02.0	5.74 ^c \pm 0.03	6.04 ^a \pm 0.06	NS	**
ST.(mm)	0.364 \pm 0.005	0.368 \pm 0.006	0.363 \pm 0.006	0.363 \pm 0.001	0.365 \pm 0.001	0.367 \pm 0.003	NS	NS
PS	9.77 \pm 0.14	9.88 \pm 0.14	9.66 \pm 0.15	9.61 ^c \pm 0.03	9.67 ^{bc} \pm 0.04	10.04 ^a \pm 0.09	NS	**

Means in a row without a similar superscript differ significantly ($P < 0.05$).

**= $P < 0.01$ NS= not significant.

FI = feed intake/day; EW= egg weight; SI= shape index; SG= specific gravity; SR= shell reflectivity; EBS (N)= egg breaking strength in Newtons; SW (g)= shell weight in grams; ST (mm)= shell thickness in millimeters; PS= percent shell.

IV. CONCLUSIONS

Feed consumption, egg weight, egg specific gravity, egg shape index and shell colour decreased when daily ambient temperature increased from 22°C to 35°C and drinking water was maintained at 20° to 26°C. Eggshell breaking strength increased when ambient temperature increased. The addition of Barnyard Grit or Eggshell 49 to the diet did not affect the quality of eggs produced under these conditions. Maintaining drinking water at 20° to 26°C appeared to prevent the deleterious effects of hot weather on eggshell quality.

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DEVELOPMENT OF A NON-INVASIVE TEST FOR STRESS IN LAYING HENS

J.A. DOWNING, D.R. FRASER and W.L. BRYDEN

The egg industry faces continued criticism of the ethics of husbandry practices used in egg production, especially the welfare of hens kept in cages. Assessment of hen welfare is difficult because it encompasses many factors. Physiological changes can be a sensitive measure of welfare as these relate to changes in the stress-axis. Many of the responses to stressors highlight the importance of the adrenal gland in regulating physiological changes (Downing and Bryden, 1999). As a general adaptive response corticosterone increases gluconeogenesis and blood glucose, increases catabolism of muscle tissue, increases fatness and depresses immunity. Short-term responses to stress result in catecholamine release from the adrenal. There are inherent difficulties with the interpretation of circulating hormone concentrations because of intrinsic patterns and changes occurring in response to sampling. Non-invasive techniques of measuring levels of stress hormones would reduce these problems. Hormone levels in the egg could provide a non-invasive method for measuring stress levels in hens and prove helpful in identifying conditions responsible for poor welfare. The gradual accumulation of albumen over 6 h during egg formation potentially provides an accurate reflection of circulating hormones over this time. The overall objective of this study was to develop procedures for measuring stress hormones in egg albumen and then to assess whether albumen levels of these hormones reflect stress experienced by laying hens.

Assays to determine corticosterone and catecholamines (adrenaline and noradrenaline) in albumen samples have been developed and validated. In a series of studies the relationship between circulating concentrations of stress hormones (corticosterone and catecholamines) and their sequestering into egg albumen was determined under normal conditions and experimentally-induced stress. Heat stress (32°C) of hens increased egg albumen corticosterone concentrations compared to hens held at 18°C (Downing and Bryden, 2001). However, there was no effect on adrenaline levels. Handling in various forms is stressful to hens. When hens were handled there was an increase in egg albumen corticosterone levels especially during early episodes of handling. It is possible hens adjust to the handling procedures and differences in corticosterone levels abate. While handling is known to stimulate catecholamine release there were no differences in albumen catecholamine levels following handling.

From these studies it appears that corticosterone levels in egg albumen reflect the exposure of the hen to stressors. Moreover, corticosterone is easily measured in albumen and sample processing is relatively inexpensive, both important aspects of a rapid assay method. In contrast, catecholamines are difficult to measure and no evidence was found to indicate that egg albumen levels of these hormones were increased by stressors which influence corticosterone levels. It is concluded that catecholamine levels in albumen fail to provide a non-invasive measure of stress in hens.

These studies have demonstrated that a more extensive evaluation of albumen corticosterone concentrations as a measure of stress in hens is warranted and should provide a useful tool in assessing the effects of husbandry and housing on hen welfare.

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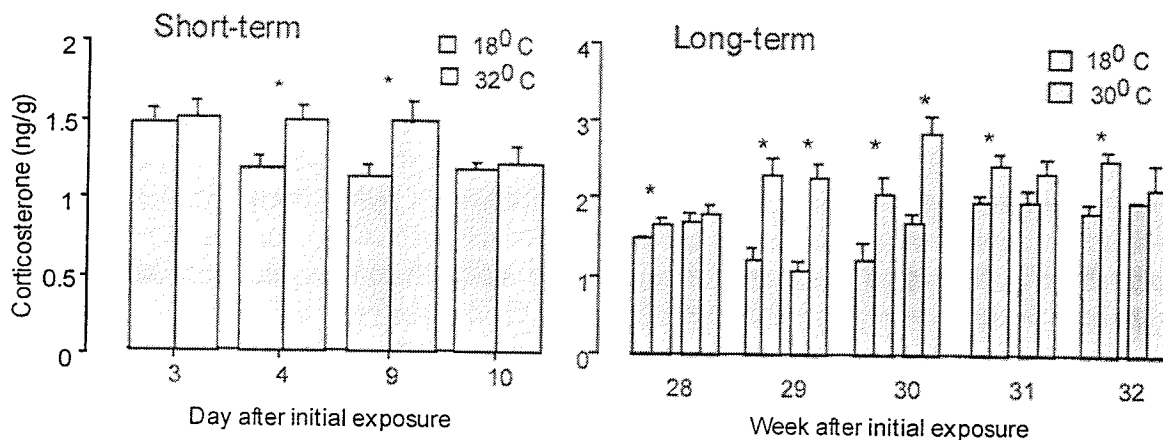
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EGG ALBUMEN CORTICOSTERONE CONCENTRATION IN HENS EXPOSED TO HIGH TEMPERATURE

J.A. DOWNING and W.L. BRYDEN

Assessment of bird welfare is difficult but measures based on physiological changes are common and many of these are related to responses by the hypothalamic-adrenal-axis. Corticosterone is the main steroid secreted from the avian adrenal but there are difficulties with interpretation of circulating levels because of sampling complications. The egg, by providing a non-invasive means of measuring levels of stress, may help overcome these problems (Downing *et al.*, 2001). The present study examined corticosterone levels in egg albumen when hens were exposed to a high ambient temperature.

In a short-term study, hens (15/room) were housed in two rooms and the temperatures set at constant 18⁰ C or constant 32⁰ C. On days 3, 4, 9 and 10 after initial exposure, eggs (10/room) were collected, opened and samples of albumen taken. On days 5 and 10 all birds were bled. In a long-term study, hens (20/room) were housed in two rooms and the temperatures set at constant 18⁰ C or constant 30⁰ C. Twice during each of weeks 28-32 after initial exposure, 10 eggs/room were collected, opened and samples of albumen taken. All hens were bled at the end of week 32. Albumen samples and plasma were analyzed for corticosterone using a validated radioimmunoassay. Differences on individual sampling days were assessed by unpaired student's t-test. Corticosterone concentrations in albumen are given in the figure. Significant ($P < 0.05$) differences are indicated by *.



Short-term, high temperature had no effect on plasma corticosterone levels but significantly increased albumen levels on days 4 and 9. Long-term, high temperature increased significantly, mean plasma corticosterone (4.5 ± 0.3 vs 2.7 ± 0.2 ng/ml) and also, egg albumen levels at most sampling times. These results indicate that corticosterone concentrations in egg albumen could provide a non-invasive measure of stress in hens.

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ARTHRITIS AND THE CHICKEN ANKLE JOINT

C.A. LUNAM¹ and M.J. GENTLE²

Pain resulting from orthopaedic disease in birds continues to be a welfare issue. Recent research has shown that arthritis results in sensitization of joint receptors which could form the basis of spontaneous pain, hyperalgesia and allodynia seen in inflammation. The relationship between the peripheral sensory afferents in the joint and the painful changes seen in arthritis are not fully understood. The aim of this work is to investigate the complex relationship between the peripheral nervous system and pain in naturally occurring orthopaedic disease. A pilot project was undertaken at the Roslin Institute, with Animal Ethics clearance, to investigate the effects of inflammation on substance P nerve fibres which are involved in inflammation and transmission of pain.

Pullets were injected with sodium urate microcrystals into the left ankle joint. The right ankle joint served as the control and received either saline or was not injected. Four hours after injection the left ankle joint developed gout and the birds were killed and the joint capsule and synovial membrane of both ankle joints were removed. After fixation of the tissue, the substance P nerve fibres were visualized using immunofluorescence. The tissue was examined using a laser confocal microscope.

Substance P containing nerve fibres were identified in both the synovial and subsynovial tissue. It is likely that some of these substance P fibres will be part of the population of fibres in which physiological responses have been investigated in both health and disease (Gentle, 1997). Fewer substance P fibres were observed in the inflamed joints compared with the controls.

These findings would support the hypothesis that during major inflammatory joint disease, substance P in the peripheral afferent nerve fibres is being released faster than it can be produced. This pilot study has for the first time shown the distribution of substance P nerve fibres in the joints of birds in both health and disease. It has been demonstrated that even short duration acute inflammation can have a profound effect on neuropeptide content of peripheral nerve fibres and this work forms the basis in which to study not only substance P but also how other neuropeptides may be affected by the disease process.

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CLAW ABRASIVES IN LAYER CAGES

P.C. GLATZ

A low-cost, non-invasive method by which the claws of caged layers could be kept short and blunt can be achieved by fitting 8-mm strips of abrasive tape on the egg guard. Bird's claws scrape against this tape while they are feeding. This reduces the effectiveness of the claws in causing injury and feather loss and reduces the risk of birds trapping their claws in the cage. Tauson (1996) suggested that in addition to abrasive strips a mixture of paint and sand might also be an effective abrasive when coated onto the egg guard. A total of 960 laying hens (Hyline Gold) were housed 5 per cage in 192 Harrison 'Welfare' back-to-back, single tier cages (each 500 mm wide by 545 mm deep; 545 cm²/bird) in a fan ventilated insulated laying shed with louvred windows. There were three treatments comprising; i) control cages without abrasives, ii) treatment cages with two 8-mm wide abrasive strips (3M-Safety Walk, General Purpose Black) fitted to the egg guard, and iii) treatment cages with an abrasive paint (Galaxy Abrasives G4450 Poultry Paint) applied in one 5-cm strip to the egg guard. A randomised design was used for allocation of treatments with 32 replicates per treatment. A single replicate comprised 10 birds in two adjacent cages. At 60 weeks of age the middle claws were assessed on both feet for claw sharpness (1-4 point scale) and middle claw length of both feet were measured. Mortality was recorded daily and deaths as a result of prolapse and cannibalism were noted over the 20-60 week period. The experiment was analysed using the General Linear Models procedure (using Base-SAS[®] software, 1988). The middle claw length of hens from the three treatments was significantly different ($P<0.05$) at 60 weeks. The birds using the abrasive paint had the lowest claw length and claw sharpness. Mortality from prolapse and cannibalism was significantly higher ($P<0.05$) for birds using the abrasive strips and abrasive paint (Table). Total mortality approached significance ($P=0.10$).

Claw length and claw sharpness (1, blunt; 4, sharp) at 60 weeks, total mortality and deaths from prolapse and cannibalism.

Treatment	Claw length (mm)	Claw sharpness	Total mortality %	Prolapse+cannibalism %
Control	31.7a	3.6a	4.7	1.6a
Strip	23.7b	2.2b	10.9	5.9b
Paint	13.8c	1.1c	9.4	6.3b
l.s.d.($P=0.05$)	1.0	0.2	ns	4.2

ns=not significant.

One of the reasons for reducing claw length with claw shorteners is to reduce mortality by minimising abrasions caused by the claws. Surprisingly, hen mortality from prolapse and cannibalism was significantly higher in cages fitted with abrasives. There are no other reports in the literature showing an increase in prolapse and cannibalism from hens using abrasives. It is speculated that when birds are frightened or competing for a position at the feed trough they might abrade their vent region on the paint or the strips region encouraging vent pecking. The results of this trial question whether claw shorteners should be installed in layer cages under Australian conditions. If abrasives in cages are responsible for the increase in cannibalism observed in this trial then their use cannot be recommended until further work is carried out.

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DIETARY VITAMIN E MODULATES INTESTINAL IMMUNITY

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Supplementation of poultry diets with Vitamin E (VE) has been shown to improve some aspects of immune function in the chicken, including increased macrophage phagocytosis and increased immunoglobulin G (IgG) and IgM antibody production (Tengerdy and Brown, 1976; Haq *et al.*, 1996). However, the effect of dietary VE on IgA antibody, which acts as the first line of defence of the intestinal mucosa, has not been evaluated in the chicken. Interestingly, recent studies in rats have shown a significant increase in the number of IgA-producing lymphocytes in the mesenteric lymph nodes with VE supplementation of the diet (Kaku *et al.*, 1999).

The present study was designed to investigate the impact of dietary VE on IgA antibody production in broiler chicks, with and without immunisation. From the day of hatch chicks were placed on a maize-based diet containing 50 mg VE/kg which was supplemented with either 50, 250, 1250 or 5000 mg VE [BASF, Lutavit E 50 Special]/kg. At d 21 all chickens were intraperitoneally immunised with Tetanus toxoid in Auspharm adjuvant (patent pending). Two weeks later they received an oral booster of T. toxoid. Serum samples for determination of antibody levels were collected on days 21, 35 and 42, samples of peripheral blood lymphocytes for analysis of T cell subsets were collected on d 19, 26, 33 and 40 and samples of intestinal scrapings were taken at the end of the experiment on d 42.

The effect of dietary VE supplementation on total IgA antibody and antigen-specific IgA antibody levels were determined in serum and intestinal scrapings by ELISA. Total immunoglobulin (Ig) in the serum was also measured. The percentage of circulating CD3⁺, CD4⁺ and CD8⁺ T lymphocytes and Ia⁺ cells were determined by flow cytometry.

Birds receiving 250 mg VE/kg had notably higher total IgA antibody levels in the intestinal scrapings at d 42. Total serum IgA of these birds was significantly higher than for the control birds at d 21, 35 and 42. Following immunisation with T. toxoid, birds receiving 5000 mg VE/kg had significantly higher anti-T. toxoid IgA in the intestinal scrapings at d 42. Total serum Ig was significantly increased at day 35 in birds receiving 250 mg VE/kg and at d 42 in birds 5000 mg VE/kg.

Significant alterations in T-cell subsets in peripheral blood lymphocytes were observed at d 26 when birds receiving 250 mg VE/kg had an increased percentage of CD3⁺ T lymphocytes. These birds also demonstrated higher percentages of CD4⁺ lymphocytes and Ia⁺ cells compared to the control birds.

These results demonstrate the positive impact of dietary VE on systemic and mucosal immune responses in the chicken. In particular, VE induces an increase in IgA (total and antigen-specific) antibody in the serum and intestinal mucosa.

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OPTIMISING INFECTIOUS BRONCHITIS VACCINATION PROTOCOLS FOR LAYING HENS

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The effect of age at first vaccination and route of vaccine administration in providing protection against infectious bronchitis virus (IBV) was investigated in cockerels. Day-old ISA Brown cockerels (350) were purchased from the Winton Hatchery near Tamworth, NSW and transferred to isolation pens at the University of New England, Armidale, NSW. There were seven experimental groups, each of 50 birds: Control (no vaccination), Eyedrop I (vaccination by eyedrop at day-old), Spray I (vaccination by coarse spray at day-old), Water I (vaccination in water at day-old), Eyedrop II (vaccination by eyedrop at two weeks), Spray II (vaccination by coarse spray at two weeks), Water I (vaccination in water at two weeks). For all vaccinated groups, the vaccine used was Webster's VicS strain IBV (Fort Dodge Australia).

Blood samples were taken from 10 birds from each group at 7 weeks of age and birds were then re-vaccinated at 8 weeks by the same routes of administration as at day-old or two weeks (except the control group which was not revaccinated). Blood samples were taken from 10 birds per group at 11 weeks of age and some birds were euthanased for determination of body weight and kidney weights at 9, 10 and 11 weeks of age. Fifteen birds from each group were transferred to a poultry isolation shed at 11 weeks of age and exposed to T strain IBV. At 12 weeks of age birds from all groups were euthanased and body weights and kidney weights measured. Blood samples were taken from control birds only at 13 and 14 weeks of age and some birds were euthanased at each of these ages for body and kidney weight measurements.

At 10-11 weeks of age, body weight was higher in the Control, Eyedrop I and Spray I groups than for all other groups. At 9, 10 and 11 weeks of age, prior to the exposure of birds to T strain IBV, there were no differences among the groups for total kidney weight or for kidney weight as a percentage of body weight. One week after the exposure to T strain IBV, kidney weights as a percentage of body weight were unchanged in all the vaccinated groups of birds, whereas both kidneys of the control group were significantly enlarged. However, at two and three weeks following exposure to T strain IBV, the kidney weight to body weight ratios of the control group had returned to normal. For the control group, the IB titres (measured by ELISA) remained at zero up until exposure to T strain IBV and were still at zero one week following the exposure. However, the titre levels increased at two weeks following exposure (5647), with a further increase at three weeks (9153). There was variation among the titre levels for the vaccinated groups although there were no obvious differences based on age at vaccination or route of administration of vaccine. The mean titre for all vaccinated groups which offered protection from exposure to T strain IBV was 1476.

Both ages at vaccination (day-old and two weeks), and all routes of vaccination (eyedrop, spray and water) were effective in protecting birds against the effects of exposure to T strain IBV. The unvaccinated birds reacted to T strain IBV with increased antibody titres and significant enlargement of both kidneys, an indication of the nephropathogenic effects of T strain IBV in birds which are not protected by adequate vaccination. This is the first study in Australia in recent decades which has correlated IB antibody titres with resistance to exposure to T strain IB (R.C. Chubb and R.B. Cumming, personal communication).

THE EFFECT OF SCALDING ON THE STRUCTURAL INTEGRITY OF EMU SKIN

K.A. WEIR and C.A. LUNAM

This research is the first of a series of experiments focused on improving the quality of emu skin. Emu skin is soft, has a unique grain and has high potential in the garment industry. However, less than 10% of emu skins are of A grade quality. A major problem affecting skin quality is lamination of the skin after tanning. It is known that collagen is a major component of skin but it is not known how the structure of collagen fibres may be altered as a result of skin processing prior to tanning.

This project examined the microscopic structure of emu skin and assessed the effect of the industry practice of scalding on skin structure and, in particular, on collagen. Scalding is used by process workers to make defeathering a more rapid and less labour intensive process as dry plucking requires two extra process workers. It is possible that scalding may denature collagen fibres, resulting in lamination.

Skins from 10 adult male emus, approximately 18 months of age, were examined. Three skin samples were taken from each emu, that is from the rump, back and wing regions. In order to replicate industry processing conditions emus were killed and defeathered at a commercial abattoir. Following electrical stunning and bleeding from the carotid artery, feathers were removed by either dry plucking, or via immersion of the emu in water at 60 – 62°C for two minutes, followed by plucking. The water temperature and duration of skin immersion were not continuously monitored during the scalding and, therefore, may have varied between emus. All skin samples were fixed in formalin for 7 d. Scanning electron microscopy and Masson trichrome staining at the light microscope level were used to identify general tissue structures and, in particular, the orientation, thickness and extent of any denaturation of collagen fibres within the dermis of non-scalded, compared to scalded, skins.

Treatment	Emu skin thickness		
	Rump region (μm)	Back region (μm)	Wing region (μm)
Scalded	520 \pm 64	669 \pm 165	413 \pm 79
Non-scalded	633 \pm 103	985 \pm 311	643 \pm 134

Non-scalded emu skin has a thick epidermis which was completely removed upon scalding. There was a differential distribution of collagen fibres and a difference in skin thickness depending on the region of skin examined. Collagen fibres of the rump and wing lie predominantly parallel to the epidermis with only a small proportion of fibres being vertically orientated whereas collagen fibres of back skin were often vertically orientated. Values in the table represent mean and standard deviation pooled from a minimum of 12 measurements; three measurements for each of four emus per treatment. Data were examined by repeated measures analysis of variance which showed a significant ($P < 0.05$) decrease in skin thickness after scalding. This decrease in thickness is due to both epidermal stripping and a reduction in thickness of the dermis which is likely due to shrinkage of collagen fibres. There was no evidence that scalding differentially affected each region of skin. Masson trichrome staining indicated scalding caused denaturation of collagen in skins.

This study suggests that scalding is detrimental to the structural integrity of emu skin as it promotes shrinkage and denaturation of collagen fibres. The effect on structural integrity is consistent with lamination of the skins during tanning. The results suggest best practice is to dry pluck, the increased labour required being offset by the higher skin quality attained.

PRACTICAL APPLICATION OF TOTAL AND DIGESTIBLE AMINO ACID DATA IN THE FORMULATION OF POULTRY FEEDS

M. BLAIR

Today poultry nutritionists have a wealth of resources to aid them in formulating poultry feeds on a total or digestible amino acid basis. These include extensive historical databases on the amino acid composition of feedstuffs and associated digestibility coefficients, information from time consuming amino acid analyses or expensive *in vivo* determinations or indirect *in vitro* determinations including more 'real-time' Near Infrared Reflectance Spectroscopy (NIRS). Regardless of the source of information, practical and realistic application of these data in commercial feed production or integrated poultry operations needs to be assessed for it to have value.

Whether digestibility coefficients for specific amino acids are from historical, actual, or indirect methodology, it is critical that accurate values for total amino acids are established for a given operation. An accurate digestibility coefficient can still result in an erroneous final digestible amino acid value being assigned to an ingredient if the total value is in error. Historical data for the total amino acids in an ingredient should be compared with values determined for samples of the ingredient being used by an operation (McGinnis, 1998). Use of NIRS provides a rapid tool for this determination. Provided the sample scan is similar to those spectra used in the calibration, the standard error of cross validation (SECV) from an NIRS reading can be used to assess the accuracy of the prediction for a particular amino acid. As a basic guideline, the actual level of an amino acid will fall within \pm one SECV 60% of the time and within \pm two SECV 90% of the time. A nutritionist or operation must decide what values they are comfortable with in deciding to use a NIRS predicted value *in lieu* of a historical or wet chemistry value for each key amino acid. By doing this it is possible to take advantage of realized real-time values.

Digestible amino acid formulation is becoming more common as a way of utilizing specific feed ingredients, utilizing the ideal protein concept which is based on digestible amino acids, allowing more accuracy in meeting the bird's requirement and decreasing nitrogen output. While on paper this is appealing, consideration needs to be given to the ability of the feedmill to segregate ingredients by this criterion and the nutritionist to know when a particular batch of ingredient will be used in feed mixes. It must also be realized that formulating diets on a digestible amino acid basis based on absolute amino acid requirement and ideal protein ratios may result in a higher cost per tonne of feed. A recent study initiated in the United States with broilers used feeds formulated on tabular digestible amino acid values and NIRS digestible amino acid values. The former diets were US\$3.33, 2.61 and 2.43 more expensive per tonne for the starter, grower and finisher feeds, respectively, than the latter diets.

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APPARENT METABOLISABLE ENERGY CONTENT OF NEW ZEALAND WHEATS

V.RAVINDRAN, D.V.THOMAS, B.J.CAMDEN and S.H.VOON

Wheat is the major raw material in poultry feed formulations in New Zealand. Studies from several parts of the world, including Australia, United Kingdom, France and Canada, have shown that the apparent metabolisable energy (AME) of wheat for broilers varies considerably (Choct, 1993). No published data are available on the variability in AME content of wheat grown in New Zealand. Results from AME assays on 80 wheat samples conducted over a 4-year period (1997-2000) at the Poultry Research Unit of Massey University were utilised in this paper. The rapid method of Farrell (1978) was employed in the assays with two modifications. Broiler chickens (28 d-old) were used instead of adult cockerels and assay diets containing 965 g/kg of wheat and 35 g/kg of vitamin and mineral supplements were used instead of substitution of the cereal in a sorghum-basal diet. The assays used mash diets and eight birds per sample. The AME contents (average, standard deviation and range of variation) of the wheats over the 4-year period are summarised.

Year	No of samples	Average AME \pm SD (range) (MJ/kg DM)	% samples with < 13 MJ AME/kg
1997	28	13.17 \pm 1.56 (10.20-15.96)	43
1998	24	13.49 \pm 0.53 (12.77-15.16)	9
1999	8	13.16 \pm 0.44 (12.33-13.66)	38
2000	20	13.06 \pm 0.94 (10.80-14.52)	50

The average AME contents were somewhat consistent between the years, but the extreme values ranged from 10.20 to 15.96 MJ/kg DM. The observed variability compares closely with those reported elsewhere (Choct, 1993). Interestingly, except during 1998, a significant proportion of the samples (38-50%) assayed had AME contents of less than 13 MJ/kg DM. This observation was unexpected since the incidence of low-AME wheats in New Zealand is generally believed to be low owing to the milder temperatures and relatively high rainfall in wheat growing areas. It is, however, noteworthy that most of the samples were assayed near harvest. It is likely, therefore, that the low AME contents may reflect, at least in part, the new season grain phenomenon and one can expect the energy availability to improve with storage (Choct and Hughes, 1999). The AME contents were influenced by both within-year ($P < 0.01$) and between-year ($P < 0.01$) effects. These effects probably reflect the influence of location, cultivar, environmental factors and their interactions. However, it was not possible to separate these effects in this data set. A more systematic survey is required to identify the factors causing this variation and the high incidence of low-AME wheats in New Zealand.

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INFLUENCE OF WET AND DRY FEED ON BROILER CHICKEN PERFORMANCE UNDER HEAT STRESS

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It is generally agreed that high environmental temperature reduces animal performance, mainly due to depression of food intake. Wet feeds have consistently improved feed intake and efficiency of utilisation of conventional feeds under normal environmental conditions (Yalda and Forbes, 1995). However Abasiokong (1989) found that wet feeding benefited broilers at heat-stress, but not normal, temperatures while Tadiyanant *et al.* (1991) also reported benefits when using wet feeds for layer chickens. The current experiment investigated the performance of heat-stressed broiler chickens offered wet feed.

Male broiler chickens (144) were allocated to 9 treatment groups of 4 replicates, each replicate consisting of 7 birds. Birds were allowed i) *ad libitum* access to a standard broiler diet (200g protein and 11.9 MJ ME/kg air-dry form) and drinking water (dry +); ii) the same feed mixed with water at a rate to give the same water intake as birds on Treatment i, and with drinking water provided *ad libitum* (wet +), and iii) as Treatment ii but with no access to drinking water (wet -). The temperature treatments were 26°C, 31°C and 36°C and the duration of the experiment was 5 weeks.

Performance of broiler chickens offered wet feed at different environmental temperatures

Temperature (°C)	26			31			36			s.d
	Dry +	Wet +	Wet -	Dry +	Wet +	Wet -	Dry +	Wet +	Wet -	
Water content of feed (g/kg)	70	1000	1000	70	1400	1400	70	1700	1700	
Total weight gain (g)	1698 ^d	1869 ^f	1898 ^f	1671 ^d	1761 ^e	1692 ^d	1308 ^a	1637 ^c	1603 ^b	109
Total feed intake (g DM)	3691 ^b	3893 ^c	3954 ^c	3798 ^{bc}	3913 ^c	3845 ^c	2973 ^a	3638 ^b	3643 ^b	214
Feed efficiency (%)	0.46	0.48	0.48	0.44	0.45	0.44	0.44	0.45	0.44	0.02

Means in the same row without a similar superscript differ significantly ($P < 0.05$).

Increasing environmental temperature significantly decreased weight gain and feed intake of broilers offered dry feed. When compared with broilers given dry feed, wet feeding significantly improved the performance at all temperatures, irrespective of whether or not drinking water was available. However, wet feeding did not completely overcome the depression observed at 36°C. Wet feeding could be a useful technique for overcoming some of the adverse effects of high environmental temperatures on feed intake and growth of broiler chickens.

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COMPARISON OF PROTEIN DIGESTIBILITY DETERMINED WITH EITHER
C₃₆ N-ALKANE OR ACID INSOLUBLE ASH AS INDIGESTIBLE MARKERS

X. LI¹, H. DOVE² and W.L. BRYDEN¹

Acid insoluble ash (AIA) is used routinely as an indigestible marker in poultry digestibility studies. Accurate gravimetric determination of AIA requires a large sample size (2g for diet, 1.2g for digesta). Shortage of the amount of digesta collected in some experiments can limit the number of nutrients analysed. Long-chain hydrocarbons have been successfully used to estimate digestibility and plant species selection by herbivores (Dove and Mayes, 1996). Choct and Hughes (1996) found that dry matter digestibilities in broilers estimated using the alkane, hexatriacontane (C₃₆ alkane) and AIA were similar. Only small amounts of samples (0.2-0.5g) are required for alkane analysis and quantification is by capillary gas chromatography. The objective of this study was to compare C₃₆ alkane and AIA as markers to estimate nitrogen and amino acid digestibility in different portions of the gastrointestinal tract of meat chickens.

The grower diet used in this study contained either added celite, (as a source of AIA, 20g/kg diet) or added C₃₆ alkane (200mg/kg diet). The diet was fed *ad libitum* to 6 pens of broilers (7 birds/pen) from 25 d of age. After 10 d of feeding, digesta from the jejunum and upper and lower ileum were collected from the birds following lethal injection with sodium pentobarbitone (Bryden, 1989). Diets and digesta samples were analysed for nitrogen, amino acids, AIA and C₃₆ and digestibility calculated. The results for nitrogen digestibility are summarised in the Table.

	AIA	C ₃₆	Pooled SEM	P value
Jejunum	0.593±0.046 ¹	0.605±0.018	0.0129	0.603
Upper ileum	0.719±0.036	0.719±0.029	0.0129	0.986
Lower ileum	0.744±0.035	0.759±0.019	0.0129	0.407

¹ Mean ± SD.

There were no significant differences between the two markers in the digestibilities of nitrogen, protein (N x 6.25) and all the amino acids tested. The variations within the treatments tended to be smaller for C₃₆ than AIA as indicated by the smaller standard deviations. The results of this study demonstrate that C₃₆ alkane is a suitable marker to estimate ileal digestibility in poultry.

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EVALUATION OF DIGESTIBLE AMINO ACID CONTENT OF FEEDSTUFFS WITH EMPHASIS ON NIRS TECHNOLOGY

M. BLAIR

Feed formulation for poultry has moved towards formulating on the basis of digestible nutrients, in particular crude protein and/or amino acids. Several methods have been used to determine digestible values for crude protein and key amino acids. However, these methods are either based on indirect *in vitro* methods or *in vivo* methods which are time consuming and expensive. Time is a key factor and can be a major impediment to integrators or feed manufacturers wishing to adapt a digestible nutrient formulation philosophy. Near Infrared Reflectance Spectroscopy (NIRS) can offer a solution to this dilemma.

Over the past few years, calibrations have been developed and/or expanded for total amino acids using wet chemistry results from the amino acid analyses of feedstuffs. Digestible amino acids calibrations have also been developed from *in vivo* studies determining the true ileal digestibility of amino acids in feedstuffs (Van Kempen and Simmins, 1997). However, care in applying the results from NIRS determinations in an operation must be exercised.

An example includes the NIRS evaluation of five meat and bone samples for digestible amino acids based on true ileal digestibility for laying hens. The NIRS gave acceptable results based on the expected error from actual wet chemistry analyses for all amino acids except methionine and total sulphur amino acids (two times the expected error). It is important to realize that the results for each amino acid must be evaluated even though the spectra from the five samples were similar to those spectra used in the calibration (< 3.00 for global values and < 1.00 for neighborhood H values). Results from NIRS are mathematical predictions and not actual chemical results; thus the predictions are not expected to be as accurate as the wet chemistry or *in vivo* results. These results indicate that either the equation is unsuitable for these amino acids, the wet chemistry is in error or that the product may contain added methionine that the equation does not consider. All aspects should be investigated before using the results to accept a product for use in formulation.

Another example involved the NIRS evaluation of 60 samples of soybean meal from Latin America of which most were heat stressed. The resulting predicted digestibility coefficients for lysine digestibility ranged from 22.5 to 93.8%, with 28 samples being below 80%. Applying a digestibility calibration from a set of non heat-stressed soybean meals did not predict accurately. In this case, the latter calibration needs to be expanded using *in vivo* digestibility data for heat stressed soybean meals.

Calibrations from NIRS determinations based on *in vivo* digestibility methodologies are a viable tool for a feed manufacturer, as demonstrated by calibrations based on true ileal digestible amino acids for poultry. However, it is important to be aware of the limitations on an individual amino acid basis.

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ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS IN LONG-TERM SELECTED LINES OF WHITE LEGHORNS FOR PART-PERIOD EGG PRODUCTION

D. SHARMA and D.B. REDDY

The immune response to antigens like sheep red blood cells (SRBC), provides an indication of general immunocompetence and has been used extensively in immunogenetic studies in chickens (Siegel and Gross, 1980; Martin *et al.*, 1989; Kundu, 1997). Selection for economic traits may influence immunocompetence depending upon the genetic correlation between immunocompetence and economic traits. In this study, the antibody response to SRBC has been used to study the effect of long-term selection for egg production on immunocompetence in two selected (H and I) and one control (C) lines of White Leghorns.

Lines H and I were procured from different sources and were under selection for part-period egg production using a family index as selection criteria over 25 generations. Line C, derived from line H at base generation, was maintained as a random bred pedigree population without any selection. Total antibody titre was determined by an haemagglutination (HA) test (Siegel and Gross, 1980), while the mercaptoethanol test (Martin *et al.*, 1989) was used to estimate mercaptoethanol resistant (MER) antibody titres. The mercaptoethanol sensitive (MES) antibody titres were calculated as the difference between the total and MER antibody titres. The means for antibody titres between the lines at various days post injection (PI) were compared using least square analysis (Harvey, 1975).

The presence of HA titre (1.5 to 2.0) on day zero revealed the presence of natural antibodies in all the breeds and these antibodies were primarily MES. The presence of natural antibodies was also reported by other workers such as Vander Zijpp and Leenstra (1980) and Kundu (1997). The lines showed non-significant differences for total HA, MES and MER antibody responses to SRBC at various d PI. The lines also showed similar persistency in antibody response. The highest titres (12.80 to 13.15) were observed at 5-7 d PI. Kundu (1997) also reported similar results. The non-significant differences for titres as well as for persistency of titres between lines H and C showed that long-term selection for egg production had not influenced antibody response to SRBC. While Vander Zijpp and Nieuwland (1989) reported higher egg numbers and egg weight in high titre lines to SRBC, as compared to low titre lines, Kean *et al.* (1994) reported few differences for body weight, age at first egg, 32 wk egg weight and rate of egg production. The present results revealed no significant effect on general immunocompetence from long-term selection for egg production.

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EFFECT OF EGG STORAGE TEMPERATURE AND DURATION ON HATCHABILITY

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Egg storage enables production of commercial broiler chicks to be adjusted in accordance with market demand for chicken meat. It is generally accepted that egg storage is detrimental to the viability of the developing embryo and, therefore, hatchability. The effect of storage on embryo viability is regulated by the temperature and humidity provided to the egg. However, studies report conflicting results concerning the storage temperature required to optimise hatchability during short storage conditions (Mayes and Takeballi, 1984; Meijerhof *et al.*, 1994; Reis *et al.*, 1997).

At 38 weeks of age, settable eggs laid between 0900 and 1500 h from a single commercial broiler breeder flock of 400 Cobb hens were collected twice daily over two periods of three consecutive days with a 4 d intermediate period. All eggs (1051) were randomly assigned to one of four storage experimental groups (see Table). The storage rooms were thermostatically controlled. Eggs were candled on d 11 of incubation to distinguish between infertile eggs and early embryonic death. Fertile eggs were further incubated for another 11 d. Hatchability was the percentage of hatched chicks from the total number of eggs set and egg viability was the percentage of hatched chicks determined from the total number of fertile eggs set. A chi-squared test for variance was used to determine the effects of storage temperature with storage duration on egg viability and hatchability.

Storage conditions					
Time (days)	Temperature (°C)	Humidity (%)	Hatchability (%)	Egg viability (%)	Fertility (%)
1 to 3	16.5	80.8	93.89 (262) ¹	95.35 (250) ¹	95.42 (12) ¹
	20	78.2	92.34 (261)	93.41 (244)	93.49 (17)
			P = 0.482	P = 0.329	
9 to 11	16.5	80.8	85.77 (267)	89.11 (239)	89.51 (28)
	10	87.6	90.42 (261)	92.19 (241)	92.34 (20)
			P = 0.099	P = 0.231	

¹Number of eggs.

Increased temperature during short storage conditions had no effect on either egg viability or hatchability, while reducing temperature marginally improved hatchability in eggs stored for 9 to 11 d. These results suggest that increasing the temperature during storage times not exceeding 3 d, does not improve embryonic viability: 16.5°C can be used for periods no longer than 3 d and 10 °C for eggs stored longer than 9 d. Data concerning the effects of hen age, broiler breeder line, egg laying time and storage conditions on fertile egg viability and hatchability are currently being analysed.

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INDIVIDUAL AND COMBINED SUPPLEMENTATION OF WHEAT-BASED BROILER
DIETS WITH PHYTASE AND XYLANASE

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The simultaneous inclusion of phytase and xylanase feed enzymes in wheat-based broiler diets may be advantageous (Ravindran *et al.* 1999). This study investigated the effects of adding granulated preparations with phytase (Natuphos® 120 g/t, 600 FTU/kg) and predominantly xylanase (Natugrain® Blend 120 g/t, 6,600 EXU/kg) activity, individually and in combination, to wheat-based diets formulated to be phosphorus (P) deficient. Experimental diets were analysed and contained 7.5 g calcium, 6.5 g total P with a calculated nonphytate-P content of 2.5 g/kg. Day-old male broiler chicks (Cobb) were allocated into 28 cages (7 replicates per treatment, 8 birds per cage) and fed a proprietary starter ration to 7 d of age. From 7 to 29 d of age birds received control or supplemented mash diets based on (/kg) pre-pelleted wheat (654 g), soyabean meal (218 g), cottonseed meal (60 g) and meat and bone meal (29 g). Growth rates and feed intakes were recorded from 7 to 18 d of age when two birds per cage were sacrificed to determine gut viscosities. Excreta were collected from 23 to 25 d of age for nitrogen (N) retention and AME determinations and at 29 d of age 3 birds per cage were taken for toe ash. Standard procedures were followed and the results are tabulated.

Treatment	Growth rate (g/bird)	Feed intake (g/bird)	Feed efficiency (g/g)	Intestinal viscosity (cPs)	N retention (%)	AME (MJ/kg DM)	Toe ash (%)
Nil	443 ^a	702	1.59 ^a	5.18 ^a	55.4	14.2 ^{ab}	12.3
Phytase	460 ^{ab}	681	1.48 ^b	4.04 ^b	54.0	14.1 ^a	12.0
Xylanase	453 ^{ab}	670	1.48 ^b	2.47 ^c	55.9	14.4 ^{bc}	12.6
Combination	473 ^b	681	1.44 ^b	2.37 ^c	57.6	14.6 ^c	12.7
SEM	7.04	9.85	0.021	0.334	0.918	0.099	0.277
Probability	0.055	0.170	0.000	0.000	0.072	0.006	0.389

^{a-c} Means in a column without a common superscript are significantly different ($P < 0.05$).

Treatments had no effect on feed intake nor, surprisingly, toe ash, which suggests that P was not limiting. Individually, both phytase and xylanase improved feed efficiency by 6.9%. However, the combination improved feed efficiency and growth rates by 9.4 and 6.8% respectively. The combination increased AME values and all three enzyme treatments reduced gut viscosity. Interestingly, phytase reduced gut viscosity to a lesser extent and did not increase AME. Treatments did not influence N retention. Overall, the results suggest that the combined inclusion of phytase and xylanase in wheat-based broiler diets have promise and further studies, with less than standard inclusion rates, are indicated, as are investigations into the mechanisms supporting the additive responses observed with gain and AME in this study. The apparent P adequacy of the diets was an unexpected finding; the nonphytate-P content may have been under-estimated and/or the birds' P requirement was less than usual recommendations suggest.

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LOW GLUCOSINOLATE CANOLA MEALS FOR LAYING HENS

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Canola meal (CM) production in Australia has increased dramatically in the last few years and new varieties with very low glucosinolates (GSL) and negligible amounts of erucic acid in the meal have long been used in layer diets as a source of protein. However, CM inclusion in diets has been limited to 40 – 100 g/kg due to past problems associated with feeding rapeseed meal which decreased production parameters, increased mortality and gave a 'fishy' or 'crabby' taint to the eggs (Butler *et al.*, 1982; Leeson *et al.*, 1986). This paper reports an evaluation of CMs from Melbourne (Victoria) and Pinjarra (West Australia) that were either pre-pressed, solvent-extracted or extruded. Levels of 100, 150 and 200 g/kg CM were included in diets formulated on a digestible amino acid (DAA) basis. The CM samples were first analysed for ileal DAA, apparent metabolisable energy (AME), GSL, total condensed tannins (TCT) and chemical composition. A diet containing 11.5 MJ AME/kg, based on sorghum-wheat-soyabean meal, was the control diet. Each of the seven experimental diets was fed to 25 individually housed Ingham Hisex Brown layers from 26 - 40 weeks of age in a randomised block design. Feed and water were always available. No deaths occurred during the experiment. The overall performance measured over 14 weeks is summarised in the Table.

Canola meal diets (g/kg)	Egg production (%)	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed conversion (g:g)	Hen weight (kg/bird)
Control	91.8	65.2	59.9	127.9	2.141	2.20
Melbourne 100	88.3	64.3	56.9	124.9	2.209	2.21
Melbourne 150	91.8	63.2	58.0	127.5	2.206	2.22
Melbourne 200	93.4	63.8	59.6	131.4	2.214	2.22
Pinjarra 100	91.8	62.9	57.8	124.6	2.162	2.18
Pinjarra 150	90.9	63.8	58.1	124.2	2.139	2.21
Pinjarra 200	92.7	63.9	59.1	126.8	2.158	2.22
SEM ¹	1.37	0.73	1.11	2.62	0.0393	0.048

¹ Standard error of the mean.

Increasing dietary levels of CM had no effect ($P>0.05$) on any production parameter measured. A factorial analysis within levels is in progress and will be presented. Geographical location and processing method also had no significant ($P>0.05$) effect on layer performance. Further analysis of organ weight (liver, pancreas) and results of an organoleptic evaluation of eggs will complement the results obtained. The results of this preliminary trial with laying hens indicated that CM diets formulated on a DAA basis and fed in amounts up to 200 g/kg did not have a negative effect on layer performance.

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METHODS OF BEAK TRIMMING

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A review was requested by the Rural Industries Research and Development Corporation to obtain information on the range of beak-trimming methods available or under development. Information on the effects of beak trimming on the welfare of poultry have been thoroughly covered in other reviews (e.g. Hughes and Gentle, 1995). Beak trimming is performed early in the life of commercial hens to decrease injuries caused by the behavioural vices of cannibalism, bullying, feather and vent pecking and to avoid feed wastage. It involves partial removal of the upper and lower beak using an electrically heated blade. Without a correct beak trimming program egg producers risk heavy losses of chickens and pullets from cannibalism and, in the laying stage, from protrusion and vent pecking. Beak-trimming of birds at 7-10 d is favoured by the Industry but research over the last 10 years has shown that beak-trimming at 1 d-old causes the least stress on birds. Correct beak-trimming can result in greatly improved layer performance but improper beak-trimming can be detrimental. Re-trimming is practiced in most flocks, although there are some flocks that only need one trimming. Given the continuing welfare scrutiny of using a hot blade to cut the beak, attempts have been made to develop more welfare friendly methods of beak-trimming. Despite the developments in design of hot blade beak-trimmers the process has remained largely unchanged. That is, a red-hot blade cuts and cauterises the beak. The variables in the process are blade temperature, cauterisation time, operator ability, severity of trimming, age of trimming, strain of bird and beak length. This method of beak-trimming is still overwhelmingly favoured in Industry and there appears to be no effective alternative procedures.

Sharp secateurs have been used to trim the upper beak of both layers and turkeys. Bleeding from the upper mandible ceases shortly after the operation and there is considerable beak regrowth. This method has not been used on a large scale because of anecdotal reports of cannibalism outbreaks in birds with re-grown beaks. A robotic beak-trimming machine has been developed in France, which permits simultaneous, automated beak-trimming and vaccination of 1 d-old chicks at up to 4500 chickens per hour. Use of the machine has not been successful due to weight variation in the chicks, incorrect loading causing chicks to drop off the line and variable degree of beak-trimming. Capsaicin can cause degeneration of sensory nerves in mammals and decreases the rate of beak re-growth by its action on the sensory nerves. Capsaicin is a cheap, non-toxic substance that can be readily applied at the time of less severe beak-trimming, but suffers the disadvantage of causing an extreme burning sensation in operators who come in contact with the substance during its application to the bird. A method was reported which cuts the beaks of 1 d-old chickens with a laser beam. No details were provided on the type of laser used, or the severity of beak-trimming, but there was regrowth of the beak. Feather pecking and cannibalism during the laying period were highest among the laser trimmed hens.

Liquid nitrogen has been used to declaw emu toes but was not effective. There was regrowth of the claws and the time and cost involved in the procedure limits the potential for using this process to beak-trim birds.

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Barnett, J.L.	71	John.Barnett@nre.vic.gov.au
Baron, F.	195	
Barram, K.M.	247	
Barrier-Guillot, B.	195, 208	
Bhandari, B.	224, 228	
Black, J.L.	22	jblack@pnc.com.au
Blair, M.	239, 243	Mike.Blair@aventis.com
Blight, G.W.	156	
Brake, J.	113	jbrake@ncsu.edu
Broom, D.	61, 79	dmb16@cam.ac.uk
Bryden, W.L.	232, 233, 236, 242, 246	wayneb@camden.usyd.edu.au
Butler, R.N.	168	
Butts, C.A.	160	
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Dove, H.	242	
Downing, J.A.	232, 233	jeffd@camden.usyd.edu.au
Dueling, F.	106	
Farrell, D.J.	148	d.farrell@mailbox.uq.edu.au
Fisher, C.	183	colin_fisher@rbnts.rossbr.com
Fraser, D.R.	232	
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Geraert, P.	195, 208	Pierre-Andre.Geraert@aventis.com
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Hetland, H.	191	
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Knott, L.	175	
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Pos, J.	156	
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Thomson, E.S.	212	
Timmers, J.A.	152	
Tivey, D.R.	168	
Trappett, P.C.	247	TrappeP@dpi.qld.gov.au
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