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(Australian Branch)

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AUSTRALIAN POULTRY SCIENCE SYMPOSIUM
2004

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SYD WILKINS MEMORIAL PRIZE

The Syd Wilkins Memorial Fund was set up in 1983-84 by the Australian Branch of the World's Poultry Science Association (WPSA), with the active collaboration of the Poultry Husbandry Research Foundation (PHRF) as it was known at the time, with the help of a major opening donation from Allied Feeds. The purpose of the fund was to honour the many contributions of Syd Wilkins to the Australian poultry industry. The practical use of the Fund was to provide an award, called the WPSA Syd Wilkins Memorial Award, for outstanding work by young poultry scientists working in Australia. The first annual award was made in 1984 but the definition of young has been extended twice from the initial 30 years to the present 35 years.

Syd Wilkins received his tertiary education at Hawkesbury Agricultural College and developed a career as a Poultry Officer in the NSW Department of Agriculture, becoming its Senior Poultry Officer by the late 1950's. In the mid to late 1960's he transferred to Allied Feeds, where he remained as a Senior Executive of the Allied Mills Group until his untimely death in 1982. During all this time he was very active in the affairs of the WPSA Australian Branch, including a period as President. He was also elected as one of the Vice Presidents of WPSA world body in the 1970's, a position he still held on this death. He was actively involved in the conduct of the 1974 and 1978 World's Poultry Congresses in New Orleans and Rio de Janeiro.

Syd Wilkins was also involved with the PHRF virtually from its beginning, and served many years as its Vice-President. He was the recipient of the Australian Poultry Award in 1974. Syd's career was contemporary with the application of the 20th century poultry technology revolution in Australia. In his own low-key and unassuming way he contributed very significantly to its progress.

Previous recipients of the prize are:

1984	Jennifer York	1994	No Award
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CONTENTS

POSTERS

POULTRY HEALTH

HARMONISING CONSUMER AND PRODUCER EXPECTATIONS

A TRIBUTE TO BRUCE SHELDON
A DIMINUTIVE SCIENTIST OF IMMENSE STATURE

R.A.E. PYM[1], R.W. ROBERTS[2], R. PEREZ-MALDONADO[3] AND M. SIMMONS[4]

Bruce Sheldon passed away at 73 years of age in Sydney on 29 April 2003 from heart and lung complications following a hip replacement operation. Bruce made a prodigious contribution to poultry science and the Australian poultry industries over many years as a research scientist in CSIRO in poultry genetics from 1953 to 1994, as President of the Australian Branch of WPSA from 1982 to 1992, and as Vice President of the WPSA World Body since 1984. Bruce was the main driving force in the early 1990s behind the transition of this meeting from what was the annual Sydney University Poultry Husbandry Foundation Symposia into the now internationally-acclaimed annual Australian Poultry Science Symposia. As a member of the Organising and Scientific Program committees of APSS and as an active participant in the Symposium each year, Bruce continued to make a significant contribution to the promotion of poultry science right up to the time of his passing.

I. RESEARCH CONTRIBUTION

Following graduation in Agricultural Science at the University of Sydney in 1953, Bruce was employed by CSIRO Division of Animal Genetics as a research scientist at Werribee in Victoria working in the area of poultry genetics under the direction of Dr John Morris. In 1956 he returned to Sydney University to undertake a PhD on direct and correlated response to selection using the vinegar fly *Drosophila melanogaster,* a species that played a vital role in the development and validation of quantitative genetic theory. Bruce obtained his PhD in 1960 and returned to CSIRO to work with poultry.

By the 1970s Bruce was a world-renowned scientist in the area of poultry genetics. His ground breaking early work on selection to reduce the interval between eggs within a clutch, with the aim of breaking the selection limit of one egg per 24 hours, employed the novel approach of a continuous light environment and accurate computer-linked laying time recording equipment. It had been generally assumed by poultry breeders that this was the limit to selection for increased egg production, since the endogenous circadian rhythm controlling ovulation via luteinizing hormone release is entrained to the 24 hour light-dark cycle. In a number of long-term selection experiments employing the above approach, Bruce and his colleagues reduced the mean interval to 21 hours in continuous light and 23.5 hours in the normal 24 hour light-dark cycle. The old selection limit had thus been overcome and the new limit was estimated as low as 18 hours in continuous light and below 22.5 hours in the normal 24 hour light-dark cycle. Very successful use was made in Australia and world-wide of Bruce's novel selection methodologies in commercial egg breeding programs. A very productive collaboration with Dr Bryan Yoo, his colleague at CSIRO, saw the publication of many jointly authored papers in the general area of the genetics of egg production.

Arising from the egg selection studies, one of the lines was shown to produce a high proportion of triploid intersexes. Dr Sheldon recognised that this resource provided an opportunity to study avian sex determination and together with his colleague Dr Marnie

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[4] Outback Environmental Controls Pty Ltd, 2/32 Billabong Street, Stafford, Qld. 4053.

Thorne at CSIRO, established a line of birds selected for a high incidence of triploidy. There followed a fruitful period of international collaborative study with this resource at the molecular level, resulting in numerous publications. The poultry industry is naturally very interested in the possibility of manipulating sex ratios since layer strain male chickens have minimal commercial value, and work by Bruce and others has made this a very real possibility in the not too distant future.

By the early 1990s, as a result of his efforts and insight, Bruce's group at CSIRO was at the forefront of world research in developing novel technologies for molecular genetic engineering in poultry. The group was closely associated with the international group engaged in developing a map of the chicken genome. A particular contribution that Bruce's group made to this area of study was the development and improvement of the *ex vivo* embryo culture technique, enabling chick embryonic development to proceed in surrogate shells. This permitted easy access to the avian blastoderm for micro-injection of donor totipotent blastodermal cells. His group also made a unique and valuable contribution with the identification of a novel avian cytokine and isolation of the protein responsible for maintaining the potential donor blastodermal cells in a totipotent state. Most regrettably, the research work terminated in the mid 1990s due to the withdrawal of joint industry-federal government funding for the work when the major poultry breeding companies in Australia curtailed breeding operations and began importing genetic stock. He was invited to membership of the International Standing Committee on Poultry Genome mapping in 1992 and remained on the committee until he passed away.

A significant component of his team's research over the years was adopted and applied by the commercial poultry industry here in Australia and world wide. Bruce received numerous invitations to present papers at international poultry and genetics & breeding forums, and he was very active in the organisation of poultry science and technology meetings in Australia for many years. In recognition of his outstanding contribution to poultry science and the Australian poultry industry, he received the Australian Poultry Award in 1992.

Although he retired from CSIRO as Chief Research Scientist in 1994, Bruce was irrepressibly active professionally following his retirement. At his home at Pennant Hills in Sydney up until his passing, he still ran selection experiments with *Drosophila* and visited the CSIRO library at least once a week.

II. CONTRIBUTION TO DEVELOPING COUNTRIES

As the Senior Vice president of the World body of WPSA, Bruce was a most influential and valuable member with his championing of the establishment of branches in developing countries, particularly in the Asia Pacific region, and the promotion of poultry education and training throughout the world. One of his pet projects with which he was very actively engaged right up to the time of his passing was the establishment of a family poultry farming working group in the Asian Pacific Federation of WPSA, something likely to impact profoundly on poverty and malnutrition in the developing countries of the region.

Bruce understood the importance of involving WPSA as well as the World's Poultry Breeding Companies, along with the International Network for Family Poultry Development and FAO in work aimed at improving the efficiency of family poultry production systems of poor communities, appreciating that a majority of the world's almost one billion very poor people gain food and security from their small poultry flocks. He also appreciated the flexible genetic approach needed in different parts of the developing world, the need for improvement in socio-economic factors including marketing systems in those countries, as well as further development of the commercial industry in developing countries.

During the past decade, Bruce developed a series of papers seeking to describe and evaluate the factors affecting whether desirable targets for production and consumption of poultry protein can be met in the next 50 years. He paid particular attention to factors continuing to limit the necessary expansion of poultry production and consumption in developing countries. He returned in March 2003 from Bangladesh where he gave an invited paper at the 3rd International Poultry Show and Seminar on factors affecting poultry production and consumption in developing countries.

III. PERSONAL QUALITIES

There was a total lack of pretence with Bruce, he was always eminently approachable by his colleagues, junior scientists, technicians and by students. A man of great integrity, warmth and compassion, Bruce was affectionately regarded by all who knew him. His demeanour was invariably pleasant and smiling and he was unbelievably generous with his time and energy to help those in need of his assistance, no matter what their position. His effectiveness in that capacity was profound due to his incisive mind, his great knowledge of genetics and poultry science in general, and his humility. As a consequence, he very readily assumed the role of mentor to many, and this developed in many cases to close friendships. As a committee member of the Australian branch and world body of WPSA, his encyclopaedic knowledge of procedural matters and his acute powers of recollection of fact and detail, were legendary.

As testimony to the high regard in which Bruce was held in the scientific community and poultry industry around the world, on news of his passing many messages of condolence and appreciation flowed in from friends and colleagues world wide. One such message from one of his former colleagues very eloquently, accurately and succinctly describes Bruce's qualities in this regard "Bruce was a gem of a person and a giant of a man. He was really that very special combination of knowledge, doggedness to get the tasks at hand done, incisive and an absolute gentleman". Those of us who had the good fortune to know him miss his guiding hand, his wisdom and his integrity, but most of all we miss his warm and caring nature, his great generosity of spirit and his smiling countenance.

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JOHN HORACE (JACK) INGHAM, AO
10 June 1928 - 5 August 2003

J.G. FAIRBROTHER

Jack Ingham AO, the elder of the Ingham brothers whose inspired vision created a business empire based on chicken meat, died on the 5th August 2003, after a long illness. Combining an entrepreneurial skill with a down-to-earth manner, Jack Ingham left many admirers in both the poultry industry and the thoroughbred racing industry.

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Without any doubt, the two giants who dominated the chicken meat industry from the mid 1960s to the present day were the 'Chicken Kings', as Jack and Bob Ingham were known to the media. They inherited a 45 hectare property at Casula in western Sydney, stocked with 650 pigs and 30,000 birds producing a half-million chickens a year, after the sudden death of their father in 1953. They built this into the largest integrated poultry operation in Australia, spanning all States and New Zealand yet still remaining a private company.

In 1959, Inghams entered into a franchise agreement for New South Wales with AA Tegel Pty Ltd for the supply of breeding stock. This was consolidated in 1963 when they purchased a 50 percent share in the Tegel operation. Over the next two decades this was increased to encompass the hatcheries and franchises offered by Tegel, Australia-wide. Inghams Enterprises now supplies more than half of all day-old meat chickens produced in Australia. Its main success, however, came in processing and marketing.

After Jack visited the United States and Great Britain in 1960, he and Bob built Australia's largest poultry abattoir at Hoxton Park, NSW. The hatchery was rapidly expanded and farming properties acquired as the meat and egg laying business grew. In these early years, a firm supplier/retailer bond was established with Woolworths and its Chairman, Sir Theo Kelly. This was to be the cornerstone of promoting frozen chickens, not only in New South Wales but in all other States where Woolworths had a strong retail presence. As the business expanded, a regular feed supply arrangement was established with Allied Feed Mills.

In June 1965, the partnership of Inghams Enterprises was formed into a proprietary company in Canberra. The initial shareholders were Jack and Bob Ingham, and they remained the holders of the entire ordinary share capital of the company. In 1966, they produced a 30-minute colour film called *Chicken City* based on their own operations, as a demonstration of the development that had taken place in the industry. This was premiered at the AMP Theatrette, Circular Quay, before a distinguished audience of government, industry, retail and commercial organisations. It was shortly after this that interstate expansion commenced. An offer was made to the Poultry Farmers' Co-Operative Society Ltd in Queensland for the sale of its processing plant at Park Ridge. Subsequently, the brothers purchased a hatchery at Wynnum and its associated breeding farm at Cleveland.

Kentucky Fried Chicken (now KFC) commenced operating in Australia in 1968 and a long association with Inghams resulted. Bob Lapointe and then Hilton Coops helped expand the business through company and franchised stores. Over the years, the major fast food groups have also aligned themselves with the market leader.

Australian Poultry Industries Association, Level 7, 122 Walker Street, North Sydney NSW 2060

AMATIL, through its subsidiary Associated Products and Distribution Pty Ltd, was also vigorously buying food companies throughout Australia. Realising their vulnerability in Victoria, both Inghams and AMATIL bid strongly for the Golden Poultry Farming Industries organisation controlled by Max Nelson and Don Moy. They became joint owners in what was a 20-year partnership embracing the farming and processing operations of Windsor Poultry Services and Wy Wurry hatchery in South Australia, Glenila Poultry in Tasmania and finally in 1972, by a share re-arrangement, the Diamond Foods company in Western Australia. In each instance the Tegel franchise to grow and supply day-old meat chickens was retained. A year later, Inghams further consolidated its position in South Australia with the takeover of the Chickmaster and Peter Simon hatcheries, the Whiting and Chambers feed-mill at Mile End and the processing plants of Achilles at Bolivar and Murrayland Producers at Murray Bridge.

On 30 June 1978, Inghams acquired the Mountain View Distributors business from Allied Mills Industries Limited. It also acquired the SPB layer which allowed them to develop further the market for a commercial layer.

Just as in the earlier days of their development, when the brothers realised the importance of securing their source of supply of genetically superior breeding stock, so also did they appreciate that control over feed-milling and raw material costs was essential if they were to remain competitive. Two of the most modern feed-mills in Australia were constructed at Clyde on the Westernport Peninsula in Victoria (1979) and at Cardiff in NSW (1982). These, along with five other mills around Australia, turned wheat, oats, sorghum, soya and other materials into finished feed for the billions of birds the company reared and fed.

In July 1980, the company acquired Eurunderee Stud in NSW and later that year, Inghams consolidated its position in Victoria with the purchase of Cester Poultry and Pappas Poultry Pty Ltd. The same year a decision was made to establish smallgoods and further processing operations in both Queensland and New South Wales.

During the 1980s and 90s, a number of further acquisitions and facility upgrades took place. These included a Product Development Centre with further processed production capacity in New South Wales, a quarantine farm in Bungonia New South Wales, a new processing plant in South Australia and significant upgrading of processing facilities in Victoria and New Zealand. The Company builds Australia's largest and most sophisticated processing plant at Murrarie on the outskirts of Brisbane in 2001. The Group had relocated its Head Office to a multi-story office block in Liverpool in 1987.

INDUSTRY SERVICE

In 1973 the Ingham brothers won the WPSA Australian Poultry Award for service to the poultry industry, not simply for the success of their company, but also for their tireless involvement in industry matters. The Ingham brothers were both members of the New South Wales Breeders and Hatcherymen's Association from 1954 - 1970 and Jack was President from 1961 - 1963. Established in 1944, the B & H Association was the most significant industry association during this period. It effectively spawned the NSW Chicken Meat Council in 1964 and Jack was its representative on the Council. This Council was a prime mover in the establishment of the Australian Chicken Meat Federation in 1965.

The National Federation, due partly to its structure, ran into problems that it could not solve and a substantial oversupply situation was reached throughout the industry in the late 1960s. The ultimate outcome was the formation, in 1968, of the Australian Poultry Industries Association (APIA). This was an Association of the nine major integrated chicken meat companies with representation by the managing directors. Jack Ingham was the foundation

Chairman and he chaired APIA continuously until August 2003. His ability to reach a consensus on issues was legendary, showing his great interpersonal skills and perseverance.

During this period Jack worked tirelessly for the industry generally, becoming involved in numerous delegations to Federal and State Ministers, Convention attendance and involvement in industry seminars. On one such occasion, in October 1979 he gave the opening address at an APIA seminar related to meat inspection and microbiological testing. He gave away his love for his other passion when he said "I don't know a lot about microbiological testing, but "microbiological" - what a great name for a race horse!"

The Inghams were strong supporters of poultry research from the earliest days. The poultry Husbandry Research Foundation of the University of Sydney, established in 1959 was a recipient of Ingham funding from day one, through the NSW B & H Association. Such funding has continued unabated and Inghams Enterprises is a governor of the Poultry Research Foundation. As well as establishing their own research programs - in processing, nutrition, avian health, genetics, product development and marketing, Jack and Bob were keen to support the establishment of the Australian Chicken Meat Research Council in 1969 and were prepared to support, financially and with senior staff, the formation of the Australian Poultry Co-operative Research Centre in 2002.

In 2003 Jack and Bob Ingham were both awarded the Order of Australia (AO) for, among other things, their service to the Australian poultry industry, thoroughbred racing and the community.

Jack's family saw him as devoted, supportive and immensely generous. He and his brother Bob extended this generosity to others in many ways, contributing not with fanfare but with compassion and pride to many charities and causes to help those less fortunate than themselves. They established the Ingham Institute of Medical Research at Liverpool Hospital, an Orthopaedic Fellowship at the Children's Hospital at Westmead and make annual contributions to many others.

At his funeral at St Andrew's Anglican Cathedral on the 12th August 2003 his eldest son Walter said: "Jack was a person who loved to be around people, to be with them in person or talking to them. He could not get enough contact. He wanted every opportunity to embrace life and openly share his life with you. He was a rare and special person who had the gift of instantly connecting to you with warmth that most of us can only wonder at and admire."

These words are echoed by all who knew him.

NEURAL CONTROL OF RATITE SKIN

K.A. WEIR AND C.A. LUNAM

Summary

Double-labelling immunohistochemistry combined with fast blue retrograde tracing identified subclasses of cutaneous neurons innervating specific targets within feathered emu skin. We report chemical coding in sensory and sympathetic neurons is different in ratites compared to volant birds. In the emu, Herbst corpuscles are innervated by axons immunoreactive (IR) for neuropeptide Y (NPY) and calcitonin gene-related peptide (CGRP), and negative for calbindin IR. Calbindin IR was exclusive to dorsal root ganglia neurons. Some sensory neurons displayed IR for tyrosine hydroxylase. Substance P (SP) and CGRP, although they coexisted exclusively in sensory neurons, were found individually in sensory and sympathetic neurons. Free nerve endings were identified in the superficial dermis co-labelling for SP and CGRP. The welfare implication of cutaneous innervation is discussed.

I. INTRODUCTION

The skin is the focus of welfare issues in birds such as feather pecking and abrasion. Avian skin undertakes a number of functions that include thermoregulation, protection from abrasion and infection, water loss and proprioception. Each of these functions is conducted by specialized anatomical features, whose function is under direct neural control.

Electrophysiological and anatomical studies have shown that sensory and sympathetic nerves each innervate specific targets in the skin of mammals (Iggo, 1977; Gibbins, 1997) and birds (Langley, 1904; Necker, 2000). In mammals specific classes of sensory and sympathetic nerves identified by different combinations of peptides/proteins/amines are highly target specific. This phenomena is termed “chemical coding” (Costa *et al.*, 1986). It is not known if chemical coding also occurs in avian skin.

Volant bird skin contains mechanoreceptors such as Herbst corpuscles, Grandry corpuscles, Merkel cell receptors and Ruffini endings, which are all found in the featherless regions (Necker, 2000). In feathered skin Herbst corpuscles are associated with the feathers which suggests that the feathers themselves are proprioceptive via neural connections between the Herbst corpuscle and the follicle sheath and/or by direct contact of the follicle sheath with the Herbst corpuscle.

Although the phylogeny of ratites has been the focus of many morphological, biogeographical and molecular studies for more than a century, the evolutionary relationship of ratites and volant birds remains controversial (for a review see Sibley & Ahlquist, 1990). Recent studies have indicated the importance of integumentary characteristics for phylogenetic reconstructions of bird species (Chu, 1998; Bertelli *et al.*, 2002). A known integumentary characteristic of ratites is a single, simple and unique feather type that covers the entire body excluding the legs (Huxley, 1867; McGowan, 1989). It is not known whether the presence of a unique feather type is coupled with any unique integumentary features. Furthermore, it is not known whether free nerve endings exist in the epidermis of ratites analogous to the skin of mammals (Munger and Ide, 1988) and volant birds (Sann *et al.* 1996). Sann *et al.* (1996) reported colocalisation of substance P (SP) and calcitonin

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generelated peptide (CGRP) immunoreactivity within nerve fibres in the epidermis and dermis of feathered chicken skin. Nerves of this type in other vertebrates are considered to be nociceptive, that is, capable of transmitting impulses interpreted as pain.

The current study aimed to describe structures within emu skin and to identify, using double-labelling immunohistochemistry, the different classes of cutaneous nerves that innervate them. Furthermore we aimed to determine if these nerves demonstrate "chemical coding" as in other vertebrates. We were particularly interested to identify any free nerve endings in the epidermis as well as the anatomical and neural relationship between feather follicles, Herbst corpuscles, and pennamotor muscles.

An offshoot of this work is that it may address welfare issues associated with feather removal in the domestic fowl. Feather removal as a result of pecking and abrasion in the domestic fowl, is likely to damage the follicular sheath and injure any innervating nerves. Mapping the types of nerves innervating the feather follicle and its associated structures in the ratite, assuming their chemical coding and distribution is analogous to volant species, will assist in evaluation of the effect of feather pecking and abrasion on the welfare of the birds in terms of potential pain and loss of proprioception.

II. MATERIALS AND METHODS

(a) Double-labelling immunohistochemistry within the skin

Ten emu chicks, 3 to 7 days of age, were euthanased by halothane overdose and the feathers trimmed adjacent to the wing. Skin from the trimmed region was excised and prepared for immunohistochemistry as described previously (Lunam, 1989). The tissue was rapidly frozen and sections (10 µm thickness) were then double-labelled using antibodies raised against a variety of neuropeptides/proteins/amines (Table 1). Sections were incubated with a combination of two primary antibodies at room temperature for 48 hrs. These were then removed and sections incubated with appropriate secondary antibodies at room temperature for 2 hrs [donkey anti-rabbit Cy5 (Jackson), donkey anti-rabbit FITC (Jackson), swine anti-goat fluorescein (Tago), donkey anti-goat Cy3 (Jackson), goat anti-mouse FITC (Boehringer), donkey anti-mouse FITC (Jackson)]. Primary antibody combinations were chosen according to chemical coding of different sensory and sympathetic subclasses of cutaneous nerves in other vertebrates. Specificity testing revealed no inappropriate binding between the primary and secondary antibodies.

Table 1. Primary antibodies for double-labelling immunohistochemistry.

Host	**Target antigen**	**Dilution**	**Source**
Rabbit	Substance P (SP)	1:1000	Auspep
Goat	Calcitonin gene-related peptide (CGRP)	1:1000	Arnel/Biogenesis
Mouse	Tyrosine hydroxylase (TH)	1:200	Incstar
Rabbit	Calbindin D 28k (Calb)	1:1000	Chemicon
Sheep	Neuropeptide Y (NPY)	1:1000	R. Rush

(b) Retrograde tracing

The retrograde tracer fast blue (FB) (Dr Illing GmbH & Co.) was used to trace the origin of nerves innervating the skin adjacent to the wing. Fast blue when injected into the skin is actively taken up by nerve terminals and transported retrogradely to the cell body (Köbbert *et al.*, 2000). Fast blue can then be visualized within the dorsal root ganglia and sympathetic chain ganglia using an ultraviolet filter. Three emu chicks (3 days of age) were injected with FB. The skin behind the right wing was swabbed with Betadine® and 3µl of 2%

FB in 10% DMSO in sterile water was injected into the skin using a fine glass micropipette. To allow FB to be retrogradely transported from the axon terminals in the skin to the cell bodies in the dorsal root ganglia and sympathetic chain ganglia, a survival time of 11-12 days after injection was used. The chicks were deeply anaesthetized with halothane and the dorsal root ganglia and sympathetic chain ganglia, ipsilateral to the injected skin and at the level of the spinal cord between the third and fourth ribs, were removed and the chicks then euthanased by an overdose of halothane. Preliminary work demonstrated that this level of the spinal cord has the greatest number of dorsal root ganglia and sympathetic chain ganglia nerve cells that project to the wing region.

In order to increase the levels of antigens within the nerve cell bodies of the dorsal root ganglia and sympathetic chain ganglia, the ganglia were incubated in colchicine (0.1g/L) (Sigma) in N_2 culture medium (Sigma) bubbling with medical air (2L/min) for a minimum of 20 hours at 37°C and processed for immunohistochemistry as described above. The tissue was rapidly frozen and 20µm serial sections were cut through the entire ganglia. The sections were double-labelled according to the procedure described by Lunam (1993), with the use of the following primary antibody combinations raised against SP/CGRP, SP/TH, TH/CGRP, TH/NPY and Calb/NPY (Table 1).

All sections were examined using an AX70 Olympus fluorescence microscope and micrographs were taken with a Hamamatsu ORCA cooled CCD camera and acquired using a Macintosh computer with IP Lab (Scanalytics, Inc.). Sections were examined using X4, X10, X20, X40 and X100 objective lenses. Fast blue labelling and immunoreactivity (IR) to various antigens were visualised by the use of multiple filters specific for each label. Individual neurons within the dorsal root ganglia and sympathetic chain ganglia were classified as either negative, that is no label, or positive for a single, double or triple label.

III. RESULTS

(a) Immunoreactivity within the skin

Immunoreactivity of nerves within each skin layer is shown in Table 2. No IR was present within the epidermis for any of the antigens examined. SP/- IR fibres were present within the superficial dermis (*stratum superficiale*). This layer, situated deep to the basement membrane, is approximately 20 µm thick and is composed of numerous capillaries and dense collagen bundles. Deep to the *stratum superficiale* is a layer of dense collagen approximately 200 µm in thickness, the *stratum compactum*. Both SP/- IR and CGRP/- IR nerves were found within nerve bundles in the *stratum compactum*. Individual SP/CGRP IR axons were observed within the *stratum superficiale, stratum compactum* and within nerve bundles throughout the extent of the *stratum compactum* and the *stratum laxum*. Within the *stratum superficiale,* SP/CGRP IR axons were often located adjacent to the dermal-epidermal border. The *stratum laxum* ranges from 0.4 to 1 mm in thickness and consists predominantly of adipose tissue. TH/SP IR was colocalised in individual axons within the *stratum laxum*. Calb/- IR and NPY/- IR nerves were observed within individual nerve fibres in the *stratum compactum* and within nerve bundles of the *stratum laxum*.

Table 2. Immunoreactivity of nerves within each layer of emu skin.

Immunoreactivity	**Epidermis**	***Stratum superficiale***	***Stratum compactum***	***Stratum laxum***
SP/- IR	-	+	+	+
CGRP/- IR	-	-	+	+
SP/CGRP IR	-	+	+	+
TH/- IR	-	-	-	+
SP/TH IR	-	-	-	+
Calb/- IR	-	-	+	+
CGRP/Calb IR	-	-	-	-
Calb/NPY IR	-	-	-	-
NPY/- IR	-	-	+	+
TH/NPY IR	-	-	-	-
TH/CGRP IR	-	-	-	-

+ indicates immunoreactivity present
- indicates no immunoreactivity detected

Nerves associated with structures within emu skin are shown in Table 3. TH/- IR nerves were observed within the *Mm. pennales* (pennamotor muscles). These muscles attach to the follicle sheath at various points along its extent. Muscle attachments are present in both the *stratum compactum* and *stratum laxum.* CGRP/- IR and NPY/- IR nerves were found within the *Mm. pennales* bundles and innervating Herbst corpuscles. Herbst corpuscles were located adjacent to the follicle sheath. SP/- IR was present in pennamotor muscles and perivascular nerves within both the *stratum compactum* and *stratum laxum.* SP/- IR and Calb/- IR nerves abutted the follicle sheath. No Calb/NPY IR or TH/CGRP IR was observed within emu skin. No neural connections were observed between the follicular sheath, Herbst corpuscle and pennamotor muscles.

Table 3. Immunoreactivity of nerves associated with structures within emu skin.

Immunoreactivity	**Herbst corpuscle**	***Mm. pennales***	**Perivascular**	**Perifollicular**
SP/- IR	-	+	+	+
CGRP/- IR	+	+	-	-
SP/CGRP IR	-	-	-	-
TH/- IR	-	+	-	-
SP/TH IR	-	-	-	-
Calb/- IR	-	-	-	+
CGRP/Calb IR	-	-	-	-
Calb/NPY IR	-	-	-	-
NPY/- IR	+	+	+	+
TH/NPY IR	-	-	-	-
TH/CGRP IR	-	-	-	-

+ indicates immunoreactivity present
- indicates no immunoreactivity detected

(b) Triple labelling in the dorsal root ganglia and sympathetic chain ganglia

Table 3 shows the presence or absence of each combination of antigen for each dorsal root ganglia and sympathetic chain ganglia. SP/- IR was found within neurons in either the dorsal root ganglia or sympathetic chain ganglia. No nerve cell bodies were found that showed labelling for both FB and SP in the sympathetic chain ganglia. In addition neurons showing triple labelling for FB/SP/CGRP were only observed within the dorsal root ganglia. CGRP/- IR and FB/CGRP IR were observed within neurons in either the dorsal root ganglia or sympathetic chain ganglia. No Calb/- IR neurons were found in the sympathetic chain

ganglia. Although no Calb/NPY IR was found within nerves in the skin, FB/Calb/NPY was observed within nerve cells bodies of the dorsal root ganglia.

Table 4. Labelling of neurons in the dorsal root ganglia and sympathetic chain ganglia

Immunoreactivity/fluorescence	**Sympathetic chain ganglia**	**Dorsal root ganglia**
FB/-	+	+
SP/-	+	+
FB/SP	-	+
CGRP/-	+	+
FB/CGRP	+	+
FB/SP/CGRP	-	+
SP/CGRP	-	+
TH/-	+	+
FB/TH	+	+
FB/SP/TH	-	+
SP/TH	-	+
FB/TH/CGRP	+	+
TH/CGRP	+	+
NPY/-	+	+
FB/NPY	+	+
FB/TH/NPY	+	+
TH/NPY	+	+
Calb/-	-	+
FB/Calb	-	+
FB/Calb/NPY	-	+
Calb/NPY	-	+

+ indicates immunoreactivity present
- indicates no immunoreactivity detected

NPY/- IR was observed within neurons in either the dorsal root ganglia or sympathetic chain ganglia. FB/TH/NPY IR, TH/NPY IR, FB/TH/CGRP IR, and TH/CGRP IR occurred within neurons in either the dorsal root ganglia or sympathetic chain ganglia. Triple labelling for FB/SP/TH IR was identified within neurons in the dorsal root ganglia.

IV. DISCUSSION

Chemically distinct subclasses of sensory and sympathetic neurons within the brachial dorsal root ganglia and sympathetic chain ganglia respectively were identified in the emu. The presence of FB in subpopulations of dorsal root ganglia and sympathetic chain ganglia neurons suggests that each of these neuron classes project to the injected region of skin. It is of interest that the same coding was found in six subpopulations of neurons in both the dorsal root ganglia and sympathetic chain ganglia, that is SP/, CGRP/-, TH/-, TH/CGRP, NPY/-, TH/NPY immunoreactive neurons. In contrast, Calb/-, Calb/NPY, SP/CGRP, SP/TH immunoreactive neurons were found exclusively in the dorsal root ganglia. Cutaneous axons were identified that display immunoreactivity for each of the above combinations except TH/CGRP, TH/NPY and Calb/NPY. Distinct peripheral target projection patterns of the chemically identified neurons were also demonstrated. Our findings in the emu of distinct subpopulations of target specific dorsal root ganglia and sympathetic chain ganglia neurons is consistent with similar findings in many vertebrate species (for reviews see Gibbins, 1997; Willis and Coggeshall, 1991).

In the current study individual SP/CGRP IR axons were located in the *stratum compactum* and in the *stratum superficiale* near the dermal-epidermal border. This chemical coding (SP/CGRP) was found exclusively within a subpopulation of dorsal root ganglia neurons that also showed fluorescence for the retrograde tracer fast blue. This suggests that

the SP/CGRP IR axons within the skin are afferent projections of sensory neurons. This finding is consistent with that of Sann *et al*. (1996) who reported a population of SP/CGRP IR cutaneous nerves in the domestic fowl. However in contrast to the domestic fowl (Sann *et al.*, 1996) and mammals (Munger and Ide, 1988), no SP/CGRP IR nerve fibres were observed within the epidermis of the emu. The absence of intraepidermal free nerve endings in our study may be due to a variety of reasons. These include firstly that peptide levels within the axons may be below detectable levels, secondly fibres may be scarce thus an insufficient number of sections were examined, or thirdly they may be absent. Electrophysiological studies have provided evidence that dermal/epidermal free nerve endings in vertebrates are either thermoreceptive or nociceptive (Willis and Coggeshall, 1991). Furthermore it is known that this subclass of fibre consist of either unmyelinated free nerve endings (C fibres) or small Aδ-fibres (Necker and Reiner, 1980; Willis and Coggeshall, 1991). Lunam and Gentle (2004) demonstrated that painful gouty arthritis produces a depletion of substance P from peripheral nerve fibres in the synovial and subsynovial tissue of the chicken ankle joint. As it is well established in vertebrates that these axons are projections of the smallest nerve cell bodies within dorsal root ganglia, we would expect that this subpopulation of sensory neurons within the emu would have the smallest cell bodies. Preliminary work in our laboratory using immunohistochemistry and retrograde tracing suggests that this is the case.

The follicular sheath was surrounded by three distinct subpopulations of axons, displaying SP/- IR, Calb/- IR or NPY/- IR. Calbindin positive neurons were found exclusively within the dorsal root ganglia whereas SP/- IR and NPY/- IR neurons were found in subpopulations in both the dorsal root ganglia and sympathetic chain ganglia. This suggests that the Calb/- IR axons are sensory afferents whereas SP/- IR and NPY/- IR may be either sensory or sympathetic axons.

Herbst corpuscles were innervated by axons displaying immunoreactivity for NPY or CGRP. Immunoreactivity for calbindin was never observed in nerves innervating Herbst corpuscles. This finding is in contrast to volant birds where Herbst corpuscles are innervated by calbindin positive axons (Duc *et al.*, 1993a, 1993b; Chouchkov *et al.*, 2002). This suggests that the NPY/- IR and CGRP/- IR dorsal root ganglia neurons that also label for fast blue are somatosensory neurons whose cutaneous projections innervate the Herbst corpuscles. Due to the IgG of the antibodies available to us, we were not able to label for the presence of both NPY and CGRP in the same section. However, as all nerves innervating Herbst corpuscles labelled with the appropriate antibody this supports the notion that the Herbst corpuscles are innervated by a single subclass of somatosensory neuron containing both NPY and CGRP. These are presumed to be the large diameter myelinated Aβ-fibres.

In the current study Calb and NPY were found to be colocalised in the same dorsal root ganglia neurons. This is in contrast to the domestic chicken where calbindin was found not to be colocalised with neuropeptide Y in the brachial dorsal root ganglia (Lunam, 1988). As we could not identify fibres within the skin with this chemical coding, the peripheral projections of this class of sensory neurons is unknown. However the presence of FB in Calb/NPY IR dorsal root ganglia cell bodies suggest that the neurons project into the deeper subcutaneous layers of the injected region which were not examined in this study.

As TH is the rate-limiting enzyme in catecholamine synthesis, the population of TH negative neurons within the emu sympathetic chain ganglia may be cholinergic and/or peptidergic employing acetylcholine and/or neuropeptide neurotransmitters. Consistent with this notion, sympathetic ganglia containing cholinergic and adrenergic neuronal populations have been reported in several vertebrate species (reviewed by Ernsberger and Rohrer, 1999). We speculate that a population of sympathetic chain ganglia neurons may have shifted neurotransmitter phenotype from adrenergic to cholinergic during development as described

in chick sympathetic ganglia. Current work is underway to determine whether these TH negative neurons label for choline acetlytransferase.

TH/- IR was found within a small population of neurons in the dorsal root ganglia of the emu. A similar finding was also reported in dorsal root ganglia neurons of the rat (Price and Mudge, 1983; Vega *et al.,* 1991). As these neurons were found not to contain dopamine-β-hydroxylase it was suggested that these neurons synthesise dopamine and thereby use dopamine as their neurotransmitter. In addition dopamine has been identified in dorsal root ganglia neurons and in fibres surrounding the follicular sheath of the domestic chicken (Phillipe *et al.,* 1993). In the current study we did not identify any perifollicular TH/- IR fibres suggesting that these TH/- IR afferent fibres from the dorsal root ganglia neurons do not project to the follicular sheath in the emu.

This study is the first to report chemical coding of pennamotor nerves. Axons displayed IR to TH/-, SP/-, CGRP/- or NPY/- within pennamotor muscles. Double-labelling immunohistochemistry revealed that TH was not colocalised with any of the three peptides and no fibres were found within the pennamotor muscles that contained both SP and CGRP. Therefore these axons are projections from at least three distinct subpopulations of neurons. Previous work has determined that feather movement is controlled by sympathetic neurons (Langley 1904; Jeikowski and Drenkhahn, 1981). In the current study, four distinct chemical codes were identified within neurons in both the dorsal root ganglia and sympathetic chain ganglia, therefore the nerves within the pennamotor muscles of the emu, may be either sensory or sympathetic in origin.

In conclusion, this work has demonstrated specific subclasses of sensory and sympathetic neurons that project to specific targets within emu skin. Numerous species differences in the chemical coding of neurons between ratites and volant birds were identified. Free nerve endings exist with the same chemical coding as thermoreceptive and nociceptive nerves in the domestic fowl and other vertebrates. Consequently any abrasion to the skin could result in either acute or chronic pain from stimulation of these nerves. We have identified at least one population of perifollicular sensory afferents and other populations of neurons containing either substance P or neuropeptide Y. Future electrophysiological work is required to determine the function of these perifollicular nerves and how they communicate with the adjacent Herbst corpuscle. As it is generally accepted that the relative percentage of a subclass of peripheral neurons is directly correlated with their functional importance, ongoing work in our laboratory is quantitating the percent contribution of each neuronal subpopulation of dorsal root ganglia and sympathetic chain ganglia. Furthermore, as neuronal cell body size has been shown to directly correlate with function in other vertebrates, we are determining the neuronal cell body area for each chemically identified neuron subpopulation.

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EFFECTS OF CONVENTIONAL AND NOVEL PROCESSING ON THE FEED VALUE OF CANOLA MEAL FOR POULTRY

H. L. CLASSEN, R. W. NEWKIRK and D. D. MAENZ

Summary

The desolventisation/toasting stage of pre-press solvent extraction was found to reduce the digestible amino acid content of canola meal in broilers. In addition, inconsistency in this stage of processing was also shown to be responsible for variability in this trait. Although toasting reduces the levels of toxic glucosinolates, broilers fed toasted meal had reduced performance in comparison to birds fed non-toasted meal. Neutral detergent insoluble nitrogen in canola meal samples proved to give a reasonable estimate of meal quality. *In vitro* experiments demonstrated that elevated temperature and meal moisture during desolventisation/toasting were responsible for protein damage. In conclusion, toasting that occurs during conventional pre-press solvent extraction can cause detrimental effects on canola meal quality and may not be necessary for the production of meal for poultry.

I. INTRODUCTION

The objectives of this paper are to discuss various aspects of processing that can influence the nutritional value of canola meal and to describe an alternate method of processing that has potential to increase the utilisation of canola nutrients. To set the stage for this discussion, it is appropriate to briefly describe the Canadian canola industry and the factors that led to increased research in the area of canola processing.

Rapeseed was introduced into Canada as a crop in the 1940's as a method of diversifying crop production, particularly in the prairie provinces. Production was initially designed to provide oil for industrial rather than food purposes and the seed was characterised by high levels of erucic acid (23-45% of oil) and glucosinolates (120 to 150 μmol/g total aliphatic glucosinolates on an oil free, dry basis). Post World War II, the demand for rapeseed declined dramatically and alternate uses were pursued. One of the major success stories of Canadian agricultural research was the reduction of erucic acid and aliphatic glucosinolates to less than 2% and 30 μmol/g of oil free meal, respectively. To identify this improved version of rapeseed, the term canola was introduced. This has subsequently been internationally recognised as a name for "double zero" cultivars of rapeseed. Canola is by far the most important oilseed grown in Canada and production has ranged from 5 to 9 million tonnes in recent years. Approximately 50% of seed is processed in Canada with the remainder exported. The products of Canadian processing are used within country or exported, with the majority of exports being to the United States.

The meal derived from canola seed is marketed as a feed ingredient to a wide range of animal species including chickens, turkeys, pigs and cattle. For poultry, canola meal is considered to have moderate to high protein level (34-39%), good amino acid balance, a relatively low apparent metabolisable energy (AME) and high levels of phytate (3%). Low and variable amino acid digestibility (NRC, 1994) are also characteristic of canola meal and may be responsible for its relatively lower than expected value (55-65%) in relationship to soybean, despite having approximately 75% of the protein content. The low price of canola meal in some markets, and poor and variable amino acid digestibility in non-ruminant species

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led to research on the effect of processing on the nutritional value of canola meal for broiler chickens. An alternate method of processing was also investigated that results in more effective utilisation of canola nutrients by a wider range of species.

II. CANOLA PROCESSING

Canola seed in Canada is virtually all processed using pre-press solvent extraction. This procedure uses screw presses to extract a portion of the oil and then removes the remainder of the oil by solvent extraction. An example of pre-press solvent processing of canola seed is shown in Figure 1. The initial step in processing is the removal of major non-canola seed materials (screenings, often added back to meal after processing). The seed is then pre-conditioned by drying (to 6-7% moisture) and heated to 75-78°C to prevent seed shattering and improve processing. Flaking then ruptures the seed coat and some oil cells prior to cooking (75-85°C for 20 to 60 min). The latter step denatures hydrolytic enzymes such as myrosinase and further ruptures oil cells. Destruction of myrosinase is essential to prevent hydrolysis of glucosinolates to more toxic and undesirable sulphur compounds. Pressure expelling then removes from 60 to 70% of the oil prior to solvent extraction with hexane. Meal exiting hexane extraction has low levels of oil and is laden with hexane (35%). The meal then enters the desolventisation/toasting (DT) stage of processing which uses a vertical column with multiple trays to heat the meal. Hexane is evaporated from the meal as a result of the indirect heat of the heated trays as well as by direct heat from the injection of steam (sparge steam) into the meal in the final lower trays. The temperature increases as meal proceeds from tray to tray, being relatively low at higher trays because of hexane evaporation but reaching temperatures of 100 to 110°C in the final trays due to steam injection. Condensation of steam increases the meal moisture content to 16-18%. Moisture can also enter the DT stage via water sprayed on the upper tray to control dust and water found in gums that may be returned to the meal at this stage. This stage also "toasts" the meal to reduce the level of anti-nutritional glucosinolates and possibly other undefined factors. The meal is then dried and cooled, and possibly ground and pelleted.

(a) Effect of processing stage on nutritional value of meal

In order to study the impact of various stages of processing on meal nutritional quality, samples were collected on three separate occasions from a single commercial processing plant. On each collection, samples were collected after six stages as outlined in Figure 1 and then used to study meal nutrient content as well as ileal apparent amino acid digestibility and fecal AME using broiler chicks (Newkirk *et al.*, 2003a). With the exception of AME changes associated with oil extraction, only minor effects on nutritional value were seen up to and including solvent extraction. However, DT reduced the lysine (LYS) content of the meal (6.03 to 5.50% of crude protein) and the digestibility of all amino acids except for alanine (ALA), glycine (GLN), serine (SER) and tyrosine (TYR). Of particular interest was the digestibility of LYS which decreased from 87 to 79%. In addition the coefficient of variability for LYS digestibility increased from 1.4% before to 5.6% after DT, supporting the concept that this stage of processing is not only responsible for decreasing the digestible amino acid content but also increasing the variability. Meal AME decreased as oil was removed during the expelling and solvent extraction phases and there was a non-significant reduction after the DT stage. Canola meal coloration also was affected by stage of processing with meal being yellow with black hull flecks and dark brown prior to and after DT, respectively. Maillard and protein-polyphenolic reactions are likely responsible for meal darkening.

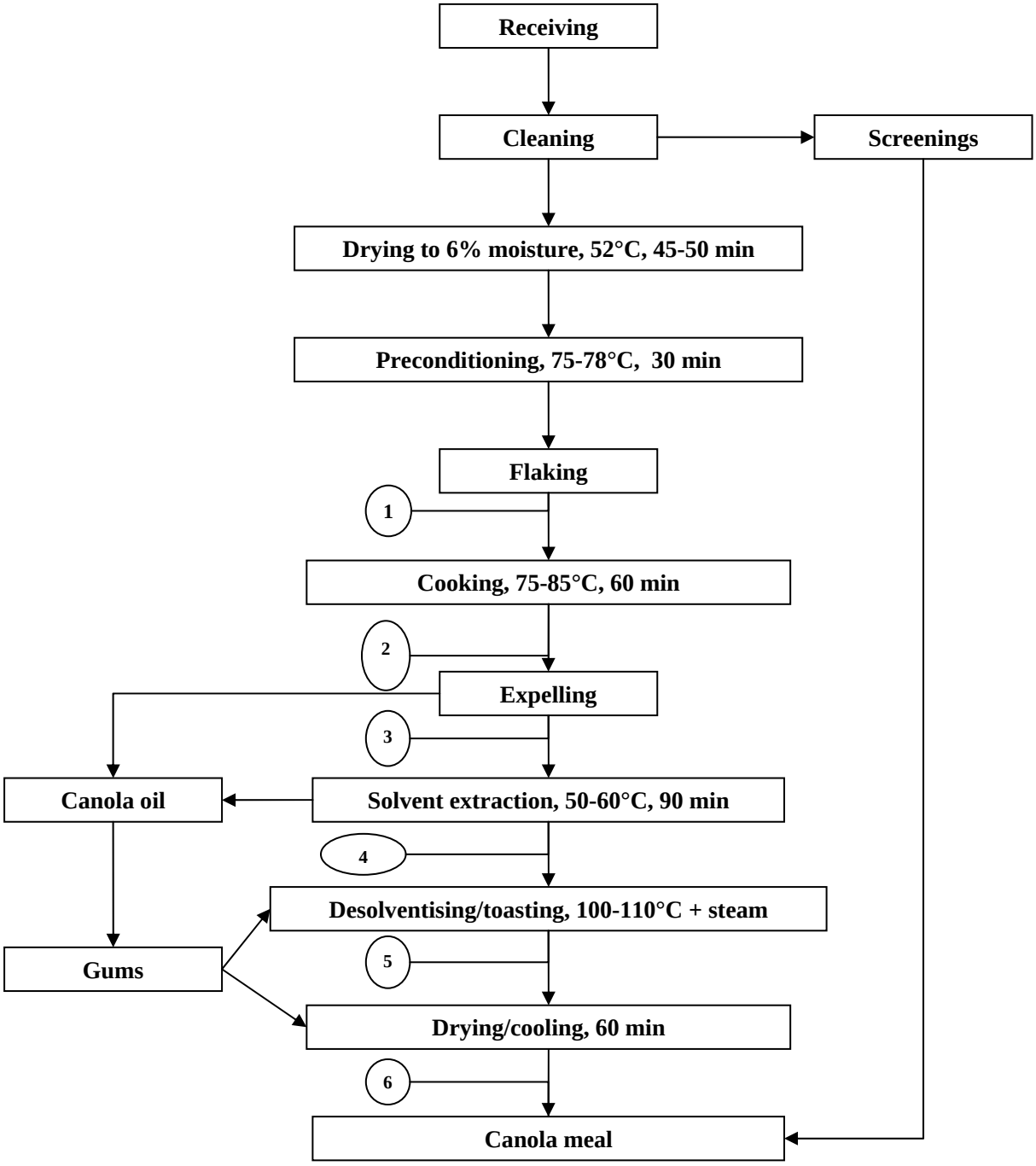

Figure 1. Example of pre-press solvent extraction of canola seed. Circled numbers represent points of sample collection for evaluation of amino acid availability (Newkirk *et al.*, 2003a).

A second experiment was completed to verify the impact of DT on meal quality in seven western Canadian processing plants and also study the effect of this process on glucosinolate content (Newkirk *et al.*, 2003b). Samples were collected before (non-toasted canola meal – NTCM) and after DT (toasted canola meal – TCM) at the same time to reduce the potential for variability due to seed source. Samples were collected on five separate occasions from each of five plants while only a limited number of samples were available

from the remaining two processors. NTCM samples were air desolventised prior to testing. Prior to DT, samples were relatively uniform in amino acid content and digestibility but as had been shown in the previous study, DT reduced the content and/or digestibility of amino acids in the meal to varying degrees. As a consequence, DT reduced the digestible content of all amino acids measured (not including TYR and TRP) with the exception of GLU. The total digestible amino acid content of the TCM samples (69.6g/16 g N; range 56.6-75.6) was on average 12% lower than for the NTCM samples (77.6 g/16g N; range 73.1-82.0). As expected, LYS was affected more than other amino acids. The average LYS content and apparent digestibility of NTCM samples was 6.0 g/16g N (range 5.7-6.3) and 89.75 (range 87-92%), respectively. After DT, LYS content and digestibility values were 5.6 g/16 g N (range 5.3-5.9) and 78.6% (range 65.5-85.7%), respectively. Significant effects of processing plant, and interactions between processing plant and DT demonstrated that considerable variability occurs both between and within plants. This is demonstrated by LYS digestibility values for all collected samples (26 non-toasted and 31 toasted) which are shown in Figure 2. DT reduced the levels of aliphatic glucosinolates from 10.5 (range 7.8-15.1) to 6.16 (range 1.2-12.5) μmol/g meal. These are well below the maximum standard set for canola meal and the nutritional significance of these levels remains to be determined but is likely low.

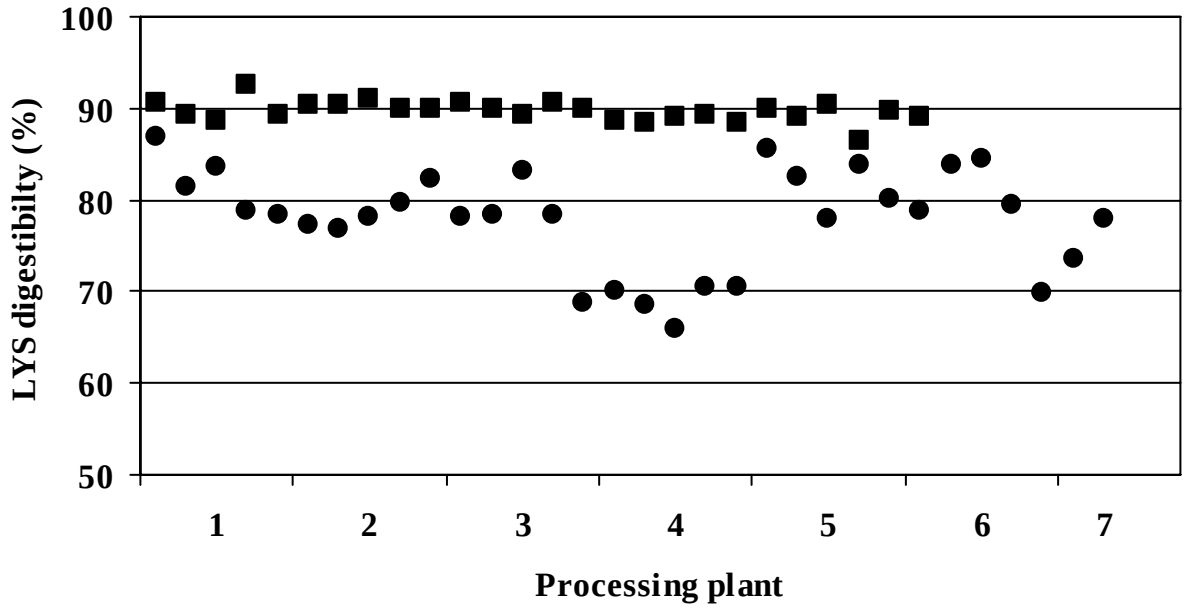

Figure 2. Apparent LYS digestibility (%) of non-toasted (■ NTCM, n=26) and toasted (● NTCM, n=31) canola meal samples.

In conclusion, the data from these experiments demonstrate the importance of DT in determining the nutritional value of canola meal, both in terms of level and variability in digestible amino acid content. The negative effects of this stage of canola processing are likely related to the temperature and moisture content of the meal during this stage and the length of time to which the meal is exposed to these conditions. The loss of content and digestibility can be affected by a number of reactions including various stages of Maillard reactions, protein/polyphenolic reactions and protein/protein reactions. Maillard reactions are likely predominant and have been broken down into early, advanced and final reactions (Mauron, 1981). Final reactions result in decomposition of involved amino acids and as such would not be detected by amino acid analysis. Products of early and advanced reactions

would be measured but would be unavailable for digestion. This corresponds with our results in terms of reduced amino acid content and reduced apparent digestibility, and suggests that various stages of Maillard reactions are occurring during DT.

III. NON-TOASTED AND TOASTED CANOLA MEAL IN BROILER DIETS

Despite the apparent nutritional advantage of non-toasted vs. toasted meal in terms of digestible amino acid content, there may be other advantages of DT that would become evident in an animal feeding trial. To provide samples to allow this comparison, NTCM and TCM samples were obtained from a commercial processor on the same day to minimise differences in originating seed (Newkirk and Classen, 2002). The NTCM sample was desolventised using a pilot scale desolventiser/toaster equipped with two steam heated trays but without the addition of steam directly into the meal. The rate of flow through the desolventiser/toaster was adjusted so that the exit temperature was approximately 100°C. It was hypothesised that the use of low heat application and no moisture being added in the form of sparge steam would result in meal that was not toasted or toasted only to a limited extent. The final meals were compared on the basis of colour (NTCM – yellow, TCM – light brown), and neutral detergent insoluble nitrogen (% of N; NTCM – 11.31, TCM – 19.73) and aliphatic glucosinolate content (μmol/g; NTCM – 11.52, TCM – 7.84). The results confirm that the NTCM was not toasted and suggest that the TCM was relatively mildly toasted.

Commercial broilers (total of 3240) were used to compare the feeding value of the NTCM and TCM meals. Meal samples replaced 0, 20, 40, 60, 80 and 100% of the soybean meal in a wheat-soybean meal based diet with diets formulated to be iso-energetic and contain the same levels of digestible LYS, methionine (MET) and cystine (CYS), and arginine (ARG). Overall, growth rate was superior for birds fed the NTCM (2.181 vs 2.148 kg at 39 days of age) as was gain to feed ratio from 0 to 19 days of age (0.642 vs 0.637). For both meal types growth rate responded in a quadratic fashion to the level of addition. Up to at least 60% soybean meal replacement produced performance equal to the control diet while higher levels of inclusion resulted in decreased growth. Feeding TCM increased proportional liver weight in comparison to NTCM. A significant interaction between meal type and level of inclusion showed that as diet level of TCM increased, proportional heart weight also increased. Similarly, serum T_3 levels increased in a linear fashion as levels of TCM increased in the diet but were unaffected by level of NTCM. Overall, NTCM was marginally superior to a mildly toasted canola meal as a feed ingredient for broiler chickens. This suggests that non-toasted canola meal with higher levels of digestible amino acids could be fed to poultry with no detrimental effect. Improved performance in chickens fed NTCM may be due to its enhanced digestible nutrient content, but the effects of TCM on liver and heart weight, and serum T_3 indicate that toasting may produce an undefined toxic factor(s). Before elimination of toasting can be recommended, its effects on feeding value must be studied in other species that may have different sensitivities to palatability and other factors in the meal.

IV. ASSESSING CANOLA MEAL QUALITY

Soybean meal processing is required to destroy anti-nutritional factors and the optimum conditions are well defined. Relatively simple assays have also been developed to assist processors and feed manufacturers in assessing both under (urease activity) and over processing (solubility in 0.2% potassium hydroxide) of the meal. The finding that canola meal processing causes variable and low digestible amino acid levels demonstrates a similar need to monitor meal quality from both a processor and meal user standpoint. To date protein solubility has received most attention and solubility does decrease in response to heat

application (Anderson-Hafermann *et al.*, 1993; Pastuszewska *et al.*, 1998). However, there is little information on how it is related to amino acid content and digestibility. Regression analysis from a limited data set from our laboratory has indicated that neutral detergent insoluble nitrogen (NDIN) may also be able to predict LYS digestibility. NDIN is commonly used to assess feed quality for ruminant species and it appears that protein that remains insoluble during neutral detergent extraction is not or poorly digestible by chickens. Near infrared reflectance spectroscopy (NIRS) is able to estimate the moisture, protein and amino acid content of feed ingredients (Williams *et al.,* 1983; Fontaine *et al.*, 2001), and appears also have potential in assessing nutrient digestibility (van Kempen, 1998). Therefore, NIRS may also have merit in assessing the nutritional value of canola meal.

Research was conducted to assess the relationship of *in vitro* analyses and amino acid digestibility (Newkirk and Classen, 2001). Protein solubility in 0.5% KOH, NDIN and NIRS were used to establish relationships using the samples collected from the western Canadian processing plants discussed above. With the exception of NIRS, regressions analyses were completed separately for all samples (NTCM and TCM) and for those which were conventionally processed (TCM). Using all samples, a linear relationship was found between LYS digestibility (R^2=0.65) and digestible LYS content (R^2=0.74), and protein solubility in 0.5% KOH. However, if only TCM samples were included in the analyses, the relationship was weak (R^2=0.17). Therefore protein solubility in KOH is not sufficiently accurate to predict the nutritional value of canola meal. NDIN was negatively associated with the digestibility and digestible content of LYS (R^2=0.78) and other essential amino acids when all samples were included in the analysis. Even with only TCM samples, the relationships for LYS digestibility (R^2=0.54) and digestible LYS content (R^2=0.58) were sufficiently strong to have value in quality assessment of canola meal. Based on this study, it would appear that meals with less than 12% of the protein as NDIN are of very good nutritional value. NIRS accurately predicted both LYS digestibility (R^2=0.92) and digestible content of LYS (R^2=0.84) when both NTCM and TCM samples were used. Unfortunately there was no opportunity to evaluate only TCM samples to determine the predictive value of NIRS using conventionally processed canola meal. Additional research is required to establish if it has value in assessing canola meal quality. Meal colour has also been mentioned a number of times in this paper as a character that distinguishes NTCM and TCM samples. However, it has not been possible to use colour as an accurate predictor, possibly because early Maillard reactions reduce amino acid digestibility without causing colour reactions. In conclusion, NDIN is the best predictor of canola meal quality at the present time but NIRS has potential to be used in the future. Because of the expense of biological testing required for calibration and validation of NIRS, it may be useful to use NIRS to indirectly predict quality by estimating NDIN content.

V. PROCESSING FACTORS AFFECTING MEAL QUALITY

It was of interest to study the conditions that are associated with reduced content and availability of amino acids as a result of DT. Based on the knowledge of Maillard reactions in other processed material, time of exposure to various temperature and moisture conditions was considered to be the most relevant for study. Therefore, air desolventised post-hexane extraction meal was exposed to various temperature and moisture conditions in a laboratory setting.

In the first experiment, meal was exposed (0-60 min) to temperatures ranging from 85 to 120°C using an isothermal autoclave. It was not possible to regulate moisture content in this experiment or accurately assess the moisture level after removal from the autoclave. At temperatures less than 100°C there was no change in NDIN regardless of time of exposure.

Exposure to higher temperatures resulted in a quadratic increase in NDIN with the degree of response temperature dependent. It was concluded that temperatures less than 100°C during desolventisation were desirable to prevent protein damage.

The effect of temperature and moisture were examined in a second experiment using NDIN, amino acid content and colour as response criteria. Temperature and moisture content ranged from 100 to 115°C and 7 to 18%, respectively, and meal was treated for 10 min. Moisture affected NDIN, amino acid content and colour of meal in a temperature related manner. At 7% moisture no effects were noted regardless of temperature, while with increasing moisture content the levels of NDIN increased, amino acid levels decreased (particularly LYS) and the meal became darker with increased temperature. The effects of moisture and temperature on NDIN are demonstrated in Figure 3. The results indicate that moisture is an important factor determining protein damage during canola meal desolventisation. The moisture level of meal exiting solvent extraction is approximately 7%. In conventional processing, moisture is added via direct injection of steam, water contained in gums that could enter at this stage, and water used to control dust in the upper tray of the desolventiser/toaster. Although it would be desirable to minimise moisture addition, other factors may delay conversion of processing plants. In the processing plants currently in use in Canada, the steam added in the final plates of the desolventiser/toaster assist in driving residual hexane though upper trays and meal prior to exiting the top of the unit. Alternate methods of removing hexane at this stage are required before sparge steam can be eliminated. Large capital investments in existing equipment will also slow adoption but alternative methods of desolventisation should be developed for the next generation of processing equipment.

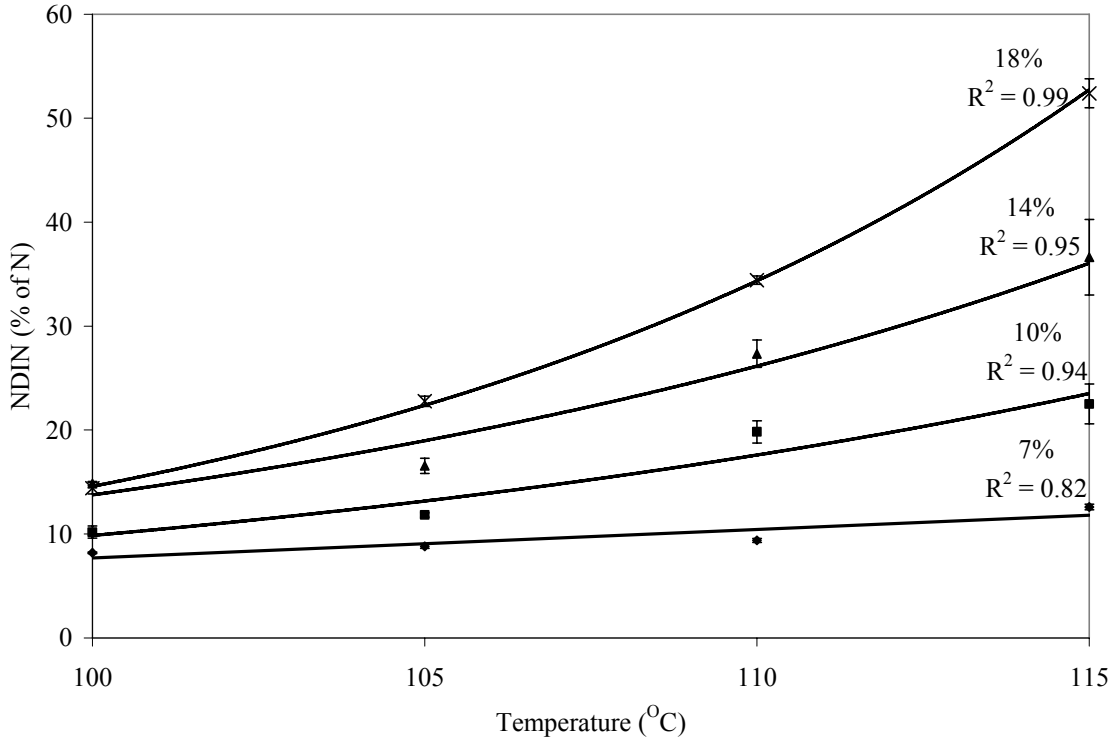

Figure 3. The effect of moisture and temperature on the neutral detergent insoluble nitrogen content (NDIN, as a proportion of N) of canola meal (± standard error) after 10 minutes of heating.

VI. NOVEL PROCESSING OF CANOLA MEAL

Canola meal is a composite of nutrients/materials that make it less than ideal for the animals to which it is fed. Canola meal has an excellent amino acid profile and its protein has low antigenicity, but high levels of fibre, phytate and possibly other anti-nutritional factors restrict it use in poultry and swine, and eliminates its potential in aquaculture. In contrast, ruminants can utilise more of the fibre and phytate phosphorus, but the high quality amino acids are an expensive source of nitrogen for rumen bacteria. As such, canola meal is non-optimised for use as a feed ingredient. This knowledge has led to research investigating the economic fractionation of canola meal into value added products that more closely match the requirements of target species. In turn, a technology company was developed that is currently entering the commercialisation phase (http://www.mcnbioproducts.com/).

The fractionation procedures are proprietary, but in general the process involves liquid fractionation. Although it is possible to obtain a number of different product lines, the three of most interest are insoluble, soluble and fibre protein fractions. The insoluble and soluble products maintain the excellent amino acid balance and low antigenicity characteristics of canola meal but concentrate the protein (650 to 800 g/kg and eliminate or reduce the levels of phytate and other anti-nutritional factors. These products are designed to compete with fish meal and high protein plant concentrates in non-ruminant and aquaculture diets. The fibre protein product retains high quality insoluble protein (34%) and is primarily designed for ruminant markets. A fourth stream from this processing system is sugar rich and contains high levels of available minerals. It is envisaged that this product may be combined with the fibre protein as a ruminant feedstuff.

The direction taken for this type of technology represents an opportunity for other feed ingredients that are undervalued and non-optimised for animal feeding. By following similar processing methods, the resulting products have the potential to generate value in excess of that derived from the original commodity. Methods of adding value to feed ingredients using new process technology are undoubtedly going to see increased prominence with more pressure for both economic and environmentally sustainable animal production.

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A POSSIBLE EXPLANATION FOR LIMITED FEED INTAKE OF WHEAT-BASED DIETS BY BROILERS

T.A. SCOTT

Summary

Bioassay research has focused on establishing nutrient levels in grains and their availability to poultry, with a major emphasis on measurement of metabolisable energy (ME). A bioassay, using young broiler chicks incorporates these measures and included measures of variability in nutrient intake and retention (growth and FCR). The most perplexing observation from these studies was that feed intake of different wheat-based diets was not associated with determined ME. It was concluded that limitation in intake was dependent on wheat source since these differences in feed intake and subsequent growth were large (>20%). Although processing (pelleting) and xylanase supplementation increased feed intake overall, these treatments did not remove the limitations to feed intake of some wheat-based diets. A hypothesis was developed that limitation in intake and acquisition of nutrients required for growth was related to differences in digesta passage rate. Digesta passage rate, and ability to obtain nutrients for growth, was improved with either change in the hydration rate of grain by pre-germination or by adding water to the wheat-based diets before feeding. In the latter case, wet-feeding resulted in increased feed intake and growth, however, for some wheat sources the increase in intake was excessive and resulted in marked increases in FCR. Both pre-germination and wet-feeding activated endogenous wheat enzymes and reduced digesta viscosity. More research is required to determine the factor(s) responsible for limitations in feed intake of wheat-based diets.

I. INTRODUCTION

A broiler chick bioassay has been used extensively to measure the feeding value of different sources of wheat fed in complete diets (80% wheat inclusion) with or without supplemental xylanases (Scott *et al.,* 1998; Scott and Pierce 2001; Scott *et al.,* 2003). Feeding value, based on this bioassay procedure, included measurements of nutrient level, availability, intake and retention (i.e., growth and feed conversion ratio). These studies have raised the serious concern that as yet unidentified factors in wheat (and barley) limit voluntary feed intake, which in turn directly limits growth rate. Within a specific series of bioassays, the between-wheat source variation in feed intake and growth rate has been greater than 20% (Scott 2000).

The variability in feed intake of the various wheat sources was not correlated to measurements of dietary apparent metabolisable energy (AME). This initiated the concern that other factor(s) inherent in these cereals was limiting feed intake and in effect preventing the broiler from attaining a desired nutrient intake to meet its genetic potential for growth (Scott 2000). Xylanase supplementation resulted in an overall increase in feed intake and growth, but the xylanase response varied between wheat source, and supplementation did little to reduce the level of variation in feed intake and growth between wheat sources (Scott 2000). Subsequent studies (Scott 2002; Scott and Silversides 2003) confirmed the variation in voluntary feed intake of different sources of wheat and suggest that there are inherent limitations of all wheat-based diets by broiler chicks and the variation previously reported is only an expression of degree.

There are many poultry bioassay evaluations of feed value of cereals; the predominant emphasis has been to measure nutrient (primarily ME) level and availability or digestibility

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(Sibbald and Slinger 1962; Schumaier and McGinnis 1967; Davidson *et al*., 1978; Mollah *et al*., 1983; and Farrell 1999 for a review). In these bioassays, the bird's preference or ability to consume the diet were either ignored or manipulated to discount variation in feed intake. This was based on the premise that the bird would voluntarily consume a diet to attain a desired energy intake, within reason. This appears to be true for individual ingredients when the diets were diluted, but fails to hold true when different sources of the same ingredient (i.e. different sources of wheat) are compared on an *ad libitum* basis.

Our observations on limitations in feed intake in effect challenge the strongly held theory that broiler chickens will consume a diet to meet its energy requirements and thereby achieve its genetic potential for growth. Similar observations pertaining to limitations in intake of Australian wheat-based diets have been reported for weanling pigs (Cadogan, 2003). Cadogan was able to explain a large portion of the variation in feed intake to be relative to non-starch carbohydrate fractions of wheat and that xylanase supplementation removed this difference. In broiler chickens, we observed an overall increase in feed intake with xylanase supplmentation, but were not able to remove variation in feed intake between wheat sources with xylanase supplementation (Scott 2000).

Now that our attention has been drawn to the possibility of factor(s) existing in wheat that limit feed intake, a concerted effort has been made to explain this phenomenon. The following presents published and unpublished data on feed intake variability of wheat-based diets and the relationship with growth, AME, and various other parameters measured on the grain and the diet.

II. OBSERVATIONS ON FEED VALUE VARIABILITY OF WHEAT

(a) The bioassay

The broiler chick bioassay has been extensively used in evaluations of feed value of ingredients and the impact of processing and/or xylanase supplementation (more than 4,000 diets have been compared using this bioassay). The bioassay was based on an 80% inclusion of wheat (Table 1) and has been formulated to meet the requirements of broiler chicks as established by the National Research Council (NRC, 1994). In the following dietary comparisons the diets were fed as a mash, those that indicate pelleting refer to pre-pelleting the wheat portion of the diet, regrinding this component, and feeding the final diet as a mash; specifically comparing the effect of the pelleting process rather than the pelleted-form with ground mash wheat-based diets. The basal diet in each case was prepared and mixed, the portion to be fed without xylanase supplementation removed and the remaining diet remixed with the appropriate xylanase.

Experimental diets were each fed to four randomly assigned cages of six male broiler chicks from 4 to 17 d of age on an *ad libitum* basis. All chicks were fed a common broiler starter diet from 0 to 4 d of age. For the 4 to 17 d period, feed intake were expressed on a bird day basis and feed conversion ratios (FCR) were determined for the same period and include weights of any mortality. The average body weight of the birds in each cage were determined at 17 d and used for body weight comparisons.

Excreta samples free of obvious feathers and feed were collected from each cage at 16 d, each sample then frozen, freeze-dried, and ground. At 17 d, the chicks were killed by cervical dislocation and the digestive tract removed and ileal digesta of all birds in each cage combined and processed in a similar manner to excreta. Finely ground diet, excreta and ileal samples were analysed for gross energy and acid insoluble ash, and used to calculate AME (MJ/kg diet) as described by Scott *et al.* (1998).

Table 1. Composition of diets used in the bioassay.

Ingredient	**/kg diet**
Wheat (g)	800.00
Tallow (g)	20.00
Isolated soy protein (g)	115.12
Corn gluten meal (g)	11.00
L-lysine (g)	1.54
DL-methionine (g)	0.44
Vitamins and minerals[1] (g)	40.90
Celite (acid insoluble ash marker) (g)	11.00
Calculated Analysis	
AME (MJ; for diets with xylanase)	13.14
Crude Protein (g)	246.00
Lysine (g)	12.50
Methionine (g)	5.50

[1] Supplied/kg diet: Vitamin A 9000 IU; cholecalciferol 1500IU; vitamin E 10 IU; vitamin K 0.5 mg; vitamin B_{12} 0.007 mg; thiamine 0.4 mg; riboflavin 6 mg; folic acid 1 mg; biotin 0.15 mg; niacin 35 mg; pyridoxine 4 mg; choline chloride 1000 mg; DL-methionine 1184 mg; ethoxyquine 0.125 g; $NaCl_2$ g; $MnSO_4$ 60 mg; $CuSO_4$ 5 mg; selenium (sodium selenate) 0.1 mg; iodine (EEI 0.35 mg; $ZnSO_4$ 50mg.

(b) Variability in voluntary feed intake of wheat-based diets

The data in Table 2 summarises the variability observed in voluntary feed intake of wheat samples evaluated in the broiler chick bioassay. A summary of the respective correlations between the three parameters (feed intake, body weight and AME of diet) for the respective samples is presented in Table 3.

The 54 wheats sampled by Scott *et al.* (1998) include nine cultivars, representing three classes of wheat, grown at three different locations, in duplicate plots, by the cereal breeding programs of the University of Saskatchewan and the Alberta Crop Development Centre. The bioassay data was collected and extensive analytical measurements of each wheat sample were measured (Classen *et al.,* 1995). Near infrared reflectance spectroscopy (NIRS) measurements of the whole grain were made and used to develop calibrations to predict AME, xylanase response, and performance of broilers (Swift *et al.,* 1998, Swift 1998).

The variability in feed intake and body weight of broilers, and AME of diets ranged from 14 to 21% (Table 2) for the 54 wheat-based diets with or without xylanase. It is apparent that feed intake, growth and AME of diets were improved by xylanase supplementation, however, there continued to be variability due to the source of wheat samples. The variability in feed intake was closely correlated to body weight (Table 3) with an r^2 of 0.79 for the 54 diets with or without xylanase. There was a significant negative correlation between feed intake and AME of diets with xylanase (r^2=-0.60), but the relationship was not significant for diets without xylanase. Classen *et al.* (1995) indicated that the numerous analytical parameters collected on the samples, provided at best only a moderate prediction of AME. Analysis of the capacity of these multiple parameters to predict performance variables (feed intake, growth and FCR) was also unsatisfactory (Scott, personal observations). Swift (1998) obtained strong correlations between NIRS (calibrations based on whole wheat) predicted and actual values for AME and between predicted and actual values for feed intake, growth and FCR. These were the first NIRS calibrations used to predict broiler performance.

Table 2. Results of two studies of the mean (± Std Dev) and range of feed intake (g/b/d from 4 to 17 d), body weight at 17 d and AME determined on excreta at 16 d collected from 208 wheat-based diets (four groups of six males fed each diet) with and without xylanase (±E) and/or with or without pelleting.

			Mean + Std dev / Range of response *min – max* (% difference)		
Bioassay Study	**No Diets**	**Treatment**	**Feed Intake g/b/d (4-17d)**	**Body Weight g 17d**	**AME MJ/kg DM**
Scott *et al.*, 1998	54	Mash / E+	39.3 ± 1.71 *35.4 – 42.6* (20.3%)	448 ± 14.0 *406 – 485* (19.5%)	13.9 ± 0.40 *12.8 – 14.6* (14.1%)
	54	Mash / E-	38.6 ± 1.60 *35.0 – 41.8* (19.4%)	433 ± 18.2 *385 – 465* (20.8%)	13.4 ± 0.46 *12.1 – 14.3* (18.8%)
Scott and Pierce 2003	25	Mash / E+	40.5 ± 1.18 *38.0 – 42.9* (12.9%)	436 ± 11.0 *412 – 456* (10.7%)	13.4 ± 0.91 *10.3 – 14.9* (44.7%)
	25	Mash / E-	39.2 ± 1.94 *35.9 – 42.6* (18.7%)	414 ± 20.2 *382 – 454* (18.9%)	12.9 ± 0.86 *10.8 – 14.5* (33.6%)
	25	Pellet / E+	44.7 ± 1.97 39.6 – 48.1 (21.5%)	476 ± 21.6 *412 – 506* (22.8%)	13.8 ± 0.53 *13.0 – 15.1* (15.8%)
	25	Pellet / E-	42.3 ± 1.53 *39.6 – 45.3* (14.4%)	432 ± 18.3 *402 – 466* (15.9%)	13.1 ± 0.80 *11.0 – 14.3* (29.7%)

Table 3. Results from two studies of correlations (r^2) between performance variables: feed intake (g/b/d 4-17 d), body weight (g at 17d), AME of diets (MJ/kg) and feed conversion ratio (FCR 4-17d) for wheat-based diets with and without xylanase (±E) and/or with or without pelleting.

Bioassay Study	**No Diets**	**Treatment**	**Variable**	**Feed Intake (FI)**	**Body Weight (BW)**	**AME**	**Feed Conversion Ratio**
Scott *et al.*, 1998	54	Mash E+	FI	1.00	0.79**	-0.60**	0.46*
			BW			-0.15	-0.09
			AME				-0.72**
	54	Mash E-	FI	1.00	0.79**	-0.15	-0.03
			BW			0.33*	-0.58**
			AME				-0.71**
Scott and Pierce 2003	25	Mash E+	FI	1.00	0.57**	-0.22	0.24
			BW			-0.04	-0.57**
			AME				-0.06
	25	Mash E-	FI	1.00	0.88**	-0.42*	0.01
			BW			-0.17	-0.45*
			AME				-0.38*
	25	Pellet E+	FI	1.00	0.91**	0.04	-0.23
			BW			0.10	-0.59**
			AME				-0.12
	25	Pellet E-	FI	1.00	0.82**	-0.02	-0.21
			BW			0.13	-0.71**
			AME				-0.22

*,** Signifies significant r^2 values at $P<0.05$ and $P<0.01$, respectively.

The data from Scott and Pierce (2003) has not been published as yet. The samples represent 25 wheat samples sourced from the 2002 crop year from various locations across the western Provinces of Canada. The 2002 crop year was considered one of the driest growing periods on record for many locations and the samples of wheat generally reflected this (i.e. small and shrunken kernels). The 25 wheat diets were either ground or ground, pelleted and reground before including in diets with or without xylanase. The 100 diets were then fed simultaneously to 100 cages of six male broilers in four separate bioassays to provide replication.

In unpelleted wheat diets, feed intake variability was lower in xylanase supplemented diets (13%) as compared to unsupplemented diets (19%); with pelleting the respective values were 21.5 and 14.4% in diets with and without xylanase (Table 2). The variability in AME was high in these samples, particularly when wheat was unpelleted (44.7 and 33.6% for diets with and without xylanase, respectively). As demonstrated previously, the correlation between measurements of voluntary feed intake and growth were high and positive, while those between feed intake and AME were not significant, with the exception of mash diets without xylanase with an r^2=-0.42 ($P<0.05$). A number of analytical parameters were also measured for these samples, but again no one parameter or multiple regression of parameters was able to provide accurate prediction of AME, feed intake, body weight or FCR.

(c) Observations on factors related to feed intake

The measurement of voluntary feed intake of cereal-based diets as impacted by cereal source, processing and supplementation with feed enzymes has proven to be an important observation. In our attempts to explain variation in voluntary feed intake of different wheat sources we have made a series of observations that have targeted more specific research initiatives. Variability in feed intake was more strongly influenced by cereal genetics than growing location. Use of alternative dietary ingredients in the basal portion of the diet did not remove variation in feed intake between sources of grain. Pelleting, and other heat treatments, did change the ranking of ingredients with respect to voluntary feed intake, body weight and AME, but did not remove variability in the measurement of these parameters. This last observation is important with respect to measuring AME, in particular. In the case of bioassay evaluations of AME it is important to consider the processing practice to which the grain will be subjected.

Several studies using this bioassay approach have provided possible explanations for differences in voluntary feed intake of wheat-based diets. Scott and Campbell (1998) evaluated the effect of pre-germinating grain on feed value. Various samples of grain were compared before and after soaking the whole grain (to initiate germination to the radical emergence stage) and then ground and dried back to an equal moisture level of untreated samples. When dry-pre-germinated grain diets were fed there were marked increases in feed intake and growth. A casual observation of interest was that there was no need for xylanase supplementation as endogenous xylanases had been activated during germination. Another observation was that after pre-germination, the grain, diets and subsequent excreta were more hydrophilic, the pre-soaking having significantly altered the water binding capacity.

Svihus *et al.* (1997) had similarly observed improvements in feed intake and growth of barley samples with soaking and germination. Samples of sprouted wheat during the 2001 harvest year were compared to unsprouted wheat from the same crop (Scott 2002, unpublished data); a significant increase in feed intake was associated with the sprouted wheat samples. Dormancy in grain is in part maintained by the level of resistance the cultivar in question would have to water uptake to initiate the germination process (Simpson 1990). This capacity to resist water uptake and initiation of dormancy decreases with storage time

and was suggested by Scott and Pierce (2001) as an explanation for increased feed intake of wheat samples after storage for six to nine months after harvest. Reduced feed intake of newly harvested grain may be one of the explanations for the "new crop phenomenon" commonly associated with poor performance of poultry fed newly harvested grain.

Swift (1998) reported the successful development of NIRS calibrations to predict nutrient level, availability, retention (AME and growth) and intake. During subsequent discussions Swift (personal communication) indicated that NIRS spectra patterns that predicted feed intake were almost identical to those used to predict moisture in grain. It is likely that moisture levels in grain would be related to physical moisture levels as well as the hydration capacity of those grains. Therefore, the moisture calibrations may be predicting water binding capacity or hydrophilic properties.

(d) Wet feeding studies

Others have reported an improvement in feed intake and growth of broilers fed diets supplemented with water (see Forbes 2003 for a review). However, there were variable responses in FCR to wet feeding. Two studies on the impact of wet feeding different sources of wheat (Scott 2002, Scott and Silversides 2003) were conducted. These studies compared cultivars of wheat from Hard Red Spring (HRS, bread wheat) and Durum (pasta) wheat classes in mash diets identical to those described for the bioassay. The wet diets were mixed with 1.2 ml water per g of dry feed each day. In both studies, feed intake and growth rate of 21 d old broilers were significantly increased by wet feeding; however there was a marked increase in FCR for the HRS wheat sources, but no change in FCR for Durum wheat-based diets fed with added water (Table 4). On dry weight basis, feed intake of wet Durum wheat-based diets was increased by 20% as compared to a 44% increase for HRS wheat-based diets; growth rate of broilers fed both classes of wheat was increased by 16%. The marked increase in feed intake of wet-fed HRS wheat-based diets resulted in a marked increase in FCR and a significant drop in AME of the same diets mirrored this. Scott (2002) suggested that the earlier concern regarding variability in FCR of wet-fed diets, reported by others (Forbes 2003), was a consequence of the source of grain used.

Table 4. A summary of the effect of water supplementation water (1.2 ml water / 1.0g feed) of Durum and Hard Red Spring wheat-based diets fed to male broiler chicks from 0 to 21 d on broiler chick performance and on dietary AME (feed intake and FCR expressed as a dry *as is* basis).

Wheat	Dry / Wet	n	Feed Intake (g/b/d)	Body Weight (g)	AME MJ/kg DM	Feed Conversion Ratio
Durum	Dry	48	34.1[d]	591[b]	12.7[a]	1.40[c]
	Wet	48	40.9[b]	685[a]	12.5[a]	1.44[c]
Hard Red Spring	Dry	48	37.2[c]	592[b]	11.2[b]	1.55[b]
	Wet	48	53.4[a]	679[a]	8.5[c]	1.80[a]

A subsequent study (Scott and Silversides 2003) confirmed this peculiarity with wet-feeding the two classes of wheat and hypothesised that this method of feeding enables the bird to increase its feed intake by increasing its rate of digesta passage through the gut. The two sources of wheat used in these studies are distinctive because of genetic selection for differences in hardness (as related to grinding), protein profile and resistance to water hydration (Canadian Wheat Board 2001), HRS wheat being significantly more hydrophilic.

This latter study (Scott and Silversides 2003) also indicated that moderate levels of feed restriction could reduce losses in FCR and AME of the wet-fed HRS wheat-based diets.

If feed intake of wheat-based diets was limited by the time it takes for a grain to become hydrated in the gut when fed in a dry form (and there are differences in rate, and possibly capacity to absorb water, between sources of wheat); then it would be possible to: a) overcome this limitation by adding water to the feed and removing some of this variation; and b) enable the broiler to use different strategies to acquire the total amount of nutrients it requires to meet its requirement for growth by either maximising digestibility or maximising throughput of diet. When Durum wheat-based diets were fed with water, feed intake and growth increased proportionally suggesting that nutrients derived from the diet were maintained, which is confirmed by the similar FCR and AME of the wet and dry treatments. However, when wet HRS wheat-based diets were fed, the broilers were able to markedly increase consumption of the diet and were not required to make the effort to maximise its capacity to digest nutrients, achieving maximum nutrient intake to support growth by simply increasing rate of passage of digesta.

Perhaps these differences in digestive strategy can explain differences in AME observed for sources of wheat with similar levels of starch? That is, in some cases would it be "easier" for the broiler to attain desired intake of energy by increasing intake rather than maximising efficiency of energy recovery. Perhaps these strategies would also explain the marked differences in performance in the field (with *ad libitum* intake) of wheat-based diets formulated for specific AME based on bioassay determinations that were, in turn, based on limitations in feed intake and forcing the bird to use a different nutrient acquisition strategy than would have been used with *ad libitum* feed access?

III. CONCLUSIONS

The significant variability in voluntary feed intake of broilers fed wheat-based diets has a direct impact on growth and minimal association with the ME measured for these sources of wheat. This indicates that the broiler chicks were unable to consume these various wheat sources to meet a constant energy intake and growth rate and that voluntary feed intake was limited by factor(s) in the wheat. Our studies, and those of others, suggest that this limitation in feed intake of broilers fed wheat-based diets were associated with limitations in digesta passage. Limitations in digesta passage were removed when the wheat-based diets were fed with added water and in other studies by changing the hydration rate of wheat by pre-germination. This would suggest that the time it takes a diet to become hydrated in the gut would directly affect rate of passage, and thereby limit intake and growth.

Although the industry finds the concept of feeding wet diets "unpalatable" and are sceptical that the costs associated with pre-treating grain (i.e. pre-germination) would not be recoverable, this does not discount the serious implication of factor(s) existing in wheat, which limit intake and growth. Further work is required to determine how the advantages of increased feed intake and growth can be maximised on a consistent basis, avoiding the excessive intake and reduced nutrient retention associated with some sources of wheat. Achieving consistent increases in feed intake and growth with no change in FCR would allow applications of wet feeding to be employed, and further allow capturing the advantage of activation of wheat endogenous enzymes observed with wet feeding. Finney (1983), a strong advocate of the use of germination (i.e., taking advantage of activation of endogenous enzymes), feels this processes (and perhaps wet-feeding, based on our studies and those of others) would have major advantages in improving the feed/food value of grains and legumes and "significantly alleviating today's food problems and avoiding tomorrow's food needs".

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INFLUENCES OF MARKET FORCES ON INGREDIENT USE AND FEED PROCESSING

P. GARLAND

Summary

The design, formulation and manufacture of livestock feeds in the EU, and in particular the UK, have undergone significant changes in the past 25 years. World trade patterns and economic policies have to some extent shaped these activities but in the more recent past social and political influences have had a more significant impact. This paper considers the changes in feed manufacture arising from *Salmonella* control, withdrawal of animal proteins, removal of used cooking oil, impact of concerns over genetic modification and the move to feeds without antibiotic digestive enhancers. The requirement for prevention of contamination of ruminant feeds with mammalian products and the specific controls employed are not discussed.

I. INTRODUCTION

It is logical to expect that the economic availability of raw materials suitable for animal feeds will be evident in the formulation of feeds. Regional policies might interfere with the economics of individual raw materials but fundamentally market forces and modern linear programming will determine the make up of rations. For example the CAP (Common Agricultural Policy) determines that, via price support mechanisms and import tariffs, the predominant carbohydrate sources in the UK and most of the EU are wheat and barley rather than maize, milo or cassava.

These economic and political effects tend to be predictable and viable alternative raw materials or feeding strategies can be developed. This then allows the livestock industry to maintain a competitive position in the market in which it operates.

However, when specific demands of powerful purchasers of feed or livestock products are met the effects might be of little impact, on a world basis, but are highly significant locally. The first signs of this occurring arose in the early 80s. A major High Street retailer in the UK issued a feed specification that was to be applied to all rations fed to broilers destined for their shelves. In this case the use of poultry offal meal and South American origin fish meal was excluded on the grounds of *Salmonella* contamination risk.

From this point onwards the restrictions imposed, usually for sound reasons, increased. The most significant impacts that occur are when a constraint is not universal across an entire market sector. The exclusion of a material from a feed miller's facility for one customer could add costs for another purchaser of feed, or having to hold two sources of a similar material has cost implications in terms of bin utilisation. Although, the technical solutions to these issues were usually straight forward, economically and commercially it is often problematic.

In 1988 the UK egg industry was dealt a devastating blow by the revelation that "sadly a high proportion of the laying flock is contaminated with *Salmonella*" (Edwina Currie, Parliamentary Under-Secretary for Health, 3rd December 1988, television interview). The repercussions were immediate and dramatic; sales of eggs fell as consumers were faced with their first real food scare. This was the first of several issues over the following years which shaped the use of raw materials as well as how animal feeds are manufactured today in the EU and in particular the UK.

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II. SALMONELLA CONTROL

The level of *Salmonella* in UK compound feeds has fallen considerably since 1988; the focus brought to bear on the topic ensured that control of the entire supply chain from raw material procurement through feed manufacture and delivery was greatly improved. The data in Tables 1 and 2 show the relative degrees of incidence of *Salmonella* contamination of feed ingredients between 1984-7 and 2001-2. The data are not directly comparable as the earlier information is from compounders' own voluntary monitoring, whilst the 2001-2 data were compiled by the government. Nevertheless this does show that *Salmonella* isolations from cereals have barely changed but those from oilseed products and imported fish meal have fallen.

Table 1. Incidence of *Salmonella* contamination in common feed ingredients 1984-1987 (Wilson, 1990)

	No of Tests	**No +ve**	**% +ve**
Cereals	1026	9	0.9
Sunflower meal	496	33	6.4
Full fat soya	339	67	19.8
Extracted soya	1167	31	2.7
S American fish	151	20	13.2

Table 2. Animal feed raw material *Salmonella* contamination rates (Evans & Kidd, 2003)

	2001			**2002**		
	No of Tests	**No +ve**	**% +ve**	**No of Tests**	**No +ve**	**% +ve**
Non oilseed meal vegetable products	14370	227	1.6	4038	26	0.6
Oilseed meals and products for feed use	14482	323	2.2	6035	258	4.3
GB and imported processed animal protein for feedstuff use	1350	33	2.4	967	33	3.4

One of the major impacts of *Salmonella* on the feed industry was that a different approach to raw material procurement had to be adopted. Monitoring materials by source became an industry standard. This allowed compounders to check the biosecurity of high risk materials and where necessary act in a concerted manner through the trade association to bring improved standards into play at suppliers providing poor quality material.

The monitoring of six rape crushing facilities between 1991 and 1993 allowed the UK feed industry, through the offices of UKASTA (United Kingdom Agricultural Supply Trade Association) to bring pressure to bear on those supplying consistently contaminated rapemeal. The threat of an industry-wide veto on a supplier is a powerful inducement to improve standards. In terms of processing of feed raw materials to reduce bacterial contamination, little has changed. The occurrence of contamination has been shown to be due to post processing recontamination and actions taken to prevent this are focused on management practices and design of material handling systems. Occasionally it might be necessary to resort to treatment of *Salmonella*-positive material with organic acids or formaldehyde based products. In the case of the latter concern has been expressed over subsequent amino acid availability but a literature review and trial studies provided by a supplier of a proprietary preparation in support of their application for use in the EU shows

no reduction in amino acid digestibility (personal communication, Anitox Ltd). Control of *Salmonella* in feed mills has had a more direct impact on nutrition and the cost of feed manufacturing. That there was a feed influence on *Salmonella* isolations there is no doubt.

Table 3. % *Salmonella*-positive finished products from a UK national feed compounder

	Cattle extruded	**Pig extruded**	**Pig meal**	**Poultry extruded**	**Poultry mash**	**Poultry heat -treated mash**	**Concentrates**
1989/90	5.1 (334)	3.2 (472)	3.6 (247)	14.2 (435)	6.4 (330)	---	3.8 (132)
2002/03	0.3 (1214)	0.25 (804)	3.4 (322)	0.1 (706)	1.9 (371)	0 (25)	2.7 (146)

In Table 3 the levels of *Salmonella* found in 1989/90 by one national compounder compared to comparable figures for 2002/3 show reductions in the detection of *Salmonella*. As with all industry-based data covering an extended period of time changes in company size and structure should be borne in mind. In this case the volume of feed manufactured more than doubled and the numbers of mills and locations changed. Whilst there is no one single element in *Salmonella* control that can be credited with the better control of *Salmonella* in feed, heat treatment has often been used as a controlling point in the feed manufacturing process. Improved hygiene standards arising from industry codes of practice have also made a significant contribution to reduced *Salmonella*.

It is quite feasible to specify conditions whereby bacteria can be virtually eliminated but the risk of reducing the nutrient value of the feed is increased. Typically feed for broiler chickens is processed at about 75-85ºC for 15-20 seconds with a moisture content of 15% before pelleting. The frictional forces in the pellet die can lift the temperature by another 10ºC. There are, though, specific customer requirements to process boiler feed at 85ºC minimum for at least 2 minutes before pelleting and our own work has shown that this can have detrimental effects on subsequent bird performance. The data in Table 4 shows liveweight and FCR of birds in a cage brooder study to 28 days where different processing conditions were evaluated at two feed mills.

The need to heat process poultry breeding rations in the late 1980s led to almost universal adoption of crumb or crumble rations. Whilst this met the immediate need for a heat processing step in the manufacture of feeds it gave a physical form that was not universally popular due to feed management difficulties at farm level. The potential for heat treated mash products became an attractive means of recapturing breeder feed volume from integrated poultry companies where it had been taken in house. In 1997 BOCM PAULS introduced two mash heat-treatment facilities, each using different processing systems. As part of the commissioning process trials were conducted to check the validity of the heat treatment employed. The conditions selected for the processes were less aggressive than those routinely requested by both retailers and poultry companies at the time. The performance data (Table 5) shows a clear advantage in terms of heat treatment for younger birds and allowed some specification modification for replacement breeder stock fed heat treated mashes.

Table 4. Effect of processing on broiler chick performance (BOCM PAULS 1995, internal data)

Feed Mill	Process conditions pre-pelleting	Liveweight 28 days (g)	FCR to 28 days
A	85-88ºC for 2-3 minutes	1435	1.613[a]
A	85ºC for 20 seconds	1452	1.515[b]
B	Expanded at 120ºC	1469	1.640[a]
B	80ºC for 15-20 seconds	1503	1.563[ab]

[ab] Means in a column without a common superscript are significantly different (P<0.001)

Table 5. Effect of heat processing on performance potential of mash feeds fed to broiler chickens to 28 days (BOCM PAULS 1997, internal data)

Mill	Process conditions	Liveweight to 28 days (g)	FCR to 28 days
C	Unheated mash	1044	2.423
C	80ºC, 2 minutes	1108	2.304
D	Unheated mash	1010	2.357
D	85ºC, 30 seconds	1219	2.118

A useful review of the effect of processing on nutritive value of feeds was given by McCraken (1999) in which the relative merits and disadvantages of heat processing of feed are discussed. From the *Salmonella* stand point it is clear that aggressive treatments may well give a better degree of confidence with respect to bacterial contamination but negative performance traits can also arise.

As a result of heat treatment in the UK the preferred means of enzyme application for both NSP and phytate substrate is post pelleting liquid application. This in itself has capital investment applications.

III. PROTEIN SOURCES

The range of protein sources available for use in poultry rations has reduced over the years. Concerns expressed by retailers over the suitability of feather meal quickly excluded this material, such that its main use was relegated to ruminant protein concentrates for home use and monogastric concentrates exported outside the EU. Similarly questions over the microbiological status of poultry offal meals removed these from the ingredient listings of independent compounders, although some integrators were able to continue using their own material until the mid 1990s. The focus on intra-species recycling from both a disease prevention and ethical perspective that followed the onset of the BSE (Bovine Spongiform Encephalopathy) outbreak excluded poultry offal from UK formulations, the industry continued to use meat and bone meal of mammalian origin until March 1996. When the UK government announced a possible link between CJD (Creudzfeldt-Jacob disease) and BSE the industry immediately stopped using meat and bone meal. There was literally an overnight change in diet formulations and the only protein sources were then fish meal, legumes, pulses, oilseeds and oilseed byproducts.

The most noticeable effect was the increase in formulation costs, these varied according to ration density but ranged from £8/tonne for high protein starter feeds to £3/tonne for chicken finisher or layer diets. A heavy reliance upon soya was concerning from the point of view of litter quality in broiler chickens and turkeys due to increased potassium and

oligosaccharide levels as well as high crude protein levels in feeds. The latter point drove those companies not already formulating to digestible amino acids to do so. There was an increase in the use of synthetic amino acids; threonine became a more attractive ingredient, as formulators sought to balance amino acid profiles in rations and previous safety margins in diet specifications were reviewed.

The benefits of meat and bone meal have been more clearly defined now that we do not have it available. Not only did we lose a source of protein which was largely of good quality but we no longer had a source of highly available phosphorous and a significant contributor to the energy level of the feed.

These key areas are readily covered by most ration specifications and alternative sources can be incorporated in formulations albeit at a cost. However, as discussed by Bedford and Fothergill (2002) there are other nutrients not routinely specified which were supplied by meat, bone and offal meals such as glycine, carnitine and conjugated linoleic acid. It is debatable whether these nutrients have become limiting but there is valid reasoning in determining exactly what a raw material might be contributing beyond the parameters covered on a formulation database. Conversely there is a risk of attributing too much to a material; great play was made of the value of meat meals in providing B vitamins and choline with recommendations made to increase supplemental levels. The lesson learnt was that there was already adequate insurance built into vitamin recommendation to accommodate lower background levels.

Strong arguments were put forward to correlate the incidence of pecking and cannibalism in laying hens with the removal of meat and bone meal from feeds. The fundamental flaw in the debate was that the material had been excluded from most free range rations on ethical grounds years previously and no change in incidence had been seen. Work published by Hadorn *et al.* (1999) supports the view that there is no link between absence of animal protein in the diet and increased pecking.

It has been desirable to use alternative protein sources in poultry feeds and in this regard rapeseed and rapeseed meals are used at significant levels in meat bird rations. The use of whole rapeseed in particular has been very effective where mills are able to handle the material as an unprocessed seed. Alternatives to whole rapeseed are expeller meals at approximately 18% oil content or blends of whole rapeseed with, typically, peas or beans processed through an expander. In the case of the latter, branded products have become well established as broiler chicken feed ingredients.

IV. GENETIC MODIFICATION

Within the EU there are requirements for non GM (genetically modified) foods and livestock products from animals fed on non GM rations. At present there are a number of categories used to define the status of materials used in feeds manufactured to meet specific market demands.

1. IP non-GM soya - identity preserved, full traceability of raw material with proof of segregation back to source of seeds planted and exact location of crop grown, <0.1% GM material.
2. Certified non-GM soya. Traceability of material back to factory/silo of origin as a minimum with PCR testing to confirm non GM status as being <1%.
3. North of Santos Soya - port of origin identified with PCR testing to confirm GM status as being <1%. (Lower cost alternative to 1 and 2 above.)
4. FAQ. No traceability beyond vessel used to ship to EU. No guarantee of status.

The costs of these different categories vary widely and are influenced by the proximity to either the US or Brasilian harvests for soya bean. At the extreme is IP material which would be used in organic rations and carries a significant premium.

Unfortunately different market sectors aim to meet differing aspirations for GM status. Therefore the biggest single issue for feed manufacturers is the availability of storage capacity at individual mills servicing a range of customers. It will be interesting to see how this area develops with the uncertainty in GM policy in Brasil since this is where all of the UK's FEMAS (Feed Materials Assurance Scheme) and North of Santos soya originates from.

So far there has been little concern over the GM status of vitamins, enzymes and oil blends except in organic feed production.

V. FATS AND OILS

As a result of the BSE announcement in 1996 the use of tallow and poultry fat in fat blends for livestock feeds all but ceased. There were initially concerns over the negative impact on carcass quality due to lower levels of tallow-derived stearic acid in added fat. This was keenly monitored in turkeys, where for the whole-bird market, a firm white finish is of importance. For the seasonal market where this factor probably outweighs liveweight gain and FCR in importance (within commercial limits) diets were formulated such that a greater proportion of energy is derived from cereal carbohydrate.

Whereas fat blends used for poultry feeds could have incorporated 50% tallow along with varying levels of soya or maize oils (usually acid oils), palm or palm fatty acid distillate and UCO (used cooking oil), there was a rapid move to blends utilising 50-60% UCO.

This has proven remarkably successful and no problems have been identified. Since the level of free fatty acids (FFA) is high in UCO such that most blends have an FFA content of 40-45% most compounders and integrators have increased Vitamin E supplementation slightly. However, we are now faced with another change in legislation which has arisen from outbreaks of swine fever in the EU and been influenced by the finding of dioxin in animal feed fats in Belgium. The use of catering and kitchen waste in animal feeds is no longer permitted in the EU. This legislation is primarily targeted at swill feeding of pigs to prevent intra-species recycling; due to wording of the legislation it means that used cooking oil from restaurants cannot be recycled to animal feed.

Currently the UK enjoys a derogation that allows the collection and use of UCO because of the high degree of traceability and quality control that exists. This will end in 2004 and we shall then rely entirely on vegetable oils, acid oils and a small volume of UCO from large food preparation facilities where the material is clearly traceable to source. The restaurant UCO is already being diverted to biodiesel production. We do not anticipate any major technical difficulties in the change in fat supply but again a cost penalty will be incurred; our EU neighbours continue to use tallow.

VI. ANTIBIOTIC GROWTH PROMOTERS

There has been, and continues to be, pressure on the use of antibiotic digestive enhancers (ADEs) from both a legislative and consumer perspective. In 1997 avoparcin was withdrawn followed by the withdrawal of tylosin, spiromycin, virginiamycin and bacitracin in 1999. This leaves only avilamycin and flavophospholipol as licensed growth promoters for use in poultry feed and these have been voluntarily withdrawn from a large part of the UK broiler chicken sector. There has not been any significant use (less than 2% of feed) of antibiotic growth promoters in the laying hen rations since the 1980s and due to market demands the use in all classes of poultry breeder feeds petered out by the late 1990s.

There is no doubt that the complete withdrawal of antibiotics from chicken feeds would have a negative effect on physical performance. Even at conservative estimates these products improve weight gain and FCR by 2-3%.

The major concern though over the withdrawal of antibiotics, is the likely impact on health and subclinical disease. In particular the higher risk of necrotic enteritis due to *Clostridia* associated with the withdrawal of the ADEs has changed the way in which chickens are fed.

There has been a surge in availability of alternative products and trials conducted by BOCM PAULS LTD (see Table 6) have demonstrated that few if any of these products can match the antibiotics in terms of performance in trial facilities. The fact that the price of these alternatives is higher than the antibiotics does further reduce the cost attractiveness.

Table 6. Summary of trials comparing non antibiotic digestive enhancers with an antibiotic control (BOCM PAULS 1995-2002, internal data)

	Results as % of Controls	
Product	**Bodyweight**	**FCR**
A Essential Oils	99	100
B Essential Oils	98	102
C Essential Oils	97	101
D Essential Oils/Herbs	99	102
E Essential Oils/Herbs	99	100
F Probiotic	93	98
G Probiotic	99	100
H Acid	99	100
I Acid	96	103
J Acids/Volatile Fatty Acids	96	104
K Volatile Fatty Acids	96	100
L Yeast by-product	103	101

The use of alternatives has been marketing led as much as science led, undoubtedly there are products which do improve performance compared to a negative control but the ability to measure this in the field is low. Therefore individual companies have made their best judgement in choosing a product but often on the basis of simply having to add something. This is not a particularly satisfactory approach when taken in isolation and the most effective results have been achieved when companies have considered all of the benefits potentially provided by the antibiotics and taken steps to find alternative means of achieving them. This has meant reviews of the whole health status of farms, tightening of biosecurity and improving cleanout standards so that all enteric disease is controlled. From a nutritional view point it has become necessary to provide feeds which give as little opportunity as possible for pathogenic organisms to get past the birds own immune system.

The following list covers most approaches taken in the UK at the moment.

1. Reduce bacterial loading of feed, it is perfectly feasible to reduce total enterobacteriacae to <10 cfu/g by steam pelleting. Field trials with acids have not been found to provide additional benefits beyond pelleting.
2. Improve digestibility of feeds to prevent supply of nutrients for lower intestine bacterial populations.

- Some re-introduction of maize at greater than 20% has been used effectively.
- Choose high quality sources of raw materials of good digestibility.
- Include oil via oil seeds rather than liquid oils.
- Incorporation of exogenous enzymes, primarily xylanase and phytase.

3. Maintain favourable bacterial population.
- Avoid swings in carbohydrate sources i.e. use wheat and barely (also maize if costs permit).
- Reduce crude protein content but maintain amino acid profile.
4. Stimulate physical activity of intestines.
- Feeding of whole grain wheat
- Coarse grist of feed before pelleting
- Meal feeding rather than *ad libitum*
- Not grinding wheat before pelleting
5. Effective control of coccidiosis and judicious use of ionophore anticoccidials

Consideration of the above along with greater emphasis on farm hygiene does allow the production of chicken without major health problems but at a cost that is not readily identifiable in terms of feed formulation.

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AMINO ACID REQUIREMENTS OF BROILERS: RELATIONSHIPS WITH GROWTH AND MEAT QUALITY

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Summary

Over the last sixty years there has been a rapid reduction in market age for broilers. This has been a direct result from genetic selection (increase in muscle yield), improved diets, and better management of the resources available to the producers. A rapidly growing broiler needs to be supplied with sufficient nutrients to meet its requirements for maintenance and for the growth of all components of the bird, including feathers. The crude protein and amino acid status of a diet can influence the carcass composition of broilers, with increased carcass protein and reduced carcass fat accompanying increases in dietary protein or essential amino acid content. Rapid increases in growth rate have been associated with improved feed conversion efficiency, while amino acid requirements as a percentage of the diet have not changed substantially. Although nutritional requirements expressed as proportions of the diet may not change linearly with improvements in performance, a greater understanding of the requirements for specific factors such as requirements for optimum immune function or breast muscle deposition requires further research.

I. INTRODUCTION

Each year the world's human population is predicted to increase by 70 – 80 million. The majority of this increase is from third world countries. With the economy of many third world countries improving, the demand for animal products and especially meat as a source of protein will increase. Chicken continues to be the least expensive meat in most of these countries. The broiler industry is ideally suited to meet this expected increase demand for animal protein with improved efficiency of production. The broiler industry in Australia and many parts of the world is relatively new compared with other livestock industries.

The broiler industry started on the east coast of the USA in 1920. By 1960, broiler meat made up 10% of the total meat consumption and increased by 5% annually to exceed the consumption of beef and pork in 1995 (Figure 1). By 2000, the total consumption of broiler and other poultry meats was 46.2% of total meat consumption (Kaku, 2001). Factors contributing to an increase in broiler production by 5% each year include genetic selection, dietary, environmental and managerial improvements, and economic factors. The broiler industry has developed into an robust business with several advantages over it competitors, these include the ease of establishing integrated operations with the adaptability for further processing and economically competitive price of poultry vs. red meats. In the USA in 2000, the price of broiler meat was cheaper than beef (32%) and pork (47%). The actual price of beef and pork has increased by 3.5 times compared to broiler meat which has increased by only 2.5 times since 1960 (Kaku, 2001). Consumers consider broiler meat healthy and inexpensive, which will only lead to further increases in demand (Ishibashi and Yonemoch, 2002).

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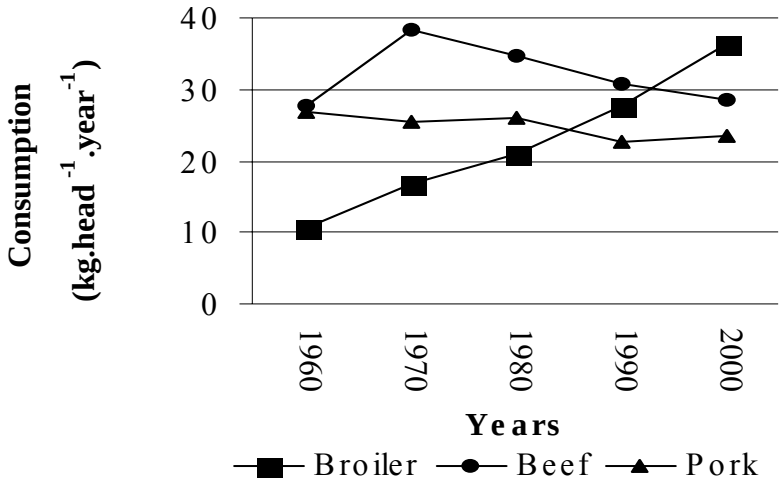

Figure 1:. Consumption of broiler, beef and pork in the USA (data from Kaku, 2001)

II. INCREASE MUSCLE GROWTH

To meet the market demands poultry selection has concentrated on growth rate and muscle mass in broiler. In the last 30 years, the production time needed to raise a 1.3 kg chicken has been halved (Dransfield and Sosnick, 1999). To meet requirements and maintain protein deposition in broilers, nutritionist must look at the demands of the rapidly growing broiler and adapt the formulated diets to meet those demands. To do this, the nutritionists has to look at two major issues -- what is the potential growth rate of the selected broiler and what are the broilers nutrient requirements to meet those needs. A rapidly growing broiler needs to be supplied with nutrients in order to meet its requirements for maintenance and for the growth of all other components of the broiler, including feathers (Gous, 1998). A good definition of potential growth is given by Gous (1998), as the maximum possible growth rate that the genotype can achieve when given perfect nutritional and husbandry conditions.

Below the requirement, breast muscle development is sensitive to dietary lysine content in the diet. Many studies citing a positive correlation between dietary lysine level and breast muscle accretion have compared diets deficient in protein and/or lysine to diets adequate (Corzo *et al*., 2002; Tesseraud *et al*., 2003). The authors are unaware of any studies in which an increase in breast muscle or growth rate is associated with increases in dietary amino acids or protein at levels well beyond the NRC requirements.

Han and Baker (1991) determined requirements of 8 to 21 day old broilers to be not greater than 1.17% lysine for maximal weight gain. This was slightly higher than the National Research Council recommendations of 1.10% (NRC, 1994). Labadan *et al*. (2001) also determined that lysine requirements for broilers up to 2 weeks of age to be 1.32% compared to 1.10% recommended by the National Research Council (NRC, 1994). Muscle protein is high in lysine and the portion of breast muscle yield has increased (through genetic selection) compared to the total carcass meat in broiler. Breast muscle contributes about 30% of total carcass meat and accounts for 50% of total edible carcass protein (Summers *et al.,* 1988); some researchers suggest that the lysine requirement has increased over time (Si *et al.*, 2001). However, the lysine requirement for maximum breast muscle accretion was similar to the requirement for growth, but greater than for feed efficiency (Labadan *et al.,* 2001).

Care must be taken when discussing changes in lysine requirements as a function of growth rate. Although broiler growth rate has increased dramatically in the past 60 years (NRC 1944; 1954; 1977; 1984; 1994), the actual lysine requirement, expressed in percentage

of the diet, has changed very little over the years. The lysine recommendation given by the National Research Council was 0.902, 0.9, 0.85 to 1.2 and 0.85 to 1.1% of the diet for the years 1944, 1954, 1984 and 1994, respectively. The ranges given for 1984 and 1994 reflect the effect of age on lysine requirement as the bird ages; lysine requirements decrease with age. Earlier versions of the NRC recommendations for poultry did not take into account the changing requirements of birds as they approached a target body weight. During the last 60 years, the growth rate of birds has increased dramatically. In 1944, a typical Rhode Island Red cockerel or pullet would reach 1.6 kg at 14 to 17 weeks, or 16 to 19 weeks, respectively. In 1944, cockerels would be expected to reach 2.27 kg in 17 to 22 weeks of age; females would reach this weight in 25 to 30 weeks (NRC, 1944). In 1954, "heavy breed" male chickens required 11, and females 14, weeks to reach 1.6 kg (ages to heavier weights were not given in the 1954 NRC). A contemporary publication suggests that New Hampshire males and females would reach 2.3 kg at 21 and 31 weeks, respectively (Titus, 1955). Although different strains of chicken were reported in each of these publications, the standard for meat-type chicken production was moving towards an increased growth rate. In 1977, broiler chickens would be expected to reach 2 kg in 8 weeks (NRC, 1977). In 1984, that body weight was reached prior to 7 weeks of age (NRC, 1984), and by 1994 a 2 kg body weight would be expected in male broilers at approximately 6 weeks. Growth rates have continued to increase since that time. Concurrent with the improvement in growth rate has been a dramatic improvement in feed efficiency. In 1944, meat-type chickens required approximately 4.1 kg of feed for each kg of gain (NRC, 1944), whereas feed conversion efficiency had decreased to 1.8 kg of feed per kg of gain by 1994 (NRC, 1994). Much of the improvement in efficiency can be attributed to the decrease in maintenance requirements due to a shorter time to a particular weight. As mentioned previously, the requirements for lysine as a percent of the diet, and presumably other amino acids, have changed very little since 1944. Expressed in terms of total lysine required to reach a particular body weight therefore, have decreased.

III. MEAT QUALITY AND INCREASED MUSCLE GROWTH

Genetic selection for growth has resulted in producing a larger broiler with a greater percent of muscle mass. Dransfield and Sosnick, (1999) found in rapidly growing broilers there is an increase appearance of morphological abnormalities, induce larger fibre diameters and a higher proportion of glycolytic fibres, and a lower proteolytic potential in the muscles. After death, there is a faster development of rigor mortis resulting in paler colour and reduced water holding capacity and poorer quality of further processed products. Little work has been done investigating the relationship between growth rate and meat quality. Presumably, these factors would decrease the consumer acceptance of such meat from rapidly growing broilers.

IV.GROWTH AND IMMUNITY OF THE BROILER CHICKENS

It has been recognised for many years that nutrient deficiency are associated with an impaired immune response and with increased susceptibility to infectious disease. In turn, infection can affect the status of several nutrients, thus setting up a vicious cycle of under nutrition, compromised immune function and infection. This recent research called "immunonutrition" has focused on looking at nutrition, infection and immunity and how they related (Grimm and Calder, 2002). Additionally, amino acid nutrition likely has more nuanced effects on immune function than has been reported for simple deficiencies. This is an exciting area of research, and one that is poised to become more important to the poultry industry as the use of growth-promoting antibiotics is changing around the world. Amino

acids such as glutamine, arginine, cysteine and taurine have been identified as important immune-modulation substrates (Suchner *et al.*, 2000). The relationship between growth and immune response in broilers has been studied while modifying dietary arginine in the diet. Both Webel *et al.* (1998) and Kidd *et al.* (2001) studied the effect of dietary arginine and determined that as long as arginine levels are kept near the National Research Council (1994) recommendations for age, the arginine at those levels should support the immune system functions in healthy chicks.

Recent studies in our laboratory have focused on the effects of growth rate of broiler chickens on inflammatory responses. The inflammatory response is of particular interest to broiler producers, as inflammation results in the diversion of nutrients away from growth and towards the non-specific mechanisms designed to ward off pathogens (Korver and Klasing, 1997, Korver *et al.*, 1997). In commercial situations, even low-level activation of the inflammatory response may result in decreased growth rate and efficiency. In our studies, modern broiler chickens were compared to two random-bred broiler lines maintained at the University of Alberta; a 1977 broiler line and a 1957 broiler line. Within each strain, birds were either allowed *ad libitum* feed consumption, or were pair-fed to the level of feed intake of the 1957 strain to eliminate the effect of feed intake on immune function. Within each strain and dietary treatment, half of the birds were subjected to an experimental cellulitis challenge by subcutaneous injection of a field isolate of *E. coli* from cellulitis lesions. As expected, the modern broilers grew faster and were more efficient than the either of the random-bred lines, in both the *ad libitum* and pair-fed treatments. Interestingly, the cellulitis challenge resulted in greater growth depression in the random-bred lines than the modern line. Associated with this response was a greater responsiveness of T cells *in vitro* from the modern birds to a uniform level of interleukin-1, indicating that modern broilers regulate their immune system differently than older, unselected lines of birds. Further research in this area is ongoing in our laboratory. An increased awareness of the mechanisms regulating immune response in broilers will become more and more important as production aids such as prophylactic antibiotics are removed from poultry feeds around the world.

V. USING THE INDICATOR AMINO ACID OXIDATION METHOD TO DETERMINE BROILER REQUIREMENTS

Traditional methods to determine amino acid requirements in broilers are based on using growth as an indicator of change in the diet. The concentration of amino acid in the diet producing the maximum growth response (or breast muscle deposition, or feed conversion efficiency) is regarded as being the requirement for the test amino acid. The determined requirement is then expressed as a fixed concentration in the diet (g/kg) (Gous, 1998).

The indicator amino acid oxidation (IAAO) technique for determining amino acids (AA) requirements has been developed and validated in pigs (Kim and Bayley 1983a; 1983b; Ball and Bayley 1984; Ball *et al.,* 1986; Ball and Bayley, 1986; Bertolo *et al*., 1986; House *et al*., 1998; Shoveller *et al*., 2003), humans (Zello *et al*., 1993; Di Buono *et al*., 2001; Kriengsinyos *et al*., 2002) and chickens (Tabiri *et al*., 2002a; 2002b; Coleman *et al*., 2003). To better understand the IAAO technique there are several good reviews based on human studies that describe the benefits and disadvantages of the IAAO method (Pencharz and Ball, 2003; Brunton *et al.*, 1998). The technique is based on the concept that a deficiency of one indispensable AA will restrict protein synthesis. Therefore, all other indispensable AA will be in relative excess and will be oxidised. As the dietary intake of the AA_{test} increases, the oxidation of all other indispensable AA decreases, corresponding to the increase in protein synthesis. If the intake of AA_{test} increases beyond the requirement, no further change in

indicator oxidation will occur (Pencharz and Ball, 2003; Brunton *et al.*, 1998). The point at which the oxidation of the indicator AA reaches a plateau is taken as the requirement provided no other nutrient is limiting. The indicator AA must have an oxidative pathway distinct from and unrelated to the AA_{test} (Pencharz and Ball, 2003; Brunton *et al.*, 1998), so that a change in dietary AA_{test} will not affect the pool size of the indicator AA. Phenylalanine and lysine have been shown to be suitable indicator AA for IAAO studies in humans (Bross *et al.*, 1998). The oxidation pattern of L-[1-14C]phenylalanine as the indicator amino acid after changes in the dietary levels of lysine has been demonstrated in broiler breeder pullets (Coleman *et al.*, 2003).

The IAAO method is ideally suited for use in poultry. Although the requirements of many amino acids for broilers are well established, this technique can be used to determine the requirements of individual birds, such that an estimate of population variability can be established. Requirements of birds under specific conditions such as inflammatory stress can be rapidly determined. There are numerous other uses for this technique as well, including determination of metabolic availability of amino acids in feedstuffs.

VI. CONCLUSION

Broiler chicken production and consumption is increasing around the world; chicken meat is an efficient, high quality source of protein that is relatively simple to produce, even with limited inputs. The increase in demand for poultry products, and the economics driving increased efficiency have led to dramatic improvements in growth rate, breast muscle deposition and feed conversion efficiency of broiler meat. Many of the improvements have been the result of genetic selection; however the nutrition of the bird must be suitable to support such rapid and efficient growth.

Amino acid requirements of meat-type chickens have been published since at least the 1940s. In that time, the amounts of various amino acids as a proportion of the diet have not changed dramatically. The efficiency of use of those amino acids has increased dramatically, largely as a function of a decrease in age to a particular body weight. Little work has been published regarding the effect of rapid growth rate of modern broilers on meat quality and consumer acceptance.

The future of amino acid research may well be in the subtle topics of nutrition-immune function interactions, and relationships between environment and amino acid metabolism. Both of these areas may be important keys in maintaining high levels of production in the absence of growth-promoting antibiotics. New techniques such as the indicator amino acid oxidation technique can play a role in elucidating these relationships.

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STANDARDISED ILEAL DIGESTIBILITY – PROPOSAL FOR A NEW SYSTEM TO DESCRIBE AMINO ACID DIGESTIBILITY OF FEED INGREDIENTS FOR POULTRY

V. RAVINDRAN

Summary

Ileal amino acid digestibility is a more sensitive approach to describe the protein quality of feed ingredients than excreta digestibility values. The relative merits of apparent and true digestible amino acid systems are discussed. The concept of standardised digestibility system as a mean of overcoming the limitations of apparent digestibility estimates is proposed. But transformation of apparent ileal digestibility values to standardised ileal values will require reliable information on estimates of basal endogenous amino acid losses at the ileal level in growing poultry and further research is warranted in this area.

I. INTRODUCTION

It is now widely accepted that caecal fermentation in poultry has significant modifying effects on protein digestion and that amino acid digestibility in feed ingredients for poultry should be determined at the ileal rather than excreta level (Ravindran *et al*., 1999). However, most published values currently available, including several compilations (Sibbald, 1986; Parsons, 1991; Rhone-Poulenc, 1995; NRC, 1994; Heartland Lysine, 1996), on digestible amino acids for poultry are based on excreta analysis. All these values have been determined with adult cockerels using the rapid assay procedure of Sibbald (1979) or modifications thereof. The attraction of this rapid assay had been its simplicity and the assay can be carried out on a large number of birds without sacrificing the birds. These values are generally applied to all classes of poultry, including growing birds. In contrast, published values on ileal amino acids digestibility values in feed ingredients are limited. Only one database on ileal amino acids digestibility for broiler chickens exists (Ravindran *et al*., 1998), along with sporadic publications reporting ileal digestibility values for selected ingredients.

A major problem faced by the users of digestible amino acids databases is the considerable confusion that exists about the terminology used to describe the amino acids digestibility estimates. For each amino acids in a feedstuff, there are at least five possible values to describe the digestibility for poultry: apparent or true for excreta (from intact or caecectomised) or ileal digestibility (Ravindran and Bryden, 1999). In particular, the issue that is often debated is which ileal digestible amino acids system is most appropriate for use in diet formulations - apparent or true digestibility values.

II. APPARENT VERSUS TRUE DIGESTIBILITY

Ileal digestibility of amino acids can be expressed as apparent digestibility or as true digestibility. The difference between these two expressions arises from whether or not the digestibility estimates are corrected for endogenous losses of amino acids. *Apparent digestibility* measures the digestibility of amino acids of both dietary and endogenous origins. *True digestibility*, on the other hand, includes a correction for endogenous amino acids secretions, and is considered to be a fundamental characteristic of the feedstuff that is relatively constant across varying dietary protein levels. Therefore the use of true digestibility data permits feed ingredients to be compared even if they are assayed under varying dietary conditions.

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The need for correction of endogenous amino acids losses in amino acids digestibility estimates has been debated for many years both by poultry and pig nutritionists. The proponents of apparent digestibility system argue that as there is no reliable method for measuring endogenous secretions under a given dietary situation, a system based on apparent digestibility is a better practical basis for diet formulations. It is also argued that correcting apparent digestibility for endogenous losses can introduce artefacts and mask important differences between feed ingredients. Although digestibility is often considered to be a characteristic of a diet or feed ingredient, it is, in reality, the property of the ingredient in relation to the animal to which the diet is given. The contention is that if a feed ingredient increases endogenous amino acids flow out of the small intestine, that represents a loss to the animal and must be realistically 'charged' against the feed ingredient as lowered amino acids digestibility.

Nevertheless, it should be noted that there are some draw backs in using apparent digestibility data in diet formulations. First, the additivity of apparent digestibility values of individual ingredients when combined in diet formulations remains questionable. Though the additivity of apparent digestibility values has been demonstrated by some studies (Angkanaporn *et al*., 1996; Bryden and Li, 2003), further research on this topic is needed, since there may be associative effects especially when high levels of poorly digestible ingredients are used. Second, for feedstuffs with low protein content (eg. cereals, grain legumes), the apparent digestibility values are underestimated relative to feedstuffs with high protein content because of the relatively greater proportion of endogenous amino acids in the digesta or excreta. Especially those amino acids present at low levels in cereals or grain legumes (e.g. lysine, threonine and tryptophan) and those present in high levels in endogenous protein (e.g. threonine) will be affected. Third, the applicability of ideal protein concept in formulation based on apparent digestibility estimates is another limitation. Because of the way in which ideal protein ratios are determined, the patterns reflect true digestibility rather than apparent digestibility (Baker, 1996).

III. ILEAL ENDOGENOUS AMINO ACID LOSSES

The reliability of available methods (Table 1) for determining endogenous amino acids losses, under a given set of dietary circumstances, had been the major issue limiting the usefulness of true digestibility estimates. All the available methods of determining endogenous losses have specific applications and shortcomings (see review, Ravindran and Bryden, 1999).

Table 1. Methods used for the determination of endogenous amino acid flows in poultry.

- Fasting of birds for 24 to 48 hours[1]
- Feeding of protein-free diet
- Linear regression, following feeding of diets containing graded levels of protein
- Guanidinated dietary protein
- Enzyme hydrolysed casein and ultrafiltration
- Feeding of highly digestible protein, e.g. wheat gluten

[1] Used only to measure flows in the excreta.

It is now recognised that endogenous amino acids losses are influenced primarily by dry matter intake and secondarily by the inherent composition of the feed ingredient or diet (i.e. fibre level, presence of anti-nutritional factors, etc). These two fractions are referred to

as basal (also known as non-specific) and specific endogenous amino acids losses, respectively. Basal endogenous losses can be defined as those inevitable losses closely associated with the metabolic functions of the animal and are independent of the diet type. These losses, therefore, represent the minimum losses that can be expected under any feeding situation.

IV. STANDARDISED ILEAL DIGESTIBILITY VALUES

The limitations of apparent ileal digestibility values, discussed above, could be overcome by standardising these estimates through corrections for basal endogenous losses, as shown below:

$$SID = AID + \frac{\text{Basal endogenous amino acid flow (g/kg DMI)}}{\text{Amino acid content of the ingredient (g/kg DM)}}$$

Where SID = standardised ileal digestibility values, AID = apparent ileal digestibility values and DMI = dry matter intake.

The SID values are independent of the method by which the AID were originally estimated and, more importantly, additive when used in practical feed formulation. This proposal to convert AID values to SID values is not a new concept. Boisen and Moughan (1996) were among the first to suggest such transformations for the pig industry. Tabulated SID of protein and amino acids in common feed ingredients for pigs have recently become available (Rademacher *et al*., 1999; Amipig, 2000; Pedersen and Boisen, 2002), wherein published apparent digestibility values have been transformed to standardised values using existing literature data on endogenous amino acid recovery in ileal digesta

The obvious advantage of the proposed system, as against the 'conventional true digestibility' assay, is that apparent digestibility and endogenous losses need not be determined in the same experiment. Standardised ileal digestibility values of ingredients for poultry can be calculated for published apparent digestibility values in the literature, but this will require an estimate of the amount of basal endogenous protein and amino acid recoveries in ileal digesta.

V. ESTIMATION OF BASAL ILEAL ENDOGENOUS LOSSES

Compared to pigs, where the literature abounds with data on ileal endogenous amino acid losses, there is a paucity of corresponding information in poultry. Clearly further research and more data are needed to obtain reliable estimates of basal ileal endogenous losses in poultry. Only when sufficient data becomes available, will it be possible to agree on a valid estimate of basal losses at the ileal level. In the case of pigs, there has been much recent discussion about how to define basal endogenous losses and how this should be determined. While some accept values following the feeding of protein-free diets as valid estimates of basal losses (Jondreville *et al*., 1995), others have averaged across data determined using different methods (protein-free diet, regression and use of highly-digestible proteins such as enzyme hydrolysed casein and wheat gluten) and calculated a weighted mean, as a measure of basal losses (Rademacher *et al*., 1999; Pedersen and Boisen, 2002).

VI. CONCLUSIONS

A new system to describe amino acid digestibility in feed ingredients for poultry is proposed. It is suggested that the development of tabulated values of SID values offers the opportunity for the poultry industry to further improve the precision of diet formulations. The transformation of available AID values to SID values, however, requires reliable information on basal endogenous amino acid losses and further research is warranted in this area.

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EFFECTS OF DIETARY METHIONINE ON BROILER FLOCK UNIFORMITY

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Summary

Graded levels of DL-methionine (DL-Met) (0, 0.4, 0.8, 1.2 g/kg) were added to basal starter, grower and finisher diets deficient in methionine and cysteine in order to evaluate the effect on body weight and breast meat uniformity of male broilers. Birds were individually weighed at days 5 (start of experiment), 17, 35 and 42. Final body and breast meat weights increased from 1831 to 2836 g and 362 to 707 g, respectively, with increasing dietary methionine content. The coefficient of variation for body weight and breast meat weight decreased with increasing dietary methionine level. Model calculations revealed that if 1800±100 g body weight and 420±25 g breast meat weight were set as production targets at 42 days of age only 26% and 30%, respectively, of the flock would meet these targets when the basal deficient diets were fed whilst 58% and 59%, respectively, would meet these targets at the highest inclusion level.

I. INTRODUCTION

It is well documented that increasing levels of essential amino acids when starting from a deficiency increase broiler performance. The response typically follows the law of diminishing returns, which means a non-linear dose response approximating an asymptote. Such equations are appropriate for determining the optimum dietary amino acid levels for maximum performance and can be combined with parameters such as feed cost and revenues from meat marketing in order to determine economically optimum dietary amino acid levels (Hoehler, 2000; Pack and Schutte, 1995). Such calculations have been based on the potential dietary effects on the average performance of a flock, but have not considered the effects of dietary amino acid imbalance on uniformity or variability within a flock. Therefore, the purpose of the present study was to examine the effect that the addition of graded levels of DL-Met to a diet deficient in methionine plus cysteine (Met+Cys) has on the variability of body weight and breast meat yield in broilers. In addition, model calculations will demonstrate the potential impact of variability on profitability assuming that variability of a delivered flock would be considered in the pay-out system.

II. MATERIAL AND METHODS

A total of 640 five-day old male Ross 308 broiler chicks were used in the experiment. Birds were equally distributed to 20 pens with 32 birds each. Average body weight and coefficient of variation (CV) within each pen were 95 g and 5.7%, respectively. Eight pens were assigned to the basal treatment while four pens were assigned to each of the remaining treatments. Maize-wheat-pea-soybean meal based basal starter (day 6 to 17), grower (day 18

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to 35) and finisher (day 36 to 42) diets were formulated to be deficient in digestible Met (2.6 g/kg, 2.3 g/kg, 2.2 g/kg, respectively) and Met+Cys (5.5 g/kg, 5.1 g/kg, 4.9 g/kg, respectively). The digestible lysine contents were 12.0 g/kg (starter), 11.3 g/kg (grower) and 10.7 g/kg (finisher).

The calculated digestible Met+Cys to lysine ratio was 0.46 while the ratios of threonine, tryptophan and arginine to lysine were 0.68, 0.18 and 1.06, respectively. Calculated amino acid levels were confirmed by analysis. Three levels of DL-Met (0.4, 0.8**,** 1.2 g/kg) were supplemented to the diet to achieve graded levels of Met+Cys. Feed and water were offered for free consumption throughout the whole experiment.

Birds were individually weighed at days 5, 17, 35 and 42. At 42 days of age all birds were slaughtered in a commercial slaughterhouse, processed and breast meat weights were recorded for each bird. Broiler performance data were checked for outliers, which were defined as values outside ± 2 standard deviations of the treatment mean. In case of outliers not only the single value, but the whole data set of the bird was excluded from the statistics. Average body weights, breast meat weights and CVs were calculated for each treatment (Table 1). ANOVA was applied using individual birds as experimental units. Comparison of means was performed according to Scheffé (SAS Institute, 1987) and $P<0.05$ was considered statistically significant.

As the achieved average maximum body weight of birds on the basal treatment at day 42 was only 1831 g, 1800 ± 100 g was chosen as the production target weight for the model calculations and CVs for 1800 g body weight were calculated using the aforementioned equations for average body weights and CVs. By means of integral calculations the proportion of the flock meeting the target window of 1700 to 1900 g body weight was determined.

Since there was only one point in time for recording carcass data after termination of the experiment at day 42, model calculations could not be standardised to a certain breast meat weight as explained for body weight. Therefore, variability found at day 42 for the four treatments (Table 1) was applied. Since across all treatments breast meat yield was on average 23.3% of empty body weight, an average breast meat weight of 420 g was assumed for a bird of 1800 g live weight.

III. RESULTS AND DISCUSSION

The development of the average body weights over time was well described by Gompertz equations [$y = a * \exp(-b * \exp(-c * (days - 5)))$; $r^2 = 0.86\text{-}0.99$] for each of the four treatments while the development of the CVs over time followed exponential functions [$y = a + b * (1-\exp(-c * (days-5)))$, $r^2 = 0.60\text{-}0.89$].

Dietary treatments resulted in an additional weight gain of 1005 g leading to an average body weight of 2836 g at day 42 in birds fed the highest DL-Met supplementation level (Table 1). The latter performance indicates excellent experimental conditions suggesting that the respective CVs might also represent relatively low variability. The responses of body weight at days 17, 35, and 42 to increasing Met+Cys levels were non-linear. However, maximum performance was probably still not achieved at the highest supplementation level.

There was a very significant increase in CV with age from 5.6% at day 5 to 16.8% at day 42 in birds fed the basal diet whereas the CV increased only slightly in birds of the highest Met+Cys level (Table 1). Thus, CV decreased from 16.8% to 6.7% with increasing dietary Met+Cys at the termination of the trial. Therefore, the magnitude of the effects on variability appears to be dependent on both the degree and the duration of the amino acid deficiency. However, the variability in body weights at day 42 decreased with increasing

body weight. Under practical conditions the broiler producer has to meet a defined average target weight plus or minus a certain deviation. Average bodyweights outside the target range result in lower revenues. Current routine in many broiler production systems is to achieve the target weight on average for the whole flock without considering variation within the flock. If variation is considered, the question about the proportion of birds meeting the defined target window arises.

According to the present data, the CVs standardised to an average body weight of 1800 g decreased non-linearly from 17.0% to 6.7% with increasing Met+Cys levels. This is similar to the final CVs at day 42, encouraging the use of the final CVs for breast meat for further model calculations (Table 2). Only 26% of the birds on the basal treatment would meet the target range of final body weight whereas this percentage would increase to 58% in birds fed the highest DL-Met inclusion level.

Table 1. Body weights at various dates and breast meat yield of male Ross 308 broilers fed graded levels of Met+Cys.

	Basal diet without DL-Met	**+ 0.4 g/kg DL-Met**	**+ 0.8 g/kg DL-Met**	**+ 1.2 g/kg DL-Met**
Body weight (g)				
Day 5 Mean	95	95	95	95
CV (%)	5.6	5.6	5.8	5.9
Day 17 Mean	328 ^{D}	405 ^{C}	434 ^{B}	454 ^{A}
CV (%)	11.0	9.7	6.9	6.9
Day 35 Mean	1319 ^{D}	1826 ^{C}	1995 ^{B}	2153 ^{A}
CV (%)	16.4	11.8	10.0	6.6
Day 42 Mean	1831 ^{D}	2452 ^{C}	2646 ^{B}	2836 ^{A}
CV (%)	16.8	11.1	9.5	6.7
Breast meat weight (g)				
Day 42 Mean	362 ^{D}	543 ^{C}	630 ^{B}	707 ^{A}
CV (%)	18.5	14.3	11.7	8.7

^{A,B,C,D} different superscripts within a row indicate significant differences according to Scheffé ($P<0.05$).

Table 2. The 1800 g body weight standardised coefficients of variation (CV) and related proportions of a broiler population meeting the production target of 1800 ± 100 g body weight and 420 ± 30 g breast meat weight

	Basal diet without DL-Met	**+ 0.4 g/kg DL-Met**	**+ 0.8 g/kg DL-Met**	**+ 0.12 g/kg DL-Met**
CV standardised to 1800 g body weight				
%	17.0	11.3	9.0	6.7
Percentage of birds with body weight of 1800 ±100 g				
%	26	37	48	58
Percentage of birds with breast meat weight of 420 ± 30 g				
%	30	38	47	59

Assuming a monetary deduction of $0.01 per kg live weight for birds outside the production target of 1800±100 g in a 10,000 birds flock, this would mean $58 higher revenue at 1.2 g/kg supplemental DL-Met compared to the basal diet. Under practical conditions the effect can be expected to be of a smaller magnitude since the Met+Cys content would not be as low as in the present basal diet. On the other hand, the variability as such might be higher in reality compared to that obtained with the experimental conditions in the present trial. This could be reduced by balancing the amino acid profile.

A similar effect would occur if revenues were based on breast meat yield (Table 2). Defining a production target of 420±30 g only 30% of a flock would meet the target when fed the basal diet while 59% of the flock would meet it at the highest Met inclusion level. Setting the financial penalty at $0.05 per kg breast meat outside the target range for a 10,000-bird flock would lead to $61 higher revenue at the 1.2 g/kg supplemented DL-Met level compared to the basal diet.

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"STANDARDISED" ILEAL AMINO ACID DIGESTIBILITY IN BROILER NUTRITION

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Summary

The "standardised" ileal amino acid digestibility of several raw materials commonly used in broiler nutrition has been determined. This was done by determining the apparent ileal amino acid digestibility and subsequent correcting for endogenous losses. In addition to the experimental data appropriate literature data were also utilised. Therefore, a basis for an improved raw material assessment has been created.

I. INTRODUCTION

One goal of modern broiler nutrition is to find out the optimum dietary nutrient and amino acid levels and to use appropriate raw materials in order to meet these requirements in a sustainable manner. However, in terms of amino acids it is well established that part of the dietary amino acids are undigested and are thus excreted. Moreover, amino acid digestibility varies between raw materials. In order to be more precise in feed formulation and to be able to predict animal performance more accurately it is meaningful to base both feed formulation and recommendations on digestible rather than total amino acid data.

Many studies have been carried out during the last two decades to determine digestibility coefficients of amino acids in a wide variety of feedstuffs for poultry. The results have been published in a number of tables. Most of the data available is based on excreta digestibility. As described by Ravindran *et al*. (1999) and Ravindran (2004) the methodology behind the excreta digestibility determination contains several drawbacks and is subject to a critical discussion. However, to overcome these limitations, determination of the ileal amino acid digestibility for growing broilers that are fed experimental diets on an *ad libitum* basis represents an alternative procedure.

Irrespective of the site for collecting the digesta or excreta, it is also well known that amino acids found in these samples are of both dietary and endogenous origin. These endogenous secretions originate from various sources including saliva, pancreatic secretions, sloughed off epithelial cells and mucin. Depending on the protein intake and protein content of the raw material the proportion of endogenous losses to the total amino acid content in the digesta samples varies. This can result in an underestimation of amino acid digestibility, especially of raw materials with low protein content. To correct for this methodological inaccuracy basal endogenous losses can be determined and the apparent digestibility coefficients can be adjusted or standardised accordingly.

In the project presented here the apparent ileal digestibility of 17 raw materials (barley, maize, sorghum, triticale, wheat, rice pollard, wheat middlings, beans, corn gluten meal, cotton seed meal, lupines, peas, rapeseed or canola meal, soybean meal, sunflower meal, feather meal, fish meal, and meat and bone meal) has been determined in growing

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broilers. In addition, data available from literature was included. Subsequently, the apparent digestibility figures were "standardised". The use of the term "standardised" instead of "true" is preferred since the data for endogenous losses was derived by compiling available literature data and thus served as best approximation. All raw materials were standardised by the same data set while "true" digestibility figures are commonly adjusted by using endogenous losses determined in the same experiment. In addition, the term "true" suggests that there is no inaccuracy or residual error possible.

II. METHODS

The amino acid digestibility of 17 raw materials has been determined in several assays. However, only the results of the four most important ones in broiler nutrition are reported here. Each assay diet was offered *ad libitum* to three pens (4 birds/pen) of male broilers from 35 to 42 days of age. On day 42, birds were sacrificed by injection of sodium pentabarbitone solution, the small intestines were obtained and the digesta in the terminal ileum, defined as the section from Meckel's diverticulum to 4cm proximal the ileo-caecal junction, was collected by flushing with distilled water. The digesta samples of each pen were pooled resulting in three replicates per experiment. The samples were freeze dried, homogenised and stored at -20°C before amino acid, crude protein, dry matter, and acid insoluble ash analysis. Amino acids were analysed according to the methods described by Llames and Fontaine (1994) and Fontaine *et al.* (1998). Different assay diets were used for cereal grains and protein meals. In case of grains (and grain by-products) the diets consisted of 918g/kg of the test substance supplemented with 42g/kg mineral and vitamin premix and 20g/kg vegetable oil. In case of protein meals the diets were based on dextrose and the test substances as the only source of protein. In each diet the proportions of test feedstuff and dextrose were varied to obtain 200 g crude protein/kg of diet. Diets containing ingredients of plant origin, blood meal and feather meal had identical calcium and phosphorus supplements, but these supplements were not included in diets containing fish meal, meat meal and meat and bone meal. Solkafloc (30g/kg) was added as a source of fibre in diets containing animal protein meals. Celite (20g/kg) was added to all diets as a source of acid-insoluble ash (AIA), which was used as an indigestible marker in the calculation of digestibility coefficients.

Apparent ileal amino acid digestibility was calculated for each observation and averaged for each ingredient. Data obtained from the literature were also included. However, papers had to meet several requirements. Amino acid analysis had to be done according to the methods mentioned above. Data had to be given as apparent digestibility. Otherwise data for endogenous losses were required in order to re-calculate the apparent digestibility. Experimental procedure had to be similar to that described above. In some cases, digestibility had been determined by the difference or substitution method which means that the digestibility of the raw material is determined by the comparison of two diets: A test diet containing the test ingredient but also other amino acid containing raw materials and a basal diet without test ingredient. The amino acid digestibility of the test diet is affected by all protein sources and is thus adjusted by using the amino acid digestibility of the basal diet in order to derive the digestibility figures of the test substance. By this approach digestibility coefficients are already corrected for the endogenous losses. However, depending on the percentage of amino acids from the test substance to total amino acids in the test diet, the effect of inaccuracies in the amino acid determination of the basal diet can be high. In cases where the latter was obvious, data sets were not considered.

In order to correct the apparent ileal amino acid digestibility data for inevitable losses a literature survey was conducted. Data sets derived by different methods (regression, N-free, wheat gluten, guanidinated casein, enzymatically hydrolised casein) are available in

literature. Advantages and draw backs of these methods have been described in detail by Ravindran and Bryden (1999). Accordingly, the enzymatically hydrolysed casein (EHC)-method is the most sophisticated one and covers most of the drawbacks of the other methods. An EHC containing diet is fed to the birds stimulating the digestion processes in a normal physiological manner. In the digesta amino acids of EHC-origin can be separated by ultra-filtration and thus endogenous and exogenous amino acids can be distinguished. Therefore, the EHC-method was found to be best and the results of five available data sets were averaged (Table 1).

Table 1. Average* ileal endogenous amino acid losses (mg/kg dry matter intake) determined by feeding enzymatically hydrolysed casein diets to broilers.

Crude protein (CP) and amino acids	**CP**	**Met**	**M+C**	**Lys**	**Thr**	**Trp**	**Arg**
mg/kg DMI	9234	79	257	255	571	82	216

* Data represent the average of five data sets.

Standardised ileal digestibility coefficients (SDC) were calculated in two steps. First, the apparent digestibility coefficients (ADC) for the crude protein and amino acids of the test diet were recalculated into apparent ileal digestibility coefficients of the test ingredient. This was also done for literature data except for figures that were derived by the so-called difference method (see above). In a second step the apparent ileal amino acid and protein digestibility of the raw materials were standardised to the following formula:

SDC = ADC + (endogenous protein or amino acid losses (g/kg DM intake) / protein or amino acid content of the raw material (g/kg DM) * 100).

III. RESULTS AND DISCUSSION

As shown in Table 1, the lowest ileal endogenous losses were reported for methionine and the highest for threonine, with losses for the remaining amino acids falling between these two extremes. This ranking is in line with data provided by other methods used to determine endogenous losses (Cremers, 2003). The relatively high threonine losses are also consistent with findings in swine nutrition (Jansman *et al*., 2002). It is assumed that mucosal secretions, such as mucin contribute to the high threonine losses since the threonine content of mucin is relatively high (about 15%).

Table 2. Standardised ileal digestibility coefficients (%) of four raw materials for broilers**.**

	CP	**Met**	**M+C**	**Lys**	**Thr**	**Trp**	**Arg**
Maize	90	94	90	92	85	81	93
Wheat	88	91	91	86	87	86	85
Soybean meal	90	91	86	90	85	89	93
Meat & bone meal	65	72	62	69	62	55	77

In Table 2 the standardised ileal amino acid digestibility of four ingredients important in broiler nutrition are shown. The number of experiments or observations varied between raw materials. Six, 11, 37, and 30 observations were available for maize, wheat, soybean meal and meat and bone meal, respectively.

With respect to maize, wheat and soybean meal the digestibility coefficients ranged between 81% and 94%, while those for meat and bone meal were between 55% and 77%. The amino acid digestibilities for maize and wheat represent the general level for grains,

whereas the values for soybean meal are relatively high compared to other plant protein sources (average 82%). The digestibility coefficients for meat and bone meal are somewhat lower than those found for fish meal but higher than those found for feather meal (Data not shown). However, it is well established that the amino acid digestibility of animal by-products are dependent on several processing characteristics like heat treatment during processing or ash content.

As expected the strongest effect of standardisation compared to apparent digestibility figures was observed for grains. The difference between standardised and apparent ileal digestible amino acids ranged between 3 and 17 percentage points for corn and wheat while it ranged only between 1 and 3 percentage points for soybean meal and meat and bone meal. Within the raw material the threonine digestibility was most affected by the standardisation, which can directly be related to the high value for endogenous losses. The standardised ileal threonine digestibility of corn, wheat, soybean and meat and bone meal was 17, 14, 3 and 3 percentage points higher, respectively, than the apparent ileal threonine digestibility. The respective values for methionine were only 4, 3, 1 and 1 percentage points.

Compared to total amino acids the relative preference of raw materials might change when diets are formulated on standardised ileal digestible amino acids. Probably even more important is that performance prediction can be done more precisely, particularly when raw materials with relatively low amino acid digestibility such as meat and bone meal, peas/beans or rice pollard are used. However, first model calculations suggested, that in complex diets including sorghum, wheat, canola meal, soybean meal and meat and bone meal the preference for wheat and soybean meal increases whilst that for sorghum, rapeseed meal, and meat and bone meal decreased. However, such effects, or at least their magnitude, are severely dependent on the prices of the raw materials and the number and levels of amino acid restrictions in the feed optimisation. In addition, it should also be recognised that switching from one (total) to the other (digestible) system affects the recommendation for optimum dietary amino acid levels which, once adapted, influence the diet composition.

IV. OUTLOOK

There are now enough data on standardised ileal amino acid digestibility available for use in practical feed formulation. However, since a feeding system only works when all parts use the same basis an adjustment of broiler amino acid requirement has to be done. The magnitude of possible changes in diet compositions might depend on several factors. For example, the new data might impact the relative preference of the raw materials when compared to the system based on total amino acids. However, such effects are strongly dependent on actual market prices of the raw materials and on the restrictions used in feed formulation.

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THE ACCURACY AND USABILITY OF THE RADIAL GEL DIFFUSION ASSAY AND A DYE-RELEASE TECHNIQUE FOR DETERMINATION OF β-GLUCANASE IN FEED.

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Summary

Two enzyme assays, the radial diffusion and the dye-release methods, were modified and tested for their suitability in measuring β-glucanase activity in poultry feed. Enzymes A and B were incorporated into a barley-based broiler starter diet at three levels (500, 1000 and 2000 g/t). The measured β-glucanase activities were 35 U/g and 49 u/g for Enzymes A and B, respectively. A comparison of enzyme recovery rates from samples containing known amounts of the enzyme and from a buffer solution containing the same amount of the enzyme revealed that a fixed percentage of the enzyme bound to feed components in a dose responsive manner. Thus, enzyme recovery rates for the feed samples were not complete and varied between 66% and 97% for the radial diffusion assay and between 76% and 91% for the dye-release assay. The two assays largely agreed with each other although at low enzyme concentrations, the dye-release assays gave higher recovery rates with lower standard errors. It may be concluded that both assays are useful in determining β-glucanase activity in feed and digesta samples, but the radial diffusion assay is cheaper to perform.

I. INTRODUCTION

Application of feed enzymes has expanded into various sectors of the feed and livestock industries. This has placed a more urgent need for rapid and reliable assays for determination of enzyme activities in feed. Currently, the dye-release method for measuring xylanase activity in feed is widely used in the pig and poultry industries around the world, but there is not an equivalent assay for β-glucanase specifically developed for the feed industry. A number of other techniques, including the viscometric technique and the radial gel diffusion method, have been used to determine the activities of non-starch polysaccharide-degrading enzymes (Rotter *et al.*, 1990; McCleary, 1995). The viscometric technique is used to determine the effect of glycanases on reducing viscosity using pure substrates, such as soluble arabinoxylans, β-glucans and pectins as standards, whereas the radial gel diffusion assay is based on the diffusion of enzyme-containing solutions from wells through a gel medium containing a substrate, e.g., β-glucan, stained with congo red. As the dyed substrate is depolymerised, Congo red which has a strong affinity to polysaccharides containing contiguous β(1→4) linked D-glucopyranosyl residues (Wood, 1981), is released and diffuses outward in a radial manner leaving a circular clear zone (Walsh *et al.*, 1995). A linear relationship between the diameter of the clear zone and log of the enzyme concentration provides the basis for activity determination. A β-glucanase assay has been developed and used routinely for the determination of β-glucanase activity in malt (McCleary and Shameer, 1987), but its suitability for use in the feed industry has not been fully evaluated.

This study investigates the suitability of the radial gel diffusion assay for determining β-glucanase activity in feed in comparison with the dye-release assay.

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II. MATERIALS AND METHODS

(a) Preparation of feed samples and enzyme extracts

Two β-glucanase sources were added at dosages of 0.5, 1.0 and 2.0 g per kg of broiler feed. Enzyme A was a dry commercial β-glucanase product (Kemin Industries, Singapore), whereas Enzyme B was a liquid lichenase of high purity (Megazyme, Co. Wicklow, Ireland). The diets were cold pelleted at 60^{o}C. The enzymes in the diets were extracted from 20.0 g of sample with 200 ml of phosphate buffer. After shaking, the flasks were allowed to stand for 10 min before aliquots of supernatant were filtered through 0.2 μm filter discs.

(b) Standard curves

The enzyme standards were prepared by adding 0, 0.025, 0.050, 0.075 and 0.100 % (w/w) of the enzyme to 10.0 g of untreated (control) feed. The enzymes were extracted with 100 ml of 0.01 M phosphate buffer (pH 6.0) with continuous orbital shaking for 15 min.

(c) Radial Diffusion Assay

Agar plates were prepared by dissolving 0.03 % β-glucan from barley (Megazyme) in boiling 0.1 M phosphate buffer (pH 6.0). Congo red and bacteriological agar were then added at 0.007 and 1.0 %, respectively, in succession and boiled until a clear solution was obtained. Approximately 25 ml of the agar solution were poured into Petri dishes (9 cm diameter). Small wells of 6 mm in diameter were made in the plates and 70 μl of feed extract and enzyme standards were loaded. The plates were then incubated at 37^{o}C for 16 h. The diffusion radius was then measured and enzyme activities calculated in relation to the standard controls.

(d) Dye-release assay

The assay developed was modified from the malt β-glucanase assay procedure provided by Megazyme. To each tube containing 0.5 ml of Azo-barley glucan substrate, 1.0 ml of feed extract (pH 6.0) was added and mixed before incubation for 10 min at 37^{o}C. Then 3.0 ml of a precipitant solution (made up of 40 g sodium acetate and 4.0 g of zinc acetate in 150 ml water) were added. The pH was adjusted to 5.0 with concentrated HCl and volume adjusted to 200 ml. This solution was finally mixed with 800 ml of methyl cellosolve (2-methoxyethanol) and left to stand for 5 min, and then centrifuged for 5 min at 1,700 g. The absorbance of the supernatant was read at 590 nm and β-glucanase activity calculated according to McCleary and Shameer (1987).

III. RESULTS

(a) Standard curves

Standard curves relating the "clear zone", as a result of enzymatic hydrolysis, and the logarithmic function of enzyme concentration were constructed with filtered and non-filtered feed extract supernatants (Figures 1a,b). A third standard curve was constructed with the same enzyme stock solution that was diluted in buffer (*i.e.*, without the addition of feed). The unfiltered extract resulted in higher hydrolysis rates than the filtered extracts. The enzyme

solution diluted with buffer gave the highest hydrolytic rate, indicating some of the enzymes binding to feed particles.

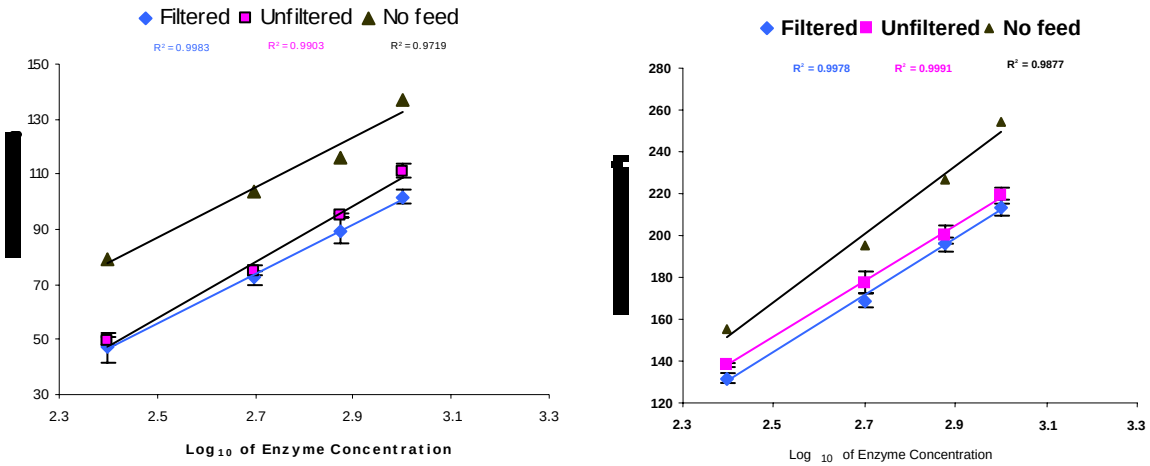

Figure 1. Standard curves for Enzyme A (Figure 1a, left) and Enzyme B (Figure 1b, right), showing the logarithmic function of enzyme concentrations with areas of "clear zone". Three curves display standard curves produced from filtered feed samples (◆), unfiltered samples (■), and no feed controls (▲).

(b) Enzyme recovery rates

The recovery rates for Enzymes A and B were above 50% of the expected values in all cases (Table 1). For most doses for both enzymes, the recovery rates obtained using the two assays were similar, although the dye-release assay gave numerically higher recovery rates when the level of enzymes in the feed was below 1000 g/t (Table 1).

Table 1. Comparison of the recovery rates of Enzymes A and B using the radial gel diffusion assay with those obtained using the dye-release assay.

Enzyme	Expected (g/t)	Radial Diffusion		Dye-Release		Assay Difference %
		g/t	% recovery	g/t	% recovery	
A	500	328 ± 64	65.6	389 ± 62	77.8	-15.7
	1000	766 ± 132	76.6	870 ± 72	87.0	-12.0
	2000	1810 ± 173	90.5	1733 ± 14	86.7	4.4
B	500	344 ± 64	68.8	378 ± 30	75.6	-9.0
	1000	970 ± 171	97.0	913 ± 179	91.3	6.2
	2000	1701 ± 64	85.1	1638 ± 22	81.9	3.9

IV. DISCUSSION

Little information is available on the survival rate of enzymes during processing (mixing, steam-conditioning, pelleting and storage) and in the digestive system (gastric acid, proteolytic hydrolysis and pH) before reaching the small intestine. Thus, the extent of "over-dosing" of supplementary enzymes, a common practice in the industry to ensure efficacious responses, is unknown. The current study assessed the effectiveness of the radial gel diffusion assay and a modified dye-release assay in determining the β-glucanase activity in feed, and possibility of developing them as a research tool for measuring enzyme activities in digesta samples.

The current study explored the possibility of using unfiltered feed samples for determination of enzyme activities in order to simplify the assay procedure further. The data from the standard curves relating the zone of hydrolysis and log of enzyme concentration for the radial diffusion assay demonstrated clearly that (a) filtering did not make any difference for the sensitivity of the assay, and (b) a portion of the exogenous enzyme was bound to feed components and was not extractable for analysis. The latter finding is consistent with McCleary, (1995) work, with the exception of the standard curves for the concentrations tested in this investigation that were almost parallel to the standard curve constructed without the presence of feed (Figure 1). This would suggest that the feed bound a fixed percentage rather than a fixed amount of the exogenous enzymes. It is, however, not known whether this linear relationship would hold over a wide range of enzyme concentrations in the feed. The results also showed that a percentage of the exogenous enzymes were bound to the filter disc membranes, highlighting the importance of constructing appropriate standard curves in order to obtain accurate recovery results.

The majority of the recovery results from the dye release assay correlated well with those from the radial gel diffusion assay (Table 1) although there were differences of up to 15% between the assays when the level of enzyme was low in the feed. Both assays are easy to conduct and do not require a large amount of samples. The dye-release assay produced less error, most likely the result of less subjectivity in measurement as compared to the radial gel diffusion assay. Another advantage of the dye-release assay is the speed with which results could be obtained. However, a drawback of this assay is the cost of the chromogenic substrate.

Radial gel diffusion and dye-release assays were developed and successfully used in the determination of supplementary β-glucanase in feed. Further research is required to refine the methods to improve their consistency.

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EFFECTS OF GERMINATION OF GRAINS ON APPARENT METABOLISABLE ENERGY VALUES AND PERFORMANCE OF BROILER CHICKENS

R.J. HUGHES[1] and R.J. van BARNEVELD[2]

Summary

In situ degradation of non-starch polysaccharide components by endogenous enzyme activity in whole grains is thought to improve the apparent metabolisable energy (AME) value of some wheats in the early post-harvest period. This study examined changes in AME and subsequent growth performance of broiler chickens fed barley, sorghum, triticale and wheat that were subjected to controlled germination for 20 or 48 hours to activate endogenous enzymes in the grains. Germination of barley for 48 h improved AME by about 1 MJ/kg, but there was improvement by germination for 20 h. Germination for 20 and 48 hours did not alter AME values for wheat, triticale and sorghum.

I. INTRODUCTION

Optimising the supply of available energy to broiler chickens is fundamental to improving production efficiency. It is already common practice to use exogenous enzymes in broiler diets as a means of increasing apparent metabolisable energy (AME) and to reduce variation in AME between samples. This is accomplished primarily by reducing the viscosity of digesta and disrupting cell walls to expose substrates such as starch to digestive enzymes (Hughes and Choct, 1999). Similarly, storage of grains between 3-6 months is also known to increase AME, possibly through *in situ* degradation of non-starch polysaccharide components by endogenous enzyme activity (Choct and Hughes, 1997). To this end, controlled germination of cereal grains prior to incorporation into poultry diets may represent a further means of exploiting endogenous enzymes to increase AME and reduce variation within and between grain types. Germination of cereal grains is a complex process and is triggered when ripe grain imbibes adequate moisture at an appropriate temperature to promote growth of the seed embryo. Growth of the embryonic axis is accompanied by the production of hydrolytic enzymes, which solubilise nutrients stored in the endosperm and can promote hydrolysis (Evers *et al.,* 1999). The aim of this experiment was to measure the influence of controlled germination of different types of cereal grain on subsequent AME values and resulting growth performance of broiler chickens. No special attention was directed towards variety of particular cereals, as the main intention was to compare responses across the main types of cereal used in poultry diets in Australia.

II. MATERIALS AND METHODS

Small samples (1 kg) of all grains were control sprouted at 20^{o}C for 0, 16, 18, 20, 22, 24, 26, 28, 40 and 48 hours. The relative α-amylase activity was determined by dye-labelling of substrate (Barnes and Blakeney, 1974). Larger quantities (20 kg) of 20 h and 48 h sprouted grain were produced for the feeding trial. Following germination, grains were dried at 40^{o}C until average moisture content was 11% then tested again to determine the relative α-amylase level to confirm that the 20 h and 48 h samples of each grain had medium and high enzyme activity, respectively.

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The AME values of grains were determined in a conventional energy balance experiment involving measurements of feed intake and excreta output as described by Mollah *et al.* (1983) with minor modifications, and subsequent measurement of gross energy values of feed and excreta by bomb calorimetry. Day-old feather-sexed broiler chickens were raised in floor pens on a commercial broiler diet to 20 d of age then transferred in single-sex pairs to metabolism cages in controlled temperature rooms to allow chickens to adapt to the cages. At 22 days of age, one bird was removed from each cage. Air temperature was maintained at 26°C at the start of the 7-day experiment and lowered daily until it was 23°C at the end. Experimental diets contained (per kg) 800 g grain, 155 g casein, 20 g dicalcium phosphate, 11 g limestone, 7 g DL-methionine, 5 g mineral and vitamin premix, 3 g salt, and 2 g choline chloride (60%). Dietary treatments were replicated six times (three cages of males and three cages of females). Cold-pressed diets were fed for seven days. The first three days enabled the chickens to adapt to the feeds. During the following four days, all excreta were collected and dried at 85°C. Moisture content of excreta voided over a 24 h period was measured. Feed intake was measured during the adaptation and collection phases of the study. Birds were weighed at the start and end of the 7-day period. Dry matter (DM) contents of samples of pelleted and milled feeds were measured. Gross energy values of dried excreta and milled feeds were measured with a Parr isoperibol bomb calorimeter. AME of the grain was calculated by subtracting from the total energy intake the energy contribution of casein, which was assumed to be 20.1 MJ/kg dry matter (Annison *et al*., 1994*).*

III. RESULTS AND DISCUSSION

The effects of type of cereal grain, germination time and sex of chicken are summarised in Table 1. The 2-way interaction between germination time and sex of chicken, and the 3-way interaction between type of grain, germination time and sex of chicken were not significant (P>0.05) for any measurement. The moisture content of excreta from birds given barley (702 g/kg) was significantly higher (P<0.05) than that from chickens given wheat (658 g/kg) and sorghum (634 g/kg). Other differences in moisture content were not significant (P>0.05). The effects of the significant (P<0.05) 2-way interaction between cereal grain and germination time on AME and dry matter digestibility (DMD) are summarised in Table 2.

Table 1. Summary of the effects of type of cereal grain, germination time (in hours) and sex of chicken on feed intake (FI, g/bird), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of grain, dry matter digestibility (DMD, g retained/g eaten), and excreta moisture (EM, g/kg).

Source of variation	**DF**	**FI**	**GR**	**FCR**	**AME**	**DMD**	**EM**
				Probability of greater F value			
Cereal (C)	3	*	*	NS	***	***	**
Germination (G)	2	NS	NS	NS	NS	NS	NS
Sex (S)	1	**	**	NS	NS	NS	NS
CxS	3	NS	*	**	NS	NS	NS
CxG	6	NS	NS	NS	***	*	NS
Error mean square		80.3	2705	0.0103	0.346	7.89×10^{-4}	27.6
Coefficient of variation		8.1	11.4	5.9	3.9	3.8	7.8
Mean		110	455	1.708	15.00	0.742	670

DF is degrees of freedom; NS P>0.05, * P<0.05, ** P<0.01, *** P< 0.001.

Controlled germination for 20 h did not improve AME or DMD (Table 2) for any of the cereal grains, nor was growth performance affected (Table 1). The significant decline in AME and DMD of wheat germinated for 20 hours is difficult to explain except to point out that two birds (one male and one female) voided large amounts of excreta with normal moisture and gross energy contents relative to other birds in the same treatment group. Hughes (2003) concluded that alteration of the balance between the host and its resident microflora (by feeding different grains, for example) can result in outcomes that are difficult to predict, particularly if the diet is not supplemented with feed enzymes or antibiotics.

Table 2. Effects of type of cereal grain and experimental germination time (in h) on apparent metabolisable energy (AME MJ/kg dry matter) of grain and dry matter digestibility (DMD, g retained/g eaten) of the diet.

Grain type	Germination time	AME	DMD
Barley	0	13.82 e	0.691 f
	20	14.26 de	0.693 f
	48	14.92 cd	0.727 de
Sorghum	0	16.36 a	0.791 ab
	20	16.68 a	0.806 a
	48	15.91 ab	0.771 bc
Triticale	0	14.29 de	0.728 de
	20	14.88 cd	0.747 cd
	48	14.80 cd	0.749 cd
Wheat	0	14.93 c	0.744 cde
	20	14.06 e	0.712 ef
	48	15.17 c	0.749 cd
	Pooled SEM	0.24	0.011

Means in a column having a common letter are not significantly different (P>0.05).

Table 3. Effects of type of cereal grain and sex of chicken on feed intake (FI, g/bird), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain).

Grain type	Sex	FI	GR	FCR
Barley	Female	108 b	431 b	1.761 ab
	Male	123 a	537 a	1.610 d
Sorghum	Female	107 b	456 b	1.647 cd
	Male	111 b	465 b	1.678 bcd
Triticale	Female	107 b	421 b	1.782 a
	Male	112 b	470 b	1.679 bcd
Wheat	Female	105 b	424 b	1.733 abc
	Male	108 b	431 b	1.776 a
	Pooled SEM	3	17	0.034

Means in a column having a common letter are not significantly different (*P*>0.05).

Controlled germination of grains for 48 hours significantly improved AME and DMD for barley only (Table 2). The 1 MJ/kg increase in AME by germination of barley is the same as the increase in ileal digestible energy reported by Svihus *et al*. (1997) who soaked barley for 24 hours at ambient temperature then germinated it for 48 hours. Svihus *et al*. (1997) also observed significant improvements in weight gain (21%) and feed conversion (16%) with this

treatment of barley. They attributed the improvements to decreases in the soluble and total β-glucan contents, and to a reduction in acid extract viscosity of the grain.

There were large differences in feed intake, growth rate and feed conversion between male and female chickens given barley (Table 3). Differences between males and females given other cereal grains were not significant for any measurement except for a significant improvement in feed conversion of the triticale diet by males compared with females (Table 3). It is possible that differences between males and females were associated with proliferation of hindgut microflora (Hughes, 2003) following the influx of relatively large quantities of undigested β-glucan from barley.

IV. CONCLUSIONS

With the exception of barley, controlled germination of cereal grains had no beneficial effects on energy metabolism or performance of broiler chickens. The increase in AME value was of the same order (approximately 1 MJ/kg DM) as that previously reported with use of feed enzyme products.

V. ACKNOWLEDGMENTS

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THE BROILER RESPONSES OF VERSATILE ENZYME ON SORGHUM DIETS

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Differing from blended enzymes, a versatile enzyme (Rovabio™) from a non-GMO fungus *Penicillium funiculosum* contains xylanase, β-glucanase, cellulase plus more than ten auxiliary enzymes (Geraert *et al.,* 2003). This versatile spectrum covers various dietary types as well as different monogastric species.

Two metabolic studies were conducted to determine responses on sorghum/wheat based broiler diets produced by a feed-miller in Australia. Rovabio™ Excel LC Liquid was sprayed onto pellets at 200 ml/t, fed to Ross male broilers, 12 replicates per treatment. Excreta were collected during days 19-22. Diet formulations and results are shown in Tables 1 and 2.

Table 1. Diet formulation

Study I, g/kg		**Study II, g/kg**	
Sorghum	510	Sorghum	367
Wheat	150	Wheat/flour 1:1	200
Soybean meal	130	Soybean meal	220
Canola meal	80	Sunflower meal	51
MBM	70	Meat meal	53
Poultry oil	20	Feather meal	12
Premix	40	Tallow	60
		Premix	37

Table 2. AME (MJ/kg DM) and protein digestibility

	Study I			**Study II**		
	Control	**Rovabio**	**Δ**	**Control**	**Rovabio**	**Δ**
AME, MJ/kg DM	13.98	14.30	+0.32	14.79	15.20	+0.41
AMEn, MJ/kg DM	13.30	13.62	+0.32	14.01	14.43	+0.41
AME, MJ/kg	12.62	12.91	+0.29	13.45	13.82	+0.37
AMEn, MJ/kg	12.62	12.91	+0.29	12.67	13.05	+0.38
Protein (%)	82.3	83.5	+1.2	88.2	91.6	+3.4

In Trial I, the apparent (AME) or N-corrected metabolisable energy (AMEn) was increased by 2.3%, or +0.32 MJ/kg DM ($P=0.06$), and protein digestibility by 1.2 percentage units ($P<0.05$). In Trial II, AME and AMEn were up by 2.7 to 3.0%, or +0.41 MJ/kg DM ($P=0.07$), and protein digestibility by 3.4 percentage units ($P<0.01$). Growth data recorded from days 12 to 22 showed the enzyme improved feed conversion by 1.2% (Study I) and reduced bird digestion variability (Study II), $P>0.05$. No other growth improvements were observed.

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THE EFFECT OF LEVELS OF COPRA MEAL AND ENZYMES ON BIRD PERFORMANCE

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Summary

Diets with different levels of copra meal (0, 10, 30 and 50 %) with or without a combination of three enzyme products (Hemicel®, Gamanase® and SSF®) were fed to meat chickens from d 4 – 14. The inclusion of copra meal depressed weight gain, feed intake, DM digestibility and increased FCR significantly. The combination of the three enzyme products significantly improved weight gain, DM digestibility and decreased FCR.

I. INTRODUCTION

Problems associated with using copra meal in poultry diets have been reviewed by Sundu and Dingle (2003). High β-mannan content (Balasubramaniam, 1976), possible heating during drying and oil extraction which could lead to "Maillard reactions" (Butherworth and Fox, 1963), coupled with its limiting amino acids, impair the quality of this feedstuff. Attempts have been made to overcome these problems through supplementation with amino acids (Thomas and Scott, 1962) and enzymes (Pluske *et al*., 1997). The use of mannanase, α-galactosidase and cellulase may assist the cleavage of copra polysaccharides which are mainly β-mannans (87 %) and cellulose (13 %). β-mannan contains mannan and galactomannan (Balasubramaniam, 1976). This experiment tested diets supplemented with an enzyme combination containing mannanase, α-galactosidase and cellulase as well as pentosanase, β-glucanase, protease, phytase, amylase and pectinase for their effects on chick performance.

II. METHODS

After an initial four-day period, 32 male Ross chicks, weighing between 54 and 76 g (66.1 ± 1.19; mean ± SE), were selected and transferred into individual cages in a controlled temperature room and exposed to 23 h light and one h dark and fed the experimental diets *ad libitum* for 10 days. Four chicks were allocated randomly to each treatment. After data collection for production parameters, three representative chicks from each treatment were then kept in the same cages for six more days. Faeces were collected for three days from days 18 to 20 to measure dry matter (DM) digestibility.

The four basal diets contained 0, 10, 30 and 50 % copra meal (CM). Half of each of the respective basal diets were treated with a combination of "Hemicel mannanase(R1)", "Gamanase(R2)" (enzyme mixture containing β-mannanase and ∝-galactosidase) and "Allzyme SSF(R3)" (source of seven combined enzymes; cellulase, pentosanase, protease, phytase, β-glucanase, amylase and pectinase). The amounts of these enzymes added were 50 mg/kg feed, 200 mg/kg feed and 20 mg/kg feed for Hemicell, Gamanase and SSF respectively.

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(R1) supplied by ChemGen Corp, Maryland, USA;

(R2) supplied by Novozymes Pty. Ltd, NSW, Australia;

(R3) supplied by Alltech Biotechnology Pty. Ltd, Victoria Australia

Each diet had four replicate single bird cages. Feed intake and weight gain were measured, at age four and 14 day of feeding the test diets. Total feed intake and faeces were recorded for the measurement of digestibility. The experimental design was a two way factorial with four basal diets and two enzyme treatments. Results were analysed by covariance analysis.

III. RESULTS AND DISCUSSION

The means of weight gain, feed intake, FCR and DM digestibility of diets fed to chicks from d four to 14 are shown in the Table. Increasing the level of CM in the diet depressed weight gain, feed intake, DM digestibility and increased FCR significantly ($P<0.05$). The weight gain of chicks was significantly depressed by adding 10 to 50 % CM to the diet. However, mean weight gain was significantly increased when the enzyme mixture was added. Feed intake reduced in response to increasing levels of CM in the diets by 17 % (10 % CM diet), 18 % (30 % CM diet) and 28 % (50 % CM diet).

Table: The effect of copra meal and enzyme on the performance of broilers from day 4 to 14

Feed / Enzyme	**Weight gain (g)**	**Feed intake (g)**	**FCR**	**DM Digestibility (%)**
0 % CM	299.1 ± 9.26^{a}	349.0 ± 6.5^{a}	1.17 ± 0.04^{a}	79.5 ± 1.2^{a}
10 % CM	202.7 ± 12.0^{b}	290.4 ± 13.3^{b}	1.45 ± 0.05^{b}	74.3 ± 1.4^{ab}
30 % CM	206.8 ± 8.8^{b}	287.5 ± 10.1^{b}	1.41 ± 0.08^{b}	67.8 ± 1.8^{b}
50 % CM	142.1 ± 11.8^{c}	250.9 ± 11.2^{c}	1.83 ± 0.13^{c}	53.7 ± 6.2^{c}
Without enzyme	200.5 ± 17.9^{a}	290.8 ± 17.9^{a}	1.56 ± 0.10^{a}	65.8 ± 4.5^{a}
With enzymes	224.9 ± 13.5^{b}	298.0 ± 9.5^{a}	1.37 ± 0.05^{b}	71.9 ± 2.1^{b}

Values with the same superscript within a column are not significantly different ($P<0.05$)

Addition of CM increased FCR significantly. The dry matter (DM) digestibilities of the diets containing 30 and 50 % copra meal were significantly lower than the digestibility of the non-copra diet and had a strong negative correlation ($R^2=0.97$; $Y= -0.50x + 80.0$, $P<0.01$) with the percentage of CM in the diet. However, the FCR was significantly decreased and the DM digestibility was significantly increased when the enzyme mixture was added.

The findings indicated that the use of copra meal in the diet of young broiler chickens depressed weight gain, feed intake, DM digestibility and increased FCR. Although the use of enzymes successfully improved weight gain, DM digestibility and decreased FCR of chicks at d 14, enzymes could not increase weight gain to the same level as that of chicks fed a maize-soybean based diet. Since many previous findings indicated that copra meal could be used at a level of 20 %, and even 40 % in an amino acid-supplemented diet, the negative effect of using smaller levels of copra meal in this current study may be due to the quality of copra meal used. Over-heating during the drying and oil extraction processes might have taken place because the colour of the copra meal was dark brown. The bulkiness of copra meal and copra NSPs may also have contributed to the poor response of the chickens (Sundu and Dingle, 2003). These current findings suggest that it may be important for the links between galactose and mannan to be cleaved by ∝-galactosidase to enable continued hydrolysis of β-mannan by mannanase.

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EFFECTS OF POTASSIUM DIFORMATE INCLUSION IN BROILER DIETS ON GROWTH PERFORMANCE AND NUTRIENT UTILISATION

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Summary

Potassium diformate (K-diformate) is a possible substitute for antibiotic growth promotants in broiler diets. Four dietary treatments of 0, 3, 6 and 12 g/kg K-diformate were offered to 192 caged broilers. The feed additive increased feed intake ($P<0.02$) and tended to increase weight gain ($P<0.07$) from 1-35 days post-hatch. From 16-35 days post-hatch, K-diformate increased feed intake ($P<0.01$) and weight gain ($P<0.05$). The effect was most evident at 6 g/kg with respective increases of 9.3 and 5.3%, respectively. K-diformate did not enhance feed efficiency although it increased ($P<0.03$) apparent metabolisable energy (AME) and nitrogen (N) retention ($P<0.05$). AME was increased by 0.60 MJ/kg (DM) at 3 g/kg and N retention by 5.6% at 12 g/kg K-diformate. K-diformate shows some promise and further evaluation appears justified.

I. INTRODUCTION

The inclusion of organic acids and their salts, or 'acidifiers', in diets with high acid binding capacities for weaner pigs to enhance growth performance is commonly practiced (Partanen and Mroz, 1999). There appears to have been less interest by the chicken meat industry in the use of organic acids for growth promotion although some investigations into their effects on bird performance and their potential to reduce carcass contamination with *Salmonella* have been completed (Byrd *et al*., 2001). With the declining use of antibiotic growth promotants, there is an ongoing need to identify effective replacements. One candidate is K-diformate (Formi®) a chemical complex, where the carbonyl group of formic acid links with the hydroxyl group of potassium formate via a hydrogen bond, which dissociates to formic acid and potassium formate in the gut. K-diformate is a crystalline powder with low corrosive properties and a K content of 288 g/kg. As K-diformate is classified as a growth promoting feed additive for pigs in Europe, the purpose of this investigation was to determine if this chemical complex has potential for poultry. With this objective, the effects of graded K-diformate inclusion levels in broiler diets on growth performance and nutrient utilisation were determined.

II. MATERIALS AND METHODS

Conventional, wheat-based, starter and finisher diets, were offered to broilers from 1-15 and 16-35 days post-hatch, respectively with K-diformate concentrations of 0, 3, 6 and 12 g/kg. Potassium levels were held constant by balancing the K content of K-diformate with potassium carbonate and adjustments to energy density and amino acid specifications were made for the diluting effect of graded levels of K-diformate, which was added mainly at the expense of wheat.

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A total of 192 day-old, male, Cobb chicks were selected on the basis of body weights and housed in electrically-heated brooders initially and then transferred to wire-floored cages in an environmentally controlled facility. Throughout the feeding period, feed and water were available *ad libitum* under continuos fluorescent lighting. The diets were offered in mash form and feed intakes and weight gains were recorded for the two feeding phases. Mortalities were monitored daily and dead bird weights were considered in feed conversion ratio calculations.

From 19-22 days post-hatch total excreta output was collected to determine the effects of dietary treatments on AME and N retention as described by Selle *et al.* (2003). Also 500g excreta samples were dried to determine treatment effects on excreta dry matter. The experimental data from eight replicates of six birds per treatment was subject to analysis of variance and regression analysis using a general linear models procedure (SPSS Inc. Chicago, IL). Where the effect of treatments met the 5% level of probability, least significant differences were calculated and, where appropriate, the significance of pair-wise comparisons between specific treatments is presented.

III. RESULTS

The results of the experiment are shown in Table 1. Growth performance of birds from 1-35 days post-hatch was satisfactory with an average weight gain of 1834 g/bird and feed efficiency of 1.58. A total of 7 birds died (3.7%), the majority of the losses occurred during the first week but the mortality rate was not related to treatment ($P>0.60$). Graded inclusion levels of K-diformate did not influence growth performance in the starter phase, from 1-15 days post-hatch. However, in the finisher phase, from 16-35 days post-hatch, K-diformate significantly increased feed intake ($P<0.01$) and weight gain ($P<0.05$); however, feed efficiency did not differ significantly ($P>0.10$) between treatments. The most pronounced effects were observed at 6 g/kg K-diformate, with a 9.3% increase in feed intake ($P<0.01$) and 5.3% in weight gain ($P=0.01$). However, at this inclusion level, K-diformate was associated with 3.7% less efficient feed conversion ($P=0.16$).

Over the entire feeding period K-diformate significantly increased feed intake ($P<0.02$) and tended to improve weight gain ($P<0.07$). Again, the most pronounced effects were observed at 6 g/kg K-diformate with increases in feed intake of 8.7% ($P<0.01$) and in weight gain of 5.8% ($P=0.01$). However, numerically feed efficiency was 2.5% inferior although this difference was not significant ($P=0.22$). K-diformate increased AME of diets ($P<0.03$). This was most evident at 3 g/kg, with an increase of 0.60 MJ/kg ($P<0.01$) and at 12 g/kg with an increase of 0.48 MJ/kg ($P=0.021$) on a dry matter basis. K-diformate enhanced N retention ($P<0.05$) with a 5.6% increase (53.8 to 56.8%) at 12 g/kg ($P=0.02$). K-diformate did not increase excreta moisture; in fact the feed additive tended to increase dry matter content of excreta ($P=0.08$).

K-diformate linearly increased N retention ($r=0.389$; $P<0.03$); however, this was the only parameter where the feed additive had a significant linear effect. The lack of linear responses to K-diformate inclusion is of interest.

Table 1: Effects of K-diformate on growth performance from 1-35 and 16-35 days post-hatch, AME, nitrogen retention and excreta dry matter.

	Potassium diformate (g/kg)					Significance
Parameter	**0**	**3**	**6**	**12**	**SEM**	**(P =)**
Growth performance						
1-35 days post hatch						
Weight gain (g/bird)	1785	1808	1889	1853	28.09	0.061
Feed intake (g/bird)	2836[a]	2800[a]	3083[b]	2898[a]	61.36	0.014[1]
FCR (g/g)	1.59	1.55	1.63	1.57	0.023	0.092
16-35 days post hatch						
Weight gain (g/bird)	1478[a]	1488[a]	1556[b]	1528[ab]	21.02	0.047[2]
Feed intake (g/bird)	2426[a]	2380[a]	2652[b]	2480[a]	55.04	0.009[3]
FCR (g/g)	1.64	1.60	1.70	1.62	0.029	0.115
AME (MJ/kg DM)	13.73[a]	14.33[b]	13.98[ab]	14.21[b]	0.138	0.024[4]
N retention (%)	53.8[a]	55.1[ab]	53.8[a]	56.8[b]	0.812	0.047[5]
Excreta dry matter (%)	29.9	31.6	33.1	31.0	0.836	0.078

[abc]within rows, means without common superscripts are significantly different ($P < 0.05$)
LSD ($P < 0.05$): [1]178, [2]60.9, [3]159, [4]0.40, [5]2.35

IV. DISCUSSION

The use of antibiotic growth promotants (AGP) to enhance pig and poultry performance is meeting increasing opposition and it is being recommended that this practice should be discontinued. The mechanisms by which AGP improve growth performance are poorly understood although antibiotic-induced modifications of the gut microflora that are favourable to the host are thought to be involved (Anderson *et al.,* 2000). As organic acids and their salts possess antimicrobial properties they have been given consideration as potential substitutes for AGP. For example, Byrd *et al*., (2001) reported that 5 g/kg formic acid in drinking water reduced the *Salmonella* population of the crop in broilers by 35% in association with a decline from 5.77 to 4.80 in crop pH.

It has been demonstrated that fumaric acid reduces the bacterial population in the small intestine (Vogt *et al*., 1981). At 1.25 g/kg fumaric acid significantly increased weight gain by 4.1% and feed intake by 5.2% of mixed-sex chicks in a 49-day feeding study under simulated commercial conditions, with birds kept on litter (Skinner *et al*., 1991). In a second experiment with male chicks, fumaric acid significantly increased gain and feed conversion by 4.3 and 4.4%, respectively. Patten and Waldroup (1988) also found that fumaric acid positively influenced bird performance but since the initial investigations by Vogt *et al.* (1979, 1981, 1982) there have been few such reports where organic acids have been shown to enhance performance. As discussed by Waldroup *et al.* (1995) the unreliable effects of organic acids in poultry may be associated with the fact that they are metabolised in the foregut (crop, gizzard, proventriculus), which would limit their antimicrobial activity in the small and large intestines.

The positive influence of K-diformate on AME and N retention at 3.0 and 12.0 g/kg is noteworthy. Similar assessments may not have been previously reported in broilers but there are precedents in swine. Formic acid enhanced total tract energy digestibility at 18 and 24 g/kg (Eckel *et al*., 1992) and improved nitrogen balance at 13.8 g/kg (Mroz *et al.,* 1997) in weaner and grower pigs, respectively. In the present study, however, K-diformate did not

positively influence nutrient utilisation at 6.0 g/kg, which appears to be associated with increased feed intakes and, in turn, reductions in feed efficiency. The overall lack of linear responses to graded inclusion levels of K-diformate is a curious observation and may indicate that the feed additive has more than one mode of action. The definition of appropriate K-diformate inclusion rates is an area that merits future investigations.

In the present study broilers were reared under 'clean' conditions in wire-floored cages. It is likely that broilers kept on litter, as was the case in the Skinner *et al.* (1991) study, would be subject to a greater microbial challenge and may be more responsive to organic acids. Thus evaluations of K-diformate may be more instructive with birds kept on litter. It is possible that K-diformate would reduce crop pH. It is interesting that Murai *et al.* (2001) reported that glutamic acid reduced crop pH from 6.0 to 5.4 and that this was associated with enhanced efficacy of exogenous phytase as assessed by bone mineralisation. The interaction between organic acids and exogenous phytases has been investigated in swine, although the results lack consistency. Nevertheless, parallel studies in broilers appear justified because organic acids may facilitate the hydrolysis of dietary phytate by supplementary phytase in the crop.

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DIET TYPE, APPARENT METABOLISABLE ENERGY AND DIGESTA VISCOSITY IN LAYING HENS OF TWO DIFFERENT AGES

J.R. ROBERTS and W. BALL

Summary

Diets based on wheat, barley or sorghum were fed to younger and older laying hens for a period of 12 weeks. Production and weekly egg mass were higher for the younger birds and feed intake and feed conversion efficiency were higher for the older hens, with no effect of diet. Egg shell quality and egg internal quality were better for the younger hens. The sorghum diet resulted in eggs with better shell quality but lighter shell colour and lower albumen quality. AME was higher for the older birds and differed among diets, with sorghum the highest and barley the lowest. Excreta moisture was higher for the wheat and barley diets than the sorghum diet, and higher for the older birds. The digesta viscosities of the jejunum and ileum were higher for the wheat and barley diets than for the sorghum diet and not different between the younger and older hens.

I. INTRODUCTION

In Australia, layer diets are usually based on wheat, sorghum, barley or a combination of these grains. Concern has been expressed about the use of barley and some wheats (e.g. new season wheats) in poultry diets, because the presence of non-starch polysaccharides (NSP) in these grains may result in anti-nutritive effects that appear to be caused by high viscosity of the contents of the digestive tract (Choct and Annison, 1992; Leeson and Summers, 2001). However, most of this research has been conducted with broilers and it is unclear to what extent the results can be extrapolated to laying hens. In addition, it is not clear whether the age of the hens has any influence on the response. A previous study showed that increased digesta viscosity resulting from the inclusion of cereal rye in a wheat-based layer diet did not have any negative effects on bird performance (Roberts *et al.,* 2003). In the present study, diets based on wheat, barley or sorghum were fed to laying hens of two different ages and production, feed intake, feed conversion ratio, egg quality, apparent metabolisable energy (AME), digesta viscosity and excreta moisture measured.

II. MATERIALS AND METHODS

Diets based on wheat, barley or sorghum were formulated by a consultant nutritionist to typical Australian commercial standards. Diets were of equal energy levels, and very similar crude protein levels (wheat 165 g/kg; barley 177 g/kg, sorghum 165 g/kg) and were manufactured at the University of New England. Each of these three diets was fed to 25 younger ISA Brown laying hens (26 weeks of age at the start of the experiment) and 25 older ISA Brown laying hens (78 weeks of age at the start of the experiment), a total of 6 experimental groups, housed in individual bird cages. The older birds had been moulted at 65 weeks of age. The diets were fed for 12 weeks during which time production and egg weight of all eggs laid were measured daily, and feed intake was measured weekly. Egg mass and feed conversion ratios (feed consumed/egg mass) were calculated weekly. Eggs production was determined for all birds prior to the commencement of the experiment and then at 4-week intervals.

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When birds were 39 and 91 weeks of age, apparent metabolisable energy (AME) was measured, in 10 birds from each of the 6 experimental groups, by the total collection procedure. At the end of the experiment, the birds used for the AME measurements were euthanased and the contents of the jejunum and ileum collected. The digesta from the jejunum and ileum were centrifuged at 15000 RPM for 15 min and the supernatant taken for measurement of digesta viscosity (Brookfield DVIII viscometer).

III. RESULTS

Diet had no effect on body weight, production, weekly egg mass or feed conversion ratio ($P>0.05$). The older birds had significantly higher body weight than the younger birds throughout the experiment. For the 12 weeks of the experiment, production ($P<0.01$) and weekly egg mass ($P=0.01$) were significantly higher in the younger birds than the older birds and feed intake ($P<0.01$) and feed conversion ratio ($P=0.03$) were higher for the older birds (Table 1).

Table 1: Effects of hen age on production, feed intake and feed conversion ratio

Measurement	Younger Hens	Older Hens
Production eggs/hens/week	$^{a}6.66 \pm 0.03$	$^{b}6.09 \pm 0.05$
Weekly Egg Mass g	$^{a}402.9 \pm 2.6$	$^{b}371.2 \pm 4.1$
Feed Intake g/hen/day	$^{b}130.0 \pm 0.6$	$^{a}141.3 \pm 0.8$
Feed Conversion Ratio	$^{b}2.31 \pm 0.02$	$^{a}2.79 \pm 0.06$

Mean ± S.E. Values in a row with different superscripts differ significantly

Table 2: Egg quality of younger and older hens on the three diets

Measurement	Hen Age	Diet			P Value		
		Wheat	Barley	Sorghum	A	D	A*D
Egg Weight	^{B}Y	62.7 ± 0.5	63.2 ± 0.5	62.1 ± 0.4	<0.01	NS	<.01
g	^{A}O	66.8 ± 1.1	67.8 ± 1.4	69.0 ± 1.1			
Shell Reflectivity	^{B}Y	$^{b}29.2 \pm 0.5$	$^{b}29.6 \pm 0.4$	$^{a}32.9 \pm 0.6$	<0.01	0.01	0.01
%	^{A}O	33.9 ± 0.7	33.5 ± 0.5	33.5 ± 0.6			
Breaking Strength	^{A}Y	41.2 ± 0.8	42.9 ± 0.8	42.6 ± 0.9	<0.01	0.01	0.01
N	^{B}O	$^{b}35.7 \pm 1.3$	$^{b}34.9 \pm 1.1$	$^{a}40.0 \pm 1.1$			
Deformation	Y	272 ± 12	274 ± 8	275 ± 9	NS	NS	NS
μm	O	281 ± 11	255 ± 11	283 ± 12			
Shell Weight	Y	6.25 ± 0.1	6.11 ± 0.1	6.23 ± 0.1	NS	0.01	0.02
g	O	$^{b}6.01 \pm 0.1$	$^{b}6.17 \pm 0.1$	$^{a}6.52 \pm 0.1$			
Percentage Shell	^{A}Y	9.99 ± 0.1	9.78 ± 0.1	10.1 ± 0.1	<0.01	0.01	NS
%	^{B}O	$^{b}8.86 \pm 0.2$	$^{b}8.87 \pm 0.1$	$^{a}9.37 \pm 0.1$			
Shell Thickness	^{A}Y	435 ± 4	425 ± 3	431 ± 5	<0.01	0.01	0.01
μm	^{B}O	$^{b}396 \pm 7$	$^{b}399 \pm 5$	$^{a}422 \pm 4$			
Albumen Height	^{A}Y	9.58 ± 0.13	9.32 ± 0.12	9.15 ± 0.1	<0.01	<0.01	NS
mm	^{B}O	$^{a}8.25 \pm 0.14$	$^{a}7.89 \pm 0.21$	$^{b}7.16 \pm 0.1$			
Haugh Units	^{A}Y	96.3 ± 0.7	95.1 ± 0.6	94.5 ± 0.6	<0.01	<0.01	0.01
	^{B}O	$^{a}88.5 \pm 0.9$	$^{a}84.3 \pm 1.5$	$^{b}79.8 \pm 1.5$			

Mean ± S.E. Y is younger hens, O is older hens, A is age, D is diet, A*D is age-diet interaction. . $^{A-B}$ indicates significant differences between young and old birds for all diets combined. $^{a-b}$ indicate significant differences among diets within an age group.

For all egg collections combined, there were significant differences in egg quality among the diets (Table 2). The sorghum diet resulted in lighter shell colour (younger birds) but higher breaking strength, shell weight, percentage shell and shell thickness (older birds) than the wheat and barley diets which were not significantly different from each other (P>0.05). For egg internal quality, albumen height and Haugh Units were highest for the wheat diet, and lowest for the sorghum diet, with barley intermediate. Egg internal quality and egg shell quality were better for the younger birds than for the older birds (Table 2), with shell colour being darker (based on reflectivity measurements) and breaking strength percentage shell, albumen height and Haugh Units being higher for the younger birds (P<0.01).

AME varied significantly among the diets (P<0.01), being highest for the sorghum diet, lowest for the barley diet, with the wheat diets intermediate (Table 3). There was also a significant main effect of bird age (P<0.01) on AME, with AME being higher in the older birds than the younger birds. There was a significant interaction between age and diet for AME. Excreta moisture measured during the AME determinations was higher for the older birds, as compared with the younger birds (P=0.02) and higher for the wheat and barley diets than for the sorghum diet (P<0.01).

Table 3: AME and Excreta Moisture for younger and older hens on the three diets

Measure-	**Hen Age**	**Diet**				**P Value**		
ment		**Wheat**	**Barley**	**Sorghum**	**ALL**	**A**	**D**	**A*D**
AME	Y	12.23	11.61	12.20	^{B}12.02	0.10	<0.10	0.10
MJ/kg		± 0.03	± 0.06	± 0.08	± 0.06			
DM	O	12.29	11.73	12.61	^{A}12.21			
		± 005	± 0.05	± 0.10	± 0.08			
	Y&O	^{b}12.26	^{c}11.67	^{a}12.41				
		± 0.03	± 0.04	± 0.08				
Excreta	Y	74.5	76.7	73.3	^{B}74.8	0.02	0.01	NS
Moisture		± 0.4	± 0.8	± 0.7	± 0.5			
%	O	77.2	77.9	74.4	^{A}76.5			
		± 0.9	± 0.7	± 1.3	± 0.6			
	Y&O	^{a}75.8	^{a}77.3	^{b}73.9				
		± 0.6	± 0.5	± 0.7				

Mean ± S.E. Y is younger hens, O is older hens, A is age, D is diet, A*D is age-diet interaction. $^{A-B}$ indicates significant differences between young and old birds. $^{a-c}$ indicate significant differences among diets.

Digesta viscosity was consistently higher (P<0.01) for the ileum than the jejunum. There were significant differences among the diets for digesta viscosity of both the jejunum (P<0.01) and the ileum (P<0.01) with digesta viscosity being similar for wheat and barley and higher for these two diets than for the sorghum diet (Table 4). However, there was no difference in digesta viscosity of either the jejunum or ileum between the older and younger hens. There was a significant inverse linear relationship between AME and digesta viscosity of both the jejunum (P<0.01) and ileum (P=0.01) for all diets combined. However, there was no significant relationship when each diet was considered separately.

Analysis of the three diets indicated that the levels of total NSP were similar for the wheat and sorghum diets (92.9 and 87.8 g/kg, respectively), with the barley diet being higher (135.7 g/kg). Insoluble NSP was also very similar for wheat and sorghum diets (83.7 and 83.6 g/kg, respectively), with barley being higher (112.6 g/kg). However, the levels of soluble NSP differed being 9.2, 23.1 and 4.3 g/kg for the wheat, barley and sorghum diets,

respectively. Extract viscosities (centipoise) of the finished diets were: wheat 4.11, barley 2.89 and sorghum 2.46.

Table 4: Digesta viscosity (cP) of the jejunum and ileum for the three diets

Diet	Digesta Viscosity Jejunum	Digesta Viscosity Ileum
Wheat	$^{a}4.89 \pm 0.50$	$^{a}9.54 \pm 0.96$
Barley	$^{a}5.77 \pm 0.61$	$^{a}9.49 \pm 1.41$
Sorghum	$^{b}2.06 \pm 0.15$	$^{b}2.86 \pm 0.23$

Mean ± S.E. Values within a row with different superscripts differ significantly ($P<0.05$).

IV. DISCUSSION AND CONCLUSIONS

Although the three diets differed in levels of non-starch polysaccharides, both the younger and older hens performed well on all diets. Although production was relatively high for the older birds, losses of eggs owing to poor shell quality resulted in a reduced egg mass. As would be expected, egg internal quality and egg shell quality were better for the younger birds. Although production, egg mass and feed conversion ratio did not differ among diets, egg shell quality was best for birds on the sorghum, particularly for the older birds. However, there were negative effects of the sorghum diet on shell colour in the younger birds and albumen quality in the older birds. It is not clear to what extent egg shell quality is correlated with the NSP levels in the diets because the wheat and barley diets differed in their levels of soluble, insoluble and total NSP but resulted in very similar egg quality. In addition, previous studies (Roberts *et al.,* 2002, 2003) found differences in egg quality between diets based on two different wheats and also when 20% cereal rye was substituted for wheat in a wheat-based diet.

These findings suggest that adult laying hens cope well with a range of dietary ingredients, provided that the diets are properly formulated. However, the results provide more evidence that the grain on which a layer diet is based can affect egg shell quality and egg internal quality by mechanisms that are not fully understood at the present time.

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THE RATE OF PASSAGE OF DIGESTA INFLUENCES ENERGY METABOLISM IN BROILER CHICKENS

R.J. HUGHES[1,2]

Summary

This study examined the relationship between digestion of energy and transit time of digesta in chickens given a wheat-based diet, with and without feed enzymes. There was a significant ($P<0.05$) positive correlation between apparent metabolisable energy (AME) value and whole tract transit time (WTTT) determined with ferric oxide marker administered in a gelatine capsule. That is, chickens with longer transit times had higher AME values. Despite genetic selection for liveweight gain and feed efficiency over many generations, the modern broiler chicken exhibits relatively wide variation in both energy metabolism and rate of passage of digesta. However, rate of passage of digesta appears not to be the reason for previously observed differences between males and females in the digestion of energy. Nevertheless, it is possible that underlying sex-dependent differences were not expressed in this particular study due to the high AME value (15.6 MJ/kg DM) of the wheat. Likewise, the high AME value also explains why there were no responses to the feed enzymes.

I. INTRODUCTION

Modern broilers have a prodigious capacity to eat (Mahagna and Nir 1996), a relatively short retention time for ingested food, and greater absorptive capacity (Mitchell and Smith 1991) compared with layers. Iskander and Pym (1987) reported that broiler chickens selected for improved feed efficiency had a slower digesta transit time and improved retention of dietary energy and protein compared with broiler chickens selected for increased feed intake. Hence it is possible that the different selection objectives and strategies used over the years to produce the current commercial lines of broiler chickens may well have produced differences between the lines in transit time, and consequently in energy metabolism. There is a lack of published data on the variation that may exist between strains and between individual birds within a strain in the relationship between rate of passage of digesta and digestion of energy. If large differences exist, it would help explain, at least in part, the wide between-bird variability in AME observed by Hughes and Choct (1997). Similarly, differences may exist between males and females in transit time, as observed in gut structure (Hughes 2003).

The following experiment tested the hypotheses that (a) AME values and digest transit time were related, and (b) the relationship between AME and transit time differed between males and females.

II. MATERIALS AND METHODS

Ross broiler chickens were raised from hatch in two rearing pens in a controlled temperature room. Male and female chickens were reared separately, and maintained on commercial starter crumbles. At 17 days of age, a total of 32 chickens were transferred in single-sex pairs to 16 metabolism cages located in a controlled-temperature room set at 25-27^{o}C initially, and given starter crumbles. The temperature setting in the room was reduced daily until it was 22^{o}C at the end of the experiment. Birds had free access to feed and water prior to and during the experimental period. Chickens were placed one per cage in 24 cages for the experiment when they were 19 days old, and continued to receive the starter crumbles.

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The basal diet comprised (in g/kg), 800 wheat (variety Oxley grown at Narrabri, NSW in 2000), 155 casein, 20 dicalcium phosphate, 11 limestone, 7 DL-methionine, 2 vitamin and mineral premix, 3 salt, and 2 choline chloride (60%). Enzyme products with xylanase activity were added to the basal diet to provide four dietary treatments comprising control (no enzyme), Avizyme 1300 (1kg/tonne), Kemzyme W1 (1kg/tonne), and Bio-Feed Wheat CT (200g/tonne). In addition to xylanase activity, Avizyme 1300 had protease activity and Kemzyme W1 had β-glucanase, protease, amylase, cellulase and lipase activities. The four experimental dietary treatments were replicated six times (three female and three male chickens) in a randomised complete block layout. Experimental diets were pelleted (4 mm diameter and 6 mm length) in a cold-press to avoid selective feeding.

On day 6 of the experiment, chickens were administered with a gelatine capsule containing ferric oxide (Fe_2O_3 200 mg/kg live-weight) as per the method of Iskander and Pym (1987). Excreta trays were examined frequently for signs of red colouration from ferric oxide in voided droppings. Whole-tract transit time (WTTT) for each chicken was taken as the time elapsed (in minutes) from time of administration of ferric oxide in a gelatine capsule to time of first observation of red colouration in droppings.

AME values for diets were determined by measurements of total feed intake and total excreta output and subsequent measurement of gross energy values of feed and excreta by bomb calorimetry. The values for AME of wheat were calculated by subtraction of energy contributed by casein (assumed to be 20.1 MJ/kg dry matter).

III. RESULTS

Effects of diet, and the interaction between diet and sex, were not significant (P>0.05) for any measurement. Males were significantly greater in live weight (in g/bird) than females at the start (948 vs 831), and at end of the experiment (1425 vs 1217), gained more weight (476 vs 386) and ate more feed (99.1 vs 84.6 g/bird/d), respectively. The mean AME value for the wheat was extremely high (15.6 MJ/kg dry matter) and ranged from 14.4 to 16.3 MJ/kg DM for individual birds. The relationship between AME of the wheat and whole tract transit time is shown in Figure1.

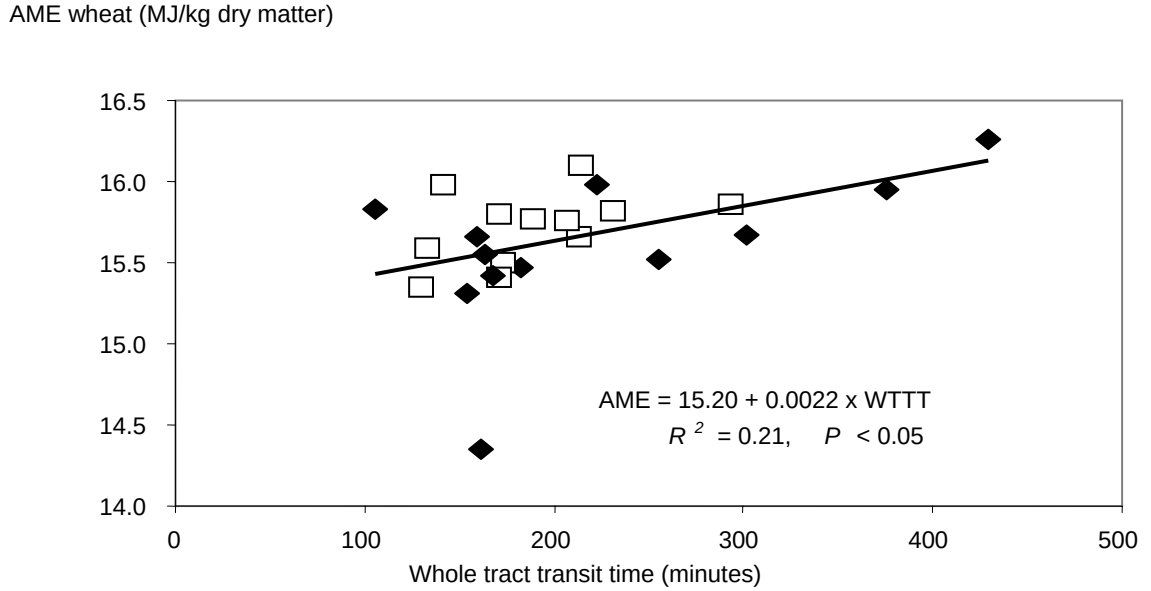

Figure 1: Plots of values for AME of wheat and whole tract transit time (WTTT) for individual chickens (n = 24). Males are represented by □ and females by ◆. Data shown here are untransformed values for WTTT. Similar statistical results were obtained for log e transformed WTTT

Analysis of covariance indicated that the linear relationship between AME and WTTT was the same for males and females. The omission of data from one female bird with the lowest AME value (14.4 MJ/kg DM) did not alter the overall result. The two chickens with longest transit time were females and are shown in Figure 2 as the points with lowest energy excretion values. The chicken with the lowest AME value in Figure 1 was also a female and had a higher energy excretion value than any other female (Figure 2).

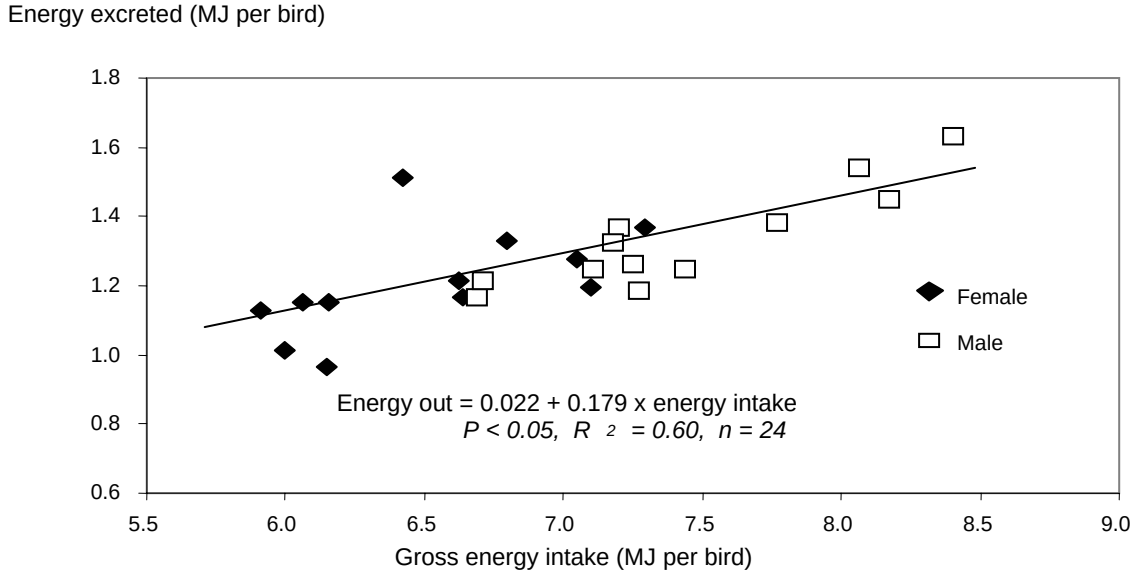

Figure 2. Relationships between energy excreted and gross energy intake for male (□) and female (◆) chickens.

Analysis of covariance indicated that the linear relationship between energy intake and energy excretion shown in Figure 2 was the same for males and females. Estimated endogenous energy loss was 22 kJ/bird/day, which is considerably less than 46 and 101 kJ/bird/day estimated for females and males, respectively, by Hughes (2003).

IV. DISCUSSION

AME increased linearly with whole tract transit time. This relationship was unaffected by the sex of the chicken. The lack of response to enzymes by chickens given the wheat-based diet was probably due to the unusually high AME value of the wheat. An AME value of 15.6 MJ/kg DM is at the high end of the ranges for Australian wheats (Hughes and Choct 1999). Repeated measurements of AME values for this wheat in subsequent experiments (data not shown) confirmed this high value.

In this experiment, chickens were given cold-pressed pellets containing 800 g/kg wheat which was not milled prior to pelleting. The resulting pellets were easily broken during feeding activity by the chickens. Svihus and Hetland (2001) pointed out that ileal starch digestibility was increased when cold-pressed pellets were crushed to mash prior to feeding, and when some of the wheat component of the diet was fed as whole grain. Hence, it is possible that the physical form of the feed contributed to the high AME value.

Although not measured, it is highly likely that this particular wheat had a relatively low concentration of soluble NSP given its very high AME value. This could explain why energy excretion by both males and females in this experiment increased linearly with increasing energy intake, and why it was comparatively lower than that for most of the chickens fed a diet based on low AME wheat as used by Hughes (2003). Svihus and Hetland

(2001) showed that overloading the digestive tract of male chickens with starch can result in incomplete digestion of starch, and impaired feed conversion, with the possibility that microbial fermentation in the hindgut could result in large losses of energy. There was no indication in the present study that a high starch intake overloaded the digestive tract of either sex in contrast to the observations made on male chickens by Svihus and Hetland (2001). This tends to suggest that it is the soluble NSP component of a high grain intake rather than the starch content *per se* that causes the impairment in starch digestion and leaves the chickens vulnerable to microbial overgrowth of the small intestine (Choct *et al*., 1996).

The relatively long WTTT of 206 minutes observed here with a wheat diet compared with WTTT of 165 minutes for a commercial broiler feed reported by Hughes *et al*. (2002) is difficult to explain on the basis of what is known about the effects of soluble NSP and addition of enzyme to the diet on digesta viscosity and transit time. The commercial broiler diet used previously and the experimental wheat diets used here all contained recommended dosages of enzymes so it seems unlikely that digesta viscosity would contribute to a difference in transit time. Similarly, it seems unlikely that soluble NSP was the cause because this would require the wheat diet to contain a higher concentration of soluble NSP than the commercial diet, which is not consistent with the very high AME value of the wheat. Perhaps the difference in transit time was associated with the physical form of the feed offered (relatively hard, steam-treated starter crumbles compared with soft cold-pressed pellets used in the present experiment), or the physical and chemical attributes of the different diets. A further possibility is the insoluble NSP content of the respective diets. Hetland and Svihus (2001) recently pointed out that inclusion of oat hulls in the diet shortened digesta transit time but did not affect nutrient digestibility. Insoluble NSP in flourmill offal added to the commercial broiler diet may have reduced the transit time.

V. CONCLUSIONS

The positive correlation between digesta transit time and retention of dietary energy in broiler chickens observed in this study is consistent with the report by Iskander and Pym (1987) on broilers selected for feed efficiency. Because the AME value of wheat was high, the work ought to be repeated with wheat of lower energy value (e.g., in the range 13 to 14 MJ/kg DM) in order to determine the relative importance of rate of passage of digesta on digestion and absorption of nutrients in individual chickens of both sexes.

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DEHULLED, 'FULL-FAT' SOYBEAN MEAL IMPROVES BROILER AND LAYER PERFORMANCE

S.B. NEOH[1] and V.RAGHAVAN[2]

Summary

The addition of dehulled, 'full-fat' soybean meal to poultry diets may improve the performance of broilers and layers in comparison to diets containing only dehulled soybean meal and an equivalent amount of vegetable oil. In broilers, increases of 7.9% in weight gain and 12.0% in feed efficiency were recorded for Avian Farms broilers. In layers, significant improvements in egg mass and a higher number of larger eggs were observed.

I. INTRODUCTION

A dehulled soybean meal produced using new technology has increased availability of nutrients in comparison to standard soybean meals and may improve the performance of broilers and layers (Neoh and Raghavan, 2002). This is supported by recent chick assays comparing this soybean meal with three other soybean meals from different sources on the basis of protein efficiency ratios (Swick, 2003). This technology has been extended to the production of 'full-fat' soybean meal in which the oil content is retained rather than extracted. The technology produces oil-extracted and 'full-fat' soybean meals in which heat-labile, anti-nutritive factors, including trypsin inhibitor, are reduced by a modified process. It is possible that the modified process is associated with better utilisation of energy and availability amino acids following their absorption from the gut. Recently, Subuh *et al.* (2002) showed that replacing soybean meal and poultry tallow with 'full-fat' soybean meal in isocaloric and isonitrogenous diets improves weight gain and feed efficiency of broilers by up to 2.5 and 5.5%, respectively. However, the total replacement of soybean meal by 'full-fat' soybean meal is not usually commercially viable due to economic and handling issues. Therefore this paper investigates the effects of incorporating 'full-fat' soybean meal into broiler and layer diets at 60 to 100 g/kg.

II. METHODS

The proximate analyses of oil-extracted and 'full-fat' dehulled soybean meals supplied by Soon Soon Oilmills of Malaysia are shown in Table 1. Feeding studies compared the growth performance of broilers offered diets formulated without and with 'full-fat' soybean meal. Six groups of straight run broilers of three different breeds were used. Each group consisted of 6 replicates of 83 birds each. Isocaloric and isonitrogenous starter and grower diets were formulated without and with 'full-fat' soybean meal. The metabolisable energy and protein levels for the crumbled starter and pelleted grower diets were 12.86 and 13.18 MJ and 21 and 19%, respectively. The control starter diet contained 250 g/kg and the finisher diet 200 g/kg soybean meal. One group of Avian Farms broilers were offered the control diet. The treatment diet was prepared using 100 g/kg 'full-fat' soybean meal to replace 80 g/kg soybean meal and 20 g/kg vegetable oil and was offered to two groups of Avian Farms (Avian), two groups of Arbor Acres (AA) and one group of Cobb birds. Growth performance and mortality rates were recorded over the 40-day feeding period.

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Table 1. Proximate analysis of 'full-fat' and oil extracted dehulled soybean meals.

Item	'Full-fat' soybean meal	Oil extracted soybean meal
Moisture, g/kg	124	115
Protein, g/kg	363	467
Fat, g/kg	210	25
Crude Fiber, g/kg	29	34
Ash, g/kg	49	65

The objective of the layer trial was to investigate the effect of using dehulled 'full-fat' soybean meal on egg mass and egg size. The trial was conducted using flocks close to peak production in three commercial farms with production ranging from 90,000 to 150,000 eggs per day from Lohman, Hisex and Golden Comet layers. Each farm was divided into two equal groups. One group was fed with feed formulated with only soybean meal. The other group was offered diets with 60 g/kg 'full-fat' soybean meal partially replacing soybean meal and vegetable oil. However, the metabolisable energy was reduced by 0.21MJ and protein level by 3 g/kg in the 'full-fat' soy diets. The diets were prepared in mash form and the nutrient specifications matrixes are listed in Table 2. The trials were conducted for a total of 10 weeks starting from 25 weeks of age during which time egg size, distribution and egg mass were recorded.

Table 2. Nutrient specification of layer diets formulated without and with 'full-fat' soybean meal.

Item (g/kg)	Feed without 'full-fat' soybean meal	Feed with 'full-fat' soybean meal
Crude protein	178	175
Metabolisable energy (MJ/kg)	11.72	11.50
Fat	65	60
Fiber	35	35
Calcium	38	38
Phosphorus	7.5	7.5
Available phosphorus	4.0	4.0
Linoleic acid	17.5	17.5
Methionine	4.5	4.5
Lysine	10	10
Methionine and cysteine	7.0	7.0

III. RESULTS

Significant improvements in performance were observed with broilers offered diets containing 100 g/kg 'full-fat' soybean meal compared to broilers offered diets containing only soybean meal and vegetable oil (Table 3). Body weights at 40 days post-hatch were increased by up to 9.9% (2028 versus 2228 g/bird) and feed efficiency was improved by up to 13.3% (1.88 versus 1.63). Avian Farms broilers (groups B and C) outperformed their control counterparts by an average of 7.9% and 12.0% for weight gain and feed efficiency, respectively. Mortality rates did not differ between treatments (P = 0.78). Subsequent field trials in commercial units with diets of similar specifications containing 100 g/kg 'full-fat' soybean meal indicated that the growth performance obtained in the experimental unit can be duplicated in the commercial situation.

Table 3. Body weight, feed efficiency and mortality rate at 40 days post-hatch for broilers offered a control diet (A) or diets containing 100 g/kg 'full-fat' soybean meal (B to F).

Group	**A**	**B**	**C**	**D**	**E**	**F**
Breed	**Avian**	**Avian**	**Avian**	**AA**	**AA**	**Cobb**
Body weight, g	2028[a]	2188[d]	2189[d]	2138[b]	2198[d]	2228[c]
Feed:Gain	1.88[a]	1.65[d]	1.66[d]	1.70[b]	1.65[d]	1.63[c]
Mortality rate (%)	3.84	3.04	3.02	3.1	2.96	2.98

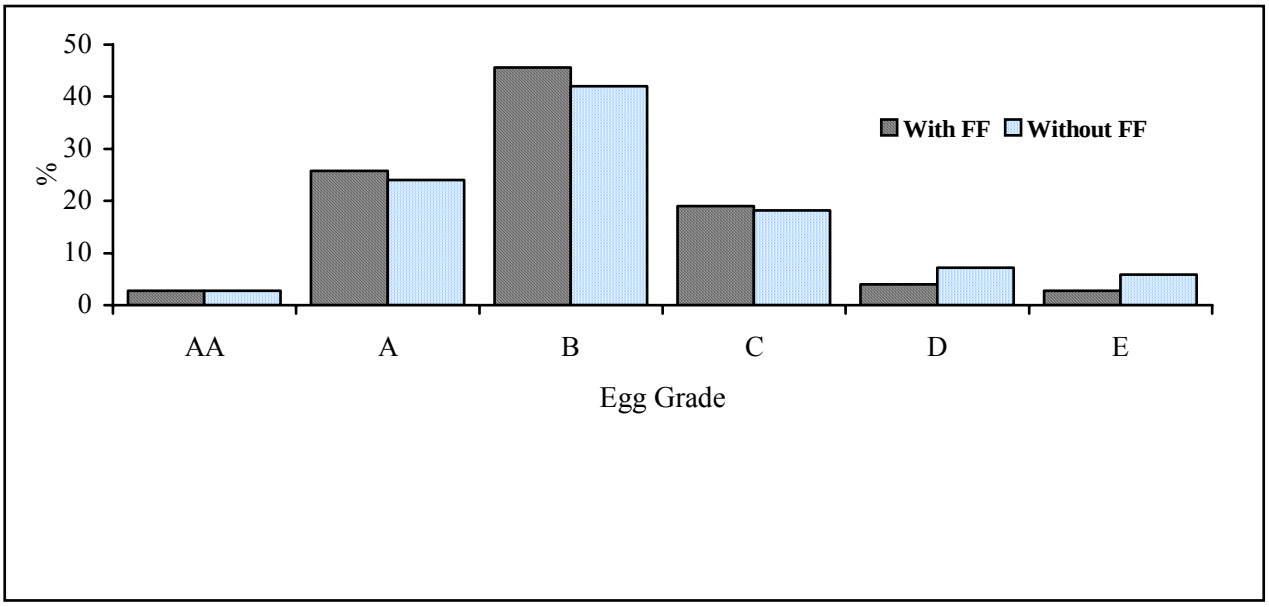
[a,b,c,d]means in a row without common superscripts differ significantly, P< 0.001

Incorporating 60 g/kg 'full-fat' soybean meal into layer diets increased (P < 0.001) egg mass by an overall average of 1.7% (64.03 to 65.11 g/h/d) as shown in Table 4. The distribution of egg weights of the various grades is AA 70-73g, A 63-68g, B 58-60g, C 56-58g, D 50-54g and E 40-43g and are illustrated in Figure 1. 'Full-fat' soy increased the percentage of A, B and C grade eggs by 1.7, 3.6 and 0.8%, respectively. On the other hand, smaller eggs such as grades D and E were reduced by 3%.

Table 4.Comparison of egg mass for layers offered diets with and without 'full-fat' soybean meal.

Treatment	**Egg Mass (g)**		
	Lohman	**Hisex**	**Golden Comet**
'Full-fat' soybean meal	65.15	65.06	65.14
Control	64.10	64.00	63.99

Figure 1. Egg size distribution for layers fed with diets with and without 'full-fat' soy.



IV. DISCUSSION

Substituting 100 g/kg 'full-fat' soybean meal for soybean meal and vegetable oil, on an isocaloric and isonitrogenous basis, can improve weight gain and feed efficiency of broilers. Similarly, layer trials using diets containing 60 g/kg 'full-fat' soybean meal resulted in heavier egg weights and an increase in the number of larger eggs although the diets containing 'full-fat' soybean meal had lower metabolisable energy and protein levels in comparison to the control diets. This suggests that 'full-fat' soybean meal has higher nutritive values in comparison to equivalent amounts of soybean meal and vegetable oil. As reviewed by Waldroup (1982) 'full fat soy is a viable feed ingredient for the poultry industry. Further studies are being conducted to quantify the additional availability of nutrients in 'full-fat' soybean meal, which may generate enhanced performance.

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THE IMPACT OF ORGANIC MINERALS ON PERFORMANCE OF POULTRY

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Summary

Organic minerals are mineral complexes formed either using a biosynthetic methods or by reacting a mineral salt with a specifically prepared mixture of amino acids and small peptides. Several studies with poultry have shown an increased bioavailability of organic minerals as compared to the inorganic form. An improvement in egg production, egg weight, feed conversion, albumen weight and consistency was observed by adding organic selenium to layer diets. Egg shell quality was also improved, but organic zinc and organic manganese were required. A higher body weight gain and feed efficiency, but not feed consumption and mortality were observed in broilers fed organic selenium. An increase in number of chicks per hen housed and an improvement of chick quality were demonstrated in breeders fed organic selenium alone or in combination with organic zinc and organic manganese.

I. INTRODUCTION

The major minerals (macrominerals) must be supplied in fairly large amounts, whereas only small quantities of the trace mineral elements are required. In general, trace minerals participate as a structural component of proteins (eg. selenium is an integral part of glutathione peroxidase) or are important components of enzymatic systems, acting as cofactors. The trace mineral (eg. zinc, copper and manganese) bind to an enzymes and alters its conformation, so that it can interact with a substrate.

For many years, nutritionists have been using minerals in an inorganic form to meet the mineral needs of the birds. Once in the gastrointestinal tract, minerals fed in an inorganic form need to be solubilised into an ionic form to be absorbed. However, charged minerals can interact with other dietary components making them partially unavailable to the animal. Besides, they can be completely complexed (eg. phytate), becoming totally unavailable to the animal. Because of these uncertainties, the levels provided in the diet are often higher than the minimum required for optimum performance, resulting in wastage and environmental impact (Close, 1998).

One alternative to overcome this unwanted effect is to use organic or chelated sources of trace elements, frequently described as proteinates. They are normally produced by first hydrolysing a protein source, which results in the formation of a hydrolysate containing a mixture of amino acids and peptides of varying chain length. The reaction of a metal sulphate with the hydrolysate under the appropriate conditions results in the formation of complexes containing chelated metal ions (Hynes and Kelly, 1995). Organic minerals can also be synthesised through a biosynthetic process, as with the formation of selenomethionine and selenocysteine. In this case, a medium containing inorganic selenium and yeast is prepared. The yeast incorporates selenium in lieu of sulphur in the methionine or cysteine.

Organic minerals may utilise peptide or amino acid uptake pathways rather than normal mineral ion uptake pathways in the small intestine. This prevents competition between minerals for the same uptake carrier. Not only is bioavailability therefore higher, but these mineral forms are more readily transported and their intestinal absorption is enhanced. They are more stable and are protected biochemically from the adverse reactions with other dietary nutrients, which could reduce their rate of absorption (Close, 1998).

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Selenium is an important component of selenoproteins, Köhrle *et al*. (2000) estimates there may be 30 to 50 such selenoproteins. Glutathione peroxidase (GSH-Px) was the first selenoprotein to be described. Selenium participates as a component of the enzyme GSH-Px. This enzyme acts on lipid peroxides and hydrogen peroxide, converting them to hydroxy acids and water, respectively. During this reaction, two molecules of reduced glutathione are converted into oxidised glutathione. Therefore, Se deficiency makes the cell more prone to oxidation, besides increasing vitamin E requirement. For years Se has been added to the diets as sodium selenite, with a relative success. However, it is important to point out a pro-oxidant effect brought about by this compound (Surai, 2002). Besides being more available (Paton *et al.*, 2002), a study conducted in Canada (Roch *et al*., 2000) indicated that the use of organic selenium results in higher GSH-Px activity in broilers, as well.

The trace mineral zinc acts as a cofactor in several enzymatic systems. It takes part in hormonal secretion, growth, reproduction, immunology and shell formation. Zinc also participates in the metabolism of nucleic acids and in keratin and collagen synthesis. Wedekind *et al*. (1992) indicated that for chicks fed corn/soybean diets, Zn bioavailability from a Zn methionine complex was 206% relative to Zn sulphate (taken as 100%).

Manganese participates in several enzymes involved with carbohydrate, lipid and protein metabolism. Manganese is essential for bone and connective tissue growth. Reproductive and immunological functions are also dependent on manganese. The availability of Mn in a proteinate form or in methionine complex has been observed to be higher than that for Mn sulphate (Henry, 1995).

II. PRACTICAL APPLICATIONS OF ORGANIC MINERALS IN POULTRY DIETS

(a) Layers

Previous studies evaluating the egg selenium content have shown that selenium yeast was more available than Se provided by sodium selenite (Cantor, 1996 and Paton *et al*., 2002). A study was conducted to evaluate the effect of addition of increasing levels of organic Se (0; 0.1; 0.2 and 0.3 ppm Se) as a supplement to a regular premix on productive performance and egg quality of ISA Brown laying hens (42-68 weeks of age). The inclusion of organic Se improved egg production, egg weight and feed:egg. An improvement of egg yolk colour and in the albumen height (Haugh units) were observed by adding organic Se to the diets. In this study, egg shell quality was not influenced by organic Se.

A second study was also conducted in our lab to investigate the effects of a combination of organic trace minerals (Se + Zn + Mn) in diets fed to ISA-Brown layers during a second cycle of production (Table 1). Egg production, feed conversion and egg shell thickness, but not egg weight, were improved by adding organic minerals to the diets. Yolk and albumen weight (data not shown) were also improved by adding organic minerals to the diets. Increases in egg production (Rapp *et al*., 2002) and in eggshell quality (Moreng *et al*., 1992 and Rapp *et al*., 2002) have been reported by adding organic Zn and Mn to layer diets.

(b) Broilers

Several studies have been conducted around the world with the use of organic Se in broiler diets. In the United States, Edens and Gowdy (2003) have reported that the use of Se-yeast resulted in a slightly better growth performance of broilers. In several other countries (Australia, Thailand and Brazil), organic Se brought about a more significant improvement in growth rate, feed conversion and carcass yield. In Australia (Naylor *et al*., 2000), in the USA

(Hess *et al*., 2003) and in Thailand (reported by Edens and Gowdy 2003) studies have shown a reduction in chicken meat drip loss by using organic Se.

Table 1. Influence of organic minerals* on performance and egg shell quality of laying hens during a post-moult period.

Organic mineral premix (g/ton)			Egg production (eggs / 28 days)	Egg weight (g)	Feed conversion (kg/egg mass)	Egg shell thickness (mm)
Se	**Zn**	**Mn**				
0.00	0	0	20.4	59.50	2.31	0.443
0.15	15	15	21.1	60.30	2.25	0.473
0.30	30	30	21.4	60.89	2.14	0.483
0.45	45	45	21.7	60.40	2.11	0.495
CV (%)			8.06	6.63	9.53	11.36
Linear Regression			$Y = 0.425x + 20.085$ $R^2 = 0.96$		$Y = 0.071x + 2.38$ $R^2 = 0.96$	$Y = 0.0163 + 0.4325$ $R^2 = 0.926$
Probability of a dietary effect			$P < 0.05$	NS	$P < 0.05$	$P < 0.05$

*supplement containing inorganic minerals: Se (0.15 ppm), Zn (60 ppm) and Mn (60 ppm)

In Brazil, two identical studies were conducted to evaluate the effect of organic Se on growth performance of broilers. The first study (Rutz *et al*., 2003) consisted of feeding all birds 0.3 ppm Se, either in the inorganic (Sei) and/or in the organic (Seo) form: T1- 0.3 ppm Sei; T2- 0.2 ppm Sei + 0.1 ppm Seo; T3- 0.1 ppm Sei + 0.2 ppm Seo; and T4- 0.3 ppm Seo. The first trial was run at an integrator poultry research facility. The inclusion of 0.1ppm Seo + 0.2 ppm Sei provided an improvement in weight gain and feed efficiency, but not feed intake and mortality. The second trial (Anciuti unpublished data) demonstrated growth performance was maximised and mortality was minimised in broilers fed diets containing 0.2 ppm Seo.

(c) Broiler breeders

Surai and Sparks (2000) have shown that dietary organic Se supplementation for broiler breeders resulted in an increase in Se content of the egg and, consequently in the embryo. In addition, organic Se has provided an increase in vitamin E, vitamin A and carotenoids in the egg. Edens (2002) reported an improvement in the fertilising capacity of sperm by feeding roosters diets containing organic Se.

A total of 300,000 Cobb breeder hens were divided in two groups, during a field trial. The control group received a corn-soybean meal basal diet, containing the levels of inorganic minerals (Se-0.3 ppm; Zn- 100 ppm and Mn- 100 ppm) necessary to meet nutrient requirements of the birds. The test group was fed the basal diet supplemented with organic minerals (Se – 0.2 ppm; Zn – 30 ppm and Mn- 30 ppm). Eggs from hens receiving the control diet (8640 eggs) and those from hens given the diet supplemented with organic minerals (9792 eggs) were evaluated using embryo diagnostic procedures. A higher fertility and hatchability and lower embryo mortality from 15-21 days of incubation were observed in birds fed organic minerals (Rutz *et al*., 2003). Paton *et al*. (2002) pointed out the importance of providing a more available Se source to the developing embryo from 13 days of incubation on: 1) Ensuring the entire lipid content of the yolk is absorbed and metabolised by the embryo during the last seven days of incubation; 2) conversion of linoleic acid to arachidonic acid; 3)

development of erythrocytes in spleen as red cells require GSH-Px; and 4) conversion of thyroxine into triiodothyronine by triiodothyronine deiodinase, a selenoprotein. In general, several other studies, conducted in the Brazilian integrator farms, have indicated an improvement from 1 to 3.6 chicks/hen-housed by using organic minerals (Rutz *et al*., 2003).

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THE RELATIONSHIP BETWEEN FEED EFFICIENCY AND VISCERA WEIGHT IN BROILERS

S.B. NEOH and L.E. NG

Gastrointestinal tract function obviously plays a crucial role in the growth performance of broiler chickens. Moreover, the gut has a disproportionately high energy requirement relative to its weight. In theory, a lighter gastrointestinal tract, with a reduced energy need, would be more efficient; resulting in improved feed conversion ratios (FCR). Preliminary experiments were conducted to test this hypothesis. In two studies, using either Cobb of Ross broilers, 6,000 chicks were offered starter and grower diets with three tiers of nutrient specifications (Table 1). At 42 days post-hatch, 10 male and 10 female birds were randomly selected from each group of 2,000 birds and euthanased. These birds were defeathered and eviscerated and the percentage of viscera to carcass weight determined. The feed conversion ratios of each group were determined from total feed consumed and total live weights of the birds. The relative viscera weights were then plotted against FCR as shown in Figure 1. Simultaneously another 2,000 birds were offered Type A diets but containing a different soybean meal. The relative viscera weights are plotted against FCR as shown in Figure 2, where there was a significant difference ($P<0.01$) in viscera weight. These preliminary results suggest there is a correlation between relative viscera weight and FCR. Reducing nutrient specifications density or using a poorer quality soybean meal were associated with heavier relative viscera weights and less efficient FCR values.

Table 1. Metabolisable energy and protein contents of experimental diets

Item	Starter diets (1-21 days)			Grower diets (22-42 days)		
	Diet A	Diet B	Diet C	Diet A	Diet B	Diet C
ME (MJ/kg)	12.97	12.59	12.33	13.39	13.07	12.69
Protein (g/kg)	215	215	206	190	190	180

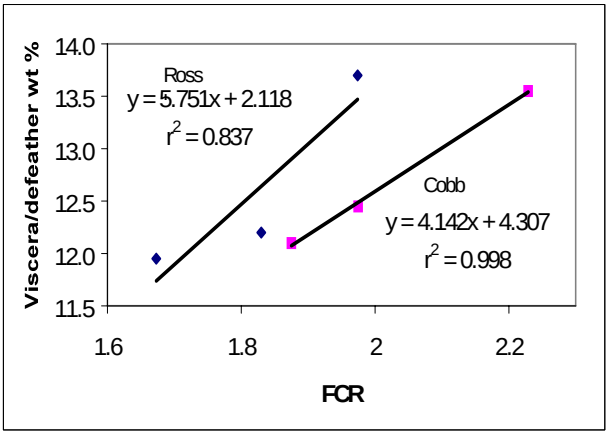

Figure 1. Relationship between relative viscera weight and FCR in diets with three tiers of nutrient specifications.

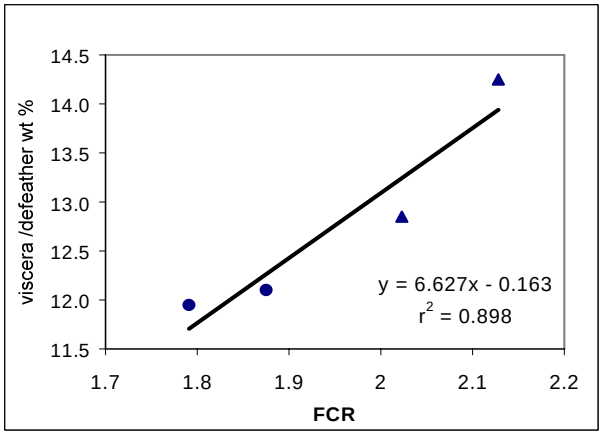

Figure 2: . Relationship between relative viscera weight and FCR in diets with different soybean meals. • Soon Soon SBM; ∇ Argentinean SBM.

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CHICKEN INTERLEUKIN-6 AS A PRODUCTIVITY ENHANCER IN BROILER CHICKENS.

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Concerns regarding antibiotic resistance have prompted authorities to review their use in livestock industries in Australia (JETACAR, 1999). Since the prohibition of in-feed antibiotics by the European Union in 1999, alternative therapies have been sought to ensure that the current productivity of the world chicken meat industry is maintained. This study investigated the immuno-stimulatory effects of chicken interleukin-6 (ChIL-6) after it was injected into broilers. Day-old male broiler chicks (Cobb) were divided into three groups of 25 birds each. Control birds received a 1 mL injection of sterile phosphate buffer solution (PBS) immediately post-hatch, intra-abdominally. Treatment group 1 (ChIL-6 Day 0) received a 1 mL intra-abdominal injection of ChIL-6 (440µg/ml in PBS). Treatment 2 (ChIL-6 Day 2) received the same treatment, but 48 hours later. After 35 days, blood was collected, and the birds were euthanased by cervical dislocation. The spleen and Bursa of Fabricus were removed and weighed (Table 1). Peripheral blood lymphocytes were measured *in vitro* for their ability to proliferate and to secrete ChIL-6 in response to mitogenic stimulation (Table 2).

Table 1. The 35-d body weight (BWT), eviscerated weight (EWT), and the relative weights (RW; % of body BWT) of spleen and bursa.

Treatment	**BWT(g)**	**EWT(g)**	**Spleen (RW)**	**Bursa (RW)**
Control	1997[a]	1753[a]	1.4[a]	2.3[a]
ChIL-6 Day 0	1953[a]	1719[a]	1.1[ab]	2.1[a]
ChIL-6 Day 2	2122[b]	1884[b]	1.1[b]	2.3[a]

[a,b] Means in a column with differing superscripts are significantly different (P<0.05).

Table 2. ChIL-6 expression and T-cell proliferation parameters.

Treatment	**ChIL-6 expression (U/ml)**	**T-cell proliferation (cpm, con A)**		
		Unstim.	10µg/mL	50µg/mL
Control	187[a]	2109[a]	9731[a]	16445[a]
ChIL-6 Day 0	209[a]	1598[a]	8612[a]	13303[a]
ChIL-6 Day 2	222[a]	1974[a]	10510[a]	20285[a]

[a] Means in a column with differing superscripts are significantly different (P<0.05).

Birds injected with ChIL-6 at 48-h post hatch had higher live (6.3%) and eviscerated weights (7.5%) and numerically higher ChIL-6 secretion and T-cell proliferation values compared to controls. Interestingly, the control birds had a significantly greater relative spleen weight than those given ChIL-6 48-h post-hatch. There was no effect on the relative bursa weight. Further investigations are required to confirm these results, and it is recommended that larger group sizes be used.

JETACAR Report (1999). http://www.health.gov.au/pubs/jetacar.htm.

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PERFORMANCE AND CARCASS TRAITS OF BROILERS FED DIETS CONTAINING YEAST EXTRACT (NuPro™)

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I. INTRODUCTION

Corn (or wheat) and soybean meal have the mainstays of broiler diets as the main sources of energy and protein, respectively. In some countries animal proteins (eg. meat meal, meat and bone meal and blood meal) have also been used as alternative sources in broiler diet formulation. However, Bovine Spongiform Encephalopathy, as well as, *Salmonella* and *E. coli* contamination have been associated with animal products. Consequently, these products have been banned in farm animals feeding in many parts of the world (Tibbetts, 2002).

Nutritionists have been looking for highly bio-available protein sources as an alternative to mammalian protein in poultry diets. A protein source derived from yeast cell contents, Nupro™ (NuPro™ Alltech Inc), appears to be a promising source of plant protein. Nupro™ is rich in inositol a potential natural growth promoter, glutamate and nucleotides. Nucleotides have been shown to have a positive impact on immune response of animals (Uavy, 1989).

The yeast cell content Nupro™ has been shown to be a successful feed ingredient in several animal species. This study evaluated the effects of Nupro™ on the growth performance and carcass traits of broiler chicks.

II. MATERIALS AND METHODS

A total of 810 day-old male Ross broiler chicks were housed in 27 floor pens. Thirty birds were randomly assigned to each pen in a completely randomized design. The animals were fed experimental diets consisting of: a practical corn-soybean meal diet (T1); with the inclusion of 20g/kg Nupro™ from 1 to 7 days of age (T2); or with the inclusion of 20g/kg Nupro™ from 1 to 7 days of age and from 38 to 42 days of age (T3). Feed and water were supplied *ad libitum*.

Body weight and feed consumption for each replicate group were measured at 7, 14, 31, 35 and 42 days of age. At the end of the trial, three birds (mean body weight of the pen) were euthanised for carcasses evaluated. Several organs were removed and weighed. Feather weight was also recorded at the end of the trial.

The data were analyzed using analysis of variance (SAS, 1997). Means were separated using Tukey's test. A probability value of less than 0.05 was necessary for differences between control and treatments to be considered significant.

III. RESULTS AND DISCUSSION

Birds fed diets containing yeast extract from 1 to 7 d of age showed higher feed intake up to 31 d of age (Table 1). However, this effect was statistically significant only up to 14 d of age. Providing yeast extract in diets to broilers from 38 to 42 d of age did not result in higher feed consumption, which suggests the yeast extract stimulates feed intake only in the early stage of chick development.

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Table 1: Feed intake (g) of broilers fed diets containing yeast extract (Nupro™)

Nupro™ (g/kg)			1 - 7	8 – 14	15 - 31	31 – 35	36 - 42
1 – 7d	8 – 37d	38 – 42d	d	d	d	d	d
0	0	0	162.6[b]	259.4[b]	920.9	1309.3	1080.8
20	0	0	177.6[a]	294.7[a]	963.5	1306.3	1101.9
20	0	20	178.4[a]	299.8[a]	948.0	1308.1	1078.8
CV (%)			4.40	4.88	5.76	3.61	6.62

Higher body weight gain was observed in broilers fed diets containing Nupro™. This effect was observed from the first week of life on (Table 2). Birds fed Nupro™ from 1 to 7 and from 38 and 42 d of age showed higher body weight ($P<0.05$) as compared to those fed control diets (69.1 g) and those fed Nupro™ only during the first week of life (49.7 g).

Table 2: Body weight gain (g) of broilers fed diets containing yeast extract (Nupro™)

Nupro™ (g/kg)			7 d	14 d	31 d	35 d	42 d
1 – 7d	8 – 37d	38 – 42d					
0	0	0	83.3[b]	354.5[b]	1686.5[b]	2055.1[b]	2562.2[b]
20	0	0	103.9[a]	384.2[a]	1713.5[ab]	2068.4[ab]	2582.6[ab]
20	0	20	108.5[a]	399.8[a]	1750.4[a]	2112.6[a]	2631.3[a]
CV (%)			5.07	4.54	3.01	2.23	2.13

Birds fed Nupro™ from 1 to 7 d and from 38 to 42 d of age showed better feed conversion (Table 3). At the end of the trial, animals fed Nupro™ consumed 40 g less per kilo of body weight gain, as compared to the control group. However, this effect was not statistically significant ($P>0.05$).

Table 3:. Feed conversion of broilers fed diets containing yeast extract (Nupro™)

Nupro™ (g/kg)			7 d	14 d	31 d	35 d	42 d	1- 42 d
1 – 7d	8 – 37d	38 – 42d						
0	0	0	1.31[a]	1.21	1.18	1.62[ab]	2.14	1.77
20	0	0	1.22[ab]	1.25	1.23	1.65[a]	2.15	1.78
20	0	20	1.20[b]	1.20	1.19	1.61[b]	2.08	1.73
CV (%)			6.48	3.86	3.45	1.78	7.99	2.40

Birds fed Nupro™ showed numerically higher carcass yield, drumstick, thigh, wing and breast weight but were not statistically different. Organ weights were not statistically influenced by the experimental diets. Feathering was not statistically influenced by the experimental diets). However, a numerically higher feather weight was observed in birds fed Nupro™.

IV. CONCLUSION

The use of Nupro™ in broiler diets from 1 to 7 d and from 38 and 42 d of age resulted in improvement of growth performance of the birds. On the other hand, feeding Nupro™ only from 1 to 7 days of age resulted in lower magnitude of improvement in growth performance.

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THE EFFECTS OF A MULTI-ENZYME IN WHEAT AND BARLEY BASED DIETS ON BROILER PERFORMANCE

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Summary

Broilers were fed medium or high levels of wheat and barley (WB) diets with and without a multi-enzyme product from 1 to 47 d, to determine the effect of the enzyme on performance. The WB levels in the medium diets were 5% and 5% in the starter, 20% and 15% in the grower, and 35% and 30% in the finisher, respectively. The corresponding contents for the high diets were 15% and 10% in the starter, 30% and 25% in the grower, and 40% and 40% in the finisher. Increasing the WB levels with supplementation of the enzyme increased weight gain to day 21. At the same age, feed consumption of all WB dietary treatments were significantly better than positive control. At d 42 and 47, birds that were fed the highest level of WB, with enzyme addition, were heavier and had better feed consumption. Birds with enzyme addition in their diets had both lower percentage of vent pasting and litter moisture compared to the non-enzyme supplemented groups.

I. INTRODUCTION

At present, enzyme supplements are used extensively in wheat and barley based poultry diets. Puchal and Mascarell (1999) have reported that enzymes may also be useful in diets based on corn and soybean meal. In addition, Douglas and Parsons (2000) have shown that chick growth performance was greatly influenced by soybean source and that both soybean source and enzyme affected ileal digestible energy. The soluble non starch polysaccharides (NSP), pentosans and mixed-linked β-glucans, cannot be hydrolyzed by endogenous enzymes and result in poor feed conversion, reduced body weight gain and wet litter conditions (Ravindran, 2003). These compounds which are present in some grains such as wheat, barley, and rye will interfere with the digestion of all nutrients in the diet, particularly of fat, fat-soluble vitamins, starch, and protein. Bedford (2002) has reported that birds fed rye or barley based diets are prone to fat soluble vitamin deficiencies, with rickets particularly common, because fat soluble vitamins are absorbed with the fat micelles. Therefore, the inability of young birds to digest NSP indicates the potential for an effective enzyme supplement.

Early attempts regarding enzyme utilization date back to the early 1950's. However, in more recent years a lot has been discovered on use of enzymes in animal nutrition. These studies, particularly in the discipline of poultry nutrition have presented a very significant breakthrough in our knowledge in this area. The objective of this study was to compare under farm conditions broiler performance when fed diets with increasing amounts of wheat and barley supplemented with enzyme.

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II. MATERIALS AND METHODS

Day-old broiler chicks were obtained from a commercial hatchery and 25 chicks were placed in each of 25 floor pens, including five pens per dietary treatment. The chicks were *ad libitum* fed mash feed and water. A photoperiod of 23L:1D was given for the first three days and 2L:2D thereafter. The intermittent lighting program was given only at nights. Five experimental diets were formulated with wheat and barley in the presence or absence of enzyme (Table 1). Proximate analysis of the wheat and barley used was determined prior to the start of the experiment. A corn/soybean meal diet was used as a positive control. Diets were formulated to specifications recommended by NRC (1994). Enzyme containing 12,000 FXU/g xylanase activity and 5,000 BGU/g β-glucanase activity was added at the suggested concentration level of 500 g/tonne. The group weight of each pen was determined on days 1, 21, 42, and 47. At the 47 days of age, each pen was visually evaluated for litter quality. On day 47, three birds per replicate were weighed, killed, and their abdominal fat content was removed to determine carcass quality. Data were subjected to one way analysis of variance using the General Linear Models procedure of SAS™.

III. RESULTS AND DISCUSSION

Vent pasting from days 1-10 tended to be higher in the wheat + barley diets, particularly at the higher levels of these cereals. Addition of enzyme reduced vent pasting to the level found in the corn based control. This indicated that the enzyme added reduced the viscosity caused by the presence of pentosans and ß-glucans by partially degrading these fiber components. Moreover, at the end of study birds with enzyme addition in their diets had better litter condition compared to the non-enzyme supplemented wheat + barley controls. Berg (1959) found that birds fed a barley diet consumed up to 28% more water than those fed with a corn diet.

Overall, bird losses due to mortality and culling were at a low level, and were not affected by diet or enzyme addition. As shown in Table 2, the groups fed wheat/barley diets supplemented with enzyme grew better during the first 21 days of the trial, and had better feed conversion efficiencies ($P<0.05$).

This finding indicated that young birds are particularly sensitive to non-starch polysaccharide. Therefore, enzymatic digestion of NSP improves performance and allows more efficient use of nutrients. By 47 d, there was no difference in live weight except for the enzyme supplemented higher wheat/barley diet, which gave heavier weights than all other diets ($P<0.05$). At 47 d, the higher wheat/barley diet, supplemented with enzyme, gave better feed conversion efficiency than the corn-soy diets ($P<0.05$). During all phase feeding periods those birds, which received no enzyme supplement in their diets consumed less feed ($P<0.05$) compared to the other groups. The anti-nutritional factors of wheat and barley are closely associated with the lower feed intake of the broilers. These pentosans become soluble during their passage through the digestive tract, as a result they form a viscous and gelatinous intestinal fluid, which inhibits the digestive process.

Overall, there were no significant differences in abdominal fat. The multi-enzyme employed in this trial contains mainly pentosanase and ß-glucanase, activities, and thus is capable of breaking down the main fiber components in both barley and wheat. Addition of this enzyme partially degraded the fibers present in the wheat/barley diets in this trial, reducing vent pasting and leading to better nutrient digestion. This better nutrient digestion was expressed as better feed conversion efficiency in the enzyme supplemented diets. This trial clearly shows that enzyme supplemented wheat/barley based diets can give the same

performance in broilers as corn based diets, with none of the litter problems often associated with such feeding such grains.

Table 1. Composition of Starting (0-21d) growing (22-42 d) and finishing (43-47d) diets; low (LowWB) and high (HiWB) wheat/barley (WB) fed with or without enzyme.

	0-21 d Starter			**22-42 d Grower**			**43-47 d Finisher**		
Composition	**Ctrl**	**LowWB**	**HiWB**	**Ctrl**	**LowWB**	**HiWB**	**Ctrl**	**LowWB**	**HiWB**
Corn	650	572	418	681	340	149	720	151	0
Soy	281	244	239	260	237	209	210	111	102
Fish meal	30	50	50	17	17	27	10	40	40
Wheat	0	50	150	0	200	300	0	350	400
Barley	0	50	100	0	150	250	0	300	400
Corn oil	0	0	9	7	20	30	0	14	25
Limestone	10	5.5	7.0	10.4	9.5	8.0	8.3	5.0	5.2
Dical Phosphate	18.0	18.0	18.0	14.0	15.0	16.0	19.0	17.0	17.0
DL-Methionine	1.60	1.35	0.92	1.40	1.60	1.60	0.90	1.20	1.30
Lysine-HCl	0.40	0.20	0.00	0.00	0.70	1.00	0.00	0.60	0.60
NaCl	3.60	3.56	3.00	4.00	4.00	3.40	4.50	3.50	3.00
Vitamin premix	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Mineral premix	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin D3	0.20	0.25	0.08	0.20	0.20	0.00	0.00	0.00	0.00
Sand	0.20	0.14	0.00	0.00	0.00	0.00	22.3	1.70	0.90
Enzyme (0.5g/kg)		+/-	+/-		+/-	+/-		+/-	+/-
Calculated analysis (as is basis)									
ME (MJ/kg)	12.10	12.10	12.10	12.29	12.29	12.29	12.06	12.13	12.13
Crude protein (%)	20.0	20.0	20.0	18.5	18.5	18.5	16.0	16.0	16.0
Lysine (%)	1.10	1.10	1.08	0.96	0.09	1.00	0.80	0.80	0.80
Methionine (%)	0.50	0.50	0.45	0.46	0.46	0.46	0.36	0.36	0.40
Met + Cys (%)	0.79	0.77	0.72	0.75	0.75	0.74	0.63	0.63	0.63
Calcium (%)	1.00	1.00	1.00	0.90	0.90	0.90	0.90	0.80	0.83
Phosphorus Av. (%)	0.40	0.46	0.46	0.40	0.40	0.40	0.48	0.40	0.40
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

Table 2. Effect of a multi-enzyme addition on broiler diets with low (LowWB) and high (HiWB) inclusion levels of wheat and barley on broiler performance to 47 d of age.

	Body Weight (g)				**Feed Conversion Ratio (g:g)**				**Feed intake (g/bird)**		
	1d	**21d**	**42d**	**47d**	**0-21d**	**22-42d**	**43-47d**	**0-47d**	**0-21d**	**22-42d**	**43-47d**
Control	40.7	576a	1617b	1890a	1.67b	2.17a	2.33b	2.26b	903	2263a	637b
LowWB No E	39.8	589b	1588a	1863a	1.50a	2.27b	2.41b	2.34c	933	2174b	600b
LowWB @ E	40.7	612b	1 5 1 a	1877	1 . 5 4	2.30b	2.50b	2.28ab	942	2515a	820a
HiWB No E	39.8	563a	1519a	1819a	1.59a	2.25b	2.40b	2.26b	896	2172b	725b
Hi WB @ E	40.2	622b	1665b	2000b	1.57a	2.07a	2.17a	2.10a	920	2520a	760a

[a-c] Means within the same column not sharing a common superscript differ significantly (P<0.05).

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EFFECTIVENESS OF ALTERNATIVE FEED SUPPLEMENTS TO BROILER DIETS USING A NECROTIS ENTERITIS CHALLENGE MODEL

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Summary

The effects of two alternative feed supplements (enzyme and organic acids) on mortality, growth performance and intestinal lesions of broiler chickens using a NE challenge model were investigated. The effects of these supplements were evaluated against a negative control (no additives) and a positive control (with monensin and Zn-Bacitracin). Birds fed Alternative A (enzyme) had bodyweights and FCR similar to the positive control group and had significantly higher bodyweight and improved FCR compared to the birds fed the negative control diet. There were only small numerical improvements observed compared to the negative control group when Alternatives B or C (organic acids) were added.

I. INTRODUCTION

The general fear of the development of antibiotic resistant "super-bugs" as a result of the overuse of antibiotic growth promotants (AGP) in animal production led to an increasing demand by consumers to ban AGP for use in animal feed. In 1999 the European Union (EU) restricted the use of AGPs and in late 2003 the EU announced a complete ban on all AGPs in animal feed. Although in Australia growth promotants are still registered, it has been recommended that "alternatives to antibiotics be researched and developed to control bacterial diseases and improve feed utilisation" (Grimes, 2000).

One of the most common and financially devastating bacterial diseases in modern poultry flocks is necrotic enteritis (NE). Necrotic enteritis was first described by Parish (1961) and is caused by the α- toxin of *Clostridium perfringens* (*CP*) types A and C. Damage to the intestinal mucosa through coccidial infection or a change in the normal intestinal microflora predisposes birds to the proliferation of *CP* (Al-Sheikhly and Al-Saieg, 1980). In its clinical form the disease results in high mortality and has to be prevented or treated immediately with antibiotics such as penicillin, virginiamycin, bacitracin, or tylosin (Watkins *et al.*, 1997). However, in its subclinical form the disease is much more financially damaging for the producer. Symptoms such as wet litter, diarrhoea and a small increase in mortality of less than 1% are often overlooked. However damage to the intestine and the subsequent reduction in digestion and absorption can reduce weight gain by more than 200g (van der Sluis, 2000) and feed conversion ratio at 35 days of age by up to 10 conversion points (Kaldhusdal and Løvland, 2000).

It has been documented that nutritional management such as lowering the inclusion rate of fishmeal, wheat or barley in the diet may prevent NE (Ficken and Wages, 1997). Furthermore, Hofacre *et al.* (2003) reported that the inclusion of a lactic acid producing bacterial culture and complex carbohydrates in broiler diets can effectively reduce the severity of an NE infection and can reduce the subclinical effects of a *C. perfringens* infection on broiler performance and mortality. These effects are believed to be the result of selective modulation of the natural gut microflora and the inhibition of the proliferation of *CP.*

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There are numerous supplements commercially available which claim to be effective in altering the intestinal microflora. However, there has been little work to determine the effectiveness of alternative supplements to prevent the occurrence of subclinical NE.

The objective of the current study was to evaluate the effects of four supplements on mortality, growth performance, intestinal lesions and the occurrence of *C. perfringens* in the small intestine of broiler chickens using a NE challenge model.

II. MATERIALS AND METHODS

A total of 150 male broiler chickens (Cobb) were purchased from the local hatchery, weighed in groups of 30 and placed in six floor pens in a climate controlled room. The treatment groups were: 1) negative control (no additives); 2) positive control (with monensin and Zn-Bacitracin); 3) Alternative A (enzyme) 0.5kg/t, 4) Alternative B (acidifier A) 2.5 kg/t; and 5) Alternative C (acidifier B) 2.5kg/t. All supplements were kindly supplied by Kemin Industries, Singapore.

Birds were fed five experimental basal diets based on wheat (47%), oats (15%), peas (7.5%) and SBM (19%) from d 0 to 7. From d 8 to 14 birds received a high protein diet based on 40% fishmeal, 45% wheat and 10% oats with the full dosage of additives. On d 14 all high protein feed was removed and replaced with the experimental basal diets.

The challenge model used in this study consisted of serial oral inoculation with *Clostridium perfringens* (approx. 2-5x10^8cfu/ml) on days 14, 15 and 16. All birds were wing tagged and bodyweight gain was measured individually on d8, d14, d21, d28, d35 and d42. Feed consumption and feed conversion ratio was measured on a pen basis on days 8, 14, 21 28, 35 and 42. Necropsies of all mortalities were conducted to determine the cause of death.

On d 18 and 21 six birds from each pen were killed by cervical dislocation. Ileal digesta from two birds was pooled and the numbers of colony forming units of *CP* (cfu) per g of digesta was determined. At the same time the small intestine of each bird was examined for the level of NE intestinal lesions according to the scoring table of Prescott (1979).

SAS systems (SAS Institute Inc., 2001) was used to perform the statistical analyses in this study. Data were analysed according to the GLM procedure for ANOVA. Duncan's multiple-range test was used to separate means when significant effects ($P<0.05$) were detected by analysis of variance.

III. RESULTS AND DISCUSSION

Birds in the negative control group without a supplement had significantly lower ($P<0.05$) bodyweight and numerically increased FCR compared to birds in the positive control group with monensin and Zn-Bacitracin (Tables 1 and 2). Furthermore, mortality as well as intestinal *CP* counts and NE lesion scores were significantly higher ($P<0.05$) in the negative control compared to the positive control group (Table 3). These findings are consistent with data from the literature which showed that inoculation with *CP* and the subsequent production of the α-toxin results in reduced growth, increased feed conversion and higher mortality (Kaldhusdal and Løvland, 2000, Köhler, 2000).

There were significant differences among treatments for bodyweight gain and average daily weight gain. Birds fed Alternative A (an enzyme product) had significantly higher ($P<0.05$) and numerically lower FCR than the negative control and comparable to those fed monensin and Zn-Bacitracin (positive control). It has been demonstrated that the inclusion of xylanase in wheat-based diets significantly reduced the bacterial population in the small intestine (Apajalahti and Bedford, 2000) and in particular the numbers of *CP* (Choct and Sinlae, 2000). The results of this study confirm that the addition of an enzyme supplement

(Alternative A) reduced digesta viscosity in the small intestine which significantly inhibited the proliferation of *CP* and subsequently reduced the severity of the NE challenge (Table3).

Table 1. Effects of supplements on bodyweight and average daily weight gain

Treatments [1]	**Bodyweight (g/bird)**			**Average daily weight gain (g/bird)**		
	8d	**21d**	**42d**	**0-14d**	**14-18d**	**18-21d**
Positive Control (PC)	155^{c}	658^{a}	2298^{ab}	21.2^{d}	49.2^{a}	57.9^{a}
Negative Control (NC)	150^{c}	525^{c}	1977^{c}	21.4^{d}	28.3^{c}	45.8^{c}
Alternative A	187^{a}	656^{a}	2373^{a}	26.8^{a}	39.7^{b}	55.9^{ab}
Alternative B	175^{b}	608^{b}	2096^{bc}	23.7^{bc}	35.5^{b}	50.7^{bc}
Alternative C	172^{b}	597^{b}	2038^{c}	25.2^{ab}	32.6^{bc}	46.0^{c}
Means	166.0	602	2141	23.5	36.9	50.8
SEM	3.6	18.4	91.9	1.01	2.59	2.53
Source of variance	Probability of greater *F* value in analysis of variance[2]					
Diet	***	***	**	***	***	***

[a,b,c] Values with unlike superscripts differ significantly (P<0.05)

2 ** P<0.01, *** P<0.001

Table 2. Effects of supplements on feed conversion ratio and mortality (measured/ pen)

	FCR				**Mortality**
	0-8d	**8-21d**	**21-42d**	**0-42d**	**NE related**
Positive Control (PC)	1.379	1.474	1.827	1.726	0/30
Negative Control (NC)	1.434	1.760	2.141	2.028	3/30
Alternative A	1.377	1.680	1.838	1.778	1/30
Alternative B	1.372	1.637	2.066	1.931	0/30
Alternative C	1.361	1.626	2.047	1.913	1/30
Means	1.364	1.630	2.008	1.891	total 5/180

Birds fed Alternative B had similar bodyweight to the positive control however feed conversion was not improved. The addition of both alternatives A and B resulted in a significant reduction in the number of *CP* in the small intestine immediately after the challenge (18d). Alternatives C had only small numerical increases in bodyweight and FCR compared to the negative control, and these were also seen for the intestinal *CP* count and NE lesion score (18d).

Table 3. Effects of supplements on intestinal viscosity, intestinal count of *C. perfringens* and lesion score on d18 and d21

	Ileal	**18d**		**21d**	
	Viscosity cP	**cfu/g digesta *10^8**	**lesion score**	**cfu/g digesta**	**lesion score**
Positive Control (PC)	10.6^{bc}	>0.01^{c}	0.04^{b}	>0.01	0.00^{b}
Negative Control (NC)	13.6^{ab}	2.03^{a}	1.71^{a}	1.23	0.88^{ab}
Alternative A	3.1^{c}	0.86^{b}	0.79^{ab}	1.10	1.46^{a}
Alternative B	19.5^{a}	0.85^{b}	0.96^{ab}	0.65	1.13^{a}
Alternative C	12.8^{ab}	1.86^{a}	1.13^{a}	1.87	1.13^{a}
Means	11.7	1.24	0.99	0.92	0.92
SEM		0.48	0.37	0.47	0.34
Source of variance	Probability of greater *F* value in analysis of variance[2]				
Diet	*	***	**	0.06	*

[a,b,c] Values with unlike superscripts differ significantly (P<0.05)

[2] * P<0.05, ** P<0.01, *** P<0.001

Based on the results of this study it appears that the reduction in broiler growth performance as a result of a sub-clinical *Clostridium perfringens* infection could be partially overcome when feed enzymes (Alternative A) are added to the diet. However, NE is a complex multifactorial disease with many unknown factors and any of the alternative feed supplements will have to be combined with strict hygiene management and good husbandry practices to maintain broiler performance and control the occurrence of the disease.

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FEED ENZYMES IMPROVE THE NUTRITIVE VALUE OF FABA BEANS

R.J. HUGHES[1], G.M. ROSS[2] and G. HARGREAVE[3]

Faba bean is a useful ingredient for inclusion in broiler diets (Perez-Maldonado *et al*., 1997) which may benefit from the use of enzyme products (Wiryawan *et al*., 1995). This study was undertaken in response to inquiries from nutritionists and feed millers about the nutritive value of faba beans and the usefulness of combinations of enzymes.

A blend of three samples of faba bean was used in this study. The basal diet contained (in g/kg) sorghum 575, soybean meal 320, sunflower oil 60, dicalcium phosphate 20, limestone 11, methionine 7, salt 2, choline chloride (60%) 2, and vitamins and minerals 2. The test diet contained faba bean 250 g/kg and basal diet 750 g/kg. All diets were cold-pelleted. Feed enzyme supplements were 500 g/tonne Bio-Feed Plus (B; multi-carbohydrase activities), 500 g/tonne Energex (E; multi-carbohydrase activities including hemi-cellulase and pectinase) and 100 g/tonne Bio-Feed Pro (P; protease activity). The 7-day energy balance experiment commenced when the chickens (Cobb 500) were 22 days of age. Each diet was fed to 2 cages of male chickens and 2 cages of female chickens (with 5 birds per cage). The first 3 days were for adaptation followed by 4 days of total excreta collection.

The table below summarises the effects of faba bean and enzymes on feed intake (FI, g/bird 22-29 days), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), dietary AME (MJ/kg dry matter), and dry matter digestibility (DMD, g retained/g eaten). Means with a common postscript letter are not significantly different (P<0.05).

Diet	**Enzyme**	**FI**	**GR**	**FCR**	**AME**	**DMD**
Basal		117.6 a	515 abc	1.598 cd	15.33 a	0.724 ab
Basal	B	124.1 a	548 a	1.586 d	15.35 a	0.724 ab
Basal	E	123.7 a	529 ab	1.638 bcd	15.18 a	0.710 b
Basal	B + E	120.4 a	525 ab	1.610 bcd	15.27 a	0.727 a
Basal	B + E + P	122.9 a	525 ab	1.647 bcd	15.25 a	0.719 ab
Faba bean		123.8 a	497 bc	1.756 a	13.70 c	0.646 d
Faba bean	B	114.7 a	473 c	1.748 a	13.82 c	0.658 d
Faba bean	E	121.2 a	488 bc	1.738 a	13.94 c	0.662 d
Faba bean	B + E	122.3 a	511 abc	1.676 abc	14.41 b	0.680 c
Faba bean	B + E + P	122.9 a	512 abc	1.684 ab	14.28 b	0.677 c
Pooled SEM		1.2	6	0.012	0.04	0.002

Improvement in AME of the faba bean diet with a combination of enzyme products B and E indicates successful degradation of anti-nutritive components in faba bean since none of the enzyme products or combinations improved the AME value of the basal diet. Addition of protease to the enzyme cocktail in the faba bean diet resulted in no further improvement in dietary AME. AME of faba bean was 13.2 MJ/kg DM without enzyme supplementation.

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REGULAR REVACCINATION FOR INFECTIOUS BRONCHITIS VIRUS IN LAYING HENS: ADVANTAGES AND DISADVANTAGES

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Summary

Different vaccination protocols for infectious bronchitis virus (IBV) were administered to ISA Brown laying hens during rearing and half the birds were revaccinated regularly during lay. At 57 wks of age, half of the birds were placed into an induced moult (moulted prior to revaccination), all birds were then revaccinated for IB and the other half of the birds moulted (moulted following revaccination). Production and egg quality were lower in the birds that were revaccinated regularly during lay, especially from 18 to 56 weeks. IB antibody titres increased at 6 and 16 weeks, then decreased and remained relatively constant from 27 to 77 weeks, increasing markedly following exposure to T-strain IBV. Egg shell quality was better in the birds that were revaccinated prior to moult. There appears to be little advantage, and some disadvantage, of regular revaccination during lay, provided that the birds have been effectively vaccinated during rearing.

I. INTRODUCTION

In Australia, live infectious bronchitis virus (IBV) vaccines have been widely used since 1966 (Cumming, 1969). At the present time, the main effect of IBV on layer flocks are reduced production ("egg drop") and reductions in egg quality (Chubb, 1987). Therefore, it is relevant to investigate the suitability of current vaccination protocols in protecting birds against challenge in the field. Some producers do not revaccinate for IBV once the birds have come into lay. However, increasingly, poultry veterinarians are recommending regular revaccination, usually every 8 weeks throughout lay.

Vaccination at 1-day-old with Vic S-strain IBV provided a limited degree of protection against a heterologous challenge with T-strain IBV at 15 d of age in broilers (Afanador & Roberts, 1994). In addition, a study using VicS IB vaccine strain with ISA Brown cockerels found that vaccination at either day-old or two weeks of age, by eyedrop, coarse spray or water vaccination, protected birds against the effects of exposure to T strain IBV (Sulaiman *et al.,* 2001).

The objectives of this experiment were to determine the effect of regular revaccination for IB during lay (versus no revaccination during lay), and the timing of moult in relation to revaccination late in lay, on production performance in laying hens. In addition, the effect on IBV antibody titres and kidney histology, of exposure to T-strain IBV at the end of lay, was assessed.

II. MATERIALS AND METHODS

Day-old ISA Brown hens (625) were purchased from the Winton Hatchery near Tamworth, NSW and transferred to isolation pens at the University of New England, Armidale, NSW. There were seven experimental groups, each of 89 birds, based on the vaccine strain at day-old and the route of vaccine administration: Control (No vaccination), VicS eye, VicS spray, VicS water, A3 eye, A3 spray, A3 water. Birds were revaccinated at 4

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weeks with the opposite strain of vaccine, via the same routes as day old. The Control Group remained unvaccinated until 14 weeks of age when all birds were vaccinated by VicS via eyedrop. Blood samples were taken from the same ten birds from each group (a total of 70 birds) at 4, 6, 16, 27, 35, 43, 49, 58, 65, 77, 79 and 80 weeks of age. Half of the birds were revaccinated every 8 weeks (from 14 weeks) with VicS vaccine strain by coarse spray whereas the remaining birds (in a separate shed) were not revaccinated beyond 14 weeks of age.

At 57 weeks of age, birds from all treatment groups were moved to individual cages for revaccination either before or after an induced moult. Half of the birds were moulted at 57 weeks, all birds were revaccinated at 62 weeks of age by coarse spray with VicS IBV and the other half of the birds were then moulted.

Egg production, egg weight and the external appearance of the eggs were recorded daily from the start of lay until the end of the experiment (80 weeks). Faecal moisture was measured 1 and 2 weeks post revaccination. Every 4 weeks, eggs were collected for egg and egg shell quality measurements (egg weight, shell reflectivity, shell breaking strength, deformation, shell weight, shell thickness, percentage shell, albumen height, Haugh Units, yolk colour score).

At 77 wks of age, birds were exposed to T strain IBV by eyedrop of 1 bird in 5. The challenge virus was purchased from Dr. Jagoda Ignjatovic of the CSIRO Australian Animal Health Laboratory, Geelong. Serum samples and kidneys were collected 1-2 wks before and 1, 2, 3, 4, and 5 wks after the challenge. Body weight and kidney weight were recorded. Histological sections were prepared from the left cranial division of each kidney and stained with haematoxylin and eosin prior to viewing under a light microscope. Any signs of abnormality in the kidney tissue were recorded.

Analysis of Variance was used to test the effect of treatment on each measured parameter. Fisher's protected LSD was utilized to separate means when significant effects were observed. Statements of statistical significance were based on $P<0.05$.

III. RESULTS

There was a significant effect of hen age ($P<0.01$) on egg production from 18 to 56 wks of age, with production increasing to a peak of 94.5 eggs/100 hens/day at 29 weeks and then decreasing gradually to 83.8 eggs/100 hens/day at 56 wks of age. There was a small but statistically significant effect ($P<0.01$) of regular revaccination on egg production between 18 and 56 weeks of age, with production during this period being higher for the birds that were not revaccinated regularly (Table 1).

There was a significant effect on hen-day production of regular revaccination from 57 to 73 weeks of age (including the moult period). The birds that had been revaccinated regularly for IBV during lay had slightly lower production at 57-73 weeks (57.9 eggs/hen/100 days) than the birds that had not been revaccinated (59.2 eggs/hen/100 days). For birds that had been revaccinated regularly during lay, production was higher when moult was induced after revaccination at 62 weeks, whereas for birds not revaccinated regularly, production was higher when moult preceded revaccination. However, there was no significant main effect of timing of moult on overall hen-day production.

There were effects of regular revaccination on egg quality from 18 to 56 wks of age (Table 1). Egg weight was higher and shell breaking strength, percentage shell, shell thickness were lower in the birds that were revaccinated regularly during lay. However, when the collections taken at 72 and 78 weeks of age were considered together, there were no statistically significant differences between the birds that were revaccinated regularly during lay and those that were not. Although shell breaking strength, shell weight, percentage shell

and shell thickness were all higher at 78 wks than at 72 wks, there was a statistically significant interaction between age and vaccination treatment only for shell breaking strength which, at 78 wks, was higher for the birds that were not revaccinated regularly during lay.

Table 1. Effect of regular revaccination on production and egg quality at 18-56 weeks (eggs/100 hens/day)

Egg and Egg Shell Quality	**Revaccinated Regularly**	**Not revaccinated during lay**
Hen-day production (%)	^{B}86.6 ± 0.4	^{A}88.1 ± 0.4
Egg weight (g)	^{A}60.4 ± 0.2	^{B}59.4 ± 0.2
Shell reflectivity (%)	33.4 ± 0.1	33.4 ± 0.1
Shell breaking strength (Newtons)	^{B}41.2 ± 0.2	^{A}42.5 ± 0.2
Shell deformation (µm)	270.5 ± 2.2	266.2 ± 2.0
Shell weight (g)	6.01 ± 0.02	6.03 ± 0.02
Percentage shell (%)	^{B}9.98 ± 0.02	^{A}10.18 ± 0.03
Shell thickness (µm)	^{B}430.4 ± 0.9	^{A}435.3 ± 0.9
Albumen height (mm)	7.34 ± 0.04	7.38 ± 0.03
Haugh Unit	84.5 ± 0.3	85.2 ± 0.2
Yolk colour score (Roche Scale)	11.24 ± 0.03	11.22 ± 0.03

Values are Mean ± S.E. Within a row, values with different superscripts are significantly different from each other.

There was no significant difference in excreta moisture or IBV antibody titre levels between birds that were revaccinated regularly during lay and those that were not. However, titres were significantly higher for all treatment groups at 6-16 weeks of age than at any other age up to 77 weeks.

Following exposure to T-strain IBV, IBV antibody titres increased markedly in all treatment groups at 79 and 80 weeks. Figure 1 shows the IBV titres of all initial vaccination treatment groups in birds, separated into those that were revaccinated regularly during lay and those that were not. There was no significant effect of exposure to T strain IBV on body weight, or total kidney, right kidney and left kidney weights expressed as a percentage of body weight, irrespective of vaccination protocol.

Haematocrit value and the plasma concentrations of sodium, potassium and calcium were not significantly affected by regular revaccination. Histological sections of kidney showed that most kidneys were normal with only a small number of kidneys showing signs of mononuclear cell infiltration. In addition, this incidence was no higher following challenge with T-strain IBV than it had been prior to challenge.

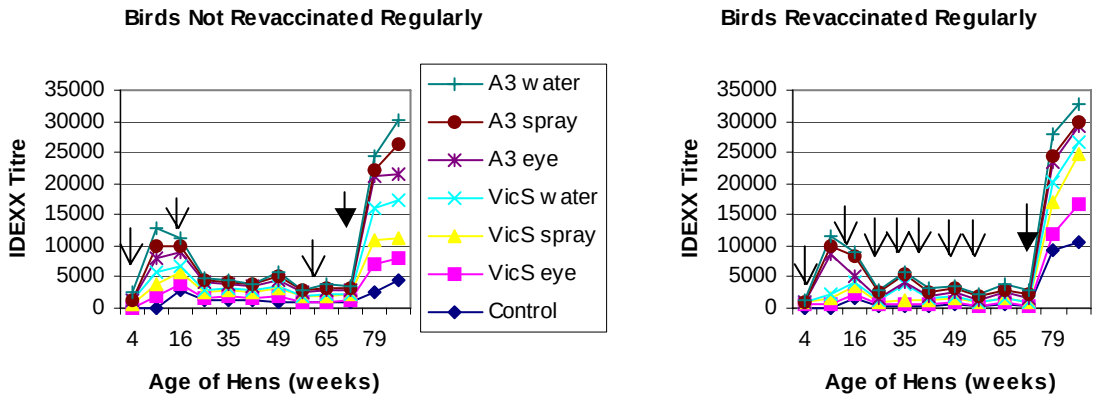

Figure 1. IBV antibody titres of initial vaccination treatment groups for birds that were not revaccinated regularly during lay (left) or revaccinated every eight weeks during lay (right). Arrows indicate vaccination, the solid arrows indicate the time of challenge.

IV. DISCUSSION AND CONCLUSIONS

Regular revaccination during lay resulted in a small reduction in production throughout the laying life of the flock and had some deleterious effects on shell quality, particularly in the early to peak lay period. IBV antibody titres remained relatively low in all groups from 27 to 77 weeks of age, in spite of revaccination, although titres increased markedly following exposure to T-strain IBV. These results may indicate that, immunologically, there is no benefit in revaccinating throughout lay, as there is limited ongoing upregulation of the immune response in these layers. It appears that there is little advantage, and some disadvantage, in revaccinating laying hens regularly during lay, if they have been effectively vaccinated during rearing. A complete vaccination/challenge study may be required to accurately relate the antibody titres seen to infection following challenge.

V. ACKNOWLEDGEMENTS

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INFLUENCE OF MAREK'S DISEASE VIRUS INFECTION ON THE HAEMATOGRAM OF BROILER CHICKENS

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Summary

Marek's disease virus (MDV) infection in chickens is very common. We report the effects of MDV infection with a very virulent Australian isolate on various haematological parameters of broiler chickens. Packed cell volume (PCV), total erythrocyte count and the haemoglobin concentration were reduced by MDV infection. PCV was reduced the most. HVT vaccination provided limited or no protection against these MDV-induced effects. These data confirm that MDV induces pathological effects on myeloid as well as lymphoid cells. As PCV is simple to measure and depression occurs early in infection, it may provide an additional diagnostic tool for evaluating MDV status.

I. INTRODUCTION

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by an α-herpesvirus. The disease is immunosuppressive, causing destruction of lymphoid and haematopoietic tissues of chickens (Jokowski *et al*., 1970; Gilka and Spencer, 1995; Islam *et al*., 2002). A significant decrease in erythrocyte (RBC) population (Neilsen and Anderson, 1971) and a marked decrease in packed cell volume (PCV) or haematocrit value after two to four weeks of MD virus (MDV) infection has been reported (Vickers *et al*., 1967; Jokowski *et al*., 1970; Gilka and Spencer, 1995; Spencer *et al*., 1996). The mechanism of pathology produced in the hematopoietic system by MDV infection is not clear but it may be due to reduced hematopoiesis (Jokowski *et al.*, 1970) or due to erythrophagocytosis by hyperactive reticuloendothelial cells of the liver and spleen of infected chickens leading to extravascular haemolytic anaemia (Gilka and Spencer, 1995).

However, reduction in RBC is a non-specific condition and has been reported in broiler chicken following stress such as feed restriction (Maxwell *et al.,* 1991; Hocking *et al*., 1994). Reduced PCV was also observed in broiler chickens following physiological stressors such as feeding of mycotoxin-contaminated feed (Aruvind *et al*., 2003).

HVT vaccine is routinely used in the Australian poultry industry to vaccinate broiler chickens. Vaccination with herpesvirus of turkey (HVT), recombinant HVT and bivalent vaccines is reported to protect against reduction in PCV following MDV challenge (Spencer *et al.*, 1996). HVT vaccination is also partially protective against immune organ damage due to MDV infection (Morimura *et al*., 1998; Islam *et al*., 2002). The primary objective of this study was to evaluate the effects of Australian MDV isolates on the hematological parameters of broiler chickens and the role of HVT vaccine in protecting against these effects.

II. MATERIALS & METHODS

Two experiments were conducted; experiment 1 included three treatments (Control, HVT and MDV infection) in a completely randomized block design with three replications of each treatment. A total of 378 chickens were randomly divided into three equal groups (126 per group). The first group (Control) was not inoculated with any virus. The second group

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(Vaccine) was vaccinated sub-cutaneously with 4000pfu of live cell-associated HVT vaccine and the third group (MDV) was infected intra-peritoneally with 50pfu of MDV.

Birds of each treatment were reared separately in nine positive pressure isolation units. At intervals of 3-7 d up to 35 d of age, five randomly selected chickens were removed from each isolator (replicate), weighed, blood sampled and euthanased for other studies.

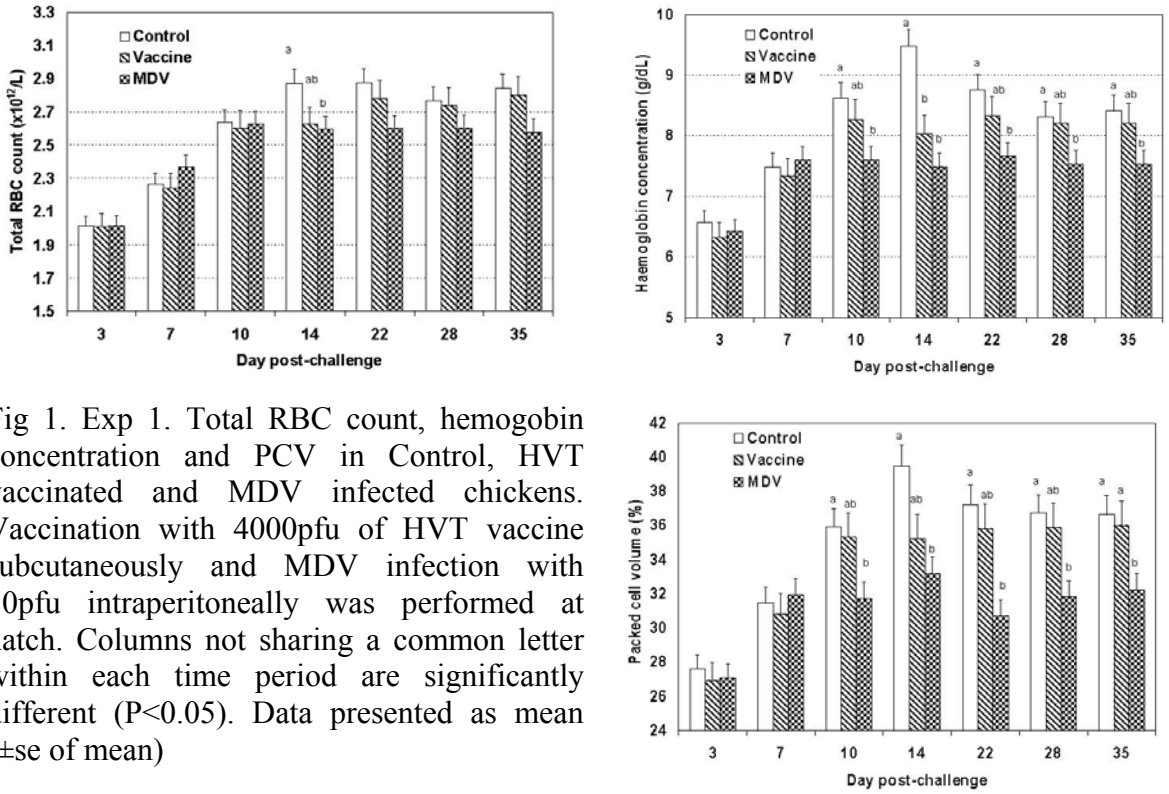


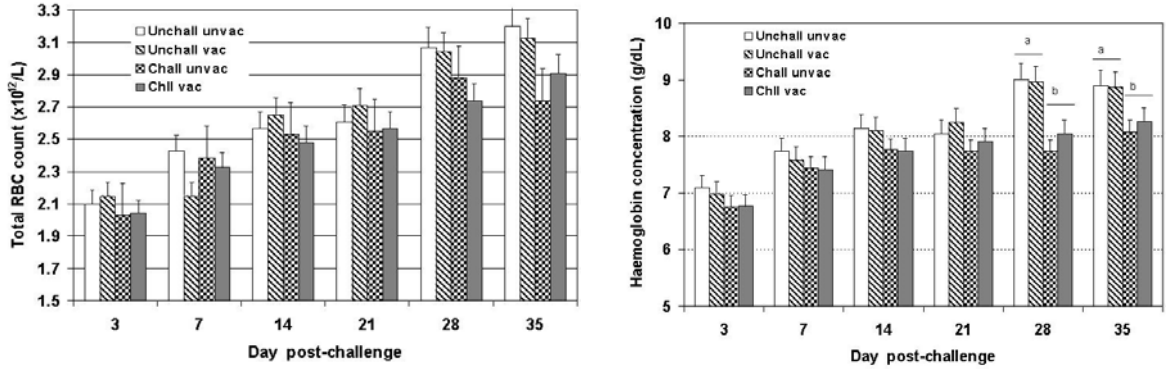
Fig 1. Exp 1. Total RBC count, hemogobin concentration and PCV in Control, HVT vaccinated and MDV infected chickens. Vaccination with 4000pfu of HVT vaccine subcutaneously and MDV infection with 50pfu intraperitoneally was performed at hatch. Columns not sharing a common letter within each time period are significantly different ($P<0.05$). Data presented as mean (±se of mean)

Experiment 2 utilized a 2x2 factorial design with two levels of vaccination with HVT (4000 or 0pfu) and two levels of MDV Challenge (100 or 0pfu) with four replicates of each treatment combination (N=360). Vaccination was performed sub-cutaneously at hatch and vaccinated chickens were permanently identified by foot web marks. Approximately equal numbers of vaccinated and unvaccinated chickens were placed in each of eight isolators (45/isolator). Chickens in four of the isolators were injected intra-peritoneally with 100pfu of MDV at three days of age while chickens in the remaining four isolators were sham challenged. At each of sample collection days 3, 7, 14, 21, 28 and 35 post-challenge, 12 randomly selected chickens from each treatment combination were weighed, blood sampled and euthanased for other studies.

Commercial feather-sexed female Cobb broiler chickens were used in both experiments. The parent flocks of the chickens were vaccinated against MD with serotype 1 MDV vaccine (Rispens CVI988), so the chickens had homologous maternal antibody to MDV. A cell-associated preparation of HVT strain FC126 (The Marek Company, Victoria, Australia) was used and the challenge virus was Australian very virulent strain of MDV (MPF 57) free of contaminants such as chicken anaemia virus.

Blood samples were collected in acid citrate buffer tubes. Absolute (total) counts of red blood cells (RBC) were made using an automated Cell-DYN® 3500 Haematology Analyser (Abbott, Norfolk, VA USA) calibrated for chicken blood. The analyser also measured the PCV (hematocrit) and percent haemoglobin concentration (Hgb).

Variables were analysed by analysis of variance (ANOVA) using Statview (SAS Institute Inc USA) with mean separation by Duncan's New Multiple Range Test. The statistical model tested the main effects of Treatment (Control, Vaccine and MDV), Day (post challenge) and their interaction in Exp 1 and the main effects of Challenge (MDV+ or MDV -), Vaccination (HVT+ or HVT-) and Day with all interactions in Exp 2.



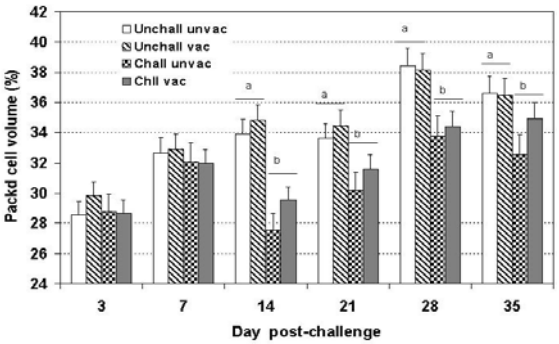


Fig 2. Exp 2. Total RBC, haemoglobin concentration and PCV of HVT vaccinated or unvaccinated and MDV challenged or unchallenged chickens. Vaccination with 4000pfu of cell-associated HVT vaccine sub-cutaneously at hatch, challenge with 100pfu of MDV occurred at day 3 post-vaccination. Columns not sharing a common letter within each time period differ significantly (P<0.05). Data presented as mean (±se mean)

III. RESULTS

(a) Experiment 1

There was a significant effect of Day (P<0.0001) and Treatment (P<0.001) on total RBC count with significant interaction between the effects of Day and Treatment (P<0.023). Overall, the RBC count increased with age (day) of chickens and was reduced by MDV infection. The interaction was significant because of the RBC number was reduced by MDV infection at days 14 and 22 but not at other time points (Fig 1A). There were significant effects of Day and Treatment (P<0.0001) and their interaction (P<0.0001) on Hgb concentration. Hgb increased with age of chickens up to day 14 and then remained relatively constant. MDV infection reduced Hgb from day 10 onwards but Vaccination had little effect on Hgb concentration (Fig 1B). here were significant effects of Day, Treatment (P<0.0001) and their interaction on the PCV (P<0.0001). There was an increase in PCV with age up to day 10 after which PCV remained steady. MDV infection reduced PCV from day 10 onwards but HVT vaccination did not affect it significantly (Fig 1C).

(b) Experiment 2

There was a significant effect of Day and Challenge (P<0.0001) but not Vaccination (P=0.06) on total RBC count. There was also significant interaction between the effects of Day and Challenge (P<0.005). There was a steady increase in RBC count to day 28.

Challenge with MDV decreased overall RBC count with HVT vaccination providing little protective effect. This effect was significant at all times other than day 21 (Fig 2A).

There was a significant effect of Day, Challenge ($P<0.0001$) and their interaction ($P<0.006$) on Hgb concentration. Vaccine had no effect on this variable. There was steady increase in Hgb with age up to day 28. Challenge reduced Hgb from days 14 to 35 (Fig 2B).

There was a significant effect of Day ($P<0.0001$), Challenge ($P<0.0001$) and Vaccine ($P<0.01$) on PCV with significant interaction between the effects of Day and Challenge ($P<0.0001$). Overall, PCV increased with age and MDV challenge reduced it from days 14-35. Vaccination provided partial protection against the reduction of PCV due to MDV challenge at days 14 and 35 only (Fig 2C).

IV. DISCUSSION

This study demonstrated that the Australian very virulent strain of MDV, MPF 57 markedly reduced the haematological parameters of total RBC count, haemoglobin concentration and PCV of commercial broiler chickens and that HVT vaccination provided very limited protection against this pathology. A 17% reduction in PCV due to MDV infection was reported in layer chickens (Spencer *et al*., 1996) but the reduction in our study was only about 10%. A very large reduction in RBC population (30% or more) has been reported following MDV challenge (Nielsen and Anderson, 1971) but reduction of both RBC and Hgb was about 6% in the current study. This variation might be due to strain differences in the viruses used or the chickens. In the report of Nielsen and Anderson (1971) freedom from chicken anaemia virus CAV) was not stated and so CAV must be considered as a possible contributory factor. The mechanism of destruction of haemopoitic tissue was not studied here but it may be due to extravascular hemolysis as suggested before (Gilka and Spencer, 1995).

Vaccination with HVT and a recombinant HVT vaccine was found to be protective against anaemia produced following MDV infection in layer chickens (Spencer *et al*., 1996) but HVT was not protective against reduced RBC, PCV and haemoglobin concentration in the present study. Among the variables measured, PCV was the most affected due to MDV infection. Reduction of PCV was even more consistent than reduction in lymphoid organ weights in the same experiment (Islam *et al*., 2002). Therefore PCV may be used as an early indicator of MDV infection but the use of this indicator as a marker of vaccine protection may not be useful as has been suggested previously (Spencer el al., 1996).

V. ACKNOWLEDGEMENTS

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EFFECT OF WHEAT TYPE, PROCESSING AND PHYTASE SUPPLEMENTATION ON NITROGEN AND PHOSPHORUS DIGESTIBILITY OF BROILER CHICKS

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The combined supplementation of phytase and processing (based on heat treatment and particle size) in wheat-based broiler diets may synergistically improve nitrogen (N) and phosphorus (P) digestibility. The effect of two wheat cultivars [Hard Red Spring (HRS) and Durum] with three particle sizes (630, 560 and 470µm) without or with heat treatment (oven dry at 80°C for 45 min) before supplementation with phytase (Natuphos® 0 or 600 FTU/kg) on N and P digestibility were investigated in a 2×3×2×2 factorial arrangement. The 24 diet treatments contained 0.5 g Avizyme 1302/kg (Danisco Animal Nutrition, Marlborough, UK). Diets (80% wheat, 20% basal diet) were formulated to supplying nutrients necessary to meet the requirements of broiler chicks, except for P (2.7 g/kg); all diets contained 1% Celite™, an acid insoluble ash marker. Day old male broiler chick (Hubbard) were allocated into groups of six males in four cages/treatment and fed the test diets to 21 d. An excreta sample was collected from each cage at 20 d of age for N and P digestibility determination. In addition, the endogenous phytase and xylanase activity in both Durum and HRS with and without processing was also analysed by Danisco Animal Nutrition. Endogenous phytase and xylanase activity of HRS were 44 and 29% higher compared to the Durum samples, respectively. Inactivation of endogenous phytase in Durum by heating was 60% and in HRS 38%. The broilers fed HRS-based diets had significantly better P digestibility than those fed Durum-based diets. This was attributed to the higher endogenous phytase and NSPase activity in HRS compared to Durum. There was no main-effect of particle size on N and P digestibility. Heat treatment decreased N and P digestibility. Phytase increased N and P digestibility. Wheat type interacted with particle size, heat treatment and phytase on N and P digestibility. The interaction demonstrates a consistent improvement in N and P digestibility of coarse and fine Durum-based, and coarse and medium HRS-based diets with heat treatment. The interaction shows a markedly higher N and P digestibility of Durum and HRS wheat-based diets when diets were fed with no heat treatment and added phytase than with heat treatment. The lack of response to phytase in heat treatment Durum and HRS with different particle sizes indicates that heat treatment can cause significant denaturing of phytic acid (Summers *et al*., 1966) or that the response is attributed to the denaturing of endogenous phytase and NSPase activity (Summers *et al*., 1968) and the lack of synergistic effect between endogenous and exogenous phytase and NSPase. Nitrogen and P digestibility were significantly increased with medium ground, heat-treated Durum without enzyme and fine unheated HRS than the other two particle sizes.

In summary, phytase, heat treatment and particle size are effective strategies to reduce N and P excretion from broiler chicks. Improvement in N and P digestibility by phytase and heat treatment were dependent of wheat type and particle size. Therefore, effectiveness of phytase appeared highest with coarse or fine unheated Durum wheat and medium unheated HRS-based diets, indicating that wheat type particle size should be considered before phytase application. Combined these factors may synergistically improve N and P digestibility and reduce the environmental impact of N and P excretion by poultry.

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Table The effect of wheat type (Durum vs Hard Red Spring (HRS), grind size (GS; coarse, medium and fine), heat treatment (HT; unheated vs oven dry) and phytase supplementation (PE;0, 600 FTU/kg) on measurements of protein and phosphorus digestibility.

			Nitrogen digestibility (%)			Phosphorus digestibility (%)		
Wheat Type			**Total**	**Durum**	**HRS**	**Total**	**Durum**	**HRS**
Total				**71.2**	**70.3**		**59.6** ^{b}	**64.2** ^{a}
Heat Treatment (HT)								
	Unheated (NT)		73.5 ^{A}	72.0 ^{b}	75.0 ^{a}	63.8 ^{A}	59.8 ^{b}	67.8 ^{a}
	Ovendry (OT)		68.0 ^{B}	70.3 ^{c}	65.6 ^{d}	60.0 ^{B}	59.4 ^{b}	60.7 ^{b}
Grind Size (GS)								
	Coarse (C)		70.7	70.5 ^{b}	70.9 ^{b}	61.7	59.1 ^{c}	64.3 ^{ab}
	Medium (M)		71.3	73.1 ^{a}	69.6 ^{b}	62.4	62.2 ^{b}	62.6 ^{b}
	Fine (F)		70.1	69.9 ^{b}	70.4 ^{b}	61.6	57.5 ^{c}	65.8 ^{a}
Phytase Enzyme (PE)								
	0		66.4 ^{B}	66.7	66.7	56.0 ^{B}	54.0 ^{d}	57.9 ^{c}
	600		75.1 ^{A}	75.6	75.6	67.8 ^{A}	65.2 ^{b}	70.5 ^{a}
HT	GS	PE (Interaction)						
NT	C	0	62.5 ^{E}	60.6 ^{j}	64.4 ^{hi}	47.7 ^{D}	45.9 ^{k}	49.5 ^{k}
NT	C	600	81.7 ^{A}	81.0 ^{b}	82.4 ^{b}	75.2 ^{A}	72.1 ^{cd}	78.3 ^{b}
OT	C	0	69.0 ^{B}	68.1 ^{gh}	70.0 ^{fg}	61.9 ^{B}	57.0 ^{ij}	66.7 ^{ef}
OT	C	600	69.7 ^{B}	72.5 ^{ef}	66.8 ^{gh}	62.1 ^{B}	61.5 ^{ghi}	62.7 ^{fgh}
NT	M	0	68.8 ^{B}	76.5 ^{cd}	61.1 ^{ij}	57.8 ^{C}	66.9 ^{ef}	48.8 ^{k}
NT	M	600	80.6 ^{A}	73.7 ^{de}	87.5 ^{a}	72.6 ^{A}	61.7 ^{ghi}	83.4 ^{a}
OT	M	0	66.0 ^{CD}	67.3 ^{gh}	64.7 ^{h}	57.5 ^{C}	57.3 ^{hij}	57.6 ^{ghij}
OT	M	600	69.9 ^{B}	74.8 ^{de}	64.9 ^{h}	61.7 ^{B}	62.8 ^{fg}	60.6 ^{ghi}
NT	F	0	67.6 ^{BC}	60.4 ^{j}	74.9 ^{de}	55.3 ^{C}	40.7 ^{l}	69.9 ^{de}
NT	F	600	79.5 ^{A}	79.6 ^{bc}	79.5 ^{bc}	74.1 ^{A}	71.5 ^{de}	76.7 ^{bc}
OT	F	0	64.1 ^{DE}	67.4 ^{gh}	60.9 ^{j}	55.7 ^{C}	56.3 ^{ij}	55.2 ^{j}
OT	F	600	69.2 ^{B}	72.0 ^{ef}	66.4 ^{gh}	61.3 ^{B}	61.4 ^{ghi}	61.2 ^{ghi}

$^{a-k}$ Different letters superscript mean values indicate significant (P<0.05) differences between mean values of interactions (grain source x heat treatment; grain source x grind size; grain source x phytase enzyme and grain source x heat treatment x grind size x phytase enzyme).

$^{A-E}$ Different letters indicate significant (P<0.05) differences in total values for main effects of heat treatment and phytase enzyme, and interactions between heat treatment x grind size x phytase enzyme.

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STUDIES ON THE PREVENTIVE ASPECTS OF AN HERBAL PREPARATION NEPHTONE AGAINST GENTAMICIN INDUCED TOXICITY IN BROILERS

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Gentamicin, an aminoglycoside is being used extensively in hatcheries with the objective of preventing early chick mortality despite its potential toxic effects on liver and kidney (Jayanthi, 2000). NephTone (Indian Herbs, Saharanpur, India) is a multiherbal formulation containing the standardised extract of *Tinospora cordifolia, Boerhaavia diffusa, Tribulus terrestris, Berginia ligulata, Crataeva nurvala, Andrographis paniculata, Solanum nigrum, Eclipta alba and Terminalia chebula*, all proven to have beneficial effects on liver health and to help regeneration of damaged renal tubular epithelial cells and optimisation of renal functions to maintain physiological homeostasis (Veerender Kumar, 1996). In view of the above considerations, the present study was planned to determine the effect of NephTone in prevention of gentamicin induced toxic effects including clinical signs and biochemical alterations in broilers.

Gentamicin was administered in day old broiler chicks at the dose rates of 15mg and 20mg/kg body weight (BW) in first trial. Three injections of gentamicin were given subcutaneously on day 0, 1 and 2 of the experiment. The birds injected with gentamicin showed clinical signs of inappetence, dullness and depression, and significant alterations ($P<0.05$) in biochemical parameters including increased serum levels of alanine transaminase, aspartate transaminase and uric acid at both doses. Bird *et al.* (1983) noticed similar changes in red tailed hawks. The gross lesions in liver and kidney were congestion, Petechiae and blood clots on surface. Histopathologically, marked vacuolar degeneration, loss of architecture in liver, loss of brush border of PCT (Proximal convoluted tubule), desquamation and degenerative changes in lining epithelium of tubules were prominent in both the groups. The severity of clinical signs, biochemical alterations, gross lesions and histopathological lesions were greater in birds injected with gentamicin at 20mg/kg BW.

In a second trial (therapeutic trial), NephTone was added in feed at two doses of 1.5 and 3.0 g/kg feed from day 0 to day 42 of the experiment, along with gentamicin administration (at both doses of 15mg and 20 mg/kg BW) on day 0, 1 and 2 of the experiment, such birds exhibited similar clinical signs, biochemical alterations in serum parameters, gross and histopathological lesions as were present in birds treated with gentamicin alone, but the duration and severity were markedly reduced. Brush border of PCT were fond to be intact. Das and Agrawala (1998) observed the similar findings. NephTone at 3.0g/kg feed was found to be more effective than the lower dose, 1.5 g/kg feed in alleviation of gentamicin toxicity.

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SPONTANEOUS AND STRESS INDUCED MYOPATHIES IN MODERN MEAT BIRDS: A CAUSE FOR QUALITY AND WELFARE CONCERNS

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Summary

Modern, rapidly growing strains of meat poultry exhibit an elevated incidence of spontaneous or idiopathic myopathy and an increased susceptibility to stress induced myopathy. These pathologies are attributable to alterations in intracellular calcium homeostasis and consequent changes in sarcolemmal integrity and may result from excessive myofibre hypertrophy and inadequate development of support tissues and vascular supply. These myopathies may have, in turn, a range of implications for both product quality and bird welfare.

I. INTRODUCTION

Artificial genetic selection for improved body weight gains and food conversion has resulted in rapid muscle growth in commercial broiler chickens and turkeys. Current commercial strains of meat type poultry appear to exhibit an increased incidence of idiopathic and stress induced myopathies compared to their slower growing counterparts or genetic predecessors (Mitchell, 1999). The mechanisms of these pathologies have not been fully elucidated but understanding the patho-physiological basis of this muscle damage is important in relation to both bird productivity and welfare. Thus derangements of ante-mortem muscle cell metabolism and alterations in sarcolemmal integrity and tissue structure associated with the presence of myopathy may have profound implications for meat quality and the incidence of specific conditions such as Pale, Soft Exudative (PSE)-like meat. Also it may be suggested that muscle dysfunction may lead to problems of altered locomotor capability and therefore behavioural changes and reduced welfare. This situation may be further compounded if the observed myopathies are accompanied by muscle discomfort or pain. The present review addresses these issues and examines current knowledge of the causes and mechanisms of myopathy in poultry and the consequences for productivity.

(a) Muscle abnormalities in poultry

Muscle abnormalities including inherited muscular dystrophy, deep pectoral myopathy, dietary deficiency myopathies and toxic myopathies have long been recognised and described in poultry. Reports of an increased incidence of Pale, Soft Exudative meat (PSE) in modern, commercial turkeys and broilers (Barbut, 1997ab) and the recognition of muscle damage associated with thermal stress and catching and transport in broilers (Mitchell, 1999) have focused attention upon the possible link between rapid growth rates, stress induced myopathy and product quality in poultry. The patho-physiological mechanisms of such myopathies and other muscle abnormalities have been the subject of an extensive review (Mitchell, 1999). A common feature of all myopathic and dystrophic conditions is the leakage of the intracellular muscle enzyme creatine kinase (CK) into the circulation. Thus increased plasma activity of CK is a useful diagnostic indicator of muscle pathology and altered sarcolemmal integrity.

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(b) Growth rate and muscle damage

In both broiler chickens and turkeys, plasma CK is elevated as body size increases (Mitchell, 1999) and it is proposed that this is indicative of a growth-associated myopathy. It is hypothesised that this increased occurrence of muscle abnormalities may be attributable to tissue growth ultimately exceeding metabolic, physiological or anatomical limits (Mitchell, 1999), although such mechanisms have yet to be fully characterised. Selection for growth rate has resulted in more and larger muscle fibres in slow, tonic and fast twitch muscles of the broiler chicken without any effect on fibre typing (Rémignon *et al.,* 1996ab). It is apparent that, in order to support the increased growth rate of the "demand organs" such as muscle, bone, fat, skin and feathers, appropriate adaptations must occur in the "supply organs" including the cardiovascular and respiratory systems, intestine and liver. It may be postulated that failure of the supply systems to meet the demands of a disproportionately large growth rate may underlie many of the current pathologies and welfare problems encountered in commercial broiler production. Whilst divergent selection for breast meat yield in broiler chickens was without effect upon histological character and meat quality attributes of breast and leg muscles in the studies of Rémignon *et al.* (1996ab), in another study a comparison of fast growing broiler lines with laying lines with a slower growth rate has revealed "significant changes of structural, metabolic and functional parameters in skeletal muscle" in meat type birds (Soike and Bergmann, 1998). Disseminated muscle fibre degeneration and hypercontraction were particularly prevalent in the breast muscle of these broiler chickens.

In addition it may be suggested that the apparent increased susceptibility to "stress" of the modern broiler fowl may be attributable to the exploitation of the genetic potential for growth in these birds in the absence of compensatory development of the corresponding homeostatic and regulatory mechanisms (Mitchell, 1999). Such a phenomenon may occur during the rapid skeletal muscle growth in the current commercial broiler chicken, resulting in alterations in tissue characteristics.

(c) Genetic origins of idiopathic myopathy

Recent studies have examined the effects of genetic selection for rapid growth rate upon muscle membrane integrity or "leakiness", using plasma CK activity as an index of the extent of pathology. Two lines of broiler chickens were compared. These lines were designated "selected" and "relaxed". The selected line represents a current commercial broiler strain, which has been continuously selected for growth rate and high feed conversion efficiency. The slower growing birds were selected for growth rate until 1978, when selection was discontinued or "relaxed", and thus represent a control line. The findings demonstrated that muscle enzyme (CK) loss increased with age in both lines of broilers but was always much greater, and thus muscle damage more extensive, in the highly selected, rapidly growing birds than in their slower growing counterparts. This was true even when actual body weight was the same in birds from the two lines (at 70 days of age in the relaxed line and 42 days in the selected birds). No differences in muscle enzyme content were found which could explain this disparity. It thus appears that the elevated enzyme efflux and muscle damage in the selected line broilers are associated with increased growth rate and not body size (Mitchell, 1999). Similar findings have been reported in comparisons of slow growing traditional line turkeys and a more rapidly growing commercial male line (Mills *et al*., 1999). Other studies have also demonstrated that rapidly growing broiler lines are more susceptible to stress-induced myopathy than genetically slower growing ones (Sandercock *et al.,* 2001). Myopathy as evidenced by elevated plasma CK, is associated with demonstrable histo-pathological changes in muscle tissue. The condition is characterised by histological changes

indicative of muscle degeneration including hyaline (hypercontracted) fibres, fatty infiltration, fragmentation of the sarcoplasm, mononucleocyte infiltration and focal necrosis. Indicators of tissue regeneration such as basophilic fibres and internalised nuclei have also been observed (Mahon, 1999). The onset of pathological changes appears to correlate with the attainment of a specific fibre diameter regardless of age or body weight suggesting a limit for fibre hypertrophy beyond which muscle function may be compromised (Mills *et al*., 2000).

Complementary studies have examined the development of idiopathic myopathy in selected and control line birds beyond normal slaughter age and through to mature body weight. The relative body weights of the selected and relaxed lines of broilers increased over the experimental period up to sexual maturity with the selected line being significantly heavier throughout. Consistent with the earlier studies, plasma activities of CK were found to increase throughout development in both lines but with a much more rapid rate of rise in the selected commercial broilers, plasma CK being four-fold higher at mature body weight in the fast growing birds (Mitchell and Sandercock, 1996). It was concluded that this reflected cumulative muscle damage associated with the continuing high body weight gains up to maturity. Indeed the final body weight of the selected broilers was about 6.0 kg as opposed to less than 3.5 kg in their relaxed counterparts.

Such investigations of the effect of genetic selection on idiopathic myopathy in poultry have been confined to a relatively small number of studies comparing small numbers of genetically divergent lines (e.g. Rémignon, *et al.,* 1996ab; Soike and Bergmann, 1998). The prevalence and the extent of genetic variation for this condition in chicken lines have only been addressed recently. Estimates of genetic variation in skeletal muscle status can be achieved using a multi-breed approach employing a large number of pure-lines but testing only a small number of individuals per line. This approach has been previously used to assess the extent of genetic variation for economically important production traits in poultry (Hocking *et al.,* 1985).

A study was undertaken in the authors' laboratory to correlate the extent of idiopathic muscle damage (as determined by plasma CK activity) with measurements of live weight (LW), total skeletal muscle weight (MW) and the weight of breast, thigh and drumstick muscle in 37 different chicken lines representative of 3 line categories (broiler [B], layer [L] and traditional [T]). CK activity, live weight and total muscle weights were assessed by analysis of variance. The relationship between CK activity and live weight and muscle composition was analysed by multiple regression analysis of natural logarithms (ln) of the variables. Transformations to ln were necessary to normalise residual errors. The maximal model included effects for age, category, live weight and breast, thigh and drumstick muscle.

Combined category means (6 and 10 weeks of age) for live weight, muscle weights and plasma creatine kinase (CK) activity are presented in Table 1. Broiler lines were significantly ($P<0.001$) larger, contained proportionally more breast muscle as a fraction of total muscle weight and exhibited higher plasma CK activities than the L and T lines. Weights and plasma CK activities increased from 6 to 10 weeks ($P<0.001$) whereas the relative muscle proportions did not change with age. Plots of log (ln) transformed live weight versus CK activity at 6 and 10 weeks of age in B, L and T-lines are shown in Figure 1. Plasma CK activity was higher at the same live weight in B-lines compared with L and T lines ($P<0.01$). Elevations in CK activity with increased live weight were also greater in B-lines than L and T lines ($P<0.01$).

Table 1. Category means at 6 and 10 weeks of age for creatine kinase (CK) activity, live weight, total muscle weight and breast, thigh and drumstick muscle as a proportion of total muscle.

Variable	Broiler	Layer	Traditional	Sed
Live weight (g)	3559	852	933	84.6
Total muscle (g)	1333	203	235	39.6
Breast, g/kg total muscle	532	431	426	4.3
Thigh, g/kg total muscle	295	333	334	3.2
Drumstick, g/kg total muscle	173	236	240	3.5
Creatine kinase (IU/l)	1017	237	247	20.6

The production data and corresponding plasma CK activities reported in this study were consistent with those described in previous studies (Mitchell, 1999; Sandercock *et al.,* 2001), with all lines examined exhibiting age dependent increases in plasma CK. At both ages, B-lines were on average four times heavier than the L and T lines and exhibited greater total muscle (six-fold), breast (eight-fold), thigh (six-fold) and drumstick (five-fold) yields. Multiple regression analysis showed the strongest correlation (smallest residual mean squares) for plasma CK activity was obtained by fitting category and the regression of CK on LW within category ($P<0.001$). These results suggest that increases in plasma CK activity were positively associated with increases in muscle mass and were not affected by changes in the relative proportion of breast meat. Comparisons of the differences in slopes and intercepts of the three line categories suggested that the observed differences in plasma CK activity between B and the L and T lines could not be explained by changes in LW alone. In addition, increases in plasma CK activity with LW were markedly higher in the B-lines compared with the L- and T-lines. This suggests that detrimental alterations in muscle function and membrane integrity exist in the B-lines that may be attributable to genetically induced changes in muscle fibre status. Sosnicki and Wilson (1991) have postulated that, in turkeys, selection for rapid growth and meat yield may have resulted in the growth of the muscle fibres outpacing the supporting capillary supply and connective tissue, leading to an increase in myo-degenerative features associated with focal ischaemia.

Figure 1. Regression analysis of log (ln) live weight versus log CK activity at 6 and 10 weeks of age in meat, layer and unselected

(d) Mechanisms of myopathy in poultry

Whilst it seems likely that the fundamental mechanisms mediating muscle cell damage and characterised in mammals, will pertain in birds, until recently there has been little work examining these processes in relation to genetic selection programmes and modern poultry production practices and problems. *In vitro* preparations of isolated skeletal muscle from broilers have been employed to elucidate the mechanisms of both stress induced myopathy and monensin myo-toxicity (Mitchell, 1999; Sandercock and Mitchell, 2003ab). These studies have examined both the uptake of radio-isotopic calcium (Ca^{45}) and the efflux of CK. The results demonstrate that elevating intracellular calcium either by increased entry of external calcium (by calcium specific ionophores) or release from sarcoplasmic stores, results in altered membrane integrity and efflux of enzymes particularly CK. Both sodium and calcium overload will induce enzyme efflux and membrane disruption. The mechanism of membrane damage involves activation of phospholipase A_2 (PLA_2) probably as a direct consequence of the raised intracellular calcium as described for mammalian muscle. CK efflux, following raised intracellular calcium, can be reduced by inhibitors of PLA_2 (Mitchell, 1999; Sandercock and Mitchell, 2003a). In response to monensin treatment increased entry of sodium, by sodium-proton exchange (Sandercock and Mitchell, 2003b) into muscle cells promotes calcium entry by the sodium-calcium exchange mechanisms in the sarcolemma in addition to releasing calcium into the myoplasm via the sarcoplasmic reticulum calcium channel or ryanodine receptor (Mitchell and Sandercock, 1997; Mitchell, 1999). Monensin induced CK efflux from isolated muscle can be inhibited by dantrolene, an agent which specifically blocks the ryanodine receptor, confirming the role of this channel in the process. These findings offer a complete explanation for the myotoxic effects of monensin in broilers. Increased entry of extracellular calcium or release of calcium from intracellular stores such as the sarcoplasmic reticulum, initiate the cascade of events which culminates in changes in sarcolemmal or muscle membrane integrity and the associated increase in enzyme efflux. On the basis of additional experiments in which acute heat stress induced increases in plasma CK

in broilers could be inhibited by dantrolene it has been proposed that similar mechanisms involving altered ion balance and calcium release through the sarcoplasmic reticulum, ryanodine sensitive, calcium release channel (SR-RSCRC) may mediate the myopathy induced by stress and thermal challenge (Mitchell, 1999). This mechanism may also underlie the progressive muscle damage seen in rapidly growing birds and the concomitant increased sensitivity to heat stress-induced myopathy.

It thus appears that the disturbances in ion balance contributing to the aetiology of various myopathies in poultry are consistent with the mechanisms proposed in mammals. The suggested mechanism would have parallels with the well-recognised and characterised Porcine Stress Syndrome (PSS) and the incidence of PSE in pigs.

(e) Stress, pathophysiological mechanisms, myopathies and meat quality

It has been proposed that artificial genetic selection for growth rate and the existence of overt muscle pathology result in changes in muscle cell structure and function resulting in alterations in histological characteristics which may have important implications for meat quality (Rémignon *et al.,* 1996ab; Soike and Bergmann, 1998). In addition some of the commercial procedures and environmental challenges known to induce muscle damage or myopathy in poultry have been linked with alterations in meat quality (McKee and Sams, 1997). Susceptibility to pre-slaughter stressors may be genetically determined and lines of quail selected for high and low fear exhibit differences in adrenocortical response (plasma corticosterone), muscle damage (plasma CK activity), drip loss and pH of breast meat following exposure to acute stress in a crush cage (Rémignon, 1998). Genetically determined changes in muscle function may predispose the tissue to stress related damage and further effects upon meat quality. Thus, pre-slaughter heat stress negatively affects meat shrink loss, colour and toughness in broilers. Acute pre-slaughter heat stress accelerates the rate of pH decline in turkey meat and increases the likelihood of PSE and may thus explain the higher incidence of this condition during the summer (McKee and Sams, 1997). Extended transportation of broilers can induce a number of disturbances in physiological variables and in some lines this may be associated with a tendency towards reduced meat quality and a PSE like condition. It has been suggested that PSE or a condition closely approximating that encountered in mammalian livestock is becoming more common in turkeys and broilers (Barbut, 1997ab). Broiler breast and leg meat colour and other meat quality variables may be adversely affected by pre-slaughter holding conditions and transportation (Kannan *et al*., 1997), although in this latter study overall meat quality was unaffected. The exact physiological mechanistic basis of these problems and the relationship between muscle cell function, myopathic change and meat quality are still, however, poorly understood. Thus, whilst numerous instances of altered meat quality in poultry have been described, any patho-physiological characteristics or responses in live muscle directly responsible for changes in the post-mortem attributes of the tissue have not been identified. For example, in the case of PSE, the high rate post-mortem glycolysis induced by acute ante-mortem stress and leading to a rapid fall in tissue pH is responsible for the meat quality problem (Barbut, 1997ab; McKee and Sams, 1997). However, it has not been previously established if alterations in ante-mortem cell calcium homeostasis, PLA_2 activity, lipid peroxidation and sarcolemmal permeability predispose to this condition as is recognised in pigs (Klont *et al*., 1994). Recently, however, Soares *et al.* (2003) have reported that mitochondrial PLA_2 activity in chicken muscle increases with age and is correlated with elevated sarcoplasmic calcium content and the incidence of PSE. Current studies in the present authors' laboratory are examining the possible relationships between ante-mortem physiological stress and post-mortem meat quality attributes in broiler chickens. Preliminary findings indicate that marked

pre-slaughter hyperthermia (body temperature increased by 2.2°C) during three hours of simulated transport is associated with disturbances in acid-base balance (hypocapnic alkalosis), elevated plasma creatine kinase activity (+40%) and a significant reduction in pH, a significant increase in drip loss, an elevated haemorrhage score and a paler appearance of the breast fillet (Sandercock *et al.,* 2001). The role of sarcoplasmic reticulum calcium release and other aspects of myoplasmic calcium regulation are to be characterised in parallel investigations in lines of broilers differing in susceptibility to physiological stress and growth associated myopathy.

II. FUTURE DEVELOPMENTS

It may be proposed that selection for improved growth rate and feed efficiency in meat birds has resulted in changes in cellular regulation of free calcium distribution and concentration. The consequences of these derangements include changes in intracellular enzyme activity, hydrolysis of membrane lipids, peroxidation and altered sarcolemmal permeability. These biochemical responses may underlie detrimental effects upon *in vivo* muscle cell function and peri-mortem metabolism and meat quality parameters. It is thus suggested that identification of the genes associated with idiopathic myopathy, calcium homeostasis and the control of muscle cell regeneration and repair constitute important routes for progress in the alleviation and prevention of growth associated and stress induced myopathies in commercial poultry.

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MODERN POULTRY PRODUCTION AND AVIAN BONE BIOLOGY

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Summary

Modern poultry production methods have allowed for increased levels efficiency of production for both meat- and egg-type poultry. Although advances in genetics, nutrition and management have largely allowed this progress, care must be taken by the poultry industry to ensure a balance between the desire for greater and more efficient production and the health of the birds. As has been observed in the past with broilers and turkeys, and is currently the case with laying hens, the pursuit of increased performance without concurrent attention paid to the metabolic and physiological needs of the animal leads to decreased health, performance and welfare. The broiler and turkey breeders have addressed many of the skeletal problems of the past through intensive selection for skeletal health; the breeders of egg-type hens have begun to address these problems as well.

New techniques to assess bone development and integrity in vivo will allow researchers to more closely monitor individual birds throughout production, and identify factors that may be useful in allowing future increases in production to take place with minimal impact on skeletal health.

I. INTRODUCTION

Genetic selection and improvements in nutrition and management have led to dramatic increases in potential for growth in meat-type birds and egg production in laying hens. Table-egg layers are typically small-framed birds bred for high levels of egg production. The huge demand for calcium for eggshell formation, the typically small appetite of many strains of layers, and the effects of bird age can all have a negative impact on bird skeletal health. In the case of meat-type birds, rapid growth can lead to abnormal skeletal development; intense efforts by primary breeders in recent years have minimised these problems, although continued effort is required to keep ahead of the problem. Broiler breeder hens are typically feed-restricted, which can interfere with the normal mechanisms used by egg-laying birds to maintain calcium balance and support both skeletal integrity and eggshell formation.

II. LAYING HEN SKELETAL ABNORMALITIES

Laying hens are typically the most sensitive type of poultry to perturbations in Ca metabolism, and subsequently, skeletal disease. The requirement of the hen for Ca for eggshell formation places a huge demand on the relatively small frame of the hen. A table-egg-type hen deposits approximately 2.3 g of Ca in an eggshell, which represents about 10% of the total Ca present in the skeleton of the hen (Etches, 1987). By the end of the production cycle, the hens are susceptible to osteoporosis (Whitehead and Fleming, 2000), with up to 30% of hens having broken or healed broken bones at depopulation (Gregory and Wilkins, 1989).

The hens have a natural storage compartment of labile Ca called medullary bone. Medullary bone is typically formed approximately 2 weeks prior to the onset of lay (Hurwitz, 1964), in response to an increase in circulating estrogen. This type of bone is deposited in the

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shafts of long bones, and is readily mobilised when Ca demand for eggshell formation is greater than Ca supply from the diet. Medullary bone is rapidly deposited from dietary Ca during periods of low Ca demand (Van de Velde *et al*, 1985).

During periods of high demand (such as near peak egg production), and as the hen ages (when efficiency of Ca absorption and deposition decreases), the hen may not be able to mobilise sufficient Ca from the medullary stores, and cortical (structural) bone may be mobilised. In addition, endosteal surfaces not covered by a layer of medullary bone result in cortical bone degradation by osteoclasts, further weakening the bones (Whitehead, 2003). Although total bone density may actually increase by the end of a production cycle, almost all of the new bone is medullary bone (Whitehead, 2003), which has little inherent strength (Fleming et al., 1998).

III. BROILER AND TURKEY SKELETAL ABNORMALITIES

Meat-type birds (broilers and turkeys) have been genetically selected for many generations for rapid growth rate, mainly as skeletal muscle, and efficiency. At times over the years, the capacity of the skeletal system of these birds has lagged behind the ability of the birds to deposit skeletal muscle. Skeletal problems are often related to the rate of growth; many of the compensatory growth (eg. light restriction) programs developed in the 1980s and early 1990s decreased the incidence of skeletal abnormalities as a consequence of slowed early growth (Classen *et al*., 1991; Riddell and Classen, 1992). Nutrition plays both direct and indirect roles in the development of skeletal disease in meat-type poultry, but genetics are also very important (Riddell, 1992). In recent years, poultry primary breeding companies have substantially decreased the incidence of metabolic and developmental skeletal disease through intensive selection of stock with rapid growth rate, good feed efficiency and a resistance to development of skeletal disorders.

IV. BROILER BREEDER HEN SKELETAL ABNORMALITIES

Broiler breeder hens not only face many of the same issues of Ca supply and demand as table-egg layers, but additional challenges brought on by standard breeder management techniques. Breeder hens are typically feed-restricted to control body weight; the breeders have similar potential for growth as broilers, and must be nutrient-restricted in order to prevent obesity and associated reproductive difficulties. In most management situations, breeder hens are fed a limited amount of feed, early in the morning. This presents a challenge to the hens, as most eggshell formation takes place at night (Etches, 1987). As the Ca from the diet is available for only a brief period during shell formation, Ca must be sequestered in the medullary bone stores to make up for the lack of dietary Ca during much of the shell-forming period within a day.

Maintaining flock uniformity is extremely important in breeder hen management, especially in terms of the age at sexual maturity. A non-uniform flock is likely to have an extended period in which individual hens lay their first egg. This will result in some hens being capable of depositing medullary bone, and also having increased Ca requirements for eggshell formation many weeks before the last hens to lay. Hens are not capable of developing medullary bone reserves until approximately two weeks prior to sexual maturity, and a change from a low Ca grower diet to a high Ca breeder diet will necessarily not meet the requirements of the entire population if the flock is not uniform. Research at the University of Alberta has shown that increasing dietary Ca to breeder pullets to soon (before they are capable of forming medullary bone), as well as too late (six weeks after photostimulation) can both lead to decreased egg production, shell quality and bone quality at 31 weeks of age (Petruk and Korver, unpublished observations). Over an extended period of

time, the hens with impaired Ca reserves may be susceptible to the same perturbations in Ca metabolism as laying hens. Thus, increased flock uniformity and appropriate timing of increased dietary Ca will decrease the number of hens that are switched to a high Ca diet either too soon or too late to support maximum production and bird health.

V. NEW METHODS FOR MEASUREMENT OF BONE QUALITY IN POULTRY

In the past, most assessments of skeletal health in poultry involved removing a sample of birds from the study population at various times during production, killing the birds and conducting destructive tests such as breaking strength, dried bone weight, ash and specific mineral measurements. Patterns of bone accretion and remodelling were followed on a population basis, with the assumption that the sampled birds reflected the state of the entire population. Obviously, this approach limited the ability of researchers to correlate individual bird traits to entire production cycles, especially if the birds were sampled before the end of the cycle. Although much of our current knowledge of avian bone biology has been obtained using these techniques, newer, more sophisticated methods have been adapted for use in poultry.

Recently, a symposium on avian osteoporosis was held in Madison, WI at the 92nd Annual Meeting of the Poultry Science Association. Some of the newer techniques discussed include digitised fluoroscopy (DF), amplitude-dependent speed of sound (Ad-SoS) ultrasonography, dual-emission x-ray absorptiometry (DEXA) and peripheral quantitative computed tomography (pQCT). DF uses video digitisation of radiographic image intensity to create an image suitable for computer analysis of bone density (Fleming *et al*., 2003). Ad-SoS ultrasonography measures the speed of sound through a bone across a precisely-measured distance to quantify bone density; the greater the density, the higher the velocity of sound (Fleming *et al*., 2003). DEXA analysis employs a system in which x-rays at two distinct energies are passed through the bone of interest, the attenuations are recorded and used to calculate a two dimensional radiographic density (Hester *et al*., 2003). Analysis of bone by pQCT involved passing an x-ray through the bone at numerous angles in a single plane; the attenuation of the x-ray at each angle is used to calculate a three dimensional, volumetric density (Korver *et al*., 2003). Each of these techniques has advantages and limitations, but all can be used on live birds. By keeping the birds alive, an individual bird can be followed through an entire production cycle, and factors such as feed intake, egg production, body weight and bone density can be correlated at multiple ages, giving a much more powerful data set than can be generated by removing birds for production as is required with the traditional methods.

These newer techniques will not replace the traditional methods by which so much of our current knowledge has been gained. However, some of these newer techniques will allow researchers to follow individual birds throughout the entire production cycle and identify factors related to skeletal health or disease. Ultimately, fewer birds will be sacrificed prematurely, and the rate of advancement of avian bone biology will increase, and hopefully keep pace with advances in productivity.

VI. CONCLUSIONS

The skeleton is an essential support element of poultry production; providing support for the body, allowing movement and in the case of egg-laying birds, providing an essential store of Ca for eggshell formation during times of low dietary Ca supply. Many of the production expectations placed on modern poultry put the bird at risk for diseases of the skeletal system. Advances in genetics, nutrition and management have and will continue to play a role in maximising productivity and minimising skeletal abnormalities. The use of

traditional analytical methods for bone quality will be used in conjunction with the newer techniques to rapidly advance the knowledge of avian bone biology.

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DAY LENGTH AFFECTS PERFORMANCE, HEALTH AND CONDEMNATIONS IN BROILER CHICKENS

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Summary

Research was completed to examine the effects of day length (12L:12D; 16L:8D; 20L:4D) on the performance and health of broiler chickens as assessed from day of hatch to the end of processing. Growth was reduced in a linear fashion with decreased day length while feed efficiency improved and the incidence of mortality and condemnations (cellulitis, dark carcasses) decreased. The data support the use of shorter day lengths in broiler production, both from an economical and animal welfare standpoint.

I. INTRODUCTION

Lighting programs for broilers have been examined many times over the last 30 years, with many different program types using variable lengths of dark periods and a wide range of patterns (Classen and Riddell, 1989; Gordon, 1994; Buys *et al.*, 1998; Rozenboim *et al.*, 1999; Scott, 2002). Some general concepts tend to hold true in comparisons of lighting regimes which provide darkness to continuous or near continuous light. Exposure of birds to darkness: 1) reduces early body weight but compensatory growth frequently results in equal weight at marketing; 2) improves feed efficiency (reduced metabolism and activity during darkness, a more concave growth curve with less maintenance requirement, less carcass fat); and 3) improves bird health with emphasis on metabolic problems such as sudden death syndrome (SDS), ascites and leg disorders. Research using a wide range of species including poultry has also demonstrated that dark exposure, likely through the hormone melatonin, improves immune function (Kirby and Froman, 1991). This suggests that darkness exposure may also benefit the bird's ability to combat infectious disease.

Despite the extensive research, there is a need to re-examine lighting principles using modern genotypes that have been selected for improved health and hence may not benefit from dark exposure to the same degree. In addition, most if not all lighting research, has failed to provide comprehensive data on losses beyond the farm gate (death in transit and lairage, condemnations). Since lighting programs affect bird health on farm and approximately one third of birds dead at shackling (DAS) are the result of farm based pathology (Classen *et al.*, 2002), it is probable that lighting programs can affect DAS. Epidemiological evidence has indicated that darkness exposure may be one of a number of factors increasing the incidence of cellulilltis (Elfadil *et al.*, 1996). Cellulitis is the most important cause of condemnations in Canada and has increased markedly in the last decade (Kumor *et al.*, 2001). Therefore, understanding how management affects its incidence has important economic implications. The objectives of this research were to compare the effects of day length on broiler performance and welfare using the Ross 308 broiler (considered to have high health standards) and in particular to pay attention to the post farm-gate response criteria such as losses in transit to slaughter and due to condemnations at the slaughtering plant.

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II. MATERIALS AND METHODS

Three lighting treatments were compared to investigate the impact of day length on response criteria. They consisted of 12L:12D, 16L:8D and 20L:4D. A total of 9000 Ross 308 chicks were randomly assigned 1000 per room (500 males and 500 females) to one of nine rooms. Rooms were environmentally independent and each lighting treatment was assigned to three rooms (3 replications per lighting program). All broilers were initially given 23L:1D but were switched to experimental day lengths on day 4 of the experiment. Light intensity was initially 20 lux and was reduced to 10 lux on day 7 where it remained for the remainder of the 35 day experiment.

Birds were fed starter (0.7 kg/bird; 12.6 MJ AME/kg; 12.50 g total LYS/kg), grower (1.1 kg/bird; 13.2 MJ AME/kg; 11.25 g total LYS/kg) and finisher (to trial end; 13.3 MJ AME/kg; 10.00 g total LYS/kg) diets *ad libitum*. Broiler management followed industry convention for temperature and ventilation. Birds were given 0.09 m^2 floor space per bird. Feed was supplied in tube feeders (0 to 28 days, 13 per room, 35 cm diameter; 28 to 35 days, 13 per room, 43.75 cm diameter) and water using Plasson waterers (12 per room, 43.75 cm diameter).

Birds were weighed as a room at 0, 14 and 35 days of the trial while sample weights of 100 birds per room were taken at 7, 21 and 28 days. Feed intake was measured on a room basis. Dead birds were recorded, weighed and examined for cause of death. At 33 days, 50 birds per room were randomly selected to assess flock uniformity (individual weights) and bird mobility (subjective gait score; Kestin *et al.*, 1992). On day of slaughter, lights came on in rooms 10 h before loading, and feed and water were withdrawn 4 and 8 h after lights came on, respectively. Final body weights were obtained within moving modules approximately 10 to 12 h after lights on. During this same time, sample birds were assessed for digestive tract clearance and skeletal gross pathology (16 birds per treatment). Birds were kept separate according to room during transit to and at the processing plant to allow collection of DAS and condemnation data.

Statistical analysis was conducted as a one way ANOVA using the Proc GLM procedure of SAS Institute Inc (1990). Duncan's Multiple Range Test was used to separate means when the ANOVA was significant and regression analysis was used as appropriate. Differences were considered significant when $P<0.05$.

III. RESULTS

Data from the experiment are shown in Tables 1 and 2. Birds on the 12L:12D lighting program were smaller at 14 days than broilers given the other lighting programs. At 35 d, there was a positive linear effect of day length on body weight with the difference between the two extremes (12 and 20 h) being 85 g or approximately one day's growth. The reduction in growth rate was associated with the reduced feed intake seen with shorter day lengths. Feed efficiency as measured as feed to gain ratio, and feed to gain ratio with mortality correction, improved in a linear fashion as day length decreased. There was a negative relationship between percent mortality (SDS, ascites) and day length. Bird mobility was poorer for birds on the 20L:4D treatment but the values were very low for all treatments indicating good skeletal quality. This was supported by a low incidence of culls due to leg disorders. Bird uniformity, within 10 or 15% of the mean was unaffected by lighting treatment. Digestive tract clearance and skeletal pathology in birds examined on day of slaughter were not affected by lighting program (data not shown). Bird death during transport and lairage was not affected by treatment but the loss for the 20L:4D treatment was nearly twice as high as the other treatments. The increased number of deaths was primarily due to

chronic heart failure. Total condemnations increased with longer day length (Table 2), with the majority of the increase due to cellulitis and dark carcasses.

Table 1. Effect of day length on the broiler performance and health

	Lighting Program				
	12L:12D	**16L:8D**	**20L:4D**	**SEM[1]**	**P**
Weight, 15d, kg	0.449[b]	0.483[a]	0.479[a]	0.0055	0.0002
Weight, 35d, kg	2.003[c]	2.046[b]	2.088[a]	0.0128	0.0009
Feed intake/bird, 0-15d, kg	0.458[c]	0.518[b]	0.555[a]	0.0143	0.0001
Feed intake/bird, 15-35d, kg	2.794[b]	2.850[b]	2.960[a]	0.0257	0.0012
Feed intake/bird, 0-35d, kg	3.266[c]	3.387[b]	3.542[a]	0.0410	0.0001
Feed to gain, 0-35d	1.663[c]	1.689[b]	1.731[a]	0.0100	0.0001
Feed to gain[M2], 0-35d	1.631[b]	1.649[b]	1.677[a]	0.0072	0.0026
Mortality %, 0-35d	4.27[b]	5.60[ab]	7.80[a]	0.617	0.0278
Bird mobility, 33d[3]	0.64[b]	0.67[b]	1.01[a]	0.0704	0.0198
Bird uniformity 10%, 33d[4]	62.7	62.7	62.0	1.324	NS
Bird uniformity 15%, 33d[5]	83.3	78.7	81.3	1.338	NS
Dead at shackling[6]	0.30	0.37	0.80	0.135	0.2903
Condemnations[6]	0.47[b]	1.30[a]	1.90[a]	0.258	0.0066
Total Losses[7]	5.07[b]	7.15[ab]	11.20[a]	1.154	0.0361

[1] Standard error of the mean. [2] Feed to gain mortality corrected. [3] Kestin *et al.* (1992). [4] % within 10% of the mean. [5] % within 15% of the mean. [6] % of birds placed. [7] Total losses include mortality, dead at shackling and condemnations. [a,b,c] Means within a row with different letters are significantly different ($P<0.05$).

IV. DISCUSSION

This research confirms earlier work that decreased day length decreases growth rate, but increases feed efficiency and bird health (reducing SDS, ascites), even in a modern genotype with decreased losses due to metabolic disease. Therefore, it points to the continued value of dark exposure in today's broiler industry. However, it was of interest that there was only a minor effect of day length on bird mobility and no effect on skeletal quality as judged by mortality/culls and gross pathology. This suggests selection for improved skeletal condition is successful in this genotype despite continued increases in growth rate.

The effect of lighting on the incidence of condemnations and in particular cellulitis was dramatic and appears to contradict epidemiologic research. It is often assumed that scratches are the origin of cellulitis and that increased darkness exposure increases scratches. Although scratches are likely an important risk factor in the development of cellulites, this research indicates a benefit of darkness exposure which may be the result of an associated increase in immunological capability. More research is required to confirm this effect but for the time being, it can be stated that darkness exposure is one of the factors that influences cellulitis and it should be used to help reduce the incidence of this important class of condemnations. The effect of day length on dark carcass condemnations is probably associated with chronic heart disease. This mechanism is supported by the reduction in ascites seen during the broiler production cycle. The failure of lighting treatment to affect digestive tract clearance is of interest and indicates that the use of dark periods does not pose a problem of increased contamination during slaughter if birds are given an appropriate feed withdrawal period, preferably on farm.

Table 2. Effect of day length on the incidence (% of placed) of condemnations at the slaughtering plant.

	Lighting program						
	12L:12D	**16L:8D**	**20L:4D**	**SEM[1]**	**P**	**Reg P values[2]**	**R^2**
Ascites	0.13	0.05	0.10	0.044	0.8025	-	-
Cellulitis	0.30[b]	0.80[b]	1.40[a]	0.199	0.0146	0.0019	0.8772
Dark carcass	0	0.30	0.35	0.080	0.1002	0.0405	0.6014
Salpingitis	0	0	0.05	0.014	0.3403	-	-
Bruising	0	0.05	0	0.014	0.3403	-	-
Pendulous crop	0.03	0.10	0	0.030	0.5155	-	-
Total	0.47[b]	1.30[a]	1.90[a]	0.258	0.0066	0.0008	0.9118

[1] Standard error of the mean. [2] Regression P values.
[a,b,c] Means within a row with different letters are significantly different ($P<0.05$).

In conclusion, day length affects productivity of broiler chickens by affecting growth, feed efficiency and bird losses due to mortality, death during transit and lairage and condemnations. While increased dark exposure reduced growth rate, it had positive effects on feed efficiency, and bird well being and welfare.

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EFFECT OF EARLY FEEDING AND GRAIN TYPE ON GROWTH AND PERFORMANCE OF BROILERS

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Summary

The effects of grain type and early feeding on growth and performance of broilers were investigated by offering four diets formulated with wheat, barley, sorghum and corn as energy source and soybean meal as the protein source. Two feeding regimens were used which included: (a) immediate access to feed and water post-hatch (FED); (b) access to both feed and water 48 h post-hatch (HELD). FED birds were heavier ($P<0.05$) throughout the entire 33-d experimental period. The HELD birds were more efficient in feed conversion at 21 days of age ($P<0.05$), but this effect became less apparent as the birds grew older. The birds on sorghum and corn diets were heavier and more efficient throughout the entire experimental period. Birds on wheat diet tended to have higher breast yield and lower drip loss, while the barley diet gave a numerically lower breast yield and higher drip loss. Feeding time had no effect on breast yield and drip loss. There was no effect of feeding time on uniformity of final body weight at day 21 and 33, while birds given sorghum and corn diets showed better uniformity at 33 days of age, compared to those given wheat and barley diets ($P<0.05$). HELD birds tended to drink more water during the first two weeks of their life, but this effect was not statistically significant. Also, the birds with early access to the barley diet tended to have a higher mortality ($P<0.05$).

I. INTRODUCTION

It was widely accepted that the early access to feed and water post-hatch has a major impact on immediate and long-term development of chicks (Uni, 1998). Reports showed that delayed access to nutrients can result in depressed immune response (Casteel *et al*., 1994; Dibner and Knight, 1998), increased early mortality and reduced overall performance (Fanguy *et al*., 1980). However, in the typical commercial practice chicks are held for 24-48 h before placement, without access to feed and water (Noy and Sklan, 1999) because of the hatchery handling and often long distance transportation. There are also some arguments that the birds should be held in the incubator for a certain period deliberately because the immune system is not fully developed at hatch and is not ready for environmental challenge (Dibner and Knight, 1998). The newly hatched birds need to transit from dependence on yolk to exogenous nutrients after hatch (Sklan and Noy, 2000). Because the digestive organs are not well development at hatch, the source of nutrients determines their maximum availability to the bird (Lilburn, 2002). Since our knowledge of early nutrition for broilers is mostly based on maize-soybean diets (Ravindran, 2003), while sorghum, wheat and barley are widely used in broiler diets in Australia and world wide, the effects of early feeding of these grains on the live-long productivity of need to be defined. This study investigated the effects of grain type and early nutrition on the growth performance of broilers.

II. MATERIALS AND METHODS

A four by two factorial design was used in this trial. Four dietary treatments consisted of a wheat, barley, sorghum and corn-based diets, respectively. The birds were fed either immediately after hatch or held without access to feed and water for 48 h.

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The birds were fed starter diets for three weeks and then changed to finisher diets. Monensin and Zn-bacitracin were supplemented at the recommended levels. No feed enzymes were used in the experimental diets. Three hundred and sixty (360) day-old Cobb male broiler chicks were allocated to three replicates of 15 birds per treatment. At the end of the second week, all the birds were transferred into AME cages. The birds from one brooder cage were randomly allocated into two AME cages to make four birds per cage. All the birds were fed *ad libitum* throughout the experiment. Body weight, feed intake and water consumption by cage were recorded weekly. Body weight gain, feed conversion ratio and mortality rate were determined weekly.

III. RESULTS

The birds given sorghum and corn diets showed significantly heavier body weight and better feed efficiency at 21 and 33 days of age ($P<0.001$) (Table 1). Bird with early nutrition had significantly heavier body weight at 21 and 33 days of age, comparing to their HELD hatch mates ($P<0.05$). HELD birds had improved FCR at 21 days of age as compared to the FED birds ($P<0.05$). However, the effect on FCR at 33 days of age became less apparent. The feeding time had no effect on uniformity of body weight at 21 and 33 days of age. The wheat diets gave the poorest uniformity, followed by the barley diet, and sorghum and corn diets gave the better flock uniformity, at both 21 ($P<0.01$) and 33 days ($P<0.05$) of age.

Table 1: Effects of grain type and feeding time on body weight, uniformity and feed conversion ratio of birds at 21 and 33 days of age[1].

Treatment	21-d			33-d		
	Body Weight		FCR (g/g)	Body Weight		FCR (g/g)
	Mean (g)	CV (%)		Mean (g)	CV (%)	
Diet						
Wheat	793^{b}	5.6^{a}	1.46^{a}	1746^{b}	7.3^{a}	1.66^{a}
Barley	625^{c}	4.8^{ab}	1.48^{a}	1436^{c}	6.4^{ab}	1.75^{a}
Sorghum	873^{a}	3.0^{bc}	1.34^{b}	1880^{a}	3.1^{b}	1.52^{b}
Corn	891^{a}	1.9^{c}	1.31^{c}	1882^{a}	3.4^{b}	1.54^{b}
SE	11.8	0.78	0.010	35.6	1.19	0.038
Holding Time						
FED - 0 h	835^{a}	3.9	1.41^{a}	1777^{a}	4.6	1.62
HELD - 48 h	757^{b}	3.7	1.38^{b}	1694^{b}	5.5	1.62
SE	8.4	0.55	0.007	25.2	0.84	0.027
F test and level of significance[2]						
Diet type (DT)	106.1***	4.51**	67.38***	34.89***	3.15*	7.85***
Holding time(HT)	42.74***	0.05	10.18*	5.41*	0.56	0.02
DT x HT	0.82	3.66*	1.26	0.23	1.65	0.07

[1]Means of 12 replicates for diets and 24 replicates for feeding time; [2] $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; ^{ab} Means within columns with different superscripts are significantly different.

Birds given the barley diet had numerically higher mortality (10.0%), compared to those given wheat (3.33%), corn (1.33%) and sorghum diets (0.00%). FED birds had a numerically higher mortality rate (4.44%) than HELD birds (2.78%), with the highest mortality occurring in the FED birds given the barley diet (15.6%) ($P<0.05$).

HELD birds lost 9.7% of the initial body weight during the first 48 h post hatch, while the FED gained 32.4% of the initial body weight during the same period (Table 2). However, a higher growth rate occurred in HELD birds immediately after the birds were given access to

nutrients from day 3 till day 21 (P<0.01). Birds given wheat, sorghum and corn diets showed an early growth during the first two weeks post hatch (P<0.01) and then a compensatory growth occurred in birds given the barley diet (P<0.01).

The FED birds had numerically higher breast yield and lower drip loss at 33 days of age (Data not shown). The wheat diet gave the numerically highest breast yield and lowest drip loss, followed by sorghum and corn, and the barley gave the numerically poorest breast yield and highest drip loss. The HELD birds tended to drink more water, especially during the first two weeks post hatch (data not shown), but this effect was not significant.

Table 2: Effects of grain type and feeding time on the relative growth rate (%) of birds during the first 3 weeks post hatch[1].

Treatment	**1-3 d**	**3-5 d**	**5-7 d**	**7-14 d**	**14-21 d**
Diet					
Wheat	13.3	52.0^{a}	65.5^{b}	190.8^{b}	125.5^{b}
Barley	10.2	41.9^{b}	51.6^{c}	165.3^{c}	145.5^{a}
Sorghum	10.1	55.8^{a}	76.6^{a}	217.4^{a}	116.3^{b}
Corn	11.9	56.9^{a}	75.2^{a}	220.0^{a}	116.9^{b}
SE	2.7	2.0	1.6	3.5	3.1
Holding Time					
FED - 0 h	32.4^{a}	43.3^{b}	62.17^{b}	186.7^{a}	119.8^{a}
HELD - 48 h	-9.7^{b}	60.0^{a}	72.26^{a}	210.0^{b}	132.3^{b}
SE	1.9	1.4	1.15	2.5	2.2
F test and level of significance[2]					
Diet type	0.31	11.20***	50.20***	53.06***	18.95***
Holding time	240.1***	67.08***	38.36***	43.57***	16.07***
Diet x Holding time	0.54	1.22	4.40*	3.18	0.08

[1]Means of 6 replicates for diets and 12 replicates for feeding time;
[2] *P<0.05; **P<0.01; ***P<0.001;
[ab] Means within columns with different superscripts are significantly different.

IV. DISCUSSION

A heavier bird on placement in the shed usually results in a heavier slaughter body weight (Wilson, 1991) and feed intake has been found to be the main factor affecting final BW in individual broiler chicks (Pinchasov, 1991). Data from various studies indicated that an immediate access to nutrients upon hatch, in solid or in liquid form, improved the BW (Moran, 1990; Pinchasov and Noy, 1993), breast muscle yield at marketing (Noy and Sklan, 1998), and the uniformity of chicks (Sklan *et al*., 2000) up to 21 days after placement, than in held chicks. The feed efficiency was found to be unaffected (Noy and Sklan, 1998).

Newly hatched chicks could lose BW in the first 24 h post-hatch, even with immediate access to feed and water (Pinchasov, 1991). In this study, we observed an average of 5.2g (12.1%) of body weight gain for FED birds during the first 24h post hatch, while the HELD lost an average of 2.3g (5.4%) of body weight in the same period. FED birds were heavier than HELD birds for the experimental period. Contrary to previous findings, HELD birds had a better FCR and a superior growth rate when given access to nutrients during the first three weeks than the FED birds. Moreover, the breast yield and flock uniformity were not affected by early feeding.

In this study, we also observed that the small intestine and digestive organs of HELD birds still grew even when the birds were losing body weight. Thus after the HELD birds

were given access to nutrients, they seemed to be more efficient in digesting and absorbing exogenous nutrients. This might explain why the HELD birds showed a greater growth rate and improved feed conversion, especially during the first three weeks post hatch.

The digestive system of the newly hatched is immature, so its nutrient assimilation depends largely on the chemical and physical properties of feed (Moran, 1990; Noy and Sklan, 1998; Sklan *et al.*, 2000). In this study, birds with early access to nutrition seemed to have higher mortality, which was also supported by our previous study (Ao and Choct, 2003), especially in the FED birds given the barley diet. Corn, sorghum and wheat diets seemed to be able to trigger earlier growth and development of supply (GIT and digestive organs) and demand organs (muscle), compared to the barley diet, which had the poorest growth performance. It is well-known that ß-glucan in barley impedes bird performance and supplementation with appropriate enzymes can ameliorate its anti-nutritive effect. The wheat diet seemed to improve breast muscle yield and reduce drip loss, but its mechanism of action needs to be further elucidated. Post-hatch holding time also altered water-drinking pattern of birds during the first weeks of their life. Information on these areas of research is scarce and reason is not understood.

This study was conducted under an experimental condition and it is possible that under a commercial situation the outcome could be different.

V. ACKNOWLEDGEMENTS

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PERFORMANCE OF TWO COMMERCIAL BROILER STRAINS FED DIETS FORMULATED ON TOTAL OR DIGESTIBLE AMINO ACIDS

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Summary

Feed intake, weight gain, feed conversion efficiency and body protein proportions were significantly increased when diets were formulated on a digestible amino acid basis. Both genotype and sex also had significant effects on growth performance and body composition. There was a significant ($P<0.05$) sex x diet interaction for weight gain which was due to a greater response in the males to formulation on the basis of digestible amino acid composition.

I. INTRODUCTION

Amino acids in most ingredients are not totally digested and absorbed by birds and knowledge of such efficiency is important in formulating diets (Ravindran and Bryden, 1999). Feed formulation based on digestible amino acids has been shown to increase weight gain and feed intake and improve body composition in broilers (Li *et al.,* 2002). Studies investigating differences in formulation of poultry diets on a total or digestible amino acid basis between broiler chickens differing in their sex and genotype have not been reported. The aim of this study were to determine the individual bird response to 4 different diets formulated on: 1) total amino acids; 2) digestible amino acids (book values; Ravindran *et al.,* 1998) using the same ingredients as diet 1; 3) digestible amino acids values determined as used in diet 1; and 4) digestible amino acids but formulated commercially. Also, to determine if there are differences between genotypes and between male and female birds.

II. METHODS

The experiment was a factorial design with two commercial broiler (strains A and B), two sexes, four diets and 20 individual bird replicates per genotype x sex x diet sub group. The chicks were housed from days 1 to 21 in two standard four-tiered electrically heated brooding cages, and fed *ad libitum* on a commercial broiler starter crumble diet. On day 21, 80 birds of each sex x genotype were transferred to 320 individual grower cages arranged in two banks of two tiered back-to-back rows of 40 cages. Within each of the eight rows of 40 cages, there were ten birds of each sex per genotype, two or three of which were given one of the four diets. The diets, which have been described (Li *et al.,* 2002), consisted mainly of sorghum, canola meal, cottonseed meal, meat and bone meal, soybean meal, vegetable oil, lysine, methionine, threonine.

At the start of the experiment (21 days of age) individual birds from each strain were weighed and sexed, and then randomly allocated to the allotted cages for that strain x sex group. Body weight gain and feed intake were recorded. At 41 days of age, the whole bodies (including feathers) of the chickens were minced and dried and then reground for determination of fat and protein.

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III. RESULTS AND DISCUSSION

(a) The effect of genotype on performance and carcass composition of broiler chicks

Feed intake and weight gain of strain B birds were significantly (P<0.001) higher than strain A. However, strain B had a significantly higher feed conversion ratio (P<0.05) than strain A. Fat expressed as a proportion of the whole body was higher in strain B birds. The results are shown in Table 1.

Table 1. Performance and carcass composition (means ± SEM) of broiler chickens from strain A and strain B averaged over diet and sex

	Strain A	**Strain B**	**P value**
Feed intake (g/day)	2264 (± 20.7)[a]	2444 (± 21.1)[b]	<0.001
Weight gain (g)	1276 (± 10.2)[a]	1347 (± 10.4)[b]	<0.001
FCR (g/g)	1.78 (± 0.013)[a]	1.82 (± 0.013)[b]	0.035
Protein (%)	17.9 (± 0.1)[a]	17.9 (± 0.1)[a]	0.095
Fat (%)	12.1 (± 0.1)[a]	12.9 (± 0.1)[b]	<0.001

a,b values in the same row with different superscripts differ significantly (P<0.05).

These differences are most likely due to the developmental characteristics of these two genotypes. Faster growing broilers would exhibit a higher feed intake. The results agree with most studies (Smith *et al.,* 1998) where faster growing strains also consume more feed. In the present study, however, the faster growing strain B was less efficient in converting feed into body weight, due most likely to its increased propensity to fatten.

(b) The effect of sex on the performance and carcass composition of broiler chicks

Male broilers were significantly faster growing, consumed more and were more efficient in converting feed into body weight than females. Females had a higher percentage of body fat than males (Table 2).

Table 2. Performance and carcass composition (means ± SEM) of female and male broilers averaged over genotype and diet

	Female	**Male**	**P value**
Feed intake (g/day)	2252 (± 20.9)[a]	2456 (± 20.8)[b]	<0.001
Weight gain (g)	1234 (± 10.3)[a]	1390 (± 10.3)[b]	<0.001
FCR (g/g)	1.82 (± 0.013)[a]	1.77 (± 0.013)[b]	0.031
Protein (%)	17.7 (± 0.10)[a]	18.1 (± 0.10)[a]	0.071
Fat (%)	12.9 (± 0.10)[a]	12.1 (± 0.10)[b]	0.020

a,b values in the same row with different superscripts differ significantly (P<0.05).

This result is generally in agreement with the findings of Waldroup *et al.* (1990) where male broilers grew faster and had better feed efficiency than females. Females begin

to deposit fat at an earlier age and at a greater rate than males. These differences suggest that the amino acid requirements of males might be higher than for females.

(c) The effect of diet on performance of broiler chicks

As shown in Table 3 dietary formulation on a digestible amino acid basis (diets 2, 3 and 4) improved growth and feed conversion in comparison to formulation on a total amino acid basis (diet 1).

Table 3. Performance of broiler chickens (means ± SEM) fed diet formulated on total and digestible amino acid basis and averaged over genotype and sex

Diet	Feed intake (g/day)	Weight gain (g)	FCR (g/g)
1	2278 (± 29)^{b}	1216 (± 14)^{b}	1.88 (± 0.018)^{b}
2	2368 (± 29)^{a}	1340 (± 14)^{a}	1.77 (± 0.018)^{a}
3	2362 (± 29)^{a}	1342 (± 15)^{a}	1.76 (± 0.018)^{a}
4	2409 (± 30)^{a}	1349 (± 15)^{a}	1.79 (± 0.018)^{a}

a,b values in the same column with different superscripts differ significantly (P<0.05).

Feed intake of chicks fed diet 1 was significantly (P<0.05) lower than those given diets 2, 3 and 4, with maximum intake on diet 4. Weight gain of broilers fed diet 1 was significantly lower than weight gain of those fed diets 2, 3 and 4. Feed conversion ratio of broilers fed diets formulated on digestible amino acids was also significantly better than those fed the diet formulated on total amino acids.

Body protein content was significantly (P<0.05) higher in birds fed diets (2, 3 and 4) formulated on a digestible amino acid basis. However, body fat content in birds was not significantly different between diets.

Table 4. Body composition of whole chickens (means ± SEM) fed diets formulated on total and digestible amino acid basis and averaged over genotype and sex.

Diet	Protein (%)	Fat (%)
1	17.4 (± 0.21)^{a}	12.4 (± 0.18)^{a}
2	18.1(± 0.21)^{b}	12.4 (± 0.18)^{a}
3	18.5 (± 0.21)^{b}	12.6 (± 0.18)^{a}
4	18.5 (± 0.21)^{b}	12.7 (± 0.18)^{a}

a,b,c values in the same column with different superscripts differ significantly (P<0.05).

There was an interaction between sex and dietary treatment for weight gain (Table 5).

Table 5. Sex by diet interaction for weight gain (means ± SEM)

Diet	Weight gain (g)	
	Females	**Males**
1	1181 (± 20)^{aA}	1251(± 20)^{bA}
2	1257 (± 20)^{aB}	1424(± 20)^{bB}
3	1248(± 20)^{aB}	1437(± 20)^{bB}
4	1251(± 21)^{aB}	1448(± 20)^{bB}

a,b values in the same row with different superscripts differ significantly (P<0.05).
A,B, values in the same column with different superscripts differ significantly (P<0.05)
LSD = 57.49

Whilst, males always grew faster than females, the difference was much greater for those given the digestible amino acid formulated diets. The results suggest that the greater genetic potential for growth in males than females is influenced by the basis of dietary amino acid formulation and that apart from higher absolute requirements; the males are more sensitive to digestible, rather than total amino acid balance. There is a suggestion of possible between sex variability in amino acid digestibility.

In conclusion, the results of this study further confirm earlier studies (Ravindran and Bryden, 1999) that formulation of poultry diets on a digestible amino acid basis is superior to formulation of diets on a total amino acids basis. Moreover, the results indicate that genotype and sex differences in performance and body composition should be taken into account in implementing nutritional programs to maximise performance of broiler chickens. This will also improve the economic return from broiler production.

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DETERMINING INDIVIDUAL AMINO ACID REQUIREMENTS IN POULTRY BY THE INDICATOR AMINO ACID OXIDATION TECHNIQUE: A REVIEW

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Summary

A full review is provided of the strengths and limitations of the indicator amino acid oxidation (IAAO) technique to determine amino acid (AA) requirements in chickens. Traditionally, AA requirements have been determined using growth assays or nitrogen retention that involved feeding graded levels of a test amino acid (AA_{test}) to the subject and looking for a clearly definable change in a relevant biological parameter (Pencharz and Ball, 2003). However, these methods are expensive, time consuming and require large numbers of animals. The indicator AA must have an oxidative pathway distinct from and unrelated to the AA_{test} so that a change in dietary AA_{test} will not affect the pool size of the indicator AA (Pencharz and Ball, 2003). L-[1-^{14}C]phenylalanine has been shown to be a suitable indicator AA for IAAO studies in poultry (Coleman *et al.*, 2003). This IAAO method is suitable for determination of AA requirements for all essential AAs (Pencharz and Ball, 2003), as well as the availability of AAs in feedstuffs (Leslie et al, unpublished data).

The IAAO technique is based on the concept that a deficiency of a single essential AA will restrict protein synthesis. Therefore, all other essential AAs will be in relative excess and will be oxidised. As the dietary intake of the AA_{test} increases, the oxidation of all other essential AAs, including the indicator AA decreases, corresponding to the increase in protein synthesis. If the intake of AA_{test} increases beyond the requirement, no further change in oxidation of the indicator AA will occur. The point at which the oxidation of the indicator AA reaches a plateau is taken as the requirement provided no other nutrient is limiting.

The IAAO technique has been in use in humans and pigs for years. As poultry are in many ways very different metabolically from either of those species, a series of development and validation steps were undertaken by our group to ensure validity of the method (Coleman *et al.,* 2003). Additionally, many of the validation steps were repeated for different types and ages of birds (eg. broiler breeder hens, broilers of various ages, Leghorn roosters). The IAAO allows for determination of AA requirements for specific ages and types of birds over a short period of time. Developmental research is underway to allow individual daily determination of AA requirements in rapidly growing broilers (Leslie *et al.*, 2003). The results of all of the validation procedures undertaken will be presented.

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THE EFFECT OF AGE AND DIETARY AMINO ACID LEVELS ON PROTEIN DEPOSITION IN BROILER CHICKENS

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Enhanced protein deposition is a major objective of both broiler breeding and nutrition programs. In this study the influence of age and digestible lysine intake on protein deposition was examined. The dietary treatments consisted of four levels of apparent ileal digestible lysine (6, 8, 11 and 13 g/kg) with the same energy level, based on wheat, sorghum, canola and soybean meals. All other digestible essential amino acids were balanced using the Illinois ideal protein ratio to digestible lysine (Baker, 1997). In the experiment each diet was fed to 6 groups containing 6 male broilers (Cobb) aged from either 0-18 days in part A or 18-42 days in part B. Carcass composition of de-feathered whole carcasses was used for determining protein deposition and the results are shown in the Figure.

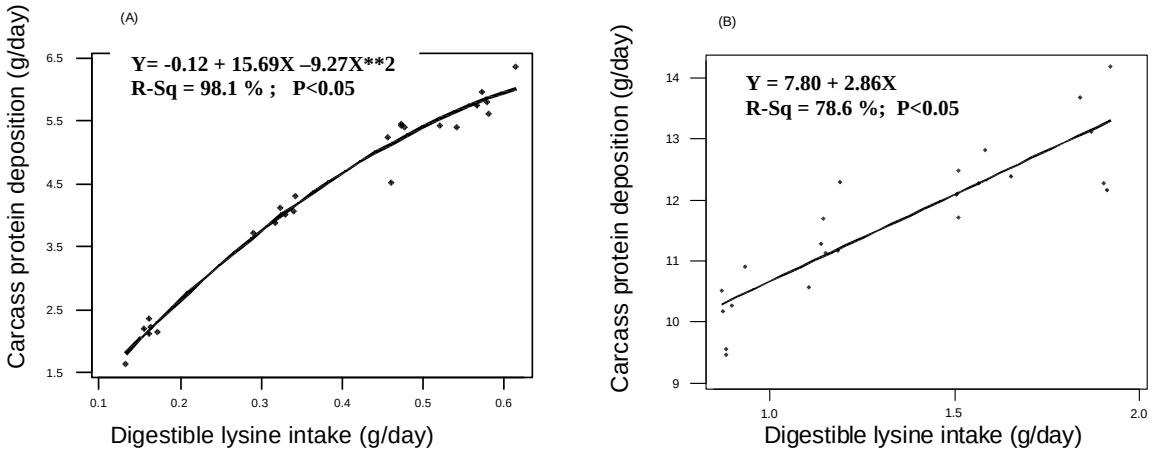

Figure- Protein deposition as a function of digestible lysine intake at 18(A) and 42(B) days of age.

Protein deposition at 18 days was a curvilinear function of digestible lysine intake, and is in agreement with Johnson and Campbell (1991). The maximum daily deposition of 6.5g was estimated from a quadratic model. At 42 days, protein deposition was a linear function of digestible lysine intake, which indicates that protein deposition as a function of digestible lysine intake was constant throughout a wide range of digestible lysine intakes and did not reach a plateau. To optimise dietary amino acid utilisation and lean tissue accretion, protein deposition may become one of the main factors for determining dietary amino acid requirement.

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EVALUATION OF MODIFIED GLUCOMANNAN (MYCOSORB®) AND HYDRATED SODIUM CALCIUM ALUMINOSILICATE TO AMELIORATE THE INDIVIDUAL AND COMBINED TOXICITY OF AFLATOXIN AND T-2 TOXIN IN BROILER CHICKENS.

C.K.GIRISH and G.DEVEGOWDA

Summary

The effects of modified glucomannan and hydrated sodium calcium aluminosilicate on individual and combined toxicity of aflatoxin and T-2 toxin were evaluated using twelve dietary treatments (four x three factorial) on broiler chickens from 0-5 weeks of age. Aflatoxin (2 mg/kg) and T-2 toxin (1mg/kg) significantly reduced feed intake, weight gain and feed efficiency. Further aflatoxin increased the relative weights of liver, kidney, gizzard and spleen whereas T-2 toxin increased the relative weights of liver and gizzard. Aflatoxin also reduced the relative weights of thymus and bursa of Fabricius while T-2 toxin reduced relative weight of the thymus. Aflatoxin and T-2 toxin also reduced antibody titres against Newcastle disease and infectious bursal disease. Significant interaction between aflatoxin and T-2 toxin were observed for their additive effects on body weight, feed intake, feed efficiency, relative organ weights and antibody titres. Modified glucomannan (1 kg/ton of feed) significantly ($P \leq 0.05$) improved body weight, feed intake, decreased relative organ weights and improved antibody titres, bursa of Fabricius and thymus weights. Hydrated sodium calcium aluminosilicate (10 kg/ton of feed) showed improvement against AF, however no beneficial effect was shown against T-2 toxin.

I. INTRODUCTION

Among the known mycotoxins, aflatoxin, ochratoxin and T-2 toxin are most important to poultry. Aflatoxin B1 (AF) is the most potent hepatotoxic and immunosuppressive where as T-2 toxin has been reported to cause oral lesions and decreased feed intake in broiler chickens. These mycotoxin-contaminated feedstuffs when consumed produce a range of devastating effects on the general well-being and productivity of poultry (Devegowda *et al*., 1998a).

Practical methods to detoxify mycotoxin contaminated grain on a large scale and in a cost effective manner are currently not available. At present, one of the most promising and practical approaches is the use of adsorbents. Research indicates that a number of adsorbents are capable of adsorbing aflatoxin B_1 and reducing its toxic effects.

A natural product called glucomannan, a cell wall derivative of *Sacchromyces cerevisiae* [1026], has received much attention in minimising mycotoxins present in the contaminated diets of livestock and poultry (Devegowda *et al*., 1998b; Whitlow *et al*., 2000; Smith *et al*., 2000)

The objectives of the trial were to evaluate the individual and combined effects of aflatoxin (AF) and T-2 toxin (T-2) on performance, organs morphology and antibody titers against Newcastle disease and infectious bursal disease with and without supplementation of modified glucomannan (MGM) and hydrated sodium calcium aluminosilicate (HSCAS).

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II. METHODS

A total of 720 day-old sexed commercial broiler chicks were divided at random into 36 replicates of 20 chicks each having an equal number of males and females. Two dietary levels each of AF (0 & 2mg/kg), T-2 toxin (0 & 1mg/kg), MGM (0 & 1kg/ton) (Mycosorb®, a proprietary product of Alltech Inc., Nicholasville, KY, USA) and HSCAS (0 & 10 kg/ton) were tested in a four x three factorial from 0-5 weeks of age. Organ morphology, antibody titres for Newcastle disease (ND) and infectious bursal disease (IBD) at 35 days, and performance parameters weekly from 0-5weeks were measured. The data obtained were analysed with SAS, GLM procedure and means were compared with Duncan multiple range test.

III. RESULTS

AF and T-2 toxin individually depressed body weight and feed efficiency (Table 1). AF increased relative weights of liver (21.7%), kidney (26.4%), and gizzard (16.8%), where as T-2 toxin showed increase in relative weights of liver (20.0%) and gizzard (10.8%). Reductions in relative size of thymus [by AF (23.3%) and T-2 toxin (18.6%)], bursa [by AF (30.1%)] and ND and IBD titres (by individually AF and T-2 toxin) were noted. The mycotoxins interacted in an additive manner and caused significant reductions in body weight and feed intake. Significant interaction between AF and T-2 toxin were observed for their additive effects on relative organ weights (Table 1) and antibody titres.

IV.DISCUSSION

Body weight was depressed in both individual and combined mycotoxins fed group, where AF & T-2 acted in an additive manner and showed the greatest effect. The increased growth depression observed with the simultaneous feeding of more than one mycotoxin may be due to additive toxic effects of individual toxins (Raju and Devegowda, 2000). A significant additive interaction was seen between two mycotoxins for their effects on feed intake and feed conversion ratio (FCR). Decreased feed consumption during combined mycotoxicoses has been reported (Kubena *et al.,* 1997; Raju and Devegowda, 2000). The lower FCR was noted with these mycotoxins have been mediated through decreased nutrient utilisation.

HSCAS supplementation improved body weight, feed intake and FCR in the AF fed groups. These data agree with previous results of the protective effects of HSCAS compound (Ledoux et al., 1999). The addition of HSCAS at 1 % resulted in no significant protection against toxicity of T-2 agrees with previous work of Chestnut *et al.,* 1992.

MGM significantly improved body weight, feed intake and FCR. These beneficial effects might be attributed to its growth promotive effect and ability to trap the mycotoxins irreversibly (Devegowda *et al*., 1996).

Liver, kidney and gizzard weights were increased by AF and AF+T-2. T-2 increased the relative weights of liver and gizzard. The results are in accordance with findings of Raju and Devegowda, 2000; Arvind *et al.*, 2003. The increase in the relative weight of gizzard is in accordance with earlier studies (Kubena *et al.,* 1990), which may be due to the results of severe inflammation and thickening of mucosal layer.

Table 1. Effect of individual and combined toxicity of AF and T-2 toxin with and without Mycosorb and HSCAS on body wt, FCR and relative organ wt (g/kg live wt) at 35 days of age.

Mycotoxin	Mycosorb (1 Kg / ton)	HSCAS (10Kg/ ton)	Body wt (g)	FCR	Liver	Kidney	Thymus
-	-	-	1495^{a}	1.86^{fg}	27.53^{ed}	8.06^{e}	3.77^{b}
-	+	-	1511^{a}	1.84^{g}	26.75^{e}	8.17^{e}	4.15^{a}
-	-	+	1497^{a}	1.86^{fg}	28.01^{ed}	8.20^{e}	4.29^{a}
AF	-	-	1311^{ed}	2.06^{bc}	33.51^{ab}	10.19^{bc}	2.89^{e}
AF	+	-	1398^{bc}	1.97^{de}	29.88^{cd}	9.46^{d}	3.71^{b}
AF	-	+	1373^{cd}	1.99^{cde}	30.97^{bc}	9.63^{d}	3.65^{b}
T-2	-	-	1390^{bc}	1.96^{de}	33.06^{ab}	8.09^{e}	3.07^{de}
T-2	+	-	1453^{ab}	1.92^{ef}	27.97^{ed}	8.20^{e}	3.61^{bc}
T-2	-	+	1398^{bc}	1.96^{ed}	33.04^{ab}	8.21^{e}	3.15^{de}
AF+T-2	-	-	1253^{e}	2.14^{a}	35.16^{a}	11.84^{a}	2.04^{f}
AF+T-2	+	-	1361^{cd}	2.09^{bcd}	31.81^{bc}	9.94^{cd}	3.30^{cd}
AF+T-2	-	+	1311^{de}	2.14^{ab}	30.00^{ab}	10.71^{b}	3.14^{de}

$^{a-f}$ Means within each column followed by common superscript do not differ significantly ($P<0.05$)

Relative weights of thymus and bursa of Fabricius were significantly reduced by dietary treatments. These reductions in size of these organs might have been due to necrosis and cellular depletion by the mycotoxins. The two mycotoxins exerted potentiated depressing effects on bursa and thymus weight when fed in combination than in isolation, suggesting additive toxic effects among them on lymphoid organs (thymus and bursa); similar results were reported by Raju and Devegowda (2002).

The mode of action of MGM in restoring the organ weights is not clear. It is thought to trap the mycotoxin molecule in its glucomannan matrix, which prevents its absorption from GIT and thereby minimises its toxic effects. The basic mechanism for protection against the toxicity of AF appears to involve sequestration of AF in the GIT and chemisorption of AF. Improvement was highly significant in MGM supplemented groups. HSCAS was found effective in counteracting adverse effects of AF only on organ weights. Chestnut *et al.* (1992) reported the ineffectiveness of HSCAS against T-2 on organ weights. The antibody titres of ND and IBD were significantly reduced in all mycotoxin treated groups. MGM significantly improved antibody titres against both vaccines. Raju and Devegowda (2002) reported the similar results. HSCAS supplementation significantly improved the ND and IBD titre in only AF fed groups. The counteraction results against ND and IBD by HSCAS for AF earlier reported by Barmese *et al*. (1990).

V. CONCLUSION

The results indicate that supplementation of Mycosorb is beneficial in preventing the individual and combined toxicity of aflatoxin and T-2 toxin in commercial broilers, while HSCAS is only beneficial against aflatoxin.

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EFFICACY OF ALTERNATIVES TO AGPS IN BROILERS CHALLENGED WITH *CLOSTRIDIUM PERFRINGENS*

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Summary

The effects of two alternative feed supplements (Additive A, a prebiotic, and Additive B an organic acid-based product) alone or in combination were investigated on the growth performance of broiler chickens using a NE challenge model. The effects of these supplements were evaluated against a negative control (no additives) and a positive control (with monensin and Zn-Bacitracin). The positive control birds had a numerically higher bodyweight and significantly ($P<0.05$) higher FCR compared to the negative control birds. The addition of the prebiotic (Additive A) had a marked beneficial effect ($P<0.05$) on FCR as well as the relative weight of the spleen. However, there were no further improvements when the Additive A was added in combination with the organic-acid based product (Additive B).

I. INTRODUCTION

One of the most common and financially devastating bacterial diseases in modern poultry flocks is necrotic enteritis (NE). The disease is caused by the α-toxin of *Clostridium perfringens* (CP) types A or C. In its acute form, birds often die without clinical signs. However, in its subclinical form the disease is much more financially damaging for the producer. The commonly observed symptoms of the disease vary with age of the birds (van der Sluis, 2000) and early signs of an NE outbreak such as wet litter, diarrhoea and a small increase in mortality of less than 1% are often overlooked.

Outbreaks of NE can be effectively treated or prevented with antibiotics such as virginiamycin, bacitracin, penicillin, tylosin or flavomycin (Watkins *et al.*, 1997). When included at subtherapeutical dosage these antibiotics can be very effective in controlling and preventing NE by selectively modifying the gut flora, suppressing bacterial catabolism and reducing bacterial fermentation. All these changes lead to increased nutrient availability for the animal and increased growth performance. In 1999, the European Union has placed a partial ban on the use of anti-microbial growth promotants (AGP) and it was recently announced by the EU that a total ban all of AGP will be banned in the future. Therefore alternative methods to control NE will become inevitable.

It has been recognised that modulations of the natural bacterial population in the intestine of broilers through the use of alternate feed supplements such as prebiotic (oligosaccharides), probiotic or organic acids have some effect in controlling the proliferation of *CP*. This paper reports the result of a study investigating the effect of manno-oligosaccharides (Additive A: Bio-MOS®) and an organic-acid-based product with lactic acid bacteria, organic acids, enzymes and electrolytes (Additive B: Acid-Pak 4-Way™) on the performance of broiler chickens challenged with *Clostridium perfringens*.

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II. MATERIALS AND METHODS

A total of 150 male and 120 female broiler chickens (Cobb) were purchased from the local hatchery and raised in brooders on commercial starter crumbles. At 14d the birds were weighed in nine single sex groups of 6 birds (n=6) and transferred to 45 metabolism cages located in two adjacent rooms. Five experimental diets based on wheat (70%), SBM (17%) and fishmeal (5%) replicated nine times (5 male and 4 female) were fed from 14 to 42d.

The treatment groups were: 1) negative control (no antibacterial or anticoccidial additives); 2) positive control (with monensin and Zn-Bacitracin); 3) Additive A 1kg/t; 4) Additive B 2 kg/t; and 5) Additive A 1kg/t plus Additive B 2kg/t. All supplements were kindly supplied by Alltech, Australia.

The challenge model used in this study consisted of a dual infection with *Eimeria. spp.* on 18d followed by the challenge with *Clostridium perfringens* (d20, 21 and 22). From 14 to 18d birds received a mix of experimental diets and fishmeal (1:1) with the full dosage of the respective additives. On d 18 all feed was removed and replaced with 100% of the experimental diets. At this point all birds, including the positive control were challenged with 10,000 each of sporulated oocysts of *E. acervulina* and *E. brunetti* using a commercially available crop needle. On d 20 birds were starved overnight (~20h) and fed a 1:1 of experimental diets and broth culture containing approximately 10^8-10^9CFU of *C. perfringens* type C. This procedure was repeated twice, however on d 21 and 22 feed was withheld for 3-4 h only. The inoculum was prepared daily using a commercially available broth (Thioglycollate broth, Oxoid, Heidelberg, Australia). Once all birds had eaten the inoculated feed, free access to experimental feed was restored. On d 28 half of the birds per cage were killed by cervical dislocation and the spleen and bursa of Fabricius were collected and weighed.

Feed intake and weight gain were recorded on a weekly basis throughout the experimental period. Mortality was recorded daily and a likely cause of death was given. SAS systems (SAS Institute Inc., 2001) as was used to perform the statistical analyses used in this study. Data were analysed according to the GLM procedure for ANOVA. Duncan's multiple-range test was used to separate means when significant effects ($P<0.05$) were detected by analysis of variance.

III. RESULTS AND DISCUSSION

In the current study birds did not die or develop a clinical form of clinical necrotic enteritis. However, the negative control birds (without antibacterial or anticoccidial feed additives) had a numerically lower bodyweight at 42 d of age and a significantly increased FCR in comparison to the positive control birds (Table 1). These findings are consistent with findings from the literature which showed that damage to the small intestine caused by *C. perfringens* will result in a reduction in bodyweight and FCR of approximately 5% (Corpet, 1999; Kaldhusdal and Hofshagen, 1992; Kaldhusdal and Løvland, 2000; van der Sluis, 2000). It was concluded that the challenge model used in the present study was effective in inducing a subclinical form of NE.

There were no significant differences among treatments for body weight (Table 1). However, significant differences ($P<0.05$) were found among treatments for FCR. Birds fed manno-oligosaccharides (Additive A) had FCR similar to the positive control and significantly different to the negative control. It is known that manno-oligosaccharides can reduce unwanted enteric pathogens such as *Salmonella* or *Campylobacter* by blocking the type-1 fimbriae which enables the pathogens to attach to the intestinal lining (Dawson and Pirvulescu, 1999). Although Clostridia do not express type 1 fimbriae it was previously reported that the addition of manno-oligosaccharides to diets fed to birds challenged with *CP*

had some effects in reducing mortality as well as reducing secondary effects of NE on feed conversion ratio (Hofacre, 2001, Hofacre *et al.*, 2003).

Table 1. Effects of manno-oligosaccharides and combination probiotic of on feed conversion ration ratio and relative weight of spleen and bursa of Fabricius

	Bodyweight	**FCR**			**Spleen**	**Bursa**
	g/bird (42d)	**13-d28**	**28-d42**	**13-d42**	**%BW**	**%BW**
Negative Control (NC)	2176.0	1.780^{b}	1.980^{a}	2.150^{a}	0.138^{bc}	0.238
Positive Control (PC)	2271.5	1.611^{a}	1.792^{b}	1.960^{c}	0.123^{c}	0.221
NC + Additive A[1]	2243.4	1.728^{b}	1.862^{b}	1.999^{bc}	0.156^{a}	0.227
NC + Additive B[2]	2208.8	1.769^{b}	1.908^{ab}	2.038^{ab}	0.148^{ab}	0.213
NC + Additives A+B	2192.6	1.757^{b}	1.850^{b}	1.951^{bc}	0.141^{ab}	0.217
Female	2064.3	1.749	1.967^{a}	2.157^{a}	0.142	0.217
Male	2341.7	1.713	1.808^{b}	1.910^{b}	0.141	0.231
SEM	28.47	0.016	0.020	0.030	0.003	0.005
Source of variance	Probability of greater *F* value in analysis of variance[3]					
Diet	NS	**	***	*	*	NS
Sex	***	NS	***	***	NS	NS
*Diet*Sex*	*NS*	NS	NS	*	NS	NS

[a,b,c] Values with unlike superscripts differ significantly (P<0.05)

[1] Additive A: Bio-MOS®, Alltech, Australia; [2] Additive B: Acid-Pak 4-Way, Alltech Australia

[3] * P<0.05, ** P<0.01, *** P<0.001

The addition of Additive B had little effect on bodyweight or FCR compared to the negative control. This is somewhat in contrast with reports by (Hofacre *et al.*, 2003) who found that the addition of a lactic acid producing bacterial culture alone or combined with manno-oligosaccharides was effective in reducing *C. perfringens* related effects on the feed conversion ratio. It appears that Additive B, a combination product with lactic acid bacteria, exogenous enzymes, organic acid and electrolytes was less effective in modifying the intestinal flora in comparison to a product with a purer culture thus allowing *CP* to proliferate in the small intestine.

The severity of necrotic enteritis and the reduction in growth performance is among other factors, influenced by the bird's immune capability (D'Assonville, 2001). It has been demonstrated that in physiologically challenged chickens the relative weight of immunological organs such as the spleen or the bursa of Fabricius decreases (Puvadolpirod and Thaxton, 2000). There was a significant difference (P<0.05) among treatment groups in the relative weight of the spleen. The addition of monensin and Zn-Bacitracin in the positive control group may have reduced the numbers of *CP* in the intestine, and hence reduced the need for the bird's own immune system to act, resulting in a reduction in the size of the immunological organs. On the other hand, the addition of Additive A alone resulted in a significant increase in the relative weight of the spleen, which can be associated with an increase in the cell-mediated immune response.

Based on the results of this study it appears that the reduction in broiler growth performance as a result of a sub-clinical *Clostridium perfringens* infection could be partially

overcome when manno-oligosaccharides (Additive A) are added to the diet. There appear to be no further benefits when the same product is added in combination with an organic-acid based product. However, NE is a complex multifactorial disease with many unknown factors and future research has to focus on the understanding of the disease itself and the development of reliable and repeatable disease models to investigate nutritional manipulation in detail.

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THE INFLUENCE OF DIETARY ASCORBIC ACID ON MUCOSAL IMMUNITY

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A number of studies have investigated the impact of dietary supplementation of ascorbic acid (AA) on the immune response in chickens. Ascorbic acid has been shown to assist in alleviating environmental stress and its effects on the immune system (Gross, 1988; Pardue *et al*., 1985). Dietary AA improved the immune response in birds vaccinated for infectious bursal disease virus, as seen in increased serum antibody titres (Amakye-Anim *et al.,* 2000) and an increased number of IgG and IgM secreting cells in the spleen and bursa of Fabricius respectively (Wu *et al.,* 2000). Birds fed an AA supplemented diet had reduced levels of pericarditis following *E. coli* challenge (Gross *et al.,* 1988) and improved resistance to a combined Newcastle disease virus and *Mycoplasma gallisepticum* infection (Gross, 1992). However, the effect of dietary AA on local immunity, and in particular IgA antibody production, has not been assessed in chickens. IgA antibody is preferentially produced at mucosal surfaces such as the intestine, where it acts as the first line of acquired immune defense. Procedures that increase IgA antibody production at mucosal surfaces may improve resistance to invading pathogens (Muir *et al.,* 2000). Therefore, this study investigated the impact of dietary AA on IgA antibody production in chickens.

At the day of hatch, 160 broiler chicks were randomly assigned to a maize-based diet (containing less than 30 mg/kg AA) which was supplemented with either 60, 110, 200 or 400 mg AA [Roche, Rovimix C-EC]/kg. Bird weights, feed consumption and feed conversion ratios (FCR) were assessed throughout the seven-week study. At day 28 all chickens received an intraperitoneal immunisation of tetanus toxoid (T. toxoid) in a vegetable oil based adjuvant, followed two weeks later with an oral booster of T. toxoid. Samples of blood were collected from each chicken on days 28, 42 and 48. At the end of the experiment samples of bile and intestinal scrapings (IS) were also collected from each chicken. Total IgA and anti-T. toxoid IgA antibody titres in serum, IS and bile were determined by ELISA.

Average bird weight, feed consumption and FCR were similar for all treatment groups. Birds fed diets containing either 60 or 110 mg AA /kg had higher average total IgA antibody titres at 28 days of age than the other groups. Following immunisation with T. toxoid, birds receiving 110 mg AA/kg diet had higher mean anti-T. toxoid IgA antibody titres in serum at day 42 and in serum and IS at day 48. However, there were no notable differences between the anti-T. toxoid IgA antibody titres in the bile at day 48.

This study has demonstrated some potential for supplemental dietary AA to increase local IgA antibody production at the intestinal surface in chickens. Increased serum IgA antibody titres were also observed. Further studies are required to assess the impact of AA supplementation on IgA antibody production in chickens under stress-inducing conditions, and any enhanced protective benefit in disease needs to be explored.

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PERFORMANCE AND WELFARE OF BROILERS AS AFFECTED BY STOCKING DENSITY AND IN-FEED ANTIBIOTIC SUPPLEMENTATION

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Summary

The influence of stocking density (16, 20 and 24 birds/m^2) and zinc bacitracin (0 or 100 mg/kg) on the performance, carcass characteristics and selected welfare indicators of broilers were examined in a 35-day trial. Treatments had no effects ($P>0.05$) on broiler performance during the starter phase (1-21 days). Weight gain and feed intake were increased ($P<0.05$) by zinc bacitracin during the finisher phase (22-35 days) and over the 35-day trial period. During the finisher phase and over the whole trial, feed/gain of birds at the highest density (24 birds/m^2) was lower ($P<0.05$) than those at the other two densities. Zinc bacitracin had no effect on feed/gain. Carcass characteristics were unaffected by stocking density or zinc bacitracin. No treatment effects were observed on the dry matter content of the litter, gait score, hock burn scores, and the feather score.

I. INTRODUCTION

In the broiler industry, one of the major welfare concerns is the effect of high stocking densities on the welfare of birds, especially during the final weeks of the growing period when the body weight per unit area is high. In Australasia, the average stocking density used in commercial farms vary between 20 and 22 birds per square metre (or 36-38 kg per square metre). This level of density has been dictated largely by economic considerations and is made possible, in part, by the low-level inclusion of in-feed antibiotics in poultry diets. It has been speculated that, with the possible removal of antibiotics, the stocking densities used in commercial farms may have to be re-evaluated.

There are many reports in the literature on the effect of stocking density on broiler performance and welfare parameters of chickens (Shanawany, 1988; Cravener *et al*., 1992; Marttrenchar *et al*., 1997; Feddes *et al*., 2002), but none have examined these effects in relation to the in-feed antibiotic supplementation. In the present study, the influence of stocking density and zinc bacitracin, an in-feed antibiotic, on the performance, carcass characteristics and selected welfare indicators of broilers were studied. Gait scores (or walking ability) and hock burns were considered as indicators of welfare of the birds. Sorensen *et al.* (2000) have shown that poorer walking ability was associated with greater incidence of leg weakness in birds housed at higher densities. These researchers also suggested that hock and footpad burns make a significant contribution to the overall prevalence of poor walking ability.

II. MATERIALS AND METHODS

The trial was conducted as a 3 x 2 factorial arrangement of treatments, involving three stocking densities (16, 20 and 24 birds/m^2) and two levels of in-feed antibiotic (without or with 100 mg/kg zinc bacitracin). The study was conducted in floor pens in an environmentally controlled room. The floor was cemented and covered in a 5 cm deep layer of wood shavings. The room was divided into 24 identical 2.6 m^2 pens, with partitions of

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solid wood/wire mesh between the pens, and one bell drinker and one feed hopper in the same location per pen. Room temperature was maintained at 32 ± 1°C during the first week of the study and gradually decreased to 24°C by the end of the third week. Temperature control was achieved through thermostatically controlled fans and wall-mounted electric heaters. The room was ventilated by four exhaust fans, mounted in pairs on opposite walls

Day-old male broiler (Ross) chicks were weighed on arrival and allocated to 24 pens so that there were four replications per treatment. The birds, raised under normal commercial management practices, were fed *ad libitum* in a two-phase broiler-feeding regime (starter phase, 1-21 days and finisher phase, 22-35 days). Body weights and feed intake were recorded on a pen basis at weekly intervals. Mortality was recorded daily and feed/gain ratios were adjusted for mortality.

Three random samples of litter were collected during the final week from the area around the feeder in each pen. Litter samples were not taken near the waterers due to some spillage in some pens. The samples were oven-dried at 70 ^{o}C for 72 h, and the percentage dry matter calculated. On Day 35, some birds were assessed for gait and feather scores, foot and hock burns, and carcass data. The gait score, or walking ability, was assessed in six randomly selected birds per pen on a six-point scale (Kestin *et al*., 1992) ranging from 0 (normal gait, walking freely) to 5 (bird unable to walk). Feather score, or the degree of feather coverage over the breast, was recorded in 10 birds per pen, on a three-point scale (1 = no visible skin, complete feather cover; 2 = relatively small amount of skin showing; 3 = relatively large amount of skin showing). Hock burns were also scored in these 10 birds using a three-point scale (1 = no burns; 2 = mild burns; 3 = severe burns).

Carcass data were collected from two randomly selected birds per pen. The birds were weighed, and then killed by cervical dislocation, followed by exsanguination. After the removal of feathers, viscera, shanks and neck, the individual weights of the eviscerated hot carcass, abdominal fat pad and breast muscle were measured for each bird. The carcass was scored for breast and hip lesions on a three-point scale (1 = no lesions; 2 = mild lesions; 3 = severe lesions).

Two-way ANOVA was employed to determine the effects of stocking density and zinc bacitracin and their interaction using the General Linear Models procedure (SAS, 1999).

III. RESULTS AND DISCUSSION

The performance data over the 35-day trial period is summarised in Table 1. Overall mortality during the study ranged between 1.3 and 3.6 %, but was not related to any treatment. Neither the stocking density nor zinc bacitracin influenced ($P>0.05$) the weight gain, feed intake and feed/gain of birds during the starter phase (1-21 days).

During the finisher phase (22-35 days) and over the 35-day period, weight gain and feed intake were increased ($P<0.05$) by zinc bacitracin, whereas stocking density had no effect. The lack of effect of stocking density is in contrast to previous published data (Shanawany, 1988; Cravener *et al*., 1992; Marttrenchar *et al*., 1997; Feddes *et al*., 2002), wherein increasing the number of birds per unit area was reported to depress growth rate and feed intake. The narrow range of stocking densities employed in the present study may explain the observed discrepancy.

Table 1. Influence of stocking density and in-feed zinc bacitracin supplementation on the weight gain (g/bird), feed intake (g/bird) and feed/gain (g/g) of broiler chickens.

	16 birds/m^2		20 birds/m^2		24 birds/m^2		SEM
Zinc bacitracin	**-**	**+**	**-**	**+**	**-**	**+**	
1-21 days							
Gain	818	853	834	843	845	836	16.2
Feed intake	1051	1119	1088	1093	1111	1087	17.8
Feed/gain	1.308	1.320	1.311	1.309	1.330	1.314	0.010
22-35 days							
Gain[a]	1210	1271	1231	1265	1188	1236	21.5
Feed intake[a]	1889	1979	1945	1995	1940	1995	28.8
Feed/gain[b]	1.584	1.587	1.603	1.609	1.655	1.642	0.012
1-35 days							
Gain[a]	2028	2124	2065	2108	2033	2072	29.3
Feed intake[a]	2940	3098	3033	3088	3051	3082	46.0
Feed/gain[b]	1.498	1.502	1.498	1.503	1.538	1.517	0.010

[a] Zinc bacitracin effect ($P<0.05$); [b]Density effect ($P<0.05$).

During the finisher phase and over the whole trial, feed/gain of birds at the highest density (24 birds/m^2) was lower ($P<0.05$) than those at the other two densities. Zinc bacitracin had no effect on feed/gain. The negative effects on feed/gain at the highest density were unexpected and are in contrast to published data. In previous evaluations involving stocking density ranges of 10 to 20 birds per m^2, increasing population density had no influence on feed/gain (Proudfoot *et al*., 1979; Shanawany, 1988; Cravener *et al*., 1992; Martrenchar *et al*., 1997). Interestingly, in a recent study by Feddes *et al.* (2002), the feed/gain of birds reared at densities of 12, 18 and 24 birds per m^2 were found to be similar.

Carcass characteristics were unaffected by stocking density or zinc bacitracin (Table 2). Carcass recovery and the relative weights of breast meat and abdominal fat were similar among the different treatments. No case of breast or hip lesions was observed in carcasses from any of the groups.

Table 2. Influence of stocking density and in-feed zinc bacitracin supplementation on the carcass characteristics and selected welfare indicators of broiler chickens[1].

	16 birds/m^2		20 birds/m^2		24 birds/m^2		SEM
Zinc bacitracin	**-**	**+**	**-**	**+**	**-**	**+**	
Carcass weight, % BW	71.0	70.5	70.3	70.8	70.5	71.2	0.68
Breast meat, % BW	19.7	19.3	19.6	19.5	19.2	20.1	0.59
Abdominal fat pad, % BW	0.96	0.96	1.10	1.14	1.20	1.16	0.07
Litter dry matter, %	63.7	62.9	61.4	60.4	60.5	61.5	2.20
Gait score	2.03	1.92	2.03	1.75	2.38	2.03	0.12
Hock burn score	1.69	1.92	1.95	1.88	2.08	1.98	0.11
Feather score	2.25	2.42	2.51	2.36	2.35	2.46	0.07

[1] Treatment effects were not significant ($P<0.05$) for any of the parameters.

The dry matter content of the litter was not influenced within the range of stocking densities tested (Table 2). Similarly, under the conditions of the current study, no treatment effects were observed on the gait score, hock burn scores, and the feather score. These findings are in disagreement with published data showing that hock burn scores are increased at high stocking densities (Cravener *et al*., 1992; Sorensen *et al*., 2000). The higher incidence of hock burns has been attributed partly to higher moisture levels and poorer quality of litter at high densities. Reduced mobility at high densities can also be a contributing factor, as this increases the time that the hocks are in contact with the litter.

In conclusion, the present data showed that the weight gains, feed intake, liveability and carcass characteristics of broilers grown at densities of 16, 20 and 24 birds per m^2 were similar. There were no welfare implications at these population densities as indicated by lack of effect of density on gait scores and the incidence of hock burns. Inclusion of zinc bacitracin improved weight gain and feed intake of broilers at the stocking densities evaluated, but had no effect on feed/gain. It is possible that the small group size have been a factor reducing the impact of stocking density in this trial and some caution must be exercised in extrapolating this current data to commercial broiler farming conditions where typical flock size is 20,000 or more birds per house.

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MANAGEMENT, NUTRITION AND PRODUCTS OF DOMESTIC GEESE: A REVIEW

D. FARRELL

Summary

Geese production has grown rapidly globally, but not in Australia, over the last decade. They are farmed for their meat, fatty livers and down. They are fast-growing and yield larger amounts of breast meat than other poultry. Ganders can reach over 5 kg and geese over 4 kg at 49 days. They can survive on pasture alone. When fat under the skin is removed, the carcass has only about 110g fat/ kg. A 5kg goose will produce up to 400g feathers and down per year. When force-fed, livers may weigh over one kg and yield over 50% fat. Egg production is generally only 40-60 eggs/goose/year and gosling number about 60/100 eggs set. Examples of layer and grower diets are given.

I. INTRODUCTION

Geese were domesticated over 5500 years ago, probably by the Egyptians. Commercial breeds belong to the genus *Anser* and are descended from the wild Swan goose, *Anser cygnoides* found mainly in Asia, and the Grey Lag goose, *Anser anser*, found mainly in Asia (Romanov, 1999). The latter type are distinguished by a large, prominent, hard knob at the base of the beak. In some European countries geese are traditional Christmas fare. They are highly adaptable, grow rapidly and some breeds reach a mature body weight of over 15 kg, with the gander much heavier than the goose. They are farmed for their meat, liver fat, down and feathers. Geese make excellent watch dogs when they lower their head at strangers. They are also used to control weeds in a variety of crops and have been particularly effective in controlling the rapidly-spreading water hyacinth. Geese are hardy, resistant to many avian diseases, easy to manage and may live beyond 15 years. A disadvantage is their generally low reproductive rate and low fertility. China is by far the largest producer of goose meat with over 200 million birds producing 1.93 million metric tonnes (mt) out of a world production of 2.10 mt. In 2001, Ukraine produced 97200 mt, Egypt 42200 mt, Hungary 40000 mt and Taiwan 30000. Production in developing countries is declining.

II. BREEDS AND BREEDING

There are at least 96 breeds of geese found mainly in the Eastern European countries. The European and Asian domesticated breeds probably originated from two species of the greylag goose, the western (*Anser anser anser*) and the eastern (*Anser anser rubriostris*) (Kozak *et al*., 1997a). The most common breeds are the giant White Emden (10 to 15 kg), the Grey Toulouse, the African, and the small White and Brown Chinese geese weighing only 4.5 to 5.5 kg but with high egg numbers (100/season). The smaller Huoyan breed can produce over 200 eggs/season. Geese do not come into lay until around 40 to 50 weeks of age. Production is highly seasonal, and most heavy breeds lay only 30 to 60 eggs/season of about six months. Landes geese have been selected in France for their ability to produce fatty livers and are now found in a number of other European countries. The large Grey goose is also popular in Europe. It grows rapidly and can be made to produce large, fatty livers.

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Egg weight (120-170 g) varies according to breed but increases during the laying season. For breeding, the ratio of ganders to geese is 1:4 to 6. They should be run together for six weeks, preferably in small flocks, before they will mate. Egg numbers remain constant for about four laying seasons. Fertility, often less than 70%, is low compared to other poultry species, while hatchability in incubators is normally above 80%. These two factors, combined, means that only about 60 goslings hatch from 100 eggs but can be influenced by weight at sexual maturity through restricting feed intake two months prior to lay, stocking density when managed under intensive conditions, diet composition and lighting regime. In a fully-enclosed housing system, 9 hours of light/day gave superior egg production and goslings per goose than 11 hours/day (Sellier and Rousselot-Pailley, 1997). Geese are excellent mothers. If allowed, they can incubate 9 to 12 eggs hatching out in 31 days. Several components of reproductive performance can be improved by selection (Kozak *et al*., 1997a).

III. GROWTH RATE

Growth rate of geese is the most rapid of poultry species. At 4 weeks of age, they are 40% of their adult weight compared with 15% for meat chickens and 5% for turkeys. Despite this, they are not fully mature until almost two years of age. Shown in Table 1 are body weights and feed conversion ratios of three genotypes; medium (M), heavy (H) and crossbred of M (maternal) and H, and the two sexes, raised under intensive conditions, given a high-quality diet (230 kg CP and 12.3 MJ AME/kg) and grown to 105 days.

Table 1. Live weight (g) of geese of three different genotypes and the two sexes at different ages (Wittmann, 1997).

Age (days)	**Medium**	**Crossbred**	**Heavy**	**Males**	**Females**
0		111		95	124
21	1657	1646	1959	1808	1688
49	4579	4843	5693	5318	4495
63	5012	5267	6197	6224	4952
77	6112	6179	7952	6954	6154
105	6800	6944	8739	7710	6879
Feed conversion ratio					
D 1-21	1.38	1.41	1.43		
D 22-105	5.45	5.42	4.90		

The most rapid growth in males and females occurs at 30 – 35 days of age of genotypes maturing at 6.9 kg for ganders and 5.7 kg for geese

IV. GEESE PRODUCTS

Goose meat is fatter than in other poultry species; fat is mainly in the skin (>500 g/kg) with only 110 g/kg in the carcass at seven weeks of age (Nitsan *et al.,* 1981). Breast meat reaches a maximum at about 55 days in both sexes. At seven weeks of age, it is about 9.5% of body weight, increasing to 18% (1.3 kg) at 16 weeks in geese weighing 6.9 kg but only about 75% is meat; the rest skin and bone. Breast meat yield and carcass characteristics (g/kg) of the geese at 105 days (Table 1) are presented in Table 2.

Table 2. Carcass composition (g/kg) of three breeds of geese and the two sexes at 105 days.

	Medium	**Crossbred**	**Heavy**	**Male**	**Female**
Carcass weight (g)	4479	4613	5799	5067	4668
Dressing	658	668	664	654	681
Breast	288	280	282	284	283
Thigh	218	212	208	207	222
Abdominal fat	76	64	68	69	69

Dressing % is lower than that found in chickens (72%) and breast meat yield considerably higher although these comparisons are made at different physiological ages. Carcass analysis of ganders and geese weighing 6.53 and 5.68 kg respectively gave similar fat (281-299 g/kg) and crude protein contents (145-150 g/kg) (Stevenson, 1983).

(a) Liver fat

Pate de fois is produced by force-feeding geese giving liver weights of 600-1000g. Because of their quiet temperament, Landes and Toulouse are the preferred breeds. Force-feeding of both ducks and geese, practised in Ancient Egypt 4500 years ago, is now banned in several countries, including Australia (Primary Industries Standing Committee, 2002). The aim is to produce a liver of about 600g from which pate de fois gras is manufactured. This delicacy has unique organoleptic properties enhanced by the low melting point of the fat. Goose liver is said to be of superior quality for making foie gras than duck liver. Liver weights from geese can exceed 1 kg. France is the largest consumer of liver fat at 15000 mt/year, but mostly from ducks. Around 800000 geese are killed for their fatty livers in France each year. Hungary, France and Israel are large producers; the latter mainly from 700000 geese annually. China is poised to be a very big producer of liver fat in a joint venture arrangement with France.

Goslings are force-fed several times a day for 14-35 days when nine to 20 weeks of age. Diets range from only moist maize to a more balanced one with protein sources and oil to assist feed passage. An example is given in Table 3 of a liver production trial in Israel in which goslings were force-fed a wet mash for 23 to 24 days from nine weeks old (Katz *et al*., 1997 – this is not in the list of references).

Table 3. Liver and body weight (g) of goslings force-fed a wet mash at 9 weeks for 23-24 days.

	Body weight		**Weight gain**	**Liver (g)**	**Fattening time (days)**	**Feed intake (kg)**
	Initial	**Final**				
Males	3763	7582	3818	862	24	19.44
Females	3270	6760	3490	808	23	17.96

The lipid content of liver from force-fed geese varies greatly but increases as liver weight increases, to well over 50%. In young goslings normal liver weight is about 80-100 g and contains 5 g of lipid.

(b) Down

Geese are also kept for their down and feathers. A goose weighing 5 kg will produce about 30 g of down and 100-130 g feathers, but factors such as genotype, age, diet and frequency of plucking, will determine yield. Geese moult at 9-10 weeks. China is the biggest producer and exporter of feathers and down (35%) largely to Taiwan, the US and Japan.

Hungary is next with 7% of global market share, estimated to be over 60000 mt/year (Kozak *et al.*, 1997b). There may be three or more pluckings at intervals of six weeks, and yield increases as geese get older.

V. MANAGEMENT AND NUTRITION

Geese are excellent survivors and can be farmed out-of-doors with a minimum of shelter and care. There are many small gaggles free ranged on small holdings throughout the world, and often with ponds which also provide them with a refuge from foxes. Geese in China are managed in small and large numbers with freedom to go out-of-doors. If eggs are artificially incubated, the goslings need a source of heat for the first two weeks. Indoor lighting should be low to stop feather stripping. Geese utilise grass and weeds very effectively and can survive with a minimum of supplementary feeding, or sometimes none. Production under these circumstances will be low. In Europe, particularly in Hungary and Poland, geese are produced in intensive systems on deep litter (straw), on slatted floors and in pens outdoors. Houses may be fully enclosed or have an outside fenced yard.

There is some debate as to how well geese can utilise forage. Their very large caeca suggest that they have the capacity to ferment dietary fibre extensively but this apparently may not be the case. The determined apparent metabolisable energy (AME) and digestible components of rye grass with 157 g crude protein/kg when offered to geese, Peking ducks and their cross (mulards) are shown in Table 4.

Table 4. Apparent metabolisable energy (AME, MJ/kg DM)) and apparent digestibility of components (g/kg) in rye grass given to geese and ducks (Jeroch *et al*., 1997).

Species	**Organic matter**	**Crude protein**	**Crude fibre**	**NDF**	**AME**
Geese	395	541	163	16.3	6.1
Peking ducks	396	282	211	21.1	7.2
Mulards	458	191	221	26.1	6.7

It appears that it is not the goose's ability to digest fibre in the diet that allows it to survive so well at pasture, but protein. Fibre digestion is limited and it follows that if they are going to grow and lay on pasture, it must be of high quality. Pingel *et al.* (1997) determined the apparent digestibility of young Italian rye grass to be only 30% when geese were given 514 g of fresh material /bird. The AME value declined with increasing intake from 8.5 to 5.5 MJ/kg DM. They also utilise grass seeds, and scavenge for insects, snails, larvae etc. Semi-intensive systems in which good-quality pasture is supplemented with grain or concentrate allow excellent growth of goslings. It is not uncommon to restrict the amount of concentrate by up to 40%. The remainder is from pasture; although this may result in one kg lower body weight at slaughter, the saving in concentrate may be about 10 kg giving a leaner carcass. Others (Romanov, 1999) have reported no difference in the final body weight of geese when fed concentrate (220-250g/goose) daily, or amounts of maize (130g-150 g/goose) and allowed to graze rye grass. All groups were fed concentrate *ad lib* for the final two weeks.

Little is known about the nutrient requirements of geese for meat and egg production. Growth rate, and protein deposition of goslings is very rapid, especially during the first four weeks, reaching almost 3 kg, but their diets have lower specification than those of broiler chickens in the starter period (1-21 d); crude protein of 158 g/kg and energy of 10.9MJ AME/kg. Examples of diets for goslings of different ages are given in Table 5.

Protein retained in feathers (80-90 g/kg body weight) is considerably higher than in chickens and turkeys. At 2-4 weeks of age, protein retained in feathers is 33% of that of the total, and 58% in the carcass (Nitsan *et al.*, 1981). From research in the USA, recommendations for crude protein were 200, 160 and 140 g/kg from 0-4, 5-6 and 7-9 weeks respectively, and much higher that that suggested by Leclerq *et al.* (1987).

Stevenson (1983) undertook an experiment with goslings to examine the influence of energy (11, 12 and 13 MJ AME/kg) in starter diets (0-4 weeks) and finisher diets (5-9 weeks) with similar CP contents of 200 g/kg and 160 g/kg respectively. At four and nine weeks mean body weights were not different although with increasing dietary energy content, food intake declined, energy intake increased and efficiency of energy utilisation also increased. The starter diet did not influence performance in the grower period. Goslings were therefore able to cope with a wide range of dietary energy concentrations. Eviscerated carcass yield was similar between the sexes at 630 g/kg, as was breast meat yield (140-150 g/kg). Requirements have been published for a few essential nutrients. Expressed as a percentage of the diet, they are generally lower than for broilers reflecting differences in body composition.

Geese breeder diets are also lower than those of high-producing laying hens. And there are recommended specifications in the literature, although these may not have been determined. Diets as low as 120 g CP/kg can be fed without a significant effect on reproductive performance (Buckland and Guy, 2003). Specifications for two layer diets, and diets for goslings, are shown in Table 5.

Table 5. Nutrient specifications (g/kg) for laying geese and goslings of different ages (Leclerq *et al*., 1987).

	Layers		**Goslings (weeks)**		
			1-3	**4-6**	**7-12**
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 2
Energy (MJ)	9.2	10.5	11.7	11.3	11.3
Crude protein	130	148	170	116	102
Lysine	5.8	6.6	9.5	5.8	4.7
Methionine	2.3	2.6	4.2	2.9	2.5
Sulphur aa	4.2	4.7	8.5	5.6	4.8
Tryptophan	1.3	1.4	1.8	1.3	1.2
Calcium	26	30	8.0	7.5	6.5
Phosphorus (total)	5.6	6.0	7.0	6.2	5.7
Phosphorus (available)	3.2	3.6	4.5	3.7	3.2
Sodium	1.2	1.4	1.5	1.4	1.4
Chloride	1.2	1.4	1.4	1.3	1.3

Geese are out of production for much of the year and are then given a low quality maintenance (holding) diet which normally includes a grain and a forage component. Sometimes they graze without any concentrate**.**

Duck breeder diets fed to geese when supplemented with additional vitamins, including A, D and E, improved fertility in young and old geese from 72 to 81%, and 40 to 55% respectively; hatchability also increased considerably (Olver, 1971).

There is no commercial goose production in Australia. With the change in the ethnic mix of the Australian population, there is little doubt that in the not too distant future demand for goose meat will increase but the production of pate de fois is unlikely on welfare grounds. It is hoped that this review may stimulate interest.

In summary, information on the nutrition and management of geese is scarce, mainly because it is a small and specialised industry in the western world, while the majority of geese are farmed in China and mostly in traditional systems. Under these circumstances, geese production is as much an art as a science.

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A MODEL FOR MAREK'S DISEASE TRANSMISSION IN BROILER CHICKENS

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Summary

A preliminary model for Marek's disease (MD) transmission among broiler chickens in one shed is presented. The model is based upon estimated correlation between the main contributing host-pathogen-environment factors influencing the transmission of MD in broiler chickens. The model requires parameter estimates for all main contributing factors to MD such as challenge level, population density, host vaccinal immunity, etc., and provides a clear explanation of how the contributing factors act to influence MD infection rate. The model is only a conceptual framework at this stage but initial simulations point to its utility. The applied example presented shows that this model makes biological common sense. The estimation of all parameters and validation of modelled relationships need to be confirmed in the field and under experimental conditions.

I. INTRODUCTION

Since 1907 when Professor Józef Marek first described Marek's disease (MD, Hirai, 2001), it has been studied by many authors and a lot of research documents based on observation and experiments have been produced on the disease. The complexity of MD, however, makes the full understanding of the dynamical nature of MD very difficult. In this paper we present a preliminary model of MD spread amongst broiler chickens taking into account the known MD host-pathogen-environmental contributing factors for the disease. Our ultimate objective in developing the model is to make predictions about the impact of different levels of vaccinal protection and other management factors on the incidence and spread of MD in broiler flocks.

II. METHODS

A mathematical model is proposed describing the change in numbers of broiler chickens in the different disease stages of MD over time in one shed under different scenarios. The framework of the model is described by differential equations. The mathematical software package MATLAB is used to simulate the scenarios. The flow chart of Marek's disease in broiler chickens is shown in Figure 1.

III. RESULTS AND DISCUSSION

(a) Model description

The mathematical model is based on correlations between host-pathogen-environmental factors and MD infection via airborne feather follicle epithelium (FFE).

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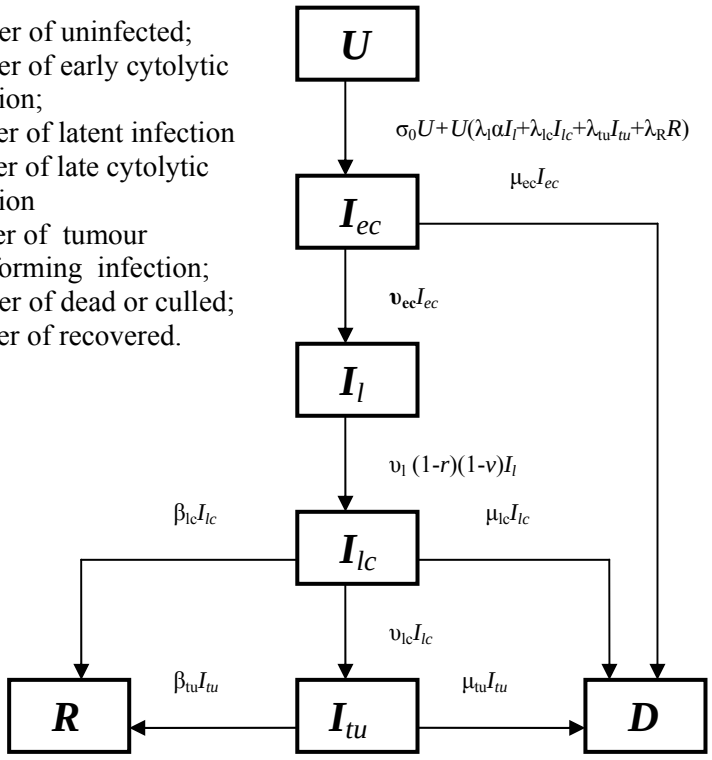
We assumed that once a chicken is infected with MD virus, it will go through four different infection stages: early cytolytic infection, latent infection, late cytolytic infection and tumour transforming infection (Hirai, 2001). There are seven classes of broiler chickens in the model: uninfected (***U***); early cytolytic infection ($\boldsymbol{I}_{ec}$); latent infection ($\boldsymbol{I}_{l}$); late cytolytic infection ($\boldsymbol{I}_{lc}$); tumour transforming infection ($\boldsymbol{I}_{tu}$); recovered (***R***); and dead or culled (***D***). The total chicken population is denoted by N so $N = \boldsymbol{U} + \boldsymbol{I}_{ec} + \boldsymbol{I}_{l} + \boldsymbol{I}_{lc} + \boldsymbol{I}_{tu} + \boldsymbol{R} + \boldsymbol{D}$.

Variables
U = number of uninfected;
$\boldsymbol{I}_{ec}$ = number of early cytolytic infection;
$\boldsymbol{I}_{l}$ = number of latent infection
$\boldsymbol{I}_{lc}$ = number of late cytolytic infection
$\boldsymbol{I}_{tu}$ = number of tumour transforming infection;
D = number of dead or culled;
R = number of recovered.



Parameters
σ_0 = infection rate incurred by initial challenge level;
λ = infection parameter;
α = fraction of latent infected chickens shedding FFE with cell-free MDV;
μ = death rate;
β = recovery rate;
$1/\upsilon$ = stage duration;
v = protection of vaccinal immunity;
r = genetic resistance.
The parameter's subscript specifies at which stage the infection is. *ec* = early cytolytic infection; *l* = latent infection; *lc* = late cytolytic infection; *tu* = tumour transforming infection.

Figure 1. Flow chart for Marek's disease in broiler chickens

The model's biological assumptions and equations are as follows. The infected chickens ($\boldsymbol{I}_{ec}$) at early cytolytic infection stage are assumed to be not infective because they do not shed virulent FFE. A fraction α of latent infected birds ($\boldsymbol{I}_{l}$) can shed virulent FFE so they infect uninfected chickens at rate $\alpha\lambda_l$. The infected birds at late cytolytic ($\boldsymbol{I}_{lc}$) and tumour transforming ($\boldsymbol{I}_{tu}$) infection stage contribute to infection at the maximal rate. Although the recovered chickens (***R***) are assumed to be infective, their infectivity λ_R is low since the level of their virulent FFE shedding is very low. All main contributing factors are employed in the infection parameters: λ_l, λ_{lc}, λ_{tu} and λ_R. The infection parameter σ_0 is incurred by initial challenge level. The following equation describes the rate of change ($d\boldsymbol{U}/dt$) in the number of uninfected broiler chickens:

$$d\boldsymbol{U}/dt = -\sigma_0\boldsymbol{U} - \boldsymbol{U}(\lambda_l\alpha\boldsymbol{I}_l + \lambda_{lc}\boldsymbol{I}_{lc} + \lambda_{tu}\boldsymbol{I}_{tu} + \lambda_R\boldsymbol{R}). \quad (1)$$

Chickens which are infected by an initially contaminated shed environment, $\boldsymbol{I}_{l}$, $\boldsymbol{I}_{lc}$, $\boldsymbol{I}_{tu}$ and ***R*** enter the early cytolytic infection class. They leave the community at average rate υ_{ec}, or they become dead from early mortality syndrome (Calnek, 1997) at rate μ_{ec}. Thus the number of early cytolytic infected birds, $\boldsymbol{I}_{ec}$, obeys the equation:

$$d\boldsymbol{I}_{ec}/dt = \sigma_0\boldsymbol{U} + \boldsymbol{U}(\lambda_l\alpha\boldsymbol{I}_l + \lambda_{lc}\boldsymbol{I}_{lc} + \lambda_{tu}\boldsymbol{I}_u + \lambda_R\boldsymbol{R}) - \upsilon_{ec}\boldsymbol{I}_{ec} - \mu_{ec}\boldsymbol{I}_{ec}. \quad (2)$$

After the duration ($1/\upsilon_{ec}$) of early cytolytic infection, early cytolytic infected birds enter the latent infection stage at rate υ_{ec}. They leave the latent community at rate $\upsilon_l(1-r)(1-v)$. The size of latent population is therefore governed by:

$$d\boldsymbol{I}_l/dt = \upsilon_{ec}\boldsymbol{I}_{ec} - \upsilon_l(1-r)(1-v)\boldsymbol{I}_l. \quad (3)$$

The infected birds after latent infection period enter the late cytolytic infection class $\boldsymbol{I}_{lc}$. They leave the community after they complete the late cytolytic infection to progress to tumour transforming infection at rate υ_{lc}. Birds at this stage recover from late cytolytic infection at rate β_{lc} and have death rate μ_{lc}. The equation for $\boldsymbol{I}_{lc}$ is:

$$d\boldsymbol{I}_{lc}/dt = \upsilon_{l}(1-r)(1-v)\boldsymbol{I}_{l} - \upsilon_{lc}\boldsymbol{I}_{lc} - \mu_{lc}\boldsymbol{I}_{lc} - \beta_{lc}\boldsymbol{I}_{lc} . \quad (4)$$

Incidence of tumour transforming infection is at rate $\upsilon_{lc}\boldsymbol{I}_{lc}$. Birds have death rate μ_{tu} and recover at rate β_{tu}. The fifth equation of the model is therefore:

$$d\boldsymbol{I}_{tu}/dt = \upsilon_{lc}\boldsymbol{I}_{lc} - \mu_{tu}\boldsymbol{I}_{tu} - \beta_{tu}\boldsymbol{I}_{tu} . \quad (5)$$

The number of recovered chickens increases at rate $\beta_{lc}\boldsymbol{I}_{lc} + \beta_{tu}\boldsymbol{I}_{tu}$. Thus we have:

$$d\boldsymbol{R}/dt = \beta_{lc}\boldsymbol{I}_{lc} + \beta_{tu}\boldsymbol{I}_{tu}. \quad (6)$$

The size of dead or culled population ($\boldsymbol{D}$) is governed by the final equation of the model:

$$d\boldsymbol{D}/dt = \mu_{ec}\boldsymbol{I}_{ec} + \mu_{lc}\boldsymbol{I}_{lc} + \mu_{tu}\boldsymbol{I}_{tu}. \quad (7)$$

(b) Applied example

Table 1. Definitions and estimated values of parameters

Parameter	Value	Definition
N	10,000	Total number of broiler chickens in one shed
A	700	Area of the shed (m^2)
d	15	Population density (15 chickens per m^2 , $d=N/A$)
t_0	0	First day of broiler chickens reared
τ_0	35	Period of disease from infection to tumour for vvMDV1
τ	55	Period of shedding virulent FFE of an infected chicken
v	0	Degree of protection by host vaccinal immunity
δ	0.10	Females are δ (%) more susceptible that males
c_l	0.05	FFE shedding rate for latent infection (5%)
c_{lc}	1	FFE shedding rate for late cytolytic infection (100%)
c_{tu}	1	FFE shedding rate for tumour transforming infection (100%)
c_R	0.02	FFE shedding rate for recovered chickens (2%)
ρ	1	Ratio of female to male
b	1	Ratio of the lowest MD incidence to the highest in a year
α	0.5	Proportion of latent chickens shedding virulent FFE
$1/\upsilon_{ec}$	5	Duration of early cytolytic infection (days)
μ_{ec}	0.01	Death rate of early cytolytic infected chickens
$1/\upsilon_{l}$	7	Duration of latent infection (days)
$1/\upsilon_{lc}$	12	Duration of late cytolytic infection (days)
μ_{lc}	0.01	Death rate of late cytolytic infected chickens
β_{lc}	0.005	Recovery rate of late cytolytic infected chickens
μ_{tu}	0.02	Death rate of tumour transforming infected chickens
β_{tu}	0.0025	Recovery rate of tumour transforming infected chickens
P_0	10^{-6}	Infection probability caused by initial challenge level
P	5×10^{-4}	Infection probability caused by one day's FFE shedding of an infected
L	60	Lifespan of broiler chickens (days)
r	0.10	Protection provided by genetic resistance
m	0.30	Protection provided by maternal immunity

An applied simulation was run to test the effects of varying host vaccinal immunity on the incidence and spread of the disease in a shed of 10,000 broiler chickens. The simulation was extended to 90 days, well in excess of the normal lifespan of broiler chickens (approx.

40-60 days). The definitions and estimated values of parameters are summarised in Table1. Parameter estimates are based on current understanding of the disease but will require subsequent refinement and validation. It was supposed that at initial time (first day of broiler chickens reared), there is only one infected chicken at latent infection stage. The simulation of the model shows that increased host vaccinal immunity reduces the new infection cases and delays the date of peak incidence of new infections (Figure 2 and 3). Figure 3 (B) shows that a MD epidemic will ultimately occur even if no chickens are infected initially and infection probability incurred from a contaminated environment is very low.

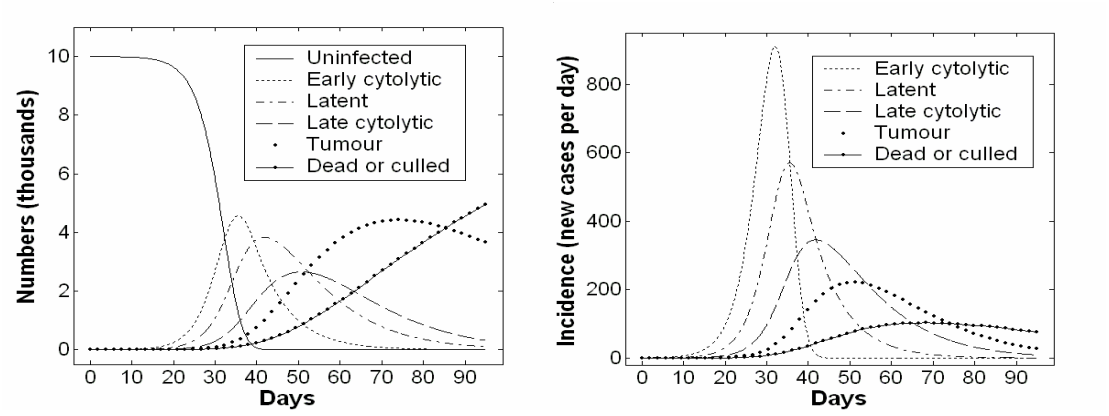


Figure 2A. Simulation of the numbers of broiler chickens in various stage of MD based on parameter values in Table 1 (v = 0). **Figure 2B**. Incidence (new cases /day) for MD stage from the same simulation. The maximum incidence of early cytolytic infection is 910 cases at day 32.

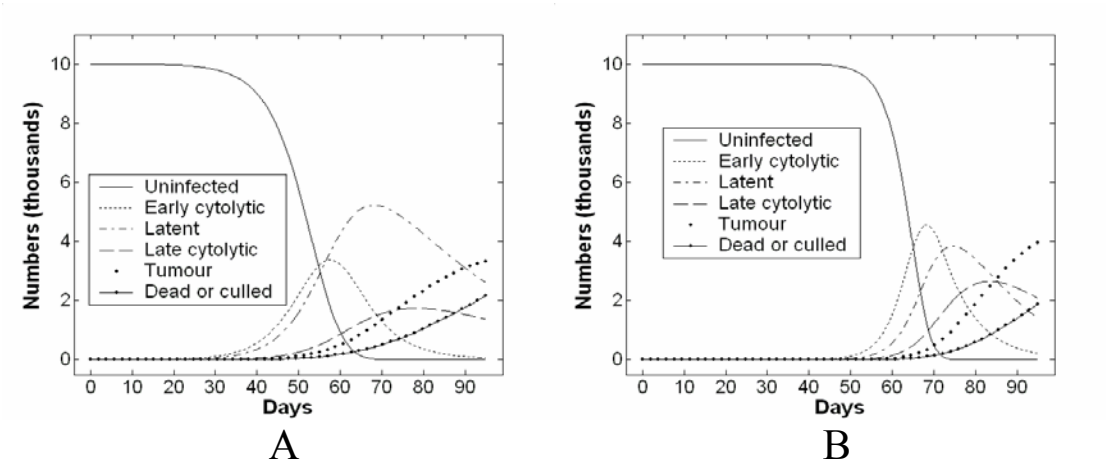


Figure 3A. Simulation of the numbers of broiler chickens in various stages of MD based on parameter values in Table 1 but with v = 0.5. The peak incidence of early cytolytic infection (cases/day) is 557 chickens at day 53. **Figure 2B**. Simulation of the numbers of broiler chickens in various stages of MD based on parameter values in Table 1 but with infection probability caused by initial challenge level set at 10^{-8} and no infected bird in the initial population. The peak incidence of early cytolytic infection is 910 at day 65.

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MONITORING MAREK'S DISEASE VIRUS IN BROILER FLOCKS USING STANDARD AND REAL-TIME QUANTITATIVE PCR OF SPLEEN AND DUST SAMPLES.

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Summary

We report the monitoring of Marek's Disease virus (MDV) on two unvaccinated commercial broiler farms using standard and real-time polymerase chain reaction (PCR). Standard (non-quantitative) PCR for MDV in spleen samples from five birds per shed (three sheds per farm), sampled at approximately weekly intervals, was compared with standard PCR of DNA extracted from shed dust, and quantitative real-time PCR (qPCR) of the same dust samples. The two farms could not be easily differentiated on the basis of standard PCR tests of spleen with the first positive spleens detected at days 27 and 25 on Farms A and B respectively, and 100% positives in a shed reached on days 41 and 32 respectively. Dust became positive to standard PCR at days 34 and 19 (one shed only) respectively for the two farms suggesting a greater MDV load on Farm B. Real-time qPCR of dust samples detected MDV in all dust samples (earliest day 19) and showed an exponential increase in virus content over time. It also revealed clear differences between farms with dust from Farm B containing approximately 10 times more MDV than that of Farm A. This was associated with reduced performance on Farm B as determined by FCR (2.086 v 2.203 for A and B respectively) and late mortality after day 28 (1.96 v 4.03%). These preliminary data suggest that q-PCR of poultry dust will prove useful for the routine monitoring of MD status in broiler flocks.

I. INTRODUCTION

Marek's disease is a ubiquitous disease of broiler chickens only partially controlled by vaccination with herpesvirus of turkeys (HVT), the vaccine currently used to control the disease in Australia. Incomplete protection is associated with immunosuppression and depressed performance during mid to late batch grow-out with appearance of lymphoid tumours late in the batch in severe cases (Islam *et al*., 2002). Monitoring of flock MD status is complicated by the fact that vaccination does not preclude infection and that infection does not equate with disease. Monitoring for MD therefore is not merely an issue of detecting MDV in chickens, but detecting it in amounts that are significant in terms of disease. Due to the co-existence of vaccinal and wild strains of MDV in the host, differentiation of the MDV serotypes involved is also an important component of effective monitoring systems.

In the past any monitoring for MD in broilers was based largely upon detection of vaccinal virus viraemia several weeks after vaccination using virus isolation from infected lymphocytes in cell culture. More recently, serotype-specific PCR which allows differentiation between HVT and MDV serotype 1 (MDV1) has been used to monitor both post-vaccinal viraemia and presence of MDV1 in peripheral blood lymphocytes and or spleen tissue (Walkden-Brown *et al*., 2003). However such tests are at best semi-quantitative (eg. percentage of birds positive) and require either bird sacrifice or blood sampling to access

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lymphocytes, the primary target cell of MDV. However after the initial cytolytic and latent phases of infection, MDV is shed in cell-free form from the feather follicle epithelium in dander (from about day 14 post infection) and detection and quantification of MDV in this material on a shed basis should provide a good indicator of flock MD status. The application of real-time PCR to quantify viral load (review: Mackay *et al*., 2002) has the potential to overcome most of the current limitations to routine monitoring of MD status in broiler flocks by enabling differentiation and quantification of different serotypes of MDV in chicken tissues or in poultry dust containing dander. This paper presents the results of a preliminary study aiming to determine whether: a) MDV can be detected and quantified effectively in poultry dust from commercial operations using PCR; b) PCR analysis of shed dust offers advantages over PCR analysis of spleen tissue; and c) Differences in MDV load in chickens or dust are related to differences in chicken performance in the field.

II. MATERIALS & METHODS

The experiment involved sample collection from two commercial broiler operations in Victoria (Farms A and B). Each farm had three sheds and operated on an all-in all-out basis and both were placed with mixed sex Cobb broilers from parent flocks vaccinated for MD using the Rispens CVI988 vaccine. Farm A was placed with 44,000 birds in early October 2002 while Farm B was placed with 117,000 birds in late October 2002. Flocks were killed out at final ages of 55 and 57 d, respectively. Chicks were spray-vaccinated against infectious bronchitis at hatch but were not vaccinated against MD. Marek's disease had been noted as a problem in unvaccinated flocks at this time.

On Farm A, spleens were collected from five birds/shed on d 27, 34, 41 and 48 after placement and shed dust sampled on d 27, 34, 41 and 48. On Farm B spleens were collected from five birds/shed on days 12, 19, 25, 32, 41, 47 and 53 and shed dust sampled on d 19, 25, 41, 47 and 53. Dust was collected from various surfaces in the shed (curtains, feeder lines, receiving area, etc.). Spleens and dust samples were frozen at –20˚C until dispatch to UNE for DNA extraction and PCR analysis. Routine performance data for each placement of chickens was recorded.

DNA was extracted from 10mg of spleen or 5mg of dust into 200µl or 100µl of elutent respectively using the DNeasy® tissue kit (Qiagen Pty. Ltd. Clifton Hill, Vic.) and stored at –20^{o}C until use. Serotype-1 specific PCR for MDV using 1ul of extracted DNA template was performed as previously described (Walkden-Brown *et al*., 2003). Prior to use in the q-PCR assay extracted DNA was quantified using spectrophotometric analysis (BIO-RAD, SmartSpec TM 3000) and diluted to a concentration of 5ng/µl. Real-time PCR to detect the target viral sequence (meq gene of MDV-1) and a reference host gene (α2 VI collagen) using 5µl of template was as described by Islam *et al*., (2004a,b).

Positive reactions to standard PCR scored 1-3 for band intensity. Viral load determined from real-time PCR data was measured in three different ways: a) relative to the amount of chicken DNA in the dust sample (relative abundance or RA method; Livak 1997); b) relative to the amount of DNA in the dust sample (Arbitrary units); and c) relative to the MDV1 content of a standard infected spleen sample (MDV1-70) on a weight for weight basis. Method a) relates target gene number to the host reference gene number while method b) simply is the calculated concentration of MDV1 read off the MDV-1 standard curve based on the standard spleen sample MDV1-70 serially diluted from 50ng/µl to 0.005µl of total DNA. Method c) takes into account the extraction efficiency of DNA from the dust and all dilutions to directly relate the amount of viral DNA in the dust sample to that in the standard spleen sample on a weight for weight basis, with a value of 100 meaning the dust sample has the same viral content as that contained in the same weight of wet standard spleen.

III. RESULTS

PCR results for spleen and data samples are summarised in Table 1 while production data are summarised in Table 2. MDV1 was detected in chicken spleens and poultry dust in all sheds on both farms by all methods used.

Table 1. Detection of MDV1 in unvaccinated broiler chickens on two farms in spleen and shed dust, using standard PCR and quantitative real-time PCR. Blank cells indicate lack of a sample on that day.

Farm	Shed	Day	Spleen PCR	Dust PCR	Viral load in dust detected by q-PCR		
			Pos./total	Pos./Neg[1]	RA[2]	AU[3]	RTS[4]
A	1	27	1/5	–	0.9	67	0.041
		34	0/5	+	0.6	38	0.016
		41	3/5	+	71.6	357	0.197
		48	4/5	++	93.1	4,467	24.486
	2	27	0/5	–	1.8	81	0.039
		34	1/5	+	13.0	100	0.059
		41	4/5	++	70.1	1,402	1.134
		48	5/5	++	671.3	2,406	12.288
	3	27	0/5	–	106.3	58	0.028
		34	0/5	+	0.9	36	0.019
		41	5/5	+	16.9	220	0.089
		48	5/5	++	227.8	3,159	2.739
B	1	12	0/5				
		19	0/5	–	0.1	4	0.002
		25	0/5	–	1.0	33	0.007
		32	3/5				
		41	5/5	++	261.0	2,879	0.751
		47	5/5	+++	4,423.1	12,866	3.111
		56	5/5	++	9,561.6	48,892	14.213
	2	12	0/5				
		19	0/5	+	3.4	125	0.036
		25	1/6	–	NE[5]	11	0.007
		32	5/5				
		41	5/5	+++	6,474.3	19,753	16.257
		47	3/5	–	NE[5]	8,684	2.428
		56	4/5	+++	6,925.0	35,396	12.476
	3	12	0/5				
		19	0/5	–	0.1	5	0.003
		25	0/5	+++	7.3	110	0.033
		32	5/5				
		41	5/5	++	339.6	3,201	1.032
		47	4/5	+++	2,575.7	17,303	6.849
		56	5/5	++	5,625.1	23,181	4.933

[1]– negative, + positive, band intensity (+ to +++)
[2]Relative abundance. Relative to chicken DNA in dust. Method a) in Materials and Methods
[3]Arbitary units (x1000). Relative to total DNA content of dust. Method b) in Materials and Methods
[4]Relative to spleen. Relative to viral content of same weight of standard spleen. 100 = same. Method c) in Materials and Methods.
[5]Not Estimable. No chicken DNA detected in sample.

Table 2. Performance data on Farms A and B in the study

Farm	Cumulative mortality (%)			Average adj. age (d)	Average weight (kg)	Adj FCR (feed:gain)
	Day 7	Day 28	Final			
A	2.80	5.13	7.09	47.04	2.489	2.097
B	1.17	3.04	7.07	48.63	2.332	2.171

Quantitative PCR revealed that the amount of MDV1 present increased dramatically over time on both farms, irrespective of the units of quantification, and that Farm B had approximately 10-fold more MDV overall in shed dust, than Farm A. The increase in viral load over time was detected by standard PCR to some extent, in terms of increased proportion of positive spleens and increased band intensity for dust samples, but these measures did not illustrate the exponential nature of the increase in viral load, nor did they allow differentiation of viral load between the two farms. Farm B exhibited significant late batch mortality (4.03% mortality after d 28, v. 1.96% for Farm A) and markedly inferior FCR relative to Farm A. Severe MD lesions were not noted on either farm although enlarged spleens, proventriculitis and wet droppings were noted.

IV. DISCUSSION

With regards the first objective, this study has demonstrated that detection and quantification of MDV1 in poultry dust collected under commercial farm conditions is feasible. DNA extraction from poultry dust using a commercial kit was technically straightforward and yielded DNA concentrations in the range 29-120 ng/µl. With regards the second objective this study suggests that q-PCR offers significant advantages over standard PCR because it allows sensitive and accurate quantification of viral load. In this study very large differences in dust viral content were able to be detected (RA range 0.1 to 6925; AU range 4 to 48,892). Real-time PCR requires a higher initial capital investment than standard PCR, and if a reference gene is measured in addition to the target gene the cost of the assay is also considerably greater. However, if a reference gene is not measured, the non-capital costs of q-PCR are little different from those of standard PCR. With regards the third objective, the results of this study support the proposition that chicken performance may be inversely related to MDV load in chicken dust. Farm B in this study had very much higher MDV content in shed dust and also considerably poorer performance. We do not know if this relationship is causal and further work is required to establish this. We plan to undertake such research and to optimise the q-PCR assay so that viral load can be quantified in SI units. The results of this preliminary experiment strongly suggest that q-PCR of poultry dust will have a major role in the routine monitoring of MD status in chicken flocks into the future.

V. ACKNOWLEDGEMENTS

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DETECTION AND QUANTIFICATION OF MAREK'S DISEASE VIRUSES USING REAL-TIME POLYMERASE CHAIN REACTION IN SEPARATE AND DUPLEX ASSAYS

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Summary

Development and initial validation of quantitative real-time PCR (qPCR) assays for the three serotypes of Marek's Disease Virus (MDV) are described. Also described is the development of an internal control qPCR assay that detects the chicken α2 (VI) collagen gene. To reduce costs, a duplex assay for MDV1 and the internal control was also developed. The MDV qPCR assays were specific to their target gene and more sensitive than standard PCR when compared using Australian field and vaccine strains of MDV. All assays were found to have acceptable reproducibility. Relative abundance (RA) of MDV1 and HVT viruses was quantified using the relative standard curve method in twenty experimentally infected chickens over a period of 7-35 days post infection and was shown to vary during the course of the infection. These qPCR assays will be useful for reliable differentiation and quantitation of MDV for a variety of applications.

I.INTRODUCTION

Marek's disease viruses (MDVs) are classified into three serotypes namely serotype 1, 2 and 3 (also known as Herpes virus of turkey, HVT). These serotypes are distinguished using polyclonal or monoclonal antibody tests, polypeptide pattern and DNA analysis (Payne, 1999). Conventional qualitative, semi-quantitative, and quantitative PCR assays are also used to detect and serotype MDV (Bumstead *et al*., 1997; Reddy *et al*., 2000; Handberg *et al*., 2001). In recent years, real-time quantitative PCR (qPCR) has emerged as an important technique for the analysis and quantification of viral nucleic acids (Mackay *et al*., 2002).

A reliable method for quantification and differentiation of MDV in chickens and their environment will greatly facilitate investigations into the epidemiology and pathogenesis of this disease and may lead to new monitoring systems for MD. This paper describes the development and validation of four TaqMan qPCR assays for the quantification and differentiation of the three MDV serotypes.

II. MATERIALS AND METHODS

Sequence-specific primers and a dual labelled TaqMan probe for each assay were designed using Primer Express v 3.1 software (Applied Biosystems, Foster City, USA) to run on a Rotor Gene 3000 real-time PCR machine (Corbett Research, Sydney Australia). The

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selected genes were Meq for MDV1, DNA-Polymerase for MDV2, SORF1 for HVT, and α2 (VI) collagen for chicken. These were sourced from GenBank and aligned using Sequencer V3.1.1 (Gene Codes Corporation).

Details of primers and probe sequences, reaction and cycling parameters, and duplex assay conditions are described by Islam *et al*. (2004) and are available on request. The relative standard curve method described by Livak (1997) was used for viral DNA quantification. This method determines the relative abundance (RA) of the target viral sequence in relation to a control host sequence scaled against a single standard sample. Standard curves for all four assays were based on 10 fold serial dilutions (250ng - 0.025ng total DNA) of samples of known high content of the target gene. MDV1 and MDV3 standards were from spleen of infected birds. For MDV2 the standard was from a vaccine preparation (Maravac vaccine, Fort Dodge Australia Pty Ltd.) and for α2 (VI) collagen it was from spleen DNA from a specific pathogen free (SPF) chicken.

Specificity of MDV primers and probes was assessed by testing a range of Australian field and vaccine strains of MDV and comparison with standard PCR results. To confirm the precision and reproducibility of each qPCR assay, the intra-assay co-efficient of variation was determined from replicates within each qPCR run. Inter-assay variation was investigated in two or three separate runs performed on different days using freshly prepared reagents.

To test the ability of the MDV1 and HVT assays to quantify virus in samples from infected chicken a total of 20 spleen samples was analysed in duplicate from 7-35 d after infection with either MDV1 or HVT (n=10). The development of a duplex MDV1/Collagen assay involved halving the concentrations of primers and probes for each in the combined reaction. The assay was further optimised by limiting the collagen primer concentrations to 0.037 μM and evaluating the ability of the duplex assay to accurately detect and quantify MDV1 in samples of known high, medium and low MDV1 content.

All DNA samples were extracted using the QIAamp DNA mini Kit according to the manufacture's instructions (Qiagen, Clifton Hill, Australia) and stored at –20^{o}C. Extracted DNA was quantified using spectrophotometric analysis (BIO-RAD, SmartSpec TM 3000) before use.

III. RESULTS

Linear-log standard curves for the 4 assays produced a strong linear fit with $\log_{10}$ DNA concentration (R^2>0.99). Standard curves for each assay were highly reproducible with no significant difference in slopes between different runs of the same assay (P<0.05). The limit of detection was 0.025ng total DNA (host plus viral) for MDV1 and collagen assays and 0.25ng for HVT and MDV2.

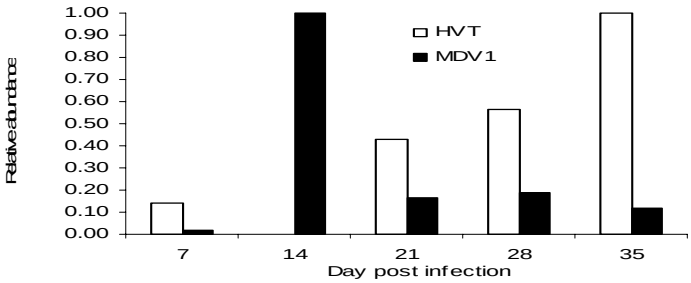

Figure 1. Quantification of MDV1 and HVT viruses in spleen tissue of broiler chickens from days 7-35 post infection with either MDV1 or HVT (n=10).

The serotype-specificity of each MDV assay is illustrated in Table 1. The superior sensitivity of the qPCR assays over the standard PCR assays is evident in several samples as is the ability to detect MDV1 and HVT in the same sample. Assay precision/reproducibility as determined by intra- and inter-assay coefficients of variation for both Ct value and calculated concentration of the 4 separate assays and the MDV1/Collagen duplex assay is shown in Table 2.

Table 1. Comparison of specificity of qPCR assays with standard PCR using Australian field and vaccine strains of MD viruses (Positive= +, Negative= -) (Normal PCR results from Walkden-Brown *et al*., 2003).

	MDV 1		MDV 2		MDV 3/HVT	
MDV Strains & Sources[(a-f)]	**PCR**	**qPCR**	**PCR**	**qPCR**	**PCR**	**qPCR**
MPF 145/1(MDV1) [a]	-	+	-	-	-	-
MPF 145/3(MDV1) [a]	+	+	-	-	-	-
MPF 145/9(MDV1) [a]	+	+	-	-	-	-
MPF 146/9(MDV1 & HVT) [a]	+	+	-	-	+	+
MPF 146/14(MDV1 & HVT) [a]	+	+	-	-	-	+
Rispens CVI988 (MDV1) [b]	+	+	-	-	-	-
BH16 (MDV1-live atte.) [b]	+	+	-	-	-	-
Rispens CVI988(MDV1) [c]	+	+	-	-	-	-
MD 19 (Maravac)(MDV2) [d]	-	-	+	+	-	-
FC126 (Cell free)(HVT) [b]	-	-	-	-	+	+
FC126 (Cell assoc.)(HVT) [c]	-	-	-	-	+	+
MPF 57(ISO4/76)(MDV1)[e]	+	+	-	-	-	-
MPF 57(ISO4/84)(MDV1) [e]	+	+	-	-	-	-
MD 19 (Iso2/2)(MDV2) [e]	-	-	+	+	-	-
FC126 (ISO1/ 286)(HVT) [e]	-	-	-	-	+	+
FC126 (ISO3/ 161)(HVT) [e]	-	-	-	-	+	+
MPF57& FC126 (ISO5/3) [e]	-	+	-	-	+	+
MPF57& FC126 (ISO3/121) [e]	-	+	-	-	+	+
SPF spleen [f]	-	-	-	-	-	-

Virus Sources: [a] RMIT University Ffield isolates), [b] Intervet Australia Pty Limited (Vaccine), [c] The Marek's Co. (Vaccine), [d] Fort Dodge Australia Pty Limited (Vaccine), [e]University of New England (Experimental samples), and [f] Steggles Pty Limited.

Table 2. Overall mean intra-assay and inter-assay coefficients of variation (CV) based on mean Ct values and calculated concentration (CC) of standards.

	Mean Intra-assay CV		Mean Inter-assay CV	
Assays	**Ct**	**CC**	**Ct**	**CC**
MDV1	0.61%	13.04%	0.99%	18.33%
MDV2	0.89%	18.04%	3.92%	13.98%
HVT	0.83%	17.96%	1.87%	31.80%
Collagen	0.64%	6.37%	2.26%	16.29%
Collagen (Duplex)	1.21%	23.36%	1.52%	6.12%
MDV1 (Duplex)	0.43%	7.99%	2.16%	14.50%

The RA of MDV1 and HVT in spleen samples from experimentally infected broiler chickens is shown in Figure 1. MDV1 and HVT exhibited a differential pattern of RA over

time clearly demonstrating the ability of the assays to usefully quantify virus during the course of a normal chicken infection. After optimisation, the duplex assay produced standard curves that were parallel with those produced by the single assays for the same target. Parallelism and ability to accurately detect both the target (MDV1) and control (collagen) sequences was maintained over a very wide range of concentrations of MDV1 (Ct 22 to Ct 37, approx. 32000 fold difference in MDV1) giving a range in RA of MDV1 from 0.000142 to 1.

IV. DISCUSSION

This is a preliminary report on three MDV qPCR assays and an internal control assay that permits the quantification of host DNA. These assays appear sensitive and specific. However, wider testing of specificity and determination of absolute sensitivity in terms of viral DNA detection thresholds are desirable and these activities are under way in our laboratory. Reproducibility of all assays with regards to Ct value appears very good but for some assays the CV of calculated concentration needs to be reduced. The use of robotic systems rather than hand pipetting may resolve the issue. Combining two of the assays into a single duplex assay was successfully achieved and will be extended to the other MDV assays.

These assays have application for the quantification of MDV in chicken tissues and the environment (eg. Walkden-Brown *et al*., 2004). This will assist studies on the epidemiology and pathogenesis of MD and may ultimately prove valuable for the routine monitoring of MDV.

V. ACKNOWLEDGEMENTS

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SEROLOGICAL METHODS FOR INFECTIOUS BRONCHITIS IN LAYING HENS

J.R. ROBERTS, W. BALL, R. CHUBB, A. SULAIMAN and M. JOLLY

Summary

Measurements of infectious bronchitis virus (IBV) antibody titres by IDEXX IBV antibody ELISA were compared with the results of agar-gel precipitation (AGP) and serum neutralisation (SN) tests. The percentage of samples testing positive by AGP and SN increased as the ELISA IBV antibody titre increased. Although it is not clear at what antibody titre level birds are protected against intercurrent infection, a mean IBV antibody titre measured by IDEXX ELISA of 439 correlated with a high level of protection against exposure to T-strain IBV. A comparison of two different IBV antibody ELISA kits showed a significant linear correlation although individual samples did not always correlate closely.

I. INTRODUCTION

Infectious bronchitis virus (IBV) and its deleterious effects were first recognised in the United States in the early 1930s (Cavanagh & Naqi, 1997) and the presence of this virus in Australian flocks was first identified by Cumming (1963) as the infectious agent associated with respiratory disease and uraemia. All layer flocks are vaccinated against IBV although it is recognised that drops in egg production and reduced internal quality and egg shell quality may occur as the result of exposure of vaccinated flocks. It is essential that the Australian egg industry has tools for surveillance of layer flocks for effects of IBV. The level of protection afforded by vaccination is usually measured in the form of IBV antibody titres. The most common method of doing this is to use commercially available IBV antibody ELISA kits. There are currently three available: KPL ProFlok; IDEXX and TropBio. The TropBio kit is the only one that was developed specifically for Australian conditions. However, the other two kits are also in common commercial use, especially IDEXX. Other methods of measuring IBV antibody titres include serum neutralisation (SN), agar gel precipitation (AGP) and haemagglutination inhibition although these tests are not always readily available commercially and are more time-consuming and expensive than ELISA. A number of studies have compared different methods for suitability. Monreal *et al.* (1983) concluded that ELISA was more suitable for monitoring antibody responses to vaccination than HI and AGP because of its higher sensitivity. A preference for ELISA, as compared with other tests, has been expressed by a number of other authors (Mockett & Darbyshire, 1981; de Wit *et al.,* 1992). However it is important to correlate the IBV antibody titres obtained using ELISA with other ways of measuring IBV antibody levels and also with the level of protection of the bird to IBV challenge.

This study compared the IBV antibody titres obtained using an IDEXX IBV antibody ELISA kit (quantitative) with the positive/negative results obtained from AGP and SN. It also compared the results of two ELISA kits, IDEXX and TropBio.

II. MATERIALS AND METHODS

Plasma samples were obtained from a trial as reported previously (Sulaiman *et al.,* 2003). Samples were taken from the same 70 birds on 12 occasions over the laying life of the flock. Some samples were negative for IBV antibodies (unvaccinated birds at weeks 4 and 6)

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whereas the samples taken at 79 and 80 weeks of age followed challenge with T-strain IBV at 77 weeks of age. Although one group of birds had not been revaccinated regularly during lay, all birds were vaccinated with VicS strain IBV at 62 weeks of age, 15 weeks prior to the exposure to T-strain IBV.

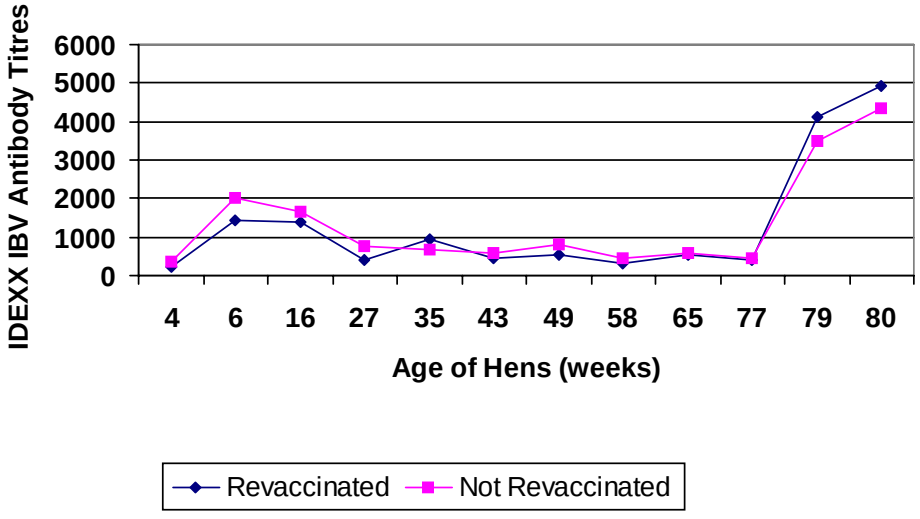
IBV antibody titres were measured using an IDEXX IBV antibody ELISA kit and were conducted by Birling Avian Laboratories. AGP tests, which give a positive/negative result, were conducted after the method of Woernle (1966) and Chubb and Cumming (1971). SN tests were conducted after the method of Fabricant (1951) to identify positive or negative only.

A series of plasma samples, again ranging from negative for IBV antibodies to high positive, were obtained from a separate experiment in which unvaccinated birds were exposed to challenge viruses. The IBV antibody titres were determined on these samples, by the International Avian Health Group at the University of Melbourne, using both IDEXX and TropBio ELISA kits. The data were then compared.

III. RESULTS

The IBV antibody titres obtained for all the plasma samples of the main experiment are shown in Figure 1 and are separated into those from birds that were revaccinated regularly during lay and those that were not. The comparison of IBV antibody titres measured by IDEXX IBV antibody ELISA and the positive/negative results obtained from AGP and SN tests is shown in Figure 2. The ELISA antibody titres have been grouped to allow for the calculation of percentage positive results for AGP and SN. The sample sizes for the groupings are: 0-100, 33 samples; 101-200, 34 samples; 201-500, 48 samples; 501-1000, 38 samples; 1001-2000, 35 samples; 2001-4000, 30 samples; 4001-6000, 21 samples; 6001-8000, 19 samples; 8001-15000 12 samples. There is a general increase in the percentage of samples testing positive for AGP and SN, as the ELISA antibody titre increases. The mean IBV antibody IDEXX ELISA titre of the birds, just prior to exposure to T-strain IBV was 439. This titre level correlated with a high level of protection of the birds (see Sulaiman *et al*. in this volume).

A comparison of the results of IBV antibody titres obtained, using both IDEXX and TropBio ELISA, on the same samples, is shown in Figure 3. Although there is a statistically significant linear relationship between the two sets of titres (P=0.0003), the fit is not close (R^2=0.301) and there is not always a good match for individual samples.



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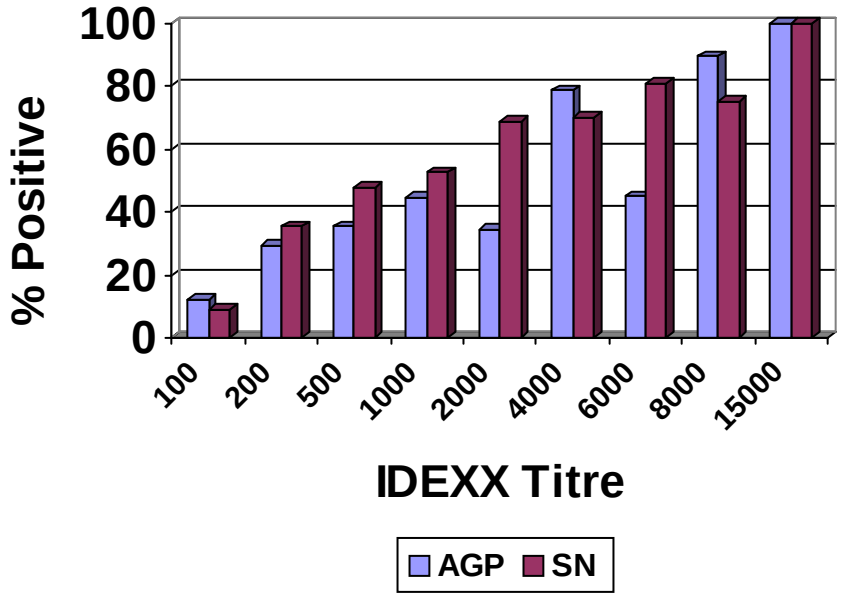

Figure 2: Comparison of IBV antibody titres obtained with IDEXX ELISA kit and percentage of samples in the titre range positive by AGP or SN test

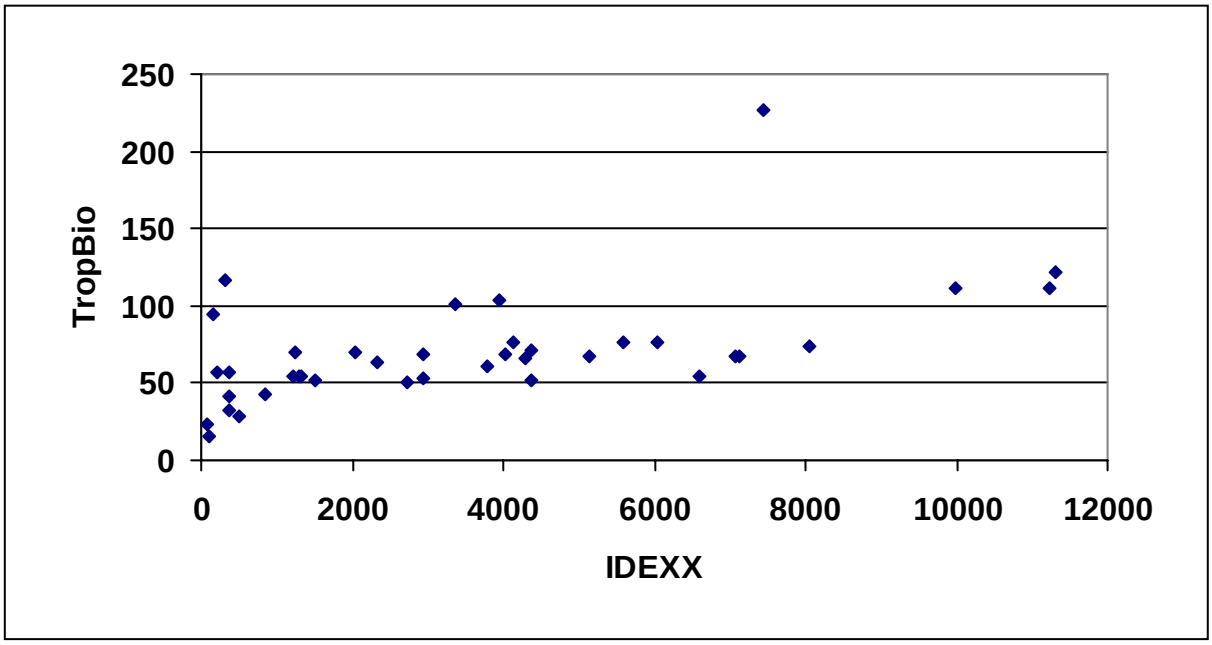

Figure 3: Comparison of IBV antibody titres obtained from IDEXX and TropBio ELISA kits

IV. DISCUSSION AND CONCLUSIONS

The different methods of measuring IBV antibody levels actually measure different types of antibodies. Therefore, it is not surprising that the results obtained are not identical. However, it is very important for the egg industry to have a reliable means of determining if flocks are adequately protected against the possibility of an IBV intercurrent infection. At

the present time, it is difficult for producers to interpret the IBV antibody titres obtained from ELISA testing. There is also some uncertainty about which ELISA kit is most appropriate under what circumstances. Further information is required to address the need for better surveillance against IBV in layer flocks.

V. ACKNOWLEDGEMENTS

This project was supported by Australian Egg Corporation Limited.

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PEAK OF LAY INFECTION WITH INFECTIOUS BRONCHITIS VIRUS – ITS IMPACT ON EGG QUALITY PARAMETERS OF FOUR STRAINS OF LAYING HEN.

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Summary

The eggshell quality of vaccinated hens challenged with infectious bronchitis (IB) virus in-lay at 41 weeks of age was found to decline. These effects were observed in hens vaccinated only during the growth phase and not revaccinated during the lay period. T strain virus was found to have a more prolonged impact while the antigenically-different N1/88 strain produced a greater decrease in egg quality parameters. There was an unexpected increase in internal quality of the virus-exposed groups during the challenge period. While the genetic strain of the hen had no significant effects on the response to challenge in terms of egg quality, there were inherent strain differences in egg quality unrelated to the challenge and these are briefly discussed.

I. INTRODUCTION

IB virus has been widely reported as a cause of dramatic declines in egg production and egg quality of laying flocks. The egg quality impacts usually associated with IB infection include a lightening of the shell colour, an increased number of broken eggs and a thinning of the albumen (Cavanagh and Naqi, 1997). All of these potential effects would have a negative impact on the commercial viability of an infected laying flock. Consequently all commercial flocks in Australia are vaccinated against IB virus. The trial described here is one component of a larger project aimed at identifying the impact of IB on the egg quality of vaccinated laying hens and determining the optimum vaccination protocol for laying hens. The current trial was designed to investigate the impact of challenge with two antigenically-different (Wadey and Faragher, 1981; Ignjatovic and McWaters, 1991; Ignjatovic *et al*., 1997) Australian strains of IB virus on four different genetic strains of commercial laying hen that had not been vaccinated beyond the growth phase.

II. METHODS

Sixty-four pullets of each of four commercial strains of laying hen were obtained from commercial hatcheries as day old chickens. The birds were raised on the floor until they were 14 weeks of age, at which time they were transferred to wire laying cages in large isolation sheds. The birds were vaccinated against IB virus at day old, 4 weeks and 14 weeks with a commercial vaccine administered by coarse spray. One or both vaccines of serotypes C and B were used to produce four vaccination treatment groups that were equally distributed throughout strain and challenge groups. Data for the vaccination treatment group is still being analysed and will be presented at a later date.

At 41 weeks of age (peak of lay) the birds were divided into three groups. One group of 128 birds was inoculated by eye drop with the serotype C (Wadey and Faragher, 1981) T-strain IB virus; the second group (118 birds) received the serotype L (Ignjatovic and McWaters, 1991) N1/88 IB virus via the same route and the last group (128) birds was not challenged (the control group). Both viruses were obtained from Dr. Jagoda Ignjatovic, Australian Animal Health Laboratory, Geelong.

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Egg collections were made immediately prior to challenge with the virus and then at weekly intervals for five weeks, ten eggs per group per week. Eggs were analysed for shell deformation, breaking strength, shell reflectivity, egg weight, albumen height, Haugh units, yolk colour, shell weight, percentage shell and shell thickness.

Analysis of variance was used to test the effect of challenge, bird strain and vaccination group on the egg quality parameters measured, with significance indicated by $P<0.05$. Fishers protected LSD was used to distinguish between means when significant effects were seen.

III. RESULTS

Statistically significant effects of the challenge treatment across all collection times were observed in egg weight, shell weight, percentage shell, shell thickness, albumen height, Haugh units and yolk colour (Table 1). Egg weight, shell weight and yolk colour score were all significantly lower for both groups of challenged birds. N1/88 exposed birds had significantly lower shell thickness and percentage shell. Unexpectedly, albumen height and Haugh units were found to be significantly lower for the control groups. There were no significant effects of challenge group on shell reflectivity, breaking strength or deformation.

Table 1. Significant effects of challenge group on egg and egg shell quality.

	Control	**T-strain**	**N1/88**	**P Value**
Egg Wt g	$62.7^{a} \pm 0.1$	$59.7^{b} \pm 0.3$	$60.0^{b} \pm 0.4$	<0.0001
Shell weight g	$6.07^{a} \pm 0.01$	$5.95^{b} \pm 0.02$	$5.97^{b} \pm 0.02$	<0.0001
% Shell	$9.68^{ab} \pm 0.02$	$9.72^{a} \pm 0.03$	$9.64^{b} \pm 0.03$	0.0366
Shell Thickness μm	$425.0^{a} \pm 0.8$	$424.6^{a} \pm 1.0$	$422.0^{b} \pm 1.0$	0.0098
Albumen Height mm	$8.98^{c} \pm 0.03$	$9.07^{b} \pm 0.04$	$9.41^{a} \pm 0.04$	<0.0001
Haugh Units	$93.4^{c} \pm 0.1$	$94.3^{b} \pm 0.2$	$95.8^{a} \pm 0.2$	<0.0001
Yolk Colour Score	$10.9^{a} \pm 0.01$	$10.8^{b} \pm 0.02$	$10.9^{b} \pm 0.02$	0.0027

Mean ± S.E. Across a row means with different superscripts are significantly different.

There were significant interactions between time after challenge and challenge group for some egg quality parameters (Table 2). Egg weight was significantly lower than the controls for both challenge virus groups at 1, 3 and 4 weeks post exposure, and for the T birds only at 2 and 5 weeks post challenge. When compared to the other treatment groups at the same time after infection, deformation of the shell was significantly lower for the T group only at 1 week after challenge, then higher for the N1/88 group at three and five weeks post exposure. The group that received N1/88 virus had significantly lower percentage shell at week three after challenge, while the T group had a lower percent shell at 5 weeks. Shell weight, albumen height also indicated significant interactions, mainly as a result of changes in egg weight and Haugh units respectively. Shell thickness and breaking strength were not significantly different between the challenge treatment groups across the time frame of the trial.

There were no significant differences among the measured parameters of any of the four strains of hen, challenged with different antigenic strains of IB viruses. However, there were some differences among the strains for each of the egg quality parameters that were consistent across time, challenge group and vaccination group (Table 3). The ISA Brown and HiSex strains had significantly darker, stronger, heavier, thicker shells that constituted a greater percentage of the egg and the HiSex strain had a significantly more flexible shell. The tinted egg layer the Hyline gray, had significantly smaller and paler eggs. Both Hyline strains had

significantly higher albumen height and Haugh unit measurements. The yolk colour of the ISA Brown and Hyline Brown strains was significantly higher than the other two strains of bird.

Table 2. Effect of challenge on shell deformation, egg weight, percentage shell and Haugh units at weekly intervals.

		Before	1 Wk	2 Wks	3 Wks	4 Wks	5 Wks	P Value
Deform µm	Control	275.9^{a} ±3.8	275.3^{a} ±5.2	261.2^{a} ±3.3	260.3^{b} ±3.2	266.3^{a} ±3.0	251.6^{b} ±2.1	0.0040
	T	274.8^{a} ±6.7	257.0^{b} ±3.6	268.4^{a} ±7.3	254.5^{b} ±3.9	264.9^{a} ±3.2	267.1^{a} ±5.1	
	N1/88	274.6^{a} ±6.5	277.4^{a} ±7.3	262.3^{a} ±4.6	275.6^{a} ±7.3	261.9^{a} ±3.7	268.7^{a} ±4.2	
Egg Wt g	Control	61.27^{a} ±0.36	62.68^{a} ±0.26	62.76^{a} ±0.26	63.01^{a} ±0.24	63.30^{a} ±0.27	62.99^{a} ±0.25	<0.0001
	T	62.34^{a} ±0.34	58.87^{b} ±0.83	60.36^{b} ±0.62	57.55^{b} ±1.10	59.42^{b} ±0.92	59.51^{b} ±0.92	
	N1/88	61.50^{a} ±0.63	55.71^{c} ±1.51	61.96^{a} ±0.34	59.15^{b} ±1.05	59.51^{b} ±0.99	61.98^{a} ±0.50	
% Shell	Control	9.33^{a} ±0.06	9.55^{a} ±0.04	10.05^{a} ±0.05	9.74^{a} ±0.04	9.72^{a} ±0.04	9.70^{a} ±0.04	0.0010
	T	9.35^{a} ±0.07	9.87^{a} ±0.07	10.15^{a} ±0.07	9.76^{a} ±0.06	9.70^{a} ±0.06	9.53^{b} ±0.06	
	N1/88	9.34^{a} ±0.08	9.64^{a} ±0.07	9.95^{a} ±0.07	9.67^{b} ±0.06	9.73^{a} ±0.06	9.50^{ab} ±0.05	
Haugh Units	Control	95.55^{a} ±0.31	94.05^{b} ±0.32	91.35^{b} ±0.32	92.98^{b} ±0.37	93.28^{b} ±0.35	93.38^{b} ±0.67	<0.0001
	T	94.96^{a} ±0.40	94.10^{b} ±0.44	94.98^{a} ±0.43	92.99^{b} ±0.48	94.16^{b} ±0.44	94.53^{b} ±0.49	
	N1/88	95.89^{a} ±0.38	95.72^{a} ±0.46	95.21^{a} ±0.47	94.66^{a} ±0.46	96.09^{a} ±0.47	97.16^{a} ±0.46	

Mean ± S.E. Means in columns within a parameter with different superscripts are significantly different. P values given are for the interaction between time and challenge treatment.

Table 3. Effect of strain of hen on egg quality parameters.

	ISA Brown	Hyline Brown	Hyline Grey	HiSex	P-Value
Egg Wt. (g)	62.48^{a} ±0.27	61.77^{ab} ±0.31	59.39^{c} ±0.29	61.34^{b} ±0.26	<0.0001
Shell Reflect. (%)	35.56^{c} ±0.22	36.59^{b} ±0.18	60.84^{a} ±0.28	35.08^{c} ±0.24	<0.0001
B.S. (N)	42.31^{b} ±0.24	40.55^{c} ±0.21	40.52^{c} ±0.20	44.59^{a} ±0.49	<0.0001
Def. (mm)	257.3^{c}±1.98	265.2^{b}±2.06	267.4^{b}±2.28	274.7^{a}±2.24	<0.0001
Shell Wt. (g)	6.37^{a} ±0.02	5.93^{c} ±0.02	5.63^{d} ±0.02	6.13^{b} ±0.02	<0.0001
% Shell	10.07^{a} ±0.03	9.43^{c} ±0.03	9.33^{d} ±0.02	9.88^{b} ±0.03	<0.0001
Shell Thick. (µm)	445.5^{a} ±0.93	414.8^{c} ±0.94	399.6^{d} ±0.80	436.4^{b} ±0.93	<0.0001
Alb. Ht. (mm)	8.56^{c} ±0.04	9.49^{a} ±0.04	9.51^{a} ±0.03	8.88^{b} ±0.04	<0.0001
Haugh Units	91.05^{d} ±0.22	95.97^{b} ±0.17	96.69^{a} ±0.15	93.22^{c} ±0.17	<0.0001
Yolk Colour scale	11.03^{a} ±0.02	11.05^{a} ±0.02	10.55^{c} ±0.02	10.94^{b} ±0.02	<0.0001

Mean ± S.E. Means in rows with different superscripts are significantly different.

IV. DISCUSSION

Challenge at 41 weeks of age after not having been vaccinated since 14 weeks of age had negative effects on the egg quality of the birds. Over the trial period as a whole, the birds exposed to N1/88 had a greater drop in shell quality parameters. This strain of IB virus had deleterious effects on shell thickness and percentage shell in addition to the declines in egg weight, shell weight and yolk colour that were also significant in the birds challenged with T-strain.

When the egg quality parameters are compared at each of the collection times (before and weekly after challenge) the timing of the declines in shell quality can be seen. In general the egg weight for the T group declined at 1 week and stayed low for the duration of the trial, while the N1/88 group had a decline to the first few weeks after challenge and then a return to the level of the control group. At weeks three and five post exposure, the group that received the N1/88 virus had higher deformation than the other treatment groups, as well as a lower percentage shell and, while not statistically significant, the shell thickness tended to be lower. These factors together probably indicate that the thinner, lighter shell was more flexible, however, the declines in the quality of these parameters has apparently not affected the breaking strength of the shell at any of these collection times.

One of the signs classically associated with IB is a decline in internal quality of the eggs (Cavanagh and Naqi, 1997). Unexpectedly in this trial, the albumen height and Haugh units of the control group was lower than that of the infected groups. This apparent increase in Haugh units due to challenge is due largely to the significantly higher values for the N1/88 group across almost all collections. The reason for this increase is unclear, although a steady but slight decrease in the Haugh units of the control group has possibly contributed more to this difference than an increase in the infected birds. However, either way, it can be said that the lowest mean Haugh unit recorded for the control birds was 91.35 reported in the second week after the other groups were exposed. This value is still at a very high level well above the USDA AA grade level of 80.70 (Jones *et al.*, 2002).

In general, there were declines in egg shell quality with challenge at peak lay, with T strain having a more prolonged effect, but N1/88 having the more severe reduction in quality.

There were no significant differences between the reactions of the different strains of laying hen to challenge with either of the IB strains used in this trial. There were however, consistent differences between the performances of the layer strains after having been raised to this age under identical environmental conditions. In general the shell quality of the ISA Brown and HiSex strains was superior to that of the Hyline strains, while the internal quality as measured by Haugh units and albumen height was better for the two Hyline strains. The higher mean yolk colour score for the ISA Brown and Hyline Brown birds is probably largely due to these two strains being the largest in body size and therefore eating more pigment-containing feed, per egg laid. The significant shell quality and Haugh unit effects are probably indicative of inherent differences among the four strains, giving each layer strain its advantages and disadvantages in terms of egg quality parameters.

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HAEMATOLOGICAL PARAMETERS OF HENS HOUSED IN DIFFERENT LAYING SYSTEMS

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Recent developments in poultry welfare in Europe, America and Australia require improvements in laying hen management, especially the modification or construction of new housing systems (Stewart, 2001). To obtain quantitative Australian information an industry supported facility for layer management research has been established at the University of Queensland. This facility provides a unique resource to investigate the effects on birds of housing under four different housing systems: conventional Australian cages (Cage3 and Cage6); European cages in environmentally controlled conditions (CE); housing in a barn system; and housing under free-range conditions (FR).

ISA Brown laying pullets were placed in the different housing systems and their productivity, health and welfare has been monitored using a range of parameters. In this paper we report the initial results from this on-going study. Apart from production data, different haematological and immunological indicators (hematocrit, differential leukocyte count and heterophil to lymphocyte ratio, and cuntaneous basophile hypersensitivity for cell-mediated immunity (Wattle Index) were used.

SYSTEM	**HDP**	**H/L RATIO**	**H**	**L**	**M**	**E**	**B**	**PCV**	**WI**
Barn	78.76^{a}	0.53^{a}	30.47^{a}	58.30^{a}	5.70^{a}	3.03^{a}	2.50^{a}	28.40^{a}	2.09^{a}
CE	82.31^{a}	0.41^{b}	26.29^{b}	65.11^{b}	5.31^{a}	1.94^{b}	1.34^{b}	31.35^{b}	2.71^{b}
Cage3	81.18^{a}	0.36^{c}	24.47^{b}	69.20^{b}	4.33^{b}	1.13^{c}	0.87^{c}	34.43^{c}	2.85^{b}
Cage6	80.29^{a}	0.39^{bc}	25.92^{b}	65.67^{b}	4.78^{ab}	1.86^{bc}	1.64^{bc}	29.82^{a}	2.62^{b}
FR	81.56^{a}	0.24^{d}	17.17^{c}	72.13^{c}	5.97^{ac}	2.97^{abc}	1.77^{bc}	33.12^{bc}	3.39^{c}

HDP: Hen/Day/%; **H/L**: Heterophil/Lymphocyte; **M**: Monocyte; **E**: Eosinophils; **B**: Basophils; **PCV**: Packed Cell Volume; **WI**: Wattle Index;

At 34 weeks of age there were no significant differences in egg production ($P>0.05$) among the different housing systems. The haematological-immunological tests suggest that hens within the free-range system are better adapted ($P<0.001$) to their environment than hens in the other systems. These birds will be assessed to 70 weeks of age so that responses to the alternate housing systems can be monitored through different seasons of the year. These studies will be followed by further flocks of birds that will also be monitored throughout their yearly laying cycle. The outcome will be to develop strategies to improve the different laying systems.

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BIOLOGICAL COST OF STRESS AND ITS MEASUREMENT IN LAYING HENS

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The way in which production practices affect the welfare of food-producing animals such as laying hens cannot be evaluated by an animal's mental state. Indirect measures of are needed to make scientific assessments. Most scientists agree that in birds measuring the biological cost of stress in a "pre-pathological state" i.e., measuring the adrenal gland response and its consequences, such as reduced immune competence and/or suppression of an endocrine response fundamental to reproduction (e.g. failure to ovulate) is more acceptable than measuring changes in physiological variables such as glucocorticoid secretion (Moberg, 1996). This study is part of a research program that examines the effects of environmental stressors encountered by laying hens. Under production conditions several measurements have been used to determine stress-induced changes. Our question in this phase was whether exogenous stressors might impact on the endocrine and immune systems as well as production at peak of lay. In order to induce physiological changes similar to those of stress we employed adrenocorticotropic hormone (ACTH) saline solution 20IU/kg/d, i.m., and handling, of 33 wks old hens (Downing and Bryden, 2002). The control birds received an equal volume of 0.9% saline or were not handled. Alterations of stress and immune response, such as Adrenal/Body Weight (A/BW; mg/100g), Spleen/Body Weight (S/BW; g/kg), and Heterophil to Lymphocyte (H/L) ratios, as well as production traits were recorded. Data were analysed using the SAS GLM procedure (Version 8.2). Significant differences among groups were determined using protected t-tests, and correlations between different measures were determined using correlation coefficient.

The results showed that exogenous stressors alter significantly A/BW ($P<0.0001$), S/BW ($P<0.005$), and H/L ($P<0.005$) ratios. There was also a 10% decrease in egg production ($P<0.005$) in ACTH treated hens, but not in handled or control hens. A non significant effect on egg weight ($P<0.03$), and shell thickness ($P<0.09$) were also observed.

Therefore, it is not surprising that productivity measures have received little attention as welfare indicators. Classical stressors affect the neuroendocrine system together with other widespread effects on reproduction, immunity, and metabolism. Hence, combining results from different indicators will allow an estimate of biological cost of stress to be made. The initial results from this on-going study were able to show in a single experiment all major adaptive stress response of laying hens. Further investigation is required to develop practical measures (i.e. non-invasive tests) that can be used to indicate the stress status of a laying hen.

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ENERGY REQUIREMENTS OF ISA BROWN AND HY-LINE BROWN LAYERS

D.N. SINGH, P.C. TRAPPETT, T. NAGLE and K.M. BARRAM

Summary

ISA Brown and Hy-line Brown hens were fed 10 diets representing five metabolisable energy (ME) levels and two densities. ME ranged from 10.3 to 12.2 MJ/kg and density was either fixed or allowed to float. Daily energy intake increased as the energy concentration of the diet increased even though daily feed consumption decreased as the energy concentration of the diet increased. Clearly, the birds were not able to fully compensate to a constant energy intake as the energy level of the diet increased. This extra energy intake did not influence bird performance. Over the whole experimental period (18–66 weeks age), ISA birds laid heavier eggs ($P<0.05$), had lower feed intake ($P<0.01$) and the feed conversion was better although just outside the 5% level of significance ($P=0.056$). The ME intake of ISA birds was lower than Hy-line birds ($P<0.01$). Thus, overall the ISA Brown birds consumed less feed to produce the same number of heavier eggs, resulting in better feed conversion efficiency than Hy-line Brown birds. Changes in feed intake were attributed to dietary ME level. Diet density had significant ($P<0.01$) influence on energy intake but had no effect on feed intake. Birds on fixed density diets consumed 125 kJ/d more than birds on floating density diets.

I. INTRODUCTION

The energy requirements of laying hens have been widely studied and reviewed. One of the first equations relating feed intake of layers to dietary energy level was devised by Hill (1956). Hill's work showed that, over a wide range of dietary energy concentrations, the regulation of energy intake was quite precise in (what were then considered to be) "high-producing" White Leghorns. However Morris (1968) showed that the adjustment of feed intake to maintain the same energy intake was far from perfect. The ability to adjust feed intake was shown to be strain dependent; the degree of overconsumption was correlated with the 'characteristic calorie intake' of the strain. Heavier strains with a high energy intake adjusted their feed intake less efficiently than lighter strains with a low energy intake. Thus Morris concluded that the widely held principle that birds adjust their feed intake to maintain a constant energy intake is not tenable and this in turn will affect the formulation of a diet designed to minimise the cost of feeding.

Gous *et al.* (1987) showed that energy concentration has no effect on egg production other than via its effect on feed intake resulting in a change in the intake of the first limiting amino acid. On the other hand these authors reported that feed intake may be affected by changes in the levels of amino acids as well as by energy concentration. Despite the findings of Gous *et al.* (1987) there have been some recent reports (e.g. Jackson *et al.*, 1999) suggesting that diets with ME levels lower than approximately 11.9 MJ/kg are inadequate to support maximum production of modern White Leghorn strains during the early and middle stages of lay. In an Australian trial using ISA Brown birds (Balnave and Robinson, 2000), the apparent effect of dietary ME level on feed intake was partly attributed to changes in diet density (or bulk) which occurred concomitantly with changes in ME level. Although at the lowest energy level and density the ME intake appeared to be well below what is normally considered to be the daily requirement, egg output was not significantly affected.

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There appears to be little other information on dietary ME concentrations or ME requirements relevant to ISA Brown and other modern brown egg layers. Harms *et al.* (2000) fed diets with ME levels of 10.5, 11.7 and 12.8 MJ/kg to four strains of bird, including Hy-line Brown. Hens fed the low energy diet consumed 8.5% more feed than those on the medium diet, while hens on the high energy diet consumed only 1.5% less feed, indicating that "hens are more sensitive to lowering the energy than increasing the energy in the diet". Egg production was unaffected by energy level. Most studies of the relationship between energy requirements, energy intake and dietary energy level have been done using diets which undoubtedly varied in bulk and fibre content as well as ME (Cherry *et al.,* 1983). Although it is known that dietary bulk (volume/weight) or density (weight/volume) has an important role in the regulation of feed intake of poultry, this characteristic is rarely considered in the formulation of diets for laying hens. The trial described here compared different diet densities and metabolisable energy levels in two strains over a 48-week period.

II. METHODS

The birds were fed wheat, sorghum, soybean meal, sunflower meal, meat and bone meal and tallow based diets ranging from 10.3 to 12.2 MJ/kg (five ME levels) and 19-20% protein. All diets were balanced for amino acids requirements as per breeder specifications. Two series of diets were used: (1) with bulk and fibre increasing as ME level declines; (2) with bulk and fibre held constant at approximately 1.3 litres/kg and 40 g/kg, respectively. The two extreme diets for each density were first designed and prepared, representing the two combinations of the lowest and highest ME level and fixed or floating density. The other three diets were then made by mixing appropriate proportions of the extreme diets. ME of the diets were determined prior to the commencement of the experiment by the total collection method and using ISA Brown and Hy-line Brown birds. Specified densities were also arrived at by using ingredient density values entered in the Feedmania Least Cost Diet program matrix. The values used were "effective densities", determined by measuring the change in diet density when a typical proportion of the ingredient is included in a diet consisting of a typical blend of ingredients. The actual densities of all the experimental diets were verified by weighing a fixed volume of the prepared diet. Amino acid levels were adjusted to ensure that the daily requirement of every essential amino acid was met.

There were thus 2 x 5 x 2 treatments, each represented by six 8-bird replicates (1 replicate = four two-bird cages). Sixty groups of eight ISA Brown (brown) and sixty groups of eight Hy-line Brown pullets were housed in layer cages at eighteen weeks of age, each group consisting of four adjacent two-bird cages. Six groups of each strain were allotted to each of the ten dietary treatments in a randomised design. The diets were fed for four consecutive periods of approximately twelve weeks. Measurements including feed intake, egg production, mortality and sample egg weights were recorded for each period; and body weights were recorded at 18 and 66 weeks of age. Egg production data are on the basis of five-days' records per week.

III. RESULTS

The results reported here are based on cumulative data from 18 to 66 weeks (Table 1). ISA Brown birds consumed less feed and energy, laid larger eggs and gained less weight than the Hy-line Brown birds (all $P<0.01$). Conversion of feed to egg mass was more efficient by ISA brown birds but this was just outside significance level ($P<0.056$). Each increase in dietary ME level resulted in decreased feed intake, increased energy intake and body weight gain and improved feed conversion ($P<0.01$). Dietary ME had no effect on rate of lay, egg

weight and egg mass. Diet density, whether floating or fixed, had an effect only in energy intake were birds on fixed density diets consumed more energy (P<0.01). There was also a trend toward increasing feed intake and body weight gain and improved feed conversion when birds were offered diets with fixed density (P<0.05). There were significant energy by density interactions for feed intake and feed efficiency (P<0.05). At the two lowest ME level (10.3 and 10.8 MJ/kg), the floating density diets resulted in higher feed intake than the highest energy and float or fixed density diets. There was no difference in feed intake when diets were either fixed or floating and had an ME of 11.2 MJ/kg (120g/d) or 11.6 MJ/kg (115g/d). However, at the highest ME level (12.2 MJ/kg) birds on fixed density diet consumed 3g/d more feed than birds on floating density diet. The responses of the two strains to changes in dietary ME and density were quite similar for all parameters except body weight gain. With increasing ME, the differences between means for body weight change of ISA bird was 86g compared to 120g for Hy-line birds. The increase in energy intake with increasing dietary ME level was similar for both strains. However, there was little variation in energy intake with dietary ME levels in the range 10.8-12.2 MJ/kg for both strains.

Table 1. Layer performance 18–66 weeks of age.

	Feed intake (g/d)	**ME intake (MJ/d)**	**Rate of lay (%)**	**Egg wt (g)**	**Egg mass (g/d)**	**FCR (FI/egg mass)**	**Body wt change (g)**
Strain							
ISA brown	118.10	1.364	86.75	64.30	55.77	2.13	437.4
Hy-line	120.31	1.386	87.05	63.68	55.44	2.17	595.0
LSD(P<0.05)	1.43	0.016	NS*	0.57	NS	0.05	48.34
LSD(P<0.01)	1.89	0.022					64.01
Dietary Energy (MJ/kg)							
11.0	123.37	1.325	85.73	63.44	54.38	2.28	437.3
11.3	123.01	1.371	87.22	63.99	55.81	2.21	519.4
11.6	120.70	1.372	87.23	64.36	56.12	2.16	554.8
11.9	116.06	1.399	87.36	63.77	55.69	2.08	528.9
12.2	112.88	1.408	86.99	64.37	56.02	2.02	540.8
LSD (P<0.05)	2.26	0.026	NS	NS	NS	0.07	76.44
LSD (P<0.01)	2.99	0.034				0.09	101.21
Density							
Float	118.90	1.39	87.40	64.00	55.93	2.14	504.1
Fixed	119.51	1.36	86.41	63.97	55.28	2.17	528.4
LSD(P<0.05)	1.43	0.016	NS	NS	NS	NS	NS
LSD (P<0.01)		0.022					

*NS Not Significant

IV. DISCUSSION

The results indicate that dietary energy content, but not density, is an important determinant of feed and energy intake. A simple interpretation of the results is that both strain overconsumes energy at dietary ME levels above 11.2 MJ/kg and under consumes

energy at dietary ME levels below 10.8 MJ/kg. The "typical" ME intake of these strains appears to be about 1400 kJ/d. These intakes were more or less met by diets with ME values in the range 11.6-12.2 and 10.8-12.2 MJ/kg, respectively, for ISA Brown and Hy-Line Brown birds. The energy intake of the two strain are in close agreement with the figure of 1370-1380 kJ/d interpolated from data of Harms *et al.* (2000) for Hy-line Brown and Grobas *et al.* (1999) for ISA Brown birds and a value of 1403 kJ/d recorded by Balnave and Robinson (2000) for ISA Brown birds on a diet containing 11.4 MJ/kg. Egg mass output appears not to be affected by increase in dietary energy but feed conversion efficiency improved with increase in dietary energy. This was for both strains. The diets with the highest ME level (12.2 MJ/kg) were the most efficient in terms of feed-to-egg mass. In both strains, the lower egg mass output at the lowest ME level (10.3 MJ/kg) resulted in poorer feed conversion than with diets of higher ME, but energy conversion was similar to diets containing 11.6 or 12.2 MJ/kg ME. In these strains energy conversion was optimised with diets containing11.2MJ/kg. The feed intake/ dietary ME plots of both strains together are consistent with an S-shaped model which assumes that each strain of bird has a characteristic range of dietary ME levels over which energy intake is relatively constant, while outside this range energy is under or over consumed. This model is consistent with the data of Harms *et al.* (2000), who showed that feed intake of ISA Brown hens decreases by only 1.5% when ME intake increases from 11.7 MJ/kg to 12.9 MJ/kg (compared with an 8.5% increase between 11.7 and 10.5 MJ/kg), resulting in considerable over consumption of energy. It would be convenient to suppose that the characteristic ME range (over which energy intake remains relatively stable) is related to the typical body weight of the strain, but data from other sources suggest this may not be the case. The pattern of energy intake found by Robinson (2001) for Hy-line Brown is much higher than in the present study. ISA Brown and Hy-line Brown strains were both efficient at adjusting feed intake to maintain energy intake when fed diets varying in ME content and either floating or fixed density over a limited range. Daily energy intake increased as the energy level of the diet increased even though daily feed consumption decreased as the energy level of the diet increased. Clearly, the birds were not able to fully compensate to a constant energy intake as the energy level of the diet increased. This extra energy intake did not influence bird performance. Both strains 'over consumed' energy when given diets containing 11.2 MJ/kg. A reasonable interpretation of the results is that changes in feed intake were mainly attributable to dietary ME level while diet density had little influence on feed intake. The apparent effect of density on other performance criteria was probably due to differences in fat content of the diets.

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EVALUATION OF AUSTRALIAN CANOLA MEAL FOR PRODUCTION AND EGG QUALITY IN TWO LAYER STRAINS

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Summary

During 1999-2000, canola meal (CM) from Newcastle (Australia) was evaluated at Department of Primary Industries (DPI), Poultry Research and Development Centre for production performance and egg quality in ISA brown and White Supertint birds. During the 14-week experimental period CM gave satisfactory performance in both strains, fed at 100, 150 and 200 g CM/kg with no mortalities occurring. Diets containing 150 and 200 g CM/kg produced fishy odour in fresh eggs only from ISA brown birds. But this taint (odour) was substantially reduced when eggs were stored at 10°C for 2 to 5 weeks. White Supertint layers did not produce 'fishy' eggs at any CM level. Sensory evaluations found no 'fishy' taint in cooked eggs for any strain at any CM level.

I. INTRODUCTION

Canola meal (CM) is often limited to relatively low dietary inclusion levels in layer diets (40-80 g/kg) due to problems associated with feeding rapeseed meal diets which can affect performance, produce thyroid, skeletal and liver abnormalities, and generate a 'fishy' or 'crabby' taint in the eggs of brown layers (Butler *et al*., 1982; Leeson *et al*., 1986). Australia has bred improved canola lines in the last 10 years, with low levels of anti-nutritional factors in the meal which are generally less harmful. These CM are widely used for laying hens with levels as high as 10% causing no apparent adverse effects. The development of new strains of laying birds in the poultry industry, and the improved new canola varieties in combination with better industry procedures for oil extraction provide new grounds for investigating increased CM inclusion levels in poultry diets. This paper reports on the evaluation of a solvent extracted CM from Newcastle fed to two layer strains for production performance and egg quality.

II. MATERIALS AND METHODS

Solvent extracted Newcastle CM produced by Cargill Australia from the 1999 and 2000 crop were first analysed for chemical composition, non-starch polysaccharides, tannins, glucosinolates, and sinapine. Ileal digestible amino acid (AA) and apparent metabolisable energy (AME) bioassays were determined using broilers aged between 37-42 days and individually caged layers, respectively (Perez-Maldonado, 2003). Results of analyses and bioassays are presented in Table 1. Following chemical analysis and bioassays, four layer diets (11.5 MJ AME/kg) with levels of 0, 100, 150 and 200 g CM/kg were formulated with varying levels (g/kg) of sorghum (481-598), wheat (107-177), meat and bone meal (48-50), soybean meal (16-97), full fat soybean meal (38-41), sunflower meal (20-70), di-calcium phosphate (0.5-0.8), and limestone (74-76). A commercial mineral and vitamin pre-mix with a yolk pigment was added to all diets that were formulated on a digestible AA basis, and were steam pelleted (70-80°C). Each of the four experimental diets was offered to individually

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housed ISA brown and Inghams White Supertint layers from 26 weeks of age during the 14-week experimental period. Layers were housed in single-level cages in sheds, with adjustable shutters ridge-vent and thermostatically controlled fans and water misters, and provided with food and water *ad libitum* and light for 15.5 h/d. There were eight treatments comprising a control diet plus all combinations of three levels of CM (100, 150, and 200 g/kg) fed to two strains of bird (ISA brown and Inghams White Supertint) with 24 birds for each treatment in a randomised block design. Data were analysed as 4 levels (control, 100, 150, 200 g CM/kg) x 2 strains (brown and white) x 24 replicates/block. The analysis of variance and treatment means were compared using the protected LSD method at P=0.05. The strains main effect means are presented in Table 2.

During week 8 of the experiment, eggs from each hen were collected and evaluated on the same day (fresh eggs) for yolk colour and egg odour by three experienced DPI operators. During week nine, similar egg collections were performed with half of these eggs cool-stored at 10°C for two weeks while the other half remained cool-stored for five weeks, after which yolk colour and odour were evaluated by three experienced DPI operators. The treatments mean results for odour and yolk colour were analysed as a frequency percent where an egg was deemed to have fishy odour if either 2/3 or 3/3 operators agreed that the egg had an odour. Results are presented in Table 3.

During week 14, 15 eggs from each of the control, 100 and 200 g CM/kg levels were sent to the University of Queensland for an egg sensory evaluation test using the traditional triangle test method to determine differences between the control eggs and the various treatments. Results are presented in Table 4.

Approval for this trial was obtained from DPI's Animal Research Institute Animal Ethics Review Committee.

III. RESULTS AND DISCUSSION

Samples of CM from the 2000 harvest had less crude protein, fat and NDF compared with the 1999 harvest with no major differences found in AME and AA composition (Table 1). However, the AA digestibility coefficients substantially differed in both harvests indicating the need for evaluating CM in each harvest season.

Feeding Newcastle CM to ISA brown (IB) and Inghams White Supertint (IWS) at 100, 150 and 200 g/kg (not presented in this report) did not affect production performance at any level of inclusion with no mortalities occurring during the 14-week period. However, IB birds laid more ($P<0.05$) eggs with lower weights, consuming less feed, thus having better feed efficiency than IWS birds which laid heavier eggs producing similar egg mass as the brown strain (Table 2).

The observations made by the three experienced DPI operators on fresh and stored eggs derived from two strain hens fed on graded levels of Newcastle CM indicated that in IB layers, CM treatments led to production of 'fishy' odour ($P<0.05$) in fresh eggs at 150 and 200 g CM/kg but not at 100 g CM/kg. All CM generated fishy odour in stored eggs from brown hens but only significant ($P<0.05$) at 200 g CM/kg with no detrimental effect on yolk colour at any CM level in the diet. However, the panel did not detect any 'fishy' odour from eggs from IWS birds (Table 3).

This problem of 'fishy' taint in eggs laid by brown hens consuming high levels of CM is due to sinapine which is present in the meal at about 12 g/kg DM. Sinapine is unable to be absorbed and metabolised by hens and passes through the intestine where it is metabolised by enteric bacteria to form choline, and further trimethylamine (TMA). Most brown birds are unable to metabolise TMA, thus subsequently divert it into the ova, producing a 'fishy' taint in eggs (Butler *et al*., 1984). Eggs produced from IWS layer strain did not produce 'fishy'

eggs, indicating that in these birds the TMA produced from sinapine was effectively metabolised by TMA oxidase. This indicates that although high levels of CM support good egg production in brown birds, no more than 100 g CM /kg in the diet should be added without risking generation of 'fishy' taint in eggs. However, IWS hens were able to support similar good performance to brown strains with up to 200 g CM/kg in the diet without affecting their egg quality.

Table 1. Composition (g/kg DM), amino acid (AA) digestibility and AME of Newcastle canola meal obtained from the 1999 and 2000 harvests.

Evaluations	**1999**	**2000**	**AA Digestibility**	
			1999	**2000**
Dry matter	905	906		
Crude protein	414	394		
Phosphorus	12.0	11.8		
Calcium	8.4	7.8		
Sulphur	7.0	7.4		
Fat	49.4	39.0		
Free condensed tannins	34.2	36.4		
Bound tannins	10.1	10.8		
Sinapine	11.8	11.7		
Glucosinolates (µmol/g)	2.0	3.4		
NDF	327	284		
Arginine	25.3	27.7	0.64	0.85
Leucine	24.9	24.5	0.69	0.75
Lysine	18.8	19.6	0.63	0.73
Methionine	8.1	5.5	0.77	0.86
Phenylalanine	13.6	14.7	0.69	0.75
Cystine	9.4	8.5	0.72	0.67
Glycine	17.8	17.3	0.67	0.72
Histidine	8.8	9.4	0.74	0.81
Isoleucine	13.4	14.7	0.63	0.71
Threonine	15.8	16.0	0.56	0.66
Tryptophan	4.9	5.3	0.67	0.80
Valine	16.6	17.6	0.62	0.70
Layer AME (MJ/Kg DM)	11.0	11.7		
Layer AMEn	10.4	10.0		

Table 2. The main effect of bird strain on egg production (lay %), egg weight (g), egg mass (g/d), feed intake (FI (g/d)), feed conversion ratio (FCR; (FI/egg mass)), bird weight (kg), specific gravity (gravity), liver and pancreas weight (g/100 g body weight) when hens were offered Newcastle canola meal diets.

Bird Strain	**Lay**	**Egg wt**	**Mass**	**FI**	**FCR**	**Bird wt**	**Gravity**	**Liver**	**Pancreas**
ISA brown	92.8[a]	62.4[a]	57.9	127.4[a]	2.21[a]	2.04	1.083	2.22[a]	0.193
White ST	90.6[b]	63.6[b]	57.6	131.3[b]	2.29[b]	2.08	1.084	2.60[b]	0.179
LSD P=0.05	2.0	1.1	1.6	3.3	0.06	0.06	0.002	0.30	0.036

Values within a column with different superscripts are significantly different (P<0.05); ST=supertint; wt.= weight

The sensory evaluation of cooked eggs performed at The University of Queensland showed that in all five treatments evaluated, neither eggs from the IB nor from the IWS strain fed 100 or 200 g CM/kg were different in flavour from the untreated control. This result indicates that the 'fishy' taint in eggs derived from brown layers fed diets containing substantial amounts of CM may disappear during cooking. Panellists were unable to detect fishy taint in cooked eggs (that was present when uncooked) and no differences were detected between eggs from control birds and birds fed CM at either 100 or 200 g/kg levels.

Table 3. Results of fishy taint (%) and yolk colour evaluations in fresh, 2 and 5 weeks (wks) stored eggs obtained from ISA Brown and White Supertint layers when offered 100, 150, and 200 g/kg of Newcastle canola meal (CM).

Treatment	**Strain**	**Odour (fresh)**	**Odour (2 wks)**	**Odour (5 wks)**	**Colour (fresh)**	**Colour (2 wks)**	**Colour (5 wks)**
Control	ISA brown	0 [b]	0	0 [b]	12.2	11.9	12.1
100 g CM/kg	ISA brown	0 [b]	8	8 [ab]	12.2	12.2	12.2
150 g CM/kg	ISA brown	17 [ab]	8	0 [b]	12.4	12.3	12.1
200 g CM/kg	ISA brown	42 [a]	33	30 [a]	12.1	12.1	12.1
LSD	(P=0.05)	27	26	23	0.3	0.3	0.3
Control	White ST	0	0	0	12.3	12.1	11.8
100 g CM/kg	White ST	0	0	0	12.4	12.2	12.2
150 g CM/kg	White ST	0	0	0	12.4	12.3	12.1
200 g CM/kg	White ST	0	0	0	12.4	12.3	12.1
LSD	(P=0.05)	NA	NA	NA	0.4	0.3	0.4

NA= not analysed. ST= supertint

Table 4. Results of sensory evaluation test of cooked eggs obtained at week 14 from ISA brown and White Supertint strains when offered 100 and 200 g/kg of Newcastle canola meal. Significance of the comparison of canola meal levels with untreated control.

Layer consuming	**Bird Strain**	**Probability**
100 g CM/kg	White Supertint	0.956 (ns)
200 g CM/kg	White Supertint	0.521 (ns)
100 g CM/kg	ISA brown	0.848 (ns)
200 g CM/kg	ISA brown	0.848 (ns)

ns= not significant P>0.05

IV. ACKNOWLEDGEMENTS

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THE POULTRY TRANSPORT THERMAL ENVIRONMENT - MATCHING "ON-BOARD" CONDITIONS TO THE BIRDS PHYSIOLOGICAL REQUIREMENTS.

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Summary

Modern broiler production systems involve the rearing of large numbers of birds on geographically dispersed sites and their subsequent transportation on purpose-built modular vehicles to centralised processing plants. Transport represents a potential risk to bird welfare and product quality. Fuller understanding of the origins of transport stress and characterisation of the birds' biological requirements allow the design and development of improved methods of transportation. This review considers the role of the transport thermal micro-environment in the etiology of physiological stress. Physiological modeling has been employed to define acceptable ranges and limits for thermal variables upon the vehicle. Improved broiler transport vehicles (mechanically ventilated) have been developed from this knowledge using sound engineering principles, thus matching micro-environments to the birds' physiological needs.

I. INTRODUCTION

Global production of poultry for meat increases year upon year. Thus, in 2002, world production of broiler chickens was approximately 45.5 billion birds and for turkeys 661 million (FAOSTAT, 2003). Whilst the major producer nations are the USA, China and Brazil, substantial contributions are made by many other countries. The European Union had broiler and turkey outputs of 5 billion and 245 million, respectively, during the same period whilst production in Australia and the United Kingdom reached their respective peaks at 416 million and 808 million broilers, the corresponding figures for turkeys being 7.4 million and 23 million. A striking feature of the geographical distribution of meat bird production is the range of climatic zones and conditions in which highly successful industries have developed. Thus, poultry are reared in temperate, equatorial and sub-artic nations under tropical and arid conditions with a very wide spread of average, minimum and maximum temperatures. This success is partly attributable to the provision of suitable birds by the major broiler breeder companies but perhaps the most important factor is the matching of the "broiler house" environments and nutritional regimes to the birds' requirements. This allows expression of the birds' genetic potential for growth and efficiency. Good control of the "in house" thermal environment is essential and ensures minimisation of physiological stress and suppression of desirable production traits. In the production cycle of a broiler flock, regulation of imposed thermal loads and thus stress may be ensured throughout the growth period. However, at the point of slaughter the journey from the site of production to the processing plant may constitute a substantial risk to the well-being or survival of the birds and may compromise productivity through mortalities, down gradings and reduced product quality. A primary cause of these problems is the thermal micro-environment to which the birds are subjected in transit (Mitchell and Kettlewell, 1998; Mitchell *et al.,* 2001). Whilst environmental control in the poultry house is usually well managed and efficient, little attention has been paid in the past to the transport thermal micro-environment. The vagaries of "passive" vehicle

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regimes, coupled to an economic necessity to transport large numbers of birds on each journey, often leads to heat stress even in relatively temperate external conditions. Similarly, poorly controlled and very heterogeneous ventilation regimes, resulting in both over ventilation and under ventilation under low external temperature climates, can precipitate extreme cold stress or paradoxical heat stress in birds in those locations in the bio-load which are most vulnerable (Mitchell *et al.,* 2001; Mitchell and Kettlewell, 2002). In order to address these issues a collaborative research project exploiting the complementary disciplines of animal physiology, environmental science and bio-engineering has pursued the objective of matching the broiler transport conditions to the birds' physiological requirements.

II. METHODS

Physiological modeling: All experiments were performed upon 6-week old broiler chickens. Birds were held in commercial transport crates and placed in controlled climate chambers for a period of three hours (typical of commercial journeys). Chamber temperature and relative humidity were controlled throughout the experimental period to ± 0.2°C and ± 5%. Various combinations of temperature-humidity were employed in the range 10-35°C and 30-95%. Temperature and humidity within the transport containers were continuously monitored using Tinytalk data loggers. Bird rectal temperatures were taken immediately prior to and following exposure to each thermal load. Blood samples were obtained at these times and pH and pCO_2 determined immediately by means of an automated blood gas analyser (Ciba-Corning 238). Thermoregulatory success (deep body temperature) and thermoregulatory effort (blood pH and gas disturbances) were correlated with the actual imposed thermal load (temperature-humidity combination).

"Apparent Equivalent Temperature" (AET) was used as an index of thermal load. This parameter is derived from the temperature, water vapour pressure and the psychrometric constant and describes the total heat exchange between a wetted surface and the environment.

$$\theta^*_{app.} = \mathbf{T + (e/\gamma^*)}$$

where θ^*_{app} = AET
T = absolute temperature (K)
e = water vapour pressure (mbar)
γ^* = corrected psychrometric constant (mbar K^{-1})

$$\gamma^* = \gamma \mathbf{(r_v/r_h)}$$

where r_v = the resistance to water vapour transfer (sm^{-1}) and r_h = the resistance to heat transfer (sm^{-1}).

Using the AET approach the combinations of temperatures and humidities which produce equivalent biological effects were determined (Mitchell and Kettlewell, 1998; Mitchell *et al*., 2001). The relationships between change in deep body temperature, blood gas and acid-base parameters, indicators of cell pathology and AET were established. The response patterns to thermal load allow definition of temperature-humidity combinations imposing mild, moderate and severe physiological stress. This approach has allowed identification of “safe”, “alert” and “danger” combinations of temperature and humidity which equate to mild, moderate and severe physiological stress. The model thus permits the definition of thermal comfort zones for broilers in transit as presented in Figure 1.

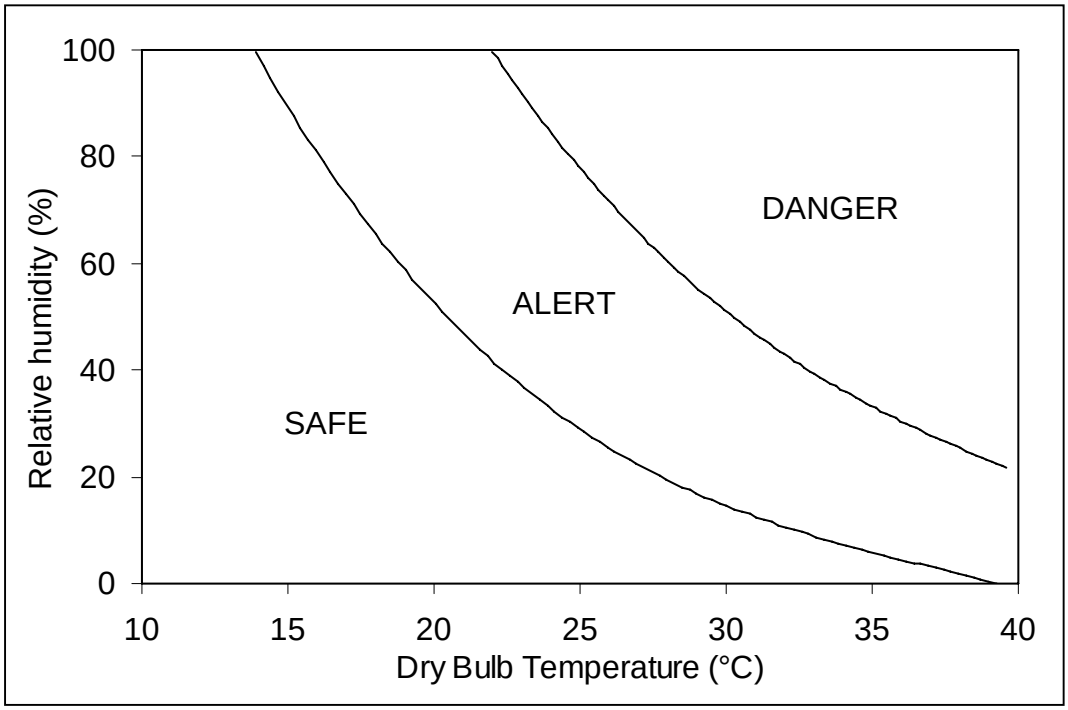

Figure 1 "Thermal Comfort Zones" for broiler transport Safe limit AET = 40°C ; danger limit AET = 65°C or greater

At temperature-humidity combinations yielding AET values between of 40-45°C or less thermal stress will be minimal in transit. At temperature-humidity combinations giving AETs between 40-45°C, moderate thermal stress will occur with some degree of hyperthermia and acid-base disturbances. At AETs of 65°C or greater, physiological stress may be deemed severe and mortalities will increase. Such thermal loads must be considered unacceptable. Under practical conditions, where relative humidities in the transport containers rarely fall below 70% because of obligatory water loss from the birds, it can be recommended that the maximum "in-crate" allowable temperature, compatible with good welfare and productivity therefore, should be 26-27°C. In parallel studies, a similar modeling approach has been adopted to define the lower limits for dry bulb temperature consistent with good welfare and productivity in birds exposed to air movement, with both dry and wet feather cover (Mitchell *et al.,* 2001; Mitchell and Kettlewell, 2002). Further studies have measured metabolic heat and moisture production of broilers under a range of thermal conditions upon vehicles (Kettlewell *et al.,* 2001ab)). Integration of the defined acceptable thermal envelope with knowledge of heat and moisture output has allowed calculation of ventilation volume flows necessary to dissipate heat and moisture to maintain acceptable "on-board" thermal environments under different climatic conditions. These findings have formed the basis for the design, development and implementation of mechanical ventilation systems for commercial broiler transport vehicles (Kettlewell and Mitchell, 2001ab).

In conclusion, physiological response modeling has been employed to define acceptable ranges and limits for thermal conditions during the transportation of broiler chickens. This novel experimental approach has provided data specific to transportation conditions and which are directly applicable to commercial practice. The optimum thermal envelope for broiler carriage has been defined in terms of the "physiological thermal comfort zones" and factors precipitating or contributing to the incidence of thermal stress have been identified. The findings have been employed as the sound scientific basis for improvements in vehicle design (in particular ventilation systems) and transport practices.

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CONSUMER CONCERNS AND FEED INDUSTRY RESPONSE TO THOSE CONCERNS

PATRICK GARLAND

Summary

The UK livestock and associated supply industries have faced several testing challenges in the form of food safety scares over the last 20 years. Not only have there been issues which impact on human health, there have been concerns expressed about potential risks associated with novel technology in the form of genetic modification (GM) as well as the use of antibiotics in animal feeds and welfare of intensively farmed livestock. This paper summarises the steps taken by the UK industry to restore confidence in food production and in particular the response of the feed industry to demonstrate the safety of animal feeds.

I. INTRODUCTION

Despite the high profile food safety issues experienced in the UK since the late 1980's (*Salmonella* in eggs, bovine spongiform encephalopathy (BSE) and *E.coli* in beef) the UK consumer has a high degree of confidence in the safety of food offered for sale. In detailed consumer studies some 75% of respondents expressed confidence in the food available and there was a general view that whilst the link between Creutzfeldt-Jakob Disease (CJD) and BSE was shocking, over two thirds of consumers were not concerned about the risk to themselves of BSE (IGD, 2000). In a previous survey British consumers were found to regard farming and food manufacturers standards in the UK as being superior to those overseas (IGD, 1999).

However, to say that the UK consumer has no concerns about food would be incorrect, from the same series of studies it was determined that in relation to food production the single greatest area on which concern is expressed is that of hygiene at the food processing site and on farm. Following close behind were animal welfare issues then GM.

These concerns are not necessarily clearly expressed in the decision making process leading to the purchase of food since price is the single most influential factor with GM, origin and source of ingredients being relatively minor factors (IGD, 2000). This apparently confusing picture is perhaps better understood when in light of the fact that food safety is regarded as a given. Regardless of the price, status or origin the UK consumer expects all food on offer to be safe.

The apparent confidence is possibly a reflection of the efforts undertaken by government, food retailers, farming industries and the feed industry to prevent reoccurrence of crises already seen and to minimise the chances of another BSE-like situation occurring.

The response of the feed industry has been guided by a number of external factors as well as by determining its own needs. At the outset legislative requirements and government codes have provided a base line upon which other interested parties have built their own requirements.

II. INDUSTRY STRUCTURE

To an extent there has been an evolution amongst the larger compounders to develop mills dedicated to species groups whilst smaller businesses have had to decide which sectors are their core business activity and exit from less attractive areas. This began to happen in the

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wake of the *Salmonella* problems in the poultry industry, with broiler breeder feeds being taken back into integrated mills and the integrators trying to produce as much as possible of their feed requirements themselves.

Following on from *Salmonella* the BSE crisis stimulated the greatest changes in the industry. It was clear that mills handling meat and bone meal would not be looked upon favourably by the dairy sector. This encouraged compounders to specialise manufacture at individual mills even after the complete banning of mammalian protein in 1996. This had several advantages in that mammalian protein materials could be excluded from cattle mills without impact on monogastric formulations, whilst materials of a higher salmonella risk normally associated with ruminants were not present in pig and poultry mills. Also, capital investments at all plants could be directed at the target market. This has driven the current structure and distribution of the UK feed industry. The intensive livestock industry has migrated east to where the bulk of the cereals are produced and the ruminant sectors have gravitated to the west where it is economic to have pasture. Therefore we now see relatively few mills making ruminant rations in the east. Capital investment in relation to food safety issues has allowed development of heat-treated mash for poultry breeding stock, heat treatment of broiler and turkey rations and acid addition for all classes of poultry. Most poultry lines in monogastric mills are now equipped with box or bunker type coolers rather than horizontal flat deck coolers, presses and conveyors are lagged and trace heated to prevent condensation and food grade stainless steel is used downstream of mixing or heat treatment. These investments have been encouraged by the close monitoring of feed and manufacturing processes for *Salmonella* and total enterobacteriacae, the latter quickly highlights areas of weakness and the potential for undesirable bacterial contamination.

Monitoring of feeds for drug residues has also encouraged investment in facilities that reduce the risk of additive carry over between batches.

III. CODES OF PRACTICE

There have been and continue to be many codes of practice within the agricultural industry and they are continually changing to reflect consumer expectations. Initially there were government codes with regard to salmonella, and then individual retailers developed their own codes of practice. The latter evolved to address new concerns and, to some extent, compete with each other. This led to an ever increasing cost of compliance and since the retailer codes were usually directed at specific industry sectors those industry sectors developed their own codes of practice.

We now have a situation where most retailers have a code of practice for each livestock sector. These inevitably vary in degree of detail but most will address feed issues and require audits of feed mills by their own staff or representatives. These audits are usually facilitated via the retailers' suppliers who might also require access to audits.

Since the retailer codes can create some duplication and the livestock industry sectors have recognised the need to promote their products, a logical step is to try to create species codes of practice that are acceptable to all retailers. Therefore there are codes of practice for all of the main livestock groups. Again, these all contain feed controls.

In response to this situation and having a genuine desire to demonstrate the professional approach taken by feed manufacturers the compounders trade association, United Kingdom Agricultural Supply Trade Association (UKASTA) created a set of assurance schemes which cover all aspects of the manufacture and supply of livestock feeds. The schemes apply to:

- Sources of Feed Ingredients
- Producers and Distributors of Feeds
- Production and Delivery of Compound Feeds
- Storage, Haulage and Laboratory Analysis of Combinable Crops

The above are covered by three schemes, namely:

UFAS (UKASTA Feed Assurance Scheme) the first scheme upon which the set of three is based. It has additional modules which cover manufacture of non GM feeds and a major retailer's (Tesco's) additional requirements. The scheme is independently audited to the European standard EN45011.

FEMAS (Feed Materials Assurance Scheme) covers the sourcing and production of feed ingredients back to country of origin. By July 2004 all ingredients will have to be FEMAS compliant. This scheme is also accredited under EN45011.

TASCC (Trade Assurance Scheme for Combinable Crops) covers the handling of combinable crops once they leave the farm. Auditors are accredited under UKAS (UK Accreditation Service).

Both the UFAS and FEMAS schemes are based on HACCP principles (Hazard Analysis and Critical Control Point) and member companies are expected to use HACCP within their own documented operating procedures.

A significant success of UFAS is that it has been recognised as a credible and robust means of ensuring high standards of feed manufacture. In turn a number of retailers have accepted the scheme and do not require their own audit of feed facilities making rations for their suppliers. The same applies to livestock sector assurance schemes. This has reduced the number and cost of external audits that individual feed mills are expected to host and ensures that the standards required are consistently achieved. Another advantage is that the objective of UFAS is for feed mills to reach an acceptable standard rather than, as with some retailers codes, to follow prescribed procedures to meet the required level of safe feed manufacture.

IV. LIVESTOCK SECTOR CODES

Since the feed and poultry industries are inextricably linked it is appropriate to briefly discuss the three main sector codes of practice in the context of addressing consumer concerns. These are the Code of Practice for Lion Eggs, Assured Chicken Production and Quality British Turkey. In all cases full traceability of food products is available and the detailed codes or standards cover all aspects of production from parent stock, hatchery, growing or laying farms, processing and all inputs including feed. As previously mentioned, the feed element is covered by requiring producers to use feed from UFAS (or equivalent) accredited mills.

The Lion Code is the longest standing of the three and is arguably the most successful to date. It is an integral part of the egg marketing campaign run by the British Egg Industry Council with all eggs from approved producers having the Lion logo printed on them as well as a use by date. A significant investment in television and poster advertising has raised the public awareness of eggs as a healthy, nutritious and convenient food. The fact that the reduction in human salmonellosis has been correlated with the compulsory *Salmonella* vaccination of all Lion Code hens has helped reinforce the positive image of eggs. The success of the Lion Code and the marketing campaign is measured in a reversal of the long term decline in egg consumption to give an actual increase of approximately 15 eggs per capita.

The Assured Chicken Production (ACP) scheme can now boast 90% of UK produced chicken as being under its guidance. All aspects of chicken production from breeding to

processing including Poussin and Free Range are covered. It has allowed the industry to respond in a proactive manner to consumer issues by providing well balanced and logical responses to some of the media inspired criticisms of poultry farming. As with other schemes ACP accreditation allows members to display the Red Tractor logo which signifies that the product meets the requirements of the British Farm Standard, which is independently monitored by Assured Food Standards.

V. GM, WELFARE AND ANTIBIOTICS

These three areas are recognised as areas of concern for UK consumers (IGD, 2000) but in real terms there is relatively little that the feed industry can do to allay those concerns. The most practical response is to endeavour to supply, where required, feeds of non GM origin, without the inclusion of antibiotic digestive enhancers and have feeds available for extensively produced livestock. This is not without difficulty since meeting a broad range of needs for basically similar nutritional products creates product proliferation on a huge scale. Typically in a monogastric feed mill between 200 and 250 base product formulations can be offered before any medicated rations are considered. The non GM requirements of customers vary considerably: some have no concern, so that USA material can be used, through northern Brazilian, which is not meant to be GM (at present); others specify less than one percent GM with full traceability. This means that some mills will carry three different sources of HiPro soya.

The feed industry has responded to both the demands of its customers and the general sentiment of the UK consumer. It has become much more open to inspection and audit. Most companies now have an open mill policy and the routine disclosure of information regarding raw material and finished product analysis as well as microbiological status is expected.

In recent years there have been several major food safety incidents related to feed and feed ingredients in the EU, these have included dioxin contamination of fats, antibiotic residues in fishmeal, hormone contamination of molasses, herbicide in organic wheat and veterinary residues in imported chicken meat. The UK feed industry has not been involved in any of these situations and there is good reason to credit this to the successful implementation of the codes put together by the industry. The fact that EU feed legislation will enshrine much of the UFAS and FEMAS codes is also recognition of their value.

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VOLATILE FATTY ACIDS AND ESSENTIAL OILS (BIACID) IMPROVE TECHNICAL PERFORMANCE OF BROILERS

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Summary

Antimicrobial growth promoters (AGP's) will soon no longer be available to the poultry producer in Europe and a similar outcome is envisaged for Australia. This paper discusses and reports on the research and developmental efforts within Provimi to provide the poultry industry with an effective alternative to AGP's consisting of a blend of volatile fatty acids and essential oils. A basic understanding of the mode of action is presented to provide a scientific basis for the efficacy of the novel product.

I. INTRODUCTION

There is continued concern that the unrestricted use of sub-therapeutic levels of feed antibiotics may be associated with the development of antibiotic-resistant human pathogens. Within the EU, public and political pressure has led to legislation banning AGP's in animal feeds by 2006. Phasing out AGP's from commercial diets may well have some negative impact on performance and profitability of the entire animal meat producing sector including the broiler chicken industry. A review of the literature by Rosen (1996) indicated that in 12,153 trials, the addition of AGP's to animal diets increased productivity 72% of the time. On average, broilers responded with a 3.6% higher weight gain and a 3.4% improved feed conversion. The magnitude of the responses observed varied and depended on such factors as diet formulation and general health and management status on the farms. In essence, AGP's have clearly demonstrated their benefit over the years and now extensive research efforts are being conducted world-wide to find and develop appropriate alternatives. This has proven to be a complex, tedious and, at times, frustrating process.

II. DEVELOPING ALTERNATIVE PRODUCTS

(a) Comprehending the challenge

In order to develop good alternative products it is important and helpful to understand the mode of action of AGP's (for review see Thomke and Elwinger, 1998). Most common AGP's exert a beneficial effect on health and performance by modifying the microflora in the gut. They have typically a broad spectrum activity against most gram-positive microbes and pathogens. Even under normal physiological and intestinal conditions the microflora in the gastrointestinal tract of poultry is a very complex ecosystem. Poorly digested nutrients, in particular, water-soluble non-starch polysaccharides (NSP) are the main causative substrates disrupting this sensitive ecosystem. In poultry, NSP carbohydrates can only be utilised by means of fermentation and this is mainly associated with, but not limited to, an increase in population of Escherichia coli, Clostridium spp. and enterococci and that impacts negatively on bird health and performance.

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(b) Characterisation, identification and screening for effective volatile fatty acids

Volatile fatty acids (VFA) have been used as feed and drinking water additives in poultry for many years. Historically, in its traditional chemical form and texture, their beneficial effects have been mainly limited to the crop and gizzard by directly reducing bacterial growth in the ingesta but not having any significant effect in the intestinal tract since they are metabolised and absorbed. Today, technology exists to impregnate VFA into small micro-beads in order to obtain a slower and gradual release in the intestine and ceca of the chicken and, thus preserve and extent their bacteriostatic activity.

Organic acids and VFA are easily absorbed through the bacterial cell wall of the pathogen. The molecule dissociates and the released hydrogen ions cause pH changes in the bacterial cytosol that interferes with normal enzyme activity and cell replication which, ultimately, leads to cell death.

Organic acids commonly used in the feed industry include formic, acetic, propionic, butyric, lactic, sorbic, fumaric, malic, tartaric and citric acid. Normally, the industry relies on mixtures of organic acids or their salts because specific mixtures have often demonstrated additive or synergistic effects. However, according to Pinchasov and Jensen (1989) too high an administration of VFA might be associated with a decrease in feed intake and, thus, reduced weight gain. Table 1 shows the results of a study comparing an AGP and two organic acids at two concentrations on selected performance criteria of broiler chicks.

Table 1. Effect of an antibiotic growth promoter and two organic acids (Acids X and Y) on mean weight gain, feed consumption and feed conversion of male Ross 308 broiler chickens (age 7 - 21 days)

	Weight gain (g)	**Feed consumption (g/b/d)**	**Feed conversion (g/g)**
Negative control	616	73.9	1.68[a]
Positive control	617	72.7	1.65[b]
Acid X (1000 ppm)	614	73.9	1.68[a]
Acid X (2000 ppm)	609	73.1	1.68[a]
Acid Y (250 ppm)	623	73.9	1.66[ab]
Acid Y (500 ppm)	625	73.7	1.65[b]

Source: Provimi's Research M98. Statistical procedure: ANOVA, using Genstat 5 software.
[a,b] Means in a column without a common superscript are significantly different ($P<0.05$).
Number of animals: 6 replications per treatment with 20 broiler chickens per pen.
Negative control: no antibiotic growth promoter; Positive control: 10 ppm Flavomycine.

The results indicate that the positive control with the AGP improved feed conversion significantly over the negative control. There were no differences in feed consumption or weight gain. Acid X, regardless of concentration, did not affect any production parameters compared to the negative control. In contrast, Acid Y at 500 ppm concentration improved feed utilisation to the same extent as the positive control.

The beneficial effects of Acid Y from the initial study needed further conformation. A new trial was conducted in which Acid Y at 500 ppm was again compared against a positive and negative control group. The obtained data is summarised in Table 2.

The results of the second trial confirmed the performance enhancing effect of Acid Y on feed conversion. There were no differences in weight gain between the treatments.

(c) Characterisation, identification and screening for effective essential oils

Essential oils are volatile, natural vegetable products extracted from herbs and spices pre-dominantly by steam distillation methods. There are at least 2600 reported essential oils. Many of them can be produced synthetically. Numerous modes of action for their bactericidal effects have been identified. The most important ones are associated with facilitation of increased bacterial cell wall permeability and inactivation of enzyme systems. *In vivo*, the effects range from stimulating appetite, enhancing secretion of pancreatic enzymes and facilitating beneficial modifications in intestinal microflora. The use of certain essential oils in combination might have additive and/or synergistic effects although some antagonistic responses have been reported as well. Careful testing and validation is therefore critical in finding appropriate and effective commercial blends. In this regard, Provimi conducted a series of studies to identify those essential oils that stimulate the secretion of pancreatic enzymes. The most promising candidates were further screened by using *in vitro* procedures to identify those candidates that had the most significant bacteriostatic effects against common pathogens in poultry. At the end of all this work, selected products were fed to groups of broiler chickens and compared against positive and negative control animals. The results of this trial are shown in Table 3.

Table 2. Effect of an antibiotic growth promoter and an organic acid (Acid Y) on mean weight gain, feed consumption and feed conversion of male Ross 308 broiler chickens (age 10 - 28 days)

	Weight gain (g)	**Feed consumption (g/b/d)**	**Feed conversion (g/g)**
Negative control	902	92.2	1.90[a]
Positive control	944	96.0	1.83[b]
Acid Y (500 ppm)	907	93.2	1.85[b]

Source: Provimi's Research M101. Statistical procedure: ANOVA, using Genstat 5 software.
[a,b] Means in a column without a common superscript are significantly different (P<0.05).
Number of animals: 6 replications per treatment with 20 broiler chickens per pen.
Negative control: no antibiotic growth promoter; Positive control: 10 ppm Flavomycin.

Table 3. Effect of an antibiotic growth promoter and two essential oil products (Products A and B) on mean weight gain, feed consumption and feed conversion of male Ross 308 broiler chickens (age 5 - 28 days)

	Weight gain (g)	**Feed consumption (g/b/d)**	**Feed conversion (g/g)**
Negative control	1393	100.5	1.66[a]
Positive control	1401	98.1	1.61[c]
Product A	1389	99.6	1.65[ab]
Product B	1382	97.9	1.63[bc]

Source: Provimi's Research M103. Statistical procedure: ANOVA, using Genstat 5 software.
[a,b,c] Means in a column without a common superscript are significantly different (P<0.05).
Number of animals: 6 replications per treatment with 20 broiler chickens per pen.
Negative control: no antibiotic growth promoter; Positive control: 10 ppm Avilamycine.

The AGP improved feed conversion significantly over the negative control group. There were no differences on either weight gain or feed consumption. Essential oils

designated as Product A had no effect on any parameters tested whereas Product B improved feed conversion significantly. Statistically, in terms of feed conversion, Product B and the positive control group were similar. The conclusion of the trial is that carefully chosen essential oils might improve performance in broilers.

(d) Combining volatile fatty acids and essential oils

The essence of the research presented thus far is that, both, organic VFA and essential oils can independently improve broiler performance. Since VFA appear to act predominantly on the feed and in the crop and gizzard while the essential oils may be more active in the intestinal tract. Therefore, it seems logical that there may be additional benefits by combining the VFA and essential oils in one treatment group. This strategy has led to the development of a blend of VFA and essential oils (Biacid) that was tested against a common AGP and a negative control. The results of two studies are shown in Table 4.

Table 4. Effect of an antibiotic growth promoter and a mix of essential oils and volatile fatty acids (Biacid) on mean weight gain, feed consumption and feed conversion of male Ross 308 broiler chickens from 5-28 days (Trial 1) and 6-35 days (Trial 2)

	Trial	**Weight gain (g)**	**Feed consumption (g/b/d)**	**Feed conversion (g/g)**
Neg. Control	1	1393	100.5	1.66^{A}
	2	1824^{a}	109.3^{a}	1.67^{a}
Pos. Control	1	1401	98.1	1.61^{B}
	2	1894^{b}	106.3^{b}	1.63^{b}
Biacid	1	1390	97.9	1.62^{B}
	2	1890^{b}	104.2^{c}	1.60^{c}

Source: Provimi's Research M106/M131. Statistical procedure: ANOVA, using Genstat 5 software.
^{a,b} Means in a column without a common superscript are significantly different ($P<0.05$).
Number of animals: 6 replications per treatment with 20 broiler chickens per pen.
Negative control: no antibiotic growth promoter; Positive control: 10 ppm Avilamycine.

The AGP improved in both trials feed conversion significantly compared to the negative control whereas weight gain and feed consumption was only improved in the second trial. Conversely, the addition of Biacid yielded statistically a similar feed conversion ratio as the traditional AGP in trial 1. In trial two, the effect of Biacid on weight gain was also similar as the traditional AGP. However, the effect of Biacid on feed consumption and feed conversion was significantly better compared to the AGP group.

Based on the results of these laboratory trials it can be concluded that Biacid, a product consisting of a carefully selected blend of VFA and essential oils, can be an effective new feed additive for broiler chicks. Further research should elucidate the precise mode of action of this product in broiler chicks.

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TRANS-CAPSANTHIN ENHANCES EGG YOLK PIGMENTATION ON A WHEAT-SORGHUM BASED DIET

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The enhancement of yolk colour is a result of xanthophylls and associated carotenoids in the hen's diet. Saponified paprika (*Capsicum Annum*) and marigold (*Tagetus Erecta*) oleoresins are used in poultry feed for imparting red and yellow colour respectively. Trans-capsanthin and trans-lutein are the major active pigments in paprika and marigold respectively. High Roche Colour Fan (RCF) scores require both red and yellow pigments in certain compositions (Fletcher and Halloran, 1981 & 1983). This study investigated the effect of total carotenoids and trans-capsanthin levels in feed on yolk RCF scores. The quantity of yellow pigment was adjusted to accommodate the different concentrations of trans-capsanthin. ISA Brown hens were fed a wheat-sorghum based diet containing the various pigment treatments over a 4 week period from 54 to 58 weeks of age with yolk colour scoring done in the final week of the experiment. The yolk colour was measured by visual comparison with a Roche colour fan.

Effect of addition of different concentrations total carotenoids and trans-capsanthin in feed on egg yolk's RCF score

	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**	**Diet 7**	**Diet 8**
Total carotenoids g/t	10.44	10.42	11.17	11.83	13.57	12.88	18.68	18.00
Trans-capsanthin g/t	2.78	2.34	2.31	1.50	1.37	1.24	1.18	0.73
Feed intake g/hen/d	117.5^{c}	126.6^{a}	125.4^{a}	119.0^{bc}	125.8^{ab}	123.4^{abc}	123.8^{abc}	122.0^{abc}
% egg production	88.10^{ab}	87.20^{ab}	92.30^{a}	89.90^{ab}	90.50^{ab}	84.1^{b}	91.60^{ab}	87.10^{ab}
Egg weight, g	64.00^{b}	67.40^{a}	65.20^{ab}	65.60^{ab}	64.70^{ab}	64.70^{b}	65.90^{ab}	64.70^{b}
RCF Day 1	11.00^{a}	11.10^{a}	11.10^{a}	10.10^{b}	9.30^{d}	9.70^{c}	10.30^{b}	8.10^{e}
RCF Day 2	12.00^{ab}	12.10^{a}	11.80^{b}	10.70^{c}	10.10d	10.40^{d}	10.80^{c}	8.80^{e}
RCF Day 3	11.90^{ab}	12.20^{a}	11.70^{b}	10.70^{c}	9.90^{d}	10.40^{c}	10.30^{cd}	8.70^{e}
RCF Day 4	12.00^{ab}	12.10^{a}	11.80^{b}	10.70^{c}	10.00^{e}	10.30^{e}	10.20^{c}	8.60^{f}
RCF Day 5	11.80^{ab}	12.30^{a}	11.70^{b}	10.70^{c}	9.90^{c}	10.30^{d}	10.50^{cd}	8.70^{f}
RCF Score (Average)	11.74	11.90	11.62	10.58	9.84	10.22	10.52	8.58

[a-f] Means within rows with different superscripts differ ($P < 0.05$)

It can be concluded that trans-capsanthin derived from saponified paprika has a significant effect on yolk colour scores. This study shows that red carotenoids are more important than the total feed carotenoids level in achieving a higher colour score on a wheat-sorghum based diet.

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EFFECTS OF PHYTASE SUPPLEMENTATION OF LOW PHOSPHORUS DIETS ON LAYER PERFORMANCE.

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Only 30-40 per cent of phosphorus from plant sources is freely available for utilisation by poultry, the rest is in the form of phytate phosphorus. Phytate not only binds phosphorus but also exhibits other antinutritional properties in the feed (Ravindran *et al*., 1995; Camovale *et al*., 1998; Selle *et al*., 2000). For example, phytic acid may form bonds with and reduce the availability of amino acids and minerals. The current practice is to meet phosphorus needs by adding inorganic phosphorus sources to poultry feed. However, unabsorbed inorganic phosphorus and phytate phosphorus are excreted and may cause high phosphorus residues in the environment. It has been shown that dietary phytase supplementation improves the production performance of meat chickens (Ravindran *et al*.,1995; Selle *et al*., 2000) and laying hens (Um and Paik, 1999). This study was undertaken to compare the effects of different levels of two sources of phytase supplementation in a low phosphorus, sorghum soybean meal layer diet.

Experimental diets were fed to 48 week old ISA brown layers for a period of 16 weeks. Six diets were compared, standard P (3 g/kg non phytate P), low P (1.1g/kg non phytate P), low-P + phytase A at 150U, 300 U, 450 U/kg feed and low-P + phytase B at 300 U/kg feed. Each diet was offered to 24 replicates of 3 hens per cage. Daily egg production was recorded and all eggs laid in one day every four weeks were weighed. Feed intake, tibia ash, excreta phosphorus, yolk colour, haugh unit, egg breaking strength and shell thickness were measured at the end of the feeding trial.

Hens fed the low-P diet had inferior egg production performance ($P<0.05$) to those fed the standard-P diet. However, production was comparable between groups fed the different diets when the low-P diet was supplemented with phytase. Hens fed the low-P diet supplemented with phytase A at 450U/kg had a significantly ($P<0.05$) higher egg weight, total egg mass, feed intake, improved feed conversion and tibia ash than those fed the non supplemented low-P diet. Egg yolk colour was significantly ($P<0.05$) higher in the eggs laid by hens in the 450 U/kg phytase A supplemented group than in the other groups. Phytase supplementation had no significant effect on weight gain, mortality, shell thickness and egg breaking strength during the trial period. However, phosphorus excretion of the low-P groups was significantly ($P<0.05$) less than that of the birds fed the standard-P diet.

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STUDY ON HMB MICROBIAL INHIBITORY ACTIVITY

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Summary

Two studies were conducted to determine the anti-microbial potential of DL-2-hydroxy-4-(methylthio)-butanoic acid (HMB). Results showed that HMB was effective in inhibiting pathogenic bacteria, including E coli O8, O149 and O157, Clostridium perfringens, Salmonella pullorum, and fungi Aspergillus flavus and Fusarium graminum. The degree of microbial inhibition was closely related to HMB concentration and the medium pH. Equi-molar comparison suggests that HMB is less effective as an anti-microbial agent than propionic acid and potassium sorbate.

I. INTRODUCTION

DL-2-hydroxy-4-(methylthio)-butanoic acid (HMB) or liquid methionine analogue not only serves as a source of methionine but has other potential roles including microbial inhibition. HMB is a hydroxy acid, with four carbons and a methyl-thio radical, with a pKa of 3.86, similar to formic (pKa 3.75), acetic (pKa 4.76) and propionic (pKa 4.88) acid. This suggests that HMB has potential anti-microbial properties. Studies on the HMB anti-microbial role are reviewed by Dibner and Buttin (2002). With the present need to find alternatives to antibiotics, the potential role of HMB in this regard, needs to be evaluated.

(a) Study One: HMB Minimum Inhibition Concentration (MIC)

This study was conducted at the Microbiological Centre of Sichuan Agricultural University, China. In total five strains of pathogenic bacteria, i.e. E. *coli* O_{157}, O_8, O_{149}, *Salmonella pullorum*, *Clostridium perfringens*, and two species of fungi, i.e. *Aspergillus flavus* and *Fusarium graminum* were examined. These organisms were obtained from the China National Veterinary Institute in Beijing, further cultivated and diluted with saline solution to 10^6 organisms/ml, and kept in 4 ^oC prior to testing.

For the inhibition test, culture broth was prepared to nurture the organisms and liquid HMB (Rhodimet^{TM} AT 88) was introduced into the broth in gradient ppm (Table 1). Test organisms (0.1 ml) were inoculated into the broth and the tubes were incubated in 37 ^oC for 24 h, then the broth was checked for minimum inhibitory concentration (MIC). A clear broth indicated no microbial growth or total inhibition whilst a perturbed broth suggested a growth or presence. After MIC check the incubation continued for an additional 24 h, no growth represented minimum lethal concentration (MLC). For fungi, the incubation was at 28 ^oC for 72 h for MIC and 96 h for MLC. Results of bacteria inhibition are presented in Table 1.

Clearly, at a HMB concentration of 2400 ppm, all bacteria stopped multiplying. For fungi, results in Table 2 showed 2,000 ppm HMB was able to inhibit growth of *Fusarium* whilst 4000 ppm was required to stop growth of *Aspergillus*.

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Table 1. Effect of HMB concentration (ppm) on microbial growth (N=3, + means growth or presence and – means no growth)

HMB	E. *coli* O_8		E. *coli* O_{157}		E. *coli* O_{149}		C. *perfringen*		S. *pullorium*	
ppm	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
0	+	+	+	+	+	+	+	+	+	+
600	+	+	+	+	+	+	+	+	+	+
800	+	+	+	+	+	+	+	+	+	+
1000	+	+	+	+	+	+	+	+	+	+
1200	-	-	-	-	+	+	-	-	-	+
2400	-	-	-	-	-	-	-	-	-	-

Table 2. Effect of HMB concentration (ppm) on growth of fungi (N=3, + means growth or presence, – means no growth)

Fusarium *graminum*			Aspergillus *flavus*		
HMB	72 h	168 h	HMB	72 h	168 h
0	+	+	0	+	+
1,000	+	+	2,000	+	+
1,400	+	+	2,500	+	+
1,800	+	+	3,000	+	+
2,000	-	-	3,500	-	+
2,500	-	-	4,000	-	-

The effect of propionic acid (PPA) was tested under the same conditions, in which PPA at 1000 ppm stopped growth of both bacteria and fungi, its MIC and MLC should fall between 500-1000 ppm. Table 3 lists a summary of the MIC results of HMB and PPA. It should be noted that there were no PPA test concentrations between 500 and 1000 ppm. As such, the actual MIC of PPA appears to lie somewhere between 500 and 1000 ppm.

Table 3. MIC comparison between HMB and Propionic acid (PPA)

	HMB, ppm	PPA, ppm
E. *coli* 0_8	<1200	<1000
E. *coli* 0_{157}	<1200	<1000
E. *coli* 0_{149}	<2400	<1000
C. *Perfringens*	<1200	<1000
S. *pullorum*	<2400	<1000
Fusarium	<2000	<1000
Aspergillus	<4000	<1000

(b) Study Two: HMB Inhibition Zone By Petri Dish Test

The Oxford Cup method was employed to determine inhibition zone. HMB and other materials were from the same source as Study One. Petri dishes with potato dextrose agar (PDA) culture medium were prepared and sterilized. Inoculants (0.2 ml) were evenly sprayed onto the culture gel surface, the dishes were then dried at 37 ^{o}C for 30 min. HMB was diluted with buffer solution to obtain concentrations of 0, 1.0, 2.0 and 4.0 mg/g at a pH of either 4.5 or 6.5. DMSO and Flavomycin (both 500 ppm) were used as negative and positive controls.

A sterilized Oxford Cup was placed vertically on the gel surface, with no gap between the cup and the gel surface. Test solutions (HMB, DMSO and Flavomycin) were poured into the cup. The cup was full but no spillage was permitted. Since the cup had no bottom the test liquids (HMB, DMSO and Flavomycin) had a direct access to gel surface. The dishes were carefully transferred into the incubator and incubated at 37 ^{o}C for 12 h. The inhibition zone was measured around the edge of the cup. The strength of inhibition was proportional to the diameter of inhibited organism growth around the cup.

Table 4 shows the inhibition results from five bacterial inoculants at pH 4.5. The degree of inhibition exerted by HMB was concentration dependent, with 4.0 mg/g achieving the highest efficacy especially against E *coli* O_{149} and S. *pullorum*. Whilst Flavomycin displayed moderate inhibition with the *E coli* strains and *S. pullorum*, this antibiotic seemed to have lost its efficacy against C. *perfringens*.

Table 4. Inhibition zone (mm) of HMB, DMSO and Flavomycin at pH 4.5 (N=3)

Concentration mg/g	**E. *coli* O_8**	**E. *coli* O_{157}**	**E. *coli* O_{149}**	**Clostridium**	**Salmonella**	**Avg.**
HMB 0	20.3	21.7	20.3	20.7	30.7	22.74
HMB 1.0	22.0	23.3	24.7	21.7	28.0	23.94
HMB 2.0	22.0	22.0	24.0	21.0	27.3	23.26
HMB 4.0	23.3	24.7	30.7	22.3	32.7	26.74
DMSO 0.5	21.3	20.7	24.0	20.0	30.7	23.34
Flavomycin 0.5	22.7	23.7	24.0	20.3	31.3	24.40

Inhibition results at pH 6.5 were less effective and less pronounced than at pH 4.5. Positive responses were observed against E. *coli* O_{149}, but no inhibition was observed against other strains. Flavomycin inhibited 4 strains but had little effect against E. *coli* O_8.

Fungal inhibition was tested using a petri dish. The gel was prepared to contain graded levels of HMB, propionic acid and potassium sorbate and pH was adjusted to either 4.5 or 6.5. Two holes of 7 mm diameter were drilled in each dish, and fixed amounts of fungi inoculants were introduced into the holes. The dishes were incubated at 28 ^{o}C for 48 h, and inhibition zones were measured by diameter, the smaller the diameter, the stronger the inhibition was. Each treatment was repeated 3 times.

Data in Table 5 demonstrated that increase in HMB dosage led to a reduction in fungi growth zone, and higher inhibition was achieved at pH 4.5. HMB 4.0 mg/g did not completely eliminate fungal growth. In contrast, propionic acid at 2.0 mg/g achieved a total elimination of fungi regardless of the medium pH. Potassium sorbate displayed a more effective inhibition at pH 4.5 than pH 6.5, but was less effective than propionic acid.

Table 5. Comparison of fungi inhibition in petri dish test (mm)

	Aspergillus *flavus*		**Fusarium *graminum***		**Avg.**
pH	4.5	6.5	4.5	6.5	
Control	29.0	28.0	20.2	22.0	24.8
HMB, mg/g					
0.5	27.0	29.0	24.2	23.0	25.8
1.0	24.3	28.0	18.2	23.0	23.4
2.0	20.2	24.8	16.8	19.8	20.4
4.0	18.2	20.0	5.5	11.7	13.8
PPA, mg/g					
0.5	21.7	25.8	0	12.5	15.0
1.0	13.7	17.3	0	0	7.8
2.0	0	0	0	0	0
4.0	0	0	0	0	0
Potassium Sorbate, mg/g					
0.5	18.3	22.0	0	20.2	15.2
1.0	13.0	20.5	0	16.7	12.6
2.0	0	19.0	0	14.8	8.4
4.0	0	15.8	0	0	4.0

II. DISCUSSION AND CONCLUSION

Some short chain organic acids (SCOA) possess the ability to penetrate cell membranes by simple diffusion. Upon entry, the SCOA can release H^+ due to low pKa. Once in the bacterial cell, the higher pH of its cytoplasm causes dissociation of the acid, reducing the internal pH, hence disrupting the enzymatic reactions and nutrient transport systems, and impairing the normal functioning of the cytoplasm. The cells hence divert energy to pump out the extra H^+, which finally exhausts the cells, resulting in death (Cherrington *et al*., 1991). Formic, lactic, propionic and sorbic acid are widely used alone or in combination, for feed or food hygiene purposes.

The present results agree with Enthoven (2002) who studied the effect of HMB on *Salmonella enteritidis* at pH 4.5 or 6.75 in comparison with formic acid. His results showed inhibitory effects at pH 6.75, and a bactericidal effect at pH 4.5. The response was dose dependent at each pH level for both HMB and formic acid.

These studies demonstrate that HMB possesses anti-microbial properties but that in this role HMB is less effective than propionic acid and potassium sorbate. In general, commercial inclusion of propionic acid as a mould inhibitor is between 0.2-0.5 kg/t feed, whilst HMB is widely applied as a methionine source at 1.5-3.0 kg. The higher dosage of HMB may act as a partial substitute for commercial mould inhibitor. Further study is warranted to quantify HMB's efficacy in microbial inhibition.

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EFFECT OF *OCIMUM SANCTUM* (TULSI) ON INFECTIOUS BURSAL DISEASE VIRUS PATHOGENESIS IN BROILER CHICKENS

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Infectious bursal disease virus (IBD) is an acute highly contagious viral disease of young chickens between 3-6 weeks of age caused by avibirna virus and is characterised by immunosuppression as virus preferentially infects immature B-lymphocytes in the bursa of Fabricius. The available vaccines do not provide complete protection in infectious bursal disease as birds are protected clinically but not against bursal damage (Vakharia *et al*, 1993, Dybing *et al.*, 1998, Tsukamoto *et al.*, 2002). Recently alternative and traditional medicine is gaining importance worldwide. The present studies were carried out to evaluate antimicrobial and immunomodulatory activities of eco-friendly *Ocimum sanctum*, which is one of the most commonly used herb against several conditions such as cough, cold etc in India.

The dried leaf powder (DLP) and steam distilled extracted essential oil of *Ocimum sanctum* were tested in two weeks old broiler chickens (Cobb) divided in to three groups treated with DLP, oil and untreated respectively. These birds in different groups were kept under cage system separately under hygienic housing conditions. One half of birds in each group were experimentally infected with approximately $1x10^5$ $TCID_{50}$ dose of Georgia strain of IBD per bird orally on 5^{th} day of experiment so as to make six experimental groups. Chickens of DLP treated groups were fed with nontoxic dose i.e. 200 mg of DLP per bird daily for 25 days while chickens in oil treated groups were given orally essential oil of *Ocimum sanctum* @ 10µl/ bird daily for 20 days. The studies included bursal index, gross changes and histopathology of visceral organs (bursa, spleen and thymus) and were conducted at 5, 10, 15, and 20 days post infection (DPI). The virus titration and neutralising antibody titration were carried out at 5, 15 DPI. These studies revealed decrease in virus titre and neutralising antibodies responses as compared to control in DLP and oil treated birds at 15 DPI. Also there were markedly reduced gross (haemorrhages in thigh muscles) and microscopic lesions (depletion of lymphocytes and atrophy in bursal follicles) in chickens treated with DLP of *Ocimum sanctum*; on the other hand essential oil treated show enhancement of disease as evidenced by vasodilatation and haemorrhages in thigh muscles (grossly) and lymphocyte depletion in bursa (histopathologically). These findings may be of future application if the toxic component as evidenced by local vasodilatation and haemorrhages in thigh muscles of oil treated birds is identified and detoxified from the essential oil. Thus preliminary results suggest *Ocimum sanctum* could be useful for improvement of available vaccines as well as for enhancing immune response of immuno compromised chickens.

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QUALITATIVE RISK ASSESSMENT FOR THE USE OF ANTIBIOTICS IN POULTRY PRODUCTION – HUMAN HEALTH IMPLICATIONS: AVILAMYCIN RISK ASSESSMENT

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For many years there has been some measure of concern in the public health community that the use of antibiotics in food animals could select for antibiotic resistant bacteria that would cause food borne disease, or transfer their resistance genes to human pathogens, and result in decreased efficacy for similar types of human use antibiotics. In Australia, the Joint Expert Technical Advisory Committee on Antibiotic Resistance issued a report in 1999 with specific recommendations to address the issue. Recommendation 1 required a risk assessment for the use of antibiotics used as growth promoters and was to follow the Australian Pesticides and Veterinary Medicines Agency (former National Registration Authority) Part 10 qualitative risk assessment outline. Additionally, the scheduling of all antibiotics to S4 status (prescription only) was to be evaluated (Recommendation 6).

The Part 10 process of risk assessment includes hazard identification, evaluation of exposure, impact and benefit:risk assessment to guide risk management options. Avilamycin, used in poultry production to enhance feed efficiency and improve weight gain in broilers, was evaluated for potential human health impact. Avilamycin (actually compromised of several related factors) is primarily active against Gram positive bacteria. Throughout the1990s, a related human-use clinical candidate, evernimicin, was in the development phase with a leading pharmaceutical company, however, due to human toxicity, it was abandoned in 2000. The potential for cross-resistance in enterococci (which causes endocarditis in humans) between the two molecules was therefore non-existent and eliminated the potential for compromising a new antibiotic for human use. The remaining possibility that co-resistance selection in chickens (i.e., use of avilamycin could select for persistence of another resistance gene) and subsequent transfer to "human" strains of enterococci in the human intestine was the basis of the hazard identification. The exposure component of the assessment evaluated surveillance data from chickens and humans in Europe, chicken contamination and dose-response relationships and literature reports of gene transfer, as well as the prevalence of nosocomial enterococcal infections in Australia. The conclusion was that risk to human health was negligible; and that based on the benefits to animal production, the continued use of avilamycin as a non-S4 product was recommended. In the minutes of the October, 2003, National Drugs and Poisons Scheduling Committee meeting this recommendation was upheld, based on advice from the Expert Advisory Group on Antibiotic Resistance, that 1) evernimicins were not used in human medicine, 2) there was no evidence that avilmaycin promoted co-resistance with vancomycin resistant enterococci, and 3) it has a low and acceptable risk of promoting antibiotic resistance in humans. Risk assessment offers drug sponsors and regulatory authorities a defined path to provide the necessary information.

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FOOD REGULATION AND THE POULTRY INDUSTRY

A. S. HILL

Summary

While Australia has one of the highest levels of food safety in the world, we still experience more than five million cases of food poisoning each year, mostly of short-term duration and unreported. This high burden of food-borne illness is unacceptable in economic and human terms.

In response to the growing burden of food borne illness, Australia, along with many other countries, as well as the Codex Alimentarius, have identified a new approach that will more effectively manage food safety. The new approach identifies food safety areas and activities that contribute significantly to the burden of food borne disease on basis of a scientific risk assessment, and focus on preventive management strategies across the entire food chain that identify and minimise food borne hazards at the point in the food chain where the hazards are introduced.

Food Standards Australia New Zealand – also known as FSANZ – is an independent government agency responsible for developing standards for the handling, composition and labelling of food in Australia and New Zealand. FSANZ also develops food safety or food hygiene standards for Australia . New Zealand has its own processes for this purpose. FSANZ is a partnership between ten governments: the Commonwealth; Australian States and Territories; and New Zealand.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

FSANZ also has regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry; and
- the promotion of fair trading in food.

The governments of Australia decided, in 2000, to establish a new regulatory framework for food. This framework came into effect in July 2002 and has resulted in a new approach to safety in the food supply. There are four major elements of the new approach:

[1] Food Standards Australia New Zealand, PO Box 7186, Canberra BC, ACT, Australia.

- consideration of the food chain in its entirety for food safety purposes;
- a science-based assessment of risk at critical points in the food chain and the development of appropriate strategies to manage the risk and to communicate with stakeholders in the process (including industry and consumers);
- an emphasis on preventative rather than reactive measures; and
- a new shared responsibility for food safety between the primary production and health portfolios of government.

On 1 July 2002, FSANZ assumed responsibility for the development of Primary Production and Processing Standards for Australia and will commence work on the development of a standard for poultry meat in February 2004. These standards will be outcome-based and focus on food safety. The standards aim to:

- increase public confidence in the safety of food products;
- provide nationally consistent standards that will set a benchmark for industry obligations to produce safe food;
- harmonise with international standards;
- ensure that food safety is addressed across the entire food chain; and
- provide minimum impost on industry to achieve the most effective food safety outcomes

A Standards Development Committee (SDC), with representation from industry, consumer, government and research organisations will provide advice and input to FSANZ in the development of each standard. The standard-setting process will involve public consultation with all primary industry sectors, stakeholders, consumers and other interested parties.

The Primary Production and Processing Standards will, for the first time, provide nationally consistent food safety regulations that are mandatory and enforceable across Australia. They will also apply to imported foods and the Australian Quarantine Inspection Service (AQIS) will ensure that importers comply with the new regulations.

A RISK PROFILING STUDY FOR THE AUSTRALIAN CHICKEN MEAT INDUSTRY

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Summary

The aims of this project (commissioned by Agriculture Fisheries and Forestry – Australia) were to develop a risk profile for chicken meat in Australia. They focussed on the following key areas:

- Identification of pathogen(s) – food commodity combination(s) of concern
- Provide a description of current health situation/problem along the entire supply chain to the consumer
- Food production, processing distribution and consumption
- Potential public health risks in the future
- Potential overarching/high order intervention options
- Identification of data gaps

I. RISK PROFILE

Campylobacter and *Salmonella* are the two leading causes of bacterial enteric disease in Australia (NEPSS[2], 2003; OzFoodNet, 2003). Poultry meat is one, but not the only, product that is frequently contaminated with both organisms (Szabo and Eyles, 1995; Institute of Medical and Veterinary Science, 2003).

The most common *Salmonella* serovars reported from human sources and chicken/poultry do not appear to have changed substantially since the report of Szabo and Eyles (1995). *S*. Sofia has now been the predominant *Salmonella* isolated from chicken in Australia for nearly two decades (Harrington *et al.*, 1991; Heuzenroeder *et al.*, 2001). Studies have shown that *S*. Sofia can act as a natural competitive exclusion agent for some *Salmonella* serovars, but not the virulent serovar *S*. Typhimurium. Both *S*. Sofia and *S*. Typhimurium can co-colonise regardless of initial colonisation status. *S*. Sofia appears to have low virulence for humans and appears to cause little human disease (Heuzenroeder *et al.,* 2001).

This risk profiling report reviewed published and unpublished data on the incidence and types of pathogens isolated from chicken meat. This data is limited and is useful as a baseline for future survey work. One of the recommendations arising from this study was that work continues in the area of *Salmonella* ecology as this is essential to enable a rapid response to any shift towards more virulent salmonellae as has occurred overseas (ICMSF[3], 1996). Future studies on chicken carcasses and cut product should concentrate on the pathogens of concern, *Salmonella* spp. and *Campylobacter* spp. rather than indicator organisms.

[1]Food Science Australia, PO Box 52, North Ryde, NSW 1670, Australia
[2]National Enteric Pathogens Surveillance Scheme
[3]International Commission on Microbiological Specifications for Foods

The National Risk Validation Project (2002) reviewed ~250 recorded foodborne illness outbreaks in Australia over the last 10 years (collated from published, State Health Departments and OzFoodNet reports). Of a total of 38 associated with chicken meat, 12 were attributed to *Salmonella*, 8 *to Clostridium perfringens* and 3 to *Campylobacter*. These figures refer only to outbreaks which were investigated and do not include sporadic incidents with which *Campylobacter* is most commonly associated (Frost, 2001). Four incidents were attributed to *Staphylococcus aureus* and one to *Listeria monocytogenes*. In recent years, surveillance of foodborne disease across Australia has been enhanced substantially via the OzFoodNet network with 86 and 92 outbreaks reported in 2001 and 2002, respectively (OzFoodNet, 2002; 2003).

The economic burden of foodborne illness in Australia is estimated at an average cost of $630 per case (ANZFA (Australia New Zealand Food Authority), 1999). ANZFA also estimated the total annual cost of foodborne illness in Australia at over $2.6 billion. If it can be assumed that *Salmonella* infections account for approximately one quarter of these cases and chicken is the vehicle for one-sixth of these infections, then the annual cost which could be attributed to chicken-mediated *Salmonella* infection is substantial indeed (~$100 million). If *Campylobacter* infections account for 14716 of 23414 cases (OzFoodNet, 2003) then the total cost of *Campylobacter* infections in that year could be estimated at something over $1 billion. While the proportion of these cases attributable to chicken is not known, there is sufficient data available to indicate that the contribution to this figure by chicken is significant.

Of note is the limited data available on outbreaks associated with the pathogens and/or product of concern addressed in this risk profile compared to the substantial serotyping data for these products that is reported in the NEPSS Annual Reports and the Australian *Salmonella* Reference Centre (Institute of Medical and Veterinary Science, South Australia).

Salmonella serovars confirmed as being associated with foodborne salmonellosis include *S*. Typhimurium PT 126 and PT 135. These serovars are also found on chicken meat. A number of case-control studies have conclusively linked the clinical isolate and the food isolate in outbreaks and so it must be recognised that there is an association between chicken and foodborne salmonellosis. However, with the limited data available, it is only possible to approximate the number of the reported salmonellosis cases reported in Australia each year that can be attributed to consumption of chicken.

Even less data is available on foodborne outbreaks associated with *Campylobacter*. Case control studies of some outbreaks have confirmed *Campylobacter* on chicken meat as the causative agent by epidemiological and/or typing techniques. However, until more data is available, little can be concluded with respect to the role of *Campylobacter* and illness associated with consumption of chicken. It can only be assumed that because of the frequency with which *Campylobacter* is isolated from chickens, supported by the limited case-control data, there is an association between chicken meat and foodborne campylobacteriosis.

The National Risk Validation Project (2002) demonstrated that the major contributing factors to outbreaks of foodborne illness in Australia (and elsewhere) are temperature abuse and cross contamination. The Food Safety Standards 3.2.2 and 3.2.3 of the Australian Food Standards Code have the potential to address these issues in the food service and food retail sectors. It has to be accepted on the information available to us that a proportion of chicken meat in carcass or portioned form will carry either or both *Salmonella* and *Campylobacter*.

Whether these pathogens become a food safety hazard will depend in the first instance on how they are handled in the market place and in the second instance on how they are handled by consumers.

Obviously legislation cannot be used to modify consumer behaviour. Another recommendation arising from this risk profiling study was that existing programs to educate consumers in the correct way to handle all potentially hazardous foods be given stronger support.

This risk profile identified that there is insufficient data available, in the public arena, to assess how effective any risk management procedures may be. It is therefore not possible to say if changes to regulations for chicken meat processing to require the use of HACCP based food safety programmes is achieving the desired outcomes. It is recommended that support be given to on-going work such as the studies reported by King and Hornitzky (2001) and Sumner *et al.* (2003) for all States. It is only by having such baseline data that the effectiveness of risk management procedures can be ascertained (Rose *et al.*, 2002).

It is also recommended that a Working Party be established by Agriculture Fisheries and Forestry – Australia with the necessary expertise and experience of the Australian poultry industry to produce relevant Codes of Practice based on HACCP principles for all sectors of the chicken production and processing chain. These Codes must cover on-farm practices as well as processing and could be distributed throughout the industry prior to the introduction of any legislation aimed at the poultry meat industry and before any food safety objectives are defined.

It is recognised that measures which successfully exclude *Salmonella* spp. from broiler flocks may not be as successful with *Campylobacter*. However, it appears that properly implemented biosecurity systems will reduce the incidence of *Campylobacter* colonisation of flocks as well as *Salmonella*. There is also important research being conducted in Australia and overseas on the possible development of vaccines and competitive exclusion techniques to combat *Campylobacter*. These, however, would appear to offer assistance in the long term rather than the short term.

In summary, there is insufficient Australian data available to conduct meaningful quantitative risk assessments of *Salmonella* and *Campylobacter* in broiler chickens, chicken meat and chicken meat products. This study recommends that resources would be best utilised, in the short to medium term, in generating more data on the incidence, types and sources of these pathogens in the Australian situation – from farm to fork – and further quantifying their role in foodborne illness.

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AUTHORS INDEX