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# AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

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## A REVIEW OF SEX DETERMINATION AND DIFFERENTIATION IN POULTRY

M.H. THORNE

Summary

The genetic basis of avian sex determination has not been elucidated. It has been suggested that sex in birds is determined by a genetic balance in the ratio of Z chromosomes to autosomes. Yet evidence based on the phenotypic observation of individuals with aberrant sex chromosomes suggests that the W chromosome contains a major female (ovary) sex determining gene, the effect of which is reversed by more than one dose of a male (testis) sex determining gene on the Z chromosome. Molecular genetic studies are anticipated to provide major breakthroughs in the understanding of avian sex determination as the genes that regulate gonadal differentiation are identified.

## I. INTRODUCTION

The question of how genetic sex determination influences primary sexual differentiation in birds is unclear. Avian species have a ZZ/ZW sex chromosome system. In normal fowls, the male is the homogametic sex, ZZ, and the female is heterogametic, ZW. Both embryonic gonads in genetic males develop to form two identical testes. In genetic females, only the left gonad develops as a functional ovary. The right gonad develops only marginally during incubation and regresses to a microscopic vestige.

While sex determination in birds is an event of genetic programming, sex differentiation involves the development of observable phenotypic changes indicative of the specific sex. Sex differentiation is divided into two stages. The primary event is the development of the gonad which encompasses the differentiation of germ cells and somatic elements within the gonad. The secondary event involves the development of the accessory sex organ which is influenced by hormones produced by the somatic cells of the gonad. The endocrine control of secondary sexual differentiation in birds is well-understood (McCarrey and Abbott, 1979).

During early embryonic development, the gonads are sexually indifferent and can differentiate into either a testis or an ovary depending on the genotypic sex of the embryo. The period of indifferent sexual development extends from about day three to day five of incubation in the chicken, and morphological differentiation begins between day five and six of incubation (Romanoff, 1960). The early gonad is typically composed of a medulla and a cortex, and sexual differentiation results from the development of only one of these gonadal components. In birds, a testis forms from a proliferation of the medulla and regression of the cortex, while the reverse occurs for development of the ovary. Detailed descriptions of the differentiation of male and female gonads of the fowl may be found in Romanoff (1960), Gilbert (1979) and Van Krey (1990).

## II. DISCUSSION

The genetic mechanism by which sex is determined in birds has been difficult to elucidate. As mentioned, avian species have a ZZ/ZW sex chromosome system. This is the opposite situation to mammals where the male is the heterogametic sex, XY, and the female

is the homogametic sex, XX. In the chicken, the Z chromosome comprises about 7.6% of the haploid genome (Fechheimer, 1990), while the much smaller W chromosome comprises only 1.4% of the total genomic DNA in the female (Tone *et al.*, 1984). During female meiosis, a small homologous pairing region is observed between the short arm of the W chromosome and a terminal segment of the short arm of the Z chromosome (Solari *et al.*, 1988). In somatic cells the Z chromosomes are euchromatic and replicate with the autosomes, but the W chromosome is late-replicating (Schmid, 1962) and heterochromatic with a highly repetitive DNA content (Tone *et al.*, 1984). In effect, most of the avian W chromosome is genetically isolated from the rest of the genome. A similar situation exists with the mammalian Y chromosome which is also small, late-replicating and heterochromatic (Graves and Schmidt, 1992).

In mammals, sex is known to be determined by the Y chromosome which is dominant and leads to testis formation (Kent *et al.*, 1966). It has been suggested that avian sex determination is similar to mammals and that the W chromosome is dominant, containing a major sex determining gene that initiates development of the ovary in females (Bitgood and Shoffner, 1990). Alternatively, it has been proposed that avian sex determination is more closely related to the *Drosophila* mechanism involving a genic balance in which the ratio of Z chromosomes to autosomes is sex determining, rather than the presence or absence of the W chromosome (McCarrey and Abbott, 1979; Sittmann, 1984; Halverson and Dvorak, 1993).

Traditionally, most knowledge on sex determination has come from observation of the sexual phenotype of individuals with aberrant sex chromosomes. Diploid fowls with ZO and ZZW sex chromosomes would be the most informative, but critical evidence from these genotypes is lacking. ZO chickens have never been detected and evidence suggests that this genotype may be lethal (Kagami *et al.*, 1995). Only one report of the putative ZZW diploid fowl is known describing a male bird with sex-linked plumage traits indicative of a female (Crew, 1933). The male was proposed to be ZZW on the basis of the phenotype and presumptive cytology of the bird's progeny, but the Z chromosome was incorrectly identified in this early work. Further, as modern cytological techniques were unavailable to positively substantiate the presence of the W chromosome, it is not possible to assign a male phenotype with any certainty to the ZZW diploid karyotype from this single report. Other factors could also have accounted for the atypical plumage pattern of the male bird such as partial sex linkage, chimerism or Z-autosome translocation.

A genic balance sex determining mechanism has been favoured by some (McCarrey and Abbott, 1979; Halverson and Dvorak, 1993) on the basis of observations of gynandromorphs in chickens. Gynandromorphs are bilateral sex chimeras with one half of the body being male and the other half female (Hutt, 1949 for review; Cock, 1954; Abbott and Yee, 1975). It has been concluded that the chromosome constitution of the gynandromorphs was ZZ male/ ZO female based on the assumption that their origin was due to loss of a Z chromosome at the first cleavage division (Hutt, 1949; Halverson and Dvorak, 1993). The assumption that the ZO half of the gynandromorph was female is argued to be consistent with the hypothesis that sex is determined by the autosome to Z chromosome ratio. However, the ZZ/ZO chromosome complement was never verified in any of these reports. Thorne (1995) reported a cytogenetic study of a gynandromorph chicken that was found to be a ZZ/ZW diploid. The presence of the W chromosome was positively verified by C-banding which stains heterochromatin intensely. Varying proportions of ZZ and ZW sex chromosomes were found in a variety of somatic tissues analysed from both the male and female side of the body. The origin of this gynandromorph may be explained by either fertilisation of a regular gamete and the first polar body,

fertilisation of a binucleated oocyte, or fusion of two blastoderms. Autopsy revealed that the bird had an atretic left ovary, a right testis, and left and right oviducts. In this case, a dominant effect of the W chromosome on avian sex determination cannot be dismissed in favour of a genic balance system.

Observations of chimeric and polyploid chickens have provided further insights on avian sex determination. The sexual phenotype of a number of chimeric chickens with varying ploidy levels suggests that the W chromosome does have a major influence on determining femaleness in birds (Thorne *et al.*, 1987; Thorne and Sheldon, 1993). For example, viable haploid-diploid chickens with Z/ZW and Z/ZZ sex chromosome complements have female and male phenotypes, respectively, and despite the additional dose of Z-bearing haploid cells, the Z/ZW chimeras have normal female reproductive organs (Thorne *et al.*, 1987). A diploid-triploid chimera with ZZ/ZZW sex chromosomes and a low proportion of triploid cells, was observed to have a normal male phenotype. Its gonads, though, consisted of a left ovotestis and a right testis indicating that the presence of a W chromosome in only a small proportion of ZZW triploid cells was sufficient to cause some ovarian development of the left gonad (Thorne *et al.*, 1987; Thorne and Sheldon, 1993).

Studies of a large number of ZZW triploid intersex birds from a unique selected strain of chickens (Thorne and Sheldon, 1991; 1993; Thorne *et al.*, 1991) have provided significant knowledge on avian sex determination. The ZZW triploids are females at hatching and they maintain an external female phenotype until sexual maturity after which time masculinization occurs. Their adult reproductive organs consist typically of a left ovotestis, right testis, and left and right oviducts (Thorne *et al.*, 1988; Lin *et al.*, 1995a; 1995b). The initial determination and differentiation of the ZZW triploid embryo as a female is observed to be normal and complete, but masculinization of the left ovary and development of a right testis in the place of a regressed right ovary starts before hatching. The masculinization of the gonads of the ZZW triploid embryo is reversible (at least temporarily) by administering estrogen during the indifferent sexual period (Thorne *et al.*, 1992). The observations on the intersex ZZW triploids, together with those of the chimeric chickens, support the hypothesis that the W chromosome contains a major female (ovary) determining gene, the effect of which is able to be inhibited by more than one dose of a male (testis) determining gene on the Z chromosome.

#### (a) Absence of dosage compensation

In contrast to mammals, an interesting property of the avian Z chromosome is the apparent absence of dosage compensation for Z-linked genes in the homogametic ZZ male. No evidence for dosage compensation has been found for Z-linked plumage colour and pattern genes in chickens, pigeons and canaries (Cock, 1964), or for the activity of the Z-linked cytoplasmic aconitase gene in liver cells of guinea fowl (Baverstock *et al.*, 1982), or for a Z-linked recessive white skin mutation affecting blood plasma colouration in chickens (Lasher and Bitgood, 1982).

The apparent absence of dosage compensation in birds has been proposed as a sex determining mechanism itself by Chandra (1993). Chandra has suggested that both the Z and W chromosomes carry one or more homologous sex determining genes. W-inactivation would halve the effective copy number of such genes in the ZW zygote, enabling ovarian development to occur. The absence of inactivation of Z-linked genes in ZZ embryos is viewed as a means by which two copies of W-homologous sex determination genes are kept active to meet the requirements of testis determination. Thus a complementary mechanism

of W-chromosome inactivation and absence of dosage compensation of Z-linked genes would regulate the sex determining mechanism. A prerequisite of this theory is that the sex-determining region of the W chromosome must consist of facultative heterochromatin rather than constitutive heterochromatin. Additionally, the apparent absence of dosage compensation in birds requires further verification, because only a relatively small number of genes have been examined for Z-chromosome inactivation. In mammals, it is known that a number of X chromosome genes escape inactivation and show greater levels of expression in females than males indicating that balancing dosage is not critical for some genes (Jones *et al.*, 1989; Fisher *et al.*, 1990).

#### (b) Sex reversal

Another unusual feature of avian sex determination is that it appears to be partially labile and reversible. Spontaneous sex reversal occurs, usually in the adult female, but very rarely in the adult male (Hutt, 1949; Van Krey, 1990). Female sex reversal arises following atrophy of the left ovary due to disease, or after ovariectomy. In the absence of ovarian estrogen secretion, medullary tissue in the rudimentary right gonad is induced to differentiate into a testis-like gonad, secreting androgens that masculinize the female bird. If sex determination in birds involves only a Z chromosome dosage mechanism, a higher frequency of spontaneous sex reversal would be expected in males as a result of mutation, or loss of a Z chromosome. The fact that this is not observed further implicates the W chromosome with a primary role in avian sex determination.

It has also been well-established in birds that sex steroid treatment of the early embryo can cause sex-reversal. For example, administration of estradiol to the male embryo during the indifferent sexual period results in the formation of a left ovotestis (Van Krey, 1990). The estradiol apparently induces the germinal epithelium of the embryonic male left gonad to develop an ovarian cortex. Administration of testosterone, however, to genetic female embryos during the indifferent sexual period does not modify the female gonads (Romanoff, 1960; Thorne *et al.*, 1992), but grafts of embryonic testes will induce varying degrees of male gonadal differentiation in the female embryo which may remain permanently after hatching (Maraud *et al.*, 1986). In the latter case, the testis graft is thought to secrete anti-Müllerian hormone which decreases ovarian estrogen secretion and causes male gonadal development. The importance of gonadal steroid hormones in influencing the initial differentiation of the avian gonad is apparent from the cited studies.

#### (c) Molecular genetic studies of avian gonadal hormones

The timing and expression of genes involved in sex steroid synthesis in the early chicken embryo has been studied by Mizuno *et al.* (1993). The genes analysed encode the steroid 17-hydroxylase/17,20-lyase (P-450c17), which is a key enzyme in the conversion of cholesterol to testosterone, and aromatase (P-450arom) which is essential in the conversion of testosterone to estradiol-17. Transcription of the P-450c17 gene appears to begin as early as the second day of incubation in both sexes, whereas transcription of the P-450arom gene begins at day five to six of incubation in the female embryo only. The latter coincides with the morphological differentiation of the gonads, which is evident at approximately day five and a half of incubation. Elbrecht and Smith (1992) found that administering an aromatase inhibitor (which blocks the synthesis of estrogen from testosterone) to chicken embryos at a stage when their gonads were bipotential, caused genetic females to develop a permanent male phenotype. These sex reversed female chickens had the physical appearance of males

and developed bilateral testes that were capable of spermatogenesis. The results suggest that exposure to estrogen during the early phase of gonadal development is crucial for the normal development of the ovary. Regulation of aromatase enzyme activity is therefore a key element in the control of gonadal differentiation and sex determination in chickens. Aromatase presumably lies in the sex determining pathway downstream from a major avian sex determining gene or genes.

(d) Molecular genetic studies in search of avian sex determining genes

In recent years, the identification of a number of genes involved in mammalian sex determination and differentiation, has led to an increased interest in avian sex determination. The testis-determining gene on the mammalian Y chromosome, *SRY*, which was isolated by genetic analysis of sex-reversed individuals (Sinclair *et al.*, 1990) has been examined in birds. The *SRY* gene, however, was not expressed in a sex-specific manner (Griffiths, 1991; Tiersch *et al.*, 1991) indicating that it does not have a role in avian sex determination. Recently, an *SRY*-related gene, *SOX9*, has been shown to have male-specific expression during the sex determination period of both the mouse and chicken (Kent *et al.*, 1996). *SOX9* is thus the first reported testis-specific gene in birds. *SOX9* is not, however, located on either the Z or W sex chromosome, and it is expressed after the first signs of sexual dimorphism in the chicken indicating that it is not a major switch gene in the sex determining pathway, but its expression is consistent with an important role in testis and genitourinary development. Studies of its regulation may yield significant knowledge on avian sex determination.

### III. CONCLUSIONS

Studies based on the phenotypic observation of individuals with aberrant sex chromosomes implicate the W sex chromosome with a major role in avian sex determination, rather than a genic balance sex determining mechanism. Evidence suggests that the W chromosome contains a major female sex determining gene that directs development of the ovary, but its effect is reversed by more than one dose of a male sex determining gene on the Z chromosome. Detailed molecular genetic studies are expected eventually to resolve the question of avian sex determination. Identification of the sex determining genes in birds will provide greater insight into the factors regulating gonadal differentiation and will allow important evolutionary comparisons at the molecular level of female and male heterogametic sex determining systems.

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## GENES WITH SEX-SPECIFIC EXPRESSION ISOLATED BY DIFFERENTIAL DISPLAY AND REPRESENTATIONAL DIFFERENCE ANALYSIS

A.H. SINCLAIR, M.J. O'NEILL and M.D. BINDER

### Summary

Differential display (DD-PCR) and representational difference analysis (RDA) have been used to clone novel genes from chicken embryonic genital ridge. Some of these genes show sex chromosome linkage and may have a role in the development of the avian testis or ovary. In addition, these genes could be used as a rapid means of sexing birds. More importantly, these differentially expressed genes may have a conserved role in vertebrate sex determination.

### I. INTRODUCTION

Sex determination is an excellent model system for studying the process of organogenesis. The undifferentiated foetal gonad has the ability to develop either as a testis or an ovary. Thus, mutations in the sex determining pathway are not always embryonic lethal but lead to observable phenotypes.

In mammals, the Y chromosome has a dominant effect on sex determination. The Y chromosome contains a testis determining gene (TDF) which induces the indifferent embryonic gonad to develop as testis. In the absence of the Y chromosome and the TDF gene the embryonic gonad will develop as an ovary. The development of secondary sexual characteristics is largely the result of the secretion of hormones from the gonad. A gene called *SRY* (sex determining region on the Y) has been cloned from the Y chromosome in mammals and has been shown to be TDF (Sinclair *et al.*, 1990; Goodfellow and Lovell-Badge, 1993). Although *SRY* is one of the earliest acting genes in the mammalian sex determining pathway identification of downstream targets for *SRY* has proved difficult. The protein encoded by *SRY* is capable of binding to many sites in the genome and there are other genes related to *SRY* which bind to very similar sites. In addition, it is now thought that *SRY* has an indirect effect on the sex determining pathway. Another strategy now needs to be employed in order to further understand human sex determination.

The development of testis or ovaries is very similar in all vertebrates. It is likely that the major genes involved in gonad development are conserved across vertebrate evolution. Sex in chickens is determined chromosomally. The female is the heterogametic sex (ZW) while the male is the homogametic sex (ZZ). The W chromosome has a dominant effect on sex determination in birds. No ZW chickens have been observed with any degree of testicular development, and no ZZ chickens have been observed with any degree of ovarian development (Thorne, 1995). It seems likely that the W chromosome contains a gene or genes which induces ovarian development in birds and possibly all vertebrates.

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(a) The mammalian testis determining factor: *SRY*

The gene *SRY* is now known to be functionally equivalent to the testis determining factor (Sinclair *et al.*, 1990; Goodfellow and Lovell-Badge, 1993). Analysis of XY females with gonadal dysgenesis who possess *SRY* showed that up to 10% of these sex reversed patients have a mutation in *SRY*. The correlation between sex reversal and mutation in *SRY* implies *SRY* is required for testis formation and male sex determination. Direct evidence for the involvement of *Sry* in sex determination was provided by transgenic mice. *Sry* was injected into mouse embryos and was shown to induce sex reversal in chromosomally female (XX) mice (Koopman *et al.*, 1991). This evidence indicates that *SRY* is both required and sufficient for male sex determination and that *SRY* is TDF.

Importantly, although *SRY* is the "switch" gene that directs testis formation from the indifferent gonad, other genes exist "downstream" of *SRY* in the sex determination pathway. Only 10% of XY females have a mutation in *SRY* and between 10-20% of XX males lack any Y-derived sequences (Goodfellow and Lovell-Badge, 1993). Sex reversal in these patients must be due to mutations in other genes elsewhere in the pathway. In addition, the tight temporal and spatial control of *SRY* expression implies the existence of regulatory genes "upstream" of *SRY*.

(b) Unravelling the testis (sex) determining pathway

It was expected that the cloning of the testis determining gene would provide access to the rest of the sex determining pathway. However, this has not proved to be the case for a number of reasons. Firstly, *SRY* binds to a sequence found in the promoter regions of many other genes (Harley *et al.*, 1994). Secondly, *SRY* has a conserved region known as the HMG box which is found in a number of closely related genes known as *SOX* (*SRY*-related HMG box) genes, most of which are not known to be involved in sex determination. This makes it difficult to determine exactly which genes are interacting with *SRY* in the sex determining pathway. In addition, the *SRY* gene shows poor conservation between species (Foster *et al.*, 1992), and is not known to be involved in sex determination in any vertebrates other than mammals. It is, therefore, possible that *SRY* represents a mammalian specific offshoot from the conserved vertebrate sex determining pathway.

Recently, a gene related to *SRY* called *SOX9* has been shown to be involved in the vertebrate testis determining pathway. The *SOX9* gene was originally isolated from human patients that suffer from a bone deformity called campomelic dysplasia (CD). Three quarters of the XY patients with this syndrome are sex reversed females. Two groups independently cloned *SOX9* and showed that mutations in this gene could prevent normal testis development and cause the bone deformity in CD patients (Foster *et al.*, 1994; Wagner *et al.*, 1994). This implied *SOX9* has an important role in the testis determining pathway (Sinclair, 1995; Cameron *et al.*, 1996). Analysis of *SOX9* expression in more detail has shown that it is dramatically up-regulated in the male genital ridge of both mouse and chicken, confirming its important role in the vertebrate testis determining pathway (Kent *et al.*, 1996).

(c) Avian sex determination

Sex in birds is determined by a chromosomal system in which the male is the homogametic sex while the female is the heterogametic sex. Since this is the opposite to that in mammals the system is termed ZZ/ZW in birds to emphasise this difference. The W

chromosome is heterochromatic with a highly repetitive DNA content (Saitoh *et al.*, 1991). Therefore, it is thought to be unlikely to contain many genes (Thorne, 1995). Until now, no genes have been confirmed to map to the W chromosome.

The W chromosome in birds seems to act as a dominant determinant of female sexual differentiation. No ZW birds have been reported with any degree of testicular development and no ovarian development is seen in any birds lacking a W chromosome (Bitgood and Schoffner, 1990). Chickens with a ZZW triploid chromosomal constitution all have some degree of ovarian development, but since testicular development can also occur it would appear there is a complex interaction between the Z and W chromosomes. It seems likely that the W chromosome contains a gene or genes which have a dominant effect on ovarian development and, thus, on female sex determination.

Sexual dimorphism in chickens appears around 6 or 7 days of incubation (Romanoff, 1960). Prior to this time the gonads are bipotential and gonadal differentiation is dependant upon the genetic sex of the embryo. In male birds the development of the testis does not differ markedly from mammalian testicular development. However, ovarian development in the female is quite different. The left gonad develops into a normal ovary but the right gonad shows less extensive development and regresses to a rudimentary vestige after hatch. However, if the left ovary is removed or becomes non-functional then the rudimentary gonad develops into an ovotestis. This results in masculinisation of the hens since the testis-like gonad contains androgen secreting Leydig cells and small numbers of spermatozoa (van Krey, 1990). Surprisingly, this asymmetry is reflected in clinical observations of intersex and hermaphrodite patients. In these patients, testicular development is much more frequently observed on the right side while ovarian development is seen more frequently on the left side (Hutson, Paediatric surgeon, Personal communication.).

#### (d) Avian sex reversal

Unlike the situation in mammalian development sexual development in birds is complicated by the fact that hormones can have an influence on the initial development of the gonad. For example, addition of an aromatase inhibitor to early chicken embryos (blocking the synthesis of oestrogen from testosterone) causes chromosomally female birds to develop a permanent male phenotype including functional testis (Elbrecht and Smith, 1992). This indicates that aromatase is in the avian sex determining pathway, presumably downstream from any putative ovary determining factor (ODF) or TDF gene.

Transplantation of the right gonad of female embryos into gonadectomised hatched males results in the masculinisation of the right female gonad and production of fertile sperm. When genetic female embryos are given testicular grafts during the indifferent period male gonadal differentiation is induced and remains permanent after hatching. The sex reversal is believed to be in response to decreased ovarian oestrogen secretion which is a result of anti-Mullerian hormone secretion by the testis graft. Early exposure of the avian male embryo to oestrogen causes transient feminisation of the male ZZ gonad (van Krey, 1990).

#### (e) Previous attempts to isolate Avian SRY

The chicken embryo provides an ideal model system for investigating the molecular genetics of gonadal development and sex determination. Fertile eggs are readily available and developing chicken embryos are more accessible and easier to manipulate than those of

eutherian mammals. In addition, there is detailed information on chicken embryology with well characterised developmental stages, in particular, the timing and morphology of gonadal development. Attempts to isolate *SRY* from birds or other vertebrates have resulted only in the isolation of *SOX* genes (Griffiths, 1991). This is because *SOX* genes are generally more closely conserved in evolution than *SRY*. It is, therefore, difficult to determine if *SRY* is not present in birds or if it is present but masked by *SOX* genes. However, it appears likely that *SRY* is not involved in avian sex determination, either as a switch or as a general regulatory gene. In order to circumvent this problem two different methods have been used to isolate sex determining genes from chicken (*Gallus g. domesticus*) embryos. In chicken embryos it has been observed that the indifferent genital ridge starts to develop into either a testis or an ovary at or after day six of incubation. Presumably, this morphological change is due to differential gene expression from the male and female gonad. Until recently the method for isolating such differentially expressed genes involved the difficult and unreliable subtractive hybridisation technique.

## II. DISCUSSION

### (a) Differential display of mRNA by random PCR amplification of 3' ends (DD-PCR)

A new technique has been developed called differential display of mRNA by random PCR amplification of 3' ends, or DD-PCR (Liang and Pardee, 1992). Essentially the technique allows the examination of those transcripts that are differentially expressed from either male or female genital ridge. Comparisons can also be made between different stages of embryonic gonadal development. This approach does not presuppose the presence of any particular gene in the avian sex determining system, nor does it preclude the possibility of isolating female (ovary) determining genes. The DD-PCR technique has been found to suffer from a number of disadvantages.

- 1) DD-PCR involves performing a thousand PCR reactions and a few hundred display gels. This equates to a time commitment of several months to a year just in the initial phase of the technique.
- 2) An arbitrary size cut-off must be imposed when examining the DD-PCR gels such that smaller differential bands (less than 250kb) will not be detected.
- 3) Differentially expressed bands on the gel must be identified visually and this becomes increasingly difficult with large numbers of densely packed bands.
- 4) The procedure is renowned for producing false positives so additional procedures and replicates must be added, further extending the time involved.
- 5) Finally, DD-PCR is not quantitative so that only the presence or absence of a transcript will be detected and more subtle differences in the levels of transcription will be overlooked.

### (b) Representational difference analysis

More recently a new technique has been developed known as representational difference analysis, or RDA (Hubank and Schatz, 1994). RDA is essentially a process of subtraction followed by PCR amplification and is used to analyse differential gene expression. However, it differs from DD-PCR in that it is fast, reproducible, generates few false positives, requires a very small amount of starting material and is very sensitive such that quantitative differences can be detected. In addition, only difference products are

produced so that the arbitrary nature of visual identification is removed and there is no lower size limit that can be detected.

In order to establish that a particular gene is involved in sex determination it is necessary to show it is expressed in a stage-specific and sex-specific manner in chickens during primary sexual differentiation. The genital ridge was dissected from chick embryos at day 5 of incubation. The remainder of the chick embryo was used for DNA extraction and subsequent PCR analysis using primers for W-chromosome specific repeats to determine the sex. Fifty genital ridges were dissected, total RNA extracted and the embryos sexed.

The mRNA was purified away from total RNA and double stranded cDNA was made from the purified message from both sexes. For the purposes of RDA the double stranded cDNA was cut with a frequent cutting restriction enzyme, Dpn II. Enzyme digestion of the cDNA allows for highly efficient ligation to special adaptor primers necessary for PCR enrichment. Representations called "amplicons" were generated for each sex by PCR amplifying the ligated cDNA. Refinement of the technique has allowed the generation of high quality representations from 20 fold less starting mRNA than used by Hubank and Schatz (1994). One amplicon (eg. male cDNA) acts as driver and the other (eg. female cDNA) acts as tester. Two rounds of subtractive hybridization and PCR enrichment of tester fragments on the chick genital ridge cDNA's in reciprocal fashion have been conducted. Already, several bands unique to one sex or the other have been observed in a background common to both sexes. A pilot experiment has shown the high degree of sensitivity in the system. Use has been made of liver mRNA spiked with known amounts of an exogenous *in vitro* transcript (tester) which was subjected to RDA subtraction with plain liver (driver). In this experiment the exogenous cDNA unique tester fragments were detected at extremely low levels and were the only product seen after three rounds of subtraction, showing the exquisite sensitivity of this technique. RDA has been used to compare transcripts produced from male and female genital ridge at days 4, 5, and 6.

#### (c) Sequence analysis of differentially expressed transcripts

In order to obtain full-length cDNAs which correspond to differentially expressed genes a male and female chicken embryo genital ridge (day 5) cDNA library has been screened. RDA difference products were re-amplified with radioactive label and used to screen either a male or female cDNA library. Positive clones were isolated and subcloned into a plasmid vector for subsequent analysis.

Full length differentially expressed cDNA transcripts subcloned in plasmid vectors were then sequenced. The nucleotide and putative amino acid sequences of these clones were used to search the DNA and protein databases for homology with known genes or proteins.

#### (d) Spatial and temporal expression of candidate sex determining genes

Sex specific transcripts must satisfy two important criteria if they are to be pursued as candidate sex determining genes. Firstly, they should be expressed in a sex specific fashion from the developing gonads of the embryo. Secondly, they must be developmentally regulated, expressed at or just prior to the time of gonadal sex determination which is between days 4 and 5 of incubation in the chicken embryo. RNA probes from the avian candidate genes were made and used for *in situ* hybridisation to whole mount embryo

sections or tissue taken at different stages of development. This establishes the time and the tissue or cell type responsible for expression.

(e) Mapping candidate sex determining genes to chicken chromosomes

Differentially expressed genes that map to the sex chromosomes would suggest an early crucial "switch" role in regulation of the sex determining pathway. However, a gene may still be involved with the avian sex determining pathway, downstream from the "switch" and map to an autosomal location. cDNA corresponding to candidate sex determining genes was labelled and used to probe Southern blots containing male and female genomic DNA. A W-specific gene will only hybridise to the female genomic DNA and Z linkage would be detected by dosage in the male DNA.

(f) Cloning and characterising mammalian homologues

Candidate avian sex determining genes have also been used to probe mammalian DNA (human and mouse) on Southern blots. Those genes which cross-hybridise have been used to isolate human or mouse homologues isolated by screening the appropriate genomic libraries. Ultimate proof that a candidate avian sex determining gene is directly involved in sex determination or gonad development will require transgenic or possibly knockout experiments in mice. The function of any of these avian candidate sex determining genes has been examined using expression vectors to produce protein for the production of polyclonal antibodies and analysis in gel shift assays and ultrastructural studies.

(g) Sex reversed XY female patients: A test for a putative sex determining gene

To date only 10% of human XY females have a mutation in *SRY* implying that other downstream genes may have mutations which account for this sex reversal. These patients effectively act as natural gene knockout experiments and have proved to be critical in earlier studies of mammalian sex determination. Consequently, the human homologue of an avian candidate gene will be used to analyse those XY female patients which have not suffered a mutation in the *SRY* gene. If such an XY female patient is found to have a mutation in this candidate gene it would provide compelling evidence that the gene is required in the vertebrate male (testis) determining pathway.

### III. CONCLUSIONS

The evolutionary perspective inherent in this study acts as a vital filter which is necessary to identify the critical genes in the vertebrate sex determining pathway. Any genes which are differentially expressed in the genital ridge of human, mouse and chicken will have been conserved for over 300 million years and are likely to play a fundamental role in sex determination. Perhaps *SRY* is a sex determining switch that is only used in mammals. Analysis of differentially expressed genes from the genital ridge of the chicken embryo may provide an insight into the basic vertebrate sex determining pathway as well as defining those aspects unique to Aves.

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## DIFFERENTIAL EXPRESSION OF GENES INVOLVED IN THE SYNTHESIS AND FUNCTION OF OESTROGEN AND A Z CHROMOSOME-LINKED *ZOV3* GENE IN THE GONADAL DEVELOPMENT OF CHICKENS

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### Summary

Examination of transcriptional level expression of five genes involved in the synthesis of oestradiol-17 $\beta$  from cholesterol and of the oestrogen receptor gene by *in situ* hybridization to chicken embryo tissue sections with antisense RNA probes has revealed remarkable differential expression of three genes; highly preferential expression of genes for 17 $\beta$ -hydroxysteroid dehydrogenase and aromatase in the female gonads and the left gonad-specific expression of the oestrogen receptor gene. The expression of the oestrogen receptor gene in the male left gonad is limited to early-stage embryos. These results explain the oestrogen-dependent differentiation of cortex in the left gonad in the female embryo and the potential of the male left gonad to be morphologically sex-reversed by administration of oestrogen to early embryos. The Z chromosome-linked *ZOV3* gene, which encodes an immunoglobulin superfamily protein, is expressed in embryonic gonads of both sexes but its expression is restricted to the ovary in mature chickens. Immunofluorescence studies combined with *in situ* hybridization have shown that *ZOV3* protein is present in granulosa cells and a group of cells in the outer thecal layer which produce oestradiol-17 $\beta$ .

### I. INTRODUCTION

Carinatae birds possess female heterogametic sex chromosomes which are recognizable in their karyotypes; ZW for female and ZZ for male. Although a positive role for the W chromosome in the early differentiation of the female gonad has been suggested (Sheldon and Thorne, 1995), a gene triggering it has not been identified. DNA sequences and genes characterized and mapped on the chicken sex chromosomes in one of the authors' laboratories are shown in Figure 1. Searching for genes in the terminal non-heterochromatic region on the W chromosome (Saitoh and Mizuno, 1992) should be important, because the W chromosome pairs at the end of this region with the non-heterochromatic end of the Z chromosome during the pre-meiotic period (Hori *et al.*, 1996) and, thus, on the analogy of the case for the mammalian Y chromosome, this region is expected to contain pseudoautosomal genes and genes involved in sex determination (Simmler *et al.*, 1985; Sinclair *et al.*, 1990; Koopman *et al.*, 1991). Although there was no direct evidence for the latter genes, the early sex differentiation of birds is characteristically different from that of mammals in that morphological sex reversal of gonads can be induced by manipulation with a sex steroid hormone and that only the left gonad in the female develops into a functional ovary in most species. The molecular-level mechanisms for the above features are summarized as well as a possible role for a newly identified immunoglobulin superfamily gene, *ZOV3*, on the Z chromosome in the functional differentiation of the ovary.

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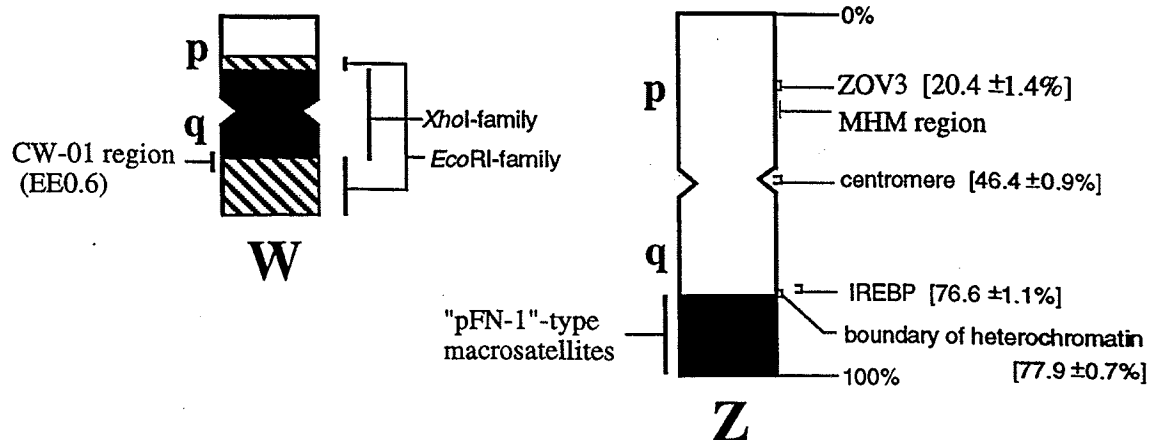


Figure 1. Genes and sequence regions on the W and Z chromosomes of the chicken which have been cloned and mapped in one of the authors' laboratory. *XhoI* and *EcoRI*-family repetitive sequences are confined to the W chromosome of domestic and jungle fowl (Kodama *et al.*, 1987; Saitoh *et al.*, 1991, Saitoh and Mizuno, 1992) and constitute W heterochromatin (Suka *et al.*, 1993). CW-01 region contains EE0.6 sequence which is widely conserved on W chromosomes of Carinatae birds (Ogawa *et al.*, In press). The MHM region is hypermethylated in both Z chromosomes of male chickens (Teranishi *et al.*, unpublished observations). IREBP is a gene for iron responsive element-binding protein (or cytoplasmic aconitase) (Saitoh *et al.*, 1993). The terminal heterochromatin of Z chromosome consists of "pFN-1"-type macrosatellite sequences in which an approximately 24-kb unit is repeated about 830 times (Hori *et al.*, 1996).

## II. METHODS

The genetic sex of an embryo was determined by slot blot hybridization of DNA extracted from the extraembryonic membrane with the digoxigenin (DIG)-labeled W chromosome-specific *XhoI*-family probe (Kodama *et al.*, 1987) as described by Mizuno *et al.* (1993). *In situ* detection of mRNA was performed by hybridizing a DIG-labeled antisense RNA probe to a tissue section 8 mm thick which had been prepared from a paraffin embedded block of embryonic tissue containing left and right gonads, and the RNA/RNA hybrids were detected by the reaction with alkaline phosphatase-conjugated sheep anti DIG antibody (Boehringer Mannheim) (Nakabayashi *et al.*, Unpublished). DNA templates for the *in vitro* synthesis of antisense RNA probes and primers for RT-PCR were all from chicken sources which were obtained from the cDNA clones or published sequences as follows: cholesterol side-chain cleavage cytochrome P-450 (P450<sub>sc</sub>) (Nomura *et al.*, 1996), 3 $\beta$ -hydroxysteroid dehydrogenase/ D isomerase (3 $\beta$ -HSD) (Nakabayashi *et al.*, 1995), 17 $\alpha$ -hydroxylase cytochrome P-450 (P-450<sub>c17</sub>) (Ono *et al.*, 1988), 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD) (Wajima *et al.*, Unpublished), aromatase cytochrome P-450 (aromatase) (McPhaul *et al.*, 1988) and oestrogen receptor (ER) (Krust *et al.*, 1986). The N-terminal peptide (N-terminus to residue 99 which is just before the



first immunoglobulin-like loop) of ZOV3 (Saitoh *et al.*, 1993) was produced in *E. coli* and the polyclonal antibody against it was raised in a mouse and affinity purified. Indirect immunofluorescence experiments were performed on a section prepared from paraffin-embedded ovarian follicles by reactions with the above antibody followed by fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse IgG (Kunita *et al.*, Unpublished).

### III. RESULTS AND DISCUSSION

#### (a) Differential expression of genes for 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD), aromatase and oestrogen receptor (ER)

Morphological differentiation of male and female gonads in chicken embryos starts between days 5 and 6 of incubation (Romanoff, 1960). In the female, the right gonad ceases its growth at about day 10 of incubation and regresses thereafter (Teng and Teng, 1977). When an aromatase inhibitor is administered *in ovo* before the seventh day of incubation, all the treated genetic females develop male-type cloacal morphology and testes are induced in some of them although they are infertile (Elbrecht and Smith, 1992). On the other hand, when oestrogen is administered *in ovo* to early genetic male embryos, they form a left ovotestis (Romanoff, 1960). These results suggest that an early exposure to oestrogen is essential for the normal development of the female left gonad and that the early left gonad in the male embryo is potentially responsive to oestrogen.

In order to elucidate the molecular mechanisms underlying these features of gonadal development in chickens, mRNA-level expressions of the five genes required for the conversion of cholesterol to oestradiol-17 $\beta$  and of the ER gene in embryonic gonads between days 7 and 14 of incubation were examined by *in situ* hybridization with antisense RNA probes. The results are summarized in Table 1 and show two interesting features of the differential gene expression: 1) the first three genes (P-450scc, 3 $\beta$ -HSD and P-450c17) of oestrogen synthesis are expressed in both left and right gonads of both sexes but the last two genes (17 $\beta$ -HSD and aromatase) are expressed only in the left and right gonads of female embryos, and 2) the expression of the ER gene is found only in the epithelium of the left gonad of both sexes. However, the expression of ER gene in the male left gonad is temporary. Judging from the intensity of *in situ* hybridization, the level of its expression is

Table 1. Detection of mRNA for the five genes involved in the synthesis of oestradiol-17 $\beta$  and for the oestrogen receptor (ER) gene in the left (L) and right (R) gonads of chicken embryos at days 7 to 14 of incubation. *In situ* hybridization to tissue sections with the antisense RNA probe was employed for all six genes. \*RT-PCR was also applied. Results are expressed as + (detectable) or - (undetectable). (+) means that mRNA was detectable at days 7 to 10 of incubation.

mRNA for	Female		Male	
	L	R	L	R
P-450scc	+	+	+	+
3 $\beta$ -HSD	+	+	+	+
P-450c17	+	+	+	+
17 $\beta$ -HSD*	+	+	-	-
aromatase*	+	+	-	-
ER*	+	-	(+)	-

comparable to that in the female at the 8th day of incubation but its intensity decreases thereafter and becomes undetectable at day 14 of incubation. When RT-PCR was applied, the amplification products from ER mRNA after 40 cycles of PCR were detectable in about 10 % of samples from individual male left gonads at day 18 of incubation but were undetectable after hatching (Nakabayashi *et al.*, Unpublished).

These results indicate that at the transcriptional level, conditions for producing oestradiol-17 $\beta$  are established in both left and right gonads in the female embryo as early as day 7 of incubation. On the other hand, oestrogen should not be produced in either left or right gonad in the male embryo because the last two genes which are required for converting androstenedione to oestradiol-17 $\beta$  are not expressed. In the female embryo oestrogen, produced presumably from both left and right gonads, acts only on the epithelium of the left gonad because the ER gene is expressed only in the epithelium of the left gonad. It should then enable the left gonad to differentiate its cortex and the primitive follicles in it. Although the differentiation of epithelium is also noted in the male left gonad as early as day 5 of incubation and the ER gene is expressed temporarily, it is no use because oestrogen is not produced. In consequence, the cortex is not formed but seminiferous codes are differentiated in both left and right gonads in the male embryo. The temporary expression of the ER gene in the male left gonad explains why the left ovotestis is differentiated in response to the early *in ovo* administration of oestrogen.

(b) Sites of expression of genes for oestrogen synthesis in embryonic female gonads

By employing two antisense RNA probes together, one labeled with DIG and the other labeled with biotin, in the *in situ* hybridization to the same embryonic tissue section containing left and right gonads, it was demonstrated that the five genes required for oestrogen synthesis are grouped into two with respect to their sites of expression in the female gonad. Genes for the first three enzymes; P-450 $\text{scc}$ , 3 $\beta$ -HSD and P-450 $\text{c17}$ , are expressed in the fat-laden cells and some medullary cord cells in the medulla of the gonad. On the other hand genes for the last two enzymes, 17 $\beta$ -HSD and aromatase, are expressed in other medullary cord cells (Nakabayashi *et al.*, Unpublished). The different sites of expression of genes for P-450 $\text{c17}$  and aromatase are shown in serial sections in Figure 2. These observations can be correlated with the situation of oestrogen synthesis in ovarian follicles: reactions of the first three enzymes are demonstrated in granulosa cells and reactions of the last two enzymes are demonstrated in a group of cells in the outer thecal layer (Johnson *et al.*, 1990).

(c) Immunological detection of ZOV3 protein in the ovarian follicle of chicken

A cDNA clone, pZOV3, was isolated from the cDNA library of 1 to 3-day old chicken left ovaries by differential screening of those clones from relatively abundantly expressed mRNA species in the ovary and its gene was located at the middle of the short arm of the chicken Z chromosome (Figure 1) (Mizuno *et al.*, 1993; Saitoh *et al.*, 1993). It has been suggested by Southern blot hybridization that the ZOV3 gene is conserved as Z-linked in a wide variety of avian species. pZOV3 contains a cDNA insert of about 4.5 kb, in which an open reading frame encoding 327 amino acid residues is present. The nucleotide sequence suggests that ZOV3 is a novel member of the immunoglobulin (Ig) superfamily and that it contains two Ig-like (C2-type) loops (L1 and L2 in Figure 3) and seven putative N-glycosylation sites in the extracellular domain, a single membrane-

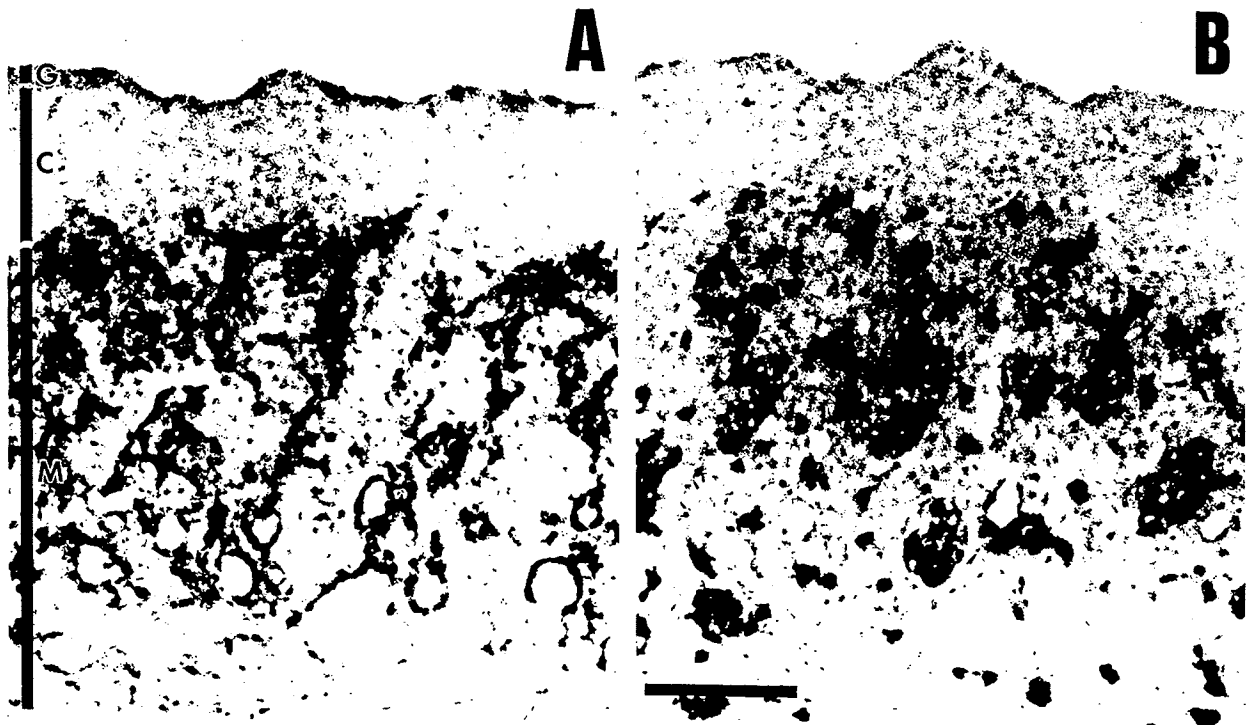


Figure 2. Expression of genes for aromatase and P-450c17 in different cell types in the left gonad of a female chicken embryo at day 14 of incubation. *In situ* hybridization with antisense RNA probes to serial sections demonstrates that both mRNAs are detected mainly in the medullary cord, but the cell types for each mRNA are not identical. In the cortex, both mRNAs are undetectable. **A:** signals for aromatase mRNA are stronger in the medullary cord cells of the superficial medulla but are weaker in the deeper medulla. Aromatase mRNA is also detected weakly in the germinal epithelium. **B:** P-450c17 mRNA is detected in the fat-laden cells and some other medullary cord cells in the medulla. Vertical bars show germinal epithelium (G), cortex (C) and medulla (M). A horizontal scale bar shows 50  $\mu$ m.

hybridization to poly(A)<sup>+</sup>RNA preparations from various tissues of 60 day-old chickens indicates that the *ZOV3* gene is expressed predominantly in the ovary and weakly in the testis. In sexually mature chickens, the expression of the *ZOV3* gene is no longer detectable in the testis and its expression is restricted to the ovary (Kunita *et al.*, Unpublished).

An N-terminal polypeptide of *ZOV3* (NT region) was produced as a fusion form with His<sub>6</sub>-tag in *E. coli*, as shown in Figure 3, and a polyclonal antibody against it was raised in mice.

Western blotting and immunofluorescence studies with the affinity purified antibody have shown that the *ZOV3* protein is produced as a membrane glycoprotein in granulosa cells and in a group of cells in the outer thecal layer of ovarian follicles. The latter cells were suggested to produce oestradiol-17 $\beta$  because *in situ* hybridization with antisense RNA probes indicated that genes for 17 $\beta$ -HSD and aromatase were expressed in those cells.

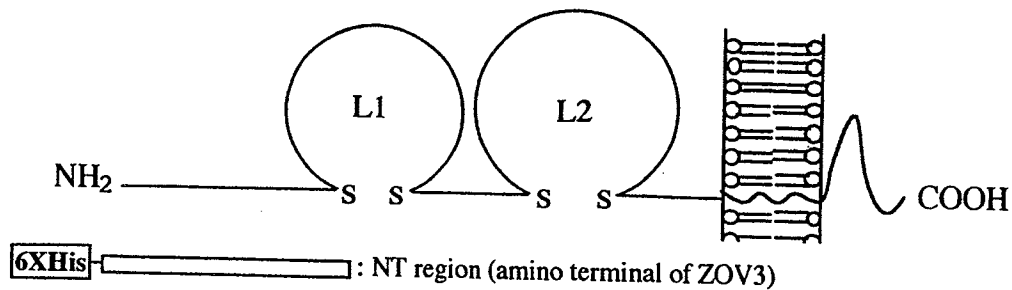


Figure 3. A structural model of ZOV3, a Z chromosome-linked immunoglobulin superfamily gene product, and position of the polypeptide with a His<sub>6</sub>-tag produced in *E. coli*.

These results imply that the ZOV3 protein plays a role in cell-to-cell contact and affects the steroidogenic function of those cell groups, but exact mechanisms remain to be elucidated. In contrast to the situation in the sexually mature chicken, the *ZOV3* gene is expressed in both male and female gonads in embryos, but its significance is unknown.

#### IV. CONCLUSIONS

The differential expression of genes for 17 $\beta$ -HSD, aromatase and ER, in the embryonic gonads of the chicken explains the molecular basis for the long-standing biologically significant features of gonadal differentiation in chickens; that is, the importance of the early exposure to oestrogen for the normal development of the female gonad, the development of only the left gonad of the female into a functional ovary, and the phenotypic sex reversal of gonads by administration of an aromatase inhibitor to the female embryo or oestrogen to the male embryo. A novel Z-linked Ig-superfamily gene, *ZOV3*, is implied to play an important role in the development of the ovary with respect to differentiation or function of sex-steroid producing cells.

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## IDENTIFICATION AND CHARACTERISATION OF A NOVEL STROMAL CELL DERIVED HAEMOPOIETIC GROWTH FACTOR

C. SIATSKAS and R. BOYD

### Summary

As a prelude to defining growth factors influencing the growth and maturation of myeloid cells, cell lines were generated from avian splenic stromal cells using Rous Sarcoma Virus as the transforming agent. One line (Splenic stromal cell line-1 [SSL-1]) was chosen as a candidate cell source because of its constitutive secretion of growth factors influencing macrophage and granulocyte differentiation. Additionally, conditioned media (CM) from this line also induced the proliferation of B-cells. Molecular analysis of RNA transcripts for the growth factors, chicken myelomonocytic growth factor (cMGF), demonstrated the absence of message for cMGF, while activities corresponding to interleukin 2 (IL-2) and interferons  $\alpha$ ,  $\beta$  and  $\gamma$  were not detected in SSL-1 CM. Intriguingly, SSL-1 CM stimulated human granulocyte and macrophage differentiation. Moreover, SSL-1 CM induced the proliferation of murine cells transfected with murine leukaemia inhibitory factor (LIF) receptors. Considering the pivotal role of mammalian LIF in maintaining embryonic stem cells in an undifferentiated state, identification of the LIF-like activity present in SSL-1 CM may provide a means to develop strategies for the generation of gene targeted birds.

### I. INTRODUCTION

The structural integrity of cells within the haemopoietic system is reliant on a small pool of stem cells which either undergo self renewal to produce more stem cells or differentiate to produce progeny which are committed to terminal differentiation. The survival, proliferation and development of all these cells is strictly dependent on extracellular signals. Among these are polypeptide regulators generally known as cytokines.

An important source of cytokines is the haemopoietic stromal milieu, which analogous to a sponge, forms a fixed interconnected matrix in which developing precursors are closely associated. Production of cytokines by the stroma can have either stimulatory or inhibitory effects and, thus, provides the homeostatic balance preventing leukaemia or cytopenia. The importance of cytokines produced by stromal cells is best exemplified in mice carrying the steel mutation. These animals do not produce stem cell factor (SCF) rendering these mice anaemic, sterile and partially or completely lacking skin pigment (Flanagan and Leder, 1990).

While the details concerning the stem cell origins and migrations of avian haemopoietic cells have been well documented little is known about the cytokine involvement associated with avian haemopoietic cell development. Although the known haemopoietic cytokines at present exceed 20 in number in the mouse (Metcalf, 1993), only five chicken specific cytokines, SCF (Zhou *et al.*, 1993), types I and II interferon (Sekellick *et al.*, 1994; Lowenthal *et al.*, 1995), chicken myelomonocytic growth factor (cMGF) (Leutz *et al.*, 1984; 1989) and 9E3/CEF-4, an IL-8 like molecule (Barker *et al.*, 1993), have been characterised at the nucleotide level.

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Considering the paucity of cloned and characterised avian cytokines, the current studies have addressed the hypothesis that the stromal cells of haemopoietic tissues provide the necessary factors for chicken haemopoietic cell development, specifically myeloid cell development. The results presented demonstrate the *in vitro* characteristics of a novel avian cytokine produced by an avian splenic stromal cell line [SSL-1] which stimulate both myeloid and lymphoid cell development. Significantly, SSL-1 CM induced the differentiation and proliferation of mammalian haemopoietic cells and signals via LIF receptors.

## II. METHODS

### a) Functional analysis of SSL-1 conditioned media

For proliferation assays, test cells were prepared according to the method of Siatskas *et al.* (1996), and aliquoted (100mL/well) in triplicate into 96 well plates at a cell concentration of  $5 \times 10^5$  cell/mL (bone marrow and spleen) and  $1 \times 10^6$  cells/mL (bursa). Cells were incubated with either SSL-1 CM, cMGF, BSL-2 CM or media alone and were pulsed-labelled with 25mL of  $^{125}\text{I}$ -Iododeoxyuridine containing  $10^{-5}\text{M}$  5-fluorodeoxyuridine (0.1mCi/well) prepared in serum free media for 24 h.

### b) Interleukin-2 assay

IL-2 dependant blasts were prepared by culturing  $5 \times 10^6$  adult spleen cells in the presence of Con A (2mg/mL) for 48 h at  $41^\circ\text{C}$ . Non-adherent blasts were subsequently washed twice in Hanks media, incubated for 15 min in the presence of 0.1M a methyl mannopyranoside (aMM), washed and resuspended in media containing at a cell concentration of  $2.5 \times 10^5$  cells/mL. Serial two-fold dilutions (100 $\mu\text{L}$ ) of test supernatants were made in DMEM-FCS supplemented with 0.1M aMM and added in duplicate to 96 well plates, followed by the addition of 100mL of blast cell suspension and cultured for 48 h at  $41^\circ\text{C}$ . In control cultures, cells were incubated with a supernatant known not to IL-2 like activities (conditioned media derived from adult spleen cells cultured for 24 h) or in the presence of a supernatant containing known IL-2 activity [Con A activated spleen cell supernatant] (Lowenthal *et al.*, 1994). Cell proliferation was assessed by pulsing cultures with 0.5 mCi/well [ $^3\text{H}$ ]-thymidine 4 h prior to harvesting the cultures onto filter mats with the incorporated radioactivity quantitated by a Trace 96 automated gas proportional beta counter.

### c) Interferon assay

Primary embryonic day 10 fibroblasts (CEF) were prepared according to the method of Lowenthal *et al.* (1995) and cultured ( $4 \times 10^5$ /mL) for 3 days at  $37^\circ\text{C}$  in media containing 5% FCS (media-FCS). Cells were subsequently harvested, washed and resuspended in media-FCS with 100  $\mu\text{L}$  seeded in 96 well plates ( $5 \times 10^4$  cells/well) and incubated at  $37^\circ\text{C}$ . After 24 h the culture medium was replaced with 100  $\mu\text{L}$  of serum free media containing two-fold serial dilutions of test supernatants which were made in duplicate. For controls, cells were cultured with a supernatant known not to contain interferon (conditioned media derived from adult spleen cells cultured for 24 h) or in a reference supernatant known to contain IFN activity [Con A activated spleen cell supernatant] (Lowenthal *et al.*, 1995). After overnight incubation at  $37^\circ\text{C}$ , culture medium was replaced with 100  $\mu\text{L}$  of medium containing SemLiki Forrest virus ( $10^3$  tissue culture units) and cultures incubated for a

further 24 h at 37°C. Cell viability was measured by uptake of neutral red dye and absorbance read at 540nm using an ELISA reader.

#### d) Human haemopoietic colony assays

Colony forming assays were performed in duplicate with  $2 \times 10^4$  normal human bone marrow cells in 1mL cultures. Semi solid medium consisted of 0.3% agar in IMDM supplemented with 25% FCS, 6pM DEAE Dextran, 0.6mM L-asparagine,  $4 \times 10^{-5}$ M 2ME, 60µg/mL benzylpenicillin and 100µg/mL streptomycin sulphate. The recombinant human cytokines used were as follows: 100ng/mL SCF, 100ng/mL GM-CSF, 500U/mL G-CSF. Cultures containing various combinations of concentrated or diluted SSL-1 CM, with mixtures of recombinant human growth factors were also established. Cultures were incubated for 14 days at 37°C in a 5% CO<sub>2</sub> incubator after which developing colonies were scored using a Zeiss dissecting microscope. Agar cultures were floated out of the petri dish, fixed onto a clean glass slide and stained with luxol fast blue and haematoxylin (Metcalf, 1984).

#### e) Bioassay of SSL-1 CM on Ba/F3 receptor inserted cell line

Screening of SSL-1 CM was performed using the established method of proliferation of cloned cell lines transfected with receptor cytokines in microwells (Metcalf *et al.*, 1994). Cloned stably transfected Ba/F3 cells, electroporated with the cDNA's encoding for the receptors for either, murine Leukemia inhibitory factor (LIF), Granulocyte-Colony stimulating Factor (G-CSF), Thrombopoietin (TPO), interleukin-6 (IL-6), parental untransfected Ba/F3 cells and FDC-P1 cells were washed three times by centrifugation in 10mL of serum free media. Cells were resuspended at a final concentration of  $2 \times 10^4$  cells/mL in DMEM containing 10% newborn calf serum and 10µL of cell suspension (containing approximately 200 cells) was aliquoted into Lux 60-well HL-A plates. SSL-1 CM was added previously in volumes of 5µL to duplicate wells in serial two-fold dilutions made using DMEM containing 10% newborn calf serum. After 48 to 72 h, at 37°C in a fully humidified atmosphere of 10% CO<sub>2</sub> in air, the microwells were scored using an Olympus inverted microscope. Unstimulated wells contained no viable cells, while the number of viable cells rose progressively with increasing concentrations of growth factor. Cell counts were not attempted when more than 200 cells were present in the wells. Cross-calibration of assays was performed by including in every assay a titration of the relevant recombinant growth factor of known biological activity.

### III. RESULTS AND DISCUSSION

#### a) In vitro properties of SSL-1 CM

Following the success of long term bone marrow cultures in both mice and humans, embryonic spleen cell line (SSL-1) was derived from the transformation of embryonic day 16 (E16) splenic stromal cells cultured under Dexter conditions (a culture system known to select for stromal cells that support myeloid cell development). Cells were characterised in terms of their overall morphology in culture, cell surface expression of myeloid cell antigens, non-specific esterase and phagocytic activities and were found to be of macrophage origin (data not shown). Using precursor cells from a number of haemopoietic tissues (spleen and bone marrow) and from different stages in ontogeny (E16, day 1, and



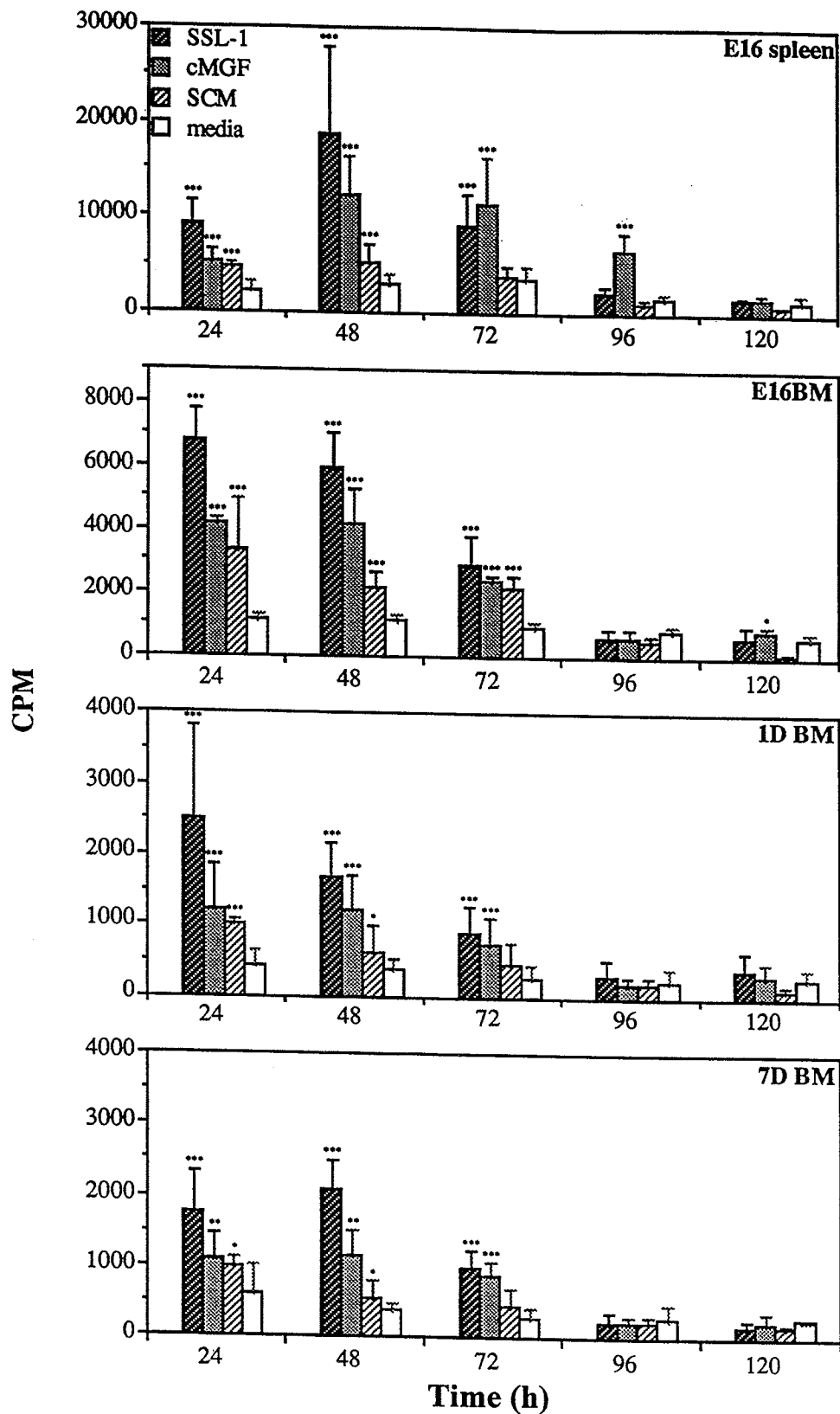


Figure 1. Proliferation of E16 spleen and BM cells from E16; day 1 and day 7 chickens cultured with SSL-1 CM recombinant cMGF, normal spleen cell conditioned media (SCM) or media alone. Cells were assessed for their proliferative activity every 24 h over a period of 120 h. Results are shown are of dilutions which gave maximal stimulation indices. Results are present as the mean $\pm$ SD of three experiments performed in triplicate.

day 7), SSL-1 CM was able to induce the proliferation and differentiation of predominantly macrophage precursors (Figure 1). Control supernatants from heterogeneous splenic stromal cultures (SCM) had similar but reduced activity while cMGF, which was used as a culture supernatant derived from transfected COS cells, induced a response that was slightly less than that seen with SSL-1 CM. Confirmation of the myeloid inducing activities present in SSL-1 CM was by flow cytometry using an extensive panel of monoclonal antibodies to chicken lymphoid and myeloid cells and analysis (morphological, histological, immunohistological and enzyme assays) of colonies grown in semi-solid media (data not shown).

To further define the activities present in SSL-1 CM, bursal derived B cells were isolated from E15 embryos and stimulated with serial dilutions of SSL-1 CM. As shown in Figure 2, SSL-1 CM induced the proliferation of B-cell precursors in a dose-dependant manner. Whether this proliferative event is coupled with the maturation of precursor B cells is unknown. Collectively these studies demonstrated that SSL-1 produce cytokines which stimulate myeloid and B cell precursors suggesting that, (i) multiple cytokine activities are present in SSL-1 CM, or (ii) a pleiotropic growth factor is secreted by SSL-1 cells.

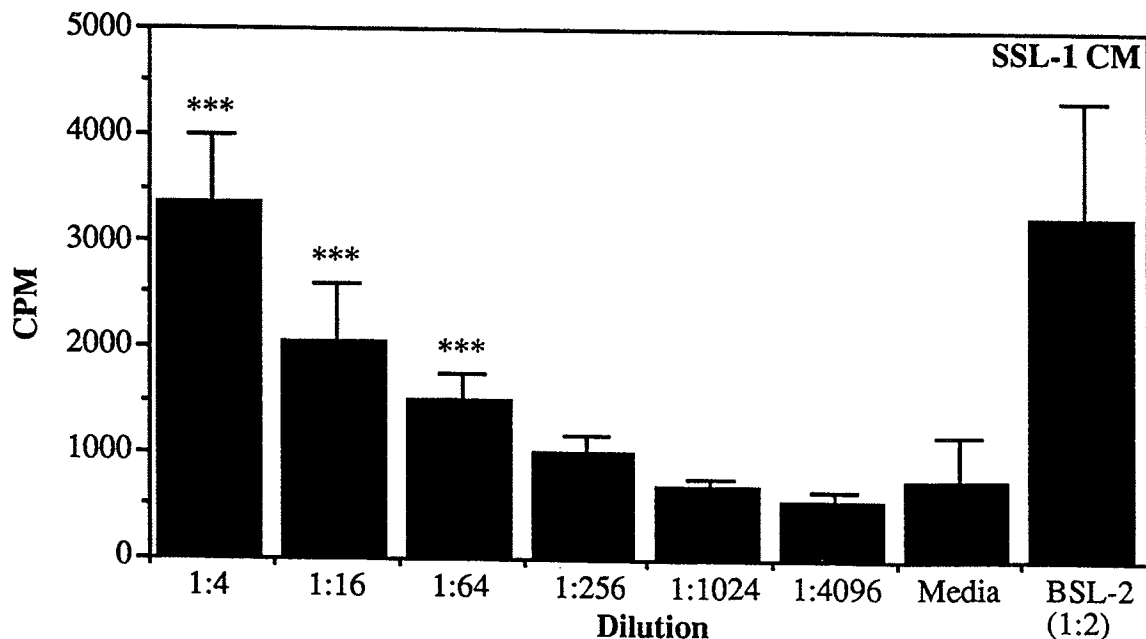


Figure 2. Proliferation of E15 bursal cells cultured with serial dilutions of SSL-1, media (negative control) or with BSL-2 CM (positive control). Values represent the mean  $\pm$  SD of three experiments performed in triplicate.

b) Are the activities present in SSL-1 known or novel?

While the properties in SSL-1 CM resembled those of cMGF, a chicken cytokine which stimulates the differentiation of myeloid precursors (Leutz *et al.*, 1984), it was important to determine whether SSL-1 produced message for cMGF. Employing RT-PCR and northern blot analysis, it was demonstrated that SSL-1 did not produce mRNA for this cytokine (data not shown). SSL-1 CM was also assayed for the presence of IL-1 and interferon. CM from adult spleen cells stimulated with ConA for 48 h all contained IL-2

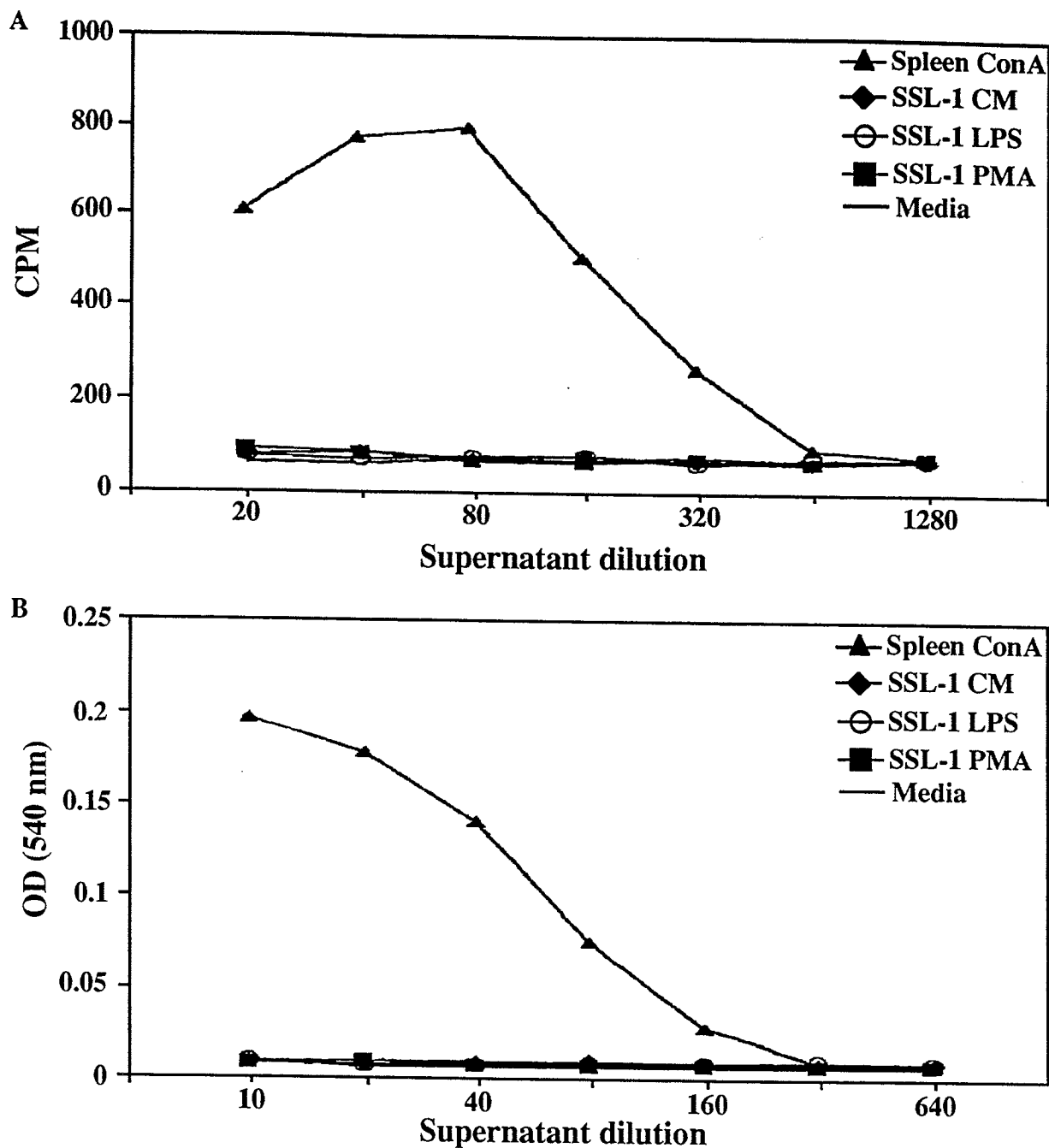


Figure 3. Quantitation of (a) IL-2, (b) interferon  $\alpha/\beta/\gamma$  production in conditioned media derived from unstimulated (SSL-1 CM) and cells stimulated with LPS (SSL-1 LPS) or PMA (SSL-1 PMA). Serial two-fold dilutions and control supernatants were prepared in duplicate for each assay. The proliferative response of IL-2 dependant blasts was used to detect the presence/absence of IL-2. Protection of chicken embryonic fibroblasts from virus mediated cytolysis was used as an indicator for interferons  $\alpha/\beta/\gamma$  activity.

and interferon activities, which exhibited dose-response kinetics. CM from unstimulated and stimulated SSL-1 cells and from normal spleen cells cultured in media for 24 h did not secrete detectable amounts of IL-2 or interferon (Figure 3). These results suggest that the haemopoietic growth factor activity produced by SSL-1 cells may be due to a novel cytokine.

c) Responsiveness of human bone marrow stimulated with SSL-1 CM

Considering SSL-1 CM was previously shown to stimulate the proliferation and differentiation of chicken haemopoietic precursors in semi-solid cultures an examination was made as to whether activities present in SSL-1 CM could induce the proliferation and differentiation of human haemopoietic cells. As shown in Table 1, in three independent experiments, SSL-1 CM demonstrated in a dose responsive manner an ability to induce colony formation of human precursor cells, with granulocyte-macrophage colonies predominating in these cultures. As controls, cells were either stimulated with the mammalian cytokines, GM-CSF, G-CSF and SCF, a combination of growth factors known to induce maximal colony numbers of granulocytes (G), macrophages (M) and granulocytes/macrophages (GM), no cytokines (saline control) or ten-fold concentrated media. In one experiment SSL-1 CM induced higher colony numbers than in cultures stimulated with the mammalian cytokines. However, this result was felt to be anomalous and not representative of the effects of the mammalian cytokines since in two other

Table 1. Colony formation of human bone marrow stimulated by individual and combinations of mammalian and avian cytokines.

Cytokine	Mean GM-CFC's/2x10 <sup>4</sup> BM cells		
	Exp 1	Exp 2	Exp 3
SSL-1 CM (x10 concentrated)	77.5	ND	ND
SSL-1 CM (x10 concentrated) (1:5 dilution)	81	ND	ND
SSL-1 CM (Neat)	ND	18.5	119
SSL-1 CM (1:5)	ND	20	79
SSL-1 CM (1:10)	ND	14.5	ND
SSL-1 CM (1:25)	ND	ND	27
SSL-1 CM (1:125)	ND	ND	3
SSL-1 CM (1:625)	ND	ND	0
avian SCF	ND	ND	0
avian SCF+SSL-1 CM (1:5)	ND	ND	62
GM-CSF+G-CSF+SCF	59	63	249
SSL-1 CM (1:1)+GM-CSF+G-CSF+SCF	ND	37	276
SSL-1 CM (1:625)+GM-CSF+G-CSF+SCF	ND	ND	240
avian SCF+GM-CSF+G-CSF+SCF	ND	ND	232
Media control (x10 concentrated)	0	ND	ND
Saline control	0	2	0

Cultures contained 2x10<sup>4</sup> bone marrow cells plus 0.1mL saline or 100ng/mL SCF, 100ng/mLGM-CSF, 500U/mL G-CSF, concentrated or diluted SSL-1 CM, or combinations of these agents as indicated. Colonies were counted and typed after 14 days incubation. Data is the mean of two replicate cultures. (ND- Not determined).

experiments cultures containing mammalian cytokines all induced significantly higher colony numbers than cultures stimulated with SSL-1. The reasons for this effect may have been due to partial degradation of the mammalian cytokines which may have led to the observed lack of colony formation. In contrast to the *in vitro* colony forming potential of SSL-1 CM, avian SCF was unable to induce the *in vitro* colony formation from human bone marrow nor did it appear to synergise with SSL-1 CM. Neither SSL-1 CM nor avian SCF, synergised with human GM-CSF, G-CSF and SCF to augment the total numbers of colonies appearing in culture. It was observed in all three experiments that the cellular content of colonies in cultures stimulated with mammalian cytokines were considerably greater than that of SSL-1 stimulated cultures, an observation which suggests that precursor cells weren't stimulated optimally in cultures containing SSL-1 CM (data not shown). Considerable variation in the number of colonies was observed between experiments. However, this may have been due to contaminating red blood cells present in the bone marrow sample which may in effect dilute out the total number of colony forming precursors added to the culture dish. Although colony formation should not ensue without the presence of a colony stimulating factor (Metcalf, 1984) in one experiment two colonies spontaneously appeared in the absence of a colony stimulating factor, a phenomenon attributed to the spontaneous proliferation of haemopoietic precursors (Metcalf, 1984).

d) Responsiveness of Ba/F3 cells electroporated with growth factor receptors and FDC-P1 cells to SSL-1 CM stimulation

In microwell cultures the response pattern of electroporated Ba/F3 cells (a murine

Table 2. Summary of experiments describing the responsiveness to SSL-1 CM stimulation of Ba/F3 cells expressing the receptors for G-CSF, IL-6, TPO and LIF.

Experiment 1				
Stimulus	Concentration in medium (ng/mL)			
	G-CSF	IL-6	TPO	LIF
SSL-1 (x10)	ND	ND	ND	2
Dexter media (x10)	ND	ND	ND	ND
Detection limit (pg/mL)	100	100	100	100

Experiment 2

Stimulus	Concentration in medium (ng/mL)			
	G-CSF	IL-6	FDC-P1 cells	LIF
SSL-1 (x10)	ND	ND	ND	12
SSL-1 (neat)	ND	ND	ND	0.4
Dexter media (x10)	ND	ND	ND	ND
Dexter media (neat)	ND	ND	ND	ND
Detection limit (pg/mL)	1000	100	100	50

SSL-1 CM was titrated out in saline containing 5% newborn calf serum and added in 5 $\mu$ L aliquots to 200 Ba/F3 cells expressing the receptors for G-CSF, IL-6, TPO or LIF. All tests were performed in duplicate in microwell cultures. Scoring of wells for proliferation was assessed 48 h post stimulation. (ND- Not detected).

pro-B cell line) with the high affinity murine receptors for granulocyte colony stimulating factor (CSF), interleukin 6 (IL-6), thrombopoietin (TPO) and leukemia inhibitory factor (LIF) were established by the addition of concentrated (x10) or non-concentrated SSL-1 CM. As controls, ten-fold concentrated or non-concentrated modified Dexter media were also assayed. As summarised in Table 2, Ba/F3 cells electroporated with the receptors for G-SCF. The IL-6 and TPO did not proliferate in response to SSL-1 CM. In contrast, in two independent experiments, Ba/F3 cells electroporated with the high affinity receptor for murine LIF proliferated in response to SSL-1 CM. In Experiment 1, using ten-fold concentrated SSL-1 CM, the concentration of LIF estimated was 2ng/mL while in Experiment 2, 12ng/mL was recorded and 0.4 ng/mL for the non-concentrated sample. These latter two values are in fair agreement considering slight errors which are inevitable in serial dilution assays. No LIF-like activity was detected in the concentrated and unconcentrated modified Dexter media controls. SSL-1 CM was also assayed on the murine myeloid factor dependant cell line FDC-P1, which requires GM-CSF or IL-3 for maintenance. As shown in Table 2, no activities corresponding to GM-CSF or IL-3 were detected in SSL-1 CM.

#### IV. CONCLUSIONS

A cell line derived from E16 splenic stroma was characterised and identified as secreting potentially a number of cytokines effecting myeloid and B cell development. Molecular analysis of RNA transcripts for cMGF demonstrated the absence of message for message in SSL-1 cells. Activities corresponding to IL-2 or interferon  $\alpha$ ,  $\beta$  or  $\gamma$  were also absent. Although generated from an avian cell source, SSL-1 CM was serendipidously found to cross react with human haemopoietic cells inducing their differentiation along the macrophage and granulocytic lineages. Moreover, SSL-1 CM induced the proliferation the murine Ba/F3 cell line transfected with the LIF receptor. This result suggests the presence of a LIF-like molecule in SSL-1 CM. The cloning and functional production of this molecule may potentially lead to strategies for the development of gene targeting technology in birds.

#### ACKNOWLEDGEMENTS

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THE PROTECTIVE 87 KILODALTON OUTER MEMBRANE ANTIGEN, Oma87, OF  
*Pasteurella multocida*

C.G. RUFFOLO and B. ADLER

Summary

The bacterial pathogen *Pasteurella multocida* causes fowl cholera in all avian species. Fowl cholera is a contagious disease that results in significant economic losses to the poultry industry world wide. In the development of vaccines against fowl cholera researchers have concentrated on the cross-protective antigens of *P. multocida*. Membrane proteins of *P. multocida* are able to elicit protective immunity but few membrane proteins have been identified.

An 87 kDa outer membrane antigen, Oma87, of *P. multocida* has been identified (Ruffolo and Adler, 1996). The *oma87* gene encoding the Oma87 protein was cloned and sequenced and found to share significant similarity with the gene encoding the D15 protective surface antigen of *Haemophilus influenzae*. Oma87 was localised to the outer membrane of the cell and Proteinase K treatment of whole cells indicated that the protein was surface exposed. Oma87 was found to be present in all 16 Heddleston serotypes of *P. multocida*.

Native and recombinant Oma87 proteins were strongly immunostained by convalescent chicken antiserum, indicating that the protein is expressed *in vivo*. Antiserum raised against Oma87 protected mice against homologous, lethal *P. multocida* challenge. These results suggest that Oma87 is a protective outer membrane antigen of *P. multocida*. Further assessment of the protective capacity of Oma87 will involve DNA vaccination studies.

I. INTRODUCTION

The animal pathogen *Pasteurella multocida* is the causative agent of a wide range of diseases including fowl cholera of all avian species, atrophic rhinitis of swine and haemorrhagic septicaemia of cattle and buffaloes. Fowl cholera is a highly contagious disease which results in significant losses to the poultry industry (Hird *et al.*, 1991). Current control strategies for this disease include the use of killed whole cell vaccines, which afford homologous protection, and attenuated live vaccines such as the CU and M9 strains. Although cross-protective immunity is elicited by the empirically derived, attenuated live vaccines, reversion to virulence occurs resulting in outbreaks of fowl cholera in vaccinated flocks (Hofacre, 1986). These attenuated live vaccines are not currently used in Australia.

Only a small number of *Pasteurella* antigens important for pathogenesis or immunity in fowl cholera have been identified. While the importance of capsules and lipopolysaccharide (LPS) remains controversial, antigens associated with the outer membrane (OM) fractions have been shown to be immunogenic. The OM fractions of *in vitro*-grown strains confer only homologous protection whereas fractions from strains grown *in vivo* can stimulate cross-protective immunity, indicating that *in vivo* expressed OM antigens are important in cross-protective immunity (Heddleston and Rebers, 1972; Rimler, 1994; Rimler and Rhoades, 1989). The major components of the OM are the OM

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proteins (OMPs) and LPS. The OMPs of *P. multocida* have been implicated as the major antigens involved in inducing a cross-protective response. In contrast LPS appears to have a minor role in inducing immunity, since immunisation with LPS alone results in partial protection (Ramdani and Adler, 1991).

To date few OMPs of *P. multocida* have been characterised. A 37 kDa OMP is considered to be the major *P. multocida* porin (Chevalier *et al.*, 1993; Lugtenberg *et al.*, 1986). A 50 kDa OMP was reported to have anti-phagocytic activity (Truscott and Hirsh, 1988) and a monoclonal antibody against a 37.5 kDa OMP has been demonstrated to be protective in animal models (Lu *et al.*, 1991). OM antigens expressed under iron limiting conditions have been identified and appear to play a role in immunity against pasteurellosis (Choi-Kim *et al.*, 1991; Ruffolo *et al.*, Unpublished data). Kasten *et al.* (1995) have identified a 16 kDa OMP which is the homologue of the *Haemophilus influenzae* P6 protective OMP. On this basis it was predicted that the *P. multocida* 16 kDa OMP was immunogenic.

Recently, a novel approach to vaccine development has involved using DNA as an immunogen. Researchers have observed that plasmid DNA containing a gene(s) that encodes for an antigen is able to elicit an immune response (Tang *et al.*, 1992). DNA vaccination has been extensively studied using influenza viral antigens and immune responses have been elicited in chickens and mice (Fynan *et al.*, 1993; Ulmer *et al.*, 1993). Immunisation with DNA appears to induce both cell mediated and humoral immune responses. This method of immunisation also has advantages over the conventional protein-based vaccines in terms of vaccine preparation, safety and cost effectiveness (Hassett and Whitton, 1996). To date, DNA from viruses and parasites have induced an immune response but the efficiency of bacterial DNA vaccines has not yet been fully determined (Barry *et al.*, 1995; Lowrie *et al.*, 1994).

In this report the identification and characterisation of an 87 kDa OM antigen, Oma87, of *P. multocida* and the gene encoding this protein, *oma87*, are discussed. Furthermore, evidence is presented that Oma87 may be a target for protective immunity and consideration is given to the *oma87* gene as being a candidate for DNA vaccination studies.

## II. CHARACTERISATION OF THE *oma87* GENE

A plasmid based genomic DNA library of *P. multocida* PBA100 (serogroup A: seotype 1) was constructed in *Escherichia coli* and screened with protective antiserum against OM fractions of *P. multocida*. The recombinant clone PBA1014 expressed an 87 kDa protein that was strongly immunostained by the protective antiserum, while further subcloning resulted in the clone pPBA1137 which contained the gene encoding the 87 kDa protein. The protein was localised to the outer membrane of *P. multocida* (see below) and thus designated Oma87, the 87 kDa outer membrane antigen of *P. multocida* (Figure 1); the gene was designated *oma87*. The nucleotide sequence and the deduced amino acid sequence of *oma87* revealed an open reading frame comprising 2,372 nucleotides which encoded a protein of 789 amino acids (data not shown).

## III. CHARACTERISATION OF THE Oma87 PROTEIN

The deduced amino acid sequence of Oma87 revealed a number of features which are common to OMPs of other bacteria. In order for OMPs to be correctly positioned in the OM of the bacterial cell, a signal peptide is required to both direct the protein towards

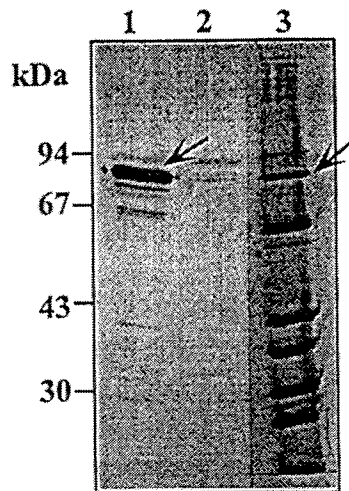


Figure 1. Immunological analysis of whole cell lysates immunostained with protective antiserum against OM fractions of *P. multocida*. Lane 1; *E. coli* PBA1137, recombinant clone expressing Oma87. Lane 2; *E. coli* control. Lane 3; *P. multocida* PBA100. The arrows indicate Oma87. The positions of standard molecular mass markers (kDa) are shown on the left.

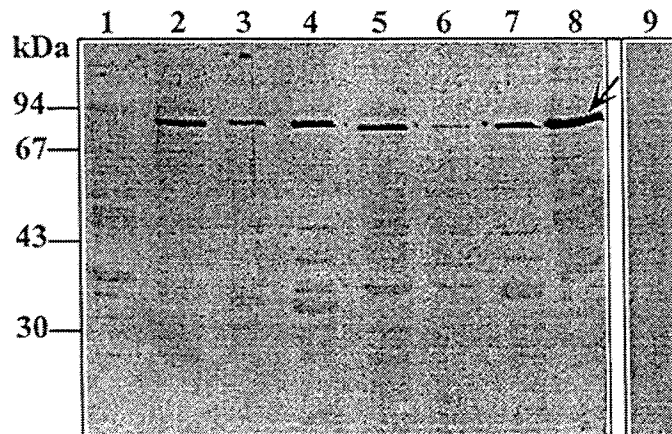


Figure 2. Immunological analysis of whole cell lysates and membrane fractions immunostained with antibodies eluted from recombinant Oma87 previously immunostained with protective antiserum against OM fractions of *P. multocida*. Lane 1; *E. coli* control WC. Lane 2; *P. multocida* PBA100 WC. Lane 3; PBA100 IM. Lane 4; PBA100 OM. Lane 5; *P. multocida* PBA101 WC. Lane 6; PBA101 IM. Lane 7; PBA101 OM. Lane 8; PBA1137, recombinant clone expressing Oma87 WC. Lane 9; PBA100 CF.

WC: whole cell lysates. IM: inner membrane fraction. OM: outer membrane fraction. CF: cytoplasmic fraction. The arrow indicates Oma87. The positions of standard molecular mass markers (kDa) are shown on the left.

the membranes and also to translocate it across the inner cell membranes towards the OM (Tomassen, 1988). Oma87 has a predicted signal peptide of 18 amino acids at the N-terminus of the protein (M1 to A18). Once an OMP is translocated across the inner membrane the signal peptide is cleaved allowing the mature protein to become properly folded in the OM. The Oma87 mature protein thus has a deduced molecular mass of 85,553 Da, which is in close agreement with the molecular mass of 87 kDa estimated by SDS-polyacrylamide gel electrophoresis. The C-terminus of OMPs also have conserved features. The last ten amino acid residues are usually hydrophobic and are thought to be important for the correct incorporation and stability of the protein into the OM (Struyve *et al.*, 1991). The last ten amino acids of Oma87 are hydrophobic consistent with a role in anchoring Oma87 in the OM.

Immunological analysis of cell fractions containing either native or recombinant Oma87 localised the protein predominantly to the OM of the cell (Figure 2). Furthermore treatment of *P. multocida* whole cells with Proteinase K abolished the reactivity of Oma87 with specific antiserum. Thus, the amino acid composition of Oma87 and the immunological analysis both indicated that Oma87 is a surface exposed OM antigen and is, therefore, a potential target for protective immunity.

In addition, immunological analysis of the 16 Heddleston *P. multocida* serotypes revealed that all serotypes expressed an 87 kDa protein, assumed to be Oma87.

Research into effective vaccines against fowl cholera has focused on identifying the cross-protective antigens of *P. multocida*. Reports have shown that antigens expressed *in vivo*, in particular membrane associated proteins, can elicit cross-protective immunity (Rimler, 1994; Rimler and Rhoades, 1989). Hence, it is likely that the cross-protective antigens of *P. multocida* are expressed *in vivo*. In order to determine if Oma87 was expressed *in vivo*, immunological studies using convalescent chicken antiserum were performed. The antiserum was able to recognise both native and recombinant Oma87 proteins (data not shown), thus indicating that the protein is expressed *in vivo* during infection and is also recognised by the chicken immune system.

#### IV. PROTECTIVE CAPACITY OF Oma87

To date, database searches have revealed that Oma87 shares extensive similarity to the D15 protective surface antigen of *H. influenzae* (Figure 3) (Flack *et al.*, 1995; Fleischmann *et al.*, 1995). Thomas *et al.* (1990) showed that affinity purified anti-D15 antibodies protected infant rats against *H. influenzae* challenge. Considering that Oma87 appears to be expressed *in vivo* and that D15 was found to be protective, an investigation into the protective immune capacity of Oma87 was carried out. Antiserum was raised against recombinant Oma87 in rabbits and was used to passively immunise mice, which were protected against a lethal challenge dose of *P. multocida*. However, mice were not protected against higher challenge doses or against a heterologous serotype (Table 1). These preliminary results indicate that Oma87 is potentially a protective antigen but immunisation studies in chickens using purified Oma87 are required. However, to obviate the need for protein purification it is proposed to use the *oma87* gene in appropriate DNA expression vectors to vaccinate chickens. The aim is to inject chickens intramuscularly with plasmid DNA containing the *oma87* gene. It has been demonstrated that foreign gene expression occurs in muscle cells which, in turn, induces an immune response (see Hassett and Whitton, 1996, for review). Provided that protection against fowl cholera is induced using this strategy DNA vaccination has two main advantages

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1
Oma87 MKKLLIASLLFG-STTAFAPFVVKDIRVDGVQAGTEGSLATLPVVRVQQRATDNDIANV 60
D15 *****T**V*****A*****GDL*QQIR*S***A**V***Y**I

61
Oma87 VRKLF↗LSGQYDDVKASREGNTLVVTVMPKPVISNVVIVGNKSIPDEAIKQNL↘DANGFKV↘G 120
D15 **S**Y**RE*****HH**DV***S*VA*SI**D*K*K*SI**T**L*****

121
Oma87 DVLNRAKLEEF↗RKGIVEHYNSVGRYNAKVDAIVNTLPNNSAEIKIQINEDDVALFKEITF 180
D15 ***I**E**N**A*SVK***A*****T*EP*****R***L*****K*KLASL**

181
Oma87 EGNEAFSSGKLADQ↗MELQTD↘SWWKLFGNKF↘DQ↘TQFNK↘DLET↘LR↘SY↘YLD↘RGYA↘QF↘QL↘LD↘TD 240
D15 K**S↘V**ST*QE*****P*****W***EGA**E***QSI**D***NN**KA**TK**

241
Oma87 VKLSDDKKEP--CLISEEGDLYTVKTRVSGGMWGGMSAELAPILETIQNLGFRRTSVLE 300
D15 *Q*N*E*TKVNV↗TIDV↘N**LQ*DLRSARII*NL*****E*L*SALH**DT**SDIAD

301
Oma87 VEQRNKS↗KLGERGYATAQVNVHPTFDEQDKTISLDFIVEAGKSYTVRQIRFEGNTSSADS 360
D15 **NAI*A*****GS*T**SV*D**DAN**L↗AITLY*↘D**RRL***L*****V***

361
Oma87 TLRQEMRQ↗QEGAWLSSELVELGKLR↘LDRTGYFESVETKTEAIPGS-DQVDVIYKVKERNT 420
D15 *****T*YN*Q*****I*****E**T**NRIDP*N**N*E***V*****

421
Oma87 G↗SINFGIGYGTESGLSYQASIKQDNFLGMSSISLGGTRNDYGT↘TVNLGYNEPYFTKDG↘V 480
D15 *****I*****V*****T*AAV*IA**K*****S*****T*****

481
Oma87 SLGGNVSFEEYDSSKSN↗TSAGYGR↘TSYGGNLT↘LGFPV↘NENNSY↘YLG↘VGY↘TYN↘KL↘KNIA↘PE 540
D15 *****F**N**N**D**SN*K**T**S*V*****V*L*H***IS*P*L*

541
Oma87 YNRDLYRQSMKY↗ND↘SWTFKSHDF↘LSFGW↘YN↘SLN↘RGY↘FPTK↘GV↘RANIG↘GRVTI↘PGSD↘NK 600
D15 ***N**I***EK↗GNG-I*↘TN**F*****K*SL*****

601
Oma87 YYKLN↗AEAQGFYPLDREHGWLSSRISAS↘FADG↘FGKRL↘PFY↘QYS↘AGGIG↘SLR↘GFAY↘GA 660
D15 ****S*DV*****D*L**V*AKA**GY*N**GN*****T*↗T*****S

661
Oma87 IGPNAIYRTRQCPD-SYCLVSSDVIGGNAMVTASTE↗LIVPTPFVADKNQNSV↘RTSLFV↘DA 720
D15 *****AEHGNGNGTEK↘KV*****IT**A*****S**S**T*****

721
Oma87 ASVWNTRWKAEDK--AKFAKLNP↗DYSD↘PSRV↘RSAG↘VALQ↘WQSP↘IG↘PLV↘FSYAK↘PLK↘KY 780
D15 *****H**SDK↗SGLD↘NNV↘LK↘SL**GKS**I**T**GF*****I***

781 796
Oma87 QGDEIEQ↗FQFSIG↘GT↘F 796
D15 EN*DV*****S*

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Figure 3. Alignment of the deduced amino acid sequences of *P. multocida* Oma87 and *H. influenzae* D15. Asterisks indicate identical amino acid residues while underlined residues indicate conserved amino acid changes. Amino acids 1 to 18 comprise the putative signal peptide. The arrow indicates the first amino acid residue of the mature protein. The ten last amino acids at the C-terminus, shown in bold, comprise a sequence which is thought important for correct assembly of the protein into the OM.

compared to conventional protein-based vaccines. Firstly, DNA cannot replicate and is, thus, safer than live vaccines which have a risk of causing disease. In addition, DNA is inexpensive to produce and is stable in a wide range of temperatures.

Table 1. Passive immunisation study using either anti-Oma87 antiserum (CR3) or control antiserum (CR6). Mice were challenged with lethal *P. multocida*, either PBA100, serotype 1 (ST1) or PBA101, serotype 3 (ST3).

Mice immunised with:	Challenge strain <sup>1</sup> and dose (x ID <sub>50</sub> )	Percentage killed <sup>2</sup>
CR3	ST1, 10 <sup>2</sup>	0
CR3	ST3, 10 <sup>2</sup>	100
CR6	ST1, 10 <sup>2</sup>	100
CR6	ST3, 10 <sup>2</sup>	100
CR3	ST1, 10 <sup>4</sup>	80
CR3	ST3, 10 <sup>4</sup>	100
CR6	ST1, 10 <sup>4</sup>	100
CR6	ST3, 10 <sup>4</sup>	100
Non-immunized control mice	ST1, 10 <sup>2</sup>	100
	ST3, 10 <sup>2</sup>	100

<sup>1</sup> The infectious doses 50 (ID<sub>50</sub>) of ST1 and ST3 were 10<sup>2</sup> and 10 colony forming units respectively.

<sup>2</sup> Groups of five mice were used for both the immunisation and control experiments.

## V. CONCLUSIONS

Our studies have been focused on identifying the individual *P. multocida* OM antigens involved in eliciting an immune response. This paper reports the identification of an 87 kDa OM antigen, Oma87, which has surface exposed regions. Oma87 shares considerable similarity to the D15 protective antigen of *H. Influenzae*. Anti-recombinant Oma87 antiserum was able to passively protect mice against a lethal homologous *P. multocida* challenge. Further studies to elucidate the protective capacity of Oma87 and its use as a vaccine are currently under way.

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## EFFECT OF BETAINE ON INVASION AND DEVELOPMENT OF THE AVIAN COCCIDIA AND GROWTH PERFORMANCE IN COCCIDIA-INFECTED CHICKENS

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### Summary

Growth performance in broiler chickens moderately infected with a mixture of avian coccidia was significantly improved by the addition of betaine to diets that contained salinomycin. Betaine was considered not to be overtly toxic to the parasite because cell culture experiments showed that levels 2- to 8-fold higher than levels in the feed had little effect on coccidial invasion or development. However, in chickens given feed containing 0.75 or 1.5 g/kg of betaine, invasion by the coccidia was significantly reduced when compared with that in control birds. Addition of 66 mg/kg of salinomycin did not further reduce invasion. Except for a moderate decrease in development by *Eimeria acervulina*, neither betaine alone nor betaine plus salinomycin markedly affected coccidial development. Electron microscopy revealed ultrastructural changes in intestinal cells of chickens fed betaine. Collectively, the data suggest that inhibition of invasion plus other physiologic properties of betaine contributed to the improvement in growth performance of the chickens.

### I. INTRODUCTION

It has been estimated that coccidiosis costs the poultry industry worldwide from 900 million to one billion dollars annually and is associated with approximately 6 to 10% of the total mortality that occurs among poultry flocks. The disease is typically linked to the intensive production practices that are used commercially today. When large numbers of young susceptible birds are placed in an environment that favors the multiplication of the coccidia, the disease rate intensifies.

Anticoccidial drugs fed from day of hatch remain the primary weapon against coccidiosis, and have provided the basis for the rapid growth of the poultry industry. However, the continuing development of drug resistance, coupled with the increasing costs of clearing new drugs, has substantially reduced the number of effective anticoccidials available today. To decrease the potential for greater losses due to coccidiosis, new control strategies that can be used as adjuncts to drug therapy must be developed.

Supplementation of basal chick diets with natural products that reduce the impact of coccidiosis on the growth performance of chickens is a promising approach. Betaine, a natural product that is present in all living organisms, has osmoprotective properties (Petronini *et al.*, 1992) that make it an attractive candidate for use against coccidiosis, an enteric disease that is associated with osmotic disorders (Crompton, 1976). Therefore, betaine was examined for its ability to enhance growth performance in coccidia-infected broiler chickens and for its effect on the parasite, *per se*.

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## II. METHODS

### (a) Growth Performance

Two replicate studies were conducted in battery cages (120 chickens per diet group per study) and one study in a floor pen facility (704 chickens per diet group). In each study, male Peterson X Arbor Acres chickens were obtained at 1 d of age and a representative group was weighed. Chickens within 5 g of the mean body weight (BW) were distributed into diet groups. The diet groups were a basal diet that met or exceeded nutritional recommendations (NRC, 1994) supplemented with 1) nothing (control), 2) 1.5 g/kg of purified betaine (1.5 bet), 3) 66 mg/kg of salinomycin (66 sal), and 4) 1.5 g/kg of betaine and 66 mg/kg of salinomycin (1.5 bet+66 sal). In the floor pen study, 2 additional supplements were fed: 44 mg/kg of salinomycin (44 sal) and 1.5 g/kg of betaine plus 44 mg/kg of salinomycin (1.5 bet+44 sal). All diets were fed *ad libitum* in crumble form from 1 to 21 d and in pellet form from 22 to 45 d of age.

At 14 d of age, the chickens in the batteries and floor pens were inoculated, via the drinking water, with a mixed culture of *Eimeria* species adjusted to deliver approximately  $1 \times 10^5$  oocysts of *E. acervulina*,  $5 \times 10^4$  oocysts of *E. maxima*, and  $1 \times 10^4$  oocysts of *E. tenella* per chicken. At 21 d of age (7 d postinoculation; DPI) in the battery and floor pen studies and 45 d of age (31 DPI) in the floor pen study, the chickens were weighed individually. Total feed consumption per cage was measured, and feed conversion ratios (FCR; feed/gain) were calculated after correction for mortality. Total mortality and day of death were recorded for each treatment group. At 21 DPI, all chickens in the battery studies and representative groups in the floor pen study were scored for intestinal lesions.

### (b) Coccidial Invasion and Development in Cell Culture

Sporozoites of *E. tenella* and *E. acervulina* were suspended in concentrations of betaine from 0 to 4.3 M, incubated for 45 min, washed twice, and resuspended in Medium 199 (M199). Aliquots of the sporozoite suspension were examined for changes in gross morphology or were inoculated onto cell cultures to evaluate their ability to invade. In addition, sporozoites of each species were suspended in M199 containing 0 to 107 mM betaine and inoculated immediately at  $3 \times 10^5$  sporozoites per culture. Cultures inoculated with *E. tenella* were fixed at 2.5, 48, and 72 h postinoculation (HPI); cultures inoculated with *E. acervulina* were fixed only at 2.5 HPI because this species develops poorly, if at all, in cell culture. All cultures were stained with hematoxylin and eosin and invasion and development were examined (Augustine, 1980).

### (c) Coccidial Invasion and Development in Chickens

Two-wk-old male Peterson X Arbor Acres chickens were fed the same 4 diets used in the battery and floor pen trials (control, 1.5 bet, 66 sal, 1.5 bet+66 sal, 0.75 bet, and 0.75 bet+66 sal). Twenty-four hours after the diets were initiated, chickens were inoculated with  $1.5 \times 10^7$  oocysts (to examine invasion) or  $3 \times 10^5$  oocysts (to examine development). The isolates of *E. tenella* or *E. acervulina* were the same as those used in the battery and floor pen studies. Tissues were taken from the middle of the caecal pouch (*E. tenella*) or the duodenal loop (*E. acervulina*) and 1) embedded in paraffin, sectioned, and labeled with parasite-specific monoclonal antibodies (Augustine and Danforth, 1984) or 2) embedded in Epon and examined by electron microscopy (Augustine *et al.*, 1992). Invasion was



quantified by counting the number of intracellular sporozoites per cross-section of intestine at 6 HPI. Development was based on the ratio of developmental stages to total parasites and on the maturity of the stages. Counts from 3 cross-sections of intestine from each bird were averaged for each data point.

#### (d) Statistical Analyses

Data from growth performance (battery and floor pen studies) and invasion in chickens were subjected to ANOVA, using the GLM procedure of SAS, in a factorial arrangement of treatments with betaine and salinomycin as the main effects. Differences at or less than  $P=0.05$  were considered to be significant.

### III. RESULTS AND DISCUSSION

#### (a) Growth Performance

Betaine in combination with salinomycin conferred protection against a moderate, mixed infection of *E. acervulina*, *E. tenella*, and *E. maxima* that was significantly greater than that afforded by betaine or salinomycin alone (Tables 1 and 2). At 7 DPI in both battery and floor pen trials, the BW of chickens fed diets supplemented with 1.5 bet plus either 44 sal or 66 sal were significantly higher than BW of chickens fed either betaine or salinomycin alone. The FCR were lower in chickens fed betaine plus salinomycin than in chickens fed betaine or salinomycin alone. Mortality was significantly decreased in chickens fed betaine plus salinomycin over that in inoculated control chickens, but not in chickens fed betaine alone. In the battery studies intestinal lesion scores were decreased significantly in chickens fed betaine plus salinomycin over those in the other diet groups. In the floor pen studies at 7 DPI lesion scores correlated well with feed conversion, being highest where feed conversion was highest (controls and chickens fed betaine alone) and lower in the other groups of birds (lesion scores not shown). The differences in lesion scores among the diet groups in the floor pen study were not always significant. At 31 DPI, floor pen-raised chickens fed diets containing betaine plus salinomycin were heavier and had lower FCR and mortality than chickens fed salinomycin alone, although the differences were not significant. There were no betaine x salinomycin interactions in either the battery or floor pen trials; therefore conclusions could not be drawn that there would be a betaine response with salinomycin alone or an additive effect of betaine and salinomycin. However, it can be concluded that, even when coccidiosis was reasonably well controlled by salinomycin, addition of betaine to the feed increased the performance of the chickens. Some compounds, such as tiamulin, have been shown to increase the efficacy of ionophores possibly by reducing the metabolic degradation of the drugs (Meingassner *et al.*, 1979). Whether betaine acts in a similar manner remains to be determined.

#### (b) Coccidial Invasion and Development

Coccidial invasion in the presence of betaine differed somewhat *in vitro* and *in vivo*. In cell culture, betaine, alone, did not alter the gross morphology of sporozoites of *E. tenella* or *E. acervulina* except at very high concentrations ( $> 1.7$  M). Moreover, betaine, alone, at levels that were roughly 2- to 8-fold higher ( $< 107$  mM) than the levels used in the battery and floor pen trials, did not adversely effect the ability to invade by either *E. tenella* or *E. acervulina* sporozoites or develop by *E. tenella* sporozoites (data not shown).

Table 1. Effect of betaine and salinomycin on growth parameters of coccidia-infected chickens (Battery study).

Betaine : salinomycin (g/kg) : (mg/kg)		Bodyweight (kg)	FCR (g:g)	Mortality (%)
0	0	0.593 <sup>cl</sup>	1.456 <sup>a</sup>	34.2 <sup>a</sup>
1.5	0	0.591 <sup>c</sup>	1.458 <sup>a</sup>	32.5 <sup>a</sup>
0	66	0.611 <sup>b</sup>	1.411 <sup>b</sup>	10.8 <sup>b</sup>
1.5	66	0.630 <sup>a</sup>	1.376 <sup>c</sup>	5.8 <sup>c</sup>
Betaine:salinomycin		F	0.690	0.596
Interaction		P	NS	NS

<sup>1</sup>Means with different superscripts differ significantly ( $P < 0.05$ ).

Table 2. Effect of betaine and salinomycin on growth parameters of coccidia-infected chickens (Floor pen study).

Betaine : salinomycin (g/kg) : (mg/kg)		Bodyweight (kg)	FCR (g:g)	Mortality (%)
<u>Day 1-21</u>				
0	0	0.580 <sup>e1</sup>	1.443 <sup>a</sup>	16.6 <sup>a</sup>
1.5	0	0.592 <sup>d</sup>	1.397 <sup>b</sup>	14.1 <sup>a</sup>
0	44	0.604 <sup>c</sup>	1.381 <sup>bc</sup>	8.4 <sup>b</sup>
1.5	44	0.617 <sup>b</sup>	1.346 <sup>cd</sup>	4.7 <sup>c</sup>
0	66	0.617 <sup>b</sup>	1.349 <sup>cd</sup>	4.7 <sup>c</sup>
1.5	66	0.640 <sup>a</sup>	1.311 <sup>d</sup>	2.7 <sup>c</sup>
Betaine x salinomycin		F	0.305	0.941
Interaction		P	NS	NS
<u>Day 1-45</u>				
0	0	1.948 <sup>c</sup>	1.977 <sup>a</sup>	19.0 <sup>a</sup>
1.5	0	1.953 <sup>bc</sup>	1.954 <sup>ab</sup>	15.9 <sup>a</sup>
0	44	1.968 <sup>bc</sup>	1.936 <sup>abc</sup>	10.6 <sup>b</sup>
1.5	44	1.982 <sup>ab</sup>	1.898 <sup>bc</sup>	7.1 <sup>bc</sup>
0	66	1.981 <sup>ab</sup>	1.920 <sup>abc</sup>	7.0 <sup>bc</sup>
1.5	66	2.004 <sup>a</sup>	1.877 <sup>c</sup>	4.3 <sup>c</sup>
Betaine x salinomycin		F	0.675	0.903
Interaction		P	NS	NS

<sup>1</sup>Means with different superscripts differ significantly ( $P < 0.05$ ).

By contrast, in chickens, betaine, alone, caused a significant reduction in invasion by both species of *Eimeria* when compared with that in control chickens (Table 3). Apparently, the characteristics of betaine that caused the inhibition of sporozoite invasion were functional in the intestinal environment but not in cell culture, suggesting that the

ingested betaine altered the intestinal epithelium or sporozoite in some way that decreases susceptibility to invasion.

Table 3. Effect of betaine and salinomycin on invasion by *Eimeria tenella* and *E. acervulina* sporozoites (mean+SEM) in chickens at 6 h postinoculation.

Betaine : salinomycin (g/kg) : (mg/kg)		n	Sporozoites/cross-section of intestine	
			<i>E. tenella</i>	<i>E. acervulina</i>
0	0	6	156 ± 43 <sup>a1</sup>	125 ± 10 <sup>a</sup>
0	66	6	6 ± 4 <sup>b</sup>	34 ± 4 <sup>c</sup>
0.75	0	6	40 ± 10 <sup>b</sup>	58 ± 4 <sup>b</sup>
0.75	66	6	68 ± 10 <sup>b</sup>	40 ± 3 <sup>c</sup>
1.5	0	6	47 ± 25 <sup>b</sup>	30 ± 3 <sup>c</sup>
1.5	66	6	53 ± 13 <sup>b</sup>	43 ± 4 <sup>c</sup>
Betaine x salinomycin		F	8.53	61.09
Interaction		P	.001	<.001

<sup>1</sup>Means with different superscripts differ significantly ( $P < 0.05$ ).

In chickens fed diets containing both betaine and salinomycin, there was a significant interaction between the supplements that impacted on invasion (Figure 1). In the absence of salinomycin, invasion by both *E. tenella* and *E. acervulina* decreased as the concentration of betaine rose from 0 to 1.5 g/kg. In the presence of salinomycin, invasion by *E. tenella* tended to increase as the concentration of betaine rose from 0 to 1.5 g/kg. Under the same conditions, invasion by *E. acervulina* increased in the presence of 0.75g/kg of betaine and then decreased slightly with 1.5 g/kg of betaine (but remained greater than invasion in the absence of salinomycin). Thus, improved performance in chickens fed both betaine and salinomycin was not caused by marked decreases in invasion as compared with invasion in chickens fed either of the supplements alone.

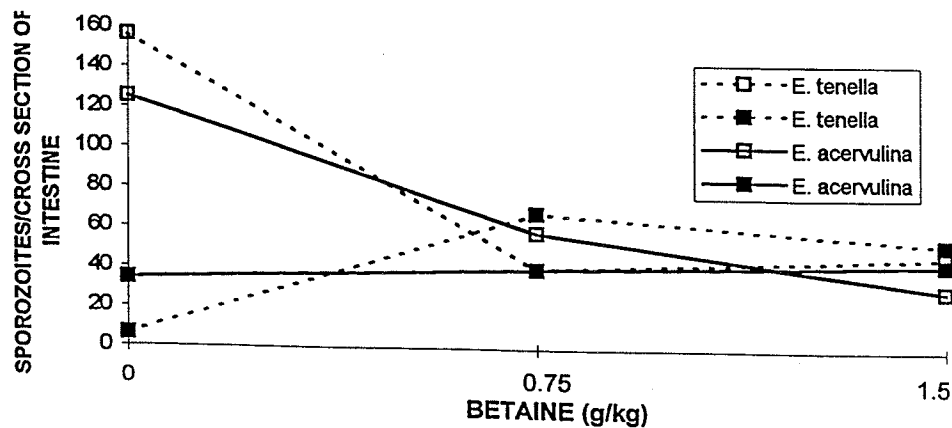


Figure 1. Effect of betaine on invasion of the intestinal epithelium by sporozoites of *Eimeria tenella* and *E. acervulina* in the presence (■) and absence (□) of salinomycin.

Betaine, alone or in the presence of salinomycin, had little effect on development by *E. tenella*. At 48 HPI, there were fewer developmental stages of *E. tenella* in chickens fed 0.75 bet+66 sal and 1.5 bet+66 sal than in chickens fed either level of betaine without salinomycin. By 96 h PI, development in chickens of *E. tenella* fed all 6 diets was similar (Table 4). Conversely, while development by *E. acervulina* in chickens fed betaine alone was similar to that in the controls at 48 HPI, by 96 HPI, development in chickens fed both betaine and salinomycin was markedly reduced over that in the other diet groups (Table 4).

Ultrastructurally, tissues from chickens fed diets containing betaine were less electron dense than tissues from chickens fed diets not containing betaine. The difference occurred in both coccidia-infected and uninfected chickens and may indicate increased retention of water in the intestinal cells. The increased capacity to retain water would be an added protection against the detrimental effects of dehydration in the infected chicken. Early developmental stages of *E. acervulina* in betaine-fed chickens were similar to those in control chickens. However, the later stages, particularly the gamonts, appeared to maintain their integrity to a greater extent in the betaine-fed chickens than in the controls. This may be in response to the observed differences in the host cells of the chickens in the 2 diet groups.

Table 4. Effect of dietary betaine and salinomycin on development of *Eimeria tenella* and *E. acervulina* in chickens at 96 h postinoculation.

<i>Eimeria</i> species	Betaine : salinomycin (g/kg) : (g/kg)	Development
<i>tenella</i>	All diet groups	Numerous stages in cross-sections. Primary stages: mature second generation schizonts and gamonts.
<i>acervulina</i>	0:0	Epithelium packed with stages. Primary stages: macrogamonts.
	0:66	A few villi heavily infected; majority lightly infected.
	0.75:0	Similar to 0/0.
	0.75:66	Development 50% of that in 0:0 and lighter than 0:66; most villi sparsely infected.
	1.5:0 1.5:66	Similar to 0:0. A few villi heavily infected and similar to 0:66; majority lightly infected or uninfected.

#### IV. CONCLUSIONS

The improvement in performance that occurred with the addition of betaine to diets containing salinomycin was not caused by overt toxicity toward the parasite or by an additive effect of betaine and salinomycin on invasion. The significant performance response in the group fed betaine alone (floor pen trial, d 1-21) could be explained by the inhibitory effect of betaine on invasion. The improved performance may have been due

also, at least in part, to a decrease in the development of *E. acervulina*. In addition, betaine also has diverse physiological properties that could potentially enhance the ability of the chickens to withstand coccidial infection. For example, betaine has been shown to stabilize cell membranes through interaction with membrane phospholipids during dehydration (Rudolph *et al.*, 1986), and to reduce faecal water content and increase the digestibility of several nutrients (Virtanen, 1995). These properties could have a direct impact on the intestinal membrane damage, dehydration, diarrhea, and maldigestion that are characteristic of coccidial infection (Crompton, 1976). Therefore, betaine may have contributed to the improved performance of coccidia-infected chickens directly, by partial inhibition of coccidial invasion and development, and indirectly, by support of intestinal structure and function in the presence of coccidial infection.

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## THE QUALITIES OF GRAIN LEGUMES FOR POULTRY

B. CARRÉ

### Summary

Five legume seeds are considered, namely peas, faba beans, white lupins, blue lupins and chick peas. The review is divided into four parts that concern proteins, metabolisable energy value, non-starch carbohydrates and other miscellaneous points. It appears from this review that many nutritional aspects of legume seeds are now well understood. However, the digestion of legume proteins has not been fully clarified and requires further studies.

### I. INTRODUCTION

The introduction of legume seeds in practical poultry diets probably began fifteen years ago and has increased regularly up to now. The nutritionists have to keep in mind that these new crops arose essentially because of the great efforts developed by plant breeders and agronomists for improving the level and regularity of yields. Plant breeders developed the nutritional quality of seeds, producing new cultivars such as alkaloid-free lupins, peas and faba beans without tannins, peas with low levels of trypsin inhibitors and faba beans without vicine and convicine. These efforts essentially concerned peas, faba beans, blue lupins and white lupins. At the same time a great deal of work was conducted in animal nutrition and biochemistry. Nowadays, much knowledge has accumulated which has enabled the introduction of these crops at up to 30 % in poultry diets (Lacassagne, 1988) provided that the qualities of these seeds have been properly estimated. The details of these qualities are reported below by the review of studies conducted on peas, faba beans, white lupins, blue lupins and chick peas. The latter seeds represent the major legume species used at present in poultry diets.

### II. PROTEINS

#### (a) Amino acids

Amino acid composition of legume proteins is an important topic to be considered because of several possible limitations. The mean compositions are presented in Table 1. These compositions vary according to protein contents. For instance, it has been clearly demonstrated that the sulphur amino acids and lysine contents of legume proteins decrease with an increase in nitrogen content of seeds (Mossé *et al.*, 1987 a, b; Baudet and Mossé, 1980).

Lysine is in excess in peas, faba beans and chick peas, but not in lupins (Table 1). Deficiency of sulphur amino acids exists for all legume proteins (Table 1). However, this is not a real problem, as a correction is easily done by addition of DL-methionine in diets. The main practical limitation perhaps concerns tryptophan (deficient for all legume proteins) if maize is used as the main cereal in diets, especially for white lupins (Table 1). Threonine deficiency is also a matter of consideration for most legume proteins, especially for white lupins (Table 1). Amino acid limitations for legume proteins may even be more

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important if dietary amino acid contents are formulated on the basis of ideal protein requirement (Table 1). Such formulations may be applied in the future for reducing nitrogen losses and pollution. With the latter system, all amino acids of legume proteins become limiting except lysine (excluding lupins) and arginine. In this condition, introduction of legume seeds will depend in part on the prices of pure amino acids, especially tryptophan.

Table 1. Essential amino-acid composition (% of crude protein) of legume seeds<sup>1</sup>.

	Requirement (starter period)		Legume seeds					
	Ideal protein <sup>2</sup>	Normal protein <sup>2</sup>	Peas	Faba beans	White lupins	Blue lupins	Chick peas	
							Kabuli	Desi
Lysine	6.6	5.2	7.5	6.6	4.8	4.8	6.8	6.7
Tryptophan	1.2	0.9	0.8	0.8	0.7	0.8	1.0	0.9
Sulphur amino acids	5.6	3.9	2.6	2.1	2.3	2.3	2.8	2.7
Arginine	7.3	5.8	9.2	9.0	11.1	10.5	9.7	9.4
Histidine	2.6	2.1	2.4	2.5	2.2	2.5	2.3	2.4
Isoleucine	4.8	3.8	4.5	4.5	4.6	4.3	4.3	4.3
Leucine	8.3	6.5	7.2	7.2	7.2	7.2	7.2	7.2
Phenylalanine + tyrosine	8.7	6.8	8.4	7.4	8.5	8.0	8.6	8.5
Threonine	4.6	3.8	3.9	3.6	3.7	3.4	3.7	3.7
Valine	5.2	4.8	5.0	4.9	4.3	4.3	4.3	4.4

1 After Mossé *et al.* (1987a,b), Baudet and Mossé (1980), Singh *et al.* (1991), P.E.A. Programme 1996, Yule and Mc Bride (1976), Hove (1974).

2 Ideal protein for low protein content (17%); Normal protein for high protein content (22 %). After Leclercq (1996) and Larbier and Leclercq (1992).

#### (b) Digestibility

Protein value depends also on digestibility. Apparent digestibilities of legume proteins often display normal values near to that of soyabean meal (80-85 %; Lacassagne *et al.*, 1988; Carré *et al.*, 1991; Brenes *et al.*, 1993). However, they can also be rather low, even with peas without tannins (Conan and Carré, 1989). There are several factors that can be responsible for low protein digestion in legumes. Condensed tannins that occur in the hulls of seeds from coloured flower cultivars of faba beans, peas and Desi chick peas have been demonstrated to decrease protein digestion (Marquardt *et al.* 1977; Lacassagne *et al.*, 1988, 1991; Singh and Jambunathan, 1981). In practice, tannins act specifically on protein digestion with no effect on starch digestion (Lacassagne *et al.*, 1991), which suggests that the effects of tannins result more from a dietary protein interaction, than from an enzyme interaction. Effects of tannins on starch digestion have only been observed with high levels of tannins (Longstaff and Mc Nab, 1991) that do not correspond to practical levels. Effects of tannins might be reduced by heating (Marquardt and Ward, 1979). However, the latter authors found that only half of the tannin effect was reduced by heating while Guillaume (1978) found no reduction of the tannin effect by heating. The most efficient ways to suppress the tannin effect are to use seeds selected for zero tannin, or to use dehulled seeds.

Table 2. Composition (%) of legume seeds and calculation of their potential metabolisable energy value. (Dry matter basis).

	Peas	Faba beans	White lupins	Blue lupins	Chick peas	
					Kabuli	Desi
Percent seed coat	10.0	13.5	20.0	24.8	7.0	16.0
Crude protein (Nx6.25)	23.7	30.3	40.0	33.0	24.3	24.9
Starch	48.2	41.8	< 1	< 1	43.6	36.3
Lipids	2.1	1.9	10.0	6.8	5.0	3.5
Sucrose	2.3	1.8	1.8	3.1	2.5	2.5
-galactosides	4.8	3.1	8.0	6.3	5.0	5.0
WICW <sup>1</sup>	14.9	18.5	34.0	42.0	13.5	22.0
NDF <sup>2</sup>	14.0	17.0	20.0	20.3	9.0	20.0
Crude fibre	7.2	9.0	12.5	15.0	4.0	11.0
Water-soluble NSP	0.7	0.9	2.0	3.0	1.2	
Ashes	3.7	4.0	4.0	3.2	3.0	3.0
Gross energy (kcal/kg)	4400	4500	4900	4750	4650	4600
Potential adult AME <sub>n</sub> value (kcal/kg)						
EC equation <sup>3</sup>	3190	3100	2600	2070	3280	2890
Theoretical CW equation <sup>4</sup>	3110	2950	2500	2140	3390	2970

1 Water insoluble cell-wall. 2 Neutral Detergent Fiber.

3  $AME_n = 37.05 \text{ CP} (\%) + 81.96 \text{ Lip} (\%) + 39.87 \text{ St.} (\%) + 31.08 \text{ sugars} (\%)$ . Fisher and Mc Nab (1987).

4  $AME_n = 0.933 \text{ GE} (\text{kcal/kg}) - 15.07 \text{ CP} (\%) - 42.82 \text{ WICW} (\%)$ . Carré (1990).

After INRA, 1984; P.E.A. Programme 1996; Evans and Cheung, 1993; Brillouet and Riochet, 1983; Singh, 1984; Quemener and Brillouet, 1983; Vose *et al.*, 1976; Cerning-Beroard and Filiatre, 1976; Naivikul and D'Appolonia, 1978; Aman, 1979; Fleming, 1981; Sosulski *et al.*, 1982; Schweizer *et al.*, 1978; Lineback and Ke, 1975; Brillouet *et al.*, 1988; Champ *et al.*, 1986; Carré and Rozo, 1990; Carré *et al.*, 1985, 1987, 1991, 1995b; Conan and Carré, 1989; Lacassagne *et al.*, 1988, 1991; Carré and Brillouet, 1986, 1989; Carré, 1984 unpublished data.

Anti-trypsin factors (TUI) may also be components that can decrease protein digestion (Garlich and Nesheim, 1966). However, this was essentially demonstrated for whole untreated soyabean seeds which contain much higher levels of TUI than peas and faba beans (Valdebouze *et al.*, 1980). Either no relationship (Carré and Conan, 1989), or only a low negative relationship ( $R^2 = 0.12$ ; P.E.A. Programme, 1996) was found between protein digestibility and TUI level of peas. Genetic selection of seeds is also a possible means of reducing TUI levels. TUI levels greatly depend on cultivar.

It has been suggested that an accessibility problem with coarse particles could be responsible for low digestibility of pea proteins (Carré *et al.*, 1991). But fine grinding instead of coarse grinding does not change legume protein digestibility (Lacassagne *et al.*, 1991; Conan *et al.*, 1992). However, the effect of grinding remains to be reassessed by testing flours with no overlap in the ranges of particle sizes.

Thermomechanical processes such as autoclaving or pelleting generally result in an improvement in protein digestibility (Marquardt and Ward, 1979; Guillaume, 1978; Carré



*et al.*, 1987, 1991; Lacassagne *et al.*, 1988; Conan and Carré, 1989). Modification of both protein and cell wall structures may be involved in the effects of the latter processes.

It is quite evident that, at present, the reasons for protein digestibility variations have not been fully clarified and, thus, require further research.

### III. METABOLISABLE ENERGY VALUES

The reviews of the compositions of legume seeds from which their ME values can be deduced are presented in Table 2. Their measured ME values are presented in Tables 3 and 4.

Table 3. AME<sub>n</sub> values (kcal/kg DM) of peas and faba beans (means  $\pm$  SD).

	Adult		Young (3 weeks)	
	Mash	Pellet	Mash	Pellet
<b>PEAS</b>				
Huyghebaert <i>et al.</i> , 1979 (oT) <sup>1</sup>	3102 (1) <sup>2</sup>	3210 (1)		
Farrell, 1983		3210 $\pm$ 179 (3)		
Carré <i>et al.</i> , 1987 (oT)	2978 (1)	3084 (1)		
Askbrant, 1988 (oT)	2920 (1)			
Conan and Carré, 1989 (oT)			2487 $\pm$ 187 (4)	
Carré <i>et al.</i> , 1991 (oT)	2757 $\pm$ 83 (2)	3011 $\pm$ 86 (2)	2682 $\pm$ 118 (2)	3007 $\pm$ 17 (2)
Conan <i>et al.</i> , 1992 (oT)	2807 $\pm$ 12 (2)	3191 $\pm$ 24 (2)		
P.E.A. Programme, 1996 (oT)	2861 $\pm$ 111 (32)	3153 $\pm$ 67 (15)	2603 $\pm$ 79 (4)	2913 $\pm$ 69 (8)
(T) <sup>1</sup>	2713 $\pm$ 166 (12)	3040 $\pm$ 82 (5)		
<b>FABA BEANS</b>				
Shanon and Clandinin, 1977(T)			2484 (1)	
Huyghebaert <i>et al.</i> , 1979 (T)	2816 (1)	3038 (1)		
Lacassagne <i>et al.</i> , 1988 (T)			2505 $\pm$ 57 (2)	2760 $\pm$ 3 (2)
(oT)			2433 (1)	2850 (1)
Lacassagne <i>et al.</i> , 1991 (T)			2285/2962 <sup>3</sup> (1)	
(oT)			2200/2700 (1)	
P.E.A. Programme, 1996 (T)	2702 $\pm$ 108 (7)	2844 $\pm$ 65 (3)		
(oT)	2894 $\pm$ 118 (5)	3059 $\pm$ 138 (3)		

<sup>1</sup> oT: seeds without tannins; T: seeds with tannins. <sup>2</sup> Number of samples. <sup>3</sup> Fine grinding.

### IV. NON-STARCH CARBOHYDRATES

Effects of non-starch carbohydrates in legume seeds need to be considered, as they differ from those in cereals. Legume seeds contain much higher levels of  $\alpha$ -galactosides than cereals (Table 2). As these components cannot be digested by endogenous intestinal enzymes (Carré *et al.*, 1994a), they can only be digested through bacterial degradation. Despite this, these components can be extensively digested by birds (Carré *et al.*, 1995 a, b) with young birds displaying a lower ability for digestion than adults (Carré *et al.*, 1995b). Using lactose as a test material, increasing their dietary level from 3 to 6 % leads to a decrease in their digestibility in young birds (Carré *et al.*, 1995 a). It can be deduced

Table 4. AME<sub>n</sub> values (kcal/kg DM) of lupins and chick peas given as mash to adult cockerels.

	White lupins	Blue lupins	Chick peas
Farrell, 1981			3220 (pellet)
Farrell, 1983		2360 (3) (pellet)	
INRA, 1984	2776		
Carré and Lacassagne, 1984 <sup>2</sup>	2470 ± 89 (4) <sup>1</sup>		
Karunajeewa and Bartlett, 1985	2340		
P.E.A. Programme, 1996	2739	2172	2897 ± 360 (2)

<sup>1</sup> Standard deviation and number of samples. <sup>2</sup> Unpublished data.

from these two studies (Carré *et al.*, 1995 a, b) that  $\alpha$ -galactosides are effectively available

to the birds and that their side effects are rather low, provided their dietary levels remain lower than 3 %. Their utilization for growth was estimated to be about 50 % of that of glucose (Carré *et al.*, 1995b).

The non-starch polysaccharides (NSP) of legume seeds contain high concentrations of pectic substances (Brillouet and Carré, 1983; Carré *et al.*, 1985). A major fraction of these pectic substances is insoluble in water (Brillouet and Carré, 1983; Carré *et al.* 1985), which explains the higher values obtained for WICW than for neutral detergent fibre (NDF) in legume seeds (Carré and Brillouet, 1986), especially in lupins (see Table 2). The high concentrations of water-insoluble pectic substances in lupins are essentially due to large amounts of linear chains of (1 4) galactans linked to the rhamnogalacturonan backbones of cotyledon cell walls (Carré *et al.*, 1985).

The occurrence of pectic substances probably explains why the water retention capacities of legume cell walls are higher than those of cereals (Carré *et al.*, 1995c). These high water retention capacities may affect the physical properties of excreta (Carré *et al.*, 1995c) and, thus, litter.

The proportions of water-soluble NSP in the total NSP of legume seeds are normal (5-10 %, Table 2). Potential viscosities of peas and faba beans are lower than the lowest values found for wheats (Carré *et al.*, 1994b). Viscosities of lupins were not measured. However, as their water-soluble NSP contents are higher than those of peas and faba beans (Table 2), higher viscosities can also be expected with values probably near to those of wheats. The positive effect of dietary enzymes on the growth performance of birds fed high lupin diets (Brenes *et al.*, 1993) probably comes from problems of viscosity in lupins. However, when fed lower amounts of lupins (30 % in diets), birds do not show significant effects of dietary enzymes on their growth performance (Annison *et al.*, 1996).

## V. MISCELLANEOUS

The mean phosphorus content of peas, faba beans and white lupins are 4.2, 6.0 and 4.0 %, respectively, about 50 % of which comes from phytic acid (Sauveur, 1989). Phytase activities are probably very low in legume seeds, as reported in peas which show an activity similar to maize (Pointillart, 1994). The mean availabilities of phosphorus in peas, faba beans and white lupins are 35, 25 and 20 %, respectively (Sauveur, 1989).

Replacing soyabean meal with white lupins requires the levels of folic acid and biotin to be adjusted, probably because of deficiencies of these vitamins in white lupins (Lacassagne, 1982).

Vicine and convicine are antinutritional factors of faba beans that need to be mentioned. These molecules are responsible for the egg weight depressing effect of faba beans (Olaboro *et al.*, 1981 a, b). So, faba bean levels in diets for laying hens should be limited to a maximum of 7 % to avoid this egg-weight depressing effect (Lacassagne, 1988). However, the selection of a faba bean cultivar free of vicine and convicine (Duc *et al.*, 1989) will probably favour the use of this crop for laying hens.

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## HEAT STABILITY OF ANTINUTRITIONAL FACTORS IN SOYABEAN MEAL

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The content and brush border membrane vesicle agglutination activity of lectins in a raw soyabean sample and twenty-five soyabean meals processed in North America and Europe were determined. Raw soyabean was found to contain 2.3 mg of lectin per g of sample. All the soyabean meals contained some carbohydrate-binding proteins, ranging from 0.22 to 1.40 mg/g of meal. Although all the carbohydrate-binding protein in the raw soyabean was agglutinating the same was not observed for the processed soyabean meal samples where large differences were found between the level of carbohydrate-binding proteins and agglutinating proteins. It appears that denaturation of the lectin by heat treatment during processing results in a dramatic decrease in the ability of the lectin to agglutinate while having a relatively minor effect on its capacity to bind to carbohydrates.

## I. INTRODUCTION

The anti-nutritional effects of lectins depends on their source and on the species or strain of animal used (Jaffe, 1980; Grant *et al.*, 1983). Some raw legume seeds, such as kidney (*Phaseolus vulgaris*), runner (*Phaseolus cocineus*) and tepary (*Phaseolus acutifolius*) beans cause weight loss and sometimes even death when incorporated into the diet (Grant *et al.*, 1983; Pusztai, 1991). The nutritional significance of soyabean lectins is somewhat confusing with some studies indicating a significant anti-nutritional effect and others failing to show any negative effect. Turner and Liener (1975) removed haemagglutinating activity from a crude extract of unheated soyabean flour by affinity chromatography and found that the performance of rats fed diets containing this haemagglutinin-free extract was not significantly different from that obtained with the original extract from which the haemagglutinin had not been removed. In contrast, Liener (1953) and Grant *et al.* (1989) found that the inclusion of soyabean lectin in diets of rats considerably inhibited their growth. Dietary lectin inclusion also induced significant enlargement of the pancreas and the jejunum (Grant *et al.*, 1989). Regardless of these findings it has been assumed that the anti-nutritional effects of soyabean lectins is completely abolished by heat-treatment during processing. However, heat treatment is not always effective. For example, Calderon de la Barca *et al.* (1991) found that most commercially available soya products contained lectins, with some containing almost as much as raw soyabeans.

Many studies have investigated the reduction in weight gain of growing animals due to dietary lectins (see reviews by Jaffe, 1980; Pusztai, 1989, 1991; Huisman and Jansman, 1991). From these studies it appears that the main effects of lectins are on the intestinal mucosa where the lectins bind to the membrane receptors of epithelial cells disorganising the microvilli and causing a reduction in the efficiency of nutrient absorption.

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The most commonly used method for the prediction of the anti-nutritional effects of plant lectins is the haemagglutination assay. However, since glycoproteins on the surface of erythrocytes may not be representative of those in the gut wall, the test probably lacks any predictive value with respect to the biological effects of the lectin. Recently, Irish *et al.* (1995) developed the Brush Border Lectin Agglutination Assay that can be used for the determination of functional lectins in animal feedstuffs. This assay is based on a measure of lectin-dependent small intestinal brush border vesicle agglutination occurring over a 16 hour incubation period. Agglutinated vesicles are pelleted by low speed centrifugation and quantified by the alkaline phosphatase activity of the resuspended pellet.

In the current study, lectins were purified from raw soyabean and processed soyabean meals by affinity chromatography and gel filtration. The quantity of carbohydrate-binding protein eluted from the affinity columns and brush border membrane vesicle agglutinating protein in the final preparation was determined.

## II. MATERIALS AND METHODS

Raw soyabean seeds and processed soyabean meal samples were gifts from processing plants throughout North America and Europe. Purified soyabean lectin and agarose activated with N-acetyl-D-galactosamine were purchased from Sigma Chemical Co., St Louis, MO, USA. All other reagents were of analytical grade.

### (a) Extraction and purification of lectins

Raw soyabean was de-fatted by overnight extraction with hexane using a soxlet apparatus. The solvent was removed by drying overnight in a fume hood. The soyabean meal samples were all analysed directly.

Finely ground (to pass through 0.5 mm screen) soyabean (2 g) was vigorously stirred with 0.9 % saline (25 ml) for 2 h at room temperature and the mixture was centrifuged at 4120 rpm for 30 min. The pellet was re-extracted in saline and the combined supernatants filtered to give the soluble protein extract. The extract was applied directly to a column containing N-acetyl-D-galactosamine-agarose and the column was washed with saline until the unbound protein peak had declined to baseline. A solution of 0.14 M galactose in saline was used to displace carbohydrate-binding material from the column. This material was assayed for protein using a modified micro-Lowry method (Sigma Diagnostics Test Kit, Sigma Chemical Co., St Louis, MO, USA), freeze-dried, resuspended in 1 ml of water and applied to a sephadex G-25 column to separate protein from galactose. The void volume from the column was freeze-dried, resuspended in 1 ml of water, assayed for protein, and stored at -20°C prior to analysis for agglutinating protein. As a control procedure 1 mg of purified soyabean lectin and a processed soyabean meal sample spiked with 1 mg of purified soyabean lectin were passed through the lectin purification scheme described above and assayed for recovery of carbohydrate-binding and agglutinating protein.

### (b) Preparation of chick small intestinal brush border vesicles

Four week old commercial broiler chicks were killed by cervical dislocation and the small intestine from the distal end of the duodenum to the ileo-caeco-colic junction was removed. The brush border membrane vesicles (BBV) were prepared from mucosal scrapings using divalent cation precipitation and differential centrifugation as described by Maenz and Patience (1992). The final vesicle pellets were resuspended, pooled, assayed for

protein, diluted to 16 mg BBV protein/ml, divided into aliquots and frozen in liquid nitrogen until time of use.

(c) Brush border vesicle lectin agglutination assay

The ability of lectins to agglutinate purified small intestinal brush border membrane vesicles was determined using the Brush Border Lectin Agglutination Assay developed by Irish *et al.* (1995). Brush border membrane vesicles were thawed and diluted with 0.9 % saline to a concentration of 0.2 mg BBV protein/ml. Lectin protein was dissolved in saline and added to 96 well microtitre plates as required for the particular experiment. Agglutination was initiated by adding BBV such that each well contained 0.005 mg of BBV protein and 250  $\mu$ l final volume. The plates were incubated overnight at 4°C to complete the agglutination process. Agglutinated BBV were pelleted by low speed centrifugation at 1100 rpm for 15 min. The supernatants were removed and the pellets resuspended with 0.150 ml water. Alkaline phosphatase activity was determined by measuring the initial rate of para-nitrophenol formation from para-nitrophenolphosphate using a Sigma Diagnostics test kit (Sigma Chemical Co., St Louis, MO, USA), .

### III. RESULTS

(a) Carbohydrate-binding and agglutinating protein recovery during lectin purification

Using the purified soyabean lectin control, 98% of the protein was recovered after affinity chromatography and 91% of the agglutinating lectin protein was recovered at the final stage of purification. (Table 1). With the spiked soyabean meal sample, 1.13 mg of protein were recovered from the affinity column and 0.955 mg of agglutinating protein were recovered at the final stage. Subsequent assays indicated 0.05 mg of agglutinating protein were recovered from 2 g of unspiked soyabean meal sample indicating that the meal itself contributed to carbohydrate-binding and agglutinating protein recovered from the spiked sample. When corrected for endogenous contribution, 90% of the pure lectin added to the soybean meal sample was recovered as agglutinating protein.

Table 1. Recovery of carbohydrate-binding and agglutinating protein from purified soyabean lectin and a spiked soyabean meal sample.

Sample	Affinity Chromatography (%)	Agglutination Assay (%)
Purified Soyabean Lectin	98	91
Spiked Soyabean Meal	91	90

(c) Carbohydrate-binding and agglutinating lectins in soyabean meal samples

Table 2 shows the mg of carbohydrate-binding and agglutinating lectin protein isolated per g of meal for 25 samples of soyabean meal obtained from various sources. All the carbohydrate-binding protein isolated from raw soyabeans functioned to agglutinate BBV. The quantities of carbohydrate-binding and agglutinating lectin protein varied considerably between samples of processed soyabean. Considerable carbohydrate-binding protein was isolated by affinity chromatography from each of the processed soyabean samples. However, in all cases, agglutinating lectin protein levels were markedly lower than the amount of carbohydrate-binding proteins in the sample.



Table 3 shows the relative distribution of carbohydrate-binding and agglutinating lectin levels in the samples using raw soyabean as a reference point. Of the 25 samples of processed meals tested, 23 had carbohydrate-binding proteins levels greater than 10% of the level obtained for raw soyabean. However, 15 of the samples had agglutinating lectin levels that were less than 3% of the level in the raw soyabean sample, and only 4 of the samples had agglutinating lectin levels in excess of 10% of that in the raw soyabeans.

Table 2. Affinity purified and agglutinating proteins in raw and processed soyabean meals (SBM).

Sample	Affinity Purified Protein		Agglutinating Protein	
	mg/g meal	% of raw	mg/g meal	% of raw
Raw	2.28	100.00	2.31	100.00
SBM 1	0.22	9.52	0.01	0.64
SBM 2	0.22	9.72	0.08	3.49
SBM 3	0.40	17.43	-0.01	-0.23
SBM 4	0.24	10.59	0.02	0.93
SBM 5	0.51	22.34	0.06	2.73
SBM 6	0.67	29.15	0.03	1.20
SBM 7	0.43	18.86	0.06	2.70
SBM 8	0.55	24.22	0.15	6.31
SBM 9	0.29	12.74	0.01	0.31
SBM 10	0.45	19.90	0.04	1.69
SBM 11	0.43	18.76	0.10	4.35
SBM 12	0.88	38.34	0.12	5.02
SBM 13	0.59	25.79	0.00	0.00
SBM 14	0.43	18.69	0.05	2.05
SBM 15	0.99	43.34	-0.04	-1.87
SBM 16	0.88	38.51	0.20	8.49
SBM 17	0.35	15.13	0.01	0.37
SBM 18	0.39	17.07	0.05	2.12
SBM 19	0.54	23.69	0.21	8.88
SBM 20	0.47	20.67	-0.05	-2.07
SBM 21	1.40	61.31	0.81	35.20
SBM 22	0.94	41.39	0.01	0.50
SBM 23	0.51	22.53	0.28	11.92
SBM 24	0.58	25.58	0.45	19.52
SBM 25	0.82	35.71	0.68	29.24

Table 3. Summary of lectin content in the processed soyabean meals.

Relative to raw soyabeans (%)	Number of samples	
	Affinity purified lectins	Agglutinating lectins
0-3	0	15
3-10	2	6
10-20	17	3
> 30	6	1

#### IV. DISCUSSION

The recovery of carbohydrate-binding and agglutinating lectin proteins in the control sample and the processed meal "spiked" with pure lectin is consistent with recovery of functional lectin during the purification process. The 9% loss of initial agglutinating lectin activity in the control sample likely represents unavoidable losses during solution transfers, freeze drying and other manipulations of the sample during purification. Recovery of pure agglutinating lectin added as a "spike" to a processed meal was the same as obtained with the control sample. Further, the quantities of carbohydrate-binding and agglutinating lectin proteins recovered from the raw soyabean sample were the same, which is consistent with 100% functional non-denatured lectin in raw soyabean meal. Therefore, the purification scheme used in this study appears to provide an accurate assessment of both carbohydrate-binding protein and small intestinal brush border agglutinating protein in unprocessed and processed soyabean meals.

Recently, Vasconcelos *et al.* (1994) isolated a novel toxic protein (soyatoxin) that was distinct from lectin or trypsin-inhibitors from raw soyabeans. The contribution of soyatoxin to the overall antinutritional properties of raw soyabeans and the significance of this protein in defining the nutritional value of processed soyabean meals is unknown at present. Soyatoxin has haemagglutination activity but can be separated from lectins by passage through a lectin-specific affinity column. Crude extracts of soyabean meals may well contain both lectins and soyatoxin which could complicate any attempt to measure lectins using an agglutination assay. These considerations bring into question results obtained from any procedure that does not isolate lectins by specific affinity chromatography prior to the measurement of agglutinating lectin in the sample.

The raw soyabean sample used in this study was found to contain 2.3 mg of carbohydrate-binding and agglutinating protein per g of meal. This is in agreement with values reported in the literature. For example, Pull *et al.* (1978) measured the affinity purified lectin levels in the raw seed meal of 102 lines of soyabeans. For 97 of the lines lectin levels ranged from 2.5 to 12.2 mg/g meal while the remaining 5 lines lacked any detectable lectins. Other studies report values of 1.45 (Vretblad, 1976), 1.6 (Lis *et al.*, 1974), 3.2 (Calderon de la Barca *et al.*, 1991) and 3.6 (Allen and Neuberger, 1975) mg of affinity purified lectin/g of raw soyabean meal.

The results of this study show that there were large differences in the amount of protein eluted from the affinity column and the actual agglutinating lectin protein in the samples of processed soyabean meal. Thus, processing the meal would appear to have a limited effect on the capacity of lectins to bind the epithelial cells. However, in 21 of 26 samples tested the agglutinating lectin level in the meal was less than 10% of the level found in the raw soyabean sample. As such, a simple measure of lectin protein eluted from an affinity column or lectin binding to an immobilised carbohydrate-binding site cannot be used as an indicator of fully-functional agglutinating lectins in a given sample.

This study provides the first indication of substantial differences in carbohydrate-binding affinity purified and agglutinating lectin levels in samples of processed soyabean meals. These differences imply an important question regarding the relative toxicity of agglutinating and non-agglutinating lectins that retain a capacity to bind to N-acetylgalactosamine. Simple binding to the brush border membrane of the intestinal epithelial cells may be sufficient to initiate endocytosis and toxic effects within the cells. Alternatively, lectins may require fully-functional agglutinating properties to damage the epithelium.

Overall, the finding of substantial levels of affinity purified and agglutinating lectins in several of the 26 samples of processed soyabean meals tested suggests the possibility that,

under certain conditions, residual lectins in soyabean-based diets could have a negative effect on the performance of production animals. Further research is required to determine the biological effects of both affinity purified non-agglutinating and agglutinating lectins to determine the importance of any residual lectin found in soyabean meals.

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## IMPROVING GRAIN LEGUME PROTEIN QUALITY THROUGH GENETIC ENGINEERING

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### Summary

Genetic engineering procedures have been used to enhance the characteristically low dietary levels of sulphur-containing amino acids found in grain legumes. A gene coding for a sunflower seed protein which is unusually rich in the S-amino acids (16% methionine, 8% cysteine) was engineered into lupins. This sunflower seed albumin (SSA) makes up 5% of the total extractable seed protein of the resultant transgenic plants. Methionine level was increased by 124% and cysteine decreased slightly but there were no significant changes in other amino acids. In rat-feeding trials, the transgenic lupins had significantly increased biological value and net protein utilisation with no change in true protein digestibility or digestible energy. This work demonstrates the feasibility of improving the nutritive value of grain legumes through genetic engineering.

### I. INTRODUCTION

Grain legumes are an important source of protein in the stock feed industry. However, in terms of the dietary needs of non-ruminant animals their seed proteins are characteristically deficient in the sulphur(S)-containing amino acids, methionine and cysteine. For example, supplementation with methionine is recommended for maximum breast meat production in poultry diets (Hickling *et al.*, 1990; Gorman and Balnave, 1995). At least three approaches have been used to overcome the problem of low methionine and cysteine in the seed protein of dicotyledonous plants. The deficiency is currently overcome by adding synthetic methionine directly to feed mixes. In 1990 Australia imported over \$11 million worth of methionine for this purpose and this figure is presumably increasing each year. Secondly, seed collections of grain legumes and their near relatives from a wide range of origins can be screened for lines that have a higher than average level of S-amino acids. This approach has not been rewarding. For example, a survey of 45 lines of *Pisum sativum* and related species found no evidence of enhanced S-amino acid levels. Any increase in one S-rich protein fraction (such as legumin) in a line was invariably accompanied by a decrease in the other major S-rich fraction (the albumins). The net effect is that the S-amino acid content, relative to the protein content, remains fairly constant (Schroeder, 1982). A third approach, which has only recently become available for testing, is to use the procedures of genetic engineering to enhance the amino acid profile of grain legumes. This approach has been made possible by the development of recombinant DNA technologies that enable the isolation, cloning and modification of the nucleotide sequences of specific genes, and the development of procedures for introducing new or modified genes into plants in such a way that they become a stable component of the plant's genome.

Three different strategies have been employed in the genetic engineering approach. One strategy has been to isolate the gene for a seed protein and to modify its nucleotide

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sequence so that it codes for a protein with an increased level of the desired amino acid. For example, the genes for  $\beta$ -phaseolin from *Phaseolus vulgaris* (Hoffman *et al.*, 1988), glycinin from soybean (Nielsen *et al.*, 1990), and vicilin from *Vicia faba* (Saalbach *et al.*, 1995) were all modified to code for additional methionine residues. In all cases the modified gene was then transferred into tobacco as the test plant. In the first two cases the modified protein proved to be extremely unstable in the transgenic seeds. In the latter case, the modified protein was stable but the level of accumulation was extremely low. A second genetic engineering approach, employed by Falco *et al.* (1995), is to isolate the gene for a feedback-insensitive key enzyme involved in the biosynthesis of the deficient amino acid and transfer it into the target plant with the aim of achieving overproduction of this amino acid. These authors isolated from bacterial sources two such key enzymes of the lysine biosynthetic pathway and introduced them into canola and soybean. This resulted in a 100 to 200-fold increase in free lysine and a two to five-fold increase in total seed lysine.

Another approach has been to seek out a protein that is naturally rich in the deficient amino acid, to isolate the gene for this protein and to introduce it into the grain legume species of interest. This strategy has only become possible in recent years with the development of tissue culture procedures for transforming some grain legumes such as soybean (Hinchee *et al.*, 1988), narbon bean (Pickhardt *et al.*, 1991), peas (Schroeder *et al.*, 1993), common bean (Russell *et al.*, 1993) and lupin (Molvig *et al.*, In press). In general, large seeded legumes have proved to be very recalcitrant in this regard compared to many other dicotyledonous plants. Two promising candidate proteins have emerged for the enhancement of S-amino acid levels in grain legumes. These are a seed protein from Brazil nut (*Bertholletia excelsa* H.B.K.) which contains 18% methionine and 6% cysteine (Altenbach *et al.*, 1987) and a seed protein from sunflower which contains 16% methionine and 8% cysteine (Kortt *et al.*, 1991). The gene coding for each of these proteins has been isolated and introduced into a grain legume. The gene for the Brazil nut protein was introduced into narbon bean and the new protein was accumulated at up to 4% of the total seed protein (Saalbach *et al.*, 1995). However, it has subsequently been established that the Brazil nut protein is highly allergenic in some human subjects (Nordlee *et al.*, 1995) and, therefore, could be an undesirable addition to any diet. A search of the scientific literature has not revealed any reports of allergenicity associated with sunflower seed proteins.

The gene for the sunflower seed albumin (SSA) protein has been introduced into narrow leafed lupins (*Lupinus angustifolius*) and this paper reports some of the properties of the resultant transgenic lupins in relation to their S-amino acid status and their nutritive value.

#### IV. METHODS

The methods used in this work have been described in detail elsewhere (Molvig *et al.*, 1997). In brief, they were as follows. All plant material (explants, transgenic plants and seeds) were from the narrow leafed lupin (*Lupinus angustifolius*, cv Warrar) and all seed analysed was from glasshouse-grown plants. Transgenic plants were produced by co-cultivating slices of the embryonic axis from developing seeds in a suspension of *Agrobacterium tumefaciens* which carried the donor DNA construct on a plasmid. The donor DNA construct contained three genes. One gene coded for the SSA protein, a second gene coded for phosphinothricin acetyl transferase (PAT), and a third gene coded for  $\beta$ -glucuronidase. The *ssa* gene was controlled by seed-specific promoter and terminator sequences from vicilin (a pea seed storage protein). The presence of the gene for the PAT enzyme enabled transformed cells in tissue culture to detoxify the phosphinothricin (PPT)

and, consequently, only these cells regenerated into viable plants.  $\beta$ -Glucuronidase served as a screenable marker for transformed plant tissues.

The presence of the SSA protein in the seeds of regenerated plants was detected by western blot procedures using antiserum developed in goats and the level of SSA was quantified by densitometric analysis of the immunoblots. The amino acid composition of lupin seed meal was determined as described elsewhere (Mason *et al.*, 1980). The samples were oxidised with performic acid prior to hydrolysis and analysis by ion exchange chromatography.

Total N was determined in an autoanalyser following Kjeldahl digestion of finely ground seed meal, and total S, oxidised S and carbon-bonded S were determined by X-ray fluorescence spectrometry (Pinkerton *et al.*, 1989). These authors demonstrated that the two latter fractions correspond to sulfate-S and amino acid-S, respectively.

Rat-feeding trials were carried out with meal from transgenic and non-transgenic plants grown under identical conditions in a glasshouse. Feeding trial procedures were as described by Eggum *et al.* (1993), with lupin meal providing all the dietary protein.

### III. RESULTS

#### (a) SSA expression in lupin seeds

Extracts of transgenic lupin seeds were fractionated by SDS-polyacrylamide gel electrophoresis and the level of SSA protein in the cotyledons was estimated by densitometric analysis of immunoblots made from these gels. The SSA levels were as high as 5% of total extractable protein. The SSA was accumulated in the same type of small protein body as the homologous lupin seed storage proteins (data not shown). Selection of subsequent generations for the most highly expressing progeny gave a line which was homologous with respect to the *ssa* gene and consistently expressed SSA at the 5% level or higher. These plants were morphologically indistinguishable from their non-transgenic counterparts.

#### (b) The composition of transgenic lupins

No significant differences were found between the transgenic lupins and their non-transgenic progenitor with respect to nitrogen content or total sulphur content (Table 1). However, analysis of the inorganic and carbon-bonded-S (corresponding to sulfate-S and amino acid-S, respectively) by X-ray fluorescence spectroscopy showed a significant redistribution of sulphur from the oxidised to the carbon-bonded pools (Table 1). This was accompanied by an increase of 124% in the level of methionine in the transgenic seeds (Table 2). There was no significant change in the level of any other amino acid, except cysteine, in which, unexpectedly, there was a slight decrease. Overall, there was a 30% increase in the total S-amino acid level in the transgenic seeds.

Table 1. Some characteristics of lupins from the non-transgenic parent line and from a homozygous transgenic line expressing sunflower seed albumin protein.

	Non-transgenic	Transgenic
N (% dry wt)	5.48	5.73
Total S (mg/kg)	4104	4104
Oxidised S (mg/kg)	1263	876
Carbon-bonded S (mg/kg)	2840	3225

Table 2. Amino acid composition (g/16 g N) of non-transgenic and transgenic lupin seeds.

Amino acids	Non-transgenic	Transgenic
Alanine	3.28	3.33
Arginine	10.46	10.33
Aspartic acid	9.57	10.04
Cystine	1.49	1.25
Glutamic acid	20.95	20.12
Glycine	4.06	4.08
Histidine	2.78	2.71
Isoleucine	4.38	4.48
Leucine	6.69	6.55
Lysine	4.60	4.63
Methionine	0.65	1.40
Phenylalanine	3.74	3.78
Proline	4.17	4.20
Serine	5.24	5.09
Threonine	3.33	3.36
Tryptophan	0.83	0.78
Tyrosine	3.53	3.76
Valine	4.02	3.95

### (c) Rat-feeding trials

The nutritive value of transgenic lupins was studied in a rat-feeding trial in which meal from either transgenic or non-transgenic lupins served as the sole protein source. Nitrogen balance determinations showed that there were no changes in true protein digestibility or digestible energy in the transgenic lupins, but there was an 11% increase in both net protein utilisation and biological value (Table 3).

Table 3. Comparison of nutritive value coefficients of transgenic and non-transgenic lupin meal in a rat-feeding trial.

	True protein digestibility	Biological value <sup>a</sup>	Net protein utilisation <sup>a</sup>	Digestible energy
Non-transgenic	0.954 (0.0020)	0.699 (0.0041)	0.667 (0.0050)	0.859 (0.0032)
Transgenic	0.950 (0.0014)	0.776 (0.0019)	0.737 (0.0270)	0.856 (0.0019)

Standard errors are shown in brackets.

<sup>a</sup>Differences between transgenic and non-transgenic samples are significant at  $< P = 0.001$ .

## IV. DISCUSSION

The introduction into lupin of a gene coding for an unusually S-rich protein from sunflower seeds resulted in significant changes in seed composition and nutritive value of the transgenic plant. The methionine level increased from 0.65 to 1.40 g/16 g N, although, at the same time, the level of cysteine fell slightly from 1.49 to 1.35 g/16 g N (Table 2). In parallel with these changes, there was a redistribution of total sulphur from the sulfate fraction to the carbon-bonded sulphur fraction (Table 1). These changes were accompanied by a marked improvement in the nutritive value of the transgenic grain as evidenced by increases in biological value and net protein utilisation. Biological value is the most sensitive indicator of amino acid imbalance among the parameters measured, because it is corrected for metabolic N in the faecal material and for both urinary N and endogenous urinary N. As far as the authors are aware this is the first demonstration of an improvement in the nutritive value of a feed grain as a result of a modification brought about by genetic engineering. Similar changes in these parameters were seen in earlier experiments in which rats were fed a diet of non-transgenic lupins supplemented with synthetic methionine (Eggum *et al.*, 1993).

The changes in S-amino acid composition in the transgenic grain were unexpected from two points of view. Firstly, despite the fact that the new protein (SSA) is rich in both methionine and cysteine, there was not a proportional increase in the level of both amino acids in the transgenic seeds. In fact, the cysteine level decreased slightly. Secondly, given that the SSA protein accounted for 5% of the total protein in the transgenic seed, the extent of the increase in methionine was not as great as expected on a theoretical basis. Analysis by X-ray fluorescence spectrometry confirmed the level of change found by analysis of individual amino acids. These results indicate that the expression of the foreign genes was accompanied by other changes in the protein or non-protein fractions of the seed. The phenotypic plasticity of the seed protein fraction has been well documented. For example, it has been shown that in response to nutritional stress during seed development, the relative amounts of the major seed storage protein fractions of legumes can change dramatically. When sulphur supply is suboptimal during pea seed development, there is a marked reduction in the content of the more sulphur-rich protein fractions (legumin and the albumins) and a corresponding increase in the sulphur-poor proteins (vicilins) (Randall *et al.*, 1979; Chandler *et al.*, 1983). It is possible that similar changes in the protein profile may occur in transgenic lupins expressing the *ssa* gene. However, in this case the changes must be more subtle because no differences were seen in the protein profile in one-dimensional SDS-polyacrylamide gels. We presume that there is a reduction in the synthesis of one or more cysteine-rich proteins in the transgenic seeds, perhaps due to competition for limiting supplies of cysteine or methionine.

In current work, which is not as advanced as the work with lupins, the *ssa* gene is being introduced into field peas and chickpeas with the aim of raising the nutritive value of these two grain crops. Feeding trials with chickens are required to see whether the results obtained with rats are also applicable to a commercially important, non-ruminant animal.

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## ADVANCES IN DEFINING THE NUTRITIVE QUALITY OF FEED INGREDIENTS

N. DALE

Summary

Assuming that nutrient requirements for poultry have been established with a reasonable degree of accuracy, optimum efficiency in feed formulation is based on an accurate appraisal of the nutritive qualities of available feed ingredients. Soyabean meal and rendered animal by-products are principle sources of protein in most commercial poultry feeds. Recent studies suggest that the metabolizable energy of meat and bone meal is substantially higher than has been traditionally reported. A proposed rapid estimation of the calcium and phosphorus contents of this ingredient may allow higher levels to be used without incurring the risks associated with variation in mineral contents.

It is widely recognized that the lysine content of soyabean meal is less available when meal has been overheated during processing. A series of studies confirmed this observation and failed to observe a response to either methionine or threonine supplementation to diets containing overprocessed meal. Estimates were made as to how much lysine needs to be supplemented to overprocessed meals in order to overcome the reduction in lysine availability.

## I. INTRODUCTION

A recognition of the nutritive content of feed ingredients is, of course, vital to successful least cost feed formulation. Standard tables of nutrient composition can, at best, give approximate levels for respective nutrients, but have neither the space nor are intended to provide in-depth information about the many feed ingredients listed. An ongoing extension project at The University of Georgia has been to better define the feeding value of ingredients available to the poultry industry. One objective of this program has been to verify "table" values for the major feed ingredients. However, equally important is the recognition that with few exceptions feed ingredients are subject to wide variations in parameters such as protein, energy, minerals and amino acids.

It has long been suspected that the metabolizable energy of meat and bone meal is substantially higher than is recorded by many tables of nutrient composition (Martosiswoyo and Jensen, 1988; Dale, 1990). In standard assay procedures for determining metabolizable energy, meat and bone meal is incorporated in test diets at a level which inadvertently includes excessive amounts of calcium and phosphorus. It is hypothesized that these high mineral levels may impede the optimum digestion/absorption of energy-containing nutrients. Several studies were undertaken to determine the metabolizable energy of meat and bone meal free of the possibly confounding effects of high mineral levels.

Variations in the calcium and phosphorus content of meat and bone meal are of concern to nutritionists as shipments particularly low in these nutrients may lead to the manufacture of feeds deficient in them. In high volume feed mills it is common for specific shipments of ingredients to be mixed into feeds and placed on farms before laboratory evaluations have been completed. A procedure is described for a rapid estimate of calcium and phosphorus in meat and bone meal. If a reliable estimate can be obtained within 5 min

of the arrival of a shipment of meat and bone meal, a decision can be made regarding acceptance of the delivery or a change in formulation.

Soyabean meal is the major protein source for poultry diets in much of the world. Following oil extraction, meals are heated to remove solvent and also to deactivate such antinutritional factors as trypsin inhibitor. In many areas it is not uncommon to occasionally receive shipments of soyabean meal which, inadvertently, have been overheated. Such overheating has long been recognized as being associated with a decrease in the availability of essential amino acids (Renner *et al.*, 1953; Warnick and Anderson, 1968; Araba and Dale, 1990). At the present time, lysine, methionine, and threonine are available to the feed industry at prices permitting their entry into broiler feeds. As part of a project to determine strategies for dealing with overprocessed soyabean meal, studies were undertaken to determine whether methionine and/or threonine supplementation, in addition to lysine, might be of value in improving the feeding value of overprocessed soyabean meal. Further studies were undertaken to determine how much amino acid supplementation can be advised when overprocessed meals must be used in feed formulation.

By-products of wheat milling have long been valuable components of poultry and livestock feed. However, extreme variation in fibre content is frequently observed not only between countries, but between flour mills and, occasionally, between batches of wheat by-product from the same mill. Understandably, a wide range in metabolizable energy values for these ingredients is reported in the literature (Hill *et al.*, 1960; Farrell *et al.*, 1967; Cave *et al.*, 1965; Summers *et al.*, 1968; Kuzmicky *et al.*, 1978). Results of a number of metabolizable energy determinations were combined with considerable data from the scientific literature to develop prediction equations for metabolizable energy content of wheat by-products.

## II. MATERIALS AND METHODS

### (a) Metabolizable energy of meat and bone meal

Four samples of meat and bone meal were separated by chloroform flotation into bone and meat plus fat fractions. Proximate composition (AOAC, 1990) and gross energy of the original meals and respective fractions are presented in Table 1, with the composition of bone and meat plus fat fractions in Table 2. True metabolizable energy determinations (Sibbald, 1986; Dale and Fuller, 1984) were made on each of the four original meals and corresponding meat plus fat fractions. The metabolizable energy of the bone fraction was

Table 1. Proximate composition (g/kg) and gross energy (MJ/kg) of meat and bone meals and of meat + fat (M+F) and bone fractions.

	Experiment 1						Experiment 2					
	Beef			Pork			Beef			Pork		
	Meal	M+F	Bone	Meal	M+F	Bone	Meal	M+F	Bone	Meal	M+F	Bone
C. Protein	486	623	278	507	600	258	498	619	228	600	665	308
Fat	103	164	16	88	106	24	97	156	17	96	122	19
Ash	317	82	580	289	118	604	307	88	605	217	91	590
Moisture	55	80	74	49	96	60	62	85	75	49	83	64
Gross												
Energy	15.57	21.66	5.61	16.59	20.46	7.59	15.31	21.64	6.91	18.55	21.27	7.80

Table 2. Composition (g/kg) of bone and meat + fat fractions of pork and beef meals.

	Experiment 1		Experiment 2	
	Beef	Pork	Beef	Pork
Bone	449	320	445	221
Meat + Fat	551	680	555	779

estimated to be 75% of the gross energy of the bone samples. Based on the relative percentages of bone and meat plus fat fractions of each meal, a metabolizable energy value could be calculated free of possible artifacts produced by high mineral levels in the samples.

(b) Calcium and phosphorus in meat and bone meals

Chloroform flotation is a tool frequently employed by feed microscopists. When chloroform (or other suitable solvents) are added to meat and bone meal in a beaker or test tube, the bone fragments sink to the bottom, the meat fraction floats toward the top, and fat is dissolved in the solvent. In order to estimate the bone content of individual samples of meat and bone meal (and thus obtain an estimate of calcium and phosphorus levels), 10 meat and bone meals were obtained from industrial renderers, with ash contents ranging from 136 to 423 g/kg. Each sample (20 g) was placed in a 100 mL graduated cylinder, with chloroform added to bring the total volume to at least 50 mL. After providing slight agitation to the floating meat layer to permit any trapped bone samples to sink, a reading was made of the volume of bone derived from 20 g of each sample. Calcium and phosphorus determinations were obtained independently on the original meals using standard laboratory procedures. The correlation analysis was conducted to determine whether this somewhat crude estimate of bone content could be used to reliably predict calcium and phosphorus levels.

(c) Amino acid supplementation to overprocessed soyabean meal

A subsample from a larger shipment of soyabean meal was cooked in an autoclave to achieve a meal with less than 40% protein solubility. This "overprocessed" meal was utilized in dietary treatments with either no amino acid supplementation, or lysine, methionine, and threonine being added singly or in all possible combinations (lysine HCl was added at 2.0 g/kg, DL-methionine at 1.0 g/kg and L-threonine at 1.6 g/kg). The control diet of the same formulation, but with normal soyabean meal from the same batch, was calculated to be marginal in all three amino acids. The various experimental diets were fed to broiler chicks from 1 to 15 days of age. Body weight and feed conversion rate were recorded at the termination of the study.

(d) Level of lysine needed to compensate overprocessing of soyabean meal

Subsamples of the same shipment of soyabean meal were heated in an autoclave to produce meals with 66% and 54% protein solubilities. Previous work (Araba and Dale, 1990) reported that protein solubilities below 75% were indicative of overprocessed meals. A control maize-soy diet was formulated to contain 11 g lysine/kg and graded levels of lysine were added to diets containing each of the two overprocessed meals. The objective of the study was to determine how much lysine needed to be added to the overprocessed meals

in order to obtain a similar growth to those chicks receiving the control soyabean meal. Once again, chicks were reared to 15 days of age on the respective diets.

(e) Metabolizable energy of wheat by-products

Fifteen wheat samples of varied origin were assayed for proximate composition, neutral detergent fibre, and nitrogen corrected true metabolizable energy. On the basis of these results, a multi-step correlation was conducted to determine which components of proximate composition could best be used to develop a prediction equation for  $TME_n$ .

### III. RESULTS

(a) Metabolizable energy of meat and bone meal

The  $TME_n$  of the original meals and fractions is presented in Table 3. As expected, the  $TME_n$  of the meat plus fat fractions was always substantially higher than the complete meal due to removal of most of the bone. The effect of assaying meat and bone meal by separate fractions as opposed to a complete meal is demonstrated in Table 4. In the sample

Table 3.  $TME_n$  (MJ/kg) of original meat and bone meal (meal) samples, and of meat + fat (M+F) fractions (as is basis).

Experiment 1				Experiment 2			
Beef		Pork		Beef		Pork	
Meal	M+F	Meal	M+F	Meal	M+F	Meal	M+F
8.95	14.13	9.77	13.77	9.31	10.83	12.41	14.88

Table 4. Sample calculation of adjusted  $TME_n$  of meat and bone meal, and corresponding results of Experiments 1 and 2.

Sample Calculation: Beef meal, Experiment 1

$TME_n$  Whole Meal (Table 3) = 8.95 MJ/kg

$TME_n$  M+F (Table 3) =  $14.13^A \times 0.551^A = 7.787$  MJ/kg

$TME_n$  Bone =  $4.21^B \times 0.449 = 1.890$  MJ/kg

	Experiment 1		Experiment 2		Average	
	Beef	Pork	Beef	Pork	Beef	Pork
$TME_n$ (MJ/kg) complete meal	8.95	9.77	9.31	12.41	9.13	11.09
$TME_n$ (MJ/kg) sum of fractions	9.68	11.19	10.86	12.89	10.27	12.04
Improvement	+8.2%	+14.5%	+16.7%	+3.8%	+12.5%	+9.2%

<sup>A</sup> Table 2

<sup>B</sup> G.E. of bone (Table 1) x 0.75 (assumed digestibility, see text).

calculation, the determined  $TME_n$  of the meat plus fat fraction is multiplied by its percent in the respective sample. The estimated  $TME_n$  of bone is multiplied by the percent bone in the meal. These two results are added to obtain a  $TME_n$  for meat and bone meal independent of the possible confounding effects of high ash content. It will be noted that for both meals in both experiments a consistent increase in energy was observed when meals were evaluated as separate fractions.

(b) Calcium and phosphorus contents of meat and bone meal

The ranges in ash, protein, calcium, and phosphorus of the 10 samples employed is presented in Table 5. On the basis of the flotation studies, prediction equations were developed to estimate percent calcium and phosphorus. The resulting equations are:

$$\% \text{ calcium} = \text{mL of bone} \times 1.69 + 0.50, \quad R^2 = 0.92$$

$$\% \text{ phosphorus} = \text{mL of bone} \times 0.76 + 0.51, \quad R^2 = 0.91$$

Table 5. Ranges (g/kg) in protein, fat, ash, calcium and phosphorus of meat and bone meal samples included in flotation study.

	g/kg				
	Protein	Fat	Ash	Calcium	Phosphorus
High	659	127	423	147	69
Low	397	82	136	34	21

(c) Overprocessed soyabean meal supplemented with amino acids

Results of this study (Table 6) show that a clear depression in body weight gain and increase in feed conversion ratio was associated with overprocessed soyabean meal. Lysine supplementation, whether alone or in combination with other amino acids, significantly improved both body weight gain and feed conversion. However, neither methionine nor threonine, singly or in combination, had a positive effect on these production parameters.

Table 6. Effect of amino acid supplementation to diets containing overprocessed soyabean meal.

	15 Day Body Weight (g)	Feed Conversion (g:g)
Control	382 <sup>a</sup>	1.45 <sup>b</sup>
Overheated	246 <sup>b</sup>	1.97 <sup>a</sup>
Overheated + Lys <sup>A</sup>	358 <sup>a</sup>	1.55 <sup>b</sup>
Overheated + Met <sup>B</sup>	253 <sup>b</sup>	1.98 <sup>a</sup>
Overheated + Thr <sup>C</sup>	237 <sup>b</sup>	1.94 <sup>a</sup>
Overheated + Lys + Met	355 <sup>a</sup>	1.66 <sup>b</sup>
Overheated + Lys + Thr	348 <sup>a</sup>	1.60 <sup>b</sup>
Overheated + Lys + Met + Thr	347 <sup>a</sup>	1.66 <sup>b</sup>
Overheated + Met + Thr	227 <sup>b</sup>	1.84 <sup>a</sup>

a,b(P < 0.05)

<sup>A</sup>2.0 g/kg lysine HCl; <sup>B</sup>1.0 g/kg DL-methionine; <sup>C</sup>1.6 g/kg L-threonine.

(d) Lysine supplementation to overprocessed soyabean meal diets

A linear growth response was observed when increasing levels of lysine were supplemented to diets containing both overprocessed soyabean meals (Table 7). In this study, the amount of lysine added which supported growth equal to that of the control diet was estimated to be the amount of lysine destroyed, and by implication, the amount which must be added to the finished feed to overcome the effects of overprocessing. Under these conditions, approximately 0.5 g/kg lysine HCl and 1.0 g/kg lysine HCl were required to overcome the growth depressing effects of soyabean meal with solubilities of 0.66 and 0.54%, respectively.

Table 7. Effect of lysine supplementation to diets containing control and overprocessed soyabean meal.

Lysine Suppl. A (g/kg)	Control		Mildly Overprocessed		Moderately Overprocessed	
	82% Prot. Sol.		66% Prot. Sol.		54% Prot. Sol.	
	Weight (g)	FCR (g:g)	Weight (g)	FCR (g:g)	Weight (g)	FCR (g:g)
0.0	391	1.41	383	1.52	361	1.55
0.5	-	-	394	1.44	381	1.42
1.0	-	-	405	1.43	393	1.50
1.5	411	1.38	412	1.36	417	1.41

<sup>A</sup>Supplementation as lysine HCl.

(e) Metabolizable energy of wheat by-products

The proximate composition, neutral detergent fibre, and TME<sub>n</sub> of the fifteen samples of wheat by-products are presented in Table 8. On the basis of these data, the following prediction equations for metabolizable energy are presented, all adjusted to reflect an 87% dry matter:

$$\text{TME}_n \text{ (MJ/kg)} = 0.0042 [3157 - 116 (\% \text{ CF})], \quad R^2 = 0.67$$

$$\text{TME}_n \text{ (MJ/kg)} = 0.0042 [3497 - 39 (\% \text{ NDF})], \quad R^2 = 0.77$$

Data from a number of previous reports in the scientific literature provided 42 determinations of AME<sub>n</sub> in which crude fibre (but not NDF) was specified. From these a third prediction equation was developed, once again based on 87% dry matter:

$$\text{AME}_n \text{ (MJ/kg)} = 0.0042 [3086 - 165 (\% \text{ CF})], \quad R^2 = 0.77$$

## IV. DISCUSSION

Metabolizable energy studies with meat and bone meal, when evaluated as separate fractions, indicate a consistent increase when determinations are made without high ash content, substantiating the hypothesis of Martosiswoyo and Jensen (1990). Thus, when meat and bone meal is included in practical feed formulae, a metabolizable energy of between 10.25 and 11.92 MJ/kg appears justified depending on the amount of bone.

Table 8. Proximate composition (g/kg), NDF (g/kg), and TME<sub>n</sub> (MJ/kg) of wheat by-product samples (87% dry matter).

Sample	Protein	Fat	Ash (g/kg)	C.F.	N.D.F.	T.M.E. (MJ/kg)
1	182	47	37	39	189	12.22
2	238	69	55	27	138	12.19
3	147	40	33	42	208	11.19
4	151	31	31	43	216	10.99
5	133	23	15	9	68	13.30
6	152	32	33	51	252	10.18
7	153	33	43	70	311	9.89
8	148	32	53	132	395	9.17
9	142	21	47	95	378	7.27
10	131	40	42	66	295	10.15
11	124	21	75	99	412	7.67
12	149	27	46	80	361	9.45
13	147	21	41	61	301	11.06
14	147	37	34	60	256	10.30
15	151	29	41	81	331	6.96
Low	124	21	15	9	68	6.96
High	238	69	75	132	412	13.30
Average	153	33	41	64	275	10.13

Prediction equations for estimating percent calcium and phosphorus on the basis of flotation techniques have given positive initial results. Additional work is in progress to confirm whether these equations will satisfactorily predict the calcium and phosphorus content of new samples. It should be cautioned, however, that use of this technique should be restricted to providing estimates, as opposed to confirmed levels, of calcium and phosphorus. Similarly, it is recommended that feed microscopy be employed to confirm that the sediment actually being measured is composed of bone and not foreign substances of mineral origin.

In spite of strict quality control parameters, the nutritionist occasionally is faced with the prospect of having to use overprocessed soyabean meal in practical diets. In developing a strategy to address these circumstances, it appears that lysine is the only amino acid currently available at competitive prices for which supplementation is indicated. Initial studies suggest that 0.5 and 1.0 g/kg of lysine HCl should be adequate to overcome the negative effects of mildly and moderately overprocessed soyabean meal, respectively. Further work is in progress to better define these levels of supplementation.

A number of factors contribute to a wide variation in the nutritive quality of wheat by-products. Equations presented should be of assistance to the nutritionist in establishing the appropriate energy value for use in formulation. It might be suggested that the TME<sub>n</sub> value obtained using Leghorn roosters might be most suitable for older birds, while the equation based on published AME<sub>n</sub> data could be more appropriate for starting pullets and broiler chicks.



## V. CONCLUSIONS

The manufacture of optimum quality poultry diets depends in large part on an appreciation of the variation in the nutritive quality of feed ingredients. In addition, strategies must be in place to deal with unexpected variability in ingredient quality. While long-term relationships with ingredient suppliers are the single most beneficial means of improving and maintaining ingredient quality, data presented herein may be of assistance to the nutritionist in dealing with situations which inevitably occur in commercial practice.

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## EFFECTS ON THE PERFORMANCE OF POULTRY OF MANUFACTURING FEED USING EXPANSION PLUS PELLETING COMPARED WITH PELLETING ALONE

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### Summary

Upstream feed processing using expansion prior to pelleting is a process being employed in the production of broiler diets. Expansion employs high pressure (20 bar) and high temperature (+120°C) for short periods of time (3-4 secs) prior to a rapid decrease in pressure to precondition feed. The advantages of this technology are poorly defined.

In two experiments the live-weight gain and feed conversion efficiency of broilers over the period from 1 to 50 days of age were improved by expansion prior to pelleting, the effects being consistent but non-significant. There was a processing x sex interaction with females responding more to the expanded diets than males. This was probably due to a reduction in the ileal digestibility of the protein and lysine in the diets that were heat treated (pelleted and expanded before pelleting). Feed processing improved the microbiological status of the feed and the pellet characteristics.

A wide range of performance and diet characteristics need to be considered in assessing the advantages of expansion.

### I. INTRODUCTION

As cereal costs continue to rise there is added incentive when manufacturing feed for poultry to use alternative raw materials, such as byproducts, and to adopt technologies which maximise the digestibility of nutrients in the feed. Furthermore, increasing the digestibility of nutrients decreases the indigestible fraction thereby reducing faecal material and contributing to a lowering in environmental pollution.

Biological processing of poultry diets by supplementation with enzymes which target specific antinutritional factors in the diet is a relatively recent innovation but which is now widely adopted. Use of enzymes offers advantages of improved energy and protein digestibility and improved bird performance. However, for the past twenty years almost all poultry feed has been mechanically processed using combinations of heat, pressure and moisture, with the simplest example being pelleting. The advantages of pelleting poultry feed is well documented but the advantages of more advanced forms of mechanical feed processing, such as expansion before pelleting, are poorly documented. The objective of expansion is to condition feed mash up-stream from the pellet press, not to process finished feed.

### II. METHODS

The objectives of the experiments were to measure broiler performance and to provide data for a cost benefit analysis of the manufacture and feeding of expanded diets. Expansion is a process whereby meal is driven through a barrel of decreasing diameter by one or two augers and water and/or steam is injected at various points. The pressure forcing the feed through the barrel and over a cone at the barrel exit creates frictional heat,

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Rhône-Poulenc Animal Nutrition, Antony, France.

producing temperatures in the region of 130 °C for a short dwell time for 3 - 4 sec which cooks or partially cooks the feed. The conditioned material is forced out over the cone in a « fluid » state. The cone is hydraulically tensioned and its movement alters the pressure in the expander and, thus, the energy imparted to the feed. The material leaving the barrel expands rapidly as the pressure is released, with consequent moisture loss and rupture of physical structure which can result in « puffing » or expansion of the feed giving a crumbly or doughy mass. Two experiments, each with the same experimental design, in which feed was either pelleted or expanded before pelleting are described.

#### (a) Animals and Management

A total of 1920 day-old sexed broiler chickens (Ross broilers) were randomly allocated by weight to one of four dietary treatments each with six replicates (6 male and 6 female) of 40 birds per replicate. The birds were reared in floor pens (4.08 m<sup>2</sup> per pen) covered with a deep litter of wood shavings. To maintain stocking density in the pens eight reserve pens containing birds of the same age receiving the same experimental diets as the birds on the experiment, were maintained. These reserve birds were used to replace birds in the trial (birds of the same sex, receiving the same diet and with similar body weight) which were lost due to ill health or mortality. The experiment was carried out in a temperature-controlled broiler house (36°C during the first 2d, and 32, 30, 28 and 25°C, respectively, to the end of the first, second, third and fourth to seventh weeks) in which relative humidity was controlled between 50 and 70%. Individual body weights were recorded on days 1, 30, 42 and 50. Food intake was measured per replicate group (days 1-30, 30 to 42 and 42 to 50). At the end of the growing and finishing periods (41 and 49 days) samples of litter were collected from each pen and stored at +4°C in sealed plastic boxes. At the end of the trial these samples were ground, mixed and analyzed for dry matter, nitrogen and free ammonia concentration.

#### (b) Experimental diets

In each experiment the four test feeds which received the different processing treatments had identical ingredient composition. Starter, grower and finisher diets were prepared for each of the test meals and all were mixed at an on-site mill. The ingredient composition of the starter, grower and finisher diets is shown in Table 1. All the feed was prepared as mash and then subjected to the different processing treatments. The starter,

Table 1. Diet compositions (g/kg).

Feed ingredients	Starter		Grower		Finisher	
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
Yellow maize	566	536	639	616	674	654
Soyabean meal	279	298	213	226	176	190
Poultry by-product meal	60	60	60	60	60	60
Meat & bone meal	20	20	20	20	20	20
Fat (animal)	47	57	43	50	45	51
Premix + minerals	27	29	26	28	24	26
AME (MJ/kg)	13.39	13.39	13.60	13.60	13.81	13.83
Crude protein (g/kg)	229	229	203	203	187	187

grower and finisher diets were offered to birds from 1 to 21 days of age, 21 to 42 days of age, and 42 to 50 days of age, respectively. Slight modifications to the management procedures during the two experiments are indicated in Table 2.

Table 2. Modifications to the experimental procedure in the two experiments.

Diets	Periods (days)			
		Starter	Grower	Finisher
Mash (A)	Exp 1	1-29	30-42	43-49
Pelleted (B)				
Expanded + Pelleted (C)	Exp 2	1-21	22-42	43-49
Expanded + Crumbled (D)				

Diet A was offered to the birds in mash form.

The remaining three diets were sent to the « Internationale Forschungsgemeinschaft Futtermitteltechnik (I.F.F.) » Germany to be processed as follows:-

Diet B preconditioned with 3% saturated steam and pelleted through a 3mm die (35 mm long).

Diet C expanded and pelleted through a 3 mm die.

Diet D expanded + crumbled.

Part of both the pelleted and expanded-pelleted diets were crumbled for birds from 1 to 7 days of age.

During production of the feed the diets were preconditioned by adding 3% saturated steam. During expansion, feeds were compacted in the expander to approximately 20 bars and the temperature of the feed increased to between 115° and 120°C just before the outlet of the expander. When the material left the expander the drop in pressure created a water evaporation equivalent to a 0.5% drop in moisture content. The specific energy requirement of the expander was 20 kWh/t.

### (c) Statistical analyses

Performance data and parameters related to litter quality were subjected to analysis of variance on the basis of group means (Statgraphics Release 5.00). Significant differences between treatments were determined using Newman-Keuls multiple range test.

## III. RESULTS AND DISCUSSION

In Experiment 1 there was no effect on feed intake as would have been expected from pelleting. Feed intake of birds in each of the four groups did not differ. Conversely, in Experiment 2, feed intake of birds given mash diets was significantly lower than that of birds given the processed diets. Total feed intake (1-49 days of age) was 4543, 4945, 5025 and 4788 g for birds given Diets A,B,C and D, respectively ( $A < D < C = B$ ;  $P < 0.05$ ).

The effects of feed processing incorporating upstream annular gap expansion on the weight gain and feed conversion efficiency of broilers over the total growth period from 1-50 days is shown in Figure 1 for both Experiments 1 and 2.

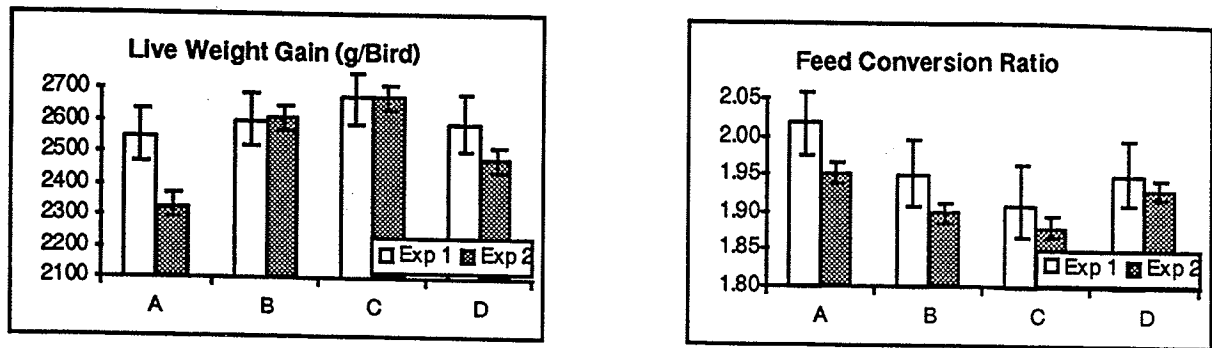


Figure 1. Performance of broilers offered either mash or pelleted diets or the same diets expanded before pelleting and grinding.

Over the period 1-50 days of age, feed processing (pelleting or pelleting plus expansion) tended to improve live-weight gain and significantly improved feed conversion efficiency. The trend was for processing to increase live-weight gain although it was only significant in Experiment 2 ( $P < 0.001$ ) (Experiment 1,  $P = 0.112$ ) as a result of the marked increase in feed intake. Compared with pelleting alone there was a trend in both experiments for weight gain to be further increased by expansion prior to pelleting. In both experiments feed conversion efficiency was improved by pelleting and further improved by expansion of the diets prior to pelleting (Experiment 1,  $P = 0.016$ ; Experiment 2,  $P < 0.001$ ). Expansion prior to crumbling also tended to improve feed conversion efficiency compared with the mash diet. In both experiments there was a significant processing  $\times$  sex interaction ( $P < 0.01$ ) with female broilers responding with significant increases in liveweight gain whereas males did not consistently respond to the processing treatment with increased weight gain. The results suggest an additional advantage from expansion compared with pelleting alone.

Table 3. Ileal digestibility of protein, lysine and methionine and microbial status of the diets.

	A	B	C	D	SEM	P Value
<b>True ileal digestible amino acid coefficients of grower feed</b>						
Protein	0.898a	0.884b	0.889b	0.887b	0.0099	0.05
Lysine	0.848a	0.832b	0.831b	0.825c	0.0196	0.001
Methionine	0.946	0.936	0.941	0.939	0.0118	NS
<b>Microbiological status of grower feed</b>						
Total aerobic/g						
Experiment 1	28000	12000	9000	1000		
Experiment 2	30000	20000	10000	60000		
Yeast and fungi/g						
Experiment 1	1590	110	180	320		
Experiment 2	4000	40	40	60		

In Experiment 1 the ileal digestibility of protein and amino acids was measured in caecotomized cockerels by the method described by Green *et al.* (1987) (Table 3). Processing of the diets using either pelleting alone or pelleting plus expansion significantly

reduced ileal digestibility of protein and lysine. There was no additional adverse affect on protein and amino acid digestibility from expansion compared with pelleting alone. Reduction in protein digestibility and, in particular, reduced digestibility of methionine and lysine may account for the treatment x sex interaction. Indeed, growth of male birds would have been more sensitive to a reduction in protein availability compared with females. The drop in amino acid digestibility was greatest for lysine (mean -1.86), probably related to the heat treatment and either denaturation of lysine or formation of Maillard type reaction products.

Counts of aerobic bacteria and yeasts/fungi all decreased significantly following processing. Yeasts and fungi showed the greatest decrease. Counts of aerobic bacteria were highly variable but generally were reduced. Expansion did not show a significant advantage over pelleting alone.

#### IV. CONCLUSIONS

Performance of these birds given maize-based diets was improved when expansion was employed upstream in the production process. Both liveweight gain and feed conversion tended to improve. The effects of heat on protein and lysine availability must be considered in order to achieve the optimum response from expansion. The microbiological quality of the feed was improved by processing although in these two experiments there was no significant advantage from expansion. Bulk density of the pellets was increased by expansion and pellet quality was improved. There was no consistent effect on litter quality (data not shown). In assessing the potential benefits from including expansion in the feed production system a wide variety of effects need to be taken into consideration in order to optimise the performance of the birds.

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## POULTRY GENETICS 1950-1997: SOME UNEXPECTED SIDE EFFECTS

P. HUNTON

### Summary

While the world's poultry breeders have provided the commercial industry with products demonstrating consistently improved performance and efficiency over the past half-century, this has not come without cost in terms of side-effects. These include ascites, obesity, leg problems and immune response problems in meat stocks, and Marek's disease and calcium metabolism in layers. While these are clearly disadvantageous, it is proposed that they may have been inevitable, given the demands of industry for ever-more-productive commercial products. Correcting the problems is being actively addressed by breeders in the last decade of the 20th century but there is little assurance that further examples will be avoided, based on the extremely competitive nature of the breeding business.

### I. INTRODUCTION

The application of population genetics to poultry breeding has made a huge contribution to the poultry industry during the past half-century. Once the initial work of pioneer animal breeders and geneticists like Hutt, Lush, Lamoreux and others was confirmed by practical experience, breeders were able to practice selection on a scientific basis with the reasonable expectation of consistent progress. This process was driven quickly by ferocious competition at the level of commercial producers of eggs, broilers and turkeys, and, latterly, ducks as well.

Breeders' horizons, however much they may have wished otherwise, were inevitably reduced to providing competitive stocks just for the next generation. They have never had the luxury of considering the long-term consequences of selection practices, even if these were predictable, due to the necessity of generating a continuously competitive product.

So it should not come as a surprise that some unexpected consequences have arisen as an indirect result of selection procedures which have been, at least in the case of meat chickens, more extreme than most of those used in fundamental research studies on which practical selection procedures were based. Whether these consequences could have been predicted is debatable: some of them may have been but, because of the competitive nature of the poultry breeding business, it is doubtful if predictions would have resulted in any change in breeding policy.

This is not intended as a criticism of the primary breeding industry: the point is that they had little choice in the breeding strategies in which they were involved. During the preparation of this paper, my attention was drawn to the book "Why Things Bite Back: Technology and the Revenge of Unintended Consequences" (Edward Tenner, Princeton Univ.) Although not quoting the poultry industry as one of his examples, which is small fry in the big technology picture, Tenner identifies the introduction of DDT as a widely used pesticide, and the introduction of the kudzu vine, now considered a weed but originally used as a soil conditioning aid, in the US.

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A further example of wisdom after the event, but valuable comment nevertheless, was that by Emmerson (1996), who urged caution in the adoption by the poultry meat industry of some of the products of molecular genetics research, and a holistic approach to future breeding developments.

## II. MAREK'S DISEASE

The classic work of Hutt and Cole at Cornell showed that resistance and susceptibility to an infectious disease could be altered by genetic selection. Over a number of generations they developed lines C and K which were resistant, and line S which was susceptible, to the complex which they described as Leukosis. As it turned out these lines exhibited resistance and susceptibility to both Lymphoid Leukosis (LL) and Marek's Disease (MD), once the two were differentiated in the late 1960's. For a review of this work see Cole and Hutt (1973).

Cole subsequently developed lines N and P, which were respectively resistant and susceptible strictly to MD (Cole, 1968). This selection program was based on a progeny test in which pedigreed chicks were exposed to a virulent MD virus at an early age. All mortalities, and remaining birds surviving to 8 weeks of age, were examined for MD lesions, and parents selected based on these data. The lines became clearly differentiated after only two generations and, as a result, several primary breeders began to undertake modified programs based on Cole's results. These programs had to be carried out at remote locations to minimize risk to valuable pedigree stocks. Sibs of the selected stocks were exposed to MD and selection of relatives based on the response of the exposed individuals. Clearly, this was an extremely expensive proposition for the breeders and when effective vaccines became available at economic prices most of these programs were quickly abandoned. The cessation of selection probably happened before resistance was fully developed. Subsequent research which identified MHC loci associated with resistance to MD was also applied by some primary breeding companies. It was shown that Cole's N-line was almost homozygous for B<sup>21</sup>, the allele associated in many studies with high levels of resistance to MD. However, there are questions concerning these so-called "resistant alleles" and some stocks containing them have been found to be relatively susceptible to MD (Hartmann, 1996)

Subsequently, variant MD viruses have been identified to which the standard vaccines afford little protection. Recent experience at one commercial egg production unit in New York state serves as an example. While it can be argued that the particular production systems in use at this location (multiple ages; no biosecurity between flocks; pullets and layers in close proximity) might predispose any stock to infection there is good evidence (see Gavora, 1989) that genetically resistant stocks perform better in the face of field challenge. However, economic and competitive pressures ensured that once vaccines were available genetic resistance to MD had a much reduced priority in breeding programmes.

The long-term effects of the use of vaccines and/or development of genetically resistant stocks are hard to define. One view might be that without them the incidence of MD would be much higher. However, another view expressed by Gavora (1996, Personal Communication) is that the evolution and emergence of highly virulent MD viruses may be partly due to the greater resistance of the birds, brought about by a combination of genetic selection and the use of vaccines.

While the new, highly virulent, MD viruses are partly controllable by the use of polyvalent vaccines, this is an expensive process. The prospects of improved vaccines



developed with the aid of molecular genetics technology, or highly resistant chicken genotypes, seem equally remote today.

### III. ASCITES

In older texts on poultry diseases, ascites is usually described as a condition restricted to high altitudes, resulting from reduced partial pressure of oxygen, leading to oxygen depletion in the birds.

In the past two decades ascites has been reported in high performance meat stocks at sea level. While still associated with oxygen demand the contemporary ascites is the result of excessive oxygen demand rather than diminished supply.

Julian (1995) has provided excellent documentation of the development and pathology of the modern condition and has described it as Pulmonary Hypertension Syndrome (PHS).

The phenomenon of extremely rapid growth, resulting from many generations of selection for growth, appetite and related traits, dramatically increases the demand for oxygen in the metabolic process. Rapid growth on its own may result in development of PHS but it will be aggravated by other factors such as low environmental temperature, sub-optimal air quality and/or ventilation and, of course, altitude.

There is good evidence of genetic variability in the incidence of PHS (Schlosberg *et al.*, 1996; McKay, Personal Communication, 1996) so there is no reason why breeders should not produce PHS-resistant varieties and, indeed, most commercial breeders are presently practicing selection for just this objective. However, this will almost certainly be at the expense of further gains in growth since the PHS seems to be at least partly a consequence of rapid growth in the first place. In addition, PHS-resistant stocks may not be much of an advantage in areas where the condition is not a problem.

### IV. OBESITY

For at least a quarter of a century, selection of meat chickens was based on mass selection with the primary criterion being weight-for-age or some variant. Secondary criteria included such traits as leg strength and body conformation. While some limited attention was paid to reproductive capability most gains were accomplished by environmental and nutritional manipulation.

In the early days of the broiler industry the vast majority of chickens were purchased whole by the consumer and abdominal fat could be easily hidden at point of sale. During the 1970's, it became apparent that the reputation of chicken as a low-fat meat choice could no longer be sustained as a generality. Large fat deposits in the subcutaneous and abdominal areas became obvious and were identified as an industry problem. The abdominal fat pad in particular was targeted for at least two reasons. First, it was clearly evident to consumers who purchased whole birds, since processors were at pains to leave as much fat with the carcass as possible to enhance "yield". Secondly, those customers purchasing cut-up chickens, primarily the fast food operations like KFC, declined to accept the abdominal fat pad as part of their purchase. Not only was it aesthetically undesirable but it also led to adulteration of cooking oil (largely unsaturated) with quantities of saturated fat with a consequent reduction in its "life".

Although body fat synthesis is "expensive" in terms of substrate required, it requires substantially less "effort" than synthesis of muscle protein. In many cases, dietary lipid becomes body lipid without degradation and re-synthesis. Since the substrates, feed

carbohydrate and fat, are limited only by appetite, those birds with the largest appetites could "afford" to lay down excessive fat in order to achieve the superior body weight demanded by the selection criteria.

During the 1970's and 1980's breeders began to address this problem in various ways. Substantial publicly funded research provided ample guidelines (e.g. Pym and Nicholls, 1979; Whitehead and Griffin, 1984; Leenstra and Pit, 1988a,b; Leenstra, 1988). The cost in nutrients meant that the most obese birds were also the least feed-efficient. So, fortuitously, breeder and consumer interests coincided and selection for improved feed efficiency and/or reduced obesity met the requirements of both parties.

However, selection for these new criteria demanded greatly increased cost and complexity in the selection programmes of meat breeders. To select for feed efficiency directly involves individual measurements of feed intake. Selection for reduced abdominal fat pad weight may involve the slaughter of subject birds and breeding from relatives. Both necessitate the use of partly or fully pedigreed populations which many breeders did not have in the period of mass selection based on weight-for-age.

## V. THE "LAZY" IMMUNE SYSTEM

Cook (1996) proposed a challenging hypothesis: "Current selection for growth and feed efficiency invariably selects for immune suppression". Maatman *et al.* (1993) has shown that broilers tend to be less responsive to antigens than Leghorns and Siegel (1996) coined the term "Lazy Immune System" to describe the phenomenon. The apparent susceptibility of broiler chickens to a wide variety of relatively mild viral and bacterial infections lends support to Cook's hypothesis. Infections such as Infectious Bronchitis, Newcastle Disease, Infectious Laryngotracheitis and *E.coli* are examples. For most of them, the consequences in broilers are much more severe than in layer stocks in comparable conditions. In addition, broilers often respond relatively poorly to vaccines administered to protect them from these pathogens.

Cook bases his hypothesis on the environment in which selection takes place. Most primary breeders attempt to simulate commercial conditions, at least as far as stocking density and nutrition are concerned, for their selection environment. While they may go to considerable lengths to ensure absence of some pathogens like *Mycoplasma*, others are undoubtedly present. But the main danger, according to Cook, is the high concentration of endotoxins present as a consequence of the birds contact with faeces and litter, which are heavily laden with bacteria. The endotoxins may be ingested, inhaled, or enter via skin abrasions to cause immune stimulation. Cook states: "The bird that grows the best and converts feed most efficiently, and thus the bird selected for the breeding programme, is the bird least likely to have an immune response to the immune stimulants."

One of the main components of the immune system affected is macrophage number and effectiveness. Cook states that immunologists have great difficulty harvesting these cells from contemporary broilers. Considerable work by Klasing in California, Cook and associates in Wisconsin, and Siegel and associates in Virginia have demonstrated interactions between nutrition and the functioning of the immune system. To add a genetic component to this equation is a natural extension and methods must be found to include some criterion of immunological function in selection programmes. When these relationships become better understood there is good reason to anticipate that progress at the commercial level could become a reality.

However, this is another reminder to us of the warnings sounded by Tenner, that it will be unwise to regard even broad immunological criteria in isolation from the overall

"success" of the birds in evolutionary terms. Attempting to move too fast has, in the past, most likely disturbed both developmental and genetic homeostasis.

## VI. LEG WEAKNESS IN CHICKENS AND TURKEYS

Dire warnings have been sounded for at least four decades that growing chickens and turkeys are becoming so heavy that their legs can no longer support their body mass. Body weights at slaughter, or at specific ages, for both species have at least doubled since serious selection began to be practiced on populations used in commercial industry. Accompanying these developments has been a steady increase in the numbers of lame and/or crippled birds, and this is amplified when, for example, broiler chickens are grown to roaster weights of 3.0 to 4.0 kg.

Breeders have always given attention to leg strength as a secondary selection criterion, usually in the form of an independent culling level. With chickens this has been important because among the breeding population natural mating must be accomplished and, while this does not apply to turkeys, selection has been undertaken strictly in order to prevent lameness. Nixey (Personal Communication, 1996) has observed that in his experience, although the modern turkey is no longer able to mate naturally, it is much more stable in terms of ability to maintain suitable posture than the contemporary chicken at an equivalent stage of development. Originally, most of the selection work aimed at leg strength and posture was undertaken by skilled handlers who were able to distinguish the desirable from the undesirable by direct observation. When the various forms of leg aberrations were distinguished more sophisticated means of identification became available. For example, the lixiscope, used by Ross Breeders, is able to identify early signs of tibial dyschondroplasia.

In view of the very large gains in body weight and the relatively low level of leg problems we may regard this as a qualified success story: things could have been a lot worse had it not been for the arduous work of the expert handlers who observed the early generations of chickens and turkeys.

## VII. CALCIUM METABOLISM IN LAYERS

Today's layer yields over 20 kg of egg mass by 500 d of age. Of this, approximately 2.0 kg is egg shell comprising >90% calcium carbonate, slightly more than the hen's body weight. In addition to synthesizing approximately 5.0 g of calcium carbonate for each day's egg the hen must also maintain the integrity of a complex skeletal system. Failure of any component of the calcium metabolism or mobilization systems may result in poor egg shell quality, cage-layer fatigue (osteoporosis) or brittle bones, in a variety of combinations.

Selection for shell quality in layers has been directed mainly at egg shell thickness or at various indexes related to it, e.g. egg specific gravity, shell deformation, breaking strength. In addition, body weight has been reduced in order to achieve improved feed conversion efficiency. The skeleton has been ignored to a large extent. As egg yields continue to rise calcium demand for this alone increases proportionately and the risk of imbalance between dietary calcium, shell demand and skeletal demand also rises.

While this has not, at this point, resulted in long-term, insoluble problems, it represents an area in which future challenges lie.

## VIII CONCLUSIONS

Commercial geneticists have wrought massive changes in the performances of laying hens, meat chickens and turkeys during the past half-century. Selection programmes are driven by the industry's insistence on continuous improvements in primary commercial traits like egg production and feed efficiency in layers, and rapid growth, body conformation and feed efficiency in meat birds.

Some of the side-effects might have been predicted, but commercial pressures may have limited the breeders' ability to respond until the effects became economically unacceptable. When this has occurred breeders have had to re-direct their selection strategies and, in most cases, add complexity to their programmes. Stocks have responded as desired but progress in the primary commercial traits will inevitably slow down. Some of the side-effects, for example the immune responsiveness of meat chickens, have yet to be addressed.

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## BROILER ASCITES SYNDROME: A REVIEW

P. GROVES

### Summary

A summary of the most recent theories on pathogenesis of the ascites syndrome in broiler chickens is presented. Methods used to experimentally reproduce the syndrome are compared, with caution expressed on extrapolation of results from studies using hypobaric conditions to field situations where the major inducer of the syndrome is hypothermia. The role of genetic selection in the increase in incidence is discussed and indications drawn from local work indicating that differences in susceptibility between broiler breeds may be most related to selection for greater muscle mass relative to cardio-pulmonary capacity. Recent research results are discussed.

### I. INTRODUCTION

The ascites syndrome or pulmonary hypertension syndrome of broilers has been the subject of an enormous amount of research over the past six to eight years, reflecting the importance that this condition has achieved in worldwide chicken meat production. An international seminar on this disease was held in Arkansas in January 1997 allowing the most up to date progress to be presented. The multifactorial nature of the ascites syndrome is readily evident just from studying the program for this meeting, covering possible interactions of genetics, hatchery management, growout management, environment, nutrition, metabolism, growth rate and other diseases.

In this paper just a few aspects of the syndrome will be reviewed, with particular emphasis on how they relate to the occurrence of the disease in Australia and recent research into its control.

### II. CURRENT THEORY ON PATHOGENESIS

Wideman and Bottje (1993) have reviewed this area comprehensively. Genetic selection for improved growth rate, feed efficiency and greater meat yield has resulted in a proportion of birds within a broiler population having an inherent potential to outgrow their cardio-pulmonary capacity. With an increase in the relative size of a rapidly growing muscle mass to organ ratio, the bird requires additional blood to be supplied by the heart to support this growth. This increased blood flow leads to an increased blood flow through the lungs (pulmonary hypertension). This increased pulmonary blood flow leads to capillary transit time reduction and the bird becomes more susceptible to an imbalance of blood perfusion and respiratory diffusion, resulting in hypoxaemia.

Hypoxaemia leads to a polycythemia as a result of erythropoietin stimulation. This increases blood viscosity, leading to the need for greater cardiac effort and a further increase in pulmonary hypertension. Progression of the inherent distension and hypertrophy of the right ventricle causes right atrio-ventricular valve insufficiency and congestive heart failure, ascites and oedema is the end result.

Wideman and Bottje (1993) further point out that this progression can be affected by two commonly encountered environmental problems. Firstly, low barometric pressure due

to altitude can result in hypoxia. In response to hypoxia, the pulmonary arterioles undergo constriction. This can substantially increase pulmonary vascular resistance and enhance pulmonary hypertension. The other potent ascites inducer is hypothermia. This stimulates increased metabolic rate and, thus, heat production. Systemic pre-capillary arterioles dilate to allow increased tissue oxygen uptake. This response requires increased cardiac output which in turn elevates pulmonary blood pressure.

### III. EXPERIMENTAL REPRODUCTION OF ASCITES SYNDROME

To evaluate hypotheses on factors involved in the ascites syndrome, it is necessary to work with an experimental system that will consistently reproduce the disease. The syndrome first became apparent in countries growing chickens at high altitudes (>1500m) [eg. Mexico, South America, South Africa, Kenya] and remained as a problem only in these areas for some years.

Appropriately, many researches adopted low barometric pressure, either by using high altitude or simulated high altitude in hypobaric chambers. Several workers (Owen *et al.*, 1990; Witzel *et al.*, 1990) developed successful models based on this method of ascites induction. Ascites levels can be exceptionally high under these conditions, especially if concurrently affected by low environmental temperatures.

Over the last 10-12 years, however, ascites has become a common problem in birds reared at lower altitudes, including Australia. The condition appears identical and often results under the hypobaric model are assumed to give identical performance under low altitude conditions. This may not always be a safe assumption, as will be alluded to below.

Ascites may be induced at low altitudes using ambient temperatures below the chicken's thermoneutral zone. Julian *et al.* (1989) produced significant increases in ascites incidence by lowering ambient temperature to 13°C after 22 days of age. In a factorial experiment Verstegen *et al.* (1989) induced ascites by gradually decreasing ambient temperature from 30°C down to 16°C by 14 days and further to 11°C by 30 days. A comparable group kept above 25°C till 14 days and then subjected to a decline to 11°C at 30 days were less affected indicating that low ambient temperatures in the first two weeks predispose to later ascites. Field observations support this finding (Groves, 1991).

Deficiencies in ventilation and, hence, questions of air quality have also been incriminated as being involved in the production of the ascites syndrome. Maxwell *et al.* (1989) described a higher incidence of cartilaginous and osseous nodules in the lungs of birds in poorly ventilated chicken houses. Lung damage has been incriminated in the aetiology of ascites and ascitic chickens have a higher number of these nodules in their lungs, but a causal association has not been demonstrated.

Ventilation deficiencies and the build up of toxic gases have been proposed as causes of ascites but this has come more from inference than from controlled experiments. Dale and Villacres (1986) noted that increased ventilation had been one of the few effective methods to reduce ascites in high altitude situations as long as cold stress was avoided. Closer to sea level, however, ventilation has been less successfully employed to control the syndrome. Julian and Wilson (1992) measured the oxygen and carbon dioxide concentrations in air in numerous pens of broilers and were unable to show any differences in gas concentrations in pens with high or low levels of ascites. In this study, oxygen concentration inside the house was only marginally reduced compared to the outside air (20.50% compared with 20.85% respectively) even though carbon dioxide levels were elevated compared to outside air (around 0.37% compared to 0.04% respectively).

Shlosberg *et al.* (1992) compared a well ventilated and an extremely poorly ventilated shed at low altitude (690m). Poor ventilation, which resulted in ammonia concentrations of 70 ppm and high humidity, was only able to decrease oxygen concentrations to 20.4% (compared with 20.7% in the well ventilated control and 20.9% outside) and no differences in ascites incidence due to ventilation differences were found.

Jones (1995) studied different oxygen concentrations without altering barometric pressure and concluded that sub-optimal oxygen concentrations did not increase ascites susceptibility.

Maxwell (1990) reported that reduced ventilation in winter coupled with increased gas-fired heating could lead to carbon monoxide concentrations of up to 70 ppm which would lead to hypoxia and hence ascites. He also suggested increased carbon dioxide, ammonia, humidity and dust could be involved.

Balog *et al.* (1994) induced ascites using an extremely low ventilation rate (beginning at 0.003m<sup>3</sup>/minute/bird at 5 weeks) and was able to somewhat reduce the disease under these conditions using ceiling fans, concluding that the fan prevented air stratification and, thus, diluted toxic gases.

These last two examples would have to be considered extreme for commercial conditions. Experience in Australia concurs with Israeli results (Shlosberg *et al.*, 1992) that ventilation plays little part in ascites and cold temperatures are the major inducer.

Ascites in Australia appears to be largely a New South Wales phenomenon with occasional problems in southern Queensland. It would be expected that the colder states would be most afflicted by ascites. Generally, shedding in South Australia and Victoria is designed to handle cooler temperatures, having a larger proportion of controlled environment sheds and making more use of hot air brooding (space heating) methods. In NSW sheds are predominantly open sided relying on natural ventilation and using radiant heaters (spot brooding) for early warmth. Queensland enjoys much milder winters and hence this type of housing copes much better. Increased ventilation under Queensland conditions often incurs only a small degree of cold stress.

Thus, it would appear that improvements in ventilation may only assist where low barometric pressure is the major inducing factor for ascites, which makes some sense as oxygen partial pressure is already reduced at altitude and further respiratory stress exacerbates the hypoxia. However, where the main factor causing ascites is low temperature (i.e. the hypoxaemia is caused by a non-respiratory demand for oxygen) where the effects on oxygen concentration will be negligible, improvements in air quality offer no decrease in the incidence of ascites.

#### IV. EFFECTS OF GENOTYPE ON ASCITES SUSCEPTIBILITY

Since the earliest emergence of the ascites syndrome it was obvious that certain strains and crosses of commercial broilers were more susceptible to the condition (Hargis and Odom, 1990; Groves 1991). It has been suggested that with intense genetic selection for improved growth rate, feed efficiency and meat yield the relative size and performance capabilities of the broiler's cardio-respiratory system has been diminished, possibly to a point where it has reached its metabolic limit.

Several studies have been undertaken to compare chickens of various types and ascites susceptibility. Vidyadaran *et al.* (1987) and Vidyadaran *et al.* (1990) compared anatomical respiratory parameters of a modern egg layer strain with the red jungle fowl, the putative progenitor of the domestic chicken. These workers demonstrated that the modern layer has a 33% smaller lung volume/body weight ratio and its blood gas tissue barrier is

28% thicker than its wild ancestor. Julian (1989) measured the lung volume of a modern broiler strain and demonstrated a decline in the lung volume/body weight ratio with age. Actual figures from these three studies have been adapted for comparison in Table 1.

Although different measurement techniques were used by these researchers, it can be judged that the lung volume/bodyweight ratios for modern layers and broilers are similar and both are reduced compared to the red jungle fowl. This observation gives credence to the theory described above on genetic selection for performance traits selecting against lung size. This is interesting as genetic selection of layer and broiler strains have taken quite different paths for many years, targeting totally different performance traits. Lung size *per se* cannot be the full answer however, as the lung volume/bodyweight ratios of broilers appears to be similar to that of layers which are not susceptible to ascites under normal chicken rearing conditions.

Table 1: Lung volume/bodyweight ratio in different genotypes.

Bird Type	Age	Lung Volume / Bodyweight (mm <sup>3</sup> /g ± SEM)	Source
Red Jungle Fowl	7 d	25.81 ± 2.80	Vidyadaran <i>et al.</i> 1987
	30 d	17.18 ± 2.69	
Commercial Layer	7 d	10.59 ± 0.93	
	30 d	15.81 ± 0.57	
Red Jungle Fowl	Adult	18.10 ± 1.90	Vidyadaran <i>et al.</i> 1990
Commercial Layer	Adult	14.65 ± 3.17	
Broiler	7 d	19.9* ± 1.76	Julian, 1989
	24 d	17.4 ± 0.87	

\* Converted to mm<sup>3</sup>/g.

A trial comparing five broiler lines available in Australia (Groves and Cross, Unpublished results) showed no significant difference in lung volume/bodyweight in normal birds at 28 days (Table 2).

Table 2: Lung size in broiler strains in Australia.

Broiler Strain	Ascites Susceptibility	Lung Volume / Bodyweight (mm <sup>3</sup> /g ± SEM)	Right Ventricular / Total Ventricular Weight Ratio
J	High	9.64 ± 0.36	0.229 ± 0.023
K	High	9.24 ± 0.18	0.214 ± 0.017
L	High	10.55 ± 0.96	0.231 ± 0.010
M	Low	10.79 ± 0.86	0.208 ± 0.013
N	Intermediate	12.25 ± 0.89	0.215 ± 0.015



Huchzermeyer *et al.* (1988) compared four broiler strains at high altitude and found that the two more ascites-susceptible strains had higher ventricular/total ventricular weights than the two lines of lower ascites susceptibility. As can be seen from Table 2, the study on broiler strains in Australia failed to show any significant differences in this parameter.

Martinez *et al.* (1992) in a little known study compared heart valve diameters of two broiler strains with a layer strain (White Leghorn) at various ages. At 3 weeks of age both atrio-ventricular valves and the aortic semilunar valve of the broilers were larger than those of the layer strain; however, the pulmonary semilunar valves of the broilers had smaller diameters than those of the layers. This area needs more research as this lack of development of the pulmonary semilunar valve could be consistent with the development of right sided congestive heart failure (pulmonic stenosis).

Other anatomical measurements were taken on the five broiler strains studied in Australia (Groves and Cross, unpublished results; Table 3).

Table 3: Anatomic and carcass characteristics of broiler strains in Australia.

Strain	Keel Length/wt 28 days	Shank Length/wt 28 days	Breast Fillet, % carcass 49 days	Thigh Fillet, % carcass 49 days	Drumsticks, % carcass 49 days
J	62.5 <sup>a</sup>	35.4 <sup>a</sup>	21.3 <sup>b</sup>	12.6 <sup>ab</sup>	14.52 <sup>a</sup>
K	66.4 <sup>ab</sup>	28.9 <sup>ab</sup>	21.7 <sup>b</sup>	13.4 <sup>b</sup>	13.4 <sup>a</sup>
L	69.4 <sup>ab</sup>	39.2 <sup>ab</sup>	20.5 <sup>ab</sup>	12.8 <sup>a</sup>	14.3 <sup>a</sup>
M	71.9 <sup>b</sup>	41.3 <sup>b</sup>	19.7 <sup>a</sup>	11.9 <sup>a</sup>	16.2 <sup>b</sup>
N	69.2 <sup>ab</sup>	39.5 <sup>ab</sup>	21.3 <sup>b</sup>	12.0 <sup>a</sup>	14.2 <sup>a</sup>

a,b - Means with different superscripts differ significantly ( $P < 0.05$ ).

The strain with the lowest ascites susceptibility (M) tended to have a larger keel and shank, and lower meat yield than the highly susceptible breeds (J, K and L). This indicates that differences in ascites susceptibility between these broiler strains has more to do with a higher muscle mass to bodyweight ratio than with variations in lung or heart capacity at this genotypic level.

Metabolic differences have also been studied in broiler lines of varying ascites susceptibility. The scientific literature reveals a number of studies comparing broiler strains which have been selected for faster growth or superior feed efficiency and leanness in relation to their thyroid function in response to lower ambient temperature. Scheele *et al.* (1991) and Scheele *et al.* (1992) provided evidence that a line selected for improved feed conversion was relatively hypo-thyroid at low temperatures compared with a line selected only for growth rate and that this line had an inability to increase its feed intake in response to cold. Decuyper *et al.* (1992) furthered the link with ascites and thyroid function and suggested that supplementing birds with thyroxine could be a method of identifying susceptible lines.

Jones (1994) working with similarly selected but different lines of birds suggested that it may be the ability of the efficient feed conversion line to maintain its growth at low temperatures without a corresponding increase in feed intake which predisposes this line to ascites.

## V. RECENT STUDIES ON ASCITES CONTROL

There have been a number of reports of improvement in the incidence of ascites by the use of medications, either in feed or water. The target of much of this work has been to improve pulmonary blood perfusion and/or oxygen diffusion capacity in the lungs. Success here would obviously benefit birds with the potential for compromised cardio-respiratory capacity.

Owen *et al.* (1994) showed that by altering the acid-base balance of the feed ascites incidence under hypobaric conditions could be altered. Acidification of feed, by adding ammonium chloride, caused a slight increase in ascites while alkalization, by adding sodium bicarbonate, significantly reduced ascites under these conditions. The mechanism of action was suggested as being a possible metabolic alkalaemia causing variable pulmonary vasodilation and a consequent decrease in pulmonary arterial pressure. A metabolic acidosis would cause the reverse response.

Wideman *et al.* (1995a) used furosemide in drinking water under cold temperature conditions and reduced mortality due to ascites. Furosemide is commonly used in mammals with congestive heart failure. It is a potent diuretic that leads to reduced pulmonary arterial pressure and also acts as a pulmonary vasodilator. The copious diuresis would present practical difficulties for the commercial use of this drug, not to mention regulatory difficulties with its use.

Wideman *et al.* (1995b) supplemented broiler feed with 1% L-arginine and were able to reduce ascites losses. L-arginine in excess over dietary requirements is required as a substrate for nitric oxide production, the latter being a potent endogenous pulmonary vasodilator, thus decreasing pulmonary arterial pressure. This is a natural product but its cost would be somewhat prohibitive for general use at present.

These reports highlight the role of reducing pulmonary arterial pressure as a means of controlling the condition.

Vanhooser *et al.* (1995) used metaproterenol, a bronchodilator, in drinking water under varying oxygen concentrations and temperatures. At lower temperatures this drug reduced ascites significantly.

Therapeutic manipulation of bronchiole and pulmonary capillary characteristics can successfully modify the incidence of the ascites syndrome, confirming the role of cardio-pulmonary compromise in the condition. Whether these findings can be employed commercially is yet to be seen. In the meantime, while genetic progress in selection against susceptibility to the syndrome is awaited management practices which can minimize the effects, such as temperature control, lighting programs and dietary measures can be implemented (Groves, 1996).

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## DIETARY ELECTROLYTE BALANCE - ITS EFFECT ON BIRD PERFORMANCE

J.D. SUMMERS

### Summary

That diet composition can influence the acid-base balance of a bird has been known for many years. However, even with an increase in the number of research reports demonstrating the importance of maintaining an optimal homeostasis in the body, there has been only a minimum amount of attention paid to the acid-base balance of the bird as affected by diet.

Acid-base balance of an animal is determined by various blood analyses. However, while such measurements may tell the acid-base status of the animal, they tell nothing of the dynamic response the animal may be going through in order to maintain this balance. Hence, it is necessary to look at the urinary excretory pattern to learn how the animal is responding to a given dietary, environmental or pathological circumstance.

### I. INTRODUCTION

Carbonic acid represents the largest source of acid in the body. However, the fixed acids, sulphuric and phosphoric, also contribute significantly to this pool. While high intake of dietary cations (eg. Na + K) and panting during hot weather can lead to an elevated pH condition in the bird, it is usually acidemia rather than alkalaemia that is likely to be a problem. Sodium and chloride are known to be alkalogenic and acidogenic respectively in poultry diets. However, as demonstrated by several workers, dietary pH has little effect on pH of the intestinal tract due to an effective buffering system.

Mongin and Sauveur (1977) reported that the balance of the dietary monovalent ions Na + K - Cl was an important consideration and suggested a balance of approximately 250 milliequivalents (mEq) as ideal for the optimum performance of young broilers. A quotation from Nesheim *et al.* (1964) demonstrates the importance of considering the balance of dietary electrolytes. "These studies show that a rather close balance of sodium and potassium to chloride and sulphate must be maintained in diets in which the forms of chloride and sulphate may increase dietary acidity. Conversely, excessive sodium supplied with a metabolizable anion can be detrimental to chicks unless it is balanced with sufficient chloride or other anion. Excesses of potassium appear to be less detrimental than sodium".

#### (a) Dietary Electrolyte Balance (DEB)

Patience *et al.* (1987), in an experiment with pigs where DEB, expressed as Na + K - Cl, ranged from -100 to +400 mEq/kg of diet showed that there was a wide range of DEB over which the performance of the animals was not affected. Thus, there is a considerable margin of safety with respect to buffering capacity before depressed performance resulting from a low DEB would be noted. Patience and Wolynetz (1990)

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defined the term dietary undetermined anion (DUA), in describing the contribution of diet to acid-base homeostasis of an animal, as the difference between the sum of the macromineral cations (sodium, potassium, calcium and magnesium) and the sum of the macromineral anions (chloride, phosphate and sulphate). This defines the balance of fixed ions in the diet. Since the diet must be electroneutral, the determination of fixed ions can be used to measure the net concentration of metabolizable ions, such as lactate, bicarbonate and acetate.

While DEB is much simpler to use and in many cases adequately describes the acid-base potential of a diet, DUA is a more precise measure. Patience and Wolynetz (1990) showed that as the DUA of a maize-soyabean basal diet decreased from 345 to 81 mEq/kg with the addition of calcium chloride the average daily gain and feed intake of pigs was reduced (Table 1). The authors remarked on the constant gain:feed ratio in the light of the reduced gain of the animals. They suggest that the effect of a low DUA is greatest in terms of feed intake. This demonstrates a major effect of appetite in the biology of the response.

Table 1. Response of pigs to chloride-mediated changes in dietary undetermined anion at high dietary potassium<sup>1</sup>.

	Dietary undetermined anion, mEq/kg					
	81	121	176	205	256	345 <sup>2</sup>
Av gain/d, kg	0.54	0.66	0.71	0.72	0.74	0.75
Av feed/d, kg	1.17	1.40	1.49	1.53	1.54	1.57
Gain:feed	0.45	0.47	0.48	0.47	0.48	0.48
Blood pH	7.04	7.08	7.12	7.13	7.15	7.15

<sup>1</sup> Calcium chloride added to a maize-soyabean (high K) diet.

<sup>2</sup> Control diet - similar to commercial standards.

## II. SOME PRACTICAL CONSIDERATIONS

### (a) Appetite

The reference by Patience and Wolynetz (1990) to the influence of acid-base balance on appetite is of interest and is an area that deserves further investigation. An improvement in feed:gain ratio is usually an indication of an enhancement in the nutritive value of a diet. Dietary treatments that result in reduced weight gain usually give poorer feed:gain ratios, eg. an amino acid deficiency. However, there are a number of situations where reduced gain is encountered with no reduction in gain:feed ratio. This would suggest that ration quality was not a factor but rather that feed intake of the animal was reduced.

The work of Patience and Wolynetz (1990) showed that pigs can decrease growth but still maintain a similar feed:gain ratio. Han and Baker (1993) showed similar weight gain but improved feed:gain ratio in chicks with methionine supplementation (5 g/kg) of their basal diet (Table 2). However, 10 g/kg methionine supplementation reduced weight gain while no difference was noted in feed:gain ratio. Also, increased levels of lysine HCl supplementation reduced weight gain but had little affect on feed:gain ratio.

Table 2. Effect of excess methionine and lysine on efficiency of feed utilization in chicks. Selected and recalculated data of Han and Baker (1993).

	Weight gain (g)	Feed intake (g)	Feed:gain (g:g)
Basal <sup>1</sup>	273	410	1.50
Basal + 5 g methionine/kg	281	407	1.45
Basal + 10 g methionine/kg	259	373	1.44
Basal + 20 g methionine/kg	187	313	1.67
Basal + 5 g L-lys HCl/kg	274	405	1.48
Basal + 10 g L-lys HCl/kg	257	386	1.50
Basal + 20 g L-lys HCl/kg	248	378	1.52
Basal + 40 g L-lys HCl/kg	240	376	1.57

<sup>1</sup> 230 g crude protein and 13.39 MJ of ME/kg.

Hens coming into production have a low feed intake (Foster, 1968), which appears to be influenced by dietary calcium levels (Meyer *et al.*, 1970). Beede (1994) pointed out the marked effect that high levels of chloride and/or sulphates present in the drinking water of cattle can have on feed intake and, hence, subsequent milk production. Fauchon *et al.* (1995) state "The importance of dietary cation:anion balance in ruminants is now well documented. Increasing dietary cation:anion improves performance by increasing feed intake and helps prevent metabolic acidosis by increasing blood pH, bicarbonate and base excess".

Can feed intake of layers coming into production be enhanced by paying more attention to the intake of dietary electrolytes and, hence, the resultant effect on the acid-base balance of the hen?

#### (b) Leg problems

An extensive study reported the effect of altering the dietary mineral balance for broiler chicks (Halley *et al.*, 1987). In general, increased levels of anions resulted in a higher incidence of tibial dyschondroplasia and twisted legs (Table 3). Orth *et al.* (1992) reported that the addition of excessive levels of cysteine to a maize-soyabean meal diet resulted in reduced weight gain and a marked increase in tibial dyschondroplasia. The additional sulphur supplementation, via the cysteine, would obviously result in an altered acid-base balance in the bird and probably accounts for the increased leg problems noted.

Table 3. Effect of cation:anion balance on chick performance.

Cation:Anion	Body weight (g)	Leg Problems	
		Tibial dyschondroplasia (%)	Varus (%)
2.20	489	6	10
1.64	568	30	51
1.31	556	28	34

Selected data from Halley *et al.* (1987).

(c) Sulphur

It is interesting that such a common and important a mineral as sulphur is monitored in very few poultry diets around the world. Summers *et al.* (1992) demonstrated that sulphur supplementation of soyabean meal diets (via H<sub>2</sub>SO<sub>4</sub>) to levels found in canola meal diets, resulted in similar performance of chicks fed the two protein supplements (Table 4). They also demonstrated the effect of increased levels of calcium in alleviating the depressed performance of the sulphur-supplemented diets.

Part of the reason for the reduced performance noted with some canola meal-supplemented diets could well be due to the higher level of sulphur present in canola meal compared to soyabean meal (11.4 vs 4.4 g/kg).

Table 4. Interaction of sulphur and calcium in canola meal and soyabean meal diets.

	Sulphur		Dietary calcium (g/kg)	Liveweight gain (g)
	Supplement (g/kg)	Diet level (g/kg)		
Canola	-	4.6	3.7	424
	2.6	7.2	3.7	371
	-	4.6	13.2	560
	2.6	7.2	13.2	481
Soyabean	-	1.4	3.7	524
	1.3	2.7	3.7	519
	2.6	4.0	3.7	479
	3.9	5.3	3.7	373
	-	1.4	13.2	635
	1.3	2.7	13.2	598
	2.6	4.0	13.2	559
	3.9	5.3	13.2	451

A further study was undertaken to investigate to what extent dietary acid-base balance was contributing to the sulphur x calcium interaction reported previously. To a 150 g/kg protein canola basal diet, supplements of calcium (via limestone) were added to give dietary levels of 4, 8 and 12 g/kg. Sulphur supplementation was added to give dietary levels of 4.8 (unsupplemented basal) and 7.2 g/kg. In order to further alter the acid-base balance of the diets, sodium and potassium carbonates were added as equal mixtures to give supplements of 0, 10 and 20 mEq/kg (Summers and Bedford, 1994).

The response to supplemental mEq from sodium and potassium carbonates was linear and paralleled that noted for increased calcium levels. However, there were several interactions and these are shown in Table 5. The addition of dietary mEq resulted in responses in the weight gain and feed consumption of the chicks. However, the magnitude of the response was much greater with the high-sulphur diet (73% for the high- compared to 12% for the low-sulphur diet for weight gain). The calcium x mEq interaction for feed:gain demonstrated a linear response to both mEq and calcium supplementation. It is interesting to note that the feed:gain results were similar at the highest levels of calcium and mEq supplementation. The above results suggest that part of the response to increased dietary calcium levels, when added to canola meal diets, is the alteration in the dietary electrolyte balance that takes place.

Table 5. Interaction of dietary mEq from sodium and potassium carbonates with sulphur and calcium.

Sulphur levels (g/kg)	mEq/kg supplementation		
	0	10	20
	Weight gain (g)		
4.8	231	253	260
7.2	132	176	228
	Feed intake (g)		
4.8	509	541	551
7.2	368	445	494
	Feed:Gain (g:g)		
4	3.09	2.58	2.18
8	2.48	2.37	2.13
12	2.23	2.18	2.19

(d) Sudden Death Syndrome in Broiler Breeders

Hopkinson *et al.* (1984) reported on a condition with broiler breeders in Australia where birds died quite suddenly at point of lay. Various studies suggested that affected birds were alkalotic and thus had a different blood acid-base balance to control birds. Blood potassium values were lower for affected flocks than for normal flocks. While the cause of the problem was never completely resolved it was noted that diets higher in potassium (ie: soyabean versus meat meal diets) were less likely to trigger the condition.

The problem appears to be similar to that seen in cattle at the onset of production and could well be triggered by an altered acid-base balance, perhaps brought on by the marked changes in blood calcium level at this time.

(e) Ascites and Sudden Death in Broilers

While sudden death syndrome (SDS) has been prevalent in broiler flocks for several decades, it has only been in the past 5 to 10 years that ascites has become a major problem in most parts of the world. SDS is an acute condition affecting mainly well developed males. Ascites is a chronic condition which usually peaks later than SDS and results in small emaciated birds, often with the body cavity filled with fluid.

During the past number of years broiler diets have changed in that more lysine is used along with higher levels of methionine and, thus, in many cases lower levels of soyabean meal. If one looks at the equation  $Na + K - Cl - S$  to calculate dietary electrolyte balance, more Cl has been added via lysine HCl, more sulphur via methionine and less K with lower levels of soya. Thus, present day diets are likely to produce a more acidic, acid-base balance in the bird.

An experiment was undertaken in our laboratory with broiler males fed a commercial type diet with an additional supplement of methionine and lysine (Summers, 1996). Weight gains and feed intakes were similar. While mortality was high for the control birds, probably due to reduced pen temperature, a marked increase in mortality was noted for the amino acid-supplemented diets (Table 6). Looking at the overall mortality by



week, it would appear that birds first died from SDS, then a combination of SDS and ascites and finally mainly ascites.

Table 6. Performance of male broilers to six weeks of age when fed a basal diet supplemented with methionine and lysine.

Treatment	Weight (g)	Feed:Gain (g:g)	Mortality (%)
1. Control	2552	1.77	10.0
2. Control + 2 g DL-methionine/kg	2569	1.76	15.5
3. Control + 4.5 g L-lysine HCl/kg	2507	1.74	16.7
4. Control + methionine + lysine	2507	1.75	21.1
SD	47	0.05	
	NS	NS	

Diet compositions are changing as are bird types. Birds with more breast meat produce increased levels of metabolic lactic acid from this white meat, more so than birds of a different genetic make up some years ago. Also, many broilers are now feather sexed and, thus, males are slow feathering. Hence, if anything, their sulphur amino acid requirements may be less than that of males of a few years ago.

#### (f) Heat Stress

Heat-stressed birds develop an alkalotic condition due to panting, which results in decreased blood CO<sub>2</sub> and increased blood pH levels.

Teeter *et al.* (1985) observed that chronically heat-stressed chicks have a respiratory cycle involving panting and non-panting phases. Blood pH of panting chicks was elevated while that of the non-panting birds was not. Supplementing drinking water with 2 g NH<sub>4</sub>Cl/L reduced the panting phase blood pH to normal levels and increased the weight gain by 23% (Teeter and Smith, 1986). Supplementing the drinking water with 1.5 g KCl/L also improved weight gain and feed utilization but had no effect on blood pH.

Birds exposed to heat stress consume increased amounts of water and are better able to withstand heat stress when the water contains a KCl or NaCl supplement (Table 7). While the main effect appears to be related to the increased water intake acting as a heat sink, acid-base balance may also be a contributing factor.

There is contradictory evidence as to the effect of these salts on enhancing performance during heat stress. However, it must be remembered that various environmental conditions, eg. temperature, humidity, duration of heat stress and level of salt supplementation and intake, could all be factors influencing the response. With the marked increase in water intake that can occur with added salts during heat stress, care must be taken to ensure that adequate watering space is available if heat-stressed birds are going to respond positively to such treatment.

### III. CONCLUSIONS

While this presentation is probably long on questions and short on answers it does highlight the marked changes that can occur in production parameters with changes in the acid-base balance of the bird. This subject deserves more research effort considering the influence that dietary electrolyte balance is known to have on the bird's acid-base balance and the effects on bird performance.

Table 7. Effect of KCl- and NaCl-supplemented water on survivability, water consumption, blood pH and plasma corticosterone of thermoneutral (TN) and heat stressed (HS) birds (35 to 45 days of age). Selected data from Deyhim and Teeter (1991).

Variable	Environment	Drinking Water <sup>1</sup>		
		Control	KCl	NaCl
Blood pH	TN	7.24	7.2	7.22
	HS	7.30	7.2	7.29
Plasma corticosterone (ng/mL)	TN	1.3	1.4	1.8
	HS	2.5	1.9	2.3
Water consumption (mL)	TN	262	269	365
	HS	353	594	477
Survivability (%)	TN	100	100	100
	HD	88	97	93

<sup>1</sup> 5 g KCl/L and 3.9 g NaCl/L.

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# THE EFFECT OF RAW SOYABEANS IN THE DIET ON INTESTINAL LESIONS AND PANCREAS WEIGHT IN BROILERS INFECTED WITH *EIMERIA ACERVULINA*

E.A. SUHARLI, A.R. EGAN and J.H.G. HOLMES

## Summary

An experiment was conducted to determine the effect of different inclusion levels of raw soyabeans in diets of broiler chickens infected with *E. acervulina* on their intestinal lesions and pancreas weights. The diets comprised three levels of raw soyabean substituting for soyabean meal at 67, 137 and 215 g/kg within a 240 g/kg crude protein diet, and a commercial coccidiostat-free diet. Two levels of infection were imposed by dosing with  $10^5$  and  $3 \times 10^5$  oocysts.

The results showed that the 215 g/kg raw soyabean inclusion reduced the number of oocysts produced. However, extended use of raw soyabean in the diets also produced hypertrophy of the pancreas, thus invalidating the advantages of their use to control coccidiosis.

## I. INTRODUCTION

Coccidiosis, which is caused by *Eimeria sp.*, has been recognised as a disease that causes many poultry farmers major economic losses. This disease is of increased significance because it also interacts with other diseases, such as infectious bursal disease and Newcastle disease, and with other gastrointestinal tract microorganisms, such as *Clostridium sp.* and *Escheria coli*, inducing more severe lesions (Ruff, 1989). Infection is induced by ingestion of sporulated oocysts by the susceptible hosts. Sporozoites are released following excystation of the oocysts and they immediately invade epithelial cells of the intestine and start to multiply and produce oocysts (McDougald and Reid, 1991).

Coccidiosis can be efficiently controlled by specific chemical products. However, the increased demand from poultry consumers for more natural products has led poultry scientists to search for other methods of controlling the disease. Among these, nutrition has long been explored because it has been demonstrated over several decades that the severity of *Eimeria sp.* infection can be affected by the nature of the diet (Ruff, 1993). Low-protein diets have been shown to reduce coccidiosis severity, as was referred to by Britton *et al.* (1964). This phenomenon is considered to have a relationship with the quantity of pancreatic enzymes released since low-protein diets are associated with low rates of trypsin secretion (Ruff, 1993; Mathis *et al.*, 1995).

Pancreatic enzymes have been demonstrated to reduce the invasion of *E. tenella* (Fuller and McDougald, 1989). Soyabean has long been known to contain heat-labile protease inhibitors, particularly trypsin and chymotrypsin inhibitors. These anti-nutritional factors have limited the use of raw soyabeans for poultry as the growth rate and protein sufficiency of the bird are sensitive to the inhibitors. As demonstrated by Mathis *et al.* (1995), soyabean is capable of reducing the severity of coccidiosis, at 50 and 100% inclusion in diets. However, long use of these levels have also produced hypertrophy of the pancreas. Therefore, this study was conducted to examine the effect of lower levels of raw

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soyabean inclusion in the diets of broiler chickens infected with *E. acervulina* at 17 days of age on intestinal lesion scores and pancreas weight.

## II. MATERIALS AND METHODS

One hundred and forty four day-old male broiler-type chickens (Strain AM 100) from a commercial hatchery were used. They were maintained under uniform conditions in 18 heated, thermostatically controlled brooder cages, with 14 birds in each cage. *Ad libitum* water and unmedicated feed were provided throughout the experiment.

At five days of age, the birds were randomly allocated to the treatment groups (Diets 1, 2, 3 and commercial coccidiostat-free feed;  $10^5$  and  $3 \times 10^5$  oocysts of *E. acervulina*) and an uninfected group given the commercial diet was used as a control. The birds continued to be given commercial coccidiostat-free diet until 12 days of age.

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

Ingredients and calculated analysis	Diet 1	Diet 2	Diet 3
Yellow maize	146	149	125
Soyabean meal (440 g CP/kg)	235	179	125
Raw soyabeans	67	137	215
Fish meal	34	35	36
Meat and bone meal	44	45	47
Wheat	415	422	441
DCP	5	5	5
Salt	3	3	3
Premix	2	2	2
Fat	48	23	0
Calculated analysis			
ME (MJ/kg)	13.2	13.2	13.2
CP	240	244	252
Fat	80	67	58
Lysine	10	11	11
Methionine	3	3	3
Available Phosphorus	8	8	8
Calcium	8	8	8
Sodium	2	2	2

Three levels of raw soyabeans (67, 137 and 215 g/kg diet) were used, substituting for heat-treated soyabean meal, which was the main protein source in the diet (Table 1).

At 12 days of age the feeds of the respective treatment groups were changed gradually, over five days, to the experimental raw soyabean diets (Diets 1, 2 and 3) to allow the birds to adjust to the dietary changes. The feed for the control and the commercial-feed treatment groups remained the same throughout the experiment. At 17 day of age, oocyst doses were given to the respective treatment groups. Thereafter, one to three chickens in each group were killed by cervical dislocation at days 5 to 7, and 11 to 14 after infection. Post mortem examination was performed by examining the serosal surface of the unopened intestine for lesions under a strong light and by scoring the lesion of the mucosal surface of the intestine after it was opened, from 0 (mild) to 4 (severe) using the scoring system of McDougald and Reid (1991). The pancreas of each bird was

separated from the duodenum and weighed. The control birds were also examined for lesions and all the birds examined in this group were scored zero.

The data were analysed by the general linear model procedure for analysis of variance (Steel and Torrie, 1980).

### III. RESULTS AND DISCUSSION

The data for the lesion scores and oocyst numbers (Table 2) showed that there was a significant difference between the control and the treatment groups ( $P < 0.01$ ). This established that the controls were free from coccidiosis and that there were no significant differences in mean lesion scores between the treatment groups.

Table 2. Mean lesion scores, faecal oocyst numbers and pancreas weights on selected days for each treatment group.

Day after infection/	Lesion Score			Oocyst no. ( $\times 10^4$ )			Pancreas weight		
	5	7	12	5	7	12	5	12	14
Control	0.00	0.00	0.00	0	0	0	1.7	1.9	4.1
T1D1 <sup>1</sup>	2.00	1.17	0.75	340	18.1	0	2.7	4.2	4.9
T1D2	2.25	1.67	0.50	230	3.2	0	3.1	4.3	7.4
T1D3	1.50	0.83	0.25	120	9.9	0	3.2	5.5	6.8
T1DC	2.50	2.17	0.75	110	4.3	0	1.8	3.3	4.1
T2D1	2.50	1.67	0.25	150	12.4	0	2.5	4.6	4.5
T2D2	1.75	1.33	0.50	110	4.3	0	3.1	4.1	6.4
T2D3	1.50	1.67	1.00	49	10.1	0	3.6	5.8	6.4
T2DC	3.00	2.67	0.50	65	25.3	0	1.6	3.1	3.1

<sup>1</sup> T1D1 =  $10^5$  oocysts diet 1, T1D2 =  $10^5$  oocysts diet 2, T1D3 =  $10^5$  oocysts diet 3, T1DC =  $10^5$  oocysts diet commercial, T2D1 =  $3 \times 10^5$  oocysts diet 1, T2D2 =  $3 \times 10^5$  oocysts diet 2, T2D3 =  $3 \times 10^5$  oocysts diet 3, T2DC =  $3 \times 10^5$  oocysts diet commercial.

There was a significant difference ( $P < 0.05$ ) between the commercial diet and Diet 3, but there were no differences among the other diets. This result suggests that the diet containing a major component of raw soyabeans (215 g/kg) reduced the severity of *E. acervulina* infection compared with the commercial diet. The competition between the parasite and the trypsin inhibitors contained in the raw soyabeans to utilise the trypsin produced by the pancreas of the host could be the reason for this effect. The proteolytic activity in the intestines of the birds was reduced and the trypsin activity, which is essential to release the sporozoites from the oocysts (Fuller and McDougald, 1989), was inhibited.

The data for pancreas weight indicated a significant effect of diets ( $P < 0.001$ ) and an interaction between days and diets. It appeared that the diets caused hypertrophy of the pancreas with extended feeding (Table 2) and that the trypsin inhibitor contained in the raw soyabeans caused the pancreas to produce more enzyme. This adverse effect would nullify the practical significance of the extended use of raw soyabeans in poultry diets for the control of coccidiosis.

#### IV. CONCLUSION

Inclusion of raw soyabeans at 215 g/kg in diets for broilers infected with *E. acervulina* was demonstrated to reduce the number of oocysts produced. However, extended use of this ingredient results in an hypertrophic pancreas which nullifies any practical advantage in reducing coccidiosis.

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## DEVELOPMENTS IN THE VACCINATION OF BROILER CHICKENS AND LAYER PULLETS AGAINST INFECTIOUS BURSAL DISEASE

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### Summary

For more than 30 years broiler chickens and layer pullets in most overseas countries have been vaccinated against infectious bursal disease (IBD) using vaccines applied through the drinking water at about 2-3 weeks of age. Vaccination has resulted in reduced mortality and improved performance in the face of the more virulent serotype 1 strains of IBD virus (IBDV). Variations in the levels of maternal antibodies sometimes create difficulties in the timing of vaccination. This paper outlines laboratory and field trials using conventional and in-ovo vaccines based on the Australian vaccine virus strain V877. The performance of the vaccines in field trials in the United Kingdom, South Africa and Australia is reviewed and the potential benefits from vaccination are discussed.

### I. INTRODUCTION

The control of infectious bursal disease (IBD) through vaccination with live vaccine administered in the drinking water has been achieved especially when combined with sound breeder vaccination programs. The latter can provide high and uniform levels of maternal antibodies and allow reasonable accuracy in predicting the correct day on which chicks should be vaccinated to avoid neutralisation of the vaccine virus. However, where variable maternal antibody levels occur vaccine failures are not uncommon and are often associated with partial neutralisation of vaccine virus in chicks with high maternal antibody levels.

The above vaccination problems can be largely overcome by the *in ovo* vaccination technique in which specific hyperimmune antiserum or antibody is mixed in the appropriate ratio with infectious bursal disease virus (IBDV) to form a virus-antibody complex that can be administered to the chicken embryo at about 18 days of incubation.

This paper summarises some of the laboratory and field studies that have been undertaken in different countries utilising the V877 strain of IBDV both as a live vaccine given in the drinking water to 2-3 week-old chickens (Jackson and Madeley, 1995) and as a component of an *in ovo* virus-antibody (IBDV-Ab) vaccine (Whitfill *et al.*, 1996).

### II. MATERIALS AND METHODS

#### (a) Live vaccine for chickens

Laboratory studies were undertaken in Australia, the Netherlands, Spain, Indonesia and Japan (Claxton and McGavin, 1993; Jackson and Madeley, 1995; Jackson *et al.*, 1996; Cyanamid Websters P/L International Registration Dossier). These studies were undertaken according to protocols required for registration in the above countries and safety and immunogenicity of the vaccine in specific pathogen free (SPF) and commercial chickens

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with maternal antibodies, and essentially consisted of studies on the antibodies to IBDV. Field trials were undertaken in The Netherlands, United Kingdom and Australia in co-operation with poultry companies. The V877 live vaccine was compared to other commercial vaccines used on concurrently or previously vaccinated flocks. Production records were maintained to compare the overall productivity gains from the use of the V877 vaccine in the face of field challenge from IBDV.

(b) In ovo vaccine

Studies were undertaken with the V877 IBDV virus - antibody complex (IBDV-Ab). All vaccinations were given *in ovo* through the air cell at a depth of 1" on day 18 of embryonation. In one experiment, broilers were vaccinated *in ovo* with either 1000 EID<sub>50</sub> V877 plus 300 antibody units, 100 EID<sub>50</sub> V877 plus 30 antibody units, 1000 EID<sub>50</sub> V877 alone or with saline. Starting at 18 days of age and every three days thereafter until 39 days of age, ten birds from each group were challenged with the very virulent IBDV strain DV86 and their bursae examined 3 days post-challenge for gross lesions. The birds were tested weekly for antibodies to IBDV.

Field trials were undertaken in South Africa. The trials in South Africa involved the use of the V877 IBDV-Ab (Bursamune *in ovo*<sup>R</sup>) on five trial sites of a poultry company. Injected eggs also received 0.2 mg gentamicin. Paired houses were used for comparison of production data. Non-injected controls were subsequently vaccinated with a live intermediate vaccine via the drinking water at 19 days of age.

### III. RESULTS

(a) Live vaccine for chickens

Laboratory studies on the protection of layer pullets vaccinated with V877 vaccine virus at 14 days of age were undertaken in Indonesia are shown in Table 1. High levels of protection were obtained with all dose rates when challenged with vvIBDV at 14,28 and 42 days post vaccination. Serological results [data not shown] indicated that the day-old ELISA titre was 9,120 and had declined to 2,510 on the day of vaccination.

Table 1. Mortality rate and % protection of layer pullets unvaccinated and vaccinated\* with V877 vaccine virus following challenge with vv IBDV.

Vaccination status	Days of age		
	28	42	56
Unvaccinated	9/20	19/20	13/20
Vaccinated:dose			
10 <sup>2.1</sup> EID <sub>50</sub>	0/20 <sup>1</sup> [100] <sup>2</sup>	0/20 [100]	0/20 [100]
10 <sup>2.6</sup> EID <sub>50</sub>	2/20 [ 78]	0/20 [100]	0/20 [100]
10 <sup>3.1</sup> EID <sub>50</sub>	0/20 [100]	0/20 [100]	0/20 [100]

Chickens were vaccinated at 14 days of age; <sup>1</sup> Mortality rate expressed as number dead from IBD/ number challenged. <sup>2</sup> Percent protection shown in parenthesis was calculated by the formula = (% mortality in unvaccinated group - % mortality in the vaccinated group divided by the % mortality in the unvaccinated group) x 100.



Results of field trials with one poultry company in the UK are shown in Table 2. On this farm location there was a marked reduction in total mortality and in IBD-associated mortality between 4 and 6 weeks of age following a single vaccination at 14 days of age. This farm had remained "a problem farm" for vvIBD despite rigorous disinfection between flocks. Overall, there was significantly better production efficiency with an average increase in Production Efficiency Factor (PEF) of 10 to 20 points.

Table 2. Field trial on a farm involving 170,000 broiler chickens in five flocks vaccinated with an intermediate IBD vaccine and V877 IBD vaccine.

FLOCK NO.	A	B	C	D	E
	INTERMEDIATE IBD VACCINE			WEBSTERS V877 IBD VACCINE	
Total mortality (%)	10.85	10.12	9.82	7.32	7.32
4-6 week mortality (%)	5.79	5.75	5.90	3.50	3.72
Age at processing (d)	49.0	50.0	50.0	46.7	47.0
Average weight at processing (kg)	2.65	2.60	2.70	2.50	2.67
Rejection at processing (%)	1.05	0.72	0.93	1.02	0.98
Feed conversion ratio	2.05	2.21	2.09	2.03	2.05
PEF <sup>1</sup>	234.38	211.25	232.00	244.17	248.91

<sup>1</sup>PEF (Production efficiency factor) =  $\frac{\text{average weight} \times \text{liveability} \times 100}{\text{FCR} \times \text{age at processing}}$

(b) In ovo vaccination

In the laboratory trial (Table 3) a high percentage (94% to 100%) of birds vaccinated with the IBDV-Ab were protected against challenge from day 18 to day 39 whereas control birds were fully susceptible to challenge by day 27 following waning of maternal antibody. Eighty percent of birds vaccinated with virus only were protected. However, there was a period of bursal susceptibility between day 24 and day 33. On days 35 and 42, 100% of birds given either of the IBDV-Ab vaccines had seroconverted.

In the field trials in South Africa (Table 4) the hatchability of eggs vaccinated with IBDV-Ab did not differ significantly from those eggs that were not vaccinated (Table 4). This result confirmed the safety of this route of vaccination. The bursal to body weight ratios indicated that the IBDV-Ab vaccine had emerged in the face of maternal antibodies whereas the live vaccine had failed to breakthrough and had not done so by day 38 (Table 5). The IBDV-Ab vaccine was also able to stimulate an earlier active antibody response with protective levels developing between days 25 and 30. In pooled production data from

the field trials IBDV-Ab vaccinated birds had 1.66% higher liveability, 2 points lower feed conversion and a 0.11 cents per kilogram lower settlement cost than the controls.

Table 3. *In ovo* vaccination of broilers with V877-antibody complex vaccine: susceptible bursae following DV86 challenge<sup>1</sup>.

Treatment Group	(Days)								Total No. Susceptible Bursae <sup>2</sup>	% Pro-tected
	18	21	24	27	30	33	36	39		
Control	0	1	6	10	10	10	-	-	37/60	38
1000 EID <sub>50</sub> V877	0	0	3	8	4	1	0	0	16/80	80
1000 EID <sub>50</sub> V877 + Ab [300]	0	0	0	0	0	0	0	0	0/80	100
100 EID <sub>50</sub> V877 + Ab [30]	0	0	2	1	2	0	0	0	5/80	94

*In ovo* vaccinations were given through the air cell at a depth of 1" on day 18 of embryonation.

<sup>1</sup>DV86 is a very virulent strain of IBDV of European origin.

<sup>2</sup>Susceptibility of bursae to challenge was determined by the presence of histopathological changes due to IBDV

Table 4. Total percent hatchability from non-injected eggs and from eggs following vaccination with IBDV-Ab vaccine *in ovo* in field trials in South Africa.

Treatment	Number set	Number hatched	Percent hatched
IBDV-Ab vaccine	251,864	212,233	84.26
Non-injected eggs	294,632	243,583	82.67

IBDV-Ab vaccine was Bursamune *in ovo*<sup>R</sup> (Cyanamid Websters P/L) together with an antibiotic gentamicin at 0.2 mg per egg on day 18 of incubation.

#### IV. DISCUSSION

These trials confirmed that both conventional live IBDV vaccine administered via the drinking water and IBDV-Ab vaccine administered *in ovo* are safe and can provide high levels of protection against challenge with vvIBDV strains. In addition, improved performance figures clearly demonstrate a significant economic gain can be achieved by vaccination in the field. Improved broiler performance was also obtained in a field trial in Australia (G. Underwood, Unpublished results). The comparative vaccine trial further showed that the IBDV-Ab vaccine was capable of providing earlier protection than vaccine applied via the drinking water when high levels of maternal antibody were present.

Attempts to overcome the neutralising effects of high levels of maternal antibody have been partially overcome by the use of more invasive vaccine strains and precise calculation of the day on which the vaccine should be administered (Kouvenhoven, 1996). However, a window of susceptibility always remains before sufficient active antibody is stimulated to protect the chicken. Vaccination *in ovo* largely overcomes this problem as the virus-antibody complex disassociates in the presence of falling levels of maternal antibody. Poultry companies with access to *in ovo* injection equipment (INOVOJECT<sup>R</sup>) should be able to effect better control of IBDV.

Table 5. Bursal to body weight ratios and ELISA antibody titres (in parenthesis) of broiler chickens in a field trial comparing IBDV-Ab vaccine given *in ovo* with an intermediate vaccine administered via the drinking water.

Treatment	Site/farm	Days of age			
		25	30	34	38
IBDV-Ab <sup>1</sup>	19/1	2.0	2.3	1.35	1.05
		[580]	[1232]	[4511]	[4826]
IBDV (DW) <sup>2</sup>	19/2	2.5	2.55	3.3	3.5
		[574]	[506]	[574]	[688]

Bursal to body weight ratios were measured at 25, 30, 34 and 38 days of age by determining the weight of the bursae and body weight of a sample of 10 chickens at each age and multiplying the ratio by 1000.

<sup>1</sup>IBDV-Ab vaccine was Bursamune *in ovo*<sup>R</sup> (Cyanamid Websters P/L).

<sup>2</sup>IBDV (DW) was an intermediate vaccine in the drinking water at 19 days of age.

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## PREVALENCE, GENETIC RELATIONSHIPS AND PATHOGENICITY OF INTESTINAL SPIROCHAETES INFECTING AUSTRALIAN POULTRY

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### Summary

The prevalence of infection with intestinal spirochaetes in chickens in Western Australia was assessed by selective culture of faecal samples. Colonisation was common, with 35.1% of layer flocks and 53.3% of broiler breeder flocks being positive. Spirochaetes were recovered significantly more frequently from flocks with diarrhoea or reduced production than from clinically normal flocks. The genetic identity and diversity of 56 selected isolates from Australia, the USA and Europe were examined using multilocus enzyme electrophoresis: these were divided into six diverse genetic groups. Three groups contained isolates previously shown to be pathogenic for chickens: (i) "*Serpulina intermedia*", (ii) an unnamed group (not identified in Australia), and (iii) *Serpulina pilosicoli*. Most pathogenic isolates from Australia were "*S. intermedia*". Day-old broiler chicks were infected orally with Australian isolates either of "*S. intermedia*" (3), a commonly isolated but unnamed group (3), or *S. pilosicoli* (1). All spirochaetes induced diarrhoea, but this occurred earlier and more birds were colonised with "*S. intermedia*" and *S. pilosicoli* strains than with strains from the unnamed group. Infection of laying hens with an "*S. intermedia*" strain caused wet faeces and reduced egg production.

### I. INTRODUCTION

Over the last 10 years infection with intestinal spirochaetal bacteria has become recognised as a problem in laying hens and pullets in Europe and the USA (Davelaar *et al.*, 1986; Griffiths *et al.*, 1987; Swayne *et al.*, 1992). Colonisation of the caecae has been associated with wet faeces, increased fat content of faeces, diarrhoea, pasty vents, reduced growth rates, delayed onset of egg laying, faecal staining of eggshells, reduced egg weight and reduced carotenoid content of eggs (Davelaar *et al.*, 1986; Griffiths *et al.*, 1987; Swayne *et al.*, 1992; Dwars *et al.*, 1990, 1992a, 1992b, 1993; Trampel *et al.*, 1994). In a European study spirochaetes were demonstrated in 27.6% of samples from 134 flocks with diarrhoea or low production, but only from 4.4% of 45 healthy flocks (Dwars *et al.*, 1989). In the USA, two of 11 flocks investigated were found to be infected and both had problems with reduced egg production (Swayne *et al.*, 1992). To date there have been no reports concerning the occurrence of intestinal spirochaetal infections in Australian poultry.

Remarkably, the spirochaetes isolated from poultry in Europe and the USA have not been identified to the species level, and it is not clear whether these various reported infections were caused by the same organism(s). Those isolates that have been examined are weakly haemolytic anaerobic organisms that resemble weakly haemolytic *Serpulina* spp. of the pig (Davelaar *et al.*, 1986; Swayne *et al.*, 1992). One isolate from the USA has been shown to belong to a distinct group within the genus *Serpulina* (Swayne *et al.*, 1995). Pigs are infected with at least four species of weakly haemolytic spirochaetes (Lee *et al.*, 1993), only two of which are considered pathogenic ("*S. intermedia*" and *S. pilosicoli* : Hampson and Trott, 1995). By analogy, poultry are likely to be colonised by a variety of intestinal spirochaetes, some of which may be pathogenic. Experimentally, certain isolates from Europe and the USA

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have been shown to induce diarrhoea, reduce growth rates and reduce egg production in infected chickens.

The purpose of the present study was i) to determine whether and to what extent intestinal spirochaetes infect Australian poultry, and their disease associations in the field, ii) to analyse available isolates to determine their genetic diversity and identity, and iii) to examine the pathogenic potential of Australian isolates in broiler chicks and layer hens.

## II. METHODS

### (a) Prevalence study

Faecal samples were collected from poultry flocks in Western Australia. A total of 410 samples were obtained from 37 layer flocks and 157 samples from 30 broiler breeder flocks. The owners/managers were questioned about the health and productivity of the flocks at the time of sampling.

Each sample was plated on Trypticase Soy agar containing 5% defibrinated ovine blood, 400 mg/mL spectinomycin and 25 mg/mL each of colistin and vancomycin. Plates were incubated at 37°C in an atmosphere of 94% N<sub>2</sub>, 6% CO<sub>2</sub> for 5 - 7 days. Spirochaetal growth was confirmed by phase contrast microscopy, and strength of beta-haemolysis was determined by comparison with porcine reference strains.

### (b) Genetic relationships study

Multilocus enzyme electrophoresis (MEE) was used to analyse genetic relationships amongst intestinal spirochaetes isolated from chickens in Australia (42), the Netherlands (7), the United States (6) and the UK (1). The technique used was based on that established for porcine spirochaetes (Lee *et al.*, 1993), and included examination of porcine reference strains. Briefly, spirochaetes were grown in broth culture, harvested, lysed to release their constitutive enzymes, and these were separated electrophoretically in starch gels. The electrophoretic mobility of 15 enzymes was measured for each isolate, and differences in mobility between isolates for a given enzyme were equated with allelic differences at the structural locus encoding that enzyme. Isolates having the same alleles at all 15 loci were placed in the same electrophoretic type (ET). Genetic distance between ETs was calculated, and a phenogram was drawn to represent genetic relationships between ETs.

### (c) Pathogenicity studies

Seven Australian isolates were tested for their pathogenic potential in day-old SPF chicks, and one was also tested in adult birds approaching lay. Three isolates belonged to MEE group b ("*S. intermedia*"), three to unnamed group d, and one was an *S. pilosicoli* isolate, recovered from a flock in Queensland (courtesy of C.P. Stephens). Groups of 20 chicks were infected by crop tube with 2 ml of broth culture (10<sup>9</sup> cells/ml) of one or other of the isolates daily for three days. Two other groups were sham inoculated with sterile broth, and one group received the non-pathogenic porcine spirochaete *S. innocens*. The chicks were killed after three weeks, and subjected to full postmortem examination and bacterial culture of caecal contents.

"*S. intermedia*" isolate HB60 was inoculated by crop tube into 10 individually-housed 14 week old commercial laying hens. Ten were sham inoculated with sterile broth. The birds were kept for 16 weeks and daily egg production and bodyweights were recorded

from 20 weeks of age. The birds were then killed and subjected to postmortem examination.

### III. RESULTS

#### (a) Prevalence study

Intestinal spirochaetes were isolated from 35.1% of the layer flocks and 53.3% of the broiler flocks. Overall, spirochaetes were isolated from 16 of 25 (64%) flocks with signs of diarrhoea or reduced production compared to 25 (28%) of flocks which were clinically normal. This difference was statistically significant ( $P < 0.02$ ). All isolates were weakly beta haemolytic, and grew as a thin haze on the plates.

#### (b) Genetic relationships

The 56 isolates were divided into 40 ETs, distributed in six major genetic groups within the genus *Serpulina*. The first group corresponded to "*S. intermedia*", and contained 16 Australian isolates, one from the Netherlands, and one from the UK. The second group corresponded to *S. innocens*, and include two Australian isolates. The third group has not previously been described, and contained 23 Australian and two Dutch isolates. The fourth unnamed group contained three pathogenic isolates from the USA. The fifth group corresponded to "*S. murdochii*", and contained one Dutch and one US isolate. The final group corresponded to *S. pilosicoli*, and contained three Dutch, two US and one Australian isolate.

#### (c) Pathogenicity studies

Infection with "*S. intermedia*" strains and *S. pilosicoli* resulted in diarrhoea within 7-9 days of inoculation. Six chicks infected the "*S. intermedia*" strains died. Birds inoculated with spirochaetes from the unnamed group developed diarrhoea after 12-13 days. Most birds inoculated with "*S. intermedia*" or *S. pilosicoli* had these organisms in their caecae at slaughter, but only 45% of those inoculated with spirochaetes from the unnamed group were colonised. The porcine *S. innocens* strain failed to colonise any bird. Colonisation with *S. pilosicoli* was associated with characteristic attachment of the spirochaete by one cell-end to caecal enterocytes, forming a dense layer over the epithelium. The other spirochaetes were unattached, and were present in large numbers in the caecal crypts and lumen.

In the adult birds infection with the "*S. intermedia*" strain resulted in a significant increase in mean faecal moisture content (80.3% compared to 77.1%:  $P < 0.006$ ). The average weight of infected birds was less than control birds but this only reached significance at week 14 of the experiment (1709 g compared to 1514 g :  $P < 0.035$ ). Infected birds produced significantly fewer eggs (0.51 eggs/hen/day compared to 0.69 eggs/hen/day:  $P < 0.023$ ). Average egg weight also was reduced in infected birds (44.56 g compared to 45.67 g :  $P < 0.003$ ). Infected birds were still colonised at slaughter, and their caecal contents were wetter and more gaseous than those of the control birds.

### IV. DISCUSSION

Infection with intestinal spirochaetes was shown to be common in layer and broiler breeder flocks in Western Australia. Rates of colonisation were higher than reported in

Europe (Dwars *et al.*, 1989) although those workers used immunofluorescence on faeces rather than culture. Given the high prevalence of infection in the current study it seems likely that some of these organisms were commensals. Nevertheless, as in Europe (Dwars *et al.*, 1989), spirochaetes were recovered significantly more frequently from flocks with diarrhoea and reduced production than from healthy flocks. Analysis of selected Australian, European and North American isolates revealed that these morphologically similar weakly beta haemolytic spirochaetes were genetically diverse, representing six different genetic groups (probably six separate species in the genus *Serpulina*). Most Australian isolates were "*S. intermedia*" (38%) or from an unnamed group (43%). The Dutch pathogenic strain 1380 (Dwars *et al.*, 1992a), and strain B230 from the UK were also "*S. intermedia*". Pathogenic strain C1 from the US (Swayne *et al.*, 1995) belonged to another genetic group not identified in Australia while another pathogenic strain from the US (Trampel *et al.*, 1994) was *S. pilosicoli*. None of the Western Australia strains were *S. pilosicoli* but this species was shown to be present in poultry in Queensland.

SPF chicks develop diarrhoea when colonised with intestinal spirochaetes. A non-pathogenic porcine strain failed to colonise. Strains of "*S. intermedia*" and *S. pilosicoli* colonised for longer than strains from the common unnamed group isolated in Australia and they also induced diarrhoea more rapidly. "*S. intermedia*" and *S. pilosicoli* strains, therefore, appeared to have more pathogenic potential than strains from the unnamed group. Previously Dutch strain 1380 (identified here as "*S. intermedia*") has been shown to cause growth depression and increased faecal fat in experimentally infected broilers (Dwars *et al.*, 1992a).

"*S. intermedia*" strain HB60 colonised birds approaching point of lay causing moist faeces and reduced egg production (number and weight). Previously, experimental infection with "*S. intermedia*" strain 1380 increased faecal fat content (Dwars *et al.*, 1992b), and caused wet droppings and reduced egg production in laying hens as well as reduced body weights in chicks hatched from eggs produced by these layers (Dwars *et al.*, 1993).

## VII. CONCLUSIONS

This study has demonstrated that infection with intestinal spirochaetes is a common problem in Western Australia, and that many of the spirochaetes, particularly strains of "*S. intermedia*", have the capacity to cause disease and associated loss of production. Further work is required to develop means to diagnose and control this newly-recognised infection.

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# INCREASING THE METABOLISABLE ENERGY OF SOYABEAN MEAL BY DEGRADING THE GALACTANS IN THE PLANT CELL WALL BY USE OF GALACTANASE AND $\beta$ -GALACTOSIDASE

I.H. KNAP, A. OHMANN and A. MULLERTZ

## Summary

The effect of adding endo-1,4- $\beta$ -galactanase,  $\beta$ -galactosidase or a combination to soyabean meal (SBM) as a means of improving the bioavailability of energy was studied in poultry. The result of a true metabolisable energy (TME) study with adult roosters was an improvement in TME of 0.1%, 1.8% and 7% respectively. In an AME broiler study, feeding 640 g sorghum/kg and 300 g SBM/kg with the same enzymes gave changes of -0.9% +1.5% and +3.9% respectively. Using only galactanase exerted a limited effect whereas  $\beta$ -galactosidase on its own did not show any improvement. These results indicate potential for improving energy utilisation in legumes by the use of enzymes able to liberate the galactose bound in the plant cell walls. However, the addition of  $\beta$ -galactosidase to cleave dimers of galactose seems important for optimisation.

## I. INTRODUCTION

Biological utilization of energy from legumes is low. For example, poultry are only able to utilize 54.4% of the gross energy from SBM and 61.4% from lupins. The high content of non starch polysaccharides (NSP) in these feedstuffs (27% in SBM and 37% in lupins) is indigestible to the birds as NSP-degrading enzymes are absent in the avian small intestine. According to Schutte (1991) the NSPs consequently pass to the hind gut. Birds can only digest the water-soluble fraction of the NSP by microbial fermentation in the hind gut.

The NSP fraction of soyabean meal and lupins contains 25% and 22% of galactose, respectively (Schutte, 1991). The galactose is present in the plant cell wall as galactans or arabinogalactans. If it was possible to make the galactose from the NSP fraction available to the bird energy utilization could be improved substantially.

Galactanase (endo-1,4- $\beta$ -galactanase) hydrolyses galactan to galactose and dimers of galactose. The objective of the present study was to determine if galactanase alone or in combination with  $\beta$ -galactosidase could improve the bioavailability of energy from SBM.

## II. MATERIALS AND METHODS

### Experiment 1: True Metabolizable Energy Study

The TME study was conducted as described by Sibbald (1976) and Dale and Fuller (1984). White leghorn roosters (55 weeks of age) were used as test animals with 10 replicates per treatment. A total of 30 g dehulled SBM was fed to each rooster and a 48 h collection period was employed.

Addition of 1 g/kg of galactanase (endo-1,4- $\beta$ -galactanase), 0.5 g/kg of  $\beta$ -galactosidase and a mixture containing both enzymes were examined together with a control of SBM without added enzyme.

### Experiment 2: Apparent Metabolisable Energy

The AME study was conducted with an experimental basal diet containing sorghum (640 g/kg) and SBM (300 g/kg). Commercial broiler chickens (Ingham IM98, 24 d of age) were used as test animals with 125 birds per treatment. Experimental diets were fed for seven days (days 1-7). The first three days (days 1 - 3) allowed the chickens to adapt to the cages and the feeds. During the following four days (days 4 - 7) feed intake was measured and all excreta collected and dried.

In this experiment galactanase (endo-1,4- $\beta$ -galactanase) was included at a dosage of 6.7 mL/kg feed, and the  $\beta$ -galactosidase was included at a dosage of 3.3 mL/kg feed. The combination was also tested.

#### Statistical Analysis

Data were subjected to GLM procedures of SAS.

### III. RESULTS

Results of the TME study are presented in Table 1 and the results of the AME study are presented in Table 2.

Table 1. True metabolisable energy (TMEn) results from Experiment 1.

Treatments	Dosage (g/kg)	Number of animals (N)	TMEn (MJ/kg)/ (% improvement)
Basal diet (B)	-	11	12.57 <sup>b</sup>
B + $\beta$ -galactosidase	0.5	11	12.58 <sup>b</sup> (0.1%)
B + galactanase	1.0	9	12.80 <sup>b</sup> (+1.8%)
B + $\beta$ -galactosidase + galactanase	0.5 + 1.0	11	13.44 <sup>a</sup> (+7.0%)

Means with similar superscripts are not significantly different at  $P < 0.05$ .

Table 2. Apparent metabolisable energy (AMEn) results from Experiment 2.

Treatments	Dosage (mL/kg feed)	Number of animals (N)	AMEn (MJ/kg DM/ (% improvement)
Basal diet (B)	-	125	12.18 <sup>bc</sup>
B + $\beta$ -galactosidase	3.3	125	12.07 <sup>c</sup> (-0.9%)
B + galactanase	6.7	125	12.36 <sup>abc</sup> (+1.5%)
B + $\beta$ -galactosidase galactanase	3.3 + 6.7	125	12.65 <sup>a</sup> (+3.9%)

Means with similar superscripts are not significantly different at  $P < 0.05$ .

### IV. DISCUSSION

A significant improvement in bioavailable energy was obtained in both studies by using a combination of galactanase and  $\beta$ -galactosidase i.e. 7% in the TME study and 13% in the AME study if the effect was considered to only reflect the SBM. Using only galactanase gave a limited effect whereas  $\beta$ -galactosidase on its own did not show any improvement. In a

previous AME study by Bryden *et al.* (1994) the use of a mixture of galactanase and  $\alpha$ -galactosidase on lupins showed an improvement of 24.6%.

These results indicate a potential for improving energy utilization in legumes by use of enzymes able to liberate the galactose bound in the plant cell walls. However, the addition of  $\beta$ -galactosidase to cleave dimers of galactose seems important for optimal effect.

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## APPLYING ENZYMES TO SORGHUM-BASED BROILER DIETS

C. WYATT, M. SOTO-SALANOVA and M. PACK

### Summary

An enzyme complex, containing amylase, protease and xylanase, has been developed for application to low viscosity poultry diets based on sorghum and maize. Three trials are reported on the application of this enzyme complex to sorghum-based broiler diets. Enzyme addition improved liveweight-corrected feed:gain ratio by 5-16 points, and also allowed a reduction in diet specification by 3% without adversely affecting broiler performance.

### I. INTRODUCTION

The use of feed enzymes is common practice in poultry and swine production in Europe, Canada and Australia where diets are often based on barley and wheat. The present paper will focus on the effects and the mode of action of a new enzyme complex targeting poultry diets based on low-viscous cereals such as maize and sorghum.

Sorghum is widely grown in arid and semi-arid areas around the world. It has a high metabolisable energy as a result of its content of starch and oil. It has a slightly lower protein content than most competitive cereals, with the same amino acid imbalance problems as maize. The major problem with sorghum, however, is considered to be its variability in tannin content. Tannins are polyphenolic compounds that have a negative effect on intestinal nutrient digestion which translates into lower ME values and amino acid digestibility (Gualtieri and Rapaccini, 1990). Australian sorghum varieties are typically low in tannins. Nevertheless, more recent findings indicate that a low tannin content does not guarantee high nutrient availability. Elkin *et al.* (1996) compiled an extensive review of sorghum varieties, confirming a wide variation in ME and amino acid digestibility in chicks even with low-tannin sorghum. In low-tannin varieties (<4g tannins/kg) ME values ranged from 14.10 to 15.82 MJ/kg and lysine digestibility from 62 to 88%.

Results from trials with an enzyme product based on protease, xylanase and amylase, and developed to improve nutrient digestibility in sorghum- and maize-based diets (Pack *et al.*, 1996), are presented below.

### II. RESULTS AND DISCUSSION

#### (a) Experiment 1

In an initial trial conducted recently at the International Institute of Animal Research, Mexico, broilers were fed sorghum (650 g/kg)-soyabean meal or sorghum (620 g/kg)-soyabean meal-rapeseed meal pelleted diets to 49 days of age. The rapeseed portion in the second set of diets was 50 g/kg in the starter and 100 g/kg in the finisher. All diets also contained around 70 g/kg of full-fat soyabeans and 50 g/kg of fishmeal. There were 8 replicates of 40 male Arbor Acres broilers per diet. The results, shown in Table 1, indicate that the addition of the enzyme complex to the diets improved weight gain and feed conversion in the birds fed the sorghum-soyabean meal formulation and the same trend could be observed for those fed the sorghum-soyabean meal-rapeseed meal combination. Also,

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Finnfeeds International Ltd, St Louis, USA, Madrid, Spain and Marlborough, UK.

enzyme inclusion allowed the addition of 5 to 10% rapeseed meal in the diet to match the performance of the broilers fed the non-enzyme supplemented sorghum-soyabean meal diet.

Table 1. Effect of an enzyme complex on performance of broilers fed sorghum-soyabean meal or sorghum-soyabean meal-rapeseed meal diets from 1-49 days.

	Sorghum-soyabean meal			Sorghum-soyabean meal-rapeseed meal		
	Control	+ Enzyme	P value	Control	+ Enzyme	P value
Weight gain (g)	2414	2481	0.02	2382	2451	0.23
Feed intake (g)	4705	4664	0.85	4825	4829	0.66
Feed:gain (g:g)	1.95	1.88	0.02	2.03	1.97	0.22
Corrected feed:gain (g:g) <sup>1</sup>	1.95	1.86	0.02	2.04	1.96	0.22

<sup>1</sup>Corrected 3 points per 100 g liveweight.

### (b) Experiment 2

In a follow-up trial at the Queensland Poultry Research and Development Centre the influence of this enzyme complex on the performance of broilers fed sorghum (567-625 g/kg)-soyabean meal (170-248 g/kg) diets was tested. The diets also contained 40-53 g/kg of cottonseed meal, 70-80 g/kg of meat and bone meal and 50 g/kg of tallow. These diets were fed to 8 replicates of 60-80 birds per diet with separate sex feeding. Enzyme addition improved ( $P < 0.03$ ) feed conversion in both males and females (Table 2). The effect was greater in the males, probably due to their higher nutrient requirements.

Table 2. Effect of enzyme addition on the performance of broilers fed diets based on sorghum and soyabean meal.

1-42 days	Male birds			Female birds		
	Control	+ Enzyme	P value	Control	+ Enzyme	P value
Weight gain (g)	2133	2255	0.07	1890	1900	0.86
Feed:gain (g:g)	1.84	1.72	0.01	1.83	1.78	0.03
Corrected feed:gain (g:g) <sup>1</sup>	1.84	1.68	0.01	1.83	1.78	0.15

<sup>1</sup>Corrected 3 points per 100 g liveweight.

### (c) Experiment 3

The alternative option to adding the enzyme directly to the control diet is to change the feed formulation to allow for the nutritive-releasing effect of the enzyme. This can reduce the cost per tonne of feed. This approach was successfully applied in a trial at the National University of Agriculture, Mexico where the reduction applied to dietary ME and crude protein content was approximately 3%. The standard ME and crude protein were 12.34 MJ/kg and 220 g/kg, respectively, during the starter period and 12.76 MJ/kg and 203 g/kg, respectively, during the finisher period. There were 7 replicates of 30 as-hatched Arbor Acres broilers per treatment. Preliminary data are summarised in Table 3. It is apparent that the energy and protein reductions resulted in reduced performance which could be restored by

addition of the enzyme, giving corrected feed conversion matching the performance on the standard diet without added enzyme.

Table 3. Effect of reducing the dietary metabolisable energy and crude protein by 3% on broilers fed sorghum diets with and without an enzyme. (Preliminary data, statistical analysis pending).

	Standard diets		Diets - 3% ME and Protein	
	Control	+ Enzyme	Control	+ Enzyme
Weight gain (g)	2393	2452	2374	2387
Feed:gain (g:g)	2.14	2.05	2.18	2.10
Corrected feed:gain (g:g) <sup>1</sup>	2.14	2.03	2.19	2.10

<sup>1</sup>Corrected 3 points per 100 g liveweight.

### III. CONCLUSIONS

Presented data indicate that appropriate enzymes can enhance the productive value of sorghum-based broiler diets. For economic benefit enzymes can be included either on top of the existing formulation to improve bird performance or in reduced specification diets to take advantage of cost savings in formulation.

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## IMPROVED HEAT STABILITY OF XYLANASES

D. PETTERSSON and P. B. RASMUSSEN

Summary

In an *in vitro* study different batches of xylanases were added to diets conditioned at different temperatures (75°C, 85°C or 95°C) before pelleting. The remaining enzymatic activity was determined by a spectrophotometric method based on the use of dyed polysaccharides.

In an *in vivo* trial broiler chickens were fed diets conditioned at 78°C or 93°C before pelleting. Diets were supplemented with enzyme preparations obtained from *Thermomyces* or *Trichoderma* and fed to broiler chickens in a balance study.

Both studies demonstrated the improved heat resistance of the newly developed *Thermomyces* xylanase measured both *in vitro* and *in vivo*.

## I. INTRODUCTION

Today there is a clear trend towards increasing pelleting temperatures and it is, therefore, of paramount importance to develop new feed enzymes with improved heat resistance.

Enzyme preparations obtained from *Humicola insolens*, has a *per se* better heat stability than preparations obtained from *Trichoderma* or *Aspergillus* organisms. It is, in addition, possible to use granulation techniques which provide extra heat resistance due to the substances used for stabilising the enzyme which, in addition, may be enclosed within a protective hydrophobic granule coating for further protection. However, a new xylanase obtained from *Thermomyces lanuginosus* has been developed and this enzyme has an improved heat resistance compared with the xylanases obtained from *Humicola insolens*.

The objectives of the current studies were to investigate the influence of pelleting temperature on residual enzyme activity measured *in vitro* and, additionally, to study the *in vivo* effects on apparent metabolisable energy and whole tract crude fat digestibility in broiler chickens.

## II. MATERIALS AND METHODS

(a) In vitro studies

Different batches of xylanases (at least two different batches of each enzyme) obtained from *Thermomyces*, *Humicola* or *Trichoderma* were added to wheat-based diets and conditioned at different temperatures (75°, 85° and 95°C) before pelleting. The diets were fed to the conditioner at 300 kg/h and steam-heated at 2 kg/cm<sup>2</sup> before pelleting in a Simon Heesen machine equipped with a 3 x 3.5 mm matrix. Samples were taken when matrix outlet temperature equilibrium was achieved for each of the conditioning temperatures used. Samples were analysed for xylanase activity (FXU/kg diet) at Novo Nordisk A/S according to internal analytical procedures based on the use of dyed polysaccharides (McCleary and Shameer, 1987).

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Novo Nordisk A/S, Krogshøjvej 36, DK-2880 Bagsvaerd, Denmark.

(b) *In vivo* study

A control diet based on wheat (590 g/kg) and soyabean meal (200 g/kg) as main ingredients was pelleted at 78°C or 93°C without or with enzymes obtained from *Thermomyces* or *Trichoderma*. The *Thermomyces* xylanase was added as a coated granulate at a level of 300 mg/kg diet and the *Trichoderma* powder preparation at 1000 mg/kg. Groups of 4 birds (16 days old) were randomly assigned to 30 pens. For each of the 6 dietary treatments 5 replicates were used. Apparent metabolisable energy (AME), nitrogen retention and total tract crude fat digestibility was determined in a balance study conducted according to Bourdillon *et al.* (1990).

## III. RESULTS

(a) *In vitro* studies

The results from the *in vitro* study are displayed in Figure 1.

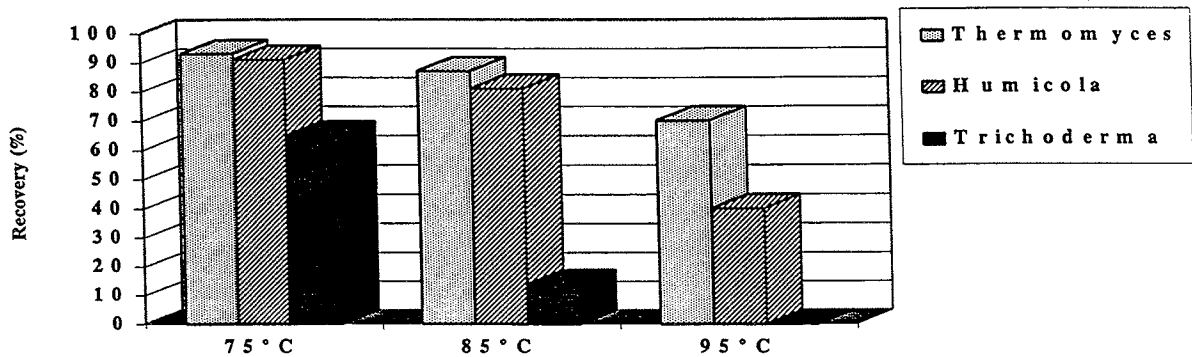


Figure 1. Recovery of xylanase activity in diets with added preparations obtained from *Thermomyces*, *Humicola* or *Trichoderma*. Diets were conditioned at 75°C, 85°C or 95°C before pelleting.

(b) *In vivo* study

The recovered activities after pelleting of the diets used in the *in vivo* study are shown in Table 1.

Table 1. Enzymatic activity measured as mg enzyme/kg diet or as percent recovery in diets.

	Initial activity	Pelleting temperature	
		78°C	93°C
<i>Trichoderma</i> xylanase	1000	660 (66 %)	Trace
<i>Thermomyces</i> xylanase	300	280 (93 %)	225 (75 %)

The results from the *in vivo* balance trial are shown in Table 2.



Table 2. Total tract crude fat digestibility (%) in control diet and enzyme supplemented diets pelleted at 78°C or 93°C and apparent metabolisable energy (AME) in MJ/kg diet and relative (%) to the respective control treatments.

	<u>Fat digestibility (%)</u>		<u>AME (MJ/kg diet)</u>	
	<u>Pelleting temperature</u>		<u>Pelleting temperature</u>	
	78°C	93°C	78°C	93°C
Control diet	73 <sup>a</sup>	70 <sup>a</sup>	11.48 <sup>a</sup>	(100%)11.52 <sup>a</sup> (100%)
<i>Trichoderma</i> xylanase	73 <sup>a</sup>	71 <sup>a</sup>	11.63 <sup>ab</sup>	(101%)
11.50 <sup>a</sup> (100%)				
<i>Thermomyces</i> xylanase	77 <sup>b</sup>	77 <sup>b</sup>	11.85 <sup>b</sup>	(103%)11.81 <sup>b</sup> (103%)

<sup>ab</sup> Means with a similar superscript are not significantly different at  $P < 0.05$  within both column and row for each measured parameter.

#### IV. DISCUSSION

The current results demonstrate the improved heat resistance of the newly developed *Thermomyces* xylanase in comparison with that of other xylanases.

In the *in vitro* study, increasing conditioning temperatures before pelleting reduced the recovery of xylanase activity for all preparations but the smallest reductions were observed with the *Thermomyces* derived enzyme.

In the *in vivo* study, conditioning at 93°C before pelleting only resulted in trace activities detected in the diet supplemented with the *Trichoderma* preparation while the diet supplemented with the *Thermomyces* xylanase still had a residual enzymatic activity corresponding to 75 % of the added activity. The results obtained in the balance study further corroborated these results. Crude fat digestibility, measured as total tract digestibility, as well as metabolisable energy content were improved ( $P < 0.05$ ) for chickens receiving the diet supplemented with the *Thermomyces* preparation.

No significant effects were registered for birds fed the diet supplemented with the *Trichoderma* preparation. Improved crude fat digestibility is usually observed, measured both as ileal or total tract digestibility in broiler chickens, when the dietary fibre effect is reduced or eliminated by enzyme supplementation (Pettersson *et al.*, 1991).

The results obtained in this study indicate that analytical methods for determining "in feed" activity are important tools for monitoring the expected efficacy of an added enzyme. When testing the efficacy of enzymes in animal experiments the actual "in feed" activity should always be determined.

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## FEED ENZYMES IMPROVE TURKEY PERFORMANCE

D. PETTERSSON

### Summary

The effects of enzyme supplementation on the productive value of turkey diets were studied in two trials. In Trial 1 diets were based on soyabean meal and wheat with a high or low content of soluble pentosans. Enzyme supplementation improved the productive value of the diet based on wheat with a high content of soluble pentosans.

In Trial 2 the effects of an  $\alpha$ -galactosidase or a pectinase preparation was studied in maize and soyabean meal based diets. On enzyme supplementation live weights at day 21 were improved for turkeys receiving either the  $\alpha$ -galactosidase or the pectinase treated diets.

### I. INTRODUCTION

The use of enzymes in poultry feeding is today common practice in Europe and there is a growing interest in the use of feed enzymes also in the North, Middle and South American states. The enzymes of interest are the arabinoxylanases (also referred to as xylanases) which degrade arabinoxylans, the major cell wall constituents in wheat, rye and triticale (Fincher and Stone, 1986; Pettersson and Åman, 1987, 1988) but also  $\alpha$ -galactosidases and pectinases that may improve the nutritive value of soyabean meal. Soyabean meal is an important ingredient in poultry diets and, in particular, in turkey diets where it may be included at levels of about 400 g/kg in order to fulfil protein requirements. The use of both wheat and soyabean meal will combine two feedstuffs rich in dietary fibre and this may cause production disturbances since turkeys, as well as other monogastric species, are not capable of degrading dietary fibre. The detrimental effects of arabinoxylans on poultry performance are well documented (Antoniou and Marquardt, 1981; Pettersson and Åman, 1988) as well as the improvements in production that may be obtained by enzyme supplementation. The use of feed enzymes in turkey diets is not, however, as well documented as in their application in broiler diets.

Soyabean meal is rich in pectin, galactans and xyloglucans and also has a high content of indigestible, but easily fermented, oligosaccharides of the raffinose series. All together this gives a range of indigestible carbohydrates from 25 % to 33 % (dry matter basis) in solvent extracted soyabean meal which accounts for the low apparent metabolisable energy content of this protein source for poultry (approximately 9 - 10 MJ/kg).

In the current trial turkeys were fed two different types of wheat that were low (cultivar Ibis) or high (cultivar Alidos) in soluble pentosan content and viscosity. Diets formulated with these wheats were fed without or with supplementation with a dietary fibre-degrading enzyme preparation in order to determine the detrimental effects of arabinoxylans on turkey performance. In addition, experiments were carried out to assess the influence on production performance of supplementing turkey diets based on maize and soyabean meal with  $\alpha$ -galactosidase or pectinase preparations.

## II. MATERIALS AND METHODS

### (a) Animals and Diets

In Trial 1 a total of 360 male turkeys were subject to a two phase feeding regimen with diets based on wheat (680 g/kg in phase 1 and 730 g/kg in phase 2). Soyabean meal inclusion was 220 g/kg (phase 1) and 180 g/kg (phase 2). There were four dietary treatments with 9 replicates of 10 birds each. Two wheats with high (cultivar Alidos) and low (cultivar Ibis) viscosity and content of soluble pentosans were used as cereal sources and diets were fed without or with supplementation of a xylanase (Bio-Feed Wheat) preparation at 225 FXU/kg diet.

In Trial 2 a total of 240 male turkeys were fed a diet based on maize (300 g/kg) and soyabean meal (500vg/kg). The control diet, calculated to be 10 % lower in metabolisable energy than NRC recommendations, was supplemented with an  $\alpha$ -galactosidase or a pectinase preparation. There were 6 replicates with 10 birds in each replicate.

The treatments were as follows;

Control diet (reduced energy level).

Control diet (reduced energy level) +  $\alpha$ -galactosidase at 500 mg/kg

Control diet (reduced energy level) + Pectinase at 125 mg/kg

Control diet (reduced energy level) + Pectinase at 250 mg/kg.

### (b) Measured parameters

Live weight gain was measured each week in both experiments as was group feed intakes, and feed conversion ratios were calculated. In Trial 1 a quantitative digestibility study was conducted and in addition viscosity was measured with a Brookfield viscometer (DV II +) on supernatants obtained by centrifugation of duodenal, jejunal and ileal digesta samples.

## III. RESULTS

### (a) Trial 1

The wheat with a high content of soluble pentosans (1.8 %) had a viscosity of 3.5 mPa s, while the wheat with a low soluble pentosan content (0.8 %) had a viscosity of 1.3 mPa s.

Table 1. Production performance in Trial 1 of male turkeys (42 days of age) fed diets based on high or low soluble pentosan wheats without (- Enzyme) or with (+ Enzyme) xylanase supplementation.

Type of pentosans in wheat	High soluble	High soluble	Low soluble	Low soluble
	- Enzyme	+ Enzyme	- Enzyme	+ Enzyme
Feed intake (g)	2430 <sup>a</sup>	2493 <sup>b</sup>	2384 <sup>c</sup>	2370 <sup>c</sup>
Live weight gain (g)	1759 <sup>a</sup>	1840 <sup>b</sup>	1777 <sup>ab</sup>	1776 <sup>ab</sup>
Feed conversion (g:g)	1.40	1.37	1.36	1.35
Ileal viscosity (mPa s)	11.0 <sup>a</sup>	2.8 <sup>b</sup>	3.8 <sup>b</sup>	3.0 <sup>b</sup>

<sup>abc</sup> Means within a row without a common superscript are significantly different at  $P < 0.05$ .

(b) Trial 2

Table 2. Production performance in Trial 2 of male turkeys (21 days of age) fed diets based on maize and soyabean meal and supplemented with an  $\alpha$ -galactosidase or pectinase preparation.

	Control	$\alpha$ -Galactosidase 500 mg/kg	Pectinase	
			125 mg/kg	250 mg/kg
Average live weight (g)	538 <sup>a</sup>	570 <sup>b</sup>	561 <sup>ab</sup>	581 <sup>b</sup>
Feed conversion (g:g)	1.62 <sup>a</sup>	1.58 <sup>a</sup>	1.68 <sup>a</sup>	1.64 <sup>a</sup>

<sup>ab</sup>Means within a row without a common superscript are significantly different at  $P < 0.05$ .

## IV. DISCUSSION

Enzyme supplementation of the diet based on wheat with a high content of soluble pentosans improved live weight by 4.6 % and feed conversion by 2 %. Enzyme supplementation of the diet based on wheat with a low content of soluble pentosans did not improve the production values. Similar results for high and low viscosity wheats have also been demonstrated in broiler chicken trials (Veldman and Vahl, 1994).

Not only the amount of dietary fibre present in a cereal influences its productive value but also the way in which the dietary fibres of the cell wall matrix are organised will affect the cell wall degradability (Cleemput *et al.*, 1993) and, thereby, the potential for release of nutrients enclosed within the endosperm cells. The solubilisation and disruption of the cereal cell walls will influence digesta viscosity and result in a delicate balance between the beneficial effects of solubilising and degrading the cereal cell walls and the detrimental effects on nutrient absorption due to an increased digesta viscosity. Wheat viscosity may not always correlate well with its nutritive value and other factors such as protein quantity and quality and available carbohydrate content may be equally important predictors for productive value in wheats that produce only a moderately increased digesta viscosity.

In Trial 2 the inclusion of an  $\alpha$ -galactosidase or a pectinase preparation both improved turkey live weights but feed conversion ratios were not significantly improved. This indicates that the nutritive value of the target substrate (soyabean meal) was improved but that the energy improvement obtained was close to the 10 % by which the diets had been adjusted. However, the effect of both the  $\alpha$ -galactosidase and the pectinase preparation on turkey performance was more pronounced at 14 days of age indicating that turkeys adapt to indigestible carbohydrates in a way similar to that of poultry receiving barley based diets (Almirall *et al.*, 1995).

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## EGG AND EGG SHELL QUALITY IN FIVE STRAINS OF LAYING HEN AND THE EFFECT OF HEN AGE

J.R. ROBERTS\*, A. M. LEARY\*, W. BALL\*\* and J.V. NOLAN\*\*

### Summary

Egg and egg shell quality were assessed in five strains of laying hen (the imported strains Isa Brown, Lohmann Brown and Hy-Line Brown and the Australian strains Tegel Super Brown, and Hy-Line-CB) at six times during the first year of lay. Egg collections were made at 35, 45, 55, 65 and 75 weeks of age. Sixty eggs from each strain at each age were collected. The imported strains laid larger eggs with darker coloured shells. Shell weight and shell thickness were highest with the imported strains although the shell weight:egg weight ratio was similar for all strains. In all strains, shell quality and Haugh units declined with increasing age.

### I. INTRODUCTION

A number of strains of brown egg layers have been imported into Australia in recent years. The advantages of the imported strains are generally regarded as being greater feed efficiency and production and the production of dark brown egg shells. These strains also tend to lay larger eggs which may or may not be an advantage, depending on the marketing practices of a particular state.

In the present study, egg shell quality was examined in five strains of layers at different times during the commercial laying life of the flock.

### II. METHODS

Sixty birds of each of five strains, the imported strains Isa Brown (ISA), Lohmann Brown (LOH) and Hyline Brown (HLB), and the Australian strains Tegel Super Brown (TSB) and Hy-Line-CB (HL-CB) were housed three to a cage in a layer shed at the University of New England's "Laureldale" farm in Armidale. The birds had been transported at day-old from their respective hatcheries to a pullet rearing farm in Moonbi, near Tamworth, New South Wales. The pullets were transported to the "Laureldale" farm at 17-18 weeks of age. Three different diets were fed: a complete ration containing ground limestone as the calcium source and the same mix minus ground limestone but with either oyster shell grit or limestone grit provided as the calcium source. The oyster shell and limestone grit were supplied when needed as judged by visual assessment. The data presented here are pooled for all three diets. Internal egg and egg shell quality assessments were conducted at 35, 45, 55, 65 and 75 weeks of hen age. Sixty eggs from each strain were measured at each age. The following measurements were made: egg weight, gross egg shell defects, egg shell pigmentation (by reflectivity and colour score), egg specific gravity (by Archimedes Principle), egg length and breadth, egg shell breaking strength (by quasi-static compression), shell weight and shell thickness (using a dial comparator gauge). Shape index (breadth/length) and percentage shell (shell weight : egg weight) were calculated. Internal egg quality was assessed by measuring the Haugh units of the albumen

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Table 1: Egg and egg shell quality in five strains of laying hens at six different hen ages. Values given are Mean  $\pm$  SEM (n = 60).

	Strain	Age Weeks					Statistical Analysis		
		35	45	55	65	75	Strain	Age	Strain x Age
Egg production eggs/hen/ 100 days (whole flock)	ISA	83.9	82.9	76.8	69.3	54.9			
	LOH	82.7	79.9	75.6	69.2	52.8			
	HLB	83.4	79.9	76.3	68.12	53.1			
	TSB	87.8	79.4	67.5	54.8	41.1			
	HL CB	88.8	82.3	70.9	64.4	55.9			
Egg Weight g	ISA	65.3 $\pm$ 0.5	67.6 $\pm$ 0.7	68.5 $\pm$ 0.7	69.3 $\pm$ 0.8	68.7 $\pm$ 0.8	< .0001	< .0001	NS
	LOH	61.7 $\pm$ 0.5	60.8 $\pm$ 0.6	65.1 $\pm$ 0.3	63.8 $\pm$ 0.4	64.4 $\pm$ 0.7			
	HLB	65.9 $\pm$ 0.7	68.1 $\pm$ 0.6	68.7 $\pm$ 0.7	69.9 $\pm$ 0.8	70.8 $\pm$ 0.9			
	TSB	60.4 $\pm$ 0.7	62.1 $\pm$ 0.6	63.4 $\pm$ 0.6	65.3 $\pm$ 0.7	66.5 $\pm$ 0.8			
	HL-CB	58.7 $\pm$ 0.5	60.0 $\pm$ 0.7	61.3 $\pm$ 0.6	63.3 $\pm$ 0.6	62.3 $\pm$ 0.6			
Shell Colour Score	ISA	3.7 $\pm$ 0.2	4.1 $\pm$ 0.2	4.2 $\pm$ 0.2	4.2 $\pm$ 0.2	4.9 $\pm$ 0.2	< .0001	.0006	NS
	LOH	3.1 $\pm$ 0.2	3.8 $\pm$ 0.2	3.8 $\pm$ 0.2	4.4 $\pm$ 0.2	3.9 $\pm$ 0.2			
	HLB	3.5 $\pm$ 0.2	4.0 $\pm$ 0.2	3.9 $\pm$ 0.2	4.1 $\pm$ 0.2	4.6 $\pm$ 0.2			
	TSB	7.0 $\pm$ 0.2	7.5 $\pm$ 0.2	7.5 $\pm$ 0.1	7.5 $\pm$ 0.2	7.8 $\pm$ 0.2			
	HL-CB	7.4 $\pm$ 0.2	7.5 $\pm$ 0.2	8.5 $\pm$ 1.2	8.5 $\pm$ 0.8	7.8 $\pm$ 0.2			
Egg Specific Gravity	ISA	1.089 $\pm$ 0.001	1.090 $\pm$ 0.001	1.085 $\pm$ 0.001	1.080 $\pm$ 0.002	1.080 $\pm$ 0.002	NS	< .0001	< .0001
	LOH	1.088 $\pm$ 0.001	1.087 $\pm$ 0.001	1.082 $\pm$ 0.002	1.080 $\pm$ 0.002	1.088 $\pm$ 0.007			
	HLB	1.088 $\pm$ 0.001	1.088 $\pm$ 0.001	1.084 $\pm$ 0.001	1.083 $\pm$ 0.001	1.080 $\pm$ 0.002			
	TSB	1.088 $\pm$ 0.001	1.086 $\pm$ 0.001	1.108 $\pm$ 0.005	1.077 $\pm$ 0.001	1.075 $\pm$ 0.001			
	HL-CB	1.086 $\pm$ 0.001	1.084 $\pm$ 0.001	1.096 $\pm$ 0.004	1.078 $\pm$ 0.001	1.076 $\pm$ 0.001			
Breaking Strength Newtons	ISA	28.6 $\pm$ 1.0	27.6 $\pm$ 1.0	24.8 $\pm$ 1.0	21.9 $\pm$ 1.2	23.2 $\pm$ 1.3	< .0001	< .0001	NS
	LOH	28.9 $\pm$ 1.2	27.5 $\pm$ 1.3	25.0 $\pm$ 0.9	24.1 $\pm$ 1.1	25.2 $\pm$ 1.2			
	HLB	31.3 $\pm$ 0.8	29.5 $\pm$ 0.9	26.9 $\pm$ 0.9	25.9 $\pm$ 1.1	26.4 $\pm$ 1.1			
	TSB	31.3 $\pm$ 1.0	24.7 $\pm$ 1.3	24.1 $\pm$ 1.1	22.7 $\pm$ 1.1	21.9 $\pm$ 1.2			
	HL-CB	27.7 $\pm$ 1.1	24.5 $\pm$ 1.0	22.5 $\pm$ 1.1	22.1 $\pm$ 0.9	20.2 $\pm$ 1.1			

Table 1 (Cont.): Egg and egg shell quality in five strains of laying hens at six different hen ages. Values given are Mean  $\pm$  SEM.

	Strain	Age Weeks					Statistical Analysis		
		35	45	55	65	75	Strain	Age	Strain x Age
Haugh Units	ISA	87.7 $\pm 1.0$	81.1 $\pm 1.3$	81.2 $\pm 1.7$	68.5 $\pm 2.1$	72.5 $\pm 2.2$	<.0001	<.0001	.0022
	LOH	85.7 $\pm 1.1$	83.4 $\pm 1.2$	85.4 $\pm 1.0$	74.4 $\pm 1.9$	69.5 $\pm 2.3$			
	HLB	88.6 $\pm 2.4$	82.5 $\pm 1.2$	80.7 $\pm 1.3$	73.3 $\pm 1.7$	72.2 $\pm 2.2$			
	TSB	83.2 $\pm 1.3$	74.8 $\pm 1.6$	71.1 $\pm 1.8$	65.3 $\pm 2.1$	58.0 $\pm 2.4$			
	HL-CB	84.0 $\pm 0.9$	79.8 $\pm 1.0$	80.1 $\pm 1.4$	68.5 $\pm 2.0$	72.9 $\pm 1.6$			
Yolk Colour Roche Scale	ISA	11.9 $\pm 0.09$	12.3 $\pm 0.08$	12.3 $\pm 0.07$	11.8 $\pm 0.06$	12.1 $\pm 0.08$	NS	.0003	NS
	LOH	11.8 $\pm 0.08$	12.2 $\pm 0.09$	12.3 $\pm 0.07$	11.8 $\pm 0.07$	12.4 $\pm 0.07$			
	HLB	11.6 $\pm 0.09$	12.2 $\pm 0.08$	12.2 $\pm 0.07$	11.7 $\pm 0.06$	12.4 $\pm 0.06$			
	TSB	12.0 $\pm 0.05$	12.3 $\pm 0.06$	12.4 $\pm 0.07$	11.8 $\pm 0.08$	12.6 $\pm 0.09$			
	HL-CB	11.9 $\pm 0.09$	12.2 $\pm 0.07$	12.4 $\pm 0.07$	11.9 $\pm 0.05$	12.2 $\pm 0.08$			
Shell Weight g	ISA	6.17 $\pm 0.07$	6.37 $\pm 0.09$	6.20 $\pm 0.10$	6.12 $\pm 0.10$	5.90 $\pm 0.11$	<.0001	.0084	.0036
	LOH	6.10 $\pm 0.06$	6.08 $\pm 0.08$	6.07 $\pm 0.08$	6.28 $\pm 0.11$	6.19 $\pm 0.11$			
	HLB	6.00 $\pm 0.07$	6.17 $\pm 0.07$	5.56 $\pm 0.19$	6.16 $\pm 0.11$	6.02 $\pm 0.12$			
	TSB	5.61 $\pm 0.10$	5.56 $\pm 0.08$	5.65 $\pm 0.07$	5.65 $\pm 0.09$	5.43 $\pm 0.12$			
	HL-CB	5.29 $\pm 0.05$	5.21 $\pm 0.06$	5.19 $\pm 0.07$	5.22 $\pm 0.07$	5.03 $\pm 0.10$			
% Shell weight	ISA	9.44 $\pm 0.09$	9.43 $\pm 0.10$	9.14 $\pm 0.13$	8.87 $\pm 0.14$	8.65 $\pm 0.19$	NS	<.0001	NS
	LOH	9.18 $\pm 0.10$	8.99 $\pm 0.11$	8.71 $\pm 0.14$	8.72 $\pm 0.14$	8.79 $\pm 0.17$			
	HLB	9.11 $\pm 0.09$	9.06 $\pm 0.07$	8.12 $\pm 0.27$	8.81 $\pm 0.13$	8.73 $\pm 0.12$			
	TSB	9.31 $\pm 0.15$	9.04 $\pm 0.12$	8.92 $\pm 0.09$	8.66 $\pm 0.11$	8.23 $\pm 0.17$			
	HL-CB	9.04 $\pm 0.10$	8.71 $\pm 0.08$	8.50 $\pm 0.11$	9.27 $\pm 1.0$	8.08 $\pm 0.15$			
Shell Thickness $\mu\text{m}$	ISA	395 $\pm 4$	397 $\pm 5$	377 $\pm 6$	387 $\pm 5$	373 $\pm 6$	<.0001	<.0001	NS
	LOH	380 $\pm 4$	373 $\pm 5$	363 $\pm 4$	384 $\pm 4$	375 $\pm 5$			
	HLB	379 $\pm 4$	378. $\pm 3$	377 $\pm 3$	384 $\pm 6$	375 $\pm 8$			
	TSB	383 $\pm 4$	362 $\pm 6$	365 $\pm 4$	378 $\pm 3$	357 $\pm 6$			
	HL-CB	358 $\pm 3$	350 $\pm 3$	342 $\pm 4$	362 $\pm 7$	337 $\pm 4$			



and the Roche colour score of the yolk. Measured data were analysed by two factor ANOVA. Fisher's (Protected) Least Significance Difference test was used to determine differences between means. Scores were analysed by the Kruskal-Wallis. Significance was assumed at  $P < 0.05$ .

### III. RESULTS

The results of the experiment are summarised in Table 1. Egg weight was highest in the imported strains and increased with age of the hen. The same trend was observed for egg length, egg width and shape index. Shell colour was lightest in the Australian strains and darkest in the imported strains. However, all strains laid lighter coloured eggs as the birds got older. Egg specific gravity was not significantly different between strains and declined with age in all strains. Shell breaking strength was greatest for the Hy-Line Brown and lowest for the Hy-Line-CB. In addition, shell breaking strength declined with age for all strains. Haugh units were generally lowest in the Tegel Super Brown and highest in the Lohmann Brown and Hy-Line Brown. Haugh units declined with bird age in all strains. Yolk colour was similar among strains and there was no consistent relationship between yolk colour and bird age. Shell weight and shell thickness were higher in the imported strains than in the Australian strains and tended to decrease with age. However, the shell weight : egg weight ratio (% shell weight) was similar for all strains although it, also, declined with age.

### IV. DISCUSSION

This study confirmed previously reported findings that there are strain differences in both internal and egg shell quality in laying hens (Buss, 1982). The imported strains had heavier and thicker shells than did the Australian strains. However, because the eggs of the imported strains also tended to be larger there were no significant differences among strains in the shell weight:egg weight ratio. There were strain differences with respect to Haugh units but not yolk colour. This study also confirmed previously reported findings that aspects of egg and egg shell quality deteriorate as the hens age (Nys, 1986). All strains showed a reduction in internal quality, as evidenced by the Haugh Units, as the hens grew older. Egg shell quality also declined with age with reductions in egg specific gravity, shell breaking strength, shell weight, shell thickness and shell weight : egg weight ratio.

### V. ACKNOWLEDGEMENTS

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## PROCEDURES FOR IMPROVING EGG SHELL QUALITY AT HIGH TEMPERATURES

S. MUHEEREZA and D. BALNAVE

Summary

Shell breaking strength of eggs from 30- and 38-week old commercial laying hens maintained from point of lay at 32°C was improved by altering the conventional lighting regimen of 16 h light:8 h dark (16L:8D) to an intermittent lighting regimen of 3L:1D. Additional improvements were achieved by supplementing the diet with sodium bicarbonate (NaHCO<sub>3</sub>). Rate of lay to 38 weeks was not significantly affected by the treatments.

## I. INTRODUCTION

Balnave and Muheereza (1996) reported the results of short-term studies which showed that supplementing a layer diet with 10 g NaHCO<sub>3</sub>/kg significantly improved the shell breaking strength of eggs from hens caged at temperatures of 30°C and 35°C in continuous light. The hypothesis examined in these studies was that a continuous lighting regimen would allow the synchronisation of egg shell formation with supplementary bicarbonate intake by the hen. The inconsistent responses to bicarbonate observed in previous studies reported in the scientific literature probably reflects the fact that under a conventional daily 16 h photoperiod (16L:8D) the bicarbonate is not consumed during the dark period, the time during which egg shell formation normally occurs.

The application of continuous lighting for an extended period is not satisfactory from a poultry welfare point of view. However, alternative lighting regimens can be used which will allow hens to rest during dark periods while still synchronising the intake of supplementary bicarbonate with egg shell formation. One possibility is the use of repetitive 3L:1D cycles. This intermittent lighting regimen is currently being examined in a long-term study using hens maintained at 32°C. The initial results from this experiment are detailed in this report.

## II. METHODS

One hundred and ninety two Tegel SuperBrown pullets were received from a rearing farm at 19 weeks of age and placed, two birds per cage, in two temperature controlled rooms maintained at a constant 32°C in a conventional 16L:8D lighting regimen. From the time of first egg (20 weeks of age) the birds in one room were continued on the conventional lighting programme while those in the other room were changed to repetitive 3L:1D cycles. Eight replicates of six hens were allocated to each of two dietary treatments in each room. These consisted of a conventional layer diet (11.96 MJ of ME and 200 g crude protein/kg) and this diet supplemented with 10 g NaHCO<sub>3</sub>/kg.

Production measurements commenced when the overall rate of lay reached 10%. Egg numbers were recorded daily and egg shell breaking strength was measured at 30 and 38 weeks of age using all eggs laid over a three day period. Egg shell breaking strength was measured using a cantilever system (Balnave *et al.*, 1992).

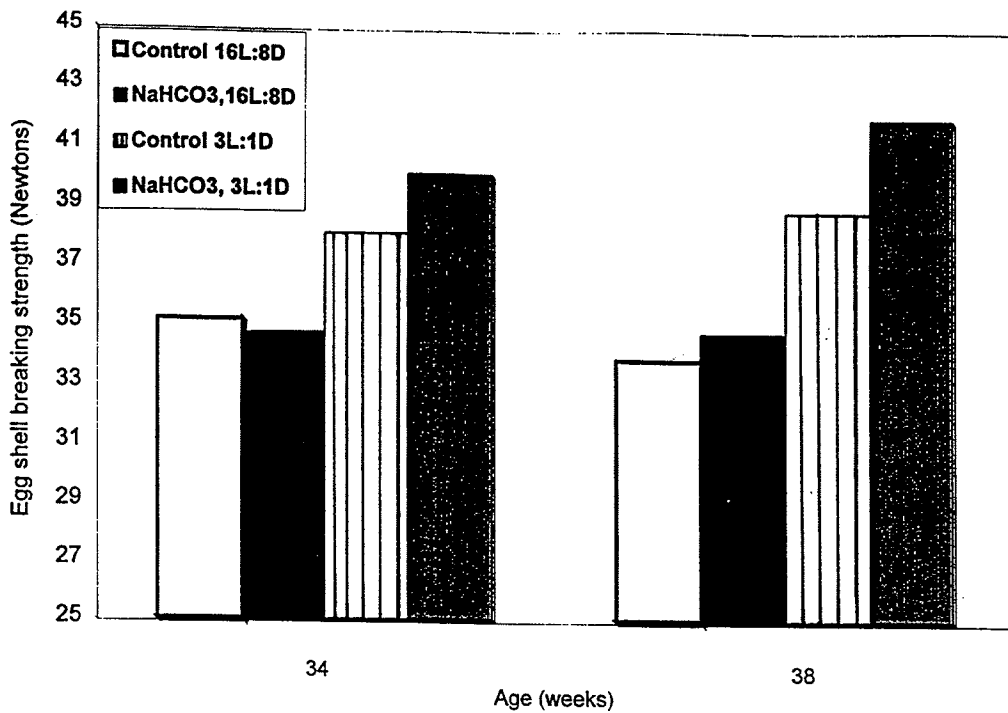


Figure 1. Egg shell breaking strength (Newtons) for hens at different ages.

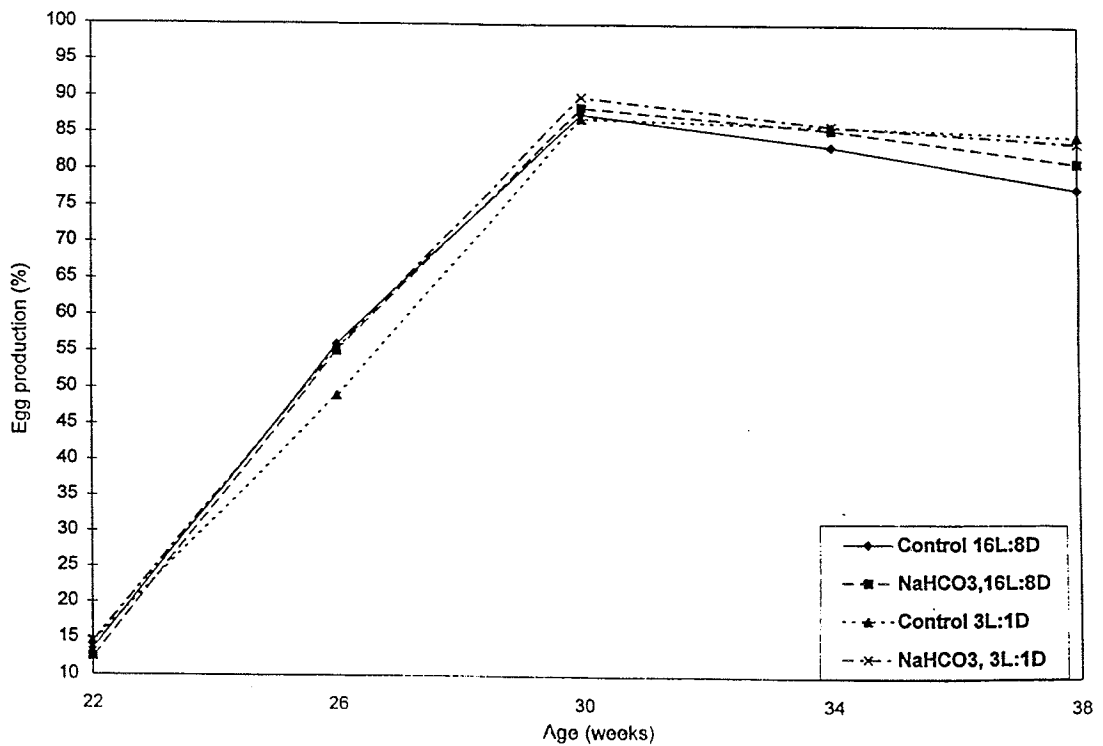


Figure 2. Egg production to 38 weeks.

### III. RESULTS AND DISCUSSION

This novel approach to improving egg shell quality has been successful in that both intermittent lighting and dietary NaHCO<sub>3</sub> supplementation have given beneficial results in terms of egg shell breaking strength.

The results for rate of lay and egg shell breaking strength are shown in Figures 1 and 2. Neither the lighting regimen nor the dietary treatment had any significant effect on rate of lay to 38 weeks. However, egg shell breaking strength was significantly affected by the lighting regimen. The improvement observed with the 3L:1D regimen was significant (P<0.001) and evident at both 30 and 38 weeks of age. The NaHCO<sub>3</sub> supplement gave improvements additional to those obtained with the 3L:1D lighting regimen (P<0.01) but had no effect to 38 weeks in the 16L:8D light regimen.

These results, reporting measures in early lay at a time when egg shell quality should be optimum, indicate that the shell breaking strength of eggs from hens maintained at high temperatures can be improved by altering the daily lighting regimen to allow hens to consume nutrients throughout the daily 24 h period. The evidence also indicates that additional improvements can be achieved by supplementing the diet with NaHCO<sub>3</sub> under the intermittent lighting regimen. The experiment is being continued to determine whether the benefits accruing from these management strategies will be more pronounced later in the laying period when egg shell quality declines with increasing age of the hen.

### ACKNOWLEDGEMENTS

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## THE EFFECT OF STEAM AND COLD PELLETING OF FOUR GRAIN LEGUMES AT DIFFERENT INCLUSION LEVELS IN BROILER STARTER DIETS

R.A. PEREZ-MALDONADO, P.F. MANNION and D.J. FARRELL

### Summary

Four grain legumes were included at 5 different levels (120-360 g/kg) in steam or cold pelleted diets of broilers grown to 21 days of age. Sweet lupins and chick peas gave inferior growth and feed efficiency to field peas and faba beans. Digesta viscosity and visual score of stickiness of excreta were higher on sweet lupin diets than on others. Increasing the inclusion of field peas and faba beans did not affect growth rate as it did for chick peas and tended to do so for sweet lupins. Steam pelleting consistently improved weight gain and feed intake when compared with cold pelleting.

### I. INTRODUCTION

Grain legume production has reached almost one million metric tonnes per year in Australia. Much of this is used by the poultry industry where there is increasing competition on a global basis for stock feed ingredients. Although many grain legumes have relatively high concentrations of energy and amino acids of good quality (Pettersson and Mackintosh, 1994), some have antinutritional factors which may limit inclusion.

Johnson and Eason (1990, 1991) reported chicken growth trials on diets with a range of grain legumes. There is still little information on response to different levels of their inclusion in diets. Effects of heat treatment by steam pelleting on bird performance are also of interest. Both inclusion level of grain legumes and steam pelleting of diets were examined.

### II. MATERIALS AND METHODS

Feather-sexed male broiler chickens of a commercial strain were used in an experimental design of 4 grain legumes, 2 pellet treatments, 5 inclusion levels and 4 replicates each of 10 chicks in a randomised block design. The chickens were housed in electrically-heated, mesh wire-floored battery brooders from one-day-old until 10 days of age. Each group then was transferred to follow-on cages in a heated, insulated room (26-29 °C) to 21 days of age. One week later the experiment was repeated using one-day-old chickens from the same source. Food and water were available *ad libitum*.

This experiment was designed to investigate the effect on bird performance of six different levels (120, 180, 240, 300 and 360 g/kg) of field peas (cv. glenroy), chick pea (cv. amethyst), lupins (cv. Gungurru), and faba beans (cv. fiord). The composition of diets with the four grain legumes included at 360 g/kg is given in Table 1. Diets containing intermediate levels of grain legumes were formulated to similar nutrient specifications by blending each of these with a basal diet without a grain legume. Half the quantity of each diet was either cold-pelleted (45°C) or steam-pelleted (70-80°C). After the pellets had thoroughly cooled, they were passed through a crumbler.

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The University of Queensland, St Lucia, Qld 4075 and the Queensland Poultry Research and Development Centre, Alexandra Hills, Qld 4161.

Group bird weight was recorded at the start and after 21 days when feed intake was also measured. At 21 days two representative birds from each cage were randomly chosen and killed by cervical dislocation and weighed. Intestinal contents from the proximal part of the small intestine of each bird were centrifuged and the viscosity of the supernatant determined in a viscometer (Model LVTCP, Brookfield Engineering Laboratories, Stoughton, USA). The length of the small intestine was measured and the liver and pancreas were excised and weighed. Excreta were visually scored for stickiness using a range of 0 (low), to 3 (high).

Table 1. Ingredient and nutrient composition (g/kg, as fed basis) of the control diet and diets containing the highest level of inclusion of each of the grain legumes.

Ingredient	Basal	Field peas	Faba beans	Lupins	Chick peas
Maize	359.20	387.84	219.44	403.44	243.03
Wheat	300.00	-	150.00	-	150.00
Legume	-	360.00	360.00	360.00	360.00
Soybean meal	244.35	147.65	148.85	91.06	142.63
Meat and bone meal	63.42	44.59	63.24	68.28	69.12
Vegetable oil	13.17	32.12	39.86	59.41	18.86
Dicalcium phosphate	-	6.77	-	-	-
Limestone	5.97	7.84	5.84	4.06	4.77
Sodium chloride	2.55	2.67	2.61	2.23	1.15
DL-methionine	2.58	3.36	3.35	3.36	3.36
L-lysine HCL	2.09	-	-	1.49	-
L-Threonine	-	0.38	0.05	-	0.41
L- Tryptophan	-	0.10	0.08	-	-
Premix	6.68	6.68	6.68	6.68	6.68
Calculated analysis					
AME (MJ kg)	12.70	12.70	12.70	12.70	12.70
Lysine	12.06	12.07	12.06	12.06	12.06
Methionine	6.03	6.16	6.13	6.03	6.22
Methionine + cystine	9.91	9.65	9.65	9.66	9.65
Threonine	8.20	8.20	8.20	8.20	8.20
Isoleucine	9.44	8.93	9.05	9.21	9.06
Tryptophan	2.64	2.29	2.29	2.48	3.01
Calcium	10.00	10.00	10.00	10.00	10.00
Available phosphorus	4.50	4.50	4.50	4.50	4.50

### III. RESULTS AND DISCUSSION

The effect of grain legume on liveweight gain, feed intake, feed conversion ratio (FCR), digesta viscosity, intestinal length, liver and pancreas weight and pen score values are presented in Table 2. When averaged over all levels of inclusions, birds fed lupins or chick peas gained less weight ( $P < 0.05$ ), tended to consume less feed and showed poorer FCR ( $P < 0.05$ ) than those fed field peas or faba beans.

Table 2. The effect of grain legumes on liveweight gain, feed intake, FCR, intestinal viscosity, intestinal length, liver and pancreas weight, and excreta score (0-3).

Treatment	Weight gain (g)	Feed intake (g)	Feed conversion (g:g) <sup>1</sup>	Viscosity (cPs)	Intestinal length (cm/100g)	Liver (g/100gWt)	Pancreas (g/100gWt)	Excreta stickiness score
Field pea	673 a	831 a	1.24 b	12.9 c	14.7 b	2.79 ab	0.33 b	0.63
Faba bean	664 a	816 ab	1.24 b	15.6 b	15.0 b	2.79 ab	0.33 b	0.77
Lupin	645 b	828 a	1.29 a	33.3 a	15.9 a	2.87 a	0.34 b	2.67
Chick pea	630 c	812 b	1.30 a	16.1 b	15.7 a	2.74 b	0.42 a	1.60
LSD	14.8	15.5	0.022	2.50	0.57	0.116	0.016	

(P=0.05)

<sup>1</sup> Corrected to dry matter. Values within a column with similar superscripts are not significantly different (P>0.05).

Table 3. The interaction of grain legumes x inclusion level on liveweight gain, bird feed intake, FCR, intestinal viscosity, intestinal length, liver and pancreas weight, and excreta score (0-3).

Treatment	Diet level (g/kg)	Weight gain (g)	Feed intake (g)	Feed conversion (g:g) <sup>1</sup>	Viscosity (cPs)	Intestinal length (cm/100gWt)	Liver (g/100gWt)	Pancreas (g/100gWt)	Excreta stickiness score
Field pea	12	664 a	818	1.25 ab	14.14 a	14.79	2.81	0.316 a	1.17
	18	672 a	828	1.25 ab	13.59 a	14.25	2.78	0.345 a	0.75
	24	686 a	838	1.23 ab	14.03 a	14.89	2.88	0.331 a	0.46
	30	687 a	839	1.22 a	10.97 a	14.68	2.64	0.329 a	0.33
	36	657 a	833	1.27 b	12.06 a	14.99	2.85	0.346 a	0.42
Faba bean	12	625 d	810	1.31 a	19.09 a	15.52	2.83	0.321 a	1.04
	18	651cd	822	1.27 ab	16.47 a	14.86	2.79	0.343 a	1.08
	24	659bc	814	1.24 b	14.66 a	14.85	2.82	0.334 a	0.63
	30	687ab	818	1.20 c	14.17 a	15.15	2.85	0.347 a	0.58
	36	699 a	818	1.17 cd	13.47 a	14.51	2.64	0.324 a	0.50
Lupin	12	650ab	848	1.31 a	21.06 a	15.24	2.94	0.334 a	2.29
	18	646ab	827	1.29 ab	31.77 b	15.46	2.83	0.342 a	2.38
	24	638ab	818	1.29 ab	32.03 b	15.66	2.80	0.325 a	2.92
	30	669 a	834	1.25 b	38.28 c	16.37	2.85	0.357 a	2.88
	36	623 b	814	1.32 a	43.19 c	16.73	2.93	0.341 a	2.88
Chick pea	12	642 a	828	1.29 ab	18.22 a	15.6	2.73	0.39 a	2.08
	18	638 a	806	1.27 a	17.97 a	16.01	2.72	0.402 ab	1.46
	24	639ab	807	1.27 a	15.0 a	15.11	2.72	0.418 ab	1.58
	30	624ab	816	1.32 b	14.34 a	15.29	2.88	0.437 b	1.42
	36	606 b	800	1.33 b	14.88 a	16.56	2.66	0.442 c	1.46
LSD (P<0.05)	33.0	34.6	0.05	5.599	1.282	0.259	0.036		

<sup>1</sup> Corrected to dry matter. Values within a column with similar superscripts are not significantly different (P>0.05).

When compared with the other treatments, intestinal viscosity was higher ( $p < 0.05$ ) in birds on the sweet lupin diet which also showed higher excreta pen score values. Relative values for gut length were also higher for sweet lupin ( $P < 0.05$ ) followed by chick pea diets. Pancreas weight was higher ( $P < 0.05$ ) in birds fed chick pea diets when compared with the other treatments.

The effects of level of grain legume inclusion on the same parameters as shown in Table 2 are given in Table 3. Feed intake, intestinal length, and liver weight, were not significantly ( $P > 0.05$ ) affected. Chick pea was the only legume to significantly ( $P < 0.05$ ) depress weight gain as dietary inclusion increased. High levels of inclusion of field pea or lupins generally produced equivalent growth to lower levels. The growth performance and FCR of chickens fed faba beans unexpectedly improved linearly as the level of faba bean increased. For legumes other than faba bean, the FCR responses to level of inclusion were somewhat curvilinear with the lowest and highest level giving poorer FCR than intermediate levels. As the level of inclusion increased, lupin was the only grain legume to exhibit significantly ( $P < 0.05$ ) higher viscosity in the small intestine of birds. This observation correlated well with high values of excreta stickiness. Pancreas weight increased ( $P < 0.05$ ) linearly on the chick pea diets as the level of inclusion was increased suggesting an antinutritional factor in chick peas of this cultivar.

The effects of hot and cold pelleting of diets on the same parameters as in Table 2 are given in Table 4.

Table 4. The effect of temperature on liveweight gain, feed intake, FCR, intestinal viscosity, intestinal length, liver weight, pancreas weight, and excreta score (0-3).

Treatment	Weight gain (g)	Feed intake (g)	Feed conversion (g:g) <sup>1</sup>	Viscosity (cPs)	Intestinal length (cm/100g)	Liver (g/100gWt)	Pancreas (g/100gWt)	Excreta stickiness score
HOT	660 a	829 a	1.27	19.9	15.2	2.81	0.358	1.34
COLD	646 b	815 b	1.26	19.0	15.5	2.79	0.355	1.38
LSD	10.4	10.9	ns	ns	ns	ns	ns	

$P < 0.05$ )

<sup>1</sup> Corrected to dry matter. Values within a column with different superscripts are significantly different ( $P < 0.05$ ).

Birds fed steam-pelleted diets gained more weight and consumed more feed ( $P < 0.05$ ) than did those fed cold-pelleted diets. None of the interactions with pelleting temperature were significant ( $P > 0.05$ ). Although sweet lupins did not demonstrate a clear reduction in performance at the highest level of inclusion both growth rate and FCR were depressed. The high levels of viscosity and the high sticky droppings score are a major concern. Chick peas did not give the anticipated response with each dietary addition and they too gave an intermediate score for sticky droppings. Faba beans which did not perform well in previous laying trials (Perez-Maldonado *et al.*, 1996) gave consistently good performance, particularly for FCR. Johnson and Eason (1991) found reduced growth when faba beans and field peas were increased from 50 to 150 g/kg. A major finding from these results was the improved growth rate and feed intake when diets were steam pelleted. Since there was no interaction this means that the effect was consistent irrespective of grain legume or level of inclusion.



#### IV. ACKNOWLEDGEMENTS

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## LOW-ME WHEAT OR LOW-ME CHICKENS? - HIGHLY VARIABLE RESPONSES BY BIRDS ON THE SAME LOW-ME WHEAT DIET

R.J. HUGHES\* and M. CHOCT\*\*

### Summary

The "low-ME" wheat phenomenon is not entirely dependent on the physico-chemical nature of wheat. It is a multi-faceted problem closely linked with the individuality of the digestive physiology of broiler chickens given diets containing high levels of arabinoxylans from wheat. Commercial glycanases offer an immediate solution to the problem. However, more effort should be put into obtaining a clearer understanding of the "low-ME" problem, particularly as to why some birds in the same flock are not affected to anywhere near the same extent as others in terms of poor nutrient digestibility and excessive output of wet, sticky excreta.

### I. INTRODUCTION

The apparent metabolisable energy (AME) of Australian wheats varies widely (10.35 - 15.9 MJ/kg dry matter) according to studies by Mollah *et al.* (1983) and Rogel *et al.* (1987). A large proportion of the variability associated with the use of wheat as the main cereal in broiler feeds can be related to between-bird variation in ability to digest "low-ME" wheats. Choct (1995) and Choct *et al.* (1995a,b) have observed that the variability in the AME of wheat increases with decreases in the measured mean values for given samples of wheat, and that reduced AME values are closely associated with increased excreta output and lower feed conversion efficiency. Commercial feed enzymes can reduce variability as well as improve AME (Bedford, 1996; Choct, 1995; Choct *et al.*, 1995a,b). Further investigations of the low-ME wheat phenomenon have been hampered by the difficulty of obtaining sufficient quantities of suitable samples for repeated digestive physiology studies.

Choct (1995) observed that low-ME wheats harvested in the 1991/92 season had high soluble and insoluble non-starch polysaccharide (NSP) contents which were associated with a hot and dry harvest season. On the basis of these observations large amounts of low-ME wheats were obtained from the Roseworthy Campus in 1994, which was also a low rainfall season in South Australia. This paper reports on two studies in which highly variable responses in AME were observed in birds given diets based on low-ME wheats collected from the 1991/92 and 1994/95 harvests. The AME of these wheats were 9.2 and 10.1 MJ/kg DM, respectively, one month post-harvest; and 12.0 and 12.7 four to six months later.

### II. MATERIALS AND METHODS

The first experiment was done in December 1993 at the Parafield Poultry Research Centre. Day-old mixed sexed broiler chickens (Ingham IM98) were raised in a floor pen on a commercial starter crumble. The birds were transferred at 20 days of age to metabolism cages (two birds per cage) and given the commercial starter diet. Birds were

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allowed two days to adapt to the new environment. At 22 days of age one bird was removed from each cage. The remaining chickens (total 40) were weighed individually and then fed the same low-ME wheat diet (1991/92 harvest). Each kg of diet contained 820 g wheat, 134 g casein, 26 g dicalcium phosphate, 11 g limestone, 5 g vitamins and minerals, 3.6 g salt and 0.4 g choline chloride. A three day period enabled the birds to adapt to the experimental feed. Feed intake was measured during this period. During the following four days excreta were collected and feed intake was measured. At the end of the 7 day experimental period all birds were re-weighed.

The second experiment took place in May 1995 at the Parafield Poultry Research Centre. Mixed-sex Steggles broiler chickens were obtained from a local commercial farm and placed in metabolism cages as described in Experiment 1. Two experimental low-ME wheat diets were fed to a total of 48 birds housed individually. Each kg of diet contained 678 g wheat (1994/95 harvest), 76 g meat and bone meal, 170 g soyabean meal, 40 g poultry tallow, 5 g vitamins and minerals, 3.6 g salt, 3.2 g methionine, 2.5 g lysine, 0.8 g choline chloride, 20 g Celite marker and 2 g hydrocarbon marker. One diet contained 1 g Avizyme 1300 (Finnfeeds International).

All chickens from Experiment 2 were killed at 31 days of age. Digesta were collected from the duodenum, jejunum and ileum, then centrifuged at 12,000 g for 30 minutes. Viscosity was determined on thawed supernatant using a Brookfield DVIII viscometer at 25°C with a shear rate 5-500 s<sup>-1</sup>. Starch was determined by the glucose oxidase and peroxidase method after enzymatic hydrolysis with amylase and amyloglucosidase.

### III. RESULTS AND DISCUSSION

Measurements of AME of wheat observed in the first experiment (Table 1) are similar to the wide range of values for Australian wheats (10.35 - 15.9 MJ/kg DM) obtained in several studies by Mollah *et al.* (1983) and Rogel *et al.* (1987). Underlying reasons for the extreme variability in the AME of the wheat variety used are indicated in Table 2. Chickens showing the poorest responses voided copious quantities of wet, sticky excreta. It was clearly evident from visual observation and by measurement of the gross energy of the excreta that wheat granules were poorly digested by many chickens. Conversely, other birds from the same flock were able to effectively utilise this particular sample of wheat (Table 2). Similar variability in the ability of individual broilers to utilise low-ME wheats was reported by Rogel *et al.* (1987).

Results from Experiment 2 (Table 3) are consistent with those of Bedford (1996) in that the use of an enzyme reduced variability as well as improving AME and performance. Analysis of covariance indicated that AME was not directly related to either jejunal or ileal viscosity, although there were significant differences between diets in AME and viscosity.

Table 1. Variable responses in chickens given a low-ME wheat diet (Experiment 1).

	Mean (n = 40)	Standard deviation	Minimum value	Maximum Value
AME of wheat (MJ/kg DM)	12.1	1.6	8.8	14.9
Liveweight gain (g/bird/d)	66	10	43	86
Feed intake (g/bird/d)	141	16	106	177
Feed conversion (feed:gain)	1.92	0.22	1.65	2.50
Dry excreta (g/bird/day)	48	13	25	82
Excreta energy (MJ/kg DM)	15.0	0.5	14.0	16.2

Table 2. Variability in performance and characteristics of excreta from groups of five chickens showing high, average and low responses to wheat (Experiment 1).

	Response to low-ME wheat diet		
	High	Average	Low
AME of wheat (MJ/kg DM)	14.7 ± 0.2	12.2 ± 0.3	9.6 ± 0.5
Liveweight gain (g/bird/d)	68 ± 8	66 ± 13	62 ± 13
Feed intake (g/bird/d)	122 ± 11	139 ± 21	153 ± 20
Feed conversion (feed:gain)	1.77 ± 0.13	1.96 ± 0.24	2.07 ± 0.22
Dry excreta (g/bird/day)	27 ± 2	47 ± 7	68 ± 12
Excreta energy (MJ/kg DM)	14.2 ± 0.2	14.9 ± 0.3	15.7 ± 0.3

Table 3. Effect of addition of commercial glycanase to a low-ME wheat diet on the AME of the diet, performance of chickens, quantity and energy content of excreta, digesta viscosity and digestibility coefficient of starch in Experiment 2. (Mean ± standard deviation with number in parenthesis).

	Control	Enzyme
AME of diet (MJ/kg DM)	13.7 ± 0.9 (24)	14.5 ± 0.3 (24)
Liveweight gain (g/bird/d)	63 ± 12 (23)	65 ± 10 (24)
Feed intake (g/bird/d)	127 ± 23 (24)	128 ± 13 (24)
Feed conversion (feed:gain)	2.07 ± 0.33 (23)	1.93 ± 0.18 (24)
Dry excreta (g/bird/day)	40 ± 9 (24)	36 ± 4 (24)
Excreta energy (MJ/kg DM)	15.6 ± 0.6 (24)	14.8 ± 0.4 (24)
Duodenal viscosity (cP)	2.9 ± 0.9 (18)	1.7 ± 0.2 (16)
Jejunal viscosity (cP)	4.6 ± 2.0 (24)	2.3 ± 0.6 (23)
Ileal viscosity (cP)	14.0 ± 8.4 (24)	3.9 ± 0.9 (24)
Starch digestibility (jejunum)	0.73 ± 0.08 (24)	0.79 ± 0.08 (24)
Starch Digestibility (ileum)	0.95 ± 0.08 (24)	0.98 ± 0.02 (24)

Likewise, there were no correlations between starch digestibility and viscosity in either the jejunum or ileum, irrespective of whether viscosity was above or below 10 mPa.s as hypothesised by Bedford and Morgan (1996). However, the AME of the diet was weakly correlated with jejunal starch digestibility in birds given the unsupplemented low-ME diet:-

$$\text{AME} = 10.2 + 4.8 (\text{starch digestibility}); P = 0.03, R^2 = 0.20$$

but not in birds given enzyme. The AME was highly correlated with ileal starch digestibility in birds with and without enzyme addition to the low-ME diet:-

$$\text{AME} = 3.5 + 11 (\text{starch digestibility}); P < 0.001, R^2 = 0.68$$

The results of both experiments clearly indicate that the low-ME wheat phenomenon is a real effect characterised by large between-bird variability in AME, poor nutrient digestibility, loss of performance and excessive output of wet, sticky droppings. The use of a commercial feed enzyme reversed these deleterious effects (Experiment 2) and reduced between-bird variability in digesta viscosity and starch digestibility (Table 3).

Lack of direct relationships between digesta viscosity and AME or starch digestibility tend to support the view that the mode of action of commercial glycanases in wheat-based broiler diets is not due solely to a reduction in viscosity but may well involve the effect of viscosity on digesta transit time or proliferation of anaerobic microflora in the

intestinal tract, as suggested by Mollah and Annison (1981), Bedford and Morgan (1996) and Choct *et al.* (1996).

In conclusion, the so-called "low-ME" wheat phenomenon is, in fact, an interaction between wheat with high NSP content and the inability of many birds in the flock to cope with these anti-nutritive factors. Further studies in this area should concentrate on why other birds are able to deal successfully with the deleterious effects of NSP.

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## β-GLUCANASE REDUCES BUT DOES NOT ELIMINATE VARIATION IN AME OF BARLEY VARIETIES

A. KOCHER\* , R.J. HUGHES\* and A.R. BARR\*\*

### Summary

The effect of a commercial β-glucanase on the variability of apparent metabolisable energy (AME) of barley was studied in two experiments. In Experiment 1, supplementation with enzyme significantly improved the mean AME value of 11 varieties of barley from  $11.35 \pm 1.24$  to  $13.80 \pm 0.56$  MJ/kg dry matter (DM), i.e., the variability between the different barley varieties measured as the standard deviation was reduced by 55% with enzyme supplementation. In Experiment 2, the mean AME value of 22 varieties with enzyme was  $14.18 \pm 0.77$  MJ/kg DM. The overall mean value for AME of all barley varieties with enzyme supplementation was  $14.06 \pm 0.73$  MJ/kg DM. The lower limit in the 95% confidence interval was 12.91 MJ/kg DM. Hence, the use of a commercial β-glucanase resulted in a highly predictable "attainable" AME value for barley for feed formulation purposes. However, from a plant breeding viewpoint, a range of 1.5-2.0 MJ/kg indicates that AME could become a selection priority.

### I. INTRODUCTION

The apparent metabolisable energy (AME) of barley as a feedstuff for poultry is strongly affected by its β-glucan content. The total β-glucan content of barley cultivars varies between 26 and 79 g/kg DM with 23 - 69 g/kg DM soluble β-glucan (Jeroch and Dänicke, 1995). β-glucan is not depolymerised by endogenous digestive enzymes. Diets containing a high level of barley β-glucan give an increased digesta viscosity and reduced performance accompanied by sticky droppings (Choct and Annison, 1991).

Nutritionists are often not able to test raw ingredients and establish a true feeding value prior to use. Variation in raw ingredients accounts for 30% of the variation in the finished product and creates multiplicative effects together with weighing errors (Fawcett and Webster, 1996). Subsequently, the diet may not reach the required specification which can result in sub-optimal performance and economic loss. Several methods of treatment of barley have improved nutritive value mainly by reducing β-glucan content (Jeroch and Dänicke, 1995). For example, addition of β-glucanase to a diet containing a high inclusion rate of barley (80%) improved AME and reduced the variability of barley varieties by nearly 50% (Bedford, 1996).

This paper reports the AME of a wide range of genetically different barley varieties. The barley varieties were chosen from a diverse set of breeding lines ranging widely in starch, lysine, β-glucan and hull content. The crops were grown at three different locations in South Australia. In the first experiment 11 varieties were tested with and without a commercial β-glucanase. In the second experiment, the "attainable" AME of 22 varieties was determined by supplementation with a commercial enzyme, as shown by Hughes *et al.* (1996) for wheat.

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## II. MATERIALS AND METHODS

### (a) Bird management and AME trial

Day-old mixed sexed broiler chickens (Ingham IM98) were raised in floor pens on a commercial starter crumble. The birds were transferred at 20 days of age, two per cage, to individual metabolism cages and given a commercial starter diet with no added commercial  $\beta$ -glucanase. Birds were given two days to adapt to the new environment. At 22 days of age, one bird was removed from the each cage. The remaining chickens were weighed individually. A three day period enabled the birds to adapt to the experimental feed. Feed intake was measured during this period. During the following four days all excreta were collected and feed intake was measured. Moisture content of excreta voided during the first day of the collection period was measured. At the end of the 7 d experimental period, all birds were weighed individually.

### (b) Diet formulation, experimental design and determination of gross energy

The first experiment included 11 barley varieties grown at Brentwood. Each variety was tested with and without enzyme present in the feed. The second experiment included 22 barley varieties grown at the Roseworthy Campus, University of Adelaide and the Charlick Experiment Station at Strathalbyn. Samples were a composite of equal weight from each site. Enzyme was added to all diets. Schooner barley grown at each site was pooled and incorporated in an enzyme-supplemented control diet used in both experiments together with a sorghum control diet without enzyme. The basal composition was barley, (500 g/kg), sorghum (320 g/kg), casein (134 g/kg) and minor ingredients (vitamin + minerals) (66 g/kg). A commercial  $\beta$ -glucanase (Avizyme 1100, inclusion rate 1 kg/t) was added directly to the minor ingredients prior to mixing. Each diet was cold pelleted and replicated four times in a randomised block design.

Gross energy of excreta and milled feed was measured using a Parr isoperibol bomb calorimeter. Dry matter content of each sample of barley and pelleted and milled feeds was determined by drying at 105°C.

## III. RESULTS

In Experiment 1, the addition of  $\beta$ -glucanase significantly improved the AME of barley. The mean value of unsupplemented samples was  $11.35 \pm 1.24$  MJ/kg DM and  $13.80 \pm 0.56$  for supplemented samples (Figure 1). The addition of enzyme significantly improved feed conversion ratio (FCR) from  $2.03 \pm 0.22$  to  $1.77 \pm 0.15$  and significantly reduced excreta moisture from  $76.36 \pm 2.61$  to  $67.58 \pm 3.64$ .

In Experiment 2, the mean value for AME calculated over all 22 samples was  $14.18 \pm 0.77$  MJ/kg DM (Figure 2). The FCR was  $1.73 \pm 0.21$  and excreta moisture was  $67.17 \pm 5.24\%$ . These results were similar to those from Experiment 1 for enzyme-supplemented diets.

## IV DISCUSSION

The inclusion of a commercial  $\beta$ -glucanase in barley-based diets reduced the variability in AME by more than 50%. These results agree with the findings of Bedford (1996). The AME results from our first experiment were confirmed in a second

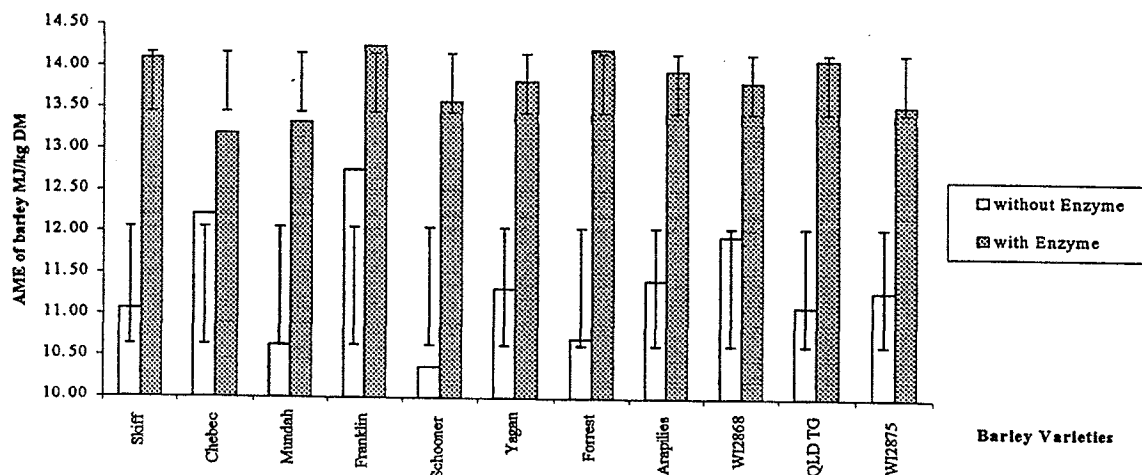


Figure 1. Increased metabolisable energy in barley and reduced variability with enzyme supplementation (vertical bars indicate one standard deviation calculated over all varieties).

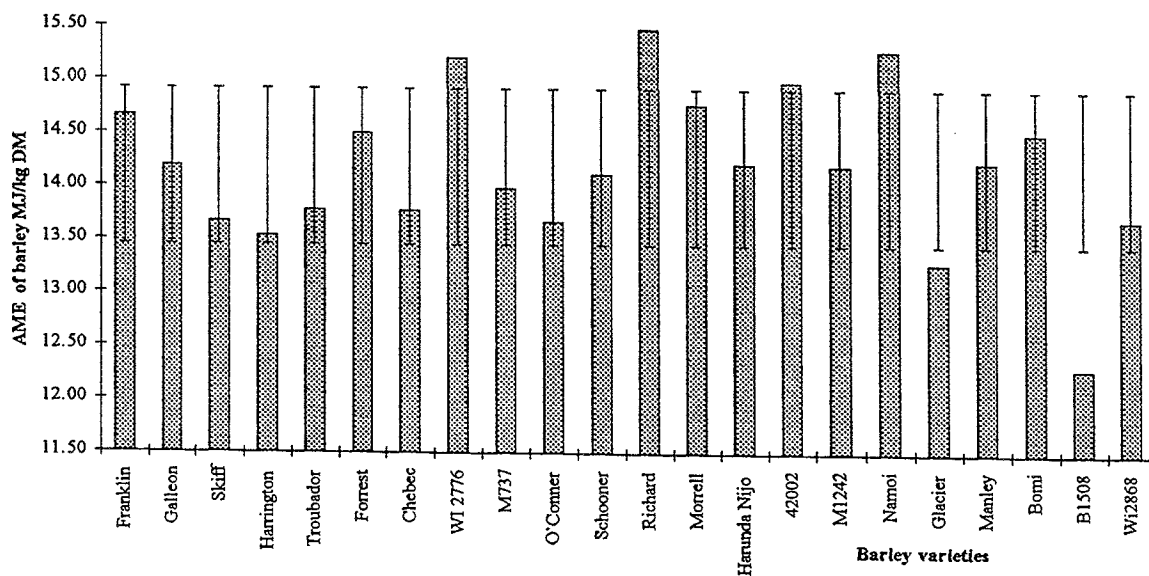


Figure 2. Variability between varieties of barley with enzyme supplementation (vertical bars indicate one standard deviation calculated over all varieties).



experiment with a wider range of barley varieties. Addition of enzyme did not improve the AME of some varieties according to pair-wise *t*-tests. The lower limit of the 95% confidence limits for the mean value of AME for barleys with enzyme was 12.91 MJ/kg DM, i.e. only one in 20 barley varieties supplemented with  $\beta$ -glucanase will be expected to be lower than 12.91 MJ/kg DM (e.g., variety B1508 which has impaired starch synthesis). The results for the barley control diet (14.43  $\pm$  0.24 MJ/kg DM for Experiment 1 and 14.69  $\pm$  0.07 MJ/kg DM for Experiment 2) indicate that the two studies were comparable. The mean value of "attainable" AME calculated over all 32 barley varieties with enzyme was 14.06  $\pm$  0.73 MJ/kg DM.

Improvements in AME and FCR together with a reduction in excreta moisture are likely to be the result of reduced intestinal viscosity brought about by depolymerisation of  $\beta$ -glucan with subsequent improvement in starch digestibility and avoidance of ileal fermentation as shown by Choct *et al.* (1996) in birds given diets enriched with non-starch polysaccharides.

Overall, the addition of  $\beta$ -glucanase improved AME but did not eliminate the variability in AME of a diverse range of barley varieties. The relatively large differences in AME remaining after addition of feed enzyme indicates wide scope for improvement by plant breeding.

#### ACKNOWLEDGEMENTS

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## FOOT LESIONS IN CAGED LAYERS: WELFARE IMPLICATIONS

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Summary

To assess the effect of foot lesions on the well-being of commercial caged laying hens (70 weeks of age) the behaviour of hens and the histopathology of the toes of hens with foot lesions were compared to a control group having no foot lesions. Hens with foot lesions had significantly more drinking bouts of less duration per bout, and spent significantly more time feather ruffling than hens without foot lesions. No significant differences in bouts of sitting, preening, hen pecking, cage pecking and eating or incidence of head scratching or dust bathing were observed between the treatments. Histology of the toes revealed a marked inflammatory response associated with the site of the lesion. Immunohistochemistry revealed nerve fibres of the type capable of transmitting pain in all toes examined. These observations indicate that the well-being of hens with foot lesions is likely to be compromised.

## I. INTRODUCTION

Laying hens are subject to lesions (hyperkeratosis) in different parts of the foot. The majority of lesions occur at the distal toe pad, with the most severe inflammation involving swelling of the foot pad from pressure resulting from standing on the wire. The aim of this study was to assess the welfare of caged hens with foot lesions compared to a control group of hens without foot lesions using behavioural and histopathology indices.

## II. METHODS

(a) Hens

Forty hens (70 weeks of age) selected from a caged flock of 2000 crossbred commercial layers were allocated in pairs to single tier laying cages (45 x 45 x 40 cm) and maintained on a layer ration. Each pair of hens was allocated to one of two treatment groups on the basis of the presence or absence of foot lesions. Treatment 1, n = 20 hens with foot lesions and Treatment 2, n = 20 with no foot lesions.

(b) Video recording of behaviour, viewing video tapes and analyses

A video recording was made for each pair of hens in each treatment post-lay from 1300h-1600h with food and water available *ad libitum*. Data on behaviour were obtained from watching video records and manually keying observations into a hand held micro-computer. The activities recorded were time and bouts of pecking at food, drinking, preening, sitting and the number of pecks made at the cage and other birds. Separate bouts of behaviour were recorded if they were separated by a pause of at least five seconds

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duration. The incidence of dust bathing, feather ruffling and head scratching were also recorded. SAS linear modelling procedures were used to analyse the effect of foot lesions on the behaviour of the hens.

(c) Histopathology and immunohistochemical labelling of nerve fibres

After video recording, all hens were killed by cervical dislocation and toes taken from each treatment group. A total of twelve toes were taken from eight hens with foot lesions and another ten toes taken from five hens with no foot lesions. The toes were fixed by immersion in Zamboni's fixative (Stefanini *et al.*, 1967) for two to four weeks at 4°C. Six toes from each treatment were processed by routine wax-embedding and 5µm-thick transverse sections stained with either haematoxylin and eosin, or Verhoeff and van Gieson, for visualisation of tissue types and any inflammatory response. Another four toes from each treatment (total of eight) were processed for immunohistochemical identification of nerve fibres labelling for substance P as described previously (Lunam, 1993).

### III. RESULTS

(a) Behaviour

Hens with foot lesions showed no significant differences in numbers of bouts of sitting, preening, eating and hen or cage pecks compared to that of hens with no foot lesions. The number of drinking bouts was significantly higher in hens with foot lesions compared to hens without foot lesions ( $P=0.006$ ) (Table 1). The incidence of hen peck bouts in birds with foot lesions was lower compared to hens without foot lesions, though this difference was not statistically significant ( $P=0.06$ ).

Table 1. Effects of foot lesions (FL) on number of bouts per hour of sitting (SB), preening (PB), hen pecking (HPB), cage pecking (CPB), eating (EB) and drinking (DB). (NFL) is no foot lesions. P = probability value in analysis of variance.

Treatment	SB	PB	HPB	CPB	EB	DB
FL	5.2	32.7	4.5	1.9	17.6	8.8
NFL	3.8	32.2	8.9	1.3	16.7	4.1
P	0.17	0.85	0.06	0.52	0.74	0.006

Table 2. Effects of foot lesions (FL) on time (seconds) spent sitting (ST), preening, (PT), eating (ET), drinking (DT) and incidence of feather ruffling (FR), head scratching (HS) and dust bathing (DB) averaged over one hour. P= probability in analysis of variance.

Treatment	ST	PT	ET	DT	FR	HS	DB
FL	1027	1016	732	146	0.25	3.7	0.9
NFL	735	949	683	187	0.03	4.4	0.7
P	0.16	0.51	0.64	0.44	0.02	0.26	0.55

Hens with foot lesions showed no significant differences in time spent sitting, preening, eating, drinking, head scratching or dust bathing compared to hens without foot lesions. Feather ruffling was the only behaviour which differed significantly between the treatments, the incidence of bouts of feather ruffling being significantly higher in hens with foot lesions compared to that of hens without foot lesions (Table 2).

#### (b) Histopathology

Macrophages and small aggregations of lymphocytes were observed in all toes with and without lesions. An inflammatory response, marked with mast cells and eosinophils, was more extensive in regions of toes with lesions than in comparable anatomical regions of toes without lesions. Immunohistochemistry revealed few freely ending nerve fibres labelling for substance P. The distribution and number of these immunolabelled nerve fibres were similar in toes with and without lesions.

### IV. DISCUSSION

These studies examined whether hens with foot lesions exhibited changes in behaviour that may indicate they were in pain. Although both treatments spent a similar average time per hour drinking, hens with foot lesions had significantly more drinking bouts, each bout of shorter duration, than hens without foot lesions. One explanation for the difference in drinking bouts is that the foot lesions become sore when additional pressure was placed upon them as the birds reached to drink from the water nipples. There was a non-significant trend ( $P=0.16$ ) for hens with foot lesions to spend more time sitting, suggesting they found it more uncomfortable to stand than hens without lesions. In addition, there was a trend for hens without foot lesions to be more aggressive, as they engaged in more bouts of feather pecking than hens with lesions. No explanation can be given as to why birds with foot lesions had a greater incidence of feather ruffling than hens without lesions.

The often extensive inflammatory response in the region of the foot lesions, marked with numerous eosinophils and mast cells, is consistent with inflammation associated with acute and/or consistent pain in mammals. The presence of free nerve endings labelling with substance P indicates that at least some nerves associated with the lesions are likely to be nociceptive, that is, they are capable of transmitting painful stimuli. These data, when considered with behavioural findings support the suggestion that foot lesions are likely to be painful and thus compromise the well-being of the hens.

### V. ACKNOWLEDGEMENT

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

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## A COMPARISON OF CEREAL AND GRAIN LEGUME DIETS ON SEVERAL DIGESTIVE PARAMETERS IN BROILERS

S.T.PETERSEN and D.J.FARRELL

### Summary

The behaviour of diets containing 700g of wheat or oats, or 250g of sweet lupins or faba beans per kg was examined in the gut of broiler chickens at 22 and 40 days of age. Digesta viscosity was highest using the wheat diet and lowest with the faba bean diet. Apparent metabolisable energy (AME) measured at the lower ileum and in the excreta showed much lower values, except for wheat, at the former site at both ages indicating considerable hindgut fermentation. This was supported by high volatile fatty acid (VFA) concentrations in caecal contents.

### I. INTRODUCTION

Poultry do not have the enzymes capable of digesting non-starch polysaccharides (NSP) such as arabinoxylans or  $\beta$ -glucans present in some cereal grains. Some NSPs may impede digestion by creating high digesta viscosity (Antoniou and Marquardt, 1981; White *et al.*, 1983). This can lead to poor weight gain, feed conversion, wet litter and consequently other problems such as dirty eggs, hock burns and breast blisters. They also contribute to the antinutritive actions by encapsulating the nutrients inside the grain. Reduced performance observed in young chicks fed such cereals has been overcome to a considerable extent by the dietary inclusion of exogenous enzymes which release nutrients and degrade viscous NSP (Bedford, 1993). Although an increase in digesta viscosity limits nutrient assimilation, it could also be argued that high viscosity allows more time for digestion by endogenous enzymes. However, the slower rate of feed transit allows intestinal bacteria to multiply and migrate to the upper reaches of the small intestine where they compete with the host for nutrients (Bedford, 1992). Recent results are presented here from an experiment undertaken to obtain a detailed chemical description of the behaviour of wheat, oats and two grain legumes within the poultry digestive system. It is hoped that this information will be useful as the basis of an *in vitro* method which will simulate digestion by the measurement of either the appearance of key metabolites or disappearance of feed components. A simple *in vitro* system which can assist with predicting any harmful effects of ANFs, especially in wheat prior to feeding, would be beneficial not only for recommendations on dietary inclusion levels but also for feed enzyme additions.

### II. MATERIALS AND METHODS

#### (a) Diets and birds

Each of the four diets used contained a major cereal or grain legume constituent and sorghum as shown in Table 1, plus 80g fishmeal/kg, 50g soybean oil/kg, 12g limestone/kg, 10g bentonite/kg, 7.5g commercial dicalcium phosphate/kg, 5.5g vitamin/mineral premix/kg and 1.5g sodium chloride/kg. Feed and water were provided *ad libitum*. At 10

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Table 1. Major ingredients in the four diets (g/kg).

Diet/	Wheat (W)	Oats (O)	Lupins (L)	Faba bean (FB)
Cereal/grain legume	700.00	700.00	250.00	250.00
Sorghum	133.50	133.50	583.50	583.50
Calculated AME (MJ/kgDM)	13.45	12.89	13.18	13.76

days of age 64 male Steggles x Ross broiler chicks were individually caged and diets were randomly assigned to 16 replicates per diet. Light was 23h/d and temperature was from 17-27°C.

### (b) Measurements and statistical analysis

The viscosity was measured in the supernatant taken from the digesta in the jejunum (fore) and ileal (hind) sections of eight birds at 22 and 40 days of age (32 birds killed per age) according to the method of Bedford and Classen (1993). Apparent metabolisable energy (AME) using 16 birds/ diet was measured by total collection at 18-21 days of age and 8 birds/ diet at 36-39 days of age. Volatile fatty acids (VFA) in caecal contents were measured according to methods adapted from Choct *et al.* (1995). Weight and length of gut/ bird were also measured at 22 and 40 days of age. Digestibilities of excreta and ileal digesta using acid insoluble ash (AIA) were analysed according to methods adapted from Dr Mingan Choct (personal communication). Statistical analysis was performed by the GLM procedure within SAS and the means were obtained and separated where appropriate by Tukey's test. Minimum significant differences (MSD) between values are given in Table 2.

## III. RESULTS AND DISCUSSION

When replicates were combined for the two ages, results of weight and length of gut/ bird showed that wheat diets produced lower weight digestive tracts, and birds on oat diets had considerably shorter gut lengths, 10-20cm less than birds on the other diets. Although not shown, feed intake over the AME collection period and weight gain from 0-22 and 0-40 days of age were also lower for birds on the wheat and oat diets. Feed conversion ratio was not calculated due to the test diets only being fed over a 7 day period prior to slaughter. This period incorporated both adaptation to the diet and collection for AME determination. Dry matter digestibility and AME calculated from excreta and ileal digesta (by the AIA method) demonstrated particularly high values for wheat diets and lower values for lupin diets. Excreta AME values were much higher than ileal data for birds at both ages fed lupins indicating increased fermentation in the caeca and proximal colon. This difference was lower for faba bean-fed birds. In older birds, differences between ileal and excreta digestibilities were similar to those of the younger birds suggesting there had been no decline in bacterial populations. This may have been due to the unusual and significant increase in viscosity of hindgut digesta despite the reduction in the dry matter of excreta with age. Volatile fatty acid concentrations were extremely high even on a dry matter basis and did not appear to decline with age, which was also demonstrated by Corrier *et al.* (1990). However, caecal contents almost doubled with age which clearly increased the total amount of VFA's. Thus, it appears that even though AME measured by total collection was high, particularly on the wheat-based diet, increased viscosity likely reduced the feed transit time allowing the proliferation of microflora in the

caeca and consequently greater VFA production. Clearly, the wheat used was of an unusually high-viscosity producing variety and although AME was high the birds may have had difficulty in assimilating the nutrients which, with low feed intake, resulted in poor body weight gain particularly at 40 days of age. Birds on the lupin and faba bean diets were better able to cope. Clearly, this was due to lower viscosity and higher feed intake. However, enough undigested material was able to reach the hindgut to be digested subsequently by the microflora.

Table 2. The effect of diet on a range of digestive parameters in broiler chickens at 22 and 40 days of age.

INTERACTION EFFECTS								
Age (day)	Diet	Excreta DM (%)	Viscosity (centipoise)		AME by collection (MJ/kg DM)	AME by AIA (MJ/kg DM)		Total VFA (umol/g DM)
			Fore	Hind		Ileal	Excreta	
22	W	40.6 a	67.8 ab	90.6 a	16.0 a	n/a	16.8 a	2099 a
22	O	40.8 a	47.1 bc	36.8 bc	15.4 bc	13.8 bc	15.2 d	2107 a
22	L	32.7 bc	14.3 d	14.1 c	14.2 d	12.3 c	13.9 e	2294 a
22	FB	37.9 ab	7.2 d	8.3 c	15.2 c	14.7 ab	15.9 bc	1865 a
40	W	36.9 abc	84.0 a	93.8 a	15.6 ab	16.2 a	16.4 ab	2110 a
40	O	32.7 bc	31.5 cd	54.0 b	15.3 bc	14.1 abc	15.5 cd	2180 a
40	L	29.9 c	15.7 d	23.4 bc	14.3 d	12.3 c	13.9 e	1898 a
40	FB	31.8 bc	9.3 d	38.8 bc	15.1 c	14.8 ab	16.00 bc	1906 a
MSD <sup>1</sup>		7.41	26.58	36.43	0.42	2.20	0.61	843
MAIN EFFECTS								
22		38.01 a	34.1 a	38.7 b	15.2 a	13.7 a	15.5 a	2091 a
40		32.86 b	35.9 a	55.2 a	15.1 a	14.2 a	15.5 a	2024 a
	MSD	2.358	8.45	10.84	0.13	0.62	0.19	268
	W	38.794 a	75.9 a	92.2 a	15.8 a	15.6 a	16.6 a	2105 a
	O	36.762 a	39.3 b	42.5 b	15.4 b	13.9 b	15.4 c	2144 a
	L	31.317 b	15.0 c	18.8 c	14.3 c	12.3 c	13.9 d	2096 a
	FB	34.865 a	8.2 c	19.8 c	15.1 b	14.8 ab	15.9 b	1885 a
	MSD	4.409	15.79	21.03	0.25	1.21	0.36	501

<sup>1</sup> Minimum significant difference at  $P < 0.05$ .

Means within a column and type of effect without a common superscript are significantly different at  $P < 0.05$ .

#### IV. CONCLUSIONS

The AME measured at the lower ileum and in the excreta was high for all diets but generally showed much lower values at the former site at both ages, which indicated considerable hindgut fermentation. This was supported by high VFA concentrations in caecal contents. However, there were considerable differences between the diets in terms of body weight gain and viscosity of digesta which suggests that different digestive processes occur throughout the gastrointestinal tract and these are dependent on the grain concerned. Replication of varieties and further investigation of the components of digestion and antinutritional factors are required in the development of an *in vitro* method for predicting performance.

## V. ACKNOWLEDGEMENTS

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## ADVERSE EFFECTS OF DIETARY SODIUM CHLORIDE ON THE BENEFICIAL RESPONSES OF BROILERS AT HIGH TEMPERATURES TO WIDER ARGININE:LYSINE RATIOS

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### Summary

Widening the dietary arginine:lysine (arg:lys) ratio from 1.05 to 1.49 improved the liveweight gain and feed conversion of 3 to 7-week-old broilers caged at 32°C. Doubling the concentration of sodium chloride in the diet from 1.2 to 2.4 g/kg negated the positive responses to wider arg:lys ratios.

### 1. INTRODUCTION

Brake *et al.* (1994) reported that wide dietary arg:lys ratios were associated with improved performance of 3-6 week old broilers at high temperatures. In particular, improvements in liveability and feed conversion were observed with wider arg:lys ratios. The response appeared to be due to differential rates of uptake of arginine by intestinal epithelium in broilers kept at thermoneutral and heat stress-inducing temperatures. Lysine uptake did not change significantly with temperature.

More recent studies with broilers at high temperatures have given equivocal results. Brake *et al.* (1996) reported a significant interaction between the arg:lys ratio and temperature for growth between 35 and 42 days of age but not at younger ages. Mendes *et al.* (1996) reported no responses to arg:lys ratio in 3-6 week old broilers and Mahmoud and Teeter (1996) also concluded that the arg:lys ratio had no effect on broiler performance.

The present study was conducted to examine whether other dietary variables, in this case sodium and chloride, might influence the response of broilers at high temperatures to wider arg:lys ratios.

### II. METHODS

One-day-old male broilers were obtained from a commercial hatchery (Inghams Enterprises Pty. Ltd., Casula, NSW). They were grown in electrically heated brooders in a controlled temperature room maintained at 25°C and fed a commercial crumbled starter diet to 14 d of age. Brooder heat was removed at 14 d of age at which time the birds were fed Diet 1 (Table 1). Birds continued on this diet until the experimental diets were introduced at 28 d of age.

At 28 d of age 120 broilers were allocated on a bodyweight basis from a larger flock to 24 replicates of 5 birds so that the mean and range in bodyweights were similar in each replicate. Eight replicates were allocated to each of three temperature-controlled rooms maintained at 32°C and one replicate randomly allocated to each of eight diets. These diets varied in arg:lys ratios (1.05, 1.20, 1.34 and 1.49) and in the concentrations of dietary sodium chloride (NaCl) (1.2 and 2.4 g/kg). The arg:lys ratios ranged from normally

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accepted ratios of 1.04 (Standing Committee on Agriculture, 1987) and 1.10 (National Research Council, 1994) to the wider ratios (1.37 and 1.43) used by Brake *et al.* (1994). The lower concentration of NaCl satisfied the requirements for sodium (1.5 g/kg) and chloride (1.5 g/kg) suggested by the National Research Council (1994).

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

Ingredients	Diet 1	Diets 2
Sorghum	357.4	357.4
Wheat	449.1	449.1
Soyabean oil	3.3	3.3
Blood meal	20.0	20.0
Fish meal	47.1	47.1
Meat and bone meal	26.1	26.1
Soyabean meal	75.0	75.0
Limestone	10.2	10.2
Sodium chloride	1.2	1.2
L-lysine HCl	0.8	0.8
DL-methionine	0.4	0.4
L-arginine base	1.5	0.0
Solka floc	3.0	4.5
Broiler premix	5.0	5.0
<u>Determined analysis</u>		
Crude protein	192	186
Lysine	10.7	10.2
Arginine	11.8	10.7
Methionine	3.9	4.2
Arginine:lysine ratio	1.10	1.05

The composition of the basal diet with the narrow arg:lys ratio (1.05) and the lower NaCl supplement is shown in Table 1 (Diet 2). The arg:lys ratio was adjusted by replacing solka floc with arginine base (1.5, 3.0 and 4.5 g/kg respectively) and the sodium chloride (NaCl) supplement (1.2 g/kg) was substituted for wheat. The calculated sodium and chloride concentrations were 1.7 and 2.0 g/kg, respectively, in the lower NaCl diets and 2.2 and 2.7 g/kg, respectively, in the higher NaCl diets. The birds were fed the diets for 3 weeks to 49 days of age.

The weekly and overall data were analysed by factorial ANOVA using the arg:lys ratio (4) and NaCl (2) as main factors. Means were compared by least significant difference (Steel and Torrie, 1982).

### III. RESULTS

The broilers on the lower NaCl diets showed improvements in feed conversion and body weight gain with increasing dietary arg:lys ratio which were not evident with broilers fed the higher NaCl-containing diets. Data for the complete 3 week experimental period

showed a significant ( $P=0.008$ ) arg:lys and a significant ( $P=0.014$ ) arg:lys x NaCl interaction for feed conversion (Table 2) while a significant ( $P=0.018$ ) arg:lys x NaCl interaction was observed for feed conversion during the final week of the study (Table 3).

Table 2. Production responses to arginine base and sodium chloride supplementation between 28 and 49 days of age.

NaCl (g/kg)	Arg:Lys ratio	L'weight gain (g)	Feed intake (g)	Feed conversion (g:g)
1.2	1.05	1059 <sup>b</sup>	2524	2.383 <sup>c</sup>
	1.20	1060 <sup>b</sup>	2448	2.294 <sup>bc</sup>
	1.34	1144 <sup>ab</sup>	2476	2.165 <sup>ab</sup>
	1.49	1237 <sup>a</sup>	2504	2.022 <sup>a</sup>
2.4	1.05	1154 <sup>ab</sup>	2551	2.188 <sup>b</sup>
	1.20	1125 <sup>ab</sup>	2552	2.262 <sup>bc</sup>
	1.34	1142 <sup>ab</sup>	2486	2.158 <sup>ab</sup>
	1.49	1085 <sup>ab</sup>	2410	2.209 <sup>b</sup>
LSD ( $P < 0.05$ )		158	299	0.151

<sup>abc</sup> Means with the same superscript are not significantly different,  $P > 0.05$ .

Table 3. Feed conversion responses to arginine base and sodium chloride supplementation between 42 and 49 days of age.

NaCl (g/kg)	Arg:Lys ratio	Feed conversion (g:g)
1.2	1.05	2.512 <sup>ab</sup>
	1.20	2.714 <sup>a</sup>
	1.34	2.320 <sup>bcd</sup>
	1.49	2.068 <sup>d</sup>
2.4	1.05	2.276 <sup>bcd</sup>
	1.20	2.393 <sup>ad</sup>
	1.34	2.344 <sup>bcd</sup>
	1.49	2.504 <sup>ac</sup>
LSD ( $P < 0.05$ )		0.339

<sup>abcd</sup> Means with the same superscript are not significantly different,  $P > 0.05$ .

#### IV. DISCUSSION

The results of this study over the complete 28-49 d period confirm the earlier report of Brake *et al.* (1994) that widening the dietary arg:lys ratio improves the efficiency of feed utilisation of finishing broilers at high temperatures. However, the trends noted with the lower NaCl diets were negated by the additional supplement of NaCl. This was shown by the significant arg:lys x NaCl interaction for feed conversion. A similar significant

interaction was obtained during the final week of the study. These results imply that the response to dietary arg:lys ratio can be influenced by other dietary nutrients. The results indicate that the dietary arg:lys ratios derived from the nutrient requirements published by the National Research Council (1994) of 1.10 (3-6 weeks of age) and 1.18 (6-8 weeks of age) and by the Standing Committee on Agriculture (1987) of 1.04 (4-8 weeks of age) are inadequate for heat-stressed broilers of these ages fed diets containing minimum concentrations of NaCl.

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# A MULTI COMPONENT CARBOHYDRASE IMPROVES THE PRODUCTION PERFORMANCE OF BROILER CHICKENS FED DIETS CONTAINING SOYABEAN MEAL IN MIXTURES OR SOYABEAN MEAL AND CANOLA MEAL AS PLANT PROTEIN SOURCES

P.B. RASMUSSEN and D. PETTERSSON

## Summary

A mixed carbohydrase, derived from the *Aspergillus niger* group, was tested in a broiler diet, based on sorghum and soyabean. The enzyme was added "on top" of the diet formulated according to the local standards in Mexico. Parallel to this, the enzyme was incorporated in a diet using increased energy digestibility figures for soyabean meal and canola meal derived from balance trials (Huyghebeart, 1995) performed according to the European Reference Method (Bourdillon *et al.*, 1990). In the "on top" trials, the enzyme significantly ( $P < 0.05$ ) improved feed efficiency by 12% and weight gain by 6%. In the canola diet the enzyme also significantly improved ( $P < 0.05$ ) feed efficiency by 11% and weight gain by 25% compared to the same diet without enzyme. Compared to the conventional diet without added enzyme the price competitive canola diet improved performance by 12-14%.

## I. INTRODUCTION

The use of enzymes to improve the utilisation of energy and nitrogen in wheat- and barley-based diets has, over the last 10 years, gained broad acceptance. The application of xylanases and beta-glucanases has gradually changed from an "on top" application to an application where the effect of the enzymes on the digestibility of, especially, energy is taken into account during feed formulation.

The use of enzymes in areas where maize and sorghum are the dominating cereals has recently gained renewed interest. Dicotyledon plants, like soy, have a cell wall and fibre structure different from that of monocotyledon plants (Chesson, 1987). The enzyme used in the current study has a range of different enzyme activities, including pectinases and several hemi-cellulases. The effect of the enzymes on the energy and protein utilisation of growing broilers has been confirmed in balance studies (Huyghebeart, 1995) and it was consequently decided to test these findings in production trials.

## II. MATERIALS AND METHODS

The trial was conducted at the Instituto Internacional de Investigacion Animal, located in Queretaro, Mexico.

Day old Arbor Acres male chicks (1,920) were randomly allotted to the dietary treatments. The birds were vaccinated against Marek's disease at the hatchery and, during the trial, against Newcastle (days 10 and 42) and infectious bronchitis (day 10).

The birds were allocated to 48 pens with a density of 10 birds per m<sup>2</sup>. The temperature was 32°C during the first week and then decreased by 2°C per week during 4 weeks until an environmental temperature of 20-24°C was achieved.

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Birds were given starter (0-21 days), grower (22-35 days) and finisher (36-49 days) diets. All diets were given as mash and nicarbazine was added to the starter diet and monensin to the grower and finisher diets.

The composition of the grower diets is shown in Table 1 and the main dietary nutrients in Table 2. The starter and finisher diets were formulated using the same principles but with nutrient contents regulated according to requirements.

Table 1. Composition of the diets in the grower phase (g/kg).

Ingredient	Conventional	Experimental
Sorghum	652.68	616.49
Soyabean Meal	215	144
Canola	-	100
Fish Meal	60	60
Tallow	20	20
Soyabean Oil	20	25
Calcium	10	10.5
Phosphate	8.0	7.0
Pigment	4.1	4.1
Sodium chloride	2.2	2.2
Vitamin premix	2.0	2.0
Methionine	1.6	1.1
Sodium bicarbonate	1.5	1.3
Mineral premix	1.0	1.0
Choline	0.9	0.9
Elancoban	0.55	0.55
Lysine	0.3	0.7
Endox	0.1	0.1
Flavomycin	0.05	0.05
Red Pigment	0.02	0.02
Total	100	100

Enzyme supplements were added (0.45 g/kg) in lieu of sorghum.

Table 2. Main nutrients in grower diet (/kg).

Nutrients		
Crude protein (g)	119.9	200.0
Lysine (g)	11.0	11.2
Methionine (g)	5.2	4.8
Met. + Cyst. (g)	8.2	8.2
Calcium (g)	9.7	10.0
Phosphorus (g)	6.4	6.8
M.E. MJ/kg	13.18	12.97

Twelve replicates, of each of four dietary treatments were randomly assigned to the 48 pens in the house. The four treatments were (see Table 1 and 2):

- 1: Conventional soyabean meal/sorghum-based diet.
- 2: Diet 1 with added Energex<sup>®</sup> CT.
- 3: Experimental canola/soyabean meal/sorghum-based diet.
- 4: Diet 3 with added Energex<sup>®</sup> CT.

During the entire trial feed intake, weight gain and mortality were recorded. Based on the feed intake and weight gain figures the feed conversion ratio (FCR) was calculated for each pen.

### III. RESULTS AND DISCUSSION

The overall results for weight gain and feed conversion ratio are shown in Figure 1. For the two groups fed the conventional sorghum/soyabean diet the enzyme improved the weight gain and the feed efficiency significantly by 6.3 and 12.2 %, respectively. The enzyme apparently improved the nutrient digestibility *per se* as the feed intake was reduced without losses in gain.

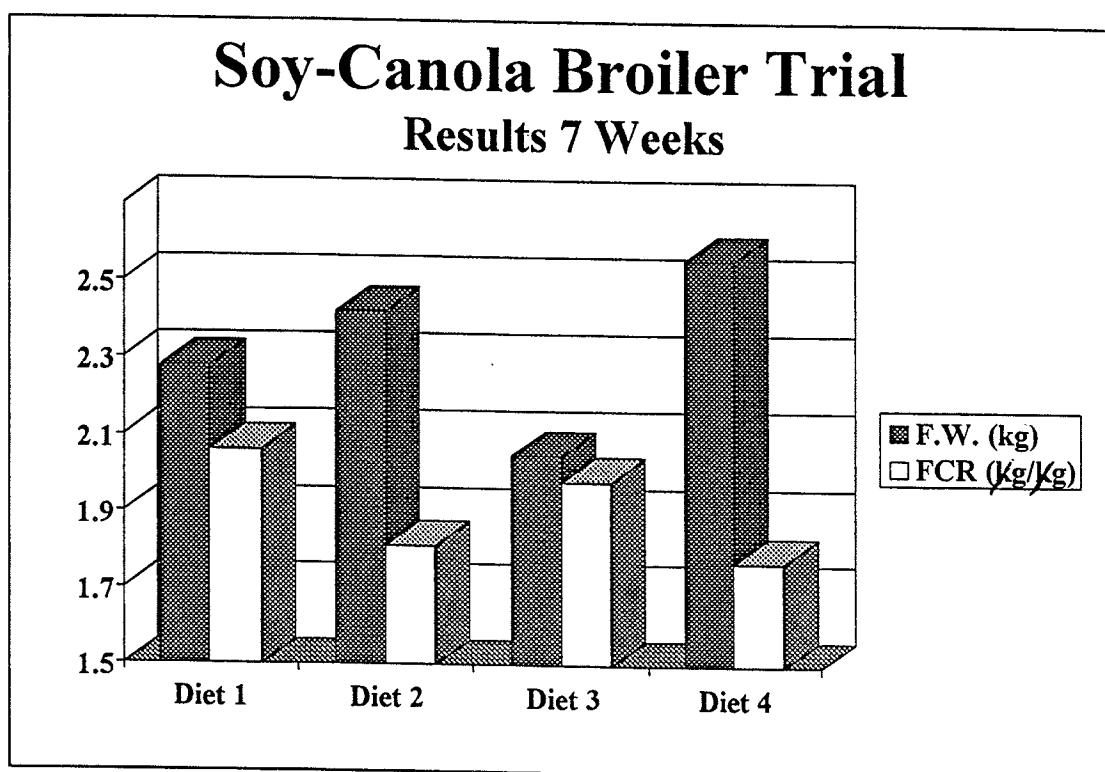


Figure 1. Final weight (FW) and Feed Conversion Ratio (FCR) for birds given the four diets.

In Diets 3 and 4 part of the soyabean meal (10.2 MJ/kg) was substituted by canola meal containing only 8.3 MJ/kg. During dietary formulation higher energy values for soyabean meal (10.6 MJ/kg) and for canola meal (9.49 MJ/kg) were used. Using these values Diet 4 was formulated to have a similar ME to Diet 1 (Table 2). Despite its lower actual ME value Diet 4 (Diet 3 with enzyme) improved the final weight by 12.5% and the feed efficiency by 14.1% compared to the conventional Diet 1. Compared to the experimental control (Diet 3) the growth rate especially was improved by 25%.

These results suggest that enzyme addition to these diets improved the energy availability in both soy and canola. Both nutrient digestibility and rate of passage seem to be affected by the enzyme as both weight gain and feed efficiency were improved.

If the enzyme for one reason or another (e.g., too extensive heat treatment) is not functional in the digestive tract, an inferior result will be obtained as may be seen from the production results on Diet 3. This diet is only 1.5% lower in energy but animal performance is very low, probably due to the anti-nutritional effects of the fibre in canola, effects which are removed by the enzyme.

#### IV. CONCLUSION

The effect of a multi-carbohydrase (Energex<sup>®</sup> CT) has been tested using a conventional soy-sorghum-based diet and an experimental energy-reformulated soy-canola-sorghum diet. The overall performance of birds given both diets was significantly improved by enzyme addition and was most pronounced with the reformulated diet. Enzyme addition appeared to overcome anti-nutritional effects associated with canola.

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## COMPARISON OF PERFORMANCE OF THREE IMPORTED AND TWO AUSTRALIAN LAYER STRAINS ON THREE DIETS

J. V. NOLAN, J. R. ROBERTS, E. THOMSON, W. BALL and R. B. CUMMING

### Summary

Egg production, egg quality and profitability of three imported and two Australian strains of commercially available layers were evaluated at the University of New England's poultry farm 'Laureldale' using three diets, i.e. a complete crumble containing ground calcium, or a calcium-deficient crumble with either oyster shell or limestone chips. Mortality from Marek's Disease and other causes was highest in imported birds which, nevertheless, in the conditions in which they were tested, were more efficient and more profitable than the more robust Australian strains.

### I. INTRODUCTION

Strains of laying hens developed outside Australia pose new questions for the Australian poultry industry. Dietary recommendations for the imported strains differ from those currently accepted for Australian strains and the ingredients used (eg. wheat and meat meal) differ in nature from those in common use in Europe and North America. Moreover, in Australia, hens are likely to be subjected to a wider range of environmental, disease and management stresses than in their countries of origin. High mortality of imported layers has been a major industry concern in recent years.

A comparison has been made at the University of New England in 1995-96 of performance and profitability between imported (ISA-Brown, Hy-Line Brown and Bartter Lohmann) and Australian (Hy-Line-CB and Tegel Brown) strains of layers up to 75 weeks of age under management conditions that are typical of many Australian poultry farms.

### II. MATERIALS AND METHODS

Birds used were hatched in December, 1994, vaccinated at the hatcheries against Marek's disease and infectious bronchitis (IB) and reared in wire-floored cages by a commercial farmer near Tamworth. At 3 weeks of age they were vaccinated for IB (A3 virus; in-contact method) and at 14 weeks for avian encephalomyelitis and IB (Vic S; in contact). The birds were beak-trimmed at 10 days and at 8 weeks of age. At 17-18 weeks of age (April, 1995), they were moved to the 'Laureldale' poultry farm (University of New England) where they were housed in single-deck, Californian-type laying cages (3 birds/cage) in a saw-tooth roofed shed. At the time of arrival the birds were accustomed to 14 h daylight. At 'Laureldale' daylength was increased in steps of 20 min /week to 16 h (at 24 weeks). Hens were selectively beak-trimmed at 28 weeks of age.

All strains were given a pre-layer diet from 14 to 18 weeks of age and a layer diet from 18 weeks until the start of the study at 20 weeks of age. All diets were fed *ad libitum*. From 20 weeks of age the 5 strains (804 birds/strain) were offered one of three crumble diets formulated to Australian layer standards (Fielders Agricultural Products, Tamworth). Diet 1 was a typical layer crumble containing cereal grain, meat meal and bone meal and fortified

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with ground limestone (16% crude protein, 3.5% calcium). Diets 2 and 3 were formulated to the same specifications but without ground limestone and hens received oyster shell grit or limestone grit mixed in the same feeder. The amount of oyster shell or limestone grit supplied was made according to visual assessment. Dietary treatments were allocated in blocks on both sides of the 6 aisles of cages so that each of the 3 dietary treatments was represented in 2 separate blocks in the shed. Blocks of 66 birds of each strain were allocated at random in each aisle. Birds that died were replaced up to 30 weeks of age. Post-mortem examination was made on all birds that died. Egg production was determined along with financial returns from sale of eggs and 'spent' birds at 75 weeks of age. Feed consumption and cost of feed were also recorded. Eggs were collected (30/diet for each of the 5 strains) at random from all treatments each week to determine changes in egg weight over time.

Results were analysed by analysis of variance.

### III. RESULTS

Cumulative mortalities for each strain are given in Figure 1. Mortalities from all causes (mainly Marek's disease and cannibalism) at 40 weeks of age were only 2-3 % for the Australian strains but 11-14 % for the imported strains. Total mortality at 40 weeks was lowest for birds on the complete diet and the diet with oyster shell grit (6.2-7.4%), but tended to be higher in birds on limestone grit (11.4%) and this trend continued as birds aged. Overall, at 75 weeks there were large differences in mortality between the strains varying from <5% (Hy-Line-CB) to 20% (Hy-Line Brown).

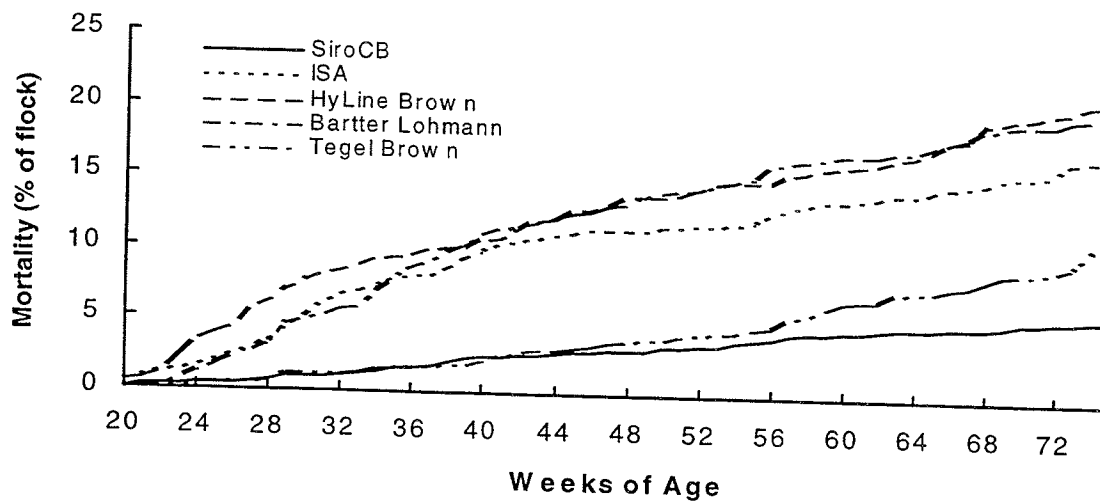


Figure 1. Cumulative mortality in each of 5 strains of layers from 20 to 75 weeks of age.

The ISA hens were first into lay, reaching 50% hen-day production at about 140 days of age. The other imported strains (Hy-Line Brown and Bartter Lohmann) reached 50% at about 149 days and the Australian strains (Hy-Line CB and Tegel Brown) about 167 days. The CB hens had the highest hen-day production but produced the lightest ( $P < 0.05$ )

eggs. The different times of onset of lay and different levels of mortality between strains both affected the hen-housed production (Figure 2). Egg weight and egg numbers were affected ( $P < 0.05$ ) by diet, but neither hen-day nor egg mass/hen-housed production differed ( $P > 0.05$ ) between diets.

Feed intake was more variable in birds given the diets containing particulate calcium (Diets 2 and 3), tending to be lower in the early part of lay but higher when hens were 40 - 75 weeks of age. Feed conversion efficiency tended to be more variable on Diets 2 and 3 (calcium grit), being sometimes superior to Diet 1 (ground calcium) and sometimes inferior. Feed conversion ratio overall was lower ( $P < 0.05$ ) in the imported strains (2.78-2.85) than in the Australian strains (3.74-3.77) which became markedly less efficient than the imported strains after about 30 weeks in lay (Figure 3). The FCR did not differ ( $P > 0.05$ ) between diets.

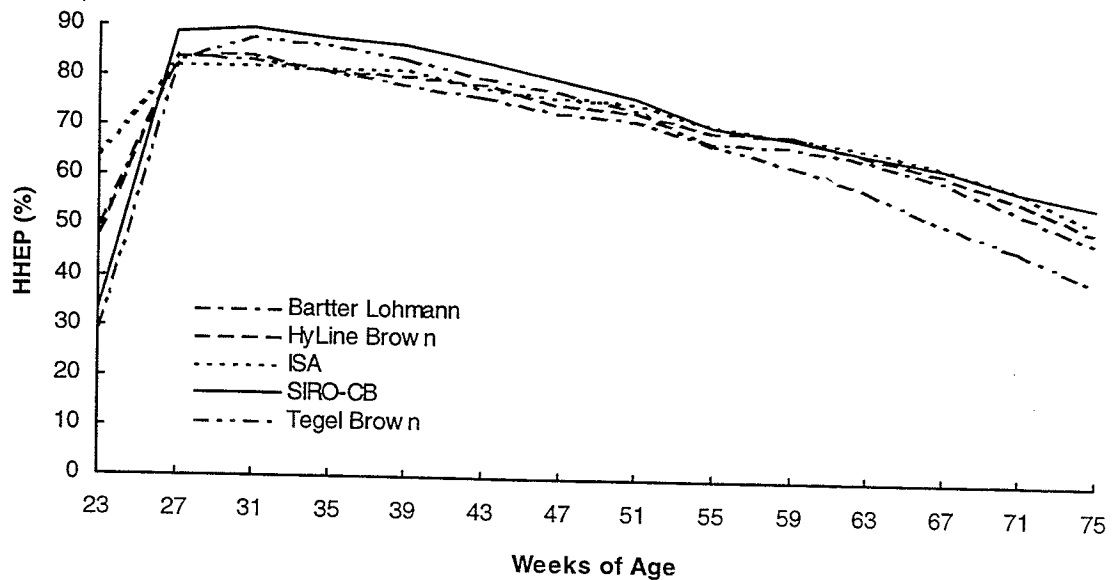


Figure 2. Strain differences in hen-housed egg production (HHEP).

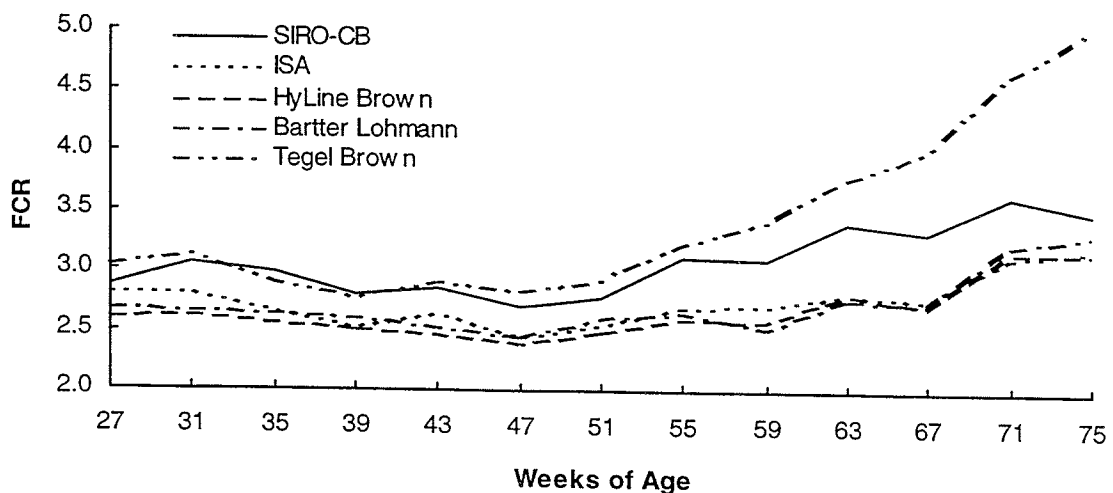


Figure 3. Differences in feed conversion ratio (FCR, g feed/g egg mass) between strains of layers.

Table 1. Hen-day production, mean egg weight and total egg production per hen housed from 20-75 weeks of age.

	ISA-Brown	Bartter Lohmann	Hy-Line Brown	Hy-Line CB	Tegel Brown
Hen-day egg production (%)	76.0	73.9	73.8	73.3	68.1
Egg weight (g)	65.0	66.0	66.0	58.6	61.4
Total eggs per hen-housed	283	270	275	282	261
Egg mass production (kg)	18.4	18.0	18.2	16.5	16.0

Profitability of the different strains (net return per bird) was assessed by taking costs of birds (including replacement of birds dying between 18 and 30 weeks of age) and feed and packaging materials (but excluding overheads, labour and running costs), and subtracting these costs from the income from egg sales (Table 2).

Table 2. Income, costs and net returns per bird.

	ISA Brown	Bartter Lohmann	Hy-Line Brown	Tegel Brown	Hy-Line CB
Total costs/bird	\$	\$	\$	\$	\$
Diet 1	25.73	25.24	24.47	24.64	26.47
Diet 2	25.60	25.02	24.72	24.68	26.23
Diet 3	24.73	23.99	23.34	23.86	26.28
Total income/bird					
Diet 1	37.61	38.54	39.13	38.76	35.16
Diet 2	36.00	36.08	37.41	37.09	35.01
Diet 3	36.52	35.96	36.66	37.62	34.92
Net Return/bird					
Diet 1	8.37	10.00	10.49	4.94	7.90
*	4.92	4.99	5.50	-0.92	0.37
#					
Diet 2	7.16	8.42	8.64	5.05	6.60
*	4.46	3.75	3.99	-0.74	-0.39
#					
Diet 3	8.04	9.59	9.23	4.93	7.93
*	4.40	3.95	5.39	-0.47	0.82
#					

\*Net return selling eggs by the dozen.

# Net return selling eggs by the kg.

#### IV. DISCUSSION

The high mortalities among imported strains confirm the Australian industry experience and our previous research results which show there is still a major mortality problem with the newly imported strains of layers. However, the imported strains are

capable of very feed-efficient egg production which will be further improved when the mortality problems are overcome. These strains appear to be more sensitive to management factors than the Australian strains. Mortality appears to be affected not only by hatchery management, hygiene and vaccination protocols for young birds but also by diet, environmental, disease and day-to-day management of hens in the laying shed. There were indications that mortality (and egg production) in imported layers was particularly sensitive to specific factors such as changes in farm staff, new batches of feed, sudden changes in housing conditions (temperature, lighting levels) or other short-term changes in layer management procedures which placed additional stresses on the birds.

The profit margin obtained for each strain depended on the method used to market eggs. Selling 'by the dozen' in Armidale was the more profitable option despite the higher packaging costs. Method of marketing affected the profitability ranking of the birds.

Therefore, continued assessment of stress-related mortality and feed efficiency will be essential as companies and producers attempt to determine the causes of mortality and the remedies. Further studies of this type will be required to provide experimentally controlled, independent and early evaluation of newly imported layers under Australian conditions.

#### V. ACKNOWLEDGEMENTS

Thanks are extended to the Egg Industry Research and Development Council for financial assistance and the companies (Bartter, Biaida, Hy-Line and Tegel) for their collaboration, advice and 'in-kind' support.

## DUCKWEED (*Spirodela punctata*) AS A PROTEIN AND PIGMENT SOURCE IN DIETS FOR LAYERS

J.V. NOLAN, R.E. BELL, E. THOMSON, D. BREMNER and W. BALL

### Summary

A study was made of egg production and egg characteristics in two strains of layers (Tegel Hi-Sex and Tegel Super Brown) when changed from a conventional layer diet to diets in which duckweed was included at 10, 30, 50, 80, 120, 200 g/kg as fed. Egg-mass production was slightly reduced in Hi-Sex hens given diets containing the higher duckweed contents and more markedly in Super Brown hens. The duckweed imparted an attractive colour to the egg yolks.

### I. INTRODUCTION

Duckweed is a small, fast-growing, aquatic plant that floats on the surface of ponds. Interest in duckweed has increased recently along with the realisation that it can extract unwanted nutrients such as aluminium, iron, magnesium, potassium and sodium from polluted water (sewerage, factory effluent) (Zirschy and Reed, 1988). However, to allow water purification to continue the duckweed must be removed along with the nutrients that it has assimilated. This duckweed is a source of potentially useful animal feed. The presence of high concentrations of minerals in the duckweed, however, could be either an advantage or a disadvantage. Calcium, which is required in the diet of laying hens, is usually present in relatively high concentrations (10-25 g/kg dry matter (DM)) (Haustein *et al.*, 1992). Duckweed also contains relatively large amounts of xanthophylls and carotene which, when ingested by layers, impart a rich yellow/orange colour to the egg yolk (Skillicorn *et al.*, 1993). However, heavy metals such as lead and cadmium may also accumulate and lead to toxicity in animals or contamination of animal products. Little is known about possible anti-nutritional effects of duckweed, although diets with more than 150 g *Lemna gibba* per kg restricted production in young broilers (Haustein *et al.*, 1992).

The present study extended previous work which confirmed the value of duckweed as a protein-rich, pigment-rich, ingredient when used in diets of laying hens (Haustein *et al.*, 1990; O'Neill *et al.*, 1996). As before, duckweed was used as a partial replacement for soyabean meal but over a wider range of inclusion. The effects on performance, egg and egg shell characteristics and yolk colour were recorded in two strains of laying hens.

### II. MATERIALS AND METHODS

Duckweed (predominantly *Spirodela punctata*) was collected from the Sewerage Treatment Works at Scone, NSW and dried as described previously (O'Neill *et al.*, 1996). Six diets were formulated to contain 11.3 MJ ME and (g/kg as fed) 160 g crude protein, 40 Ca, 10 P, with increasing amounts of duckweed in lieu of soyabean meal and wheat (Table 1). The other ingredients in all diets were (g/kg as fed): sorghum, 150; wheat bran, 50; sunflower oil, 20; limestone, 80; di-calcium phosphate, 30; sodium chloride, 5; lysine, 2.5; DL-methionine, 1.5 and vitamin/mineral mix, 5. No yolk pigments were added.

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Table 1. Dietary formulations with increasing concentrations of duckweed (g/kg as fed).

Duckweed	10	30	50	80	120	200
Wheat	526	521	511	496	476	436
Soyabean meal	120	105	95	80	60	20

Sixty Tegel Hi-Sex and 60 Tegel Super Brown hens were moved at 35 weeks of age into single-bird cages in a small shed. There were 4 rows of cages each with 30 single-bird cages. Strains were alternated in 6-cage blocks to give a uniform blocking throughout the shed. The 6 experimental diets were allocated to one of the birds in each block. The hens were fed their previous diet (commercial layer; Ridley AgriProducts, Tamworth) for one week and then offered the experimental diets from the start of week 2. All diets were offered *ad libitum* and water was freely available. Experimental measurements were made during weeks 3 to 6. Eggs were collected daily at 08.00 h and additional feed was provided if necessary. Special egg collections were made from all hens at weekly intervals on 4 occasions (*i.e.* 4 eggs/hen). Shell colour of these eggs was scored, and specific gravity, shell breaking strength (by quasi-static compression), percentage shell (shell weight x 100/egg weight), Haugh units of albumin and yolk colour (Roche scale) were also recorded. Data were analysed by two-way analysis of variance.

### III. RESULTS

The crude protein content of the duckweed was 340 g/kg DM. Analysis of the duckweed by inductively coupled plasma spectrometry gave the following (g/kg DM) P, 12.1; S, 5.82; K, 30.0; Ca, 15.5; Mg, 4.20; Na, 3.17 and (mg/kg) Mn, 745; Fe, 957; Zn, 162; Cu, 17.9; Al, 629 and B, 905. The relationship between the mineral and duckweed contents of the diets are shown in Figure 1.

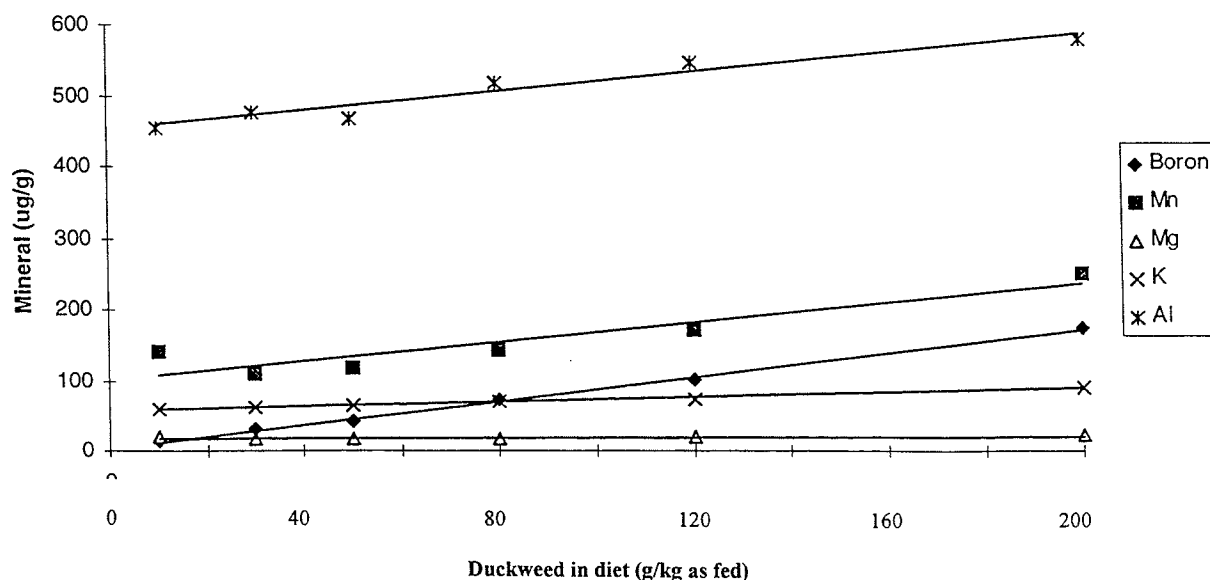


Figure 1. The concentrations of certain minerals in experimental diets for laying hens in which duckweed replaced soyabean meal.

Two Hi-Sex and 12 Super Brown birds did not readily ingest the experimental diets in week 2. Non-acceptance was not related to diet. These hens were removed from the trial and not replaced. Mean feed intake in week 3 (103 g/bird/day) was lower than in weeks 4, 5 and 6 (120 g/bird/day). Over this period mean intake was higher in the Hi-Sex than in the Super Brown hens (130 v. 102 g/bird/day) but decreased with increasing duckweed content in the diets (Figure 2). Hen-day egg production was higher in Hi-Sex hens (86.4 v. 62.9 %;  $P < 0.001$ ) and tended to decline (88 to 83 %) with increasing duckweed in the diet. Hen-day production declined significantly in the Super Brown hens from 74 to 47 %.

Mean egg weight was higher ( $P < 0.001$ ) in the Hi-Sex than in the Super Brown strain (64.7 v. 57.9 g), and was slightly lower ( $P > 0.05$ ) in the diets with higher levels of duckweed, and showed a significant strain x diet interaction ( $P < 0.001$ ).

Egg mass production decreased in both strains with increasing levels of duckweed in the diet, but the percentage decrease in egg mass production was less in the Hi-Sex than in the Super Brown birds (Figure 3). Feed conversion ratio (FCR) was lower ( $P < 0.05$ ) in Hi-Sex than in the Super Brown hens (2.29 v. 2.58) and tended to decrease with increasing duckweed content in the diets. The FCR in Super Brown birds appeared to be largely unaffected by the duckweed concentration in the diet.

Egg shell colour and yolk colour score were higher ( $P < 0.001$ ) in eggs from the Hi-Sex than the Super Brown strain, and were higher ( $P < 0.01$ ) in eggs from hens of both strains when given the diets with a higher duckweed concentration (Figure 4). Shell weight differed ( $P < 0.001$ ) between strains (Hi-Sex 5.79 v. 5.27 g) and diets ( $P < 0.001$ ) but the between-diet differences were not related to duckweed content, and there was no diet x strain interaction. Shell breaking strength and specific gravity did not differ between strains or between diets ( $P > 0.05$ ). Albumin quality (Haugh units) differed ( $P < 0.01$ ) between diets but was not correlated with duckweed content of the diets. There was a diet x strain interaction ( $P < 0.05$ ).

## V. DISCUSSION

This study clearly demonstrates the potential of duckweed to provide a source of amino acids and minerals, and yolk pigments that impart an attractive golden colour to the egg yolk.

The response to the experimental diets differed between strains. The Hi-Sex hens maintained a high intake and hen-day and egg mass production, and a good feed conversion ratio on all diets. There was a small decline in the production of Hi-Sex hens given diets with higher levels of duckweed whereas the Super Brown birds appeared to be more adversely affected as the level of inclusion of duckweed was increased and the amount of soyabean and wheat were correspondingly decreased. The diets were not specifically balanced for individual amino acids and minerals as no analysis had been done on this material at the time of diet formulation.

All the birds in this trial consumed relatively large amounts of water and produced very wet droppings. A possible reason is the relatively high concentrations of total salts arising from the presence of duckweed in the diets. Individual minerals, such as boron and aluminium, may also have had detrimental effects. However, there was no discernible trend in the wetness of droppings with level of inclusion of duckweed in the diet. (In a nearby trial, the same strains given a diet formulated using the same batch of soyabean meal also



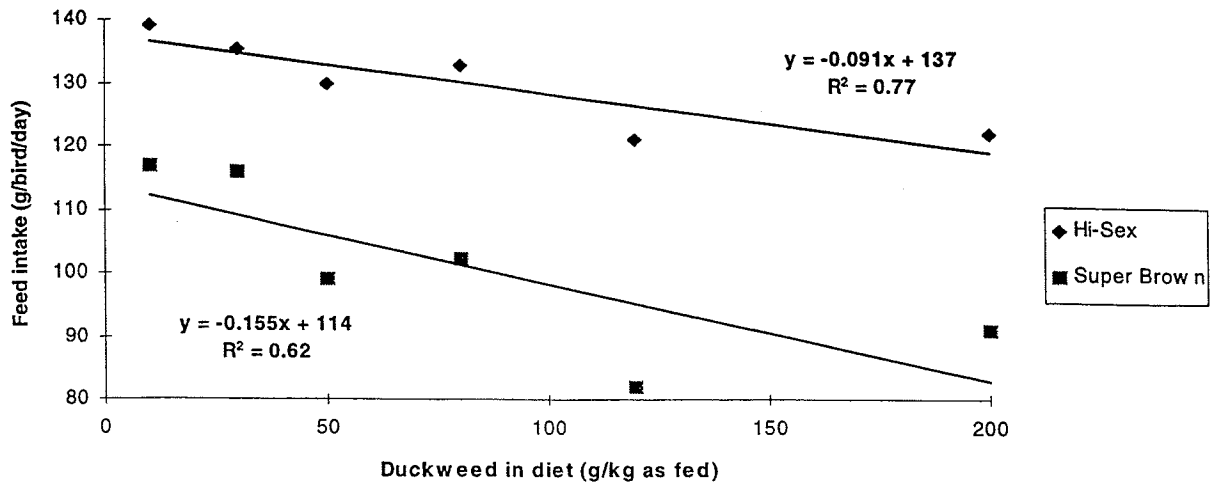


Figure 2. Relationship between feed intake of hens of two strains and duckweed concentration of the diet.

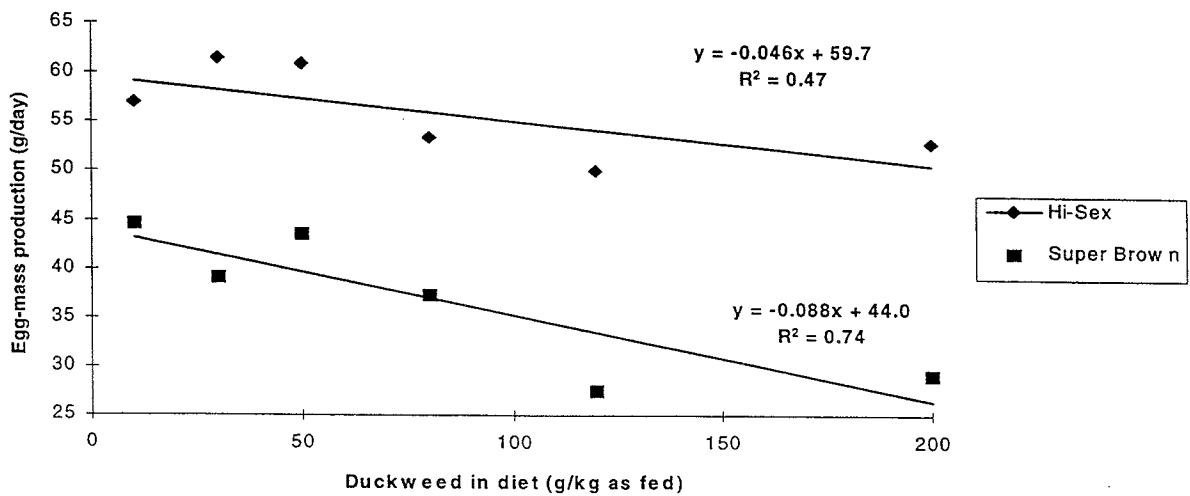


Figure 3. Egg mass production from two strains of hens given diets with different duckweed concentration.

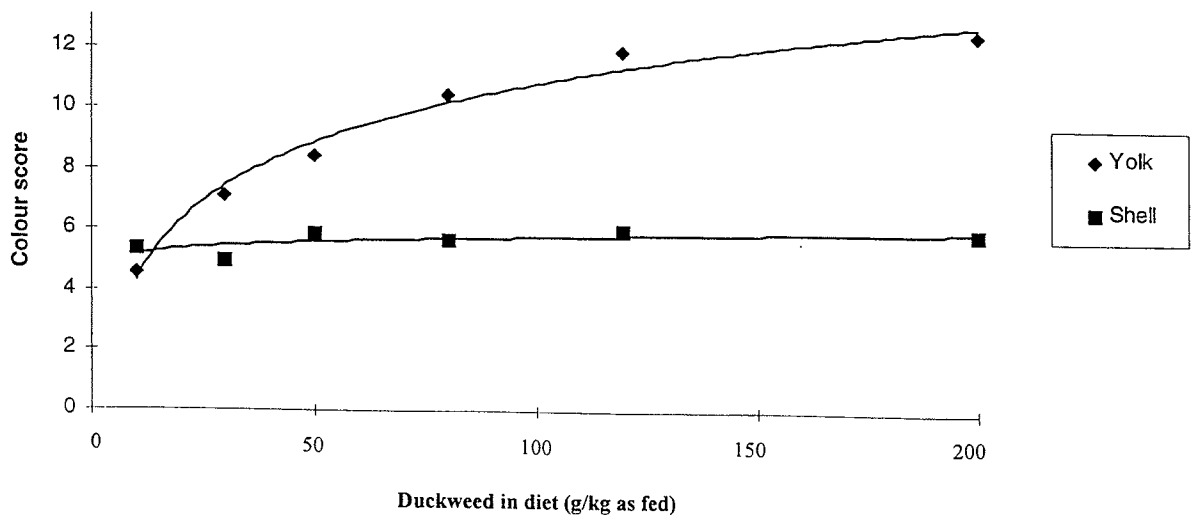


Figure 4. Relationships between shell and yolk colour scores of eggs from two strains of hens given diets with different duckweed concentrations.

produced wet droppings. The wetness declined after the birds were given a similar diet formulated with soyabean meal from a different source, although there were other possible confounding factors.) Thus, it is not possible to conclude with certainty that duckweed was, or was not, the reason for the wet droppings. In view of the high concentrations of minerals that may accumulate in duckweed, it appears to be highly desirable to know the mineral content of duckweed when diets are being formulated so that levels of all minerals can be adjusted appropriately.

## VI. ACKNOWLEDGEMENTS

We thank the Rural Industries Research and Development Corporation and the University of New England for financial support for this study.

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## FEED UTILIZATION AND BODY GROWTH OF A NEW STRAIN OF BROILER CHICKEN FROM HATCH TO 35 DAYS OF AGE

P.A. IJI and D.R. TIVEY

### Summary

The pattern of growth and feed utilization of a recently developed strain of broiler chicken (Steggles x Ross; F<sub>1</sub> generation) was investigated between hatch and 35 days of age. In early life, the rate of intestinal growth was greater than the rate of body growth, thereby priming the bird for efficient nutrient digestion and absorption. Final body weight was about 40-fold the hatching weight.

Feed was utilized with an overall efficiency of 1.68 kg per kg body weight gain. The retention of most assessed nutrients increased with age.

### I. INTRODUCTION

The Australian poultry industry has evolved from a closed nucleus flock dependent on the development of genotypes within the continent to one that now explores the utilization of stock from overseas (Nolan, 1978). The Steggles x Ross strain (Australian Poultry Ltd) represents the recent generation of birds developed in Australia, crossing existing local stock with imported strains.

Broiler chicks are hatched with relatively enhanced skeletal muscle framework (Anthony *et al.*, 1989). Therefore, broiler productivity is largely dependent on the rate of intestinal development in early life. Nutrient utilization may also be affected by the rapid genetic development imposed on most strains of broiler chickens (Dunnington and Siegel, 1995).

There is a need for more research on the growth patterns and nutrient requirements and utilization of the new broiler strains developed in Australia. This report presents observations on the pattern of intestinal and body growth, and nutrient retention, by the Steggles x Ross broiler chicks as part of a larger study on the physiological responses of the strain to dietary factors. Such data will provide information towards further genetic development and diet formulation.

### II. MATERIALS AND METHODS

Eighty mixed sex day-old broiler chicks (Steggles x Ross; F<sub>1</sub>) developed in the mid-1990s by Australian Poultry Ltd. were used for the study. The chicks were reared in battery brooder cages for two weeks prior to transfer to cages in lots of 5 - 7 chicks. Commercial starter (1-21 days) and finisher (21-35 days) diets obtained from Milling Industry Stockfeeds (Murray Bridge, South Australia) were fed during the study. Local laboratory analysis (AOAC, 1984) indicated the starter and finisher diet composition, in g/kg as: nitrogen (45.5; 44.4); fat (48.5; 49.5) and gross energy (17.5; 19.1 MJ/kg). The diets also contained (g/kg): lysine (starter, 11.6; finisher, 9.9); methionine (3.7; 3.5); threonine (8.2; 7.4); linoleic acid (17.4; 15.8); calcium (12.3; 11.4) and phosphorus (8.0;

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7.7) respectively. Feed and water were supplied *ad libitum*. Chicks were vaccinated against infectious bronchitis on day 10. Brooding and rearing temperatures varied between 40-26°C (stepwise reduction between 1 and 21 days of age) and 26.5-21°C respectively. Light was provided for 23.75 hours per day.

All chicks were weighed once a week. Feed consumption over the period was measured. Mixed faecal and urine samples were collected from all cages at the end of each week and pooled. Samples were dried to constant weight in an oven at 75°C over 24 hours. The retention of dry matter (DM), gross energy (GE), nitrogen (N), amino acids, lipid, fatty acids and minerals was estimated as difference in nutrient composition between feed and droppings.

Randomly selected chicks were slaughtered through intravenous injection of Lethabarb, 1.0 mL/kg body weight (Virbac, Australia Pty. Ltd.) on day 2 and subsequently each week. The entire carcass, gastrointestinal tract and liver were weighed.

Data on body and intestinal growth were subjected to ANOVA and mean values compared by least significant difference.

### III. RESULTS AND DISCUSSION

The empty body weight increased from 37.3 g on day 2 to 1540.7 g on day 35 (Table 1). Intestinal, gizzard and liver weights increased from 6.1; 5.3 and 2.0 g respectively on day 2 to 114.6, 44.6 and 39.5 g at 35 days of age.

Table 1. (A) Weight (g) and (B) growth rate (g/day) of body and abdominal parts of Steggles x Ross chicks at different ages.

	Age (days)						
(A)	1	7	14	21	28	35	SE
Body <sup>1</sup>	37.3 <sup>e</sup>	164.3 <sup>e</sup>	385.2 <sup>d</sup>	728.2 <sup>c</sup>	1135.2 <sup>b</sup>	1540.7 <sup>a</sup>	52.62
Intestine <sup>2</sup>	6.1 <sup>d</sup>	19.0 <sup>d</sup>	46.8 <sup>c</sup>	67.4 <sup>b</sup>	88.4 <sup>b</sup>	114.6 <sup>a</sup>	6.22
Gizzard	5.3 <sup>d</sup>	17.2 <sup>cd</sup>	22.1 <sup>bc</sup>	33.1 <sup>ab</sup>	35.5 <sup>ab</sup>	44.6 <sup>a</sup>	4.46
Liver	2.0 <sup>d</sup>	9.8 <sup>c</sup>	21.2 <sup>b</sup>	27.9 <sup>b</sup>	35.1 <sup>a</sup>	39.5 <sup>a</sup>	2.04
(B)	1-7	7-14	14-21	21-28	28-35	SE	
Body <sup>1</sup>	18.2 <sup>b</sup>	31.6 <sup>ab</sup>	49.0 <sup>ab</sup>	58.1 <sup>a</sup>	57.1 <sup>a</sup>	8.94	
Intestine <sup>2</sup>	1.8	4.0	3.0	3.0	3.7	1.01	
Gizzard	1.7	0.7	1.6	0.3	1.3	0.75	
Liver	1.1	1.6	1.0	1.0	0.6	0.39	

<sup>a-d</sup> - Values on the same row with the same superscript are not significantly different at  $P > 0.001$ .

<sup>1</sup>Body weight excluding gizzard, liver and intestines.

<sup>2</sup>Intestine, post-gizzard to the cloaca.

Body and visceral organ weight significantly ( $P < 0.001$ ) increased with age. The combined contribution of assessed visceral organ weights to body weight decreased ( $P < 0.001$ ) from 26 % on day 2 to 11 % on day 35. The rate of body growth but not the visceral organ parts increased ( $P < 0.001$ ) with age. The relative growth rate of the visceral organs was more rapid than empty body growth in early life (1-14 d), similar to the findings of Lilja *et al.* (1985) in studies on Japanese quail.

Over the five weeks of the study, and based on the total population of chicks studied, 2790 g feed/bird were ingested, with a weight gain and feed conversion ratio of 1665 g and 1.68 respectively. The diets appear to meet the nutrient requirements specified by the NRC (1994) except in methionine whose specific requirement is difficult to measure in view of the inter-relationships among the sulphur-amino acids and the sparing action choline gives to methionine.

The retention of most nutrients rose with age following the rapid growth of the intestine in early life (Table 2). Stillborn *et al.* (1994) compared crossbreds of male Ross or Steggles with female Arbor Acres and reported on the lower feed intake and body weight but higher feed conversion efficiency of the Steggles crossbreds compared to Ross crossbreds in the first 6 weeks of life. The Steggles crossbreds were also found to be less fatty than the Ross crossbreds. Crosses between Steggles and Ross stock may, therefore, be of some benefit to the poultry industry.

Table 2. Nutrient retention by Steggles x Ross chicks determined every 7 days from hatch to 35 days of age.

Nutrient	Nutrient retention (%) by age (weeks)				
	1	2	3	4	5
Dry matter	76.9	76.8	78.6	87.1	87.9
Gross energy	42.5	47.8	56.4	61.0	64.7
Nitrogen	26.3	24.5	35.9	43.2	43.8
Methionine	71.1	74.2	76.9	74.8	74.7
Lysine	72.9	89.9	89.1	92.4	86.6
Threonine	60.1	65.8	71.1	75.1	72.2
Phenylalanine	75.2	80.1	80.2	75.2	82.4
Valine	68.4	72.3	75.6	76.3	75.6
Alanine	64.8	67.6	73.0	61.8	95.7
Fat	37.4	50.4	57.9	58.4	61.8
Oleic acid	42.7	54.9	61.9	58.2	66.6
Linoleic acid	53.0	62.3	71.6	69.3	68.0
Linolenic acid	53.8	64.4	79.3	79.9	80.2
Calcium	18.6	27.0	6.1	23.3	33.6
Phosphorus	4.6	19.2	9.6	16.5	15.9

Nitrogen retention was low during early growth and reached a maximum of about 44% only in the fifth week of study. Specific amino acid retentions were, however, high, generally over 60%. The low nitrogen retention from feed, particularly in early life is attributed to the supply of protein from the yolk sac (Murakami *et al.*, 1988).

Fat retention varied between 37.4% in the first week and 62% in the fifth week. The retention of unsaturated fatty acids were higher than fat retention and increased with age. These differences may be due to the nutritional importance of the fatty acids considered in this study. Poultry may not have a major requirement for fat *per se* and this may have accounted for the low retention of fat relative to the fatty acids. Linoleic and linolenic acids are regarded as essential for monogastric animals. Calcium and phosphorus gave low retentions although it is not certain how much of these were available from the diets.

## IV. CONCLUSION

The Steggles x Ross broiler chick is endowed with a large gastrointestinal tract at hatch and the tract develops rapidly in early life. This pattern is commonly observed in other avian species and strains of chickens. The strain is also capable of extracting required nutrients from diets judging by the moderately high levels of retention of assessed nutrients following early intestinal development. Further studies are underway to determine the pattern of intestinal structural and functional development in this strain, naturally and under nutrient challenge.

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# EFFECT OF CALCIUM PRESENTATION AND FEEDING METHOD ON EXCRETA CONSISTENCY OF LAYING HENS

R.D. TAYLOR

## Summary

Two strains of commercial laying hens were subjected to complete feed or whole-wheat feeding regimes with calcium provided as limestone, either ground and included in the feed, or as grit (4.0-4.76 mm diameter) provided daily or every second day. Moisture content of excreta was measured by a total collection method at 12, 19/20, 28/29,34 and 38 weeks of age and an excreta scoring method was introduced once weekly from 22-38 weeks of age. No relationship was found between excreta moisture score or moisture content with the level of total feed or grit intake. A significant ( $P < 0.05$ ) Time x Strain x Feed effect was found for excreta score. Regression analysis indicated that the scoring method and actual excreta moisture content were poorly correlated ( $R^2$  0.16,  $P < 0.05$ ). Consistency, as determined by scoring method, was firmer in excreta produced by the whole grain feeding method.

## I. INTRODUCTION

General concern has been raised about wet excreta from layer production causing odour, water pollution and dirty egg problems. Little data is available on the effects of nutrition on moisture content of excreta produced by laying hens. In a review of feeding programs for layers, Leeson (1993) suggested that lower calcium diets would reduce excreta moisture by lowering water consumption. Leeson and Summers (1991) reported higher excreta moisture in pre-lay hens on laying hen (hence high calcium) rations. In the present trial, various methods of calcium presentation, in association with two feeding methods, were investigated and the effects on excreta moisture were monitored.

## II. METHODS

One hundred and forty four commercial layers, half an imported strain (Strain 1) and half an Australian strain (Strain 2), were introduced to two feeding methods from eight weeks of age. Both feeding methods were based on the same wheat-based feed formulation with method one provided as a complete pelleted feed (C) and method two as whole wheat and the meals/premix in mash form (W). Within each feeding method calcium was provided by the following alternatives: (1) ground limestone included in the ration, (2) limestone grit 4.0-4.76 mm diameter available daily in a separate feed trough, and (3) the limestone grit available every second day in a separate feed trough. A rearing diet formulation was used from 8-18 weeks of age and a laying diet formulation after 18 weeks. At 12 weeks of age three replicate pairs of birds per treatment were selected at random from eight-bird rearing cages and placed in laying cages. Excreta were collected daily for eight days using an aluminium foil tray, shaped to fit the edges of the cage floors and held under the floor by wire. Moisture contents of excreta were determined after samples were oven-dried at 75° C for 48 h.

Pullets were transferred to single-bird cages in laying accommodation at 16 weeks of age. Laying commenced in earnest from 21 weeks of age. At 21 weeks of age, photographs were taken of excreta in varying condition under the cages. Scores were assigned from 1, apparently "dry" separate excreta, through to 5, a wet amorphous mass. From 22 weeks of age a score was applied once weekly to the condition of the excreta on the concrete floor under all 144 experimental birds. The photographs were studied prior to scoring the excreta each week. The starting position for scoring each week was chosen randomly. At 19/20 and 28/29 weeks of age, 14-day excreta collections were made under three birds per treatment using the aluminium trays as described above. Moisture content of the excreta was determined as described above. For the final five days of the 28/29 week collection and for two consecutive days at 34 and 38 weeks of age, excreta collections were scored and the moisture content determined.

The statistical evaluations of the data were performed by repeated measures analysis using the Greenhouse-Geisser correction factor (where necessary) within the General Linear Models (GLM) procedure of SAS (SAS Institute, 1989). Where no Time by Strain or Treatment interactions were found, data were pooled and means evaluated using the GLM procedure. Where appropriate, significant Least Squares Means (LSMEANS) were separated using paired-sample t-tests and are presented in tables with appropriate standard errors (SE).

Regression analysis was performed on excreta score versus excreta moisture content and total feed and grit consumption versus excreta score and excreta moisture content using the regression analysis procedures of the statistical package Minitab (Version 7, copyright Minitab Inc.).

### III. RESULTS

The two strains of bird produced excreta with a similar moisture content at 12 weeks of age (Strain 1, 73.6%; Strain 2, 74.9 %;  $P > 0.05$ ). Moisture content of excreta was significantly different ( $P < 0.05$ ) across treatments at 12 weeks of age (Table 1). However, the excreta did not appear to be physically different across treatments. No relationship between excreta moisture content and total feed or grit consumption was found by regression analysis.

Table 1. Percentage moisture content of excreta from birds at 12 weeks of age as affected by complete (C) or whole-wheat (W) feeding and method of calcium provision.

Feed/Calcium	C 1*	C 2	C 3	W 1	W 2	W 3	SE
Moisture	69.9 <sup>a</sup>	73.3 <sup>ab</sup>	70.2 <sup>a</sup>	76.0 <sup>b</sup>	77.8 <sup>b</sup>	78.2 <sup>b</sup>	1.93

\* See text.

<sup>ab</sup> Means with similar superscripts are not significantly different ( $P > 0.05$ ).

No significant differences were found in excreta moisture content across strains and treatments at 18/19 weeks, at peak egg production (28/29 weeks) or at 34 and 38 weeks of age (Table 2). No relationship between excreta moisture content and total feed or grit consumption was found at any stage. Excreta moisture content was consistent over time ( $P > 0.05$ ) for each individual bird. A poor relationship between excreta moisture content and excreta score was found at each age ( $R^2 = 0.16$ ;  $P < 0.05$ ).



Table 2. Percentage excreta moisture content for birds of Strains 1 and 2 at 18/19 weeks, at peak production (28/29 weeks) and at 34 and 38 weeks of age.

Week	18/19	28/29	34	38
Strain 1	75.4	74.8	73.2	70.1
Strain 2	75.4	73.5	69.6	69.1
SE	0.75	1.39	1.46	1.54

The method of calcium presentation did not affect ( $P > 0.05$ ) excreta moisture score within feeding methods. Pooled data for each feeding method revealed a significant ( $P < 0.05$ ) Time x Strain x Feed effect for the excreta score. The interaction is illustrated graphically in Figures 1 and 2. Excreta moisture scores increased over the experimental period for the birds of Strain 2 as did scores for birds provided with a complete feed.

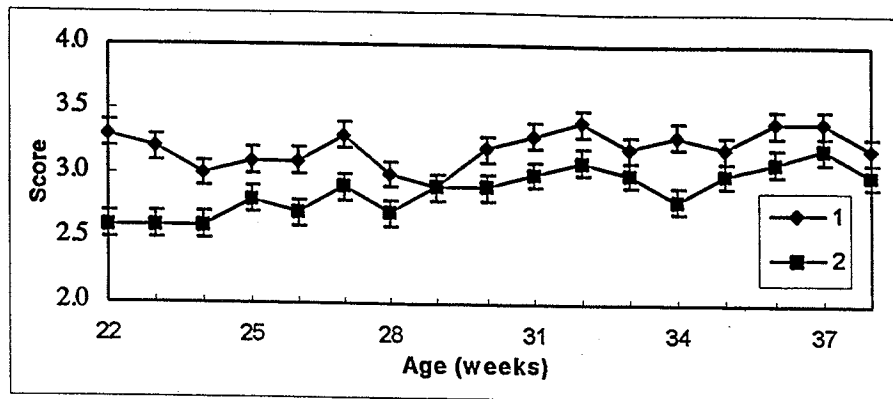


Figure 1. Excreta moisture score for Strains 1 and 2 from 22 to 38 weeks of age (Means  $\pm$  SE).

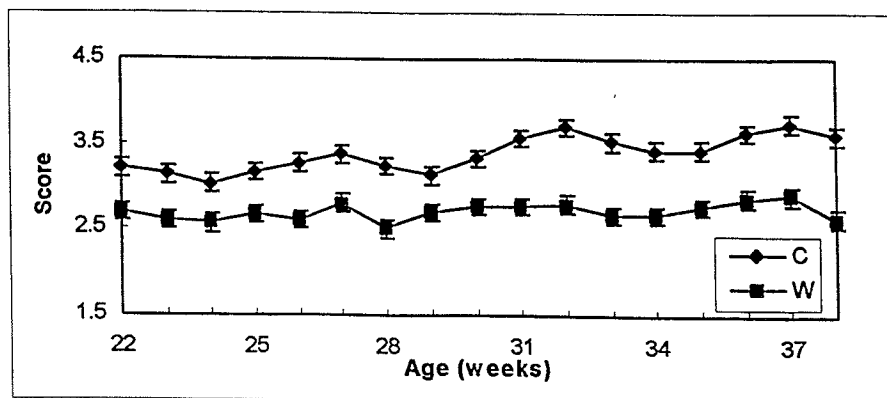


Figure 2. Excreta moisture score for complete (C) and whole-wheat (W) fed birds from 22 to 38 weeks of age (Means  $\pm$  SE).

#### IV. DISCUSSION

In the current study, excreta moisture contents were within or below the 75-80 % range which was determined as being normal by Larbier and Leclercq (1992). These authors suggested that an excreta water content greater than 80 % indicated that a bird was suffering from diarrhoea. There is concern that the various calcium presentation methods may allow, or cause, excessive calcium intakes by the birds and thus lead to wetter excreta (Leeson, 1993). However, the method of calcium presentation to the birds did not affect excreta moisture levels in the current study. No explanation is proposed for the higher moisture levels of excreta from the whole-wheat fed birds at 12 weeks of age but this difference was not carried through into the laying period. The excreta did not appear different at 12 weeks of age, but there was an obvious difference in the appearance of excreta from birds on the complete feed as the birds approached point of lay. This led to the scoring method being applied at 22 weeks. At no stage was the determined moisture content affected by the strain of bird but the excreta scoring method employed revealed a physical difference in the consistency of excreta produced by the two strains. Birds fed a complete diet produced excreta that were of a looser physical consistency than those fed on whole-wheat diets. It was apparent that feeding whole-wheat caused water to be held more effectively within the excreta.

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## THE EFFECT OF ALLZYME VEGPRO ON THE DIGESTIBILITY OF BROILER FEEDS AND ON PRODUCTION

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### Summary

This experiment was carried out to study the effect of Allzyme VegPro on broiler performance and the digestibility of nutrients when diets containing different levels of crude protein were fed to broiler chickens. Five experimental diets were formulated and fed from 18 to 53 days of age. The treatments consisted of: Treatment 1, diet containing 190 g crude protein (CP)/kg as control; Treatment 2, diet containing 180 g CP/kg; Treatment 3, diet containing 180 g CP/kg + 1 g Allzyme / kg; Treatment 4, diet containing 170 g CP/kg and Treatment 5, diet containing 170 g CP/kg + 1 g Allzyme / kg. No significant differences were observed among treatments in final body weight, body weight gain, feed consumption, feed conversion and metabolizable energy intake. No significant differences were found in apparent digestibility of crude protein and ether extract. Enzyme supplementation slightly improved weight gain, feed conversion and the apparent digestibility of crude protein and fat.

### I. INTRODUCTION

Protein and amino acid requirements of poultry vary with age, leading to the common practice of decreasing the levels of crude protein during the growing and finishing periods. Couch and Rayton (1974) reported that the protein requirement of broilers to 9 weeks of age was between 180 and 200 g/kg with the need for methionine supplementation at the 200 g/kg protein level and methionine and lysine supplementation at the 180 g/kg protein level. Salmon *et al.* (1983) used four finisher diets ranging from 166 to 207 g crude protein/kg with similar energy levels. They reported that weight gains from 4 to 8 weeks were not significantly affected by finisher protein levels, but feed efficiency of males was improved when the diet contained 189 g/kg or more of protein.

Nutrient utilization may be influenced by dietary protein concentration, as demonstrated by many investigators. Recent studies have shown that enzyme supplementation has the potential to improve the nutritive value of feedstuffs for monogastric animals (Annison, 1992). To reach their potential in improving the nutritive value of feedstuffs enzymes have to be biologically active in the gastrointestinal tract. Pettersson *et al.* (1990) found that supplementing broiler diets with enzymes allowed a reduction in crude protein level without affecting growth rate. Krzysztof *et al.* (1995) reported that a cocktail of enzymes in the diets of growing turkeys improved performance, bone mineralization and retention of phosphorus and calcium.

The present experiment was carried out: (1) to compare diets with different levels of crude protein but with similar amino acid profiles and metabolizable energy content, and (2) to evaluate the effect of enzyme supplementation of low-protein diets.

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## II. MATERIALS AND METHODS

Unsexed broiler chicks were used in this experiment. One-day old chicks were housed in batteries and provided with 23h light and 1h dark / day and were given a starter diet and water *ad libitum* up to 18 days of age at which time the experiment started. A total of 150 chicks was randomly assigned to five treatments, with three replicates. All chicks were weighed and wing banded individually. The chicks were housed in batteries with feed and water supplied *ad libitum* from 18 to 53 days of age.

Five experimental diets were formulated to contain different levels of crude protein (CP) without or with enzyme supplementation. Treatment 1 contained 190 g CP/kg as the control diet, Treatment 2 contained 180 g CP/kg, Treatment 3 contained 180 g CP/kg + 1 g Allzyme VegPro/kg Treatment 4 contained 170 g CP/kg and Treatment 5 contained 170 g CP/kg + 1 g Allzyme VegPro/kg. All diets were formulated to contain similar amino acid profiles and metabolizable energy (12.8 MJ/Kg.). The VegPro enzyme was produced by Alltech Biotechnology Center, Nicholasville, Ky 40356, USA.

During the experimental period, the criteria of response were weekly individual body weights. Feed consumption data were recorded weekly on a group basis, body weight gain and feed conversion were also calculated weekly on a group basis. All diets were tested in metabolism trial with 20 adult cocks to determine nutrient digestibilities and metabolizabilities.

Processing of data and statistical analyses were performed using statistical software (Statgraphics, Version 5.0 STSC Rockville, 1991). One-way analysis of variance was used to estimate significant differences.

## III. RESULTS AND DISCUSSION

Data of live body weight are presented in Table 1. Regarding to the entire period of study, no significant differences were found among treatments in final body weight. Enzyme supplementation increased the final body weight as compared with respective treatments which contained the same protein level without enzyme, but the differences were not significant. Average body weight gain, feed consumption, protein consumption, feed conversion, protein efficiency and metabolizable energy intake are presented in Table 2. No significant differences were observed among treatments in body weight gain, feed consumption, feed conversion and ME intake. Regarding protein consumption and protein efficiency, the differences were significant, this being due to the decrease in the protein concentrations of the diets. Enzyme supplementation improved body weight gain, feed conversion and protein efficiency, but the differences were not significant. These results may be due to a low enzyme level in the diets that was not sufficient to produce significant responses.

The average digestibility coefficients for crude protein and ether extract are shown in Table 3. Analysis of variance showed no significant differences among treatments in this data. Enzyme supplementation slightly improved the digestibility of dietary crude protein and crude fat but the differences were not significant. These results are in keeping with those obtained by Annison (1992) and Pettersson *et al.* (1990). Friesen *et al.* (1992) reported that enzyme supplementation to broiler diets increased the apparent digestibility of energy, lipid and protein of cereals. Enzyme supplementation also improved weight gain and feed conversion of broiler chickens.

Table 1. Average live body weight of broiler chicks at different ages.

T'ments/kg	Age in days					
	18	25	32	39	46	53
190 g CP	297±3	629±11	927 ± 16	1285±22 <sup>b</sup>	1738 ±29	2143 ± 39
188 g CP	278±7	590±14	916 ± 23	1269±25 <sup>b</sup>	1704 ± 34	2106 ± 47
180 g CP + Allzyme	282±5	595±11	911 ± 16	1263±22 <sup>b</sup>	1719 ± 33	2156 ± 42
170 g CP	291±5	606±14	936 ± 20	1327±28 <sup>ab</sup>	1786 ± 36	2165 ± 44
170 g CP + Allzyme	293±6	608±16	970 ± 17	1362± 24 <sup>a</sup>	1789 ± 34	2211 ± 46

<sup>a-b</sup> Means within a column with no common superscripts are significantly different at ( $P < 0.05$ ).

Table 2. Average of body weight gain, feed consumption, protein consumption, feed conversion, protein efficiency, and metabolizable energy intake for broiler chicks during experimental period from 18-53 days of age.

T'ments/kg	Body weight gain (g/day)	Feed consumption (g/day)	Protein consumption (g/day)	Feed conversion (g:g)	Protein efficiency (g/kg)	ME intake KJ/day
190 g CP	52.7±1.2	118.8±1.8	23.8 ±0.4 <sup>a</sup>	2.253 ±0.03	451 ± 7 <sup>a</sup>	1520 ± 23
180 g CP	52.2±2.7	116.4±1.6	20.9 ±0.3 <sup>b</sup>	2.237 ± 0.09	403 ± 16 <sup>b</sup>	1490 ± 21
180 g CP + Allzyme	53.5±0.5	114.6±2.7	20.6 ±0.5 <sup>b</sup>	2.140 ± 0.06	385±10 <sup>bc</sup>	1467 ± 34
170 g CP	53.5±0.8	122.7±3.0	19.6 ±0.5 <sup>bc</sup>	2.290 ± 0.02	366± 4 <sup>bc</sup>	1570 ± 38
170 g CP + Allzyme	54.8±1.4	119.0±4.0	19.0 ±0.6 <sup>c</sup>	2.179 ± 0.12	349± 20 <sup>c</sup>	1524 ± 51

<sup>a-c</sup> Means within a column with no common superscripts are significantly different at ( $P < 0.05$ ).

Table 3. Digestibility of protein and ether extract in experimental diets.

Digestion coefficient:	Treatments (g/kg)				
	190 CP	180 CP	180 CP + Allzyme	170 CP	170 CP + Allzyme
Crude protein	84.27	83.81	84.50	84.24	84.65
Ether extract	89.31	90.31	91.60	91.12	92.83

#### IV. CONCLUSIONS

The current results show that broiler performance and nutrient digestibility were not affected by decreasing the crude protein level in grower and finisher diets from 190 to 170 g/kg when amino acid levels were adjusted. Enzyme supplementation slightly improved weight gain and feed conversion of broiler chicks.

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## EFFECT OF FEEDING OATS ON THE PERFORMANCE AND LIPID METABOLISM OF WATERFOWL

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### Summary

Two feeding trials were carried out to investigate the effect of feeding 450 g oats/kg in diets for table ducks (Trial 1) and in young geese (Trial 2) on live weight, feed conversion ratio (FCR), carcass yield, chemical composition of muscle and abdominal fat pad. The blood plasma total cholesterol, triglycerides (TG) and very low density lipoprotein (VLDL) levels were studied.

It was found that oats decreased the live weight during the first 4 (table ducks) and 5 (geese) weeks of age. Oat feeding increased feed intake and FCR but in the later phase of rearing the oat-fed groups showed a more rapid growth rate. There were no significant differences in carcass yield or in the chemical composition of mixed breast and leg muscle. The abdominal fat weight was moderately lower in oat-fed table ducks and moderately higher in geese. The plasma Tg level in table ducks and the total lipid and cholesterol level in the blood plasma of geese were significantly decreased.

### I. INTRODUCTION

In recent years the nutritional role of non starch polysaccharides (NSP) in monogastric diets has been described. There are considerable differences in cereals (barley, wheat, oats) between gross and metabolisable energies and the presence of arabinoxylans and beta-glucans in oats have been associated with this difference (Aman, 1987; Knudsen and Hansen, 1991; Knudsen *et al.*, 1993a; Johanssen *et al.*, 1993). The NSP are responsible for increasing the viscosity of gut contents (White *et al.*, 1981; Broz, 1993) and making nutrients like starch and proteins less available for digestion (Knudsen *et al.*, 1993b), thereby reducing utilization and growth rate. It is well known, that some dietary fibres have a cholesterol-lowering effect. Guar gum, pectin and barley (Wang *et al.*, 1992; Martinez *et al.*, 1992) have repeatedly been shown to reduce plasma cholesterol concentrations. Ilman *et al.* (1991) and Jonnalaganda *et al.* (1993) found hypocholesterolaemic effects of oat bran in rats and hamsters. At the same time barley-based diets did not show this effect in table ducks (Vetési *et al.*, 1996).

There are several hypotheses to explain the cholesterol-lowering effect of fibre. Gallaher *et al.* (1993) found that greater viscosity in the intestinal contents is strongly associated with cholesterol reduction. At the same time the results of Ikeda *et al.* (1989) showed that the type of dietary fat significantly influenced the effect that dietary fibre exerted on lipid metabolism.

The object of the present work was to investigate the effect of an oat-based diet on performance, carcass yield and lipid metabolism of table ducks and young geese.

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## II. MATERIALS AND METHODS

### Trial 1

Experimental animals: Szarvas K-94 breed ducks, with sex ratio 1:1, with 40 birds in each group. The birds were fed to 14 days of age with pelleted starter diet and from 15 to 49 days of age with finisher diet.

#### Feeding regime

TD/1: control diet (without oats).

TD/2: 450 g oats/kg in an isoenergetic and isonitrogenous diet.

### Trial 2

Experimental animals: Landes breed geese, in 1:1 sex ratio, with 60 birds in each groups. Pelleted starter diet was fed to 21 days of age, grower diet from 22 to 35 days of age and finisher diet from 36 to 55 days of age.

#### Feeding regime

G/1: control diet (without oats).

G/2: 450 g oats/kg in an isoenergetic and isonitrogenous diet.

Daily feed intake and weekly individual weights were measured. On the final day of the experiment 12 birds from each group were slaughtered. The carcass yield and abdominal fat were measured gravimetrically and the chemical composition of mixed samples of breast and legs from each bird were determined. Blood samples were taken from 10 ducks at 14, 35 and 49 days of age and from 10 geese at 21, 35 and 55 days of age. The cholesterol and TG content of plasma were determined using an enzyme-based commercial kit (Clinsotest). The VLDL level were measured using the precipitating turbidimetric assay of Griffin and Whitehead (1982) validated for waterfowl (Mézes *et al.*, 1995).

## III. RESULTS AND DISCUSSION

Feeding 450 g oats/kg in an isoenergetic and isonitrogenous diet caused decreased growth of both table ducks and young geese during the first 4 weeks (table ducks) and 5 weeks (geese) of age. This was followed by a more rapid growth rate so that there were no differences in the finishing live weights of the birds (Table 1). The feed intake of treated birds was higher than controls and this resulted in significant increases in FCR. The FCR was a more sensitive indicator of the anti-nutritional effect of oats than was weight gain as noted previously by Bedford and Sheppy (1995).

There were no significant differences in carcass yield and chemical composition of muscle samples.

The feeding of oats had a marked effect on fat deposition in table ducks, particularly shown by an increase in liver fat content. Interestingly, the VLDL content of blood plasma also increased at the end of investigation paralleling the increases in TG and cholesterol. The results suggest that the lower density of feed caused by the higher fibre content produced higher rates of lipid transport and higher fat deposition during the late phase of fattening, especially in the liver. This effect on fat deposition was found in an experiment with chickens (Wiseman, 1988).



Table 1. Effect of feeding oats on the performance and slaughter yield of birds.

Group:	Experiment 1		Experiment 2	
	TD/1	TD/2	G/1	G/2
Finishing weight,g	2690	2705	4177	4250
Weight gain,g: 1	465	481	1262	1250
2	1545	1517	1428	1358
3	634	703	1391	1547
Cumulative weight gain	2644	2701	4081	4125
Daily feed intake,g 1 <sup>1</sup>	63	79	91	92
2	212	232	232	251
3	260	296	281	316
Mean daily feed intake,g	186	209	195	212
FCR, g:g 1	1.80	2.13	1.52	1.55
2	2.88	3.21	2.27	2.77
3	5.73	5.90	4.03	4.08
Cumulative FCR, g:g	3.44	3.80	2.62	2.60
Carcass yield (n=12/group)				
Grill weight, %	58.9	59.5	59.8	57.3
Abdominal fat pad, %	1.94	1.59	3.42	3.76
Liver weight, %	1.86	3.35	2.71	2.69

<sup>1</sup> Experiment 1, 1 = 1-14 d, 2 = 15-35 d, 3 = 36-49 d.  
Experiment 2, 1 = 1-21 d, 2 = 22-35 d, 3 = 36-55 d.

Table 2. Effect of age and oat feeding on some lipid parameters of table ducks and geese.

Group:	Experiment 1		Experiment 2	
	TD/1.	TD/2.	G/1.	G/2.
Plasma VLDL level(OD 546):				
1 <sup>1</sup>	0.08	0.09	0.08	0.10
2	0.049	0.047	0.048	0.146***
3	0.043	0.186***	0.079	0.042
Plasma TG content, mmol/L:				
1	0.869	0.537	2.56	2.17*
2	0.846	0.540**	2.99	3.32
3	0.537	1.537***	1.80	1.88
Plasma cholesterol level, mmol/L:				
1	5.48	6.76	3.43	3.23
2	2.67	3.05***	8.52	9.26*
3	1.89	2.46***	7.24	6.76*

<sup>1</sup> Experiment 1, 1 = 1-14 d, 2 = 15-35 d, 3 = 36-49 d.  
Experiment 2, 1 = 1-21 d, 2 = 22-35 d, 3 = 36-55 d.

There were no differences in the abdominal and liver fat contents due to feeding oats to geese. Otherwise, the VLDL and cholesterol content of blood plasma decreased at the end of the investigation which suggests a negative effect on lipid metabolism and transport.

The results showed a marked species difference in the response to oat feeding which was possibly caused by different rates of lipid and/or cholesterol synthesis. A cholesterol reducing capacity of oats was not found in these experiments. This was probably related to differences in lipid metabolism and transport in waterfowl species compared to other birds.

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## STUDY ON MEAT PRODUCTION ABILITY OF CHERRY VALLEY, BARBARIE AND MULE DUCK

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### Summary

In this experiment meat production ability, feed consumption and optimal slaughtering age were investigated in three genotypes.

The experiment was set up in such a way that paternal and maternal lines, being involved in producing mule ducks, were simultaneously taken as the other two studied genotypes. With this method the characterising features of close genotypes could be measured. One hundred ducks of each genotype and gender were grouped into 6 cages.

Individual live weight from one day of age was recorded weekly. Feed consumption and mortality were determined from the age of 6 weeks to the age of 12 weeks, and test cuts were also carried out in this period in order to determine the cut out and other slaughter parameters.

The experiment aimed to define the physiologically optimal slaughtering age (best cut outs) in broiler duck production.

### I. INTRODUCTION

The significance of this topic can be appreciated from the fact that it is probable that the role of duck meat products on the Hungarian market is to change considerably in the years to come; i.e., products with a low fat content and higher meat quality seem set to become predominant. The following types were examined in the present work:

1. The Cherry Valley hybrid (abbreviated in this paper to CV), a domestic duck;
2. Muscovy duck;
3. The hybrid of these, the mule duck.

Each of the three genotypes examined represents a product in its own right on the market. Only the domestic duck is marketed as a roasting duck in Hungary; muscovy ducks and mule ducks are exclusively used for the production of duck liver by means of force feeding.

The marketing of the mule duck as a roasting duck has until now been prevented by its colour. However, a white genotype now exists, although this has, as yet, no significant role on the Hungarian market. It is a disadvantage of the dark plumage that the colourings present in the feathers get under the skin during the plucking process, thus causing such an aesthetically unfavourable effect that meat products derived from such types are less widely sought by the consumer than their white-feathered counterparts. However, as mentioned previously, until now this factor had little significance, as the main product was always the liver, the fattened carcass meat being merely a by-product. Even income derived from pigmented carcass meat has been regarded solely as supplementary income.

It is known that the optimal slaughter age for the domestic duck (CV) is between 45 and 49 days. Slaughter age is certainly higher for muscovy ducks raised as roasting ducks and for mule ducks, but the optimal age for slaughter with regard to these types has not yet been determined. Consequently, the primary objective of the investigation was to examine the capacity for meat production of mule ducks. It was aimed that the biological optimum of the carcass dressing percentage for raising these types as roasting ducks be determined.

## II. MATERIAL AND METHODS

The end products of 200 Cherry Valley ducks, 200 muscovy ducks and 200 mule ducks originating from the Palotási Kacsafarm Kft. were the subject of the investigations carried out in this study.

The ducks were raised separated according to sex. Each duck was fitted with a wing tag, to enable the individual parameters to be monitored.

The ducks were accommodated in 6 cages, separated according to genotype and sex. The ground area of the boxes was 14 m<sup>2</sup>. The initial stock density of 14 birds per m<sup>2</sup> was gradually reduced to a level of 2.8 birds per m<sup>2</sup>. Lighting was provided for a period of 23 hours per day in the first week, 18 hours in the second week and 12 hours from the third week. The initial lighting intensity was 18-20 lux, being reduced gradually to 6-7 lux. This was important, particularly due to the violent temperament of the muscovy ducks. The data were processed by means of the ANOVA method of the STATGRAPHICS programme.

## III. RESULTS

The results are shown in Tables 1 and 2.

Table 1. Summary of production results.

Week of life	Geno-type <sup>1</sup>	Live weight (g)			Carcass (g)			Oven-ready Weight (g)		
6	mg:cvg	2230	2511	**	2001	2254	**	1790	2040	**
	mg:bg	2230	1369	**	2001	1198	**	1790	1052	**
	cvg:bg	2511	1369	**	2254	1198	**	2040	1052	**
	mt:cvt	1850	2453	*	1720	2201	**	1532	2008	**
	mt:bt	1850	1321	*	1720	1204	**	1532	1077	**
	cvt:bt	2453	1321	*	2201	1204	**	2008	1077	**
9	mg:cvg	3602	3334	NS	3090	2921	NS	2771	2663	NS
	mg:bg	3602	3223	*	3090	2924	NS	2771	2600	NS
	cvg:bg	3334	3223	NS	2921	2924	NS	2663	2600	NS
	mt:cvt	3220	3398	NS	2848	2967	NS	2611	2967	NS
	mt:bt	3220	2183	**	2848	1856	**	2611	1700	**
	cvt:bt	3398	2183	**	2967	1856	**	2967	1700	**
12	mg:cvg	4181	3981	NS	3598	3441	NS	3320	3160	NS
	mg:bg	4181	3567	NS	3598	2998	*	3320	2774	*
	cvg:bg	3981	3567	NS	3441	2998	NS	3160	2774	NS
	mt:cvt	3788	3613	NS	3335	3036	*	3068	2786	NS
	mt:bt	3788	2413	*	3335	2021	**	3068	1864	**
	cvt:bt	3613	2413	*	3036	2021	**	2786	1864	**

\* P < 0.05, \*\*P < 0.01

Table 2. Summary of results for meat cuts.

Week of life	Geno-type <sup>1</sup>	Breast weight (g)			Breast fillet (g)			Thigh weight (g)			Thigh fillet (g)		
6	mg:cvg	230	346	**	84	149	**	425	416	NS	247	228	NS
	mg:bg	230	107	**	84	24	**	425	272	**	247	168	**
	cvg:bg	346	107	**	149	24	**	416	272	**	228	168	**
	mt:cvt	178	372	**	58	170	**	403	422	NS	230	225	NS
	mt:bt	178	139	NS	58	39	NS	403	278	**	230	155	**
	cvt:bt	372	139	**	170	39	**	422	278	**	225	155	**
9	mg:cvg	656	725	NS	339	360	**	564	439	NS	326	234	**
	mg:bg	656	562	*	339	240	**	564	564	NS	326	338	NS
	cvg:bg	725	562	NS	360	240	NS	439	564	NS	234	338	**
	mt:cvt	591	737	**	301	337	**	516	477	NS	300	226	**
	mt:bt	591	351	**	301	189	**	516	353	**	300	203	**
	cvt:bt	737	351	**	337	189	NS	477	353	**	226	203	**
12	mg:cvg	965	813	NS	627	414	NS	653	595	*	402	288	**
	mg:bg	965	654	NS	627	385	*	653	638	NS	402	388	NS
	cvg:bg	813	654	*	414	385	*	595	638	*	288	388	**
	mt:cvt	885	703	*	556	379	NS	579	540	NS	348	269	**
	mt:bt	885	515	**	556	353	**	579	371	**	348	211	**
	cvt:bt	703	515	**	379	353	**	540	371	**	269	211	NS

\* P &lt; 0.05, \*\*P &lt; 0.01.

<sup>1</sup> mg= male mule duck  
 mt= female mule duck  
 bg= male muscovy duck  
 bt= female muscovy duck

cvg= male Cherry Valley type  
 cvt= female Cherry Valley type

#### IV. DISCUSSION

##### (a) Carcass

In the course of data evaluation it became evident that the growth phase in the CV drake lasts until the 12th week, but, as the tables indicate, this only serves the purpose of fat deposition. From weeks 9 to 12 the muscovy ducks showed only a very slight increase in weight. In the mule ducks a rapid increase in weight was observed from week 6. This increase was caused by the development of breast muscle (see below).

##### (b) Oven-ready weight

The CV type exhibited continuous growth, but this was at all points at a lower level than that observed with the mule ducks. A rapid increase in weight was, as mentioned previously, noted in the muscovy ducks, this being characteristic of the late-maturing types. The mule drakes showed a higher level of muscle development from week 9.

(c) Breast fillet

In the CV hybrid both sexes had the highest weight in weeks 6 and 9, at 159 g and 347 g respectively. In the week 9 mule ducks already exhibited good condition, at 339 g liveweight, approaching that of the CV drakes (359 g). The mule ducks outgrew their parents in the week 12.

(d) Thigh fillet

It is evident that, in this case, increase in weight was less rapid than that observed in the breast fillet. This can, for the greater part, be traced back to the physiological differences between the breeds. The early maturity of the CV type and the late maturity of the muscovy duck types can here be clearly distinguished. Mule ducks must also, in view of their capacity for meat production, be classified as late-maturing types; this is borne out by the data obtained for week 12.

(e) Valuable meat parts

The conclusions drawn from this study so far confirm that, at the age of 6 weeks, all three genotypes examined are at a similar level of development, at 41-42%. The CV type can exhibit a considerable rate of growth up to week 9 (5% growth in the valuable meat parts), but after this point this ratio declines. In the same period only an insignificant change in the performance parameters was observed in the flying ducks. The mule ducks exhibited a high rate of growth (over 5%) from weeks 9 to 12.

## CONCLUSIONS

The CV type ducks increased in weight even after day 49 of life, and carcass dressing percentage improved (by 5%) in this type until week 9. The results obtained for flying ducks could only be evaluated by taking into account the reaction to the Derzsy vaccine, which caused suffering among the drakes. A good standard of feed conversion was, nevertheless, shown. These were able to maintain their intensity of growth over a long period, but exhibited a lower carcass dressing percentage than the other two genotypes. The mule ducks raised as roasting ducks matured late. These reached their optimal slaughter weight only at the age of 12 weeks. With respect to this result, this type lags far behind the other two genotypes. Increase in growth in the mule ducks ran parallel to that observed in the CV ducks, but the former showed a substantially higher level of feed conversion than the latter. The highest carcass dressing percentage was attained in this type in week 12 of life, this being defined in this study as the optimal slaughter age. Of the genotypes examined, only the mule ducks achieved a ratio of valuable meat parts of 50% or more.

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## AMINO ACID DIGESTIBILITIES OF DIFFERENT WHEATS DETERMINED WITH POULTRY

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### Summary

Amino acid digestibilities of wheat samples, differing mainly in their protein content, were determined with 9-week old broiler chicks using the wet force feeding method. In order to calculate true digestibility values endogenous amino acid losses (EAAL) were also measured by the regression method using fasted birds after force feeding a nitrogen(N)-free diet. No significant differences between the apparent and true amino acid digestibilities of wheat samples were found. On the other hand dry matter intake increased EAAL and different methods gave different amounts of endogenous amino acid excretion. The amino acid (AA) compositions of endogenous excreta was similar. The EAAL contained substantial amounts of threonine, valine, leucine, lysine and arginine.

### I. INTRODUCTION

It is recognised that exact information on the digestibility of amino acids in different feedstuffs enables more accurate diet formulation. Since wheat plays an important role in poultry diet formulation in many countries the main objective of this study was to determine the apparent and true digestibilities of the amino acids in wheat samples which differed mainly in their protein contents.

True digestibility, unlike apparent digestibility of amino acids, appears to be independent of dietary protein level and may allow feed ingredients to be compared accurately even if they are ingested in different quantities (Donkoh and Moughan, 1994). Therefore, endogenous amino acid losses (EAAL) were also determined.

### II. MATERIALS AND METHODS

#### (a) Determination of apparent amino acid digestibility (AAAD)

Nine-week old broiler chicks were housed in individual cages. Chicks were weighed and randomly divided into two groups containing 6 birds each. Chicks were fasted for 36 h in order to ensure complete emptying of their digestive tracts and were force fed an average 40.5g of wheat containing a higher amount (126g/kg) of protein (Wheat H) and 41.5g of wheat containing a lower amount (107g/kg) of protein (Wheat L). Feeds were fed moistened. The technique and equipment were similar to those described by Lessire (1990). The ratio of wheat and water was 1:1. Excreta were collected daily during the subsequent 48 h.

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In order to avoid contamination of droppings with feathers and scurf, excreta were collected in nylon bags which were stuck around the cloaca. During experiments water was available *ad libitum*.

#### (b) Determination of EAAL with different methods

The EAAL were measured partly according to the method that is described above with the modification that in this case chicks were either force fed with moistened N-free diet (57g air dry basis; diet water ratio:1:0.5) or they were fasted for an additional 48 h. Excreta were collected in both groups during the last 48 h.

The third procedure for the measurement of EAAL was the regression method when chicks were fed graded amounts (50, 100, 150 and 200 g/kg) of dietary protein. The experimental diets were mixed from a N-free diet using soyabean meal as the sole protein source. In this experiment chicks were fed *ad libitum*. After a 3 day adaptation period food intake and the amount of excreta were measured daily during the next 3 days.

#### (c) Chemical analysis

Before analysis samples were freeze dried, weighed and ground. Amino acid contents of diets and excreta were determined after 24 h acid hydrolysis with 6 M aqueous HCl at 110 °C. Before hydrolysis, samples were oxidised with formic acid in order to avoid any losses in methionine and cystine. Tryptophan contents were not determined.

#### (d) Statistical analysis

Statistical analysis was performed, using ANOVA to detect significant differences among endogenous amino acid contents or digestibility values. Feeding graded levels of protein allowed the calculation of EAAL using linear regression equations, after regressing amino acid intake against amino acid excretion.

### III. RESULTS AND DISCUSSION

#### (a) Apparent digestibilities of amino acids

Apparent amino acid digestibilities (AAAD) in "Wheat L" were slightly higher than in "Wheat H" (Table 1), but the difference was not significant.

Comparing the digestibilities of essential amino acids (EAAD), low digestibility values (about 60%) were found for threonine, glycine, valine and lysine, while the digestibilities of methionine, phenylalanine, leucine and histidine were higher (about 80%).

#### (b) Measurement of EAAL

Different methods gave different values for EAAL. During the regression procedure the amino acids excreted increased significantly with increasing amino acid intake. The response could be described by linear regression equations and the intercepts of these equations allowed the endogenous amino acid values to be obtained with this method. All the linear equations proved statistically significant ( $P < 0.001$ ).

As expected the lowest EAAL value was measured with fasted chicks (113.6 mg/day). Feeding the N-free diet increased the daily EAAL to 245.2 mg/day. Since the



highest dry matter intake was registered during the regression method the daily EAAL was the highest in this case (395.5 mg/day). However, when the daily EAAL values were divided by the daily dry matter intakes a higher value was registered with the N-free diet (8.48 mg/g d.m.) than with the regression method (5.84 mg/g d.m.). This implied that dry matter intake increased EAAL but this response was curvilinear.

The measurement of EAAL with fasted animals does not take into account the effect of food intake. Therefore, it does not seem to be a real value. Our results support the findings of Parsons *et al.* (1983) who reported that dietary carbohydrate substantially affects the excretion of endogenous amino acids by poultry and that fasted birds may not provide accurate EAAL values. Raharjo and Farrell (1984) also found a significant effect of the acid detergent fibre content of a N-free diet on the amino acid outputs in ileal digesta and in excreta.

Comparing different methods for the determination of EAAL Siriwan *et al.* (1993) found that using fasted birds provided significantly lower EAAL than that obtained both by feeding the N-free diet and from regression analysis. On the other hand Siriwan *et al.* (1993) measured the highest EAAL by regression analysis rather than by feeding a N-free diet. The reason for this contradiction may be that in the present experiment the N-free diet was force fed whereas Siriwan *et al.* (1993) used *ad libitum* feeding. Recently, Siriwan *et al.* (1994) published results on measuring EAAL in chicks using guaidinated casein. Using this method resulted higher EAAL than the other methods.

Table 1. Apparent and true amino acid digestibility coefficients of wheat samples.

	APPARENT		TRUE (FAST)		TRUE (NFREE)		TRUE (REGR)	
	Wheat H	Wheat L	Wheat H	Wheat L	Wheat H	Wheat L	Wheat H	Wheat L
CYS	0.74	0.72	0.85	0.83	0.89	0.88	0.83	0.81
ASP	0.66	0.63	0.77	0.74	0.84	0.82	0.78	0.75
MET	0.78	0.78	0.86	0.85	0.90	0.90	0.87	0.86
THR	0.64	0.68	0.79	0.81	0.92	0.95	0.83	0.86
SER	0.77	0.75	0.87	0.85	0.91	0.90	0.86	0.85
GLU	0.91	0.91	0.94	0.94	0.96	0.96	0.94	0.95
GLY	0.60	0.59	0.73	0.72	0.79	0.78	0.69	0.67
ALA	0.60	0.59	0.73	0.71	0.79	0.77	0.70	0.68
VAL	0.59	0.58	0.66	0.70	0.88	0.91	0.76	0.80
ILE	0.70	0.81	0.79	0.88	0.83	0.92	0.91	0.99
LEU	0.77	0.80	0.86	0.88	0.88	0.91	0.88	0.90
TYR	0.68	0.70	0.81	0.82	0.84	0.85	0.80	0.82
PHE	0.81	0.80	0.88	0.87	0.90	0.90	0.88	0.87
LYS	0.58	0.62	0.74	0.76	0.80	0.82	0.76	0.78
HIS	0.79	0.81	0.86	0.87	0.88	0.90	0.87	0.89
ARG	0.73	0.77	0.82	0.85	0.84	0.88	0.84	0.87
Average	0.71	0.72	0.81	0.82	0.87	0.88	0.83	0.83
SE	0.09	0.10	0.07	0.07	0.05	0.05	0.07	0.08

#### Calculation of true amino acid digestibility (TAAD)

Endogenous amino acid outputs were used to calculate true amino acid digestibilities. The EAAL values obtained by the regression and "N-free" methods were able to convert the

apparent digestible amino acid values directly since they were expressed as mg EAAL/g d.m. intake. True digestibility values from EAAL obtained with fasted chicks were based on the daily excretion of EAAL and these daily values were divided by the actual wheat intake.

The TAAD values of the sum of the amino acids ranged from 81% to 88% (Table 1). The lowest values were obtained using the EAAL obtained with fasted chicks (81% Wheat H; 82% Wheat L) while the highest values resulted from the calculation with the EAAL from the "N-Free" method (87% Wheat H; 88% Wheat L) (Table 1).

In the endogenous excreta the dominant essential amino acids were threonine, valine, leucine, lysine and arginine. The essential amino acid pattern of the EAAL did not change with the different methods used. The only difference was the relatively low amount of glycine determined with the regression method.

Comparing the apparent and true digestibility values showed that correcting AAD to TAAD decreased the differences that existed between the apparent digestibilities of individual amino acids and improved the digestibilities of amino acids found in higher amounts in the endogenous excreta.

#### IV. CONCLUSIONS

In spite of the large differences in the protein and amino acid contents of the two wheat samples no significant differences were found in the apparent and true digestibilities of amino acids.

The exact measurement of EAAL is difficult and different methods gave different results. What is clear is that dry matter intake influenced EAAL significantly. Therefore, EAAL determined with fasted animals cannot be accepted as accurate.

Among the essential amino acids EAAL contained substantial amounts of threonine, valine, leucine, lysine and arginine. The digestibilities of these amino acids can change markedly when apparent digestibility is converted to true digestibility.

In wheat protein the apparent and true digestibilities of the individual amino acids are different. It should be taken into account during diet formulation.

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## SUBSTITUTION OF SOYABEAN MEAL BY SUNFLOWER MEAL WITH OR WITHOUT ENZYME SUPPLEMENTATION IN BROILER DIETS

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### Summary

Seven experimental diets were formulated and used for broiler chicks from 18 to 46 days of age. The control diet contained 200 g/kg of solvent-extracted soyabean meal (Treatment 1). A preparation of solvent-extracted sunflower meal (SFM) was used at levels of 150 or 200 g/kg of the diet instead of soyabean meal (Treatments 2 and 3). Treatment 4 contained 150 g/kg of SFM plus Phylacell 0.6 g/kg feed. Treatment 5 contained 150 g/kg SFM and Energex™ 0.5 g plus Bio-Feed™ pro 0.25 g/kg feed. Treatment 6 contained 200 g/kg of SFM plus Energex™ 0.5 g/kg feed. Treatment 7 contained 200 g/kg of SFM plus Kemzyme® Dry 1 g/kg feed. No significant differences were found among treatments in body weight gain, feed consumption, protein consumption, feed conversion, protein efficiency, carcass traits, and digestion coefficient of nutrients. No beneficial effects were shown from the use of enzyme supplementation with this SFM preparation.

### I. INTRODUCTION

Sunflower meal contains less protein, lysine and energy than soyabean meal. Rad and Keshavarz (1976) showed results indicating that 50% of soyabean meal protein could be replaced with sunflower meal protein in rations for broiler chicks with no adverse effects on gain and feed conversion. Chrappa *et al.* (1987) and Tsvetanov *et al.* (1988) reported that replacement of 50 % of soyabean meal by sunflower meal reduced feed utilization. The dressing percentage was not affected by the feeding of sunflower meal. Zatari and Sell (1990 a, b) have found that diets containing 100 or 200 g/kg of sunflower meal for broiler chicks had no effect on body weight gain but decreased feed efficiency. Pelleting diets containing sunflower meal increased weight gain and feed intake and improved feed efficiency. Irish and Balnave (1993) showed results indicating that broilers fed on soyabean meal grew significantly less than broilers fed diets containing 25% sunflower meal. Hegedüs and Fekete (1994) reported that extracted soyabean meal can be substituted partly or entirely with extracted sunflower meal in broiler diets when supplemented with lysine and methionine and an appropriate energy level was provided. Sherif *et al.* (1995) found that 50% of soyabean meal could be replaced with expeller sunflower meal in grower and finisher diets for broiler chicks without adverse effects on performance or carcass traits.

There are a large number of studies on the use of enzyme supplementation in diets containing cereals (wheat, barley, oats and rye). Friesen *et al.* (1992) reported that enzyme supplementation of broiler diets increased the apparent digestibility of the energy, lipid and protein of cereals. Enzyme treatment also improved weight gain and feed conversion of broiler chicks. Pettersson *et al.* (1993) reported that enzyme supplementation improved weight gain, increased feed intake and improved feed conversion of broiler chicks.

This experiment was conducted to study the effects of substituting soyabean meal with solvent extracted sunflower meal (finely ground and supplemented with lysine, methionine and energy), with or without crude enzyme preparations, on the performance of broiler chicks and nutrient digestibility.

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## II. MATERIALS AND METHODS

Ross meat-type unsexed broiler chicks were used. All chicks received a starter diet and water *ad libitum* to 18 days of age at which time the experiment commenced. A total of 210 chicks were weighed, wing banded individually and used in this study. The chicks were divided into 21 groups of 10 chicks and randomly assigned to 7 treatments with three replicates. The chicks were housed in batteries with experimental feed and water supplied *ad libitum* from 18 to 46 days of age.

Seven experimental diets were formulated and used. The control diet contained 200 g/kg of soyabean meal (Treatment 1). A preparation of solvent-extracted sunflower meal (84.4% sunflower meal finely ground after extraction, 12% sunflower oil, 2.3% lysine, and 1.3% methionine) was used at levels of 150 or 200 g/kg of the diet in place of soyabean meal (Treatments 2 and 3). For the replacement of soyabean meal the sunflower meal preparation (SFM) was supplied in lieu of equivalent amounts of soyabean meal. Treatment 4 contained 150 g/kg of SFM plus Phylacell 0.6 g/kg feed. Treatment 5 contained 150 g/kg of SFM and Energex™ 0.5 g/kg plus Bio-Feed™ Pro 0.25 g/kg feed. Treatment 6 contained 200 g/kg of SFM plus Energex™ 0.5 g/kg feed. Treatment 7 contained 200 g/kg of SFM plus Kemzyme® dry 1 g/kg feed.

The experimental diets supplemented with the SFM preparation were almost identical in amino acid content, but poorer in crude protein content and richer in energy and crude fibre content than the control diet. The sunflower meal preparation was supplied only from 18 days of age because of its high crude fibre content.

### Source of crude enzymes and contents

- Phylacell (Endo- $\beta$ -1,4-glucanase, Exo- $\beta$ -1,4-glucanase,  $\beta$ -glucoseoxidase) produced by PHYLAXIA Oltóanyagtermelő Vállalat, Budapest, Hungary.
- Energex™ (Fungal  $\beta$ -glucanase, Hemi-cellulase, Pectinase, Endoglucanase) produced by NovoNordisk, Denmark.
- Bio-Feed™ Pro (Protease) produced by NovoNordisk, Denmark.
- Kemzyme® dry ( $\alpha$ -Amylase,  $\beta$ -Glucanase, Cellulase complex, Lipase, Protease) produced by™ Kemin Industries Inc., Des Moines Iowa, USA.

During the experimental period the criteria of response were weekly body weight, body weight gain, feed consumption, protein consumption, feed conversion and protein efficiency (protein consumption g/kg body weight gain). At the end of the experiment 10 birds per treatment, with body weights near the average value of the respective treatments, were selected and slaughtered. The slaughter test was made to determine body parts and organs, abdominal fat content and total edible parts percentage. All diets were tested in a digestibility trial to determine nutrient digestibilities.

Processing of data and statistical analysis were performed using statistical software (Statgraphics, Version 5.0 STSC, Rockville, 1991). One-way analysis of variance was used to estimate significant differences.

## III. RESULTS AND DISCUSSION

Data for body weight gain, feed and protein consumption, feed conversion and protein efficiency are shown in Table 1. No significant differences were found amongstreatments in daily body weight gain, daily feed and protein consumption, feed

conversion and protein efficiency. These results are in keeping with those reported by Hegedüs and Fekete (1994) and Zafari and Sell, (1990 a, b). In our experiment substitution of soyabean meal with the sunflower meal preparation caused a slight decrease in the protein content of the diets and a slight increase in fibre content and energy. These results showed that the utilization of sunflower meal was better than that of soyabean meal. Irish and Balnave (1993) showed results indicating that broilers fed on soyabean meal grew significantly less than broilers fed on diets containing 25% sunflower meal.

Table 1. Average of body weight gain, feed consumption, protein consumption, feed conversion and protein efficiency for broiler chicks during experimental period from 18-46 days of age (means  $\pm$  SE).

Treatments g/kg	B'weight gain g/day	Feed consumption g/day	Protein consumption g/day	Feed conversion g:g	Protein efficiency g/kg
Control	54.6 $\pm$ 0.5	105.2 $\pm$ 0.8	21.5 $\pm$ 0.2	1.926 $\pm$ 0.024	393.8 $\pm$ 5.0
150 SFM	55.3 $\pm$ 1.8	110.0 $\pm$ 1.8	21.2 $\pm$ 0.3	1.995 $\pm$ 0.096	385.0 $\pm$ 18.5
200 SFM	56.9 $\pm$ 1.2	112.5 $\pm$ 3.3	21.3 $\pm$ 0.6	1.983 $\pm$ 0.096	374.9 $\pm$ 18.2
150 SFM + E*	54.1 $\pm$ 1.0	104.5 $\pm$ 1.2	20.2 $\pm$ 0.2	1.931 $\pm$ 0.024	372.8 $\pm$ 4.7
150 SFM + E*	51.5 $\pm$ 4.1	102.6 $\pm$ 7.0	19.8 $\pm$ 1.4	1.997 $\pm$ 0.030	385.5 $\pm$ 5.8
200 SFM + E*	55.9 $\pm$ 1.3	107.3 $\pm$ 1.3	20.3 $\pm$ 0.3	1.920 $\pm$ 0.021	362.9 $\pm$ 4.0
200 SFM + E*	54.5 $\pm$ 0.9	108.9 $\pm$ 1.0	20.6 $\pm$ 0.2	1.998 $\pm$ 0.016	377.6 $\pm$ 3.0

E\*: Enzyme supplement (see Materials and Methods).

Analysis of variance of the percentage body parts and organs, abdominal fat and total edible parts showed that no significant differences were observed among treatments. These results agree with those obtained by Chrappa *et al.* (1987) and Sherif *et al.* (1995) who found that dressing percentage was not affected by the feeding of sunflower meal to broiler chicks.

Values for nutrient metabolizabilities and digestibilities are presented in Table 2. No significant differences were observed among treatments in nitrogen retention or in metabolizability of dry matter, ether extract, nitrogen free extract and energy. Also no significant differences were found in the digestion coefficients of crude protein, crude fibre and organic matter.

#### IV. CONCLUSION

The sunflower meal preparation concentrated with energy and supplemented with lysine and methionine was effectively utilized in grower and finisher broiler diets in place of soyabean meal without adverse effects on production. There were no beneficial effects of enzyme supplementation with this sunflower meal preparation.

Table 2: Metabolizability and digestion coefficient of nutrients in experimental diets.

Treatments	1	2	3	4	5	6	7
Metabolizability %							
N-retention	30.21	28.84	29.05	25.87	29.55	24.62	28.41
Ether extract	88.20	88.39	89.35	87.58	86.87	88.26	88.14
N. F. E.	93.57	95.25	95.52	94.34	95.21	94.45	95.51
D. M.	72.86	73.18	73.40	72.23	73.43	72.29	73.46
ME	81.04	80.03	80.20	80.41	80.73	80.16	80.73
Digestion coefficient %							
Crude protein	85.23	86.22	85.62	86.14	86.14	86.11	86.17
Crude fibre	18.06	17.89	17.32	16.87	17.79	17.65	17.67
Organic matter	81.21	82.16	82.42	81.05	82.16	81.12	82.35

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## THE EFFECT OF SEX ON THE FEATHER PRODUCTION OF GEESE

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Summary

This study has evaluated the effect of sex on quality and quantity of raw plucked goose feathers. According to statistical analysis there is a significant relationship between live weight and quantity of plucked feathers. The relationship between live weight and down is changed by the number of pluckings in the same year and sex. The relationship between the raw plucked feathers and plucked down changes from a medium to a strong significant correlation. The relationship is stronger in females than males. The correlation between quantity of raw feathers and percentage of down is significant but the regression (b) value in the case of males is negative.

## I. INTRODUCTION

In Hungary the goose production component of total poultry exports in 1994 was 45.5 % and more than 50 million dollars was generated from goose feather exports. This money is about 30 % of the income from goose production exports (M. P., 1995). According to the International Down and Feather Bureau Incorporated (1996) Hungary by its sale of 4294 tons of raw feather and down shares 6.9 % of the world feather trade and its share in market value is 10.4 %. Hungary is the second largest feather exporter after China. These facts have justified the effort spent on research connected to factors affecting goose feather production.

The feather production of geese is mainly dependent on the frequency of plucking. The amount of raw ripe feather per plucking (cover + down feather) is determined by genetic factors, age, body size, by husbandry and feeding conditions (Tóth *et al.*, 1988), climatic conditions (Bögre and Bogenfürst, 1971), and the level of plucking (thoroughly or superficially) (Tóth *et al.*, 1988). Objective judgement of the level of plucking is difficult. A goose of 5 kg live weight has about 400 g feathers (Schneider, 1995) i.e. about 8 % of live weight is composed of feather and down. The Hungarian Upgraded goose selected for feather production can provide at first plucking 80-90 g, at the second plucking 130-155 g and at the third plucking 155-180 g of feathers.

This study deals with the effect of sex on feather production.

## II. MATERIALS AND METHODS

The feather production of young Hungarian Upgraded geese, 140 layers and 75 ganders developed at the Goose Breeding Research Station of the University of Agricultural Sciences, Gödöllő, was studied. In six families (a family consists of 4 ganders and 14 layers) the male and female progenies were tested, in another five families only the female progenies were involved. The birds were plucked three times in one year and at each plucking the live weight and the raw feather and down weight were determined individually, and the down content of feather (%) was calculated.

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Relationships between live weight and weight of raw plucked feathers (cover and down) and weight of down as well as weight of raw feathers and weight of down and the down content (%) were estimated by correlation analyses at each plucking and for the combined three pluckings.

### III. RESULTS AND DISCUSSION

The live weight of ganders as well as raw feather and down production surpassed the live weight and feather and down production of their half sib layers at each plucking and each families. These differences did not prove to be significant although in some cases (e. g. at the first plucking the ganders gave 10 g – more than 10 % – more feather and down than the layers). These differences appeared at each plucking and in each family. Tóth *et al.* (1988) found the raw feather production of ganders to be significantly better than layers of the same breed. Our data show a higher down content of the female raw feathers but this finding was also not significant.

According to our correlation analysis the live weight and raw feather weight is always significantly correlated (ganders:  $r = 0.40-0.62$ , layers  $0.30-0.50$ , Table 1). Tóth *et al.* (1988) also found positive and significant relationship in the same breed between the two traits (gander:  $r = 0.519$ , layers  $0.509$ ). Góra (1973) and Szado (1972) (see Szado *et al.*, 1995) found the following correlation coefficients: between live weight and raw feather weight  $r = 0.99$  (Góra) and  $r = 0.56$  (Szado); between live weight and down weight  $r = 0.96$  and  $0.60$ . The relationships in each case were significant. This relatively strong relationship between live weight and plucked feather weight can be explained by the fact that the feather weight is influenced first of all by body surface which has an established and constant correlation with the live weight (Tóth *et al.*, 1988). According to our findings the differences between the raw feather production of males and females were parallel with differences in live weight at each plucking and the three pluckings together. A similar conclusion was made by Bögre (1984).

There was also a correlation between live weight and down weight. The difference between sexes in this relationship showed a changing character. At the first plucking there was a closer relationship and higher regression coefficient in the ganders. At the second plucking the layers had higher regression coefficients and at the third plucking the layers maintained their advantage.

The relationship between the total plucked raw feather weight at three pluckings and the average live weight was stronger in females than males. The situation was the same in the relationship between live weight and down weight, the females having a higher correlation coefficient than males with the value of the regression coefficient being two times higher than in males.

There were significant medium-strong ( $0.62-0.63$ ) and strong ( $0.82-0.87$ ) correlations between raw feather weight and the weight of down in both sexes at the three pluckings. We could not find much difference between the correlation coefficients of the two sexes but the regression coefficient of females was higher than males owing to the higher down content of the raw feather in females.

There was a significant relationship between raw feather and down content except for the second plucking. A negative relationship (b) was found between the total raw feather production of ganders and down content.



Table 1. Relationships between live weight and raw feather weight and down weight and between raw feather weight and down weight and down percentage.

Relationships between		Sex	b	r		b	r		
live weight - raw feather weight	1st	gander	16,4250	0,6214 ***	3th	16,3530	0,5199 ***		
		layer	6,7871	0,3014 ***		15,6760	0,4979 ***		
live weight - down weight		gander	3,0801	0,3254 **		5,1430	0,3042 *		
		layer	1,2115	0,0126		6,3088	0,3438 ***		
raw feather weight - down weight		plucking	gander	0,2388		0,6672 ***	plucking	0,4319	0,8032 ***
			layer	0,2747		0,6388 ***		0,4922	0,8446 ***
raw feather weight - down %	plucking	gander	0,0494	0,1720	0,0571	0,3087 **			
		layer	0,0979	0,2296 **	0,0882	0,4207 ***			
live weight - raw feather weight	2nd	gander	11,7740	0,4002 ***	Total	44,9240		0,4916 ***	
		layer	18,4320	0,3841 ***		45,2530		0,5278 ***	
live weight - down weight		gander	2,0680	0,1353		10,1620	0,3102 *		
		layer	8,1570	0,4260 ***		18,0800	0,4316 ***		
raw feather weight - down weight		plucking	gander	0,3230		0,6223 ***	0,2245	0,6270 ***	
			layer	0,3428		0,8772 ***	0,3945	0,8247 ***	
raw feather weight - down %	plucking	gander	0,0183	0,0693	-0,0270	0,3452 **			
		layer	0,0048	0,0316	0,0213	0,2751 ***			

\*P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001

#### IV. CONCLUSIONS

From the relationship analyses the following conclusions can be drawn :

1. by increasing the live weight of geese their raw feather production will also increase. Higher increases can be predicted in the layers than in the ganders.
2. increasing the live weight of layers can also increase the amount of down with a high probability, and nearly two times more improvement can be attained in this trait in females than in males.
3. by selection of layers for higher live weight the amount of raw feather and down can indirectly be increased. In the case of ganders more important is the direct selection for raw feather production.
4. in layers, selection for raw feather production will not decrease its quality (down content) but in ganders the one sided selection for raw feather production in the long-term can result in quality problems.

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## SEASONAL EFFECTS ON HATCHABILITY TRAITS OF MEAT-TYPE PIGEON PARENTS

I. MELEG

### Summary

The reproductive performance of pigeons selected for commercial squab production and kept in an environmentally-controlled house are less affected by seasons than other domestic pigeons. In this research study breeding pairs were kept in separate pigeon cages.

A total of 8497 eggs descended from 480 Auto Sexing Texan breeding pairs was evaluated for estimating the effect of the seasons. The experiment lasted for 12 months divided into four 3 month periods. During incubation each type of loss (cracked eggs, infertile eggs and mortality to days 5, 14 and 18 of incubation) was evaluated by the seasons. Mortality was also evaluated on the basis of the sex of the embryo.

Spring was the most favourable season with respect to a lower incidence of cracked eggs and autumn was more favourable with respect to lower production of infertile eggs and lower early mortality. Late mortality was less affected by the seasons than by the sexes: it was 2-3 times higher for male than for female embryos.

### I. INTRODUCTION

Fertility can, from time to time, be very low in pigeon flocks. This considerably decreases the number of weaning squabs per pair and the commercial squab production experiences serious economic loss. Under normal conditions the eggs of all domestic pigeons - fancy, racing, and utility types - take from 17 to 19 days to hatch (Eggleston, 1921).

The percentage of pigeon eggs laid which fail to hatch is much higher than generally supposed. It can be safely estimated that it averages 15-20% of all eggs laid in well managed lofts, to a higher percentage where conditions are not so favourable.

These losses may be divided into losses from infertile eggs, from dead germs and from squabs dying in the shell. Platt *et al.* (1937) showed that of 11,583 eggs laid, 18.95% failed to hatch and 14.5% were reported infertile. Levi (1963) examined 1528 eggs which failed to hatch and found that only 375 were actually infertile, while 1033 had dead germs and 120 dead embryos of 7 days and older. Delhauer (1967) measured 774 laid eggs; 448 squabs were marketed. 131 eggs were classed as infertile and 21 as dying in the shell.

Infertile eggs are a major problem in the hatching period. Ballay (1976) found that infertile rate was 10.1% in the Auto Sexing King and 17.7% in the Auto Sexing Texan breeds. Cooper (1977) reported that the number of infertile eggs was least when 11 hours per day of artificial light was used. Bötcher *et al.* (1985) showed that increasing the crude protein content of pigeon pellets improved fertile egg production.

Numerous experiments on hatchability traits have been reported in several pigeon breeds, kept in the volier system and the seasons considerably influenced production. Information is lacking for pigeons kept in environmentally controlled, windowless houses.

## II. MATERIALS AND METHODS

In the experiment 480 Auto Sexing Texan breeding pair's hatchability traits were established. A total of 8497 eggs descended from pigeon breeding pairs were evaluated for estimating the effect of the seasons. The rate of cracked eggs, infertile eggs and mortality to days 5, 14 and 18 of incubation were measured.

The breeding flock was housed in an environmentally-controlled, windowless pigeon house. All pairs were randomly distributed to separate cages. The test period lasted for 12 months divided into four 3 month periods (spring, summer, autumn, winter). The lighting program was 12 h light and 12 h dark. The light intensity was 2.5W/m<sup>2</sup>. All pigeons were fed pellets containing (g/kg): crude protein, 174; crude fat, 24; crude fibre, 42. Water and feed were provided *ad libitum*.

## III. RESULTS AND DISCUSSION

Table 1 summarizes the hatchability traits of the Auto Sexing Texan pigeon population. The average hatchability rate of the eggs laid was 61.8%. In the literature higher hatchability rates were reported by Platt *et al.* (1937) and Cooper (1977). In the summer and autumn hatchability was 2% above the average.

The number of cracked eggs was least in spring and summer. Annual rate regarding cracked eggs was 13.1%. This was higher than reported by Cooper (1977) and Böttcher *et al.* (1985). It can improve with the right choice of nest material.

Table 1. Hatchability of meat-type pigeons depending by seasons.

Traits	Spring	Summer	Autumn	Winter	LSD (P < 0.05)
Eggs laid (total)	2197	2195	1994	2111	20.30
Cracked eggs(total)	220	244	311	336	7.98
Embryonic mortality (total):					
1-5 days	416	425	382	350	6.20
6-14 days	88	53	33	90	2.53
15-18 days-female	63	30	33	45	1.57
-male:	134	94	95	104	2.71
Infertile eggs(total)	96	110	68	115	2.84
Hatching rate of eggs laid (%)	59.68	63.50	63.69	60.33	2.50

The early mortality was higher than late mortality. The mortality result was most favourable in autumn. Late mortality was less affected by the seasons than by the sexes. It was 2-3 times higher for male than for female embryos in each season. These results were better than the experimental results reported by Levi (1963) and Delhauer (1967).

The number of infertile eggs was low in this experiment, especially in autumn. This was better than the results of systematic investigations in several pigeon breeds carried out by Platt *et al.* (1937), Levi (1963) and Ballay (1976).

#### IV. CONCLUSIONS

The hatchability traits of meat-type pigeons kept in windowless controlled houses are less affected by seasons than other pigeon populations. The hatchability rate was above average in summer and autumn. The number of infertile eggs and late mortality were much better than previously reported.

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## APPLYING ENZYME PRODUCTS TO THE FEEDING OF TABLE DUCKS

L. KISS\* and M. VETÉSI\*\*

### Summary

In Hungary more than 70 % of the costs of duck production are associated with the feed. The biggest components of duck feeds are the plant carbohydrates and proteins. In the feed of monogastric animals about 70 % of the dry matter is carbohydrate (Graham, 1991) and 13-26 % is protein. Thus, it is essential that these nutrients are utilized efficiently by ducks to maximise the economics of production.

Monogastric animals do not produce enzymes which hydrolyse the non-starch polysaccharides (NPS) of feed, but commercially produced enzymes can be added to feed. The present paper describes the effects of adding enzymes which degrade polysaccharides and proteins.

### I. INTRODUCTION

The results of research have shown that the use of enzyme products has a positive effect on the digestion of monogastric animals and this positive effect is lower in pigs than in table ducks (Chesson, 1991). The enzyme products used to increase feed efficiency have two effects: one of them is the direct utilization of nutrients while the other is the elimination of antinutritive effects (Hottern, 1993). Much better results have been obtained when lower amounts of enzymes (1-5 mg/kg) have been used (Chelsson, 1992). The advantages of using enzymes in the feed of monogastric animals are the following:

- the viscosity of hemicellulose gels decreases,
- those spatial barriers which obstruct the access of enzymes (e.g. the amylases) to nutrients are eliminated,
- soluble oligosaccharides are released which are fermented to volatile fatty acids in the posterior section of the intestines and, after absorption, ensure supplementary energy (Hottern, 1993).

To ensure the optimum effect of enzymes added to the feed three essential aspects should be considered: the specificity of enzymes, the complex character of plant NSP and the effects caused by the enzyme-induced changes in the nutritive parameters of NSP components (Hottern, 1993).

### II. MATERIALS AND METHODS

The experiments were carried out on the duck farm of the Szarvas Duck Farm Ltd. in 1996 with wheat-based and barley-based feeds and the duck variety Szarvasi K 94. There were 100 table ducks in each experimental group (with sex ratio 1:1).

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There were five groups in each experiment in which the following feeds were fed.

Experiment No.1 (wheat-based feed)

Group A	Control feed + Bio Feed Pro
Group B	Control feed + Bio Feed Plus
Group C	Control feed + Bio Feed Pro and Bio Feed Plus
Group M	Modified feed + Bio Feed Plus and Bio Feed Pro (only in starter feed)
Group K	Control feed

Experiment No. 2 (barley-based feed)

Group A	Control feed + Bio Feed Pro
Group B	Control feed + Bio Feed Beta
Group C	Control feed + Bio Feed Pro and Bio Feed Beta
Group M	Modified feed + Bio Feed Pro (only in starter feed) and Bio Feed Beta
Group K	Control feed

The Bio Feed Pro (1 mg/kg) and the Bio Feed Plus and the Bio Feed Beta (3 mg/kg) were mixed in the feed in pelleted form. The enzyme preparations were produced by Novo Nordisk. The calculated nutrient composition of the modified feed was similar to that of the control feed. The dietary compositions are shown in Table 1.

Description of added enzymes

Bio Feed Plus is a newly developed carbohydrate preparation produced by submerged fermentation of *Humicola insolens*. The enzyme hydrolyses arabinoxylans and  $\beta$ -glucans into oligosaccharides and some mono-, di- and tri-saccharides. In the IUB system the two activities are classified as: endo-1,4- $\beta$ -xylanase (No.3.2.1.8) and endo-1,4- $\beta$ -glucanase (No. 3.2.1.4). Bio Feed Plus also contains other carbohydrate activities, including cellobiase, hemi-cellulase and cellulase.

Bio Feed Beta is a recently developed multi-component enzyme preparation produced by submerged fermentation of *Bacillus amyloliquefaciens* and *Hucicola insolens*. Bio Feed Beta contains a mixture of  $\beta$ -glucanase, xylanase and  $\alpha$ -amylase. The enzyme hydrolyzes  $\beta$ -glucans (1,4- $\beta$  and 1,3- $\beta$ -glucans) into oligosaccharides and some glucose, di- and tri-saccharides. Further, the  $\alpha$ -amylase component is able to hydrolyze starch into predominantly maltodextrins together with smaller quantities of glucose, maltose and maltotriose. In addition, Bio Feed Beta contains other carbohydrate activities, including cellobiase, hemi-cellulase and cellulase.

Bio Feed Pro is a proteolytic enzyme preparation obtained by submerged fermentation of a selected strain of *Bacillus licheniformis*. The active enzyme component is subtilisin Carlsberg which is an endoprotease of the serine type and which is extensively described in the literature. In the IUB system is it classified as : subtilisin (No.3.4.21.62). No other enzyme activities are present in appreciable amounts.

The Bio Feed Plus and the Bio Feed Beta contained  $\beta$  - glucanase which was less pH-sensitive than the ones of bacterial origin. Thus, it is more efficient for feeding to poultry (Philip *et al.*, 1995).

Table 1. Composition of the applied feed mixtures (mg/kg).

## Experiment 1. (wheat-based feed)

Composition	Control		Modified	
	Starter	Fattening	Starter	Fattening
Wheat	310	609	405	655
Maize	450	195	450	230
NAPLIZ GB <sup>1</sup>	165	130	120	90
Meat meal	35	35	-----	-----
Mineral premix	26	26	20	20
Vitamin premix	5	5	5	5

## Experiment 2. (barley-based feed)

Composition	Control		Modified	
	Starter	Fattening	Starter	Fattening
Barley	319	609	405	635
Maize	450	190	450	240
NAPLIZ GB <sup>1</sup>	165	135	120	100
Meat meal	35	35	-----	-----
Mineral premix	26	26	20	20
Vitamin premix	5	5	5	5

<sup>1</sup>NAPLIZ GB is a sunflower seed mixture containing (g/kg): Crude protein, 420, crude fibre, 110, lysine 40 and methionine, 20.

Two phase feeding was applied. Each group received 1 kg starter feed per duck and then the fattening feed was provided. The birds were fed *ad libitum* in pelleted form (3 and 5 mm diameter). The brooding period was three weeks. First, the ducks were kept on a grated floor or on run-out with a swimming ditch. During the fattening period the ducks were kept on ponds. The two experiments were carried out in 10 pens each separated with fence. The individual groups were allocated at random to the pens.

During the time of the experiment the bodyweight increase and the amount of feed used were measured weekly. The dry matter content of the duck manure was measured at ten days of age. At 49 days of age 10 % of the population was slaughtered and the body mass, the amount of abdominal fat, the weight and ratio of breast and leg and the chemical features of the muscles were determined.

### III. RESULTS

The results are shown in Tables 3 and 4. There were no statistical differences between the live weights, feed conversions, dry matter contents of manure and the compositions of muscles between the two experiments at 49 days age. The results indicate that the composition of the feeds satisfied the demands of the ducks in respect to energy



Table 3. Live weight and feed conversion at 49 days, in Experiments 1 and 2.

Group	Liveweight (g)		Feed conversion (g:g)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
"A"	2899	2862	2.867	2.883
"B"	2886	2941	2.855	2.856
"C"	2954	2900	2.855	2.901
"M"	2989	2937	2.845	2.858
"K"	2872	2854	2.864	2.898

Table 4. Feed expenses for Experiments 1 and 2.

Group	<u>Experiment 1</u>		<u>Experiment 2</u>	
	Ft/kg	Ft/duck	Ft/kg	Ft/duck
"A"	130.46	378.21	131.01	374.95
"B"	130.64	377.02	130.38	383.44
"C"	132.64	390.13	131.83	382.30
"M"	123.39	368.83	123.12	361.60
"K"	128.87	370.14	129.75	370.31

content and amino-acid composition. Thus, added enzyme preparation had no measurable effect on the production parameters in groups A, B and C, i.e. they increased the feed expenses unnecessarily.

More significant and interesting results were found when the modified feeds were applied. Although the calculated nutrient composition of the modified feed was identical with that of the control feed, it is well-known that the digestibility of the energy of maize is greater than that of wheat and barley. The situation is similar in respect to amino acid digestibilities of sunflower, wheat and barley. In this case the enzymes had a greater effect as the results reached with the modified feeds were similar to the control group, but the expenses were less.

#### IV. CONCLUSIONS

The use of enzyme preparations in the control feeds had no detectable effect on broiler duck production.

In the modified feeds in which maize and sunflower were supplemented with barley and wheat, the production parameters and the other analysed parameters were identical with those of the control group.

The feed expenses decreased significantly in the group fed the modified feeds. In case of feed with wheat the expenses decreased by 4 %, while in the feed with barley the expenses decreased by 5 %.

Further analyses are required to optimise the composition of the modified feeds to further decrease production costs.

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## THE EFFECT OF KEMZYME AND PHYLACELL ENZYME PREPARATIONS ON THE UTILIZATION OF BROILER FEEDS CONTAINING SUNFLOWER MEAL

D. GERENDAI, Kh. EL SHERIF and T. GIPPERT

### Summary

Four experimental diets were formulated and fed to broilers from 20 to 47 days of age. The control diet contained 215 g/kg of soyabean meal (Treatment 1). A preparation of solvent-extracted sunflower meal (SFM) was used at level of 150 g/kg diet (Treatment 2) in place of soyabean meal. Two crude enzyme preparations were added to the diet containing the 150 g/kg sunflower meal preparation, Phylacell 0.6 g/kg feed (Treatment 3) and Kemzyme® dry 1 g/kg (Treatment 4). The experimental diets were formulated to contain almost the same crude protein and metabolizable energy (ME) as the control diet. The results showed no significant differences among treatments in body weight gain and nutrient digestibility coefficients. Feed conversion for the group fed the 150 g/kg of SFM was worse than the control group ( $P < 0.05$ ) but enzyme supplementation improved feed conversion.

### I. INTRODUCTION

There are a large number of reports regarding the use of sunflower meal as a protein supplement in poultry diets. Many of these studies have given conflicting conclusions because the nutritive value of sunflower meal depends on the method of processing. Sunflower meal contains less protein, lysine and energy than soyabean meal. In diets containing high levels of sunflower meal the lysine is the first-limiting amino acid (Rad and Keshavarz., 1976; Raya *et al.*, 1989; Gippert, 1994). Hegedüs and Fekete (1994) have reported that extracted soyabean meal can be partly or entirely substituted with extracted sunflower meal in broiler and laying hen diets if supplemented with lysine and methionine and an equal energy level is provided.

There are a large number of studies on the use of enzyme supplements in diets containing cereals (wheat, barley, oats and rye). Friesen *et al.* (1992) reported that enzyme supplementation of broiler diets increased the apparent digestibility of the energy, lipid and protein of cereals. Enzyme treatment also improved the weight gain and feed conversion of broiler chicks. Brenes *et al.* (1993a, b) obtained results indicating that enzyme addition to diets containing raw lupins, wheat or barley improved the weight gain and feed conversion of broiler chicks. Pettersson *et al.* (1993) reported that enzyme supplementation improved weight gain, increased feed intake and improved feed conversion of broiler chicks.

The following experiment was conducted to study the effects of sunflower meal (SFM) and enzyme supplementation in grower and finisher diets on the performance of broiler chicks and nutrient digestibilities.

### II. MATERIALS AND METHODS

A total of 120 (Ross meat-type) chicks were used in this experiment. At 20 days of age all chicks were weighed and wing banded individually. The chicks were divided into 12 groups of 10 birds and randomly assigned to 4 treatments, with three replicates. The chicks

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were housed in batteries with feed and water supplied *ad libitum* from 20 to 47 days of age.

Four experimental diets were formulated and used. The control diet contained 215 g/kg of soyabean meal (Treatment 1). A sunflower meal preparation (90.4% solvent-extracted sunflower meal, 8% sunflower oil, 1.5% lysine, and 0.1% methionine) was used at a level of 150 g/kg of the diet (Treatment 2) in place of soyabean meal. The sunflower meal preparation provided the equivalent amount of protein supplied by soyabean meal. Two crude enzyme preparations were added to the diet contained 150 g/kg of sunflower meal: Phylacell 0.6 g/kg feed (Treatment 3) and Kemzyme<sup>®</sup> dry 1 g/kg (Treatment 4). - Phylacell (Endo- $\beta$ -1,4-glucanase, Exo- $\beta$ -1,4-glucanase,  $\beta$ -glucoseoxidase) produced by PHYLAXIA Oltóanyagtermelő Vállalat, Budapest, Hungary. - Kemzyme<sup>®</sup> dry ( $\alpha$ -Amylase,  $\beta$ -Glucanase, Cellulase complex, Lipase, Protease) <sup>TM</sup>produced by Kemin Industries Inc., Des Moines, Iowa, USA.

The experimental diets were formulated to contain almost the same crude protein and metabolizable energy (ME) as the control diet. All diets were tested in a digestibility trial to determine nutrient digestibilities and metabolizabilities.

During the experimental period the criteria of response were live body weight, body weight gain, feed consumption, protein consumption, feed conversion and protein efficiency.

Processing of data and statistical analysis were performed using statistical software (Statgraphics, Version 5.0 STSC, Rockville, 1991). One-way analysis of variance was used to estimate significant differences.

### III. RESULTS AND DISCUSSION

Regarding the complete period of study (20 to 47 days of age) no significant differences were observed among treatments in body weight. The groups fed 150 g/kg of SFM or SFM plus enzymes had a slightly higher body weight than the control group but the differences were not significant. Mean final body weights were 1841, 1905, 1920 and 1995 g for Treatments 1, 2, 3 and 4, respectively. These results are in keeping with those obtained by Raya *et al.* (1989), Zadari and Sell (1990) and Sherif *et al.* (1995) who found no significant differences in body weight or body weight gain when sunflower meal was used to replace soyabean meal in diets for broiler chickens.

Data of body weight gain, feed consumption, protein consumption, feed conversion and protein efficiency are shown in Table 1. All groups gained more body weight than the control group but the differences were not significant. Analysis of feed and protein consumption indicated that groups fed diets containing 150 /kg of SFM or 150 g/kg of SFM plus Kemzyme<sup>®</sup> dry (Treatments 2 and 4) consumed more feed and protein ( $P < 0.05$ ) than the control group. The increases in feed consumption may be due to the increase in the fibre content of the diets and, therefore, the quicker passage of digesta through the digestive tract. These results are in keeping with those obtained by Brenes *et al.* (1993a).

Analysis of the feed conversion data showed that the group fed the diet containing 150 g/kg of SFM had a lower feed conversion ( $P < 0.05$ ) than the control group but that enzyme supplementation improved feed conversion to that of the control group. This result agrees with that of Zadari and Sell (1990) who found that diets containing 100 or 200 g/kg of SFM for broiler chicks had no effect on body weight gain but decreased feed conversion. No significant differences were observed in feed conversion between the control group and groups fed diets containing 150 g/kg of SFM plus enzymes.

Enzyme supplementation improved feed conversion and protein efficiency in Treatments 3 and 4 compared with Treatment 2 which contained 150 g/kg SFM. The

improvements in feed conversion and protein efficiency may be due to the positive effect of enzyme supplementation on digestion. These results agree with those of Friesen *et al.* (1992), Brenes *et al.* (1993a, b) and Pettersson *et al.* (1993) who found that enzyme supplementation improved feed conversion in broiler chicks.

Values of nutrient metabolizabilities and digestibilities are presented in Table 2. No significant differences were detected in nitrogen retention or in the metabolizability of dry matter, ether extract and energy. The metabolizability of nitrogen-free extract (NFE) was high in all groups compared to the control group. No significant differences were found in the digestibility coefficients of crude protein, crude fibre or organic matter.

#### IV. CONCLUSION

It can be concluded that soyabean meal can be replaced partly with solvent extracted sunflower meal supplemented with lysine, methionine and energy in grower and finisher diets for broilers without adverse effects on performance and nutrient digestibilities. Enzyme supplementation improved feed conversion and protein efficiency of broiler chicks fed diets containing a high level of sunflower meal.

Table 1. Average body weight gain, feed consumption, protein consumption, feed conversion, and protein efficiency for broiler chicks from 20-47 days of age.

Treatments	B'weight gain g/kg	Feed consumption g/day	Protein consumption g/day	Feed conversion g:g	Protein efficiency g/kg
Control	52.3 ± 1.2	117.4 ± 2.3 <sup>a</sup>	24.1 ± 0.5 <sup>a</sup>	2.247 ± 0.007 <sup>a</sup>	460.3 ± 2 <sup>a</sup>
150 SFM	54.5 ± 1.9	135.4 ± 2.9 <sup>b</sup>	27.5 ± 0.6 <sup>c</sup>	2.487 ± 0.084 <sup>b</sup>	505.4 ± 17 <sup>b</sup>
150 SFM +Phylacell	55.0 ± 2.5	124.2 ± 2.5 <sup>ab</sup>	25.2 ± 0.5 <sup>ab</sup>	2.267 ± 0.105 <sup>a</sup>	460.0 ± 22 <sup>a</sup>
150 SFM+ Kemzyme	57.8 ± 0.6	126.0 ± 1.5 <sup>b</sup>	25.6 ± 0.3 <sup>b</sup>	2.180 ± 0.025 <sup>a</sup>	443.0 ± 5 <sup>a</sup>

<sup>a-c</sup> Means ± SE within a column with no common superscript are significantly different (P < 0.05).

Table 2. Metabolizability and digestion coefficient of nutrients in experimental diets.

Diets	Control	15% SFM	15% SFM + Phylacell	15% SFM + Kemzyme
Treatments	1	2	3	4
Metabolizability %				
N-retention	36.18	36.88	32.38	32.62
Ether extract	88.36	89.71	89.47	89.92
NFE	96.42 <sup>b</sup>	99.19 <sup>a</sup>	98.67 <sup>a</sup>	99.12 <sup>a</sup>
DM	76.63	78.57	77.17	77.71
ME	80.42	81.35	81.36	80.06
Digestion coefficient %				
Crude Protein	81.81	80.16	82.69	83.48
Crude fibre	15.67	16.25	14.85	16.25
Organic matter	83.91	85.51	84.72	85.21

<sup>a-b</sup> Means within a row with no common superscripts are significantly different (P < 0.05).

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## CURRENT PROBLEMS OF INCUBATION OF WATERFOWL EGGS

F. BOGENFURST

Summary

The incubation of goose, duck and muscovy duck eggs raises numbers problems due to the particular lifestyle of these species and the technology involved in keeping them. Incubation of waterfowl eggs is much more difficult than that of hen's eggs. There are two critical factors which deviate from what is valid for hen's eggs: level of turning and periodic cooling. Good results were obtained with the oblique setting of goose eggs. Without periodic cooling the hatchability decreases.

## I. INTRODUCTION

Recent research has drawn attention to the fact that, in addition to the angle and frequency of turning of goose eggs, the axis and level of turning also play an important role in embryonic development. Also, there has been some controversy over the beneficial effect of periodic cooling and spraying of duck and goose eggs with water during incubation. In particular, Kalthoven (see Kortlang, 1985) has conducted a series of experiments in the Netherlands in order to obtain a better understanding of this process.

## II. METHODS

Studies were conducted to examine the effect of turning on the hatchability of goose eggs. In a further study eggs were incubated with and without periodic cooling in a walk-in incubator with multi-stage operation.

## III. RESULTS AND DISCUSSION

Correct turning plays a positive role in the formation and volume of the subembryonic fluid and in the creation of albumen sac and, thus, in the utilisation of protein. This is put into practice in the method elaborated with goose eggs, the essence of which is that the longitudinal axis of the egg is at an angle of 45-60° to the longitudinal axis of the tray, and thus the yolk and the developing embryo move on a lengthened, elliptical course during the 90° turning process. This is demonstrated in Figure 1. We have achieved good results when applying this method to the incubation of ostrich and emu eggs.

Due to the need to cool the eggs of waterfowl they can be incubated in a cabinet incubator. In a walk-in incubator with multi-stage operation the results are much worse, as it is not possible to cool the eggs periodically. Figure 2 illustrates the difference obtained in the experiment. It can be seen that the greatest difference was in the proportion of embryos dead in shell. The incubation results improved with periodic cooling.

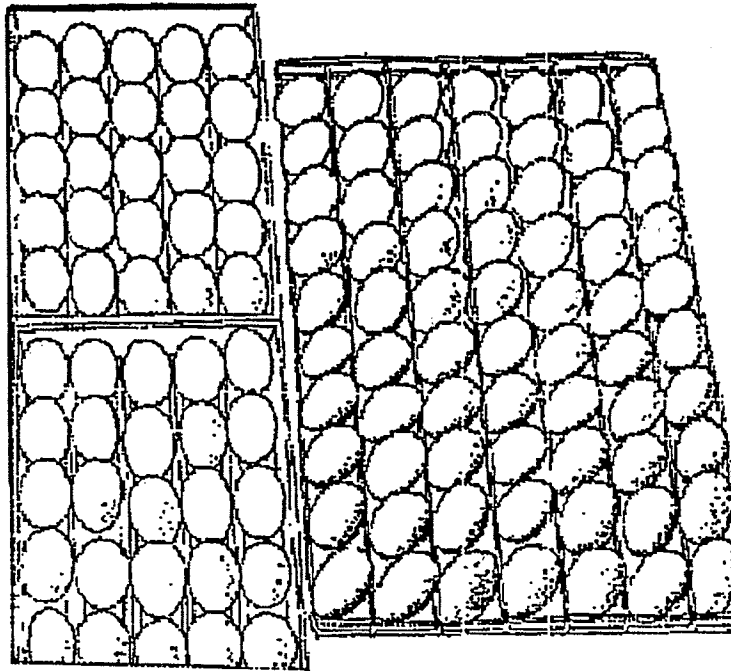


Figure 1. The traditional and new method of setting goose eggs.

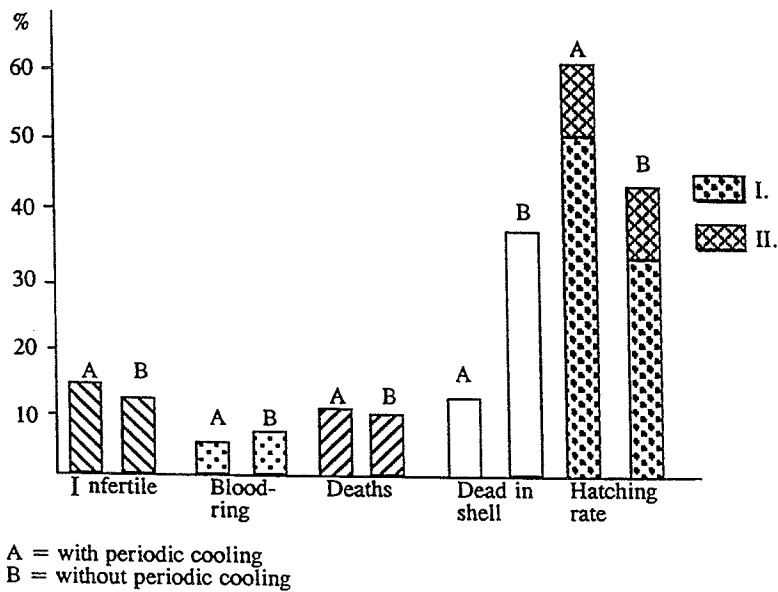


Figure 2. Incubation of goose eggs with and without periodic cooling.

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*EIMERIA* FOR THE CONTROL OF COCCIDIOSIS NOW A REALITY IN AUSTRALIA

D.G RICHARDS

There are four species of *Eimeria* in Australia associated with infections of poultry. These are *E. acervulina*, *E. maxima*, *E. tenella* and *E. necatrix*. The latter two are associated with mortalities of up to 10% in some flocks. Wild challenges are unpredictable. Mortality increases and flock uniformity decreases with challenge during rearing while mortality with associated egg drops can occur during production.

To avoid the cost of feeding anticoccidials on a whole life program, the poultry industry encourages immunity to develop in parent birds. The onset of challenge can be manipulated with feed and water-supplemented anticoccidials. A different approach used in Australia to induce immunity early in the growing cycle is controlled exposure. Joyner (1973) showed it was possible to induce immunity to *Eimeria* by feeding low doses of pathogenic strains. By modifying the concepts of Joyner (1973) and using laboratory passaged strains of *Eimeria* the poultry industry has developed an exposure and critical time medication approach. Birds were challenged with four species of *Eimeria* and strategically medicated between 9 and 12 days post challenge. The medication is necessary to overcome the variable rate of uptake of the *Eimeria* administration and to protect the birds from the rapid build up of pathogenic organisms which occurs 6 to 8 days post administration. The drawbacks of the present approach are: (1) when medication is administered too early some species can be suppressed. This causes outbreaks some days after the medication has finished. (2) mortality from the more pathogenic strains of *Eimeria* may occur if the medication is applied too late. The controlled approach has resulted in nil mortalities being achieved regularly with all species used.

Shirley *et al.* (1995) report similar problems with the use of field strains of *Eimeria* to induce immunity. The use of attenuated or precocious strains is recommended.

Jorgensen (1996) reported preparations of precocious strains of *E. acervulina* and *E. maxima*. *E. tenella* strains with lowered pathogenicity and selected strains of *E. necatrix* are now being assessed. (Jorgensen, Personal communication).

There are no commercially licensed stocks of *Eimeria* in Australia. A four-species preparation made under licence will be available to the commercial poultry industry in April 1997 in which the strains identified by Jorgensen will be included.

The work being reported will include selection criteria used to select the strains of *Eimeria*, a description of the specialised laboratory built to prepare the *Eimeria* cultures, analysis of liquid nitrogen storage and recovery of *Eimeria*, chicken inoculation testing using fractured oocysts for unwanted pathogens, seed stock preparation, seed stock storage and shelf life in final packaging.

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## EFFECT OF LIPASE SUPPLEMENTATION OF RICE BRAN ON EXCRETA ENERGY CONTENT IN ADULT COCKERELS

D.V. THOMAS\*, I.T. KADIM\*, P.J. MOUGHAN\* and S. BOURNE\*\*

Rice bran is a potentially important feedstuff for poultry, containing relatively high concentrations of crude protein (130-170 g/kg) and fat (200-230 g/kg). Rice bran also contains high levels of non-starch polysaccharides but it does not appear that these are anti-nutritive for broiler chickens (Annison *et al.*, 1995). However, the AME content of rice-bran is lower than anticipated on the basis of its gross energy content and it may be that this is related to utilization of the lipid component (Warren and Farrell, 1990; Annison *et al.*, 1995). The aim of the present study was to assess the influence of an exogenous lipase (Alltech Inc., USA) on the bioavailability of energy from rice bran. Adult Hyline Brown cockerels were housed individually in wire-bottomed cages in a temperature (18°C) and light (16h) controlled room. The birds were fasted for 48h and were then intubated with 40g of rice bran without or with (100 mg/kg) added lipase. Total excreta were collected (48h) and feed and excreta were subjected to bomb calorimetry and AME was determined (Sibbald, 1975). Results (mean  $\pm$  SE) are shown below.

Treatment	Excreta Energy (MJ/kg dry matter)	Excreta Weight (g dry matter)	AME (MJ/kg 'as is')
RB <sup>1</sup> - Lipase (n=12)	16.57 <sup>a</sup> (0.175)	20.2 <sup>a</sup> (0.33)	12.15 <sup>a</sup> (0.176)
RB + Lipase (n=11)	15.78 <sup>b</sup> (0.203)	20.7 <sup>a</sup> (0.34)	12.47 <sup>a</sup> (0.167)

Means within a column having different superscripts are significantly different at  $P < 0.05$ .

<sup>1</sup>RB = Australian full-fat rice bran.

Supplementation with lipase led to a significant ( $P < 0.05$ ) reduction in faecal energy concentration which translated to a non-significantly higher AME value. The results of a further study suggest that broiler chickens may show a greater response to lipase than cockerels. Significant ( $P < 0.05$ ) increases in the AME of rice bran-containing diets have been found as a result of supplementing broiler diets with lipase. Based on the results of these two studies it would appear that lipase supplementation may provide a useful means for increasing the bioavailability of energy in rice bran-containing diets for chickens.

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## EFFECTS OF DIETARY PHYTATE AND AVAILABLE PHOSPHORUS LEVELS ON THE RESPONSES OF BROILERS TO SUPPLEMENTAL PHYTASE

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Phytic acid (PA) is the primary storage form of phosphorus (P) in plant seeds, accounting for 60-80% of total P in these materials. The ability of poultry to utilise phytate P from plant-derived ingredients is generally assumed to be poor. In addition, the ability of PA to complex with several biologically important minerals and proteins is well known (Ravindran *et al.*, 1995) and is another nutritional concern associated with phytate. The adverse effects on nutrient utilisation and bird performance will be greater at higher dietary levels of PA. The availability of commercial phytase, an enzyme that hydrolyses PA, offers a practical means of improving the utilisation of phytate P in poultry diets. The present study was conducted to evaluate the response of broilers to microbial phytase (Natuphos®, BASF Corp., Ludwigshafen, Germany) added to wheat-sorghum-soyabean meal diets containing three levels of PA and two levels of non-phytate P (nP).

Nine hundred 7-day old male broiler chicks were used in a 3 x 3 x 2 factorial arrangement of treatments with five replicates (10 chicks/pen) to study the response to three levels of phytase (0, 400 and 800 FTU/kg diet) when given in combination with three levels of PA (10.4, 13.2 and 15.7 g/kg) and two levels of nP (2.3 and 4.5 g/kg). The 'low' PA diet was based on wheat (steam pelleted to destroy intrinsic phytase activity), sorghum and soyabean meal, and the 'medium' and 'high' PA diets were formulated by the inclusion of rice pollard. All diets contained similar levels of metabolisable energy, crude protein, lysine and sulphur-containing amino acids, and the calcium: total P ratio was maintained at 1.4:1. The diets were fed from day 7 to 25. Criteria evaluated included weight gain, feed intake, feed/gain and toe ash content.

Weight gains were lowered ( $P < 0.001$ ) by increasing dietary PA levels and increased by dietary phytase ( $P < 0.001$ ) and nP ( $P < 0.05$ ) additions. However, a nP x phytase interaction ( $P < 0.001$ ) was observed in which the magnitude of response to added phytase was greater at the lower dietary nP level. Feed intake followed a similar pattern to that of weight gains. Feed/gain was increased ( $P < 0.001$ ) by increasing PA levels but these adverse effects were overcome ( $P < 0.05$ ) by supplemental phytase. Dietary nP level had no influence on feed/gain of broilers. Toe ash contents were lowered ( $P < 0.05$ ) by dietary PA and increased ( $P < 0.001$ ) by dietary additions of phytase and nP. These results suggest that the anti-nutritive effects of PA on broiler performance and bone mineralisation can be effectively overcome by the provision of supplemental phytase.

RAVINDRAN, V., BRYDEN, W.L. and KORNEGAY, E.T. (1995). *Poult. Avian. Biol. Rev.* 6: 125-143.

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OVULATION AND OVIPOSITION PATTERNS IN JAPANESE QUAIL  
(*Coturnix coturnix Japonica*)

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It is recognised that further genetic improvement in egg production efficiency in chickens will depend on an ability to modify oviposition interval. Such modification depends on a good understanding of reproductive physiology as it relates to the regulation of, and association between, ovulation and oviposition. Japanese quail have proven to be a good physiological model for chickens in genetic studies and an understanding of the reproductive physiology of quail in this context has potential value, given the much shorter generation interval of this species. This study used *Coturnix* to obtain information on the egg formation process and on the mean and range time interval between oviposition and ovulation of the follicles. It complements the earlier study of Woodard and Mather (1964).

One hundred and sixty twelve-week-old female quail from a line selected for aspects of growth and breast meat yield were used in this experiment. The birds were placed in individual cages on a 16 h light:8 h dark day with the light period from 04.00 h to 20.00 h. A layer diet containing 11.7 MJ of ME and 170 g CP/kg was given *ad libitum*. Over a 10d period oviposition interval was determined for each bird by continuous inspection. Following this the birds were killed at different intervals after oviposition and the presence and position of the follicle in the oviduct determined. Results are given in the Table.

Time after oviposition (min)	No of birds examined	No with released follicle	Section of oviduct containing follicle
0	4	0	-
15	12	0	-
30	17	7	ostium
45	11	6	ostium, NI*
60	20	15	NI, magnum
120	17	17	magnum
240	16	16	magnum, isthmus
360	7	7	isthmus, uterus
480 - 1380	39	37	uterus

\* NI - neck of infundibulum.

The majority of eggs were laid between 14.00 and 17.00 h, i.e. between 10 and 13 h after the onset of light. This contrasts markedly with chickens, where lay is typically some 6 h earlier. The oviposition interval ranged from 23.58 to 25.06 h, with an average of 24.37 h and a standard deviation of 22 minutes. About 6 h after oviposition of the previous egg the follicle reached the uterus and remained there for shell formation for about 17 to 21 h before being laid. The results show no evidence of early ovulation in relation to oviposition in the line of quail studied, although this is perhaps not surprising given the nature of prior selection in the birds.

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## THE APPARENT METABOLISABLE ENERGY OF FEEDINGSTUFFS MEASURED IN GROWING EMUS

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O'Malley (1996) stated that the nutrient requirements of emus had not been determined and current feeding standards are based on observations rather than on scientific experimentation. Here we report the apparent metabolisable energy (AME) of six diets each based on a different major feedingstuff and measured in emus.

Repeated measurements were made in each of five individually penned emus aged 12-16 weeks using acid insoluble ash (AIA) as an indicator to calculate dry matter digestibility. Energy and nitrogen were determined on subsamples of feed and excreta.

The basal diet (g/kg) consisted of maize, 863; fish meal, 68 and soyabean meal 24 plus minerals and vitamins. Bentonite (100 g) was added to increase the AIA of feed and excreta. To 700 g basal diet was added either 300 g of millrun (a mixture of wheat pollard and bran), 300 g of meat and bone meal, 300 g of grain sorghum, 300 g of sunflower meal or 300 g of field peas. The mean AME and AME<sub>n</sub> values (MJ/kg DM) for the actual diets used and for the five ingredients calculated by substitution are shown in the Table. Typical values for adult poultry are given in parenthesis for each ingredient.

Table. Mean AME and AME<sub>n</sub> of six diets each with a different major ingredient and each offered to five growing emus (12-16 weeks of age).

	Diet AME (MJ/kg)	Ingredient AME (MJ/kg)	Diet AME <sub>n</sub> (MJ/kg)	Ingredient AME <sub>n</sub> (MJ/kg)
Basal	12.40 <sup>a</sup>		12.33 <sup>a</sup>	
Millrun	10.39 <sup>bc</sup>	5.7 (10.0) <sup>1</sup>	10.62 <sup>b</sup>	6.6
Meat and bone meal	9.56 <sup>b</sup>	2.9 (10.9)	8.99 <sup>c</sup>	2.6
Grain sorghum	11.46 <sup>a</sup>	9.3 (15.6)	11.35 <sup>ab</sup>	10.5
Sunflower meal	9.75 <sup>bc</sup>	3.6 (9.6)	9.75 <sup>bc</sup>	3.7
Field peas	11.49 <sup>ac</sup>	9.4 (13.4)	11.26 <sup>a</sup>	8.7
LSD (P<0.05)	1.561		1.509	

<sup>1</sup> Typical AME values for adult poultry (MJ/kg DM).

Although AIA may cause some bias in estimating dry matter digestibility since only 90% was recovered, the very low AME values for individual ingredients suggest that the young, growing emu is unable to utilise byproducts such as millrun, meat and bone meal and sunflower meal efficiently. Fermentation capacity at this age may be poorly developed. The AME of the basal diet is also below expectations given its high maize content. Attempts to measure AME using total collection were unsuccessful with emus and there was some variation between birds using AIA. These preliminary results suggest that it is inappropriate to use tabled AME values for poultry to formulate diets for emus and it will be necessary to undertake further AME measurements for a range of feedingstuffs using the emu. Some differences may also occur for the same ingredient in growing and adult birds.

O'MALLEY, P.J. (1996). In: *Improving Our Understanding of Ratites in a Farming Environment*. (Ed. D.C. Deming). pp 92-108. University of Manchester.

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SELECTION FOR BREAST MEAT YIELD IN JAPANESE QUAIL: EFFECTS ON  
FEED EFFICIENCY AND BODY COMPOSITION

B. POPOVIC and R.A.E. PYM

Popovic and Pym (1996) have presented results from a selection experiment in which Japanese quail were selected for three generations for aspects of growth and breast meat yield. This paper reports on responses to selection for five generations.

Birds were selected for: increased 42d live weight (line LWI); increased breast meat weight (line BWI); increased breast meat proportion (line BPI); decreased breast meat proportion (line BPD); or at random (line C). Selection for breast meat yield was based on multiple regression prediction equations incorporating measures of muscle length and width, and of muscle depth, using real-time ultrasound. Measures were made of feed efficiency on a diet containing 12.7 MJ of ME and 225g CP/kg, in a total of 320 birds from the five lines from 14 to 28 days in sex-combined cage groups and from 28 to 42 days in sex-separated groups, with 8 cages each of 8 birds per line. At 42d the birds were weighed, killed and dissected and the weight of breast muscle, thighs and drumsticks determined. Results are given in the Table.

Line	Weight (g)	FCR		Breast muscle		Drumsticks (g/kg)	Thighs (g/kg)
		14-28d	28-42d	(g)	(g/kg)		
LWI	230	2.64	4.11	44.6	194	60.5	95.7
BWI	225	2.62	4.35	47.5	206	60.8	97.3
BPI	208	2.67	4.12	43.5	220	59.7	98.1
BPD	196	2.84	4.74	32.5	162	59.0	93.0
C	203	2.64	4.23	39.2	195	60.5	95.7
LSD <sub>0.05</sub>	7	0.12	0.56	1.5	5	1.7	2.9

Liveweight was essentially unaltered in the breast proportion lines but increased similarly in the high liveweight and breast weight lines. The FCR was higher in the low breast proportion line than in the other four lines from 14 to 28d ( $P < 0.05$ ), and there was an indication of a similar, though non-significant, effect in the 28-42d period. Breast weight was increased markedly in the high breast weight line, moderately in the high breast proportion and high body weight lines and decreased markedly in the low breast proportion line. Breast proportion showed good divergent response in the two lines selected for this trait, whilst the line selected for liveweight showed no response in breast proportion. Contrary to concerns about selection for increased breast meat yield, there was no indication of a compensatory reduction in the proportion of the other saleable cuts, viz thighs and drumsticks, whether compared with the high liveweight or control lines. The results suggest considerable positive direct response to selection for increased breast meat yield and no untoward effects on either feed efficiency or other saleable cuts.

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## EFFECT OF GROUND LIMESTONE, OYSTER SHELL CHIPS OR LIMESTONE CHIPS ON PRODUCTION AND EGG CHARACTERISTICS OF LAYING HENS

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Laying hens have a high dietary calcium (Ca) requirement for egg shell formation and ground limestone is commonly included in complete diets at about 80-100 g/kg to meet this requirement. Egg shell calcification usually occurs during the dark night period when the hen is not eating. The Ca is drawn either from the intestinal tract or bones. It has been suggested that Ca absorption might be more sustained if hens were given access to particulate Ca as this might persist for longer in the intestines after eating ceases.

Hens (5 strains) were given one of 3 diets, *viz.* complete layer crumble containing ground limestone (Diet 1: Fielders, Tamworth), or the same dietary mix without added Ca but fed with either oyster shell chips (Diet 2) or limestone chips (Diet 3) added on visual assessment. Details of bird housing and management are given in Nolan *et al.* (1997). Mean values are given in the Table for feed intake, feed conversion ratio and egg production in the period from 20 to 75 weeks of age and for egg characteristics (combined values for 5 egg collections at 35, 45, 55, 65 and 75 weeks of age). There were 1,340 hens/diet.

	Diet 1	Diet 2	Diet 3	Significance
Feed intake (g/hen/day)	137	136	137	NS
Feed conversion ratio (g feed/g eggs)	3.09	3.12	3.13	NS
Hen-day production (%)	74.7 <sup>a</sup>	72.0 <sup>b</sup>	72.4 <sup>b</sup>	P < 0.001
Hen-housed production (%)	72.1 <sup>a</sup>	69.1 <sup>b</sup>	68.7 <sup>b</sup>	P < 0.001
Egg mass production (g/hen/day)	47.2 <sup>a</sup>	46.3 <sup>b</sup>	46.2 <sup>b</sup>	P < 0.001
Egg weight (g)	65.3 <sup>a</sup>	66.9 <sup>b</sup>	66.4 <sup>b</sup>	P < 0.001
Shell weight (% egg weight)	8.81	8.91	8.86	NS
Shell weight (g)	5.72 <sup>a</sup>	5.85 <sup>b</sup>	5.86 <sup>b</sup>	P < 0.01
Shell thickness (µm)	371 <sup>a</sup>	375 <sup>ab</sup>	380 <sup>b</sup>	P < 0.01
Shell breaking strength (Newtons)	25.0 <sup>a</sup>	25.3 <sup>b</sup>	26.3 <sup>b</sup>	P < 0.05
Shell reflectivity (light meter)	72.0 <sup>a</sup>	71.6 <sup>a</sup>	73.5 <sup>bc</sup>	P < 0.001
Specific gravity	1.085 <sup>ac</sup>	1.087 <sup>b</sup>	1.083 <sup>c</sup>	P < 0.001
Egg diameter (mm)	44.2 <sup>a</sup>	44.6 <sup>b</sup>	44.6 <sup>b</sup>	P < 0.001

Birds given the diets with particulate calcium laid larger eggs with thicker and stronger shells but all measures of egg production were lower and there were no differences in feed intake or in the efficiency of feed conversion between the diets. Feeding particulate calcium is an additional activity which may increase the on-farm costs of production, although the costs of feed may be lower due to the lower energy requirement to produce calcium chips rather than ground calcium. Under the conditions of this experiment, however, Diet 1 was the preferred method of feeding calcium.

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## THE EFFECT OF RELOCATION FROM FLOOR PENS TO CAGES ON EGG SHELL QUALITY IN LAYING HENS

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Studies have shown that relocation of laying hens produces calcium coated and body-checked eggs (Hughes *et al.*, 1986; Leary *et al.*, 1996). Indirect evidence that the response is attributable to stress was obtained by Hughes *et al.* (1986). They found a single injection of adrenalin to result in egg retention and increased incidence of abnormal eggs. This study examined the effect of relocation on the appearance and shell quality of eggs.

The experiment consisted of three periods. All periods of the experiment utilized the same birds from five strains: Isa Brown, Hy-Line Brown, Lohmann Brown, Hy-Line-CB and Tegel Super Brown. As many as 24 birds in each strain were used. All birds were housed in floor pens at the University's Laureldale Farm prior to the relocation. The first period of the experiment consisted of relocating half of the birds from each strain, chosen randomly, to single bird cages on the University of New England campus. The remaining birds were also moved to the university campus but were placed in floor pens. The second period of the experiment was a cross-over of the first period. The final period of the experiment consisted of moving all birds to single bird cages. Eggs were collected for internal and shell quality measurements for a 7 d control period before, and for a 14 d period after relocation. Between each relocation birds were given a 7 d rest period to ensure complete adjustment to the new conditions.

As a result of the relocation in both periods one and two, egg production dropped initially before increasing to over 100% on the second day after the presumed stress of relocation, an indication of retention of eggs in the shell gland. The incidence of calcium coated, white banded and slab-sided eggs also increased for several days after relocation. The Table summarises some shell quality results when birds were moved from floor pens to cages in periods 1 and 3.

	Period One				Period Three			
	Isa Brown		Tegel Super Brown		Isa Brown		Tegel Super Brown	
	Before*	After*	Before*	After*	Before*	After*	Before*	After*
Breaking strength (N)	29.99	26.96	27.47	16.86	28.93	26.84	21.30	24.73
P Value	±1.12	±1.75	±0.93	±5.15	±1.16	±1.16	±1.46	±1.42
Specific gravity	NS		0.0015		NS		NS	
P Value	1.089	1.081	1.084	1.079	1.089	1.086	1.078	1.082
	±0.001	±0.001	±0.001	±0.002	±0.001	±0.001	±0.002	±0.002
	0.0095		NS (0.0620)		NS (0.0832)		NS	

Significant reductions in shell quality measurements indicate that relocation had a detrimental effect on shell quality. The results of the final period of the experiment showed few changes as the result of the treatment, suggesting that the birds had habituated to the process of relocation. However, changes recorded were a reduction in yolk colour and reduced shell deformation as a result of relocation.

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## IMPROVING THE ULTRASTRUCTURAL CHARACTERISTICS OF EGG SHELLS FROM HENS RECEIVING SALINE DRINKING WATER

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Saline drinking water has been shown to have a deleterious effect on egg shell quality (Balnave and Scott, 1986). Egg shell quality may be assessed in a number of ways but most methods determine the amount of shell present. Egg specific gravity, shell thickness and shell weight percent are such methods. However, the ability of an egg shell to withstand insult is influenced not only by the total amount of shell present, but also by the quality of construction of the shell. The microscopic structure (ultrastructure) of the mammillary layer of an egg shell provides insights into how well the shell is constructed.

The aim of the present study was to determine the ability of various zinc compounds to offset the deleterious effects of saline drinking water on egg shell quality using shell ultrastructure as an indicator of quality. The egg shells used in the present study were obtained from the experiment of Balnave and Zhang (1993). Australian commercial laying hens received one of five drinking water treatments: municipal tap water, saline drinking water (2g NaCl/L), saline drinking water supplemented with either zinc ethylenediaminetetraacetic acid (NaCl + Zn-EDTA), zinc sulphate (NaCl + ZnSO<sub>4</sub>) or zinc methionine (NaCl + Zn-methionine). Shell was taken from the equator of the eggs and examined under a scanning electron microscope after the method of Solomon (1991).

The saline drinking water resulted in the production of egg shells with lower breaking strength, as compared with shells from hens receiving municipal water. The addition of the zinc compounds to the saline water restored shell breaking strength to control levels (Balnave and Zhang, 1993). The incidence of confluence, an ultrastructural feature which strengthens egg shells, was significantly reduced in the shells of birds receiving saline drinking water. The addition of Zn-methionine or ZnSO<sub>4</sub> to the saline drinking water increased the incidence of confluence. Cuffing is another ultrastructural variation which increases the strength of egg shells. The addition of Zn-EDTA or Zn-methionine increased the incidence of cuffing in birds receiving saline water. All zinc compounds decreased the incidence of changed membrane, an ultrastructural feature which is associated with poor quality egg shells.

In summary, ingestion of saline drinking water may have deleterious effects on egg shell quality, as assessed by traditional means as well as by examination of the ultrastructural features of the shells. The addition of zinc compounds to saline drinking water offsets some of the negative effects of the saline water. The mechanism by which this occurs is uncertain but it may be related to effects on carbonic anhydrase activity (Mueller and Leach, 1974).

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

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## MEETING GROWTH-RELATED REQUIREMENTS FOR DIETARY AMINO ACIDS IN DIFFERENT COMMERCIAL BROILER GENOTYPES

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The use of broiler starter, grower and finisher diets reflects the bird's changing requirements for amino acids, expressed as a proportion of the diet, as growth proceeds. The adjustment, however, is crude and only approximately allows for what is essentially a daily change in amino acid requirements. Qualitative differences in amino acid requirements between strains and sexes are also often ignored because of inadequate information. Growth models provide an opportunity to accurately determine daily requirements for amino acids for birds of a given sex and strain under defined management conditions.

The study described here involved two commercial broiler strains given 2, 4 or 8 dietary regimens from hatching to 40d, formulated using information from the Gous Growth Model to optimise performance for each strain x sex group. The strains had been characterised for purposes of the model in an earlier study (Gous *et al.*, 1996). A summit-dilution technique was used to generate the diets required. The birds were reared to 20d in 2 brooder cages each of 10 birds for each sex x line x dietary regimen group. From 20 to 40d the birds were allocated to 240 single bird cages. The birds were killed at 41d and measurements made of abdominal fat and breast meat yield. Results are shown in the Table.

Strain	Regimen	Growth rate (g/d)		FCR		Breast (g/kg)		Abd. fat (g/kg)	
		Male	Female	Male	Female	Male	Female	Male	Female
A	2	40.7	35.0	1.86	2.02	119.4	113.2	18.3	19.8
	4	40.6	36.3	1.81	2.01	111.5	107.2	13.1	17.7
	8	42.3	34.4	1.77	1.98	103.4	101.2	10.3	18.6
B	2	39.3	32.4	1.93	2.06	113.0	93.3	12.4	21.1
	4	40.9	33.2	1.88	2.04	109.4	100.6	16.0	21.9
	8	38.2	32.2	1.95	2.09	100.1	110.2	14.1	28.0
LSD <sub>0.05</sub>		4.1	3.5	0.10	0.09	10.9	11.8	4.3	5.9

Growth rates were lower than anticipated in all groups, largely due to a plateauing for about 7 days of the daily rate of gain depressed performance of the birds when placed in the single cages at 20 days. There was no effect of dietary regimen on either growth rate or FCR within the line x sex groups, possibly due to this effect. In three of the four sex x line groups there was significantly more breast meat in birds on the 2- than on the 8-dietary regimen treatment. This could be due to the higher levels of amino acids received by the 2- than the 8-dietary regimen groups during the final 10 days of the growing period. There was no consistent dietary trend in abdominal fat. The sub-optimal growth performance presents problems for meaningful interpretation of the relative performance of the different dietary regimens.

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## AMINO ACID DIGESTIBILITIES OF PLANT PROTEIN SUPPLEMENTS FOR BROILERS

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Poultry production throughout the world relies heavily on plant protein supplements to supply the major portion of dietary protein requirements. Next to cereal grains this group of feedstuffs constitutes the largest component of poultry diets. Soyabean meal is the most common plant protein supplement used by the Australian feed industry. Relative to other oilseed meals the protein in soyabean meal is well balanced in terms of essential amino acids for poultry fed diets based on cereal grains. However, the local demand for soyabean meal far outstrips the supply and the trend in the industry is to use a range of locally produced protein sources. In particular, increasing emphasis is being placed on the utilisation of canola meal and lupins. In the present paper, the apparent ileal amino acid digestibility values of soyabean meal, sunflower meal, canola meal, cottonseed meal and lupins (*Lupinus angustifolius*) for broilers are reported.

Assay diets were based on dextrose and contained the test feedstuff as the only source of protein. The proportions of dextrose and the test feedstuff were varied in each diet to obtain 200 g crude protein/kg. All diets were fortified with minerals and vitamins and contained celite (20 g/kg) as an indigestible marker. Each assay diet was fed *ad libitum* to three pens (4 birds/pen) of male broilers from 35 to 42 days of age. At the end of the trial digesta contents from the terminal ileum were collected and processed as described previously (Siriwan *et al.*, 1993). Samples of diets and digesta were analysed for amino acids and acid-insoluble ash, and the apparent ileal amino acid digestibility values were calculated.

The amino acid digestibilities in soyabean meal and sunflower meal were higher than those in other plant protein supplements. The overall mean apparent ileal amino acid digestibility coefficients were: soyabean meal, 0.839; sunflower meal, 0.858; canola meal, 0.711, cottonseed meal, 0.707 and lupins, 0.779. The mean lysine and threonine digestibility coefficients were: soyabean meal, 0.751 and 0.855; sunflower meal, 0.763 and 0.815, canola meal, 0.629 and 0.692; cottonseed meal, 0.609 and 0.510 and lupins, 0.751 and 0.804, respectively. The poor digestibility of lysine in cottonseed meal is in agreement with previous reports (Ravindran and Blair, 1992). Digestibility of threonine was usually the lowest of the essential amino acids in plant protein supplements. The relatively poor digestibilities determined for canola meal were partly due to the low digestibility values observed in one of the three samples assayed. In contrast, variations in amino acid digestibilities observed among the four soyabean meal samples assayed were small, indicating the uniformity of the product.

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SIRIWAN, P., BRYDEN, W.L., MOLLAH, Y. and ANNISON, E.F. (1993). *Br. Poult. Sci.* **34**: 939-949.

## AMINO ACID DIGESTIBILITIES OF ANIMAL PROTEIN SUPPLEMENTS FOR BROILERS

L.I. HEW, V. RAVINDRAN, G. RAVINDRAN and W.L. BRYDEN

Animal proteins are normally superior to plant proteins as sources of essential amino acids, particularly of lysine, the first limiting amino acid in cereal grains. Meat meal is the animal protein supplement most widely used by the Australian feed industry, with fish meal, blood meal and hydrolysed feather meal being the other supplements. Despite their importance in balancing the supply of essential amino acids in feed formulations published data on the digestible amino acid contents of these ingredients is conflicting. In this paper, the apparent ileal amino acid digestibilities of these animal protein supplements for broilers are reported.

Assay diets were based on dextrose and contained the test feedstuff as the only source of protein. The proportions of dextrose and the test feedstuff were varied in each diet to obtain 200 g/kg crude protein. All diets were fortified with minerals and vitamins and contained celite (20 g/kg) as an indigestible marker. Each assay diet was fed *ad libitum* to three pens (4 birds/pen) of male broilers from 35 to 42 days of age. At the end of the trial, digesta contents from the terminal ileum were collected and processed as described previously (Siriwan *et al.*, 1993). Samples of diets and digesta were analysed for amino acids and acid-insoluble ash, and the apparent ileal amino acid digestibility values were calculated.

The amino acid digestibilities in fish meal and blood meal were substantially higher than those in meat meal and feather meal. The overall mean apparent ileal amino acid digestibility coefficients were: meat and bone meal, 0.555; meat meal, 0.615; blood meal, 0.841; fish meal, 0.768 and feather meal, 0.563. The mean lysine digestibility coefficients were: meat and bone meal, 0.447; meat meal, 0.493; blood meal, 0.874; fish meal, 0.825 and feather meal, 0.540. Digestibility of threonine was usually the lowest of the essential amino acids in the animal protein meals examined. However, wide variations in amino acid digestibilities were observed among meat meal samples, highlighting significant batch-to-batch differences. In the two samples of meat meal the overall mean digestibility coefficients were determined to be 0.590 and 0.638. Corresponding figures for threonine and lysine digestibilities were 0.433 and 0.552, and 0.634 and 0.702, respectively. Interestingly, the crude protein concentrations of these two meat meal samples were similar (594 and 557 g/kg, respectively). The factors contributing to this variability in the nutritive quality of meat meal have been examined previously (Skurray, 1974) and will continue to be a concern to the feed industry.

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SKURRAY, G.R. (1974). *World's Poult. Sci. J.* **30**: 129-136.

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## TRYPTOPHAN CONTENT OF SORGHUM GRAIN VARYING IN NITROGEN CONTENT

G. RAVINDRAN and W.L. BRYDEN

Sorghum is an important energy source in poultry diets, with its cost and availability compared with wheat and maize determining the level of inclusion. The considerable variation, ranging from 70 to 170 g/kg, that has been reported in the crude protein content of sorghum grain (Williams and Wills, 1996) also influences the amino acid profile and protein quality. While a tabulation of the relationship between absolute amino acid content and grain protein content for most essential amino acids has been given (Evans, 1985), corresponding information on tryptophan is lacking. This is due probably to the problems associated with tryptophan analysis. Amino acids other than tryptophan are routinely analysed by ion-exchange chromatography following acid hydrolysis. Under these conditions tryptophan is destroyed and has to be analysed separately. With the recent establishment in this laboratory of an analytical procedure that has proved useful for the routine analysis of feeds (Ravindran and Bryden, 1996), sorghum samples with varying nitrogen (N) content were collected and analysed for tryptophan.

Twenty two grain samples, selected to cover the widest range of protein content available, were collected from a commercial feed manufacturer during the 1995/96 growing season. The analytical results for tryptophan were obtained on an absolute basis ( $T_a$ ; g/kg), from which the values were converted to a protein basis ( $T_p$ ; g/16 g N). Total N was determined by micro-kjeldahl. Linear regression analyses between N and  $T_a$  or  $T_p$  were carried out and correlation coefficients were computed. For the 22 grain samples investigated N content ranged from 9.44 to 20.80 g/kg. The  $T_a$  in the grain increased linearly ( $r = 0.82$ ;  $P < 0.001$ ) with increasing levels of N, while an inverse relationship ( $r = -0.55$ ;  $P < 0.01$ ) was observed between  $T_p$  and N content of the grain. The  $T_a$  contents for < 11, 11.1-13, 13.1-15, 15.1-17, 17.1-19 and > 19.1 g/kg N ranges were determined to be 6.70, 7.41, 7.82, 8.95, 10.28 and 11.32 g/kg, respectively. It can be calculated from  $r^2$  that at least 64% of the variation in the tryptophan content of the sorghum grains under study is accounted for by variation in nitrogen content. For the range of N investigated, the relationship between  $T_a$  and N can be described by the equation:  $T_a = 0.68 + 1.68N$ . The results indicate that while the tryptophan content of the protein declines with increasing N levels, the grain's value as an absolute source of tryptophan increases with increasing N levels. Similar trends have been reported for other essential amino acids in sorghum (Williams and Wills, 1996).

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## BIOGENIC AMINES IN MEAT MEALS

C.A. DEN BRINKER\*, C.J. RAYNER\*, M.G. KERR\* and W.L. BRYDEN\*\*

Biogenic amines are found at low concentrations in all cells and are required for normal metabolic functions (Bryden *et al.*, 1996). In processed meat meals high concentrations of biogenic amines may be present due to the action of microorganisms containing enzymes which decarboxylate free amino acids to their corresponding amines. The concentrations of the amines produced are dependent upon the availability of free amino acids, the presence of decarboxylase enzymes and conditions favouring bacterial growth (Halasz *et al.*, 1994). High concentrations of biogenic amines in rendered products are an indication of raw material spoilage. The raw material is vulnerable to bacterial decay when left at high temperatures for long periods prior to rendering. Research studies have shown that biogenic amines in animal protein meals may be responsible for toxic effects in poultry. Enlarged proventriculus, gizzard lining erosion, undigested feed in excreta and pathological changes in the gut mucosa, kidneys and liver have been observed in birds fed diets containing high concentrations of biogenic amines (Dudley-Cash, 1993). Limited information is available concerning the concentrations of biogenic amines in Australian meat meals and the concentrations that are considered safe for consumption by poultry.

In a study commissioned by the Meat Research Council 81 samples of meat meals collected from rendering plants throughout Australia were analysed to determine the concentrations of putrescine, cadaverine and histamine. The amines were determined by HPLC using a C18 reverse phase column and UV detection. Samples analysed contained concentrations of putrescine, cadaverine and histamine ranging from 2-35, 6-329 and 6-58 mg/kg, respectively. In a second study companies provided weekly samples to establish the extent of batch to batch variation. In one case total amine values varied from 83 to 558 mg/kg over a six week period.

Results from the investigation have shown that meat meals can contain varying concentrations of putrescine, cadaverine and histamine which may indicate microbial degradation of the raw material prior to processing. These variable biogenic amine concentrations may also reflect the temperatures at which the raw material was kept, the composition of raw products, rendering temperatures and the time delay between slaughter and rendering. Further studies are required to determine the acceptable concentrations of biogenic amines in animal protein meals used as poultry feeds.

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## HUMAN BEHAVIOUR AFFECTS PRODUCTION AND WELFARE IN HENS AND BROILER CHICKENS

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and G.J. COLEMAN\*\*

This paper reviews the human-animal relationship in agriculture and its implications for the poultry industries. The behaviour of humans towards animals can produce high levels of fear of humans and the nature and frequency of these interactions may have marked consequences on productivity and welfare (Hemsworth *et al.*, 1993). Observations on stockpeople in the pig and dairy industries indicate that the attitudes of stockpeople about interacting with animals were highly predictive of the behaviour of the stockpeople towards their animals. In pigs and cows, the aversive and rewarding properties of humans which increase or decrease fear of humans include, i) hits, slaps and kicks and ii) pats, strokes and the hand of the stockperson resting on the animals, respectively. In poultry, aversive properties of humans which increase fear of humans include fast speed of movement and unexpected movement or appearance of the stockperson. In laying hens high levels of fear of humans are associated with lower production in both commercial (Barnett *et al.*, 1992) and experimental (Barnett *et al.*, 1994) environments. In broilers low fear of humans is associated with improved feed efficiency (Hemsworth *et al.*, 1994).

Based on the interrelationships between the stockperson's attitudes and behaviour and the behaviour, productivity and welfare of farm animals the following general model of human-animal interactions in agriculture has been proposed. Because a stockperson's behaviour towards animals is largely under volitional control, this behaviour is strongly influenced by the attitudes that the stockperson holds about the animals. These attitudes and consequent behaviours affect the animal's fear of humans which, in turn, affects the animal's performance and welfare. While there is excellent evidence in pigs and moderate evidence in dairy cows for the model, the evidence in poultry is more limited. An experiment to manipulate human attitudes and behaviours in the broiler industry showed some short-term effects on human behaviour and bird productivity but did not appear to influence the stockperson's attitudes, bird behaviour or long-term productivity. These findings suggest our understanding of the human behaviours that affect bird behaviour are currently insufficient to take advantage of the potential benefits of improved human-animal relationships in the poultry industries.

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## BEHAVIOURAL ENRICHMENTS FOR LAYER PULLETS AND HENS

C. RUDKIN

Public perceptions of the poultry industry both in Australia and overseas are fuelling a push to larger or alternative cage systems as well as alternative systems that involve larger group sizes which may be costly to the industry. Moreover, larger group sizes often increase problems with cannibalism (Barnett, 1994). Rudkin (1991) suggested that feather pecking and cannibalism are primarily a result of a uniform environment with a lack of complex pecking stimuli. The problem may be alleviated by adding complex pecking stimuli within the present housing systems at relatively low cost. The aim of the present study was to test the effects of pecking enrichments of varying degrees of complexity on behaviour and production.

Non-beak-trimmed SIRO-CB pullets housed on litter when growing, then in cages when laying were given four different treatments: no added stimuli, mixed grain, grain and chaff, and lucerne hay. Hay was provided in racks in pens and cages, while grain or grain and chaff was scattered on the floor in pens and put in racks suspended at the front of cages. In cages, grain was combined with all other nutrients to form a complete layer mix. There were four replicates of each treatment in pens, and eight in cages.

Treatment (pens)	Basal	Grain	Chaff	Hay	Probability
Feather scores (/30)** at 16 weeks	28.5 <sup>ab</sup>	27.0 <sup>a</sup>	28.5 <sup>b</sup>	29.7 <sup>c</sup>	<0.005
Cannibalism (number of cases)	12	61	10	2	NA
% Food conversion (with hay/chaff)	12.2	12.6	12.1	12.2	NS
Weight gain / day (g)	11.0	10.8	11.0	11.2	NS
% Mortality (disease)	11.3	9.3	5.0	10.0	NS
Treatment (cages)	Basal	Grain	Chaff	Hay	Probability
Feather scores (/30)** at end of lay	14.7 <sup>a</sup>	19.1 <sup>b</sup>	20.5 <sup>bc</sup>	22.8 <sup>c</sup>	<0.0001
Cannibalism (total number cannibals)	5	3	1	0	NA
% Egg production (HDA)	81.9	81.1	79.7	81.6	NS
Egg weight (g)	58.9	58.8	59.1	59.0	NS
Albumin (Haugh units)	61.9 <sup>a</sup>	63.6 <sup>a</sup>	65.3 <sup>ab</sup>	68.4 <sup>b</sup>	<0.0333
Yolk colour (Roche)	9.9 <sup>b</sup>	9.2 <sup>a</sup>	10.3 <sup>bc</sup>	10.5 <sup>c</sup>	<0.0001
% Feed efficiency (without hay/chaff)	38.2 <sup>b</sup>	34.5 <sup>c</sup>	36.5 <sup>b</sup>	40.4 <sup>a</sup>	<0.0001
% Feed efficiency (with hay/chaff)	38.2 <sup>a</sup>	34.5 <sup>b</sup>	35.6 <sup>b</sup>	35.1 <sup>b</sup>	<0.0011
% Mortality (disease)	8.2	11.7	4.9	6.9	NS

Means in the same row without a common superscript are significantly different at  $P < 0.05$ .

NS - Not significantly different. NA - Not amenable to statistical analysis.

\*\* Higher feather scores represent better feathering.

It was concluded that the most complex enrichment, lucerne hay in racks, is a low cost method of meeting pecking needs of intensively housed birds, with no loss of production. It may also control behavioural problems of non-beak-trimmed birds in alternative housing systems. Throwing grain on the floor of litter-housed birds is not recommended until further studies separate possible nutritional and behavioural effects.

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Department of Animal Production, University of Queensland Gatton College, Lawes, Queensland, 4343.



## MEDIA STRATEGIES FOR DISSEMINATING RESEARCH TO INDUSTRY

I.A. DINNING\*, M. BOURKE\*\*, K.C. CRITCHLEY\*\*\*, P.C. GLATZ\*\*\*\*  
and C.A. LUNAM\*\*\*\*\*

This presentation includes the screening of excerpts from recent video productions and discussion of strategies for disseminating research and best practice to industry.

The video projects were initiated in response to a decline in the number of industry personnel with the expertise required to maintain high standards in certain aspects of poultry husbandry. New staff were performing tasks without adequate training and management decisions were being made without knowledge of recent research. It was assumed that these problems could be reduced through the production and distribution of appropriate media materials.

Video programs dealing with beak trimming and vaccination have been produced and responses are being sought from industry and researchers. The beak trimming video presents world leading Australian research and implicitly urges Australian producers to be leaders in stock management. The research presented assists viewers to understand the physiological, behavioural and production responses of birds to beak trimming. The vaccination video is structured to convey concepts relating to the importance of vaccination and conditions critical to effectiveness.

Although both video programs include matter of fact demonstrations, their styles are experimental. They are intended to engage viewers as active observers and interpreters of information. A multimedia approach has been used, in part, to develop program structures with potential for modularisation. The value of modularisation is being considered in ongoing project work.

Initial industry responses to the video programs have been varied. The programs have been a catalyst for discussion in which there has been some confirmation of both the need for such input and the appropriateness of using video. The inclusion of general information, research reports and demonstrations in the same video program has not been appreciated by some viewers. Some have only wanted to see practical demonstrations. The issues these viewers have raised are amongst those which demand further exploration.

Integral to this work is the ongoing development of a media project model which incorporates routines for project development, production and formative evaluation. Viewer responses suggest that it may be appropriate to also explore the incorporation of routines for economically reversioning materials to suit specific audiences and events.

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## EFFECT OF CALCIUM PRESENTATION AND FEEDING METHOD ON LOSS OF CROP CONTENTS FROM LAYING HENS

R.D. TAYLOR

Spoilage of feed and difficulties in measurements of feed remnants due to oral fluid losses by laying hens were encountered in a series of nutrition experiments. Cumming (1984) and Taylor (1996) reported problems of "fluid dribbling" from hens, especially those allowed to choice-feed.

One hundred and forty four commercial layers, half an imported strain (Strain 1) and half an Australian strain (Strain 2), were introduced to two feeding methods from eight weeks of age. Both feeding methods were based on the same wheat-based feed formulation with method one provided as a complete pelleted feed (C) and method two as whole wheat with the meals/premix in mash form (W). Within each feeding method calcium was provided by the following alternatives: (1) ground limestone included in the ration, (2) limestone grit 4.0-4.76 mm diameter available daily in a separate feed trough, and (3) the limestone grit available every second day in a separate feed trough.

From 30 weeks of age, a score was applied twice weekly to the condition of the feed for all 144 experimental birds. Scores were graded from 1, dry feed, through to 5, a wet, amorphous mass. At 39 weeks of age all birds were suspended by the legs four times a day for two days in order to collect fluid lost orally.

The statistical evaluation of the data was performed by Repeated Measures Analysis. Significant Least Squares Means (LSMEANS) were separated using paired-sample t-tests. Regression analysis was performed on wet feed score versus excreta moisture content, an excreta moisture score, total feed intake and limestone grit intake.

Strain 1 birds produced a higher wet feed score than Strain 2 birds (1.7 vs 1.5,  $P < 0.05$ ). Wet feed scores were affected ( $P < 0.05$ ) by feeding treatments. Results are shown below.

Feed/Calcium	C 2	C 3	W 1	C 1	W 3	W 2
Feed Score	1.28 <sup>a</sup>	1.41 <sup>ab</sup>	1.57 <sup>ab</sup>	1.60 <sup>abc</sup>	1.74 <sup>bc</sup>	1.92 <sup>c</sup>

<sup>abc</sup> Values without a similar superscript are significantly different at  $P < 0.05$ .

No relationship ( $P > 0.05$ ) between wet feed score and excreta moisture content, excreta moisture score, total feed intake or grit consumption was found by regression analysis. Wet feed score was consistent over time ( $P > 0.05$ ) for each individual bird. No fluid was collected by suspending the birds and subsequent videotape observation indicates that the loss of fluid is not a passive process.

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TAYLOR, R.D. (1996). M.Rur.Sc. Thesis, University of New England, Armidale, NSW.

## EFFECT OF WARM DRINKING WATER ON PRODUCTION PERFORMANCE OF HENS IN WINTER

P.C. GLATZ

The supply of cool drinking water to hens during heat waves is critical for maximising production and liveability. No information is available on the converse situation in winter, that is, the benefits or otherwise of supplying warm drinking water to hens exposed to cold conditions. This study examined the influence of warm drinking water on the production performance of a commercial laying strain exposed to ambient temperature during winter. A total of 168 hens were housed in single bird cages in rooms where the minimum and maximum temperatures recorded over the experiment were 8 and 15°C respectively. There were two treatments (drinking water at 10°C and 30°C) each of 12 replicates of 7 hens over the period from 31 to 37 weeks of age. Drinking water was provided to hens via nipple lines in which water was constantly circulated from water tanks chilled or heated to the required temperature. Eggs were counted daily and food intake measured weekly. At 32 and 36 weeks, the shell thickness of all eggs produced from Monday to Friday from 0800 to 1200 h was measured using a thickness gauge fitted with rounded jaws (Glatz and Barnett, 1996). Rate of lay, food intake and egg weight were measured between 31-37 weeks of age and shell thickness at 32 and 36 weeks of age. Results are shown in the Table.

Drinking water temp (°C)	Food intake (g/day)	Rate of lay (%)	Egg weight (g)	Shell thickness <sup>1</sup> (µm)	Shell thickness <sup>2</sup> (µm)
30	119.6	91.7	61.7	379 <sup>a</sup>	385
10	122.2	93.4	61.7	388 <sup>b</sup>	381
LSD	4.5	4.2	0.9	7	5

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

<sup>1</sup> = 32 weeks, <sup>2</sup> = 36 weeks.

Initially hens given warm drinking water produced thinner shells than those provided with cold water. By 36 weeks of age there was no difference in shell thickness between the treatments as hens became accustomed to the warm water. There was a non significant trend ( $P = 0.11$ ) throughout the experiment for the hens drinking hot water to eat less food. This study suggests that warm drinking water initially has a deleterious effect on egg shell quality which cannot be attributed to a reduction in food intake. It is likely that other metabolic factors may be responsible.

GLATZ, P.C. and BARNETT, J.L. (1996). *Aust. J. Exp. Agric.* **36**: 269-275.

EFFECTS OF MANIPULATION OF AGE AT MATURITY ON PERFORMANCE OF  
SLOW AND FAST MATURING STRAINS OF LAYER

D. ROBINSON, B.A. DAVIS, P.C. TRAPPETT and K.M. BARRAM

Two early-maturing imported strains (E1 and E2) and two late-maturing local strains (L1 and L2) were reared under three husbandry regimens designed to induce advanced (A), normal (N) or delayed (D) maturity. This was achieved by using a combination of variable daily light periods (step-up, constant and step-down lighting patterns for A, N and D respectively), different nutrient densities in the starter and grower diets (high for A and low for N and D) and different feeding schedules from nine weeks of age until 5% production depending on growth rate (A ad libitum, N mildly restricted on some days and D restricted every day by limiting time of access to feed). The changes in daylength were made in one step at ten weeks of age with a view to maximising the effect on age at maturity (Lewis *et al.*, 1992). In the laying period each strain x rearing treatment combination was represented by eight replicated groups of eight birds (four adjacent two-bird cages). Results for maturity criteria and laying performance from 17 to 53 weeks of age are shown below.

Strain/ treat- ment	Age (d) at 5% lay	Bodywt (kg) at:		Eggs/ bird (hen-d)	Mean egg wt (g)	Feed/ bird (g/d)	Feed/ eggs (g/g)	Mortality (%)
		119 d	5% lay					
E1-A	104	1.675	1.467	227.37	57.89	119.7	2.292	9.38
E1-N	133	1.587	1.748	215.41	61.32	120.1	2.292	15.63
E1-D	145	1.428	1.647	199.98	61.69	115.4	2.357	12.50
E2-A	106	1.742	1.578	221.54	58.72	118.8	2.302	14.06
E2-N	133	1.601	1.864	207.93	61.23	117.7	2.330	10.94
E2-D	143	1.405	1.708	201.25	63.00	119.9	2.383	14.29
L1-A	122	1.656	1.674	207.59	54.66	114.0	2.532	9.38
L1-N	139	1.450	1.735	202.73	56.86	117.6	2.571	7.81
L1-D	157	1.329	1.678	192.83	58.36	115.2	2.580	3.13
L2-A	128	1.678	1.773	205.11	54.49	120.1	2.707	25.00
L2-N	146	1.553	1.875	198.55	55.83	122.0	2.774	9.38
L2-D	158	1.420	1.802	185.62	58.82	121.1	2.798	8.93

Ages of reaching 5% rate of lay and bodyweights at 17 weeks of age differed markedly between rearing treatments ( $P < 0.001$ ). Treatment N birds had the highest bodyweights at point of lay. For all strains hen-day egg number decreased ( $P < 0.001$ ), average egg weight increased ( $P < 0.05$ ), egg weight at any given age increased and feed conversion tended to become poorer with increasing age at maturity. In the last four months egg production and feed conversion tended to be poorer for treatment A than for N or D. Mortality of strain L2 was higher ( $P < 0.05$ ) in treatment A than in N or D. The results indicate that by adopting a rearing program which advances maturity, total egg numbers can be increased and mean egg weight reduced in the first nine months of lay. This may be economically advantageous for imported large-egg strains of layer.

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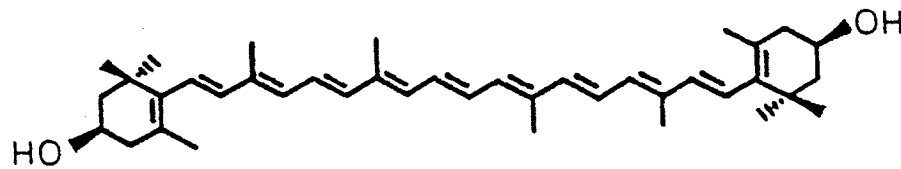
Queensland Poultry Research and Development Centre, PO Box 327, Cleveland, Queensland 4163.

## meso-ZEAXANTHIN: A POTENTIAL CAROTENOID FOR BROILER AND EGG YOLK PIGMENTATION

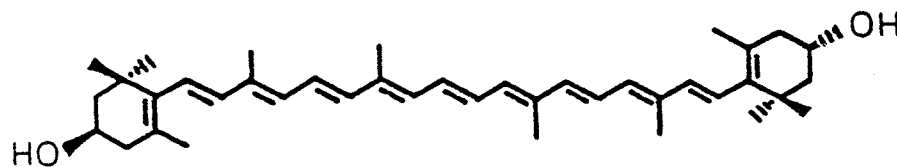
V.E. RIDAURA

Zeaxanthin is one of the most widespread carotenoids in nature. It is found in maize, maize gluten and marigold flowers together with other carotenoids at different concentrations and in different ratios. Individually, zeaxanthin gives a predominantly orange deposition in the skin of broilers and in egg yolks, more than most other natural carotenoids. Unfortunately, there is not a source of zeaxanthin with a constant concentration that can be used in the poultry industry in order to standardise the concentration of this carotenoid during feed preparation.

Recently, an industrial process has been developed in which the natural esterified yellow lutein found in the oleoresin from marigold extracts (approximately 82%) may be converted to a 25:75 mixture of free yellow lutein and orange meso-zeaxanthin. The meso-zeaxanthin has the same double bond arrangement as zeaxanthin but an opposite stereochemistry in the hydroxyl group at the 3'-ring of the carotene backbone. Therefore, the new process converts the natural ratio of high lutein/low zeaxanthin found in marigolds to high meso-zeaxanthin/low lutein.



zeaxanthin



meso-zeaxanthin

There are no reports in the scientific literature which indicate possible uses for meso-zeaxanthin in the poultry industry. Now that an efficient method for the production of meso-zeaxanthin has been developed studies are being carried out to determine the potential of this product as a feed additive for the pigmentation of broilers and egg yolk and to establish economic comparisons with other carotenoid sources presently on the market. These pigmentation studies correlate different meso-zeaxanthin concentrations as well as several inclusion rates in the feed.

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Bioquimex-Reka, Mexico and Lynteq Australia Pty Ltd, Sydney, NSW 2000.

**IGF-I, BUT NOT IGF-II, PROMOTES LEAN GROWTH AND MORE EFFICIENT  
FEED CONVERSION IN CHICKENS FROM LINES WITH DIVERSE FEED  
EFFICIENCY AND BODY COMPOSITION**

R.A. PYM\*, F.M. TOMAS\*\* and G.L. FRANCIS\*\*

There have been few reported studies of the effects of IGF-I or IGF-II in broiler chickens. IGF-I decreased abdominal fat pad (AFP) weight (Huybrechts *et al.*, 1992) but IGF-II increased the AFP weight, possibly due to suppression of plasma T<sub>3</sub> levels (Spencer *et al.* 1996). No growth responses to either IGF were seen. The present study examined the effects of IGF-I, IGF-II and combined IGF-I and IGF-II infusion to experimental lines of broilers differing in food utilisation efficiency and body fatness. The 92 birds from control (C), efficient (E) and high food intake (F) lines (lean and fat, respectively) were housed in single cages from five weeks of age ( $\approx$  550 g body weight) and given free access to a stock broiler starter ration. The chickens were randomised by sex and line to receive a 14 day infusion of either IGF vehicle (Control), IGF-I (225  $\mu$ g/kg/d), IGF-II (225  $\mu$ g/kg/d) or combined IGF-I and IGF-II (each 112.5  $\mu$ g/kg/d) delivered via an Alzet mini-osmotic pump implanted subcutaneously at the base of the neck. Growth performance, plasma hormones and carcass composition were measured. Results for IGF treatment effects are shown in the Table.

	Control	IGF-I	IGF-II	IGF-I and -II	SE <sub>diff</sub>
Weight gain (g)	331	379*	352	382*	18.5
FCR (g:g)	2.31	1.96**	2.15	2.06*	0.094
Abdominal fat pad (g/kg)	10.68	6.38*	9.72	7.75	1.55
Plasma IGF-I (ng/mL)	15.9	51.5**	13.8	33.0**	3.00
Plasma IGF-II (ng/mL)	19.3	19.2	31.0**	29.5**	1.64
Plasma T <sub>3</sub> (ng/mL)	2.79	1.88***	2.11*	1.87***	0.277

Values are least square means from 3-way ANOVA of sex, line and treatment effects.

\*, \*\*, \*\*\*; Significantly different from controls at P < 0.05, 0.01 and 0.001, respectively.

IGF-I, but not IGF-II, treatment increased the rate of weight gain, improved food utilisation efficiency and decreased AFP weight. The combined IGF-I and IGF-II treatment gave responses generally consistent with the lower dose of IGF-I. The fall in the plasma T<sub>3</sub> levels with IGF-I treatment shows that the IGF-I reduction of AFP weight is not secondary to changes in T<sub>3</sub> levels which are normally inversely related to fatness. Sexes and lines showed differences in growth, feed efficiency and body composition but all responded similarly to the IGF treatments.

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ENHANCEMENT OF GROWTH AND FOOD UTILISATION EFFICIENCY BY IGF-I  
IN A COMMERCIAL BROILER LINE IS RELATED TO REDUCED RATES OF  
PROTEIN TURNOVER

F.M. TOMAS,\* R.A. PYM\*\* and G.L. FRANCIS\*

A significant increase in growth rate and food utilisation efficiency was observed in experimental broiler lines treated with IGF-I (Pym *et al.*, 1997). Efficient feed conversion has been shown to be associated with low rates of muscle protein breakdown (Tomas *et al.*, 1991). The present study examined the growth promoting effects of IGF-I, IGF-II and combined IGF-I and IGF-II infusion in commercial broilers and included measurements of protein metabolism. Female broilers from the Ross sire line were housed in single cages from three weeks of age ( $\approx$  600 g body weight) and given free access to a stock broiler starter ration. The chickens were infused for 13 days with either IGF vehicle (Control), IGF-I (300  $\mu$ g/kg/d), IGF-II (300  $\mu$ g/kg/d) or combined IGF-I and IGF-II (each 150  $\mu$ g/kg/d) delivered via Alzet mini-osmotic pumps implanted subcutaneously at the base of the neck. Growth performance, plasma hormones and carcass composition were measured. Nitrogen balance and excretion of N<sup>T</sup>-methylhistidine, an index of muscle protein breakdown, were measured over the last two days. Protein synthesis rates in muscle were measured by incorporation of <sup>3</sup>H-phenylalanine at the end of the study. Results for IGF treatment effects are shown in the Table.

	Control	IGF-I	IGF-II	IGF-I and -II
Weight gain (g)	62.5 $\pm$ 1.8	66.0 $\pm$ 3.2*	61.9 $\pm$ 3.1	61.4 $\pm$ 1.6
Nitrogen balance (g/d)	2.23 $\pm$ 0.08	2.52 $\pm$ 0.13*	2.34 $\pm$ 0.09	2.36 $\pm$ 0.09
FCR (g:g)	1.85 $\pm$ 0.04	1.70 $\pm$ 0.02*	1.79 $\pm$ 0.03	1.79 $\pm$ 0.03
Abdominal fat pad (g/kg)	10.3 $\pm$ 0.4	9.0 $\pm$ 0.4	12.4 $\pm$ 0.6*	10.8 $\pm$ 0.6
Plasma T <sub>3</sub> (ng/ml)	1.75 $\pm$ 0.13	1.21 $\pm$ 0.18*	1.51 $\pm$ 0.12	1.47 $\pm$ 0.08
Protein breakdown (%/d) <sup>†</sup>	2.36 $\pm$ 0.17	1.85 $\pm$ 0.08*	2.13 $\pm$ 0.15	2.00 $\pm$ 0.12
Protein synthesis (%/d) <sup>†</sup>	10.6 $\pm$ 0.36	9.90 $\pm$ 0.24	10.7 $\pm$ 0.55	11.0 $\pm$ 0.34

\* Significantly different from controls at P < 0.05.

<sup>†</sup> % of protein pool per day.

IGF-I treatment increased the rate of weight gain and improved nitrogen balance and feed efficiency. IGF-II effected only an increased abdominal fat pad weight. The rate of N<sup>T</sup>-methylhistidine excretion was significantly reduced by IGF-I. Since rates of protein synthesis were not increased, the increased growth rate can be ascribed to the lower rate of protein breakdown, consistent with improved efficiency. The concomitant fall in the plasma T<sub>3</sub> levels with IGF-I treatment may be a contributing factor to the increased efficiency.

This work was supported in part by the Chicken Meat Research and Development Council.

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## IMMUNOHISTOCHEMICAL DETECTION OF CALBINDIN D28K AND OESTROGEN RECEPTOR IN THE SMALL INTESTINE OF PRE - AND POST - LAY HENS

A.A. SAKI and D. R. TIVEY

The knowledge that there is an increased requirement for dietary calcium ( $\text{Ca}^{2+}$ ) after sexual maturation of layer hens is well established. This increase is reflected in an elevated enterocyte Calbindin D28K expression (a calcium binding protein essential for calcium transport) and an increased potential for the intestinal mucosa to absorb  $\text{Ca}^{2+}$  in 25-week old hens as compared with 11 - 17 week old hens (Wu *et al.*, 1993, 1994). However, it still remains to be clarified whether there is a direct action on the intestinal mucosa of the increased oestrogen level that is associated with sexual maturation. In order for a direct cellular action of this hormone to occur enterocytes must express oestrogen receptors. Thus, this study aimed to identify the temporal and spatial expression of Calbindin D28K and oestrogen receptors (ER) to evaluate the possibility of a direct oestrogen action on the small intestine.

Ten Isa Brown hens, 15 and 26 weeks of age (5 birds per group), were obtained from a commercial producer. Birds were killed by lethal injection (1 mL nembutal/ kg of body weight) after which the small intestine was rapidly dissected and rinsed with ice cold phosphate buffered saline. Two 1cm samples were taken from each of the duodenum, jejunum and ileum and fixed in either Carnoy's or a 4% buffered formaldehyde for Calbindin D28K and ER localisation respectively. For immunolocalisation specific mouse monoclonal antibodies against Calbindin D28K (Sigma, Clone C13000) and ER (Dako, Clone ID5) were used (Wu *et al.*, 1993; Kusuhara and Tomoo, 1991). Non - specific mouse IgG was used as a negative control and all antibodies were detected using a rabbit anti-mouse IgG conjugated to horseradish peroxidase. Antibodies were used at a 1/100 dilution.

In both 15 and 26 week groups anti - Calbindin D28K immunoreactivity was detected in the enterocytes of duodenal tissue. However, in more distal regions of the small intestine the enterocyte expression of this calcium binding protein was confined to the 26 week group. In contrast, immunoreactivity against oestrogen receptors was observed in enterocytes from all tested regions of the small intestine, irrespective of the age of the bird.

In conclusion, in the distal intestine oestrogen receptor expression precedes that of Calbindin D28K thereby demonstrating that oestrogen has the potential to regulate Calbindin D28K expression in this tissue. Thus, increased oestrogen levels during sexual maturation may induce Calbindin D28K expression and hence modulate intestinal absorptive capacity for calcium.

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EFFECT OF HYPERTHERMIA DURING INCUBATION ON SUBSEQUENT  
PRODUCTION PERFORMANCE OF LAYING HENS

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Chicks which hatch following heat exposure are weak and there is an increased incidence of culls (Wilson, 1991). Information on production performance of hens previously subject to embryonic hyperthermia has not been reported. This study examined the influence of hyperthermia during incubation on the subsequent production performance of commercial laying strains housed in a naturally ventilated cage layer shed. Embryos from two commercial strains of laying hens were exposed to hyperthermia (increase of 0.5°-2.0°C) from day 10 of incubation and compared to control groups incubated at normal temperature. In the laying phase a randomised design was used for allocation of treatments with 40 replicates per treatment. Each replicate comprised three consecutive cages, each cage (45 x 45 x 40 cm) housing three birds. Hens were weighed at 18 weeks of age. Rate of lay, food intake and egg weight were measured over the period from 18 to 54 weeks of age and the results are presented in the Table.

Incubation treatment	Hatch (%)	Rate of lay (%)	Food intake (g/day)	Egg weight (g)	Bodyweight at 18 weeks (g)
Control 1	85	66.1 <sup>a*</sup>	119.9 <sup>a</sup>	56.3 <sup>a</sup>	1630 <sup>a</sup>
Hyperthermia 1	56	65.0 <sup>a</sup>	116.6 <sup>a</sup>	55.1 <sup>b</sup>	1570 <sup>b</sup>
Control 2	83	69.8 <sup>b</sup>	100.9 <sup>b</sup>	56.3 <sup>a</sup>	1360 <sup>c</sup>
Hyperthermia 2	61	67.3 <sup>c</sup>	97.2 <sup>c</sup>	55.4 <sup>b</sup>	1290 <sup>d</sup>
LSD		1.8	3.3	0.8	55

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

No statistical tests could be done on hatch %. 1=heavy strain, 2 =light strain.

Both strains subject to hyperthermia during incubation had lower body weights at 18 weeks and produced smaller eggs over the period of lay. Hens of the lighter strain subjected to hyperthermia during incubation consumed significantly less food and produced fewer eggs than their controls. These studies have demonstrated that hyperthermia during incubation induces adverse effects on the performance of mature hens, especially with lighter bodyweight strains. Maintenance of correct temperature throughout the incubation process is critical not only to achieve maximum hatchability but also to prevent sub-optimal performance from hens during the laying cycle.

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## SUSCEPTIBILITY OF COMMERCIAL BROILER STRAINS TO FUSAROCHROMANONE-INDUCED TIBIAL DYSCHONDROPLASIA

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Tibial dyschondroplasia (TD), a condition characterised by the presence of an abnormal cartilage mass in the proximal head of the tibia, is a widespread problem of fast-growing broiler chickens. It has been shown to be induced by various factors including nutrition, genetics and management (Orth and Cook, 1994). Fusarochromanone (FC), produced by some isolates of *Fusarium equiseti*, is the only mycotoxin associated with the aetiology of TD (Walser *et al.*, 1982). In the present study, the influence of FC on the incidence of TD in three commercial broiler strains, designated as Strain A, B and C, was examined.

Three separate trials, using day-old broilers, were conducted. In Trial 1, the effects of supplementing a maize-soyabean meal basal diet with either 30 g/kg of FC-contaminated rice, 10 g/kg cysteine, 4.8 g/kg DL-methionine, dietary electrolytes or dietary electrolytes plus 10 g/kg cysteine on the incidence of TD in Strain A was investigated. The FC-contaminated rice had a toxin concentration of 30 mg/kg. Each diet was fed to four pens of eight birds each. In Trial 2, broiler Strain B which has been previously observed in this laboratory to be susceptible to TD (Van Wel, 1992) was utilised. The treatments consisted of a wheat-sorghum-soybean meal basal diets and three experimental diets containing 30, 60 or 90 g/kg of FC-contaminated rice. Each diet was fed to two pens of eight birds each. In Trial 3, the influence of feeding diets containing 0, 30 or 60 g/kg of FC-contaminated rice on the incidence of TD in three commercial broiler strains (A, B and C) was compared. In all trials, performance data was recorded at days 7, 14, 21 and 28. On day 28, the birds were killed and examined for TD. In TD positive birds, lesions were scored according to the size of the cartilage plug.

In Trial 1, although the weight gain and the feed:gain ratio of Strain A broilers were influenced ( $P < 0.05$ ) by dietary treatments, none of the birds showed any indication of TD. In Trial 2, dietary inclusion of more than 30 g/kg of FC-contaminated rice lowered ( $P < 0.05$ ) weight gains of Strain B broilers but had no effect on feed intake and feed:gain. However, TD was observed and the incidence and severity of TD lesions increased with increasing levels of dietary FC levels. In Trial 3, significant differences ( $P < 0.05$ ) were observed among strains in both incidence and severity of TD. Strains B and C exhibited high susceptibility to TD while Strain A appeared to be resistant to the condition. In Strains B and C, the incidence and severity of TD increased with increasing levels of dietary FC. The present results confirm the ability of FC to induce TD and demonstrate that genetic predisposition to TD is a primary factor in the development of the disease.

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INFLUENCE OF ENZYME SUPPLEMENTATION ON APPARENT METABOLISABLE ENERGY AND AMINO ACID DIGESTIBILITY OF WHEAT, MILLRUN AND TRITICALE FOR BROILERS

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and W.L. BRYDEN\*

The addition of xylanase enzymes to poultry diets based on wheat is becoming a routine practice in Australia. While the exact mechanism(s) of action of supplemental xylanases remains unclear degradation of non-starch polysaccharides in the cell wall of the grain is thought to be the major outcome. The beneficial effects of xylanases in overcoming the anti-nutritive influence of these viscous polysaccharides on the performance and utilisation of energy and amino acids in broilers fed on wheat-based diets have been previously reported (Hew *et al.*, 1996). The efficacy of xylanase supplementation, however, may be influenced by the age of the wheat included in the diet. The present study was conducted to examine the influence of a commercial xylanase (Avizyme®, Finnfeeds International Ltd, U.K.) on the apparent metabolisable energy (AME) and apparent ileal amino acid digestibility (AAAD) of old season and new season wheats, millrun (a mixture of wheat pollard and bran) and triticale for broilers.

The AME values of the test feedstuffs were determined, with or without the enzyme, using the classical total collection method as described by Mollah *et al.* (1983). For the AAAD assays, the basal diet contained the test feedstuff as the only source of protein and was used with or without the enzyme. Celite, a source of acid-insoluble ash, was added at 20 g/kg to all diets as an indigestible marker. Each assay diet was fed to three pens (4 birds/pen) of male broilers from 35 to 42 days of age. On day 42, digesta contents from the terminal ileum were collected and processed (Siriwan *et al.*, 1993). Samples of diets and digesta were analysed for acid-insoluble ash and amino acids, and the AAAD values were calculated.

Enzyme addition improved ( $P < 0.05$ ) the AME of old season and new season wheats by 7.0 and 5.1%, respectively, but had no effect on the AME values of millrun and triticale. The AME contents (MJ/kg) of the basal and enzyme supplemented diets were as follows: old season wheat, 14.3 and 15.3; new season wheat, 13.7 and 14.4; millrun, 11.3 and 11.5, and triticale, 13.2 and 13.2. The AAAD in both wheat types was improved ( $P < 0.05$ ) by supplemental enzyme with improvements in the digestibility of individual amino acids ranging from 2 to 8 percentage units. Although numerical increases in the AAAD of millrun and triticale were seen with enzyme addition, the differences were not significant ( $P > 0.05$ ). The overall mean AAAD coefficients of feedstuffs with or without enzymes were as follows: old season wheat, 0.902 and 0.855; new season wheat, 0.903 and 0.845; millrun, 0.745 and 0.732, and triticale, 0.812 and 0.792.

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## THE NON-STARCH POLYSACCHARIDE COMPONENT OF WHEAT MILLING BY-PRODUCTS

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Wheat milling by-products are valuable sources of protein, starch and fat for broiler chickens. They also contain, however, non-starch polysaccharides (NSP) which may exert anti-nutritive activity and may contribute to the lower AME values recorded for wheat milling by-products in comparison to wheat. Dependent upon the concentration and type of NSP present in wheat milling by-products the addition of enzymes may lead to an increase in nutrient absorption. The present study aimed to characterise the NSP contents of three wheat milling fractions; bran, pollard and mill screenings. Samples of bran, pollard and mill screenings were obtained commercially (Manawatu Flour Mills, Palmerston North, New Zealand) and a representative sub-sample of each of the products was submitted to chemical analysis for soluble and insoluble NSP fractions, uronic acids, starch and protein. The by-products reflected the milling of a blend of Australian hard wheat and Australian ASW wheat (50%) with a variety of New Zealand soft wheats (50%). The extraction and analytical procedures are as described by Annison *et al.* (1995). The concentrations (g/kg dry matter) of several components are presented below.

	Bran	Pollard	Mill Screenings
Total NNSP <sup>1</sup>	182.6	125.9	88.9
Soluble NNSP	12.9	15.1	15.4
Insoluble NNSP	169.7	110.8	73.5
Uronic Acids	115.5	95.8	91.6
Starch	101.0	399.0	495.0
Crude Protein	160.0	156.6	119.4
Recovery <sup>2</sup>	55.8	75.7	79.5

<sup>1</sup>Neutral non-starch polysaccharide.

<sup>2</sup>% dry matter accounted for by analysis.

The study confirms that Australasian wheat milling by-products contain relatively high levels of NSP's and also contain soluble NSP's. Soluble NSP's from wheat have been shown to be anti-nutritional for broiler-chickens (Annison, 1991). All three by-products contained appreciable amounts of insoluble NSP although mill screenings contained less NSP but considerably more starch compared to the other two wheat milling by-products. The data highlight that successful use of by-products can only commence when full compositional analysis is complete.

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## EXOGENOUS ENZYMES IMPROVE FEED DIGESTIBILITY IN YOUNG BROILERS AS WELL AS IN LAYING HENS

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In-feed enzymes, such as xylanase and  $\beta$ -glucanase, are used to overcome the antinutritional effects of soluble non-starch polysaccharides (NSP) in cereals like barley, wheat or rye. Indeed, NSP are known to reduce performance (decreased feed efficiency) and litter quality (sticky droppings and wet litter), particularly with very young broilers. Most producers are reluctant to use high concentrations of these cereals even when supplemented with enzymes during the starting period. Moreover, Bedford and Morgan (1996) suggested that the improvement in feed efficiency with enzyme addition was often greater in the finishing period than in the growing period. Thus, it appeared important to investigate the effect of enzyme addition in relation to age. Furthermore, adult birds are assumed to be able to extract more nutrients from their feed than young birds and may not be expected to respond positively to enzyme supplementation.

Two experiments were designed to study the effect of enzyme supplementation on feed digestibility and apparent metabolisable energy (AME) in relation to age in young broilers from 8 days of age and in adult layers fed wheat-based diets. Growth and laying performance were also measured to validate any improvement in digestibility.

Ross day-old male broilers were reared in battery cages to determine AME (120 birds) or in floor pens to measure growth performance (3000 birds). They received a pelleted diet containing 500-570 g/kg of wheat with soyabean meal, extruded soyabeans, and animal or vegetable fat from day-old to 42 days of age. Xylanase (Rovabio™ Xylan) was sprayed onto the pellets (200 mL/tonne). AMEn was measured according to the European reference method (Bourdillon *et al.*, 1990) between 8-12 and 24-27 days of age. The first balance trial showed an improvement in AMEn (+2.4%) which further increased to +4% at 27 days of age. Feed efficiency was enhanced by 3.4 to 4.4% in the finishing period.

Isabrown laying hens (48 birds) received a wheat-based diet (650 g wheat/kg) from 27 to 32 weeks of age. The AME and feed digestibility were measured in *ad libitum* fed birds according to Lessire *et al.* (1995) using 12 replicates of 2 hens per cage. Xylanase (Rovabio™ Xylan) was incorporated as powder (50 g/tonne) in the mash feed. The AMEn was significantly improved by xylanase supplementation (13.14 vs 12.66 MJ/kg DM, +3.8%). Feed efficiency measured between 27 and 32 weeks of age was enhanced by more than 4% in the enzyme-treated group.

These results indicate that xylanase addition allows young broilers as early as 8 days of age and adult layers to derive more nutrients from their feed, resulting in enhanced profitability in poultry production.

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