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(University of Sydney)

and

THE WORLD'S POULTRY SCIENCE ASSOCIATION
(Australian Branch)

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AUSTRALIAN POULTRY SCIENCE SYMPOSIUM
2014

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A HISTORY OF THE AUSTRALIAN POULTRY SCIENCE SYMPOSIA

D. BALNAVE[1] and E.F. ANNISON[1]

The Australian Poultry Science Symposia evolved from the earlier successful symposia of the Poultry Research Foundation (PRF) which began in 1978. The decision to extend the previously held occasional symposia to an annual event arose at meetings of the Director of the Foundation, Professor Frank Annison, the Research Director, Associate Professor Derick Balnave and the President, Dr Balka Bains. The first of the annual symposia was held in February 1983. Professor Balnave generously accepted responsibility for organising the symposia which also involved the time consuming tasks of editing submitted manuscripts and preparing the proceedings. These tasks became increasingly onerous as the numbers of participants grew at each symposium. Professor Balnave continued to accept these increasing burdens for the first few years after the PRF joined forces with the Australian Branch of the World's Poultry Science Association (WPSA) in 1989, as discussed below. Dr (now Professor) Wayne Bryden provided invaluable support to Professor Balnave during the establishment of the PRF Symposia.

At these PRF symposia distinguished overseas scientists were invited to present the latest research findings of relevance to the Australian poultry industry. This was an important extension arm of the Foundation and the means by which many overseas scientists and commercial personnel were brought into contact with Foundation members and other Australian research scientists and postgraduate students. The conference meetings were held in Sydney on the main campus of the University and the conference dinners were organised in various distinguished locations within the University grounds.

Dr Alan Sykes from the University of London and Dr Colin Whitehead from the Poultry Research Centre, Roslin, Scotland were the invited overseas scientists at the 1983 symposium and they were supported by a range of speakers from various Australian universities and State Departments of Agriculture, CSIRO and the poultry industries in Australia. During the next five years among the invited speakers from overseas were Professors John Summers from the University of Guelph, Canada and Kenneth Washburn, University of Georgia, USA, Dr Shmuel Hurwitz from The Volcani Center, Israel, Drs Michel Larbier and Gerard Uzu, France, Dr Colin Fisher from Roslin, Scotland, and Dr Mark Whitacre from Degussa AG, West Germany.

The success of these symposia is highlighted by the fact that by the third annual symposia it was necessary to introduce a Short Communications Section to meet the growing demand of submitted papers and in 1987 a further extension of the programme was made to classify papers into specific categories for invited speakers, major submitted papers, short submitted papers for oral presentation and poster presentations. The initial two-day symposium was quickly extended by a further half day to meet the increased registrations and paper submissions.

In addition to attending the annual conference each year a distinguished invited speaker also participated in a tour of major poultry centres in various states and this enabled those unable to attend the conference to hear the latest research findings from overseas. Initially these tours were also organised by Professor Balnave who travelled with the guest speaker but with the introduction of the annual APSS in 1989 the WPSA took over these travel responsibilities.

During these and subsequent years Foundation members were most generous in providing financial support through sponsorship of speakers and in providing for other

[1] University of Sydney

conference needs. In particular, the financial support received from the Australian Chicken Meat and Egg Research Councils was extremely important in establishing these early PRF symposia and their contributions are worthy of particular mention. Until his retirement in 1985 the untiring efforts of Mr Charles Morbey in meeting and encouraging financial support from industry and Foundation sources are also worthy of recognition. Mrs Deirdre Pudney and Mrs Noelene West later assumed responsibility for the day to day conference organisation and the preparation of the proceedings. Mrs Pudney and Mrs West provided efficient and tireless support for the first thirteen years of the APSS. Mrs Pudney retired from the Foundation in 2001 followed by Mrs West in 2003. After this time their responsibilities were taken over by Mrs Jo-Ann Geist who has continued to fill this demanding and supportive role in similarly efficient terms to the present time.

In 1987 the PRF executive was approached by the President of the Australian Branch of WPSA, Dr Bruce Sheldon, with the proposal that the PRF combine resources with the WPSA to organise an annual national conference that would maintain continuity with the established PRF Symposia. Accordingly, Dr Bains, President, Professor Annison, Director, and Professor Balnave, Research Director, met for discussions with Dr Bruce Sheldon, Mr R. Brewster and Dr Ray Johnson in which it was agreed that both organizations would combine resources to host an annual Australian Poultry Science Symposium (APSS), commencing in 1989. The aim was to present an annual conference that would attain the status of a national/international meeting. To this end all submitted papers were to be refereed. A high priority was to maintain the scientific merits of the previous PRF symposia while providing information of relevance to all sectors of the poultry industry. Special provision was to be provided for the presentation by postgraduate students of current results from university research programmes. Proceedings containing the papers presented at the conference were to be provided when participants registered for the symposium and every effort was to be made to produce these to a high professional standard.

An Editorial Committee was formed to organise the 1989 symposium. This consisted of Professor Balnave, Chair, Professor Annison, Dr Bruce Sheldon, Dr Ray Johnson and Dr Malcolm McDonald. The main responsibility for the symposium remained with the PRF and the Australian Branch of the WPSA assumed responsibility for the post-symposium tour. At the same time a Programme Committee consisting of Professor Balnave, Professor David Fraser, Dr Wayne Bryden, Dr Bruce Sheldon, Dr Ray Johnson and Mr R. Brewster was formed to plan the 1990 symposium.

The first APSS was held on the main campus of the University of Sydney on 7-8 February 1989. It proved to be a resounding success with strong support being received from all sectors of the industry as well as universities, WPSA, CSIRO and State Departments of Agriculture. Professor Trevor Morris from Reading University, UK, Dr G. H. Jackson from the Royal Agricultural Society of England and Professor Robert E. Moreng from Colorado State University, USA were the overseas guest speakers. They were supported by a large number of local speakers and topics were presented in the subjects of nutrition and feeding, genetics, disease, housing, feed composition and egg shell quality.

Attendance at the annual PRF symposia in the 1980s had approximated fifty to seventy participants each year and this figure increased to approximately one hundred at the first APSS in 1989. Attendance figures continued to increase reaching approximately 170 in 1999. The increased attendance was to a considerable extent the result of overseas registrations. Over one-third of participating delegates were from overseas in 1995 and 1997 saw the attendance of the first official scientific delegation from overseas, namely Hungary. The editing of submitted papers and the preparation of the proceedings were major workloads for the small PRF conference executive and after the first three symposia the Foundation and the WPSA agreed to alternate responsibility for editorial affairs on a three yearly basis. This

greatly eased the pressure on the Foundation conference executive and Professor Balnave in particular as he was responsible initially for both the conference organization and editorial matters. It was also a major factor in maintaining the high standard of the conference proceedings. This arrangement has continued until the present time and editorial responsibilities continue to be shared at various times by the two organizations although not necessarily on a three yearly rotation. Accordingly, at different times and for different periods of time Dr Ray Johnson, Dr Bob Pym, Associate Professor David Farrell and Dr Juliet Roberts, as well as Dr Peter Selle from the PRF, have assumed responsibility for editorial affairs (see Table). However, the organisation of the symposium has remained under the auspices of the Poultry Research Foundation.

Chairpersons of the Organising and Editorial Committees of the Australian Poultry Science Symposium, 1989-2014

Year	Organising Committee	Editorial Committee
1989-91	D. Balnave	D. Balnave
1992-94	D. Balnave	R.J. Johnson
1995-97	D. Balnave	D. Balnave
1998	D. Balnave	R.A.E.Pym
1999	D. Balnave	D.J. Farrell
2000	D. Balnave	R.A.E.Pym
2001	D. Balnave	D. Balnave
2002-03	W.L. Bryden	R.A.E. Pym
2004-06	T.A. Scott	T.A. Scott
2007	T.A. Scott	R.A.E. Pym
2008	W.L. Bryden	P. Selle
2009-10	P. Groves	P.Selle
2011-13	A.J. Cowieson	J. Roberts
2014	A.J.Cowieson/P. Groves	P.Selle

The international status achieved by the APSS was shown by the comments of the respected journalist William A. Dudley-Cash writing in the US magazine "Feedstuffs" in April 2001. He stated "one of the most outstanding poultry science symposiums is sponsored by the Poultry Research Foundation of the University of Sydney and the Australian Branch of the World's Poultry Science Association". In subsequent private correspondence with Professor Balnave he stated "the Australian Poultry Science Symposium is easily in the top 1% (if not number 1) of all poultry science meetings in the world. The breadth of topics is excellent, the quality of the information is excellent."

During these early years two important honour awards were presented at the annual symposia. Shortly after his death in 1982 the WPSA with the support of the PRF introduced the WPSA Syd Wilkins Prize. Syd Wilkins was a major figure in the Australian poultry scene being President of the Australian Branch of the WPSA, a Vice-President of the world body of WPSA and for many years Deputy President of the PRF. The award was presented for excellence in poultry research conducted by a young poultry scientist in Australia as evidenced by the presentation of a paper at the APSS. The presentation of the other major award, known as the Australian Poultry Award, had been made annually by the Australian Branch of WPSA at various important poultry industry meetings. With the growing importance of the APSS the Australian Branch of WPSA arranged for this presentation to be made at the official APSS dinner. This award is made to an Australian resident who has made

an outstanding contribution to poultry science or to the Australian poultry industry and is decided by secret ballot.

As indicated above, since Professor Balnave's retirement in 2001 the responsibility for organising the symposium has devolved to a number of people (see Table) who have maintained the high status in which the symposium is held both within Australia and overseas. This has meant continual evaluation and introduction of changes in the conference organisation. However, the principles defined for the first symposium still operate. Distinguished invited overseas scientists are supported by many Australian-based academics and research workers with input also from industry personnel. Proceedings are prepared to a high standard and are available to all registrants before the opening of the symposium. As has always been the case, the major problem relates to finding time to allow the presentation of all papers within the two and a half day time period of the symposium. Particular concern relates to the presentation of the large numbers of short communications submitted each year. Various options have been tried over the twenty five years of the symposium, including poster and video presentations, but it seems that oral presentations are still the preferred method for symposium participants. The symposium dinner was initially held in St John's or St Andrew's Colleges of the University of Sydney but in recent times has vacated the University colleges and now alternates between various prestigious non-University venues in Sydney. Since 2012 the pre-registration informal Avian Science Forum on the Sunday evening prior to the official opening of the symposium, introduced in that year by the then Director of the Foundation, Associate Professor Aaron Cowieson, has proved to be a great success. The symposium then extends over the next two and a half days in conference facilities on the main campus of the University of Sydney.

The only change from the conventional symposium organisation occurred in 2008 when the 50th anniversary of the Poultry Research Foundation was celebrated with a special 50th Anniversary Seminar in which past and current members of the Foundation and members of industry presented papers outlining the history and achievements of the Foundation. This document presents a wealth of information on Foundation staff and research activities from 1959 until 2008.

At the time of writing participation in the symposium remains strong with current registrations of well over two hundred annually. Those attending come from all sections of the poultry industry and the poultry research fraternity and include many from overseas. It seems that after twenty five years the APSS is a well established and recognised international scientific meeting that has met and even surpassed the hopes of its founding organisers. Members of both the Poultry Research Foundation and the Australian Branch of the World's Poultry Science Association should be rightly proud of their contribution to the success of this international symposium.

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM – EXTENSION OF RESEARCH AND DEVELOPMENT TO THE CHICKEN-MEAT AND LAYER INDUSTRIES FOR 25 YEARS

J. McLEISH[1]

It has been my privilege to participate in 22 of the Australian Poultry Science Symposia that have been held over the past 25 years. Clearly, the importance of extension, of communicating outcomes from research conducted at universities and other institutions, to the 'coal-face' nutritionist involved in the chicken-meat and layer industries results is self-evident. Curiously, communication of technical material about products from their suppliers tends to be more rather than less and I wish I could remember who said "putting up barriers tends to lock more information out then they keep in".

One cannot give enough recognition to the short papers presented at APSS and included in the Proceedings. In many instances, there 1-page papers are precursors to full papers in peer-reviewed journals but they have already been presented at APSS, in some cases several years in advance of their full publication. A vast amount of research work has been extended to the poultry industries during the 25 years.

It is noteworthy that the Poultry Research Foundation held their first annual science meeting in 1968 and the proceedings contained the vast tally of 5 papers. The first Australian Poultry Science Symposium (PRF and WPSA) was held in 1989 and contained nine invited papers, five long papers and fifteen short papers for a total of 29 papers. Ten years later, the 1999 APSS Proceedings contained seven invited papers, twenty-two long papers, and twenty-four short papers for a total of 53 papers.

APSS 2009 presented papers by topic group, which demonstrated the increasingly complex demands on the layer and broiler industries. The topics included climate change and industry sustainability (6 papers); enzymes, feed additives and nutrition (8 papers); hot topics (8 papers); gut microflora, probiotics and immunity (7 papers); incubation (4 pages); feed processing and digestive physiology (8 papers) welfare (6 papers); chick quality and early nutrition (4 papers); Marek's Disease and food safety (7 papers). A total of 58 papers.

If one can take one or two gems from each APSS is it a great return on investment. In the last 25 years we have seen poultry in Australia advance and currently Australia is number 17 in the world for chicken-meat production. The performance of birds is comparable to USA with 2 kg birds being produced at 35 days with a FCR of 1.64 to 1. Our layer industry is producing 1 kg of egg mass from 2.1 kg of feed.

Our industry is continuing to improve it efficiency year on year. The sheer amount of recycled protein and co-products generated by other sectors of the food industry that would have been discarded at great ecological cost, are now converted with high efficiency into valuable protein in the form of chicken- meat and eggs. Our industry is advantaged by the research and development, extension and rapid adoption via APSS. In addition to this, one of the most important services performed by APSS, is to encourage the future of our industries, our young graduates, post graduate students and post doctoral researchers, by providing this vehicle for people to meet and communicate.

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WPSA INVOLVEMENT IN THE AUSTRALIAN POULTRY SCIENCE SYMPOSIUM OVER THE PAST 25 YEARS: PROMOTING THE EXCHANGE OF KNOWLEDGE

A. NAYLOR[1] and J. R. ROBERTS[2]

The previous speakers have given a wonderful outline of the history and value of the Australian Poultry Science Symposium since its inception in February 1989. The role of WPSA in the symposiums origination and conduct has been explained and WPSA's contribution to and support of the symposium has been strong and will continue to be enthusiastically pursued as one of its major activities and accomplishments in Australia.

The World's Poultry Science Association came into being as the "International Association of Poultry Instructors" in 1912 and Australia has been involved in this organization from its very first organizational meeting. This body's first World's Poultry Congress was held in the Netherlands in 1921 but it wasn't until 1928 that the name "World's Poultry Science Association" was coined. By 1946 WPSA was well established in Australia with 2 affiliate and 9 individual members.

The objectives of WPSA are aimed at the advancement of knowledge of all aspects of Poultry Science and the Poultry Industry, mainly through dissemination of knowledge among interested people throughout the world. The Australian Branch of WPSA was officially established in 1956. With goals of facilitating and promoting the exchange of knowledge in all fields of the Poultry Industry in Australia it was a natural synergism and common desire that drew the WPSA and Poultry Research Foundation into a coordinated and cooperative embrace to produce and foster the Australian Poultry Science Symposium. Dr Balnave has described the initial synthesis involved in the establishment of the symposium and we well applaud the foresight of the team that developed the APSS concept and made it a successful and valuable component of Poultry Science in Australia and of high standing in the international poultry science field.

WPSA awarded the Syd Wilkins Award between 1984 to 2005 to encourage young researchers and this developed a basis within presentations made at APSS. WPSA also presents the Australian Poultry Award, for an Australian determined to have a made long-term outstanding contribution to poultry science and/or the Australian poultry industry and this award is presented annually during the APSS symposium dinner.

APSS remains the premier Poultry Science meeting in Australasia and is held in substantial international regard and we look forward to many more years of productive science expounded through its form, fostered and supported by the PRF and , of course, WPSA. WPSA is rightly proud of its involvement APSS.

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EFFECT OF NUTRITION ON BROILER BREAST MUSCLE DEVELOPMENT: POSSIBLE TRANSDIFFERENTIATION OF MYOGENIC SATELLITE CELLS TO AN ADIPOGENIC LINEAGE

S.G. VELLEMAN[1]

Summary

Adult myoblasts, also termed satellite cells, are myogenic stem cells responsible for all posthatch muscle growth. These cells are mesodermal precursor cells which can give rise to skeletal muscle, osteocytes, chondrocytes, adipocytes, and other mesodermally derived tissues. These different cell types will arise due to the cells responding to signals directing their cell fate determination. Satellite cells are extremely responsive to the mitogenic effects of their environment including nutrition (Asakura et al., 2001; Moore et al., 2005 a,b). Because satellite cells mediate all posthatch muscle growth through muscle fiber hypertrophy, it is necessary to understand the effect of immediate posthatch nutritional regimes including feed restrictions on satellite cell fate and the development and growth of muscle to ensure the maintenance or improvement of meat quality.

I. INTRODUCTION

The development of skeletal muscle occurs as a result of the proliferation and differentiation of myoblasts that fuse to form multinucleated myotubes. The myotubes further differentiate into muscle fibers expressing muscle specific contractile proteins. After hatch, continued skeletal muscle growth is dependent upon the proliferation and differentiation of myogenic satellite cells (also termed adult myoblasts). Satellite cells are myogenic stem cells that play an important role in muscle growth by proliferating, differentiating, and fusing with adjacent muscle fibers (Moss and LeBlond, 1971). The increased number of nuclei in muscle fibers coincides with increased protein synthesis and muscle fiber size enlargement. Thus, muscle growth and development can be divided into 2 distinct periods, hyperplasia and hypertrophy. Hyperplasia occurs during the embryonic period and is characterized by an increase in muscle fiber number. Posthatch muscle growth is termed hypertrophy and results in the enlargement of existing muscle fibers.

Since satellite cells are stem cells, they are multipotential in terms of their cellular fate. Satellite cells are derived from mesodermal cells which form the cellular lineage for skeletal muscle, adipocytes, and chondrocytes. Asakura et al. (2001) demonstrated in an in vitro study that satellite cells could be induced to follow myogenic, osteogenic, or adipogenic cellular pathways depending on the culture conditions. In vivo, the immediate posthatch period when feed restrictions are used by the industry coincides with the period of maximal satellite cell activity in broilers (Halevy et al., 2000; Mozdziak et al., 2002). Satellite cells during the first week posthatch are sensitive to thermal conditions (Halevy et al., 2001) and nutritional status (Halevy et al., 2000; Mozdziak et al., 2002; Velleman et al., 2010). The closer changes in thermal and nutritional regime are to hatch, the lower the bird's compensatory growth response for both body weight and breast muscle weight accretion (Halevy et al., 2000).

In commercial poultry operations, it is common during the immediate posthatch period for chicks to rely on nutrients from the yolk during shipping. In addition, control of metabolic disorders such as those resulting in leg problems and ascites has led to the industry

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recommendation of early feed restriction during the first 2 week posthatch (Arce et al., 1992; Acar et al., 1995). The logic is that short term feed restrictions applied early in life would allow the chicken to restore balance between supply and demand organs (Katanbaf et al., 1988; Acar et al., 1995). Final processing body weights would be achieved through compensatory gain. The concept of compensatory gain has been studied with mixed results due to factors including the timing, duration, and amount of the restriction (Auckland, 1972; Cherry et al., 1978; Malone et al., 1980; Ferket and Sell, 1989; Washburn, 1990). Studies have been conducted by Velleman and colleagues to determine if breast muscle morphological structure, gene expression, and satellite cell activity is the same in birds undergoing compensatory growth after an immediate posthatch feed restriction compared to birds reared in unrestricted conditions (Velleman et al., 2010; unpublished).

II. EFFECT OF POSTHATCH FEED RESTRICTION ON BROILER BREAST MUSCLE DEVELOPMENT AND MUSCLE TRANSCRIPTIONAL REGULATORY FACTOR GENE EXPRESSION

To address the effects of an immediate posthatch feed restriction on breast muscle formation, fertile eggs were obtained from a commercial broiler breeder company and hatched. The chicks were divided into a full-fed group and an 80% feed restriction group for the first 2 week posthatch. Samples of the breast muscle were studied using histology to assay structure and RNA analysis of gene expression of MyoD and myogenin. These genes were selected because of their role in regulating satellite cell proliferation and differentiation. MyoD expression is necessary for proliferation and myogenin is required for differentiation. Halevy et al. (2003) showed that the expression of MyoD and myogenin is affected by posthatch feeding in turkeys. The expression of MyoD and myogenin was affected during the first 4 days posthatch by the feed restriction (Figure 1). The restricted birds had a significant increase in MyoD expression through 4 days posthatch compared to the unrestricted or control group (Figure 1A). Myogenin expression was reduced about 50% in the feed restricted group (Figure 1B).

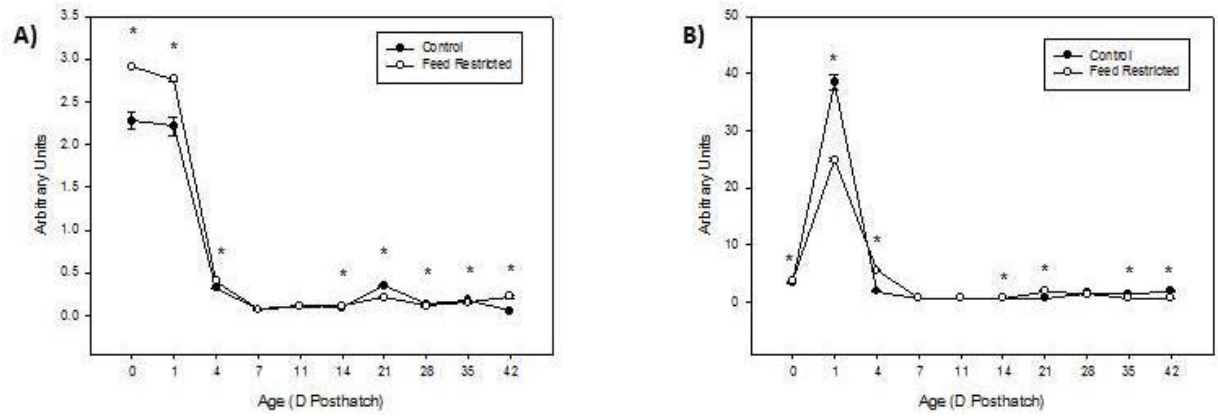

Figure 1 – Expression of A) MyoD and B) myogenin in control (full fed) and 20% feed restricted birds from hatch Day (D) through 42 D of age. The bars represent the SEM. *Indicates a significant difference ($P<0.05$).

Morphologically there was a significant difference in the development and structure of the breast muscle between the feed-restricted and unrestricted groups through the 42 days of the study. At 1 day posthatch in the control breast muscle samples, the muscle fiber bundles were well organized with well-defined connective tissue layers surrounding the bundles as well as the individual fibers. By day 7 posthatch, the individual muscle fibers and

muscle fiber bundles were evenly spaced and symmetrical in breast muscle from the full-fed birds. In the feed restricted breast muscle, the individual fibers and fiber bundles were not uniformly spaced. At day 7 rounded muscle fibers were also detected which would likely form hypercontracted fibers. This altered morphological development continued throughout the 42 days of the study with the feed restricted breast muscle also showing increased necrosis of muscle fibers compared to the full fed birds.

In addition to the altered morphological structure of the breast muscle, increased fat deposition was observed in the breast muscle of the birds with the 20% feed restriction for the first 2 week posthatch. In the feed restricted birds, intramuscular fat deposition was measurable beginning at day 28. In contrast, intramuscular fat was observed beginning at day 35 in the full-fed breast muscle samples. Furthermore, the size and the extensiveness of the intramuscular fat depots were increased in the feed restricted birds compared to the full fed birds (Figure 2 A and B).

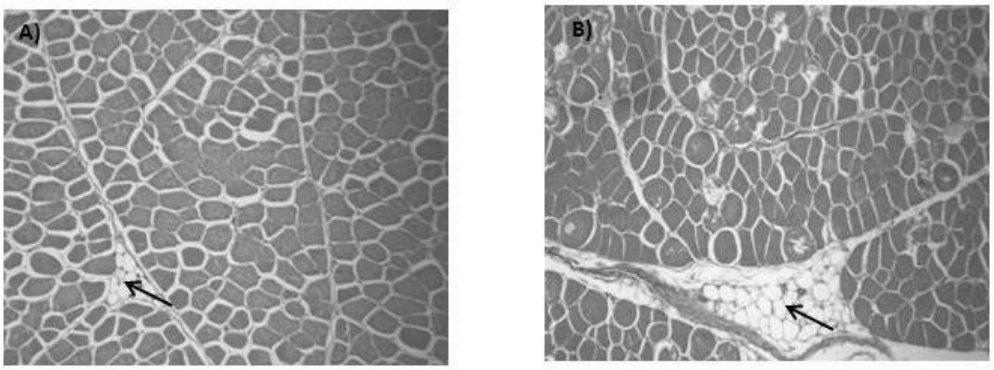

Figure 2 – Fat cell morphological distribution at 35 days of age in A) the control (full fed); and B) 20% feed restricted breast muscle. The arrows highlight the fat cells.

III. EFFECT OF THE TIMING OF THE POSTHATCH FEED RESTRICTION ON BROILER BREAST MUSCLE STRUCTURE, INTRAMUSCLAR FAT DEPOSITION, AND GENE EXPRESSION

The first week posthatch appears to be the most intense period of satellite cell activity in broilers (Plavnik and Hurwitz, 1988; Halevy et al., 2000). Halevy et al. (2000) reported, based on 48 h starvation periods during the first week posthatch in broiler chicks, that the timing of the starvation period influenced muscle and body growth potential. Feed deprivation the first 2 days posthatch had the greatest effect on growth compared to deprivation periods beginning at day 4 or 6 after hatch.

To determine if timing the feed restriction in broilers to a period after the first week posthatch or after the time of maximal satellite cell activity would positively affect breast muscle structure and fat accretion, trials were conducted with a 20 % feed restriction during the first week posthatch compared to the chicks being full-fed the first week posthatch followed by a 20% feed restriction the second week posthatch. After the feed restrictions the chicks were full fed. Limiting the feed restriction to the first week posthatch resulted in similar changes in MyoD and myogenin expression as having a 2 week immediate posthatch feed restricition. MyoD levels were increased the first 4 days posthatch and myogenin levels were significantly decreased. These data suggest that satellite cell proliferation was increased while differentiation was decreased. Interestingly, in the first week posthatch feed restricted birds the expression of the fat specific gene peroxisome proliferator-activated receptor gamma (PPARγ) was significantly increased compared to the control full-fed birds. Fat deposition in breast muscle was also increased in the breast muscle from the first week

posthatch feed restricted birds. In contrast full feeding the birds, the first week posthatch and during the second week posthatch feed restrict the birds with a 20% restriction restored MyoD and myogenin expression to normal levels and PPARγ expression was unaffected. Morphologically fat deposition was decreased in the breast muscle from the week 2 restricted birds.

These data from the in vivo studies was highly suggestive of a direct effect of nutrition on the myogenic satellite cells in terms of their proliferation, differentiation, and transdifferentiation to an adipogenic cell lineage. To address whether the satellite cells were directly affected by nutritional status, in vitro experiments were conducted with isolated broiler satellite cells so the satellite cells could be assayed without the presence of other cell types and factors.

IV. THE EFFECT OF NUTRITION ON MYOGENIC SATELLITE CELL PROLIFERATION, DIFFERENTIATION, AND TRANSDIFFERENTIATION TO AN ADIPOGENIC LINEAGE

To modify total protein synthesis in vitro methionine (Met) concentration was altered from optimal levels used in the culturing of satellite cells (Powell et al., 2013a). Methionine is the first amino acid in all proteins and is an essential amino acid (Scott et al., 1982). The availability of the essential sulfur amino acids Met and cysteine (Cys) was restricted to regulate protein synthesis during satellite cell proliferation and differentiation. To investigate the cellular response of satellite cells, six different culture conditions with altered Met and Cys concentrations were used. These treatments consisted of the control concentration of 30 mg/L Met and 96 mg/L Cys, which represents the optimal level of these amino acids present in Dulbecco's Modified Eagle Medium. Treatments consisted of one treatment of increased Met 60 mg/L Met and 192 mg/L Cys, and the following 4 restricted treatments 7.5, 3, 1, and 0 mg/L Met and 24, 9.6, 3.2, and 0 mg/L Cys, respectively.

The effect of these treatments was assayed to measure the effect on broiler breast muscle satellite cell proliferation and differentiation. Breast muscle satellite cell proliferation was decreased with both the increased and decreased Met/Cys concentration treatments (Figure 3A). Cellular morphology was also affected during proliferation (Figure 3B). As satellite cells proliferate they become elliptical in phenotype. At 72 h of proliferation just before fusion, the control cells (30/96 mg/L Met /Cys) had the most alignment, and those with higher and lower concentrations had less alignment. Without Met, there was no alignment and the cells were rounded. In terms of muscle formation, reductions in alignment will reduce multinucleated myotube formation and subsequent muscle fiber development from the multinucleated myotubes. If satellite cell proliferation is not at a maximal level, muscle mass accretion in the bird will be compromised.

Differentiation or the fusion to multinucleated myotubes was also affected by altered breast muscle satellite cell protein synthesis. Interestingly, continued exposure to the 60/192 mg/L Met/Cys treatment significantly increased differentiation compared to the control, whereas concentrations of Met/Cys lower than the control decreased differentiation and multinucleated myotubed formation. Although differentiation was increased in the 60/192 mgL Met/Cys treatment, the expression of the fat specific genes PPARγ and stearoyl-CoA desaturase (SCD) was increased (Powell et al., 2013b). The expression of fat-specific genes in pure cultures of breast muscle satellite cells is highly suggestive of the transdifferentiation of satellite cells to an adipogenic lineage as satellite cells if following a muscle-specific cell fate will only express muscle-specific genes not adipogenic-specific genes. These data support the in vivo observations of Velleman et al. (2010) of the possible transdifferentiation of satellite cells with suboptimal nutrition to an adipogenic cell fate.

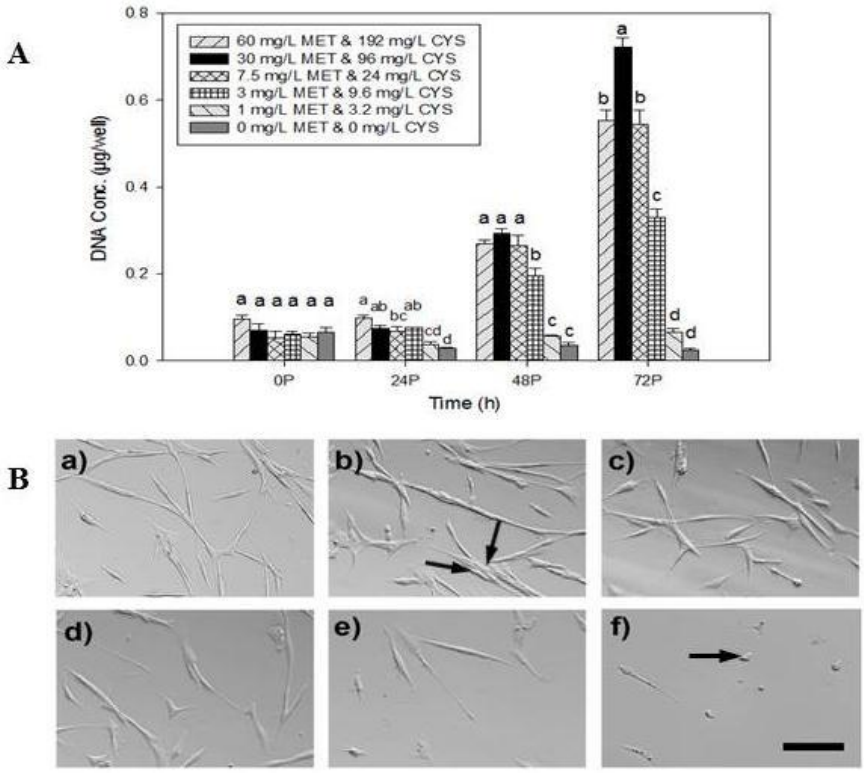

Figure 3 – Proliferation of breast muscle satellite cells (SC) cultured with variable Met and Cys concentrations (Conc.). A) Proliferation was measured from 0 through 72 h of proliferation. Bars represent the SEM. Bars without common letter within time were significantly different (P< 0.05). B) photomicrographs of the SC at 72 h of proliferation following Met and Cys treatment. a) 60 and 192 mg/L; b) 30 and 96 mg/L; c) 7.5 and 24 mg/L; d) 3 and 9.6 mg/L; e) 1 and 3.2 mg/L; and f) 0 and 0 mg/L. The arrows in b) highlight SC aligning and in f) morphological structure. The scale bar = 100µm. *Used by permission of Poultry Science. Powell et al. 2013. 92:2163-2173*

V. CONCLUSIONS

These in vivo and in vitro data demonstrate that broiler breast muscle satellite cells are sensitive to nutrition during both proliferation and differentiation, and with suboptimal nutrition can transdifferentiate to an adipogenic lineage. Decreased proliferation will reduce the available pool of satellite cells to form multinucleated myotubes and decrease differentiation potential. Reductions in satellite cell mitotic activity will decrease muscle mass accretion. In addition, satellite cells are partially differentiated and are multipotential stem cells which can follow other cell lineages especially an adipogenic pathway (Shefer et al., 2004). Thus, future studies need to be undertaken to optimize nutritional input to maximize satellite cell mitotic activity and limit transdifferentiation to an adipogenic cell fate to avoid satellite cell mediated effects on muscle structure and meat quality.

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NUTRITIONAL STATUS AND MUSCLE DEVELOPMENT IN PRE- AND POST-HATCH BROILERS

O. HALEVY[1], R. KORNASIO[1] and Z. UNI[1]

Summary

Chicks are commonly fasted for the first 36 to 72 h post-hatch due to the logistics of commercial production. Fasting for 48 to 72 h post-hatch results in retarded body weight (BW), delayed intestinal development and lower pectoral muscle weight, indicating that early feeding, up to 6 h post-hatch, is crucial for effective growth and performance on marketing day. Another way to compensate, at least in part, for the adverse effects of late feeding is the in-ovo feeding (IOF) of pre-hatch embryos. This methodology has been shown to have a long-term effect in compensating for the physiological stress and deficiency in BW and muscle growth caused by providing first feed no earlier than 36 h post-hatch.

I. INTRODUCTION

Under commercial industry practices and current standard feeding procedure, hatchlings are commonly held for 36 h to 72 h from the time of actual hatch to placement (Decuypere et al., 2001). This is due to the wide "hatching window", whereby hatcheries do not remove hatchlings until the maximum number of eggs have hatched, and to hatchery treatments such as sexing, vaccination and transport. Thus, most hatchlings are "fasted" for 48 h or more before their first access to feed and water. Holding hatchlings without food and water for over 24 h has long-lasting negative effects on both broilers and turkeys (Augustine, 1982; Noy and Sklan, 2001; Batal and Parsons, 2002; Juul-Madsen et al., 2004). Hatchlings become more susceptible to pathogens, and have decreased weight (Pinchasov and Noy, 1993; Geyra et al 2001; Bigot et al., 2003; Dibner and Richards, 2004; Dibner et al., 2008) and development of critical tissues and organs, such as intestine and skeletal muscle (Halevy et al., 2000, 2003; Mozdiak et al., 2002; reviewed in de Oliveira et al., 2008). In addition, glycogen reserves are utilized as the embryos go through the hatching process (Christensen et al., 1982; Lu et al., 2007), and they are not replenished until the newly hatched chick has full access to food. Insufficient glycogen forces the embryo to mobilize more muscle protein for gluconeogenesis, thereby reducing early growth and development (Vieira and Moran, 1999). Together, the physiological and metabolic processes and changes taking place in birds during the pre- and post-hatch periods affect hatchling quality and subsequent performance at later ages.

II. SKELETAL MUSCLE GROWTH

In vertebrates, the development and growth of skeletal muscle after birth/hatch is mainly, if not solely, dependent on the accumulation of new myonuclei of muscle progenitors, the satellite cells (also termed adult myoblasts). These cells, first identified by Mauro (1961), lie between the basal lamina and sarcolemma of the myofibers. In the chick, satellite-cell mitotic activity begins as early as embryonic day E15, peaks on day 2 or 3 post-hatch, and decreases significantly by day 8 post-hatch when the myofibers undergo terminal differentiation and fusion into myofibers (Hartley et al., 1992; Stockdale., 1992; Halevy et al., 2000, 2006a). Normally, the number of satellite cells decreases to less than 5% of total myofiber nuclei

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toward the end of the growth phase and they become largely quiescent (Halevy et al., 2004). However, upon muscle damage, these cells are capable of activating their myogenic program, and either proliferating and differentiating into myofibers or undergoing self-renewal to maintain the satellite cell pool (reviewed in Bischoff, 1994; Zammit et al., 2004; Yablonka-Reuveni, 2011). The myogenesis of these muscle progenitors is governed at the transcriptional level by the paired box proteins Pax3 and Pax7 (Conboy and Rando, 2002; Halevy et al., 2004; Zammit et al., 2004; reviewed in Wang and Rudnicki, 2011) and by the muscle-specific helix-loop-helix MyoD family of proteins—MyoD, myogenin, Myf5 and MRF4—and myocyte enhancer-binding factor 2 (MEF2) (reviewed in Naya and Olson; Shi and Garry, 2006). Several growth factors have been implicated in stimulating or inhibiting satellite-cell proliferation and differentiation in mammals and avians. Among them are members of the fibroblast growth factor family, transforming growth factor beta, platelet-derived growth factor, hepatocyte growth factor and insulin-like growth factor I and II (IGF-I and II) (Florini et al., 1996; Tatsumi et al., 1998; Zorzano et al., 2003), as well as the morphogenic factor sonic hedgehog (Shh, Koleva et al., 2005; Elia et al., 2007). Both, IGF-I and Shh have been implicated in inducing myoblast proliferation and differentiation as well as muscle hypertrophy (Rommel et al., 2001; Paul and Rosenthal, 2002; Alzghoul et al., 2004; Elia et al., 2007; Madhala-Levy et al., 2012).

Recent studies conducted by us and others have shown that approaches aimed at enhancing the pool of satellite cells in muscle during pre- and post-hatch periods in broilers have long-term effects on muscle growth (i.e., hypertrophy) and meat production until marketing day. Among these are monochromatic green-light illumination (reviewed in Halevy et al., 2006a; Rozenboim et al., 2013) and thermal manipulations. For example, incubation at 39.5^{o}C (increase of 1.7^{o}C from normal conditions) from E16 to E18 for 3 or 6 h daily increased hypertrophy and enhanced absolute muscle growth relative to controls, until day 35 of age (Collin et al., 2007; Piestun et al., 2008, 2009). Immediate and later (up to 2 weeks post-hatch) effects in terms of elevating muscle-cell proliferation relative to controls were observed (Piestun et al., 2009), suggesting that the increased hypertrophy can be attributed to a higher reservoir of myogenic progenitor cells (i.e., satellite cells) produced in response to the thermal manipulations. Moreover, in this study as well as in previous ones in which the incubation temperature was increased by only 0.7^{o}C (to 38.5^{o}C; Halevy et al., 2006a), or in which post-hatch chicks were exposed to mild heat on day 3 (Halevy et al., 2001), post-hatch IGF-I levels in the muscle tissue were higher in the treated groups than in the controls, implying a mechanism by which heat manipulations in chicks affect muscle development, with locally secreted IGF-I playing a major role.

It should be noted that in all of these studies, the hatchlings had immediate access to food and water. This was based on evidence that mitotic activity and subsequent muscle growth are irreversibly retarded through to marketing by a delay in first feeding during the first 48 h post-hatch of chicks and turkey poults (Halevy et al., 2000, 2003).

III. IN-OVO FEEDING AND ITS EFFECT ON MUSCLE GROWTH

In broilers, the digestive capacity begins to develop a few days before hatch (Uni et al., 2003), and most of the development occurs in the immediate post-hatch hours when the neonatal chick begins consuming feed (Jin et al., 1998). Since the late-term embryo naturally consumes the amniotic fluids, insertion of a nutrient solution into the embryonic amniotic fluid (in-ovo feeding, IOF) may enhance development. Indeed, our previous research demonstrated that inserting a nutrient solution which includes carbohydrates and β-hydroxy-β-methylbutyrate (HMB), a metabolite of the amino acid leucine, by IOF 3 to 4 days before hatch elevates the glycogen stores depleted during the pre-hatch period, and increases body

weight (BW) and absolute muscle growth (Tako et al., 2004; Uni et al., 2005). Similar results were obtained in turkeys and ducks (Foye et al., 2006; Hen et al., 2009). HMB has been reported to prevent exercise- and disease-induced protein degradation and muscle damage (Panton et al., 2000; Smith et al., 2004; reviewed in Portal et al., 2010). Moreover, a recent study has shown its direct promotive effect on proliferation and differentiation of cultured myoblasts and myotube fusion, as well as on myoblast survival (Kornasio et al., 2009). It has been suggested that the direct effects of HMB on myoblast differentiation and survival resemble those of IGF-I, at least in culture.

In light of these findings, we examined whether providing nutrients for the prenatal bird by IOF methodology would have a long-term impact in reducing the effects of the physiological stress and deficiency caused by the current standard feeding procedure i.e., providing first feed no earlier than 36 h post moment of hatch. Four treatment groups differing in their time of first feed (6 h and 36 h post-hatch) and administration of IOF were compared. IOF was conducted on E18 with a sterile IOF solution containing 10% (w/v)? dextrin and 0.4% (w/v)? Ca-HMB in 0.4% NaCl, as described by Uni et al. (2005). As expected, avoiding first feeding for 36 h from the moment of hatch led to irreversible growth deficiency expressed by reduced BW and muscle weight on day 35 (Kornasio et al., 2011). However, IOF procedure on E18 supported the growth of chickens subjected to 36 h-delayed access to feed and water as reflected by higher BW and pectoral muscle weight compared to their non-IOF counterparts on days 14 and 35. The supporting effects of IOF could be explained, at least in part, by the higher liver and muscle glycogen reserves found in the embryos immediately after the IOF treatment (E19) and onward. These higher reserves may have led to a promotive effect on myoblast proliferation (i.e., satellite cells; Hartley et al., 1992; Halevy et al., 2006b) on E19 and post-hatch, and later on, on hypertrophy, as evidenced by myofiber diameters on day 35 that were comparable to those of early-fed chicks (Kornasio et al., 2011). Interestingly, IOF also had a promotive effect on body and muscle weights of chicks fed early post-hatch. This study demonstrated the IOF's long-term effect in supporting BW and post-hatch muscle growth when first feed is delayed by 36 h.

IV. MYOGENIC FACTORS AND IGF-I ARE AFFECTED BY NUTRITIONAL STATE

In light of the above findings, further biochemical analyses were conducted to elucidate the mechanism underlying the supportive effect of IOF on myogenic differentiation in early- and late-fed chicks. Muscle samples were excised from the superficial regions of the proximal one-half of the left pectoralis major (Halevy et al., 2004) of the experimental embryos (control vs. IOF) and post-hatch chicks (early or late first feed alone or with IOF) at various days of age. Muscle proteins were extracted and the lysates subjected to western blotting with antibodies against myogenin, a marker for myoblast differentiation, and against locally expressed IGF-I, followed by densitometry analysis (Halevy et al., 2004). In view of the large number of samples from each time point and the multiple in-vivo repeats, samples harvested at the same time were analyzed in parallel on the same blot for each day and results were expressed as fold induction relative to the control group (non-IOF and late feed, Control-SP). Myogenin protein levels were significantly higher in the IOF-treated vs. untreated embryos (Fig. 1A), suggesting a higher number of differentiating fetal myoblasts and their fusion with the existing myofibers. In the post-hatch chicks, myogenin levels were higher in late-fed chicks which received IOF (IOF-SP) vs. their counterparts which did not receive IOF (Control-SP) on days 2 and 3; on these days, proliferation and differentiation of satellite cells are maximal (Halevy et al., 2004, 2006b; Piestun et al., 2009). Indeed, the highest myogenin levels were detected in the early-fed (EF) chicks on day 2, but these levels declined rapidly compared to those in the late-fed (SP) groups, implying a faster differentiation of satellite

cells and their fusion to myofibers. This is in agreement with the decline in the number of satellite cells found in this group (Kornasio et al., 2011). In contrast to the Control-EF group, myogenin levels in the IOF-EF group were relatively low, implying that most of the cells were still in their proliferative stage, as found by Kornasio et al. (2011).

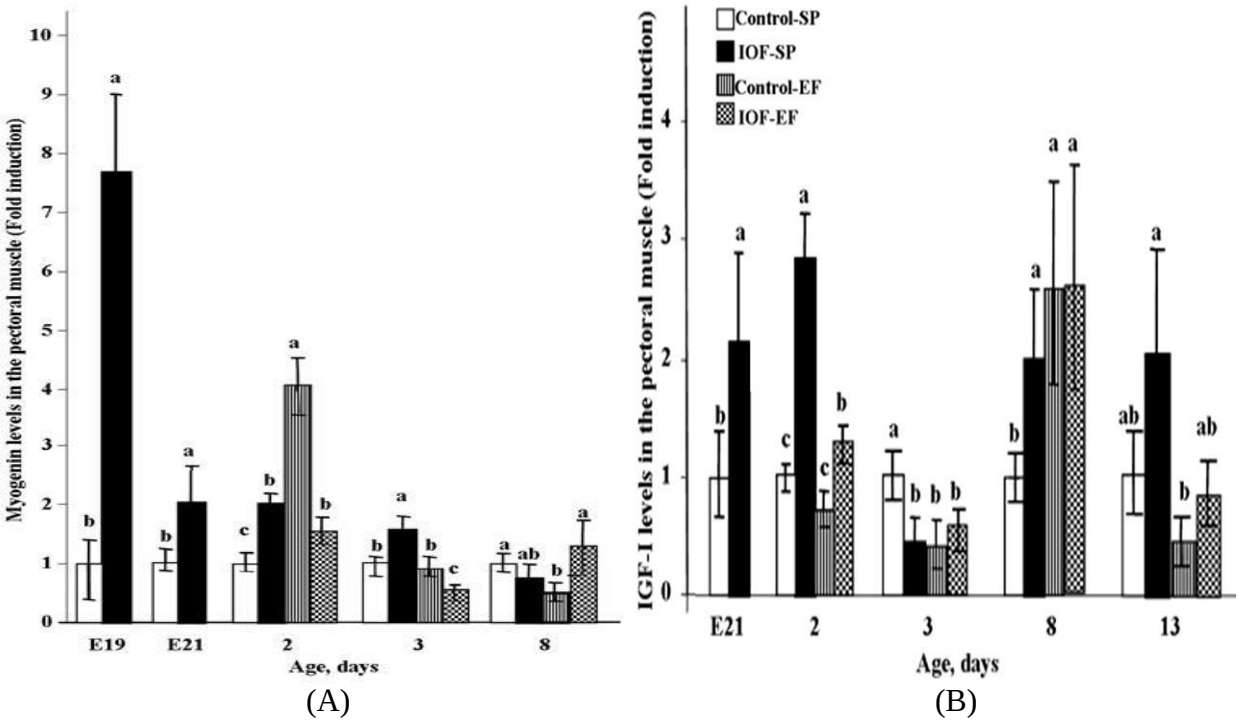

Figure 1 - Myogenin (A) and IGF-I (B) protein levels in pectoralis muscle tissue. Pectoralis muscle samples were removed in parallel from individual embryos or chicks from all groups (n = 4) on various days of age. On each day, samples derived from the four groups were run side by side on an SDS-polyacrylamide gel and protein-expression levels were evaluated by western blot analysis. Bands were quantified by densitometry relative to □-tubulin and results are means ± SE presented as fold induction of control. Data with different letters differ significantly within the same age ($P < 0.05$).

Locally expressed IGF-I has been shown by us and others to be a prime candidate for the induction of myoblast activity following muscle stress (Adams et al., 2000; Halevy et al., 2001, 2006; Piestun et al., 2009). IGF-I can promote myoblast proliferation and differentiation, as well as muscle hypertrophy (Rommel et al., 2001; Paul and Rosenthal, 2002; Madhala-Levy et al., 2012). Here, IGF-I expression in the muscle was significantly higher in all IOF groups compared to their non-IOF counterparts (either early- or late-fed) on almost all days, suggesting that IOF promotes satellite cell activity as early as pre-hatch and immediately post-hatch. The differences were more pronounced on day 13, suggesting an even higher impact of IGF-I on muscle hypertrophy; this was reflected by higher myofiber diameter and muscle weight (Kornasio et al., 2011). It is worth mentioning the long-lasting induction of IGF-I in response to IOF of late-term embryos, which is reminiscent of the effect found by heat manipulation on similar embryonic days (E16 to E18) (Piestun et al., 2009). Our best explanation for this phenomenon is that IOF during critical phases of satellite-cell myogenic activity in late-term embryos (Hartley et al., 1992) may induce epigenetic modifications (Tzschentke and Plagemann, 2006).

V. CONCLUSIONS

Accumulating evidence indicates that the nutritional status of meat-type chicks at critical phases of gastrointestinal tract and muscle development during pre- and post-hatch periods dictates their muscle growth and meat production, and their well-being during their life span until marketing. The IOF procedure can compensate, at least to some extent, for the deleterious effects of withholding immediate feed from the hatchlings on their BW and muscle growth, via enhancement of liver and muscle glycogen reserves and satellite-cell proliferation. While immediate feeding post-hatch has beneficial effects on the broiler's performance, the combination of IOF and immediate feeding produces the best results, with the most beneficial long-term effects on BW and post-hatch muscle growth.

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INFLUENCE OF NUTRITIONAL RESTRICTION ON SATELLITE CELL ACTIVITY AND ADIPOGENIC TRANSDIFFERENTIATION

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Summary

The muscle stem cells, known as satellite cells, were studied to assess the effect of varying severities of nutritional restriction on their ability to contribute to muscle hypertrophy. Satellite cells facilitate muscle hypertrophy by donating their nuclei to existing muscle fibres, allowing for increased protein synthesis within the fibres. The proliferation and differentiation of satellite cells was significantly affected in vitro by nutritional restriction, with markedly different responses based on the severity of the restriction imposed. In addition, lipid staining and adipogenic gene expression also indicated these cells may be undergoing transdifferentiation to form fat cells following severe nutritional restriction.

I. INTRODUCTION

Neonatal nutrition is an important facet of broiler production, and will only become more significant as the market age of broilers decreases. A critical component of muscle development during this period are the myogenic stem cells, called satellite cells. Muscle fibre formation is complete at hatch and subsequent muscle growth relies on hypertrophy of existing muscle fibres. Satellite cells facilitate this hypertrophy by proliferating, differentiating into myoblasts, and then fusing with and donating their nuclei to existing muscle fibres. These nuclei then enable increased protein synthesis within the fibre.

Immediate posthatch feed restrictions or deprivation, as occurs between hatch and arrival at the grow-out farm, have been shown to cause impaired satellite cell activity and subsequently affect muscle growth (Halevy et al., 2000; Mozdziak et al., 2002). Additionally, as satellite cells are stem cells, they can adopt alternate cellular lineages. In particular they have been shown to follow an adipogenic lineage to form fat cells when stimulated by nutritional manipulation (Asakura et al., 2001).

To investigate the role of nutrient restrictions on satellite cell proliferation and differentiation, isolated broiler pectoralis major (PM) satellite cells were subjected to restricted protein availability. This was accomplished in an in vitro cell culture system through methionine (Met) and cysteine (Cys) restriction. The possible adipogenic transdifferentiation of the satellite cells was investigated through real-time quantitative PCR (RT qPCR) analysis of two marker genes of adipogenesis, peroxisome proliferator-activated receptor γ (PPARγ) and stearoyl-CoA desaturase (SCD). Additionally, the accumulation of lipids within the satellite cells was determined using Oil-Red O, a fat soluble dye.

II. MATERIALS AND METHODS

Single satellite cells were previously isolated from the PM muscle of 5-wk-old female Cornish Rock broiler chickens by McFarland et al. (1997).

The chicken PM satellite cells were plated in 0.1% gelatin-coated 24-well plates for the analysis of proliferation and adipogenic activity, and 48-well plates for the analysis of

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differentiation (Greiner BioOne, Monroe, NC, USA). The cells were grown for 24 h in plating medium, as described in Powell et al. (2013a), before the addition of Met/Cys treatments. One of six proportional Met and Cys treatments were provided to the satellite cells for each assay as part of a defined feeding media during proliferation, and fusion media during differentiation, as described in Powell et al. (2013a). These six Met/Cys concentration treatments consisted of: 60/192, 30/96 (control), 7.5/24, 3/9.6, 1/3.2, and 0/0 mg/L.

To investigate the effect of Met/Cys restriction on the proliferative ability of satellite cells, the DNA concentration of each treatment was determined using Hoechst 33258 fluorochrome as described in Powell et al. (2013a). In brief, plates were removed at 24 h intervals throughout 72 h of proliferation and the DNA concentration was measured on a Fluoroskan Ascent FL (Thermo-Electron Co., MA, USA).

To investigate the rate of differentiation of the satellite cells under different Met/Cys restriction treatments, the muscle-specific creatine kinase enzyme levels were measured as per the methods of Powell et al. (2013a). In brief, the rate of thio-nicotinamide adenine dinucleotide reduction by creatine kinase was determined by absorbance measurements with a BioTek ELx800 plate reader (Biotek, Winooski, VT, USA) at an optical density of 405 nm.

Two different indicators of adipogenic activity were used as indicators of satellite cell transdifferentiation. Staining of the satellite cells with the fat-soluble dye Oil Red-O at 48 h of both proliferation and differentiation was performed, as per the methods of Powell et al. (2013b). This was quantified by elution of the stain with 100% isopropanol, and analysis by absorbance spectroscopy with a Spectronic Genesys 5 (Thermo Fisher Scientific, Waltham, MA, USA) at an optical density of 500 nm. A RT qPCR analysis of the adipogenic marker genes, PPARγ and SCD was also investigated. This was performed at 48 and 72 h of proliferation, and 24 through 72 h of differentiation. Total RNA extraction and cDNA synthesis of the samples was performed as described in Powell et al. (2013b). The RT qPCR was performed using the DyNAmo Hot Start SYBR Green qPCR kit (Finnzymes, Ipswich, MA, USA) with a DNA Engine Opticon 2 real-time system (MJ Research, Waltham, MA, USA). The PPARγ primer sequences (GenBank accession number NM_001001460) were: forward primer 5'-$_{805}$CCA CTG CAG GAA CAG AAC AA$_{824}$-3' and reverse primer 5'-$_{1053}$CTC CCG TGT CAT GAA TCC TT$_{1034}$-3' while the SCD primer sequences (GenBank accession number NM_204890) were: forward primer 5'-$_{152}$CAC GGG TGA CCA AGA ATG GG$_{171}$-3' and reverse primer 5'-$_{483}$CAG GGG CAG TGT AGC TTT GT$_{464}$-3'. The RT qPCR was performed under the conditions described in Powell et al. (2013b) and the relative level of gene expression was calculated using a standard curve for each target gene as described previously by Liu et al. (2006). The mole amount of each cDNA product was then normalized across sampling times using the expression of the reference gene glyceraldehyde-3-phosphate dehydrogenase from the control 30/96 mg/L Met/Cys treatment. Each sample was analysed in duplicate.

III. RESULTS

Pectoralis major SC proliferation and differentiation was affected by Met/Cys treatment. Both increased and decreased levels of Met/Cys caused decreased proliferation compared to the control 30/96 mg/L Met/Cys by 72 h, which became more significant as Met/Cys concentration was reduced (Figure 1A). Differentiaton rate decreased in a dose-dependent manner, as the 60/192 mg/L Met/Cys treatment had the highest differentiation rate by 72 h (Figure 2B).

Peak Oil-Red O lipid staining was observed in the 60/192 mg/L and 0/0 mg/L Met/Cys treatments during proliferation, and in the 1/3.2 and 0/0 mg/L Met/Cys treatments during differentiation (Figure 2). The expression of the adipogenic marker genes PPARγ and

SCD were also affected by Met/Cys treatment. During proliferation the control 30/96 mg/L Met/Cys treatment had the highest expression in both genes, shared with the 7.5/24 and/or 60/192 mg/L treatments (Figure 3A, B). During differentiation the peak expression of both genes was observed under the restricted Met/Cys treatments, with peak activity of PPARγ and SCD occurring under the 1/3.2 mg/L Met/Cys treatment by 72 h (Figure 3A, B). No increase in gene expression was observed under the 0/0 mg/L Met/Cys treatment for either gene (Figure 3A, B).

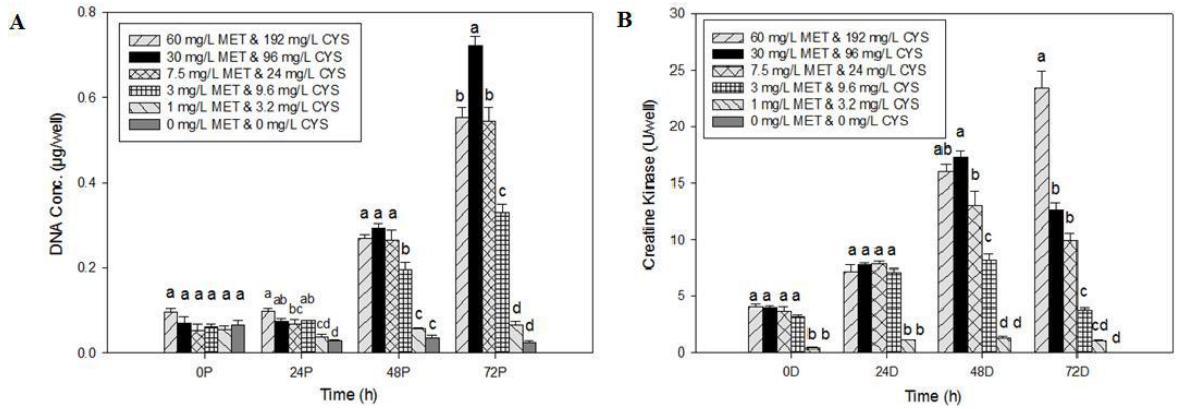

Figure 1 - The A) proliferation and B) differentiation of satellite cells cultured with variable Met and Cys concentrations. Values without a common superscript within a time point differ significantly ($P < 0.05$).[#]

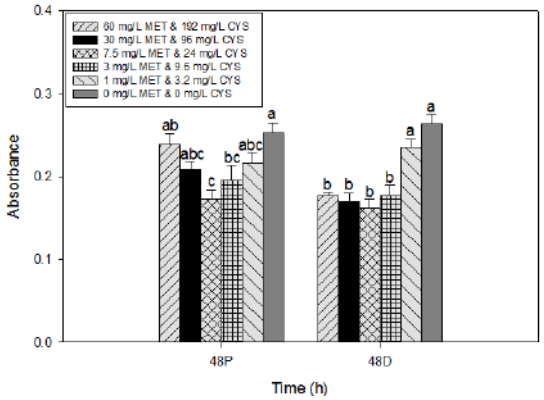

Figure 2 - Oil-Red O absorption of satellite cells at 48 h of proliferation (P) and differentiation (D) cultured with variable Met and Cys concentrations. Values without a common superscript in a time point differ significantly ($P < 0.05$).[#]

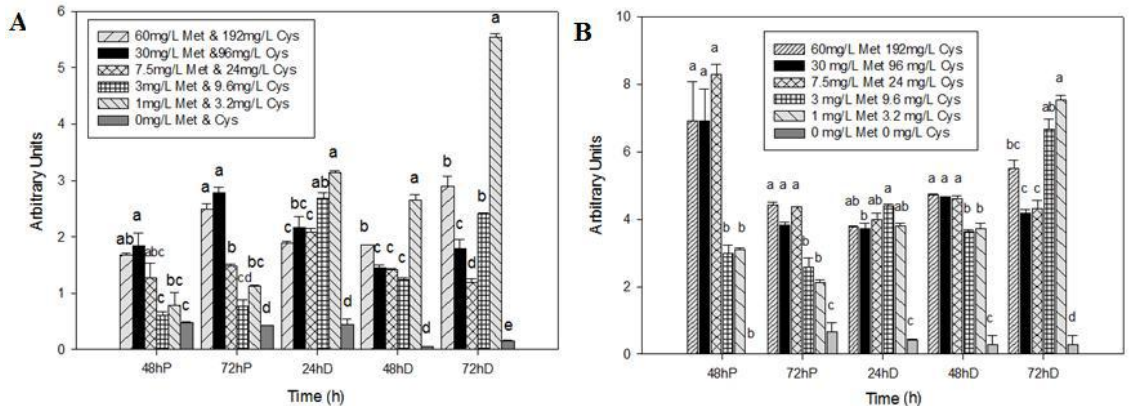

Figure 3 - Gene expression of the adipogenic marker genes A) peroxisome proliferator-activated receptor γ and B) stearoyl-CoA desaturase in satellite cells during proliferation (P) and differentiation (D). Values without a common superscript within a time point differ significantly ($P < 0.05$).[#]

[#] Used by permission of Poultry Science. Powell et al. 2013a. Poult. Sci. 92:2163-2173.

IV. DISCUSSION

These data show the significant effect of nutritional restriction on the ability of satellite cells to proceed through the myogenic differentiation process to contribute to muscle hypertrophy. The ability of the satellite cells to proliferate and differentiate was severely reduced by Met/Cys restriction, and the severity of the restriction applied was very important in modulating this response.

It was interesting to observe increases in both lipid droplet accumulation, which is essential for adipocyte differentiation (Green and Kehinde, 1975), and adipogenic marker gene expression in the severely restricted and excess Met/Cys treated PM satellite cells during differentiation. Upregulation of PPARγ and SCD indicates increased adipogenic transdifferentiation of the satellite cells to adipocytes may have occurred. This is possible as satellite cells are a stem cell population which retain a broad differentiation capacity. The down regulated gene expression of the 0/0 mg/L Met/Cys treatment is due to the inadequate amount of RNA that could be collected from these cells, owing to their extremely reduced proliferation and differentiation. Based on the gene expression of the 3/9.6 and 1/3.2 mg/L Met/Cys treatment and Oil-Red O staining of the 0/0 mg/L Met/Cys treatment, the 0/0 mg/L Met/Cys gene expression could be significantly higher.

The increased adipogenic gene expression and lipid accumulation is likely a consequence of reduced protein synthesis, proliferation, and myogenic differentation in the Met/Cys restricted satellite cells. Under these conditions excess amino acids will be available to the cells which can be converted to glucose by gluconeogenesis, and induce lipid accumulation of the satellite cells (Guillet-Deniau et al., 2008) and subsequent transdifferentiation to adipocytes (Aguiari et al., 2006). Another possible outcome from the excess amino acids is oxidation to produce pyruvate, acetyl-CoA, or intermediates of the tricarboxylic acid cycle which could also be utilized for fatty acid synthesis to facilitate lipid production.

This redirection of satellite cell fate could cause increased or earlier intramuscular fat formation in vivo, a phenomenon which has been observed by Velleman et al. (2010) following an immediate posthatch nutritional restriction. These data highlight the need to investigate the importance of posthatch nutritional guidelines to maximize myogenic activity of satellite cells and minimize their possible role in fat deposition.

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INCUBATION AND BROODING CONDITIONS ESSENTIAL FOR THE OPTIMISATION OF NEONATAL NUTRITION

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Summary

Genetic selection and improved nutrition have increased growth rates and decreased the slaughter age of broiler chickens worldwide during the last two decades. Optimization of early life conditions has become widely recognised as a major influencer on the final performance of broiler chickens in terms of body weight, feed conversion ratio (FCR), and total mortality. With industry trending towards decreased slaughter ages, the incubation and brooding period now form a larger part of the total life span of the broiler chickens and suboptimal conditions during this period have a larger impact on overall performance. As a result, there has been a concerted effort, both in research as well as industry, to focus more on neonatal nutrition. However, maximizing the benefits of neonatal nutrition is only possible when incubation and brooding conditions are optimized. Embryonic as well as post-hatch chickens are highly dependent on their environment with even small fluctuations in external conditions, i.e. temperature, having a negative impact on survival and performance in the field. In order to achieve the best results, optimization of neonatal nutrition as well as environmental conditions throughout the incubation and brooding period needs to be considered together.

I. INTRODUCTION

Growth rates of broiler chickens have increased and production cycle times have decreased by 60% in the last 40 years. This is widely agreed to be the direct result of genetic selection and improvements in poultry nutrition (Havenstein et al., 2003a). The incubation and brooding period has become a larger part of the total life span of the broiler chicken. This is expected to increase even more in the future as the slaughter age decreases (Hulet, 2007). To achieve the genetic growth potential of the bird, neonatal nutrition has become increasingly important nowadays (Havenstein et al., 2003b). Understanding and optimization of incubation and brooding conditions is essential to achieve the largest benefit from neonatal nutrition.

II. INCUBATION PERIOD

Nutrients stored inside the egg are utilized for growth and development during the 21 day incubation period of the chicken embryo (Wilson, 1997). To start embryo development, temperature is one of the most important environmental conditions (Romanoff, 1936; Decuypere and Michels, 1992; Meijerhof, 2009). The temperature that the embryo experiences during incubation is especially important and has a large effect on pre- and postnatal survival and development (French, 1997; Leksrisompong et al., 2007; Molenaar et al., 2010a). It is important to point out that the embryo temperature differs from the air temperature inside the incubator as well as the temperature displayed on the outside of the incubator (Meijerhof and Van Beek, 1993). Embryo temperature is the result of embryonic

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heat production and heat transfer between the egg and its surrounding (Meijerhof and Van Beek, 1993). Actual embryo temperature is difficult to measure without killing the developing chicken therefore eggshell temperature is often used as an indicator rarely deviating more than 0.2ºC from the actual embryo temperature (French, 1997). Eggshell temperature needs to be measured at the equator of the eggshell. A simple ear infrared thermometer is sufficient enough to get a good indication of the eggshell temperature. Different studies have shown that an eggshell temperature of 37.8°C during incubation results in the best hatchability, chick quality, and posthatch performance (Lourens et al., 2007; Leksrisompong et al. 2007, Molenaar et al., 2011). Relatively small deviations from the optimal eggshell temperature (37.8 ± 1.5ºC) can easily occur yet have a major impact on survival and (organ) development (Lourens et al., 2005, 2007). This is thought to be the result of changes in nutrient utilisation and physiological processes during incubation (Molenaar et al., 2010a, 2013).

III. BROODING PERIOD

Although the chicken embryo is fully grown at hatch, further development and maturation of the thermoregulatory, gastro-intestinal and immune system is necessary in the brooding period (Maiorka et al., 2006; Tzschentke, 2007), comprising the first 7 days of the chick's life. The sensitivity of the brooding period is highlighted by the fact that chicks are unable to regulate their body temperature posthatch; they are completely dependent on environmental temperature to maintain their body temperature (Romanoff, 1941). Ideally, body temperatures of chicks need to range from 40.0 and 41.0°C throughout the brooding period in order to achieve the lowest mortality and finest development. Comparable with the incubation period, temperature is the most important factor that needs to be controlled during the brooding period (Maatjens, 2010).

Secondly, feed intake is essential to stimulate intestinal and immunological development in the brooding period (Noy and Sklan, 1997). Time between hatch and first feed consumption can be up to 72 hours, because of variations in hatch time, chick handling, and transportation time (Dibner et al., 1998; Van den Brand et al., 2010). During this period without feed and water, the chick has to rely on water and nutrients of the residual yolk (Noy and Sklan, 1997). Early feed consumption is important because intestinal development and growth is much larger with feed than without (Geyra et al., 2001). Furthermore, early feed consumption can improve the immune status of the chick as immunoglobulins from the residual yolk can be utilized rather than acting as an energy source (Dibner et al., 1998).

Difficulties in optimizing brooding conditions and the large benefits of a good start early in life have led to the development of two commercial brooding systems for chicks.

IV. BROODING SYSTEMS

The commercial brooding systems, Patio system (Vencomatic, the Netherlands) and the HatchBrood system (HatchTech B.V., the Netherlands) have been specifically developed to optimize the early life conditions of the chickens. In the Patio system, the hatching and growout phase are combined. Eggs are transported at day 18 of incubation from the hatchery to the Patio system. Chicks hatch within the system and can eat and drink immediately after hatch. The unit contains different rows and levels to grow the chicks until slaughter age (Van de Ven et al., 2009). The Patio system is a replacement for conventional broiler farms. In the HatchBrood system, chickens are placed in cradles inside the unit after normal processing in the hatchery and they are provided with feed and water for 4 days. The HatchBrood system is used as an extension of the hatchery with the chicks delivered as 4-days-old instead of day-old to the broiler farm (Van der Pol et al., 2013). Environmental conditions and early feed

can easily be adjusted to the specific requirements of chicks during the brooding period in these brooding systems.

V. NEONATAL NUTRITION

Neonatal nutrition has gained attention recent years because of the significant benefits that can be achieved on final performance. The first application of neonatal nutrition is with the use of *in ovo* feed. *In ovo* feed has been developed to supply the chicken embryo already with additional nutrients (Uni and Ferket, 2004), but is not widely adopted in the commercial poultry industry yet (Kadam et al., 2013). An isotonic solution containing carbohydrates, proteins or a mixture of both is injected into the amnion of the embryo at around day 18 of incubation before the embryo pips the internal shell membrane and starts lung ventilation (Uni and Ferket, 2004). The solution is absorbed by the chicken embryo and can improve hatchability and hatchling quality (Uni and Ferket, 2004) This is a result of increased glycogen reserves, improved gut and muscle development, and better skeletal health (Uni and Ferket, 2004, Bello et al., 2013). The long-term effects of *in ovo* feed are not consistent (Kadam et al., 2012).

Optimal incubation conditions are very important when *in ovo* feed is applied. In practice, high eggshell temperatures (>39.5ºC) are often found in the second half of incubation as a result of poor air velocity or cooling capacity, or high temperature settings in the incubator (French, 1997; Lourens et al., 2005). High eggshell temperatures (>39.5ºC) during the second half of incubation have been found to influence embryonic nutrient utilization by an increased glucose demand and a lower protein efficiency for growth (Molenaar et al., 2010a, 2013). If *in ovo* feed is applied when embryos are experiencing high temperatures, the injected nutrients are likely to be used to compensate for nutrients losses due to this high temperature (Molenaar et al., 2010b). To achieve the highest benefits of *in ovo* feed pre- and postnatal, eggshell temperatures need to be maintained uniformly at 37.8 ± 0.2ºC in the incubator.

In the brooding period, nutrition is generally given more attention and prestarters are often provided to the posthatch chicks. Recent developments at commercial feed producers to optimize prestarters include: use of highly digestible raw materials, smaller pellet sizes (2.0 mm), and high pellet durability (Wijtten, personal communication). However, chicks need to find and start consuming the feed to benefit from early nutrition. The most important factor involved in the start of feed consumption is the body temperature of the chick. The chick needs to have a body temperature between 40.0 and 41.0ºC posthatch to show normal behaviour and start looking for feed (Molenaar, 2012). At commercial broiler farms, it is often found that the body temperature of chicks becomes too low (<40.0°C); this results in a low feed consumption and decreased development and it can lead to high mortality rates (Maatjens, 2010).

In conclusion, the greatest benefit of neonatal nutrition on chick performance can be achieved when incubation and brooding conditions are optimized. The body temperature of the embryo and chick temperature is the critical factor that needs to be controlled for optimization of neonatal nutrition.

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BETAINE INCREASES PERCENTAGE HATCHED IN BROILER BREEDERS

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Natural betaine has two roles in nutrition. As an osmoregulator it can protect cell enzymes systems and membranes from ionic inactivation during stress. As a methyl donor via transmethylation, it is more effective than other potential methyl group donors such as methionine and choline (Eklund et al., 2005). Natural betaine has been shown to benefit parameters such as bodyweight gain, feed conversion efficiency, especially during times of adverse environment for the birds such as excessive heat and coccidial challenge (Eklund et al., 2005). More recently betaine has been shown to improve embryo survival and total born, in pigs (Van Wettere et al., 2009). The hypothesis of this study is betaine will have a similar effect in broiler breeders; to increase embryo survival and percentage of eggs hatched.

The study was conducted in commercial broiler breeder sheds, at Turi Foods Bannockburn Farm. Each shed contained approximately 7,000 Ross 308 hens, with 8 males per 100 hens. The sheds are 100% deep litter with manual next boxes. The experiment was run in two blocks, with a cross-over of treatments per shed so that no one treatment was offered to the same shed. The study began when hens were 25 weeks of age, and ended when the hens were 55 weeks old. The treatments were two levels of natural anhydrous Betaine (Betafin S1, Danisco), at 0 and 2000ppm. The diet formulations were to Ross 308 recommendations, and were adjusted depending on the age, intake, bodyweight and production parameters. Standard flock performance figures were obtained twice a week and were then analysed for the actual chicks hatched percentage, chick weights and the percentage of chicks culled in the hatchery. Statistics were conducted using PASW statistics 18.

Table 1 - Performance of broilers breeders offered two levels of anhydrous betaine

Betaine level (g/kg)	Chick Weight (g)	Percentage Hatched (%)	Percentage chick culls (%)
0	40.91	84.75	0.44
2000	40.72	86.89	0.41
SEM	*0.164*	*0.302*	*0.015*
P value	0.614	0.004	0.397

The results supports the hypothesis that supplemental betaine improves the chick hatch percentage in broiler breeders (P=0.004). There was no effect of the treatment on chick weight or percentage of culls. The present results strongly suggest that betaine improves the number of hatched chicks in broiler breeders, which supports the past observations of betaine improving the number of total and born alive in pig breeders. The suggested mode of action is that betaine improves embryo survival through its osmoregulation and transmethylation properties.

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HEPATIC CONCENTRATIONS OF VITAMIN K IN CHICKS OF BROILER BREEDER HENS FED DIFFERENT FORMS OF THE VITAMIN

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Vitamin K consists of a group of structurally related compounds; phylloquinone (K1), menaquinones (MKs also known as K2) and menadione (K3) which is the synthetic form of the vitamin added to poultry diets. There is increasing evidence that vitamin K through its role as a cofactor for carboxylation of glutamate to γ-carboxy-glutamic acid (GLA) residues in proteins plays a significant role in bone metabolism, energy metabolism, spermatogenesis, apotosis and innate immunity, in addition to its recognized role in blood coagulation. The lack of a side chain restricts the activity of K3 to a role in blood clotting. The various forms of vitamin K are different not only with regard to their co-factor activity but also their absorption, transport, tissue distribution and turnover. Moreover, there are differences in vitamin K metabolism between species.

The aim of this study was to determine the efficacy of vitamin K transfer from the maternal diet to the chick. Ross broiler breeder pullets were obtained at 20 weeks of age and placed into four floor pens with each pen containing 12 hens and 2 roosters. The pair of roosters was rotated between pens on a weekly basis. A basal diet was formulated that met nutrient requirements of the hens and to it was added per kg either No vitamin K (Diet 1); 3.0 mg Vitamin K3 (Diet 2); 3.0 mg vitamin K1 (QAQ) (Diet 3) or 6.0 mg vitamin K1 (QAQ) (Diet 4) to make up the four experimental diets. Quinaquanone® (QAQ) is a patented product of Agricure Pty Ltd. It is a stabilised soluble formulation and contains both K1 and K2 (10:1). The eggs that were retained for hatching were maintained at 14^{o} C for up to seven days before being placed in a Multiplo® automatic incubator for 18 days and then transferred to a hatcher for 3 days. After hatching, chick livers were removed and frozen prior to analysis for vitamin K1 and vitamin K2 (MK4, menaquinone). MK4 was measured as it is believed that it is alkylated form of menadione in the liver.

Low hepatic concentrations of Vitamin K1 were found in chicks from hens fed diets without supplementation with this vitamin. Dietary supplementation resulted in significant ($P<0.05$) increases in hepatic concentrations that reflected levels in the diet. In contrast, concentrations of MK4 remained low and relatively constant across treatments. The vitamin K1 concentrations found in chick livers were as expected from the dietary levels. The low levels found in livers from diets 1 and 2 reflect the amounts contributed to the diets by soybean meal, canola meal and soy oil. If normal commercial ingredients are fed it is impossible to have a diet completely devoid of vitamin K (H. Regtop *pers com*.). The results for MK4 and vitamin K1 were similar to the concentrations found in livers from diets 1 and 2. It is unclear from where MK4 originates as it is only formed in mammalian tissues but little is known about the metabolism in chickens. The results from this study suggest that there is little or no conversion of vitamin K3 to MK4 and only limited conversion of vitamin K1 to MK4. The ramifications of the hepatic reserves of vitamin K from feeding hens different forms of vitamin K, found in this study, for the skeletal development and health of the chick awaits elucidation.

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RAPID ASSESSMENT OF FEED INGREDIENT QUALITY

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Summary

Near infrared spectroscopy, image analysis technology and immunoassays and enzymic colorimetric tests are now well advanced to allow rapid assessment of the nutrient composition, presence of anti-nutritional factors, mycotoxins and weed seed contamination of feed ingredients prior to diet formulation. There is a great opportunity for poultry producers to use these technologies to optimise diet formulation, improve bird performance and enterprise profitability. Recent developments in several of these technologies are discussed.

I. INTRODUCTION

Rapid assessment of the quality of feed ingredients is now crucial for the financial success of intensive livestock producers. A great deal is known about the nutrient requirements, factors determining feed intake and the negative impacts of anti-nutritional factors for all intensively reared livestock including poultry. Diets formulated for optimum performance and profitability need to meet, but not greatly exceed, the nutrient requirements for specific livestock classes and to contain low concentrations of anti-nutritional factors and mycotoxins.

There are large variations across and within feed ingredients in the quantity and availability of energy, amino acids and minerals such as phosphorous. For example, the apparent metabolisable energy (AME) content (MJ/kg) of wheat, barley and triticale has been shown to range between samples within each grain type by over 3 MJ/kg (Scott, 2004; Black et al., 2005). The intake of energy (MJ/day) by broilers varies by approximately 34% when different samples of wheat are incorporated into diets (Black, 2008). Similarly, the variation in nutrient content of Australian canola meal samples is high, with crude protein ranging from 316 to 425 g/kg, lysine from 17.7 to 21.4 g/kg, methionine plus cysteine from 14.0 to 17.3 g/kg, threonine from 13.7 to 16.5 g/kg and total phosphorus (P) from 7.9 to 11.9 g/kg with phytate bound P ranging from 67 to 95% of total P (Spragg and Mailer, 2007). Glucosinolate content also ranged widely from 2.4 to 8.9 mmole/g in these canola samples and can have negative effects on animal performance. Mycotoxin contamination of feed ingredients occurs sporadically, but when present can have a major impact on the performance of poultry (Yunus et al. 2011).

Traditional methods for assessing the nutritional value of feed ingredients such as mean values tabulated for specific feed types or assessing available energy content from test weight (kg/hl) and screening percentage are frequently inaccurate, whereas laboratory analyses are costly and generally provide results long after the ingredient has been used. However, in recent years near infrared spectroscopy (NIR), image analysis and immunoassay or enzymic colorimetric test strips have been developed to allow rapid and often real-time measurement of feed ingredients before being fed to animals. Ingredients can be selected for diet formulation to best meet animal requirements based on these rapid measurements. This

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paper discusses recent developments in NIR technology for predicting the available energy content (MJ/kg) and intake (MJ/d) of cereal grains for poultry and the available lysine content in oilseed meals. Brief reference is also made to developments of technologies for rapid assessment of other ingredient components including anti-nutritional factors and mycotoxins.

II. AVAILABLE ENERGY IN CEREAL GRAINS

Provided other nutrient requirements are satisfied, the rate of broiler production is driven by the intake of available energy (MJ/d), whereas the efficiency of production (kg product/kg feed) is driven primarily by the available energy content of the diet (MJ/kg). Consequently, both measures of energy (MJ/d and MJ/kg) are needed to fully describe the apparent metabolisable energy (AME) value of a grain for broilers. A series of experiments have been conducted at the South Australian Research and Development Institute, Roseworthy with 309 cereal grains to measure AME content and AME intake in broiler chickens. The cereal grains examined were wheat, barley, triticale, sorghum, oats, maize and rice. The grains included those grown under near normal environmental conditions, weather damaged grains due to drought, frost and pre-harvest germination, new or unusual cultivars such as high and low amylose grains, hard and soft cultivars, coloured and white endosperm grains and those with unusual NIR spectra. Each experiment included approximately 30% of grains that had been used in previous experiments to provide connectivity between experiments for statistical analysis needed to remove variation between experiments. Hence, the series of experiments could be regarded as one large experiment, with statistically corrected values for individual grains changing slightly after each experiment.

Chickens were allocated on day 22 of age and given 3 days to adapt to the cages and experimental diets. Each cage contained five birds and was made of wire mesh with dimensions of 60 cm x 45 cm and 38 cm high. Trays were fitted below each cage for collection of excreta. All cages had individual feed troughs and drinking nipples and were shielded to prevent cross-contamination of excreta. After the 3 day adaptation period, excreta was collected daily for 4 days and dried overnight at 80ºC in a fan-forced oven. The dried excreta from each cage were pooled over the four day collection period. The birds from each cage were weighed as a group when introduced into the cage and after 7 days. Cage feed consumption was measured on days 3 and 7, then converted to mean daily intake over each period.. Dried feed and excreta samples were finely ground and analysed for gross energy content using an isoperibol bomb calorimeter with benzoic acid standardisation. Diets were formulated to contain per kg: 807 g cereal grain, 155 g casein, 11 g dicalcium phosphate, 13 g limestone, 7 g DL methionine, 2 g mineral-vitamin mixture, 3 g sodium chloride and 2 g choline chloride (60%). Experiments for 175 grains (210 with connectivity grains) included treatments with and without the addition of xylanase and phytase enzymes[#].

A sample of each grain was scanned using a FOSS 6500 instrument at the time the grains were prepared into diets to be fed to birds. Scans were conducted on whole and laboratory milled grain samples. Calibrations were developed in WINISI software (FOSS, Denmark) using modified partial least squares regression and cross-validation, with scatter correction and mathematical treatments chosen to optimise calibration statistics. Calibration results presented are following omission of grains seen by the WINISI software as outliers after two passes.

[#] wheat, barley and triticale = Porzyme 93010 50 g/tonne and phytase Phyzyme TPT 50 g/tonne;
sorghum = Rovabio Excel 200 g/tonne and phytase Phyzyme TPT 50 g/tonne;
maize = Avizyme 1500 and phytase Phyzyme TPT 50 g/tonne.

a. AME predictions

Table 1 shows statistics for the NIR calibrations based on whole grain and milled grain scans for AME (MJ/kg as fed) and AME Intake Index, which is calculated by dividing each value for AME intake (MJ/day) from all experiments by the highest value and multiplying by 100 to give values from theoretically 0 to 100. The index value was used rather than the MJ/d value because the latter changes each day as a chicken grows. The index provides an estimate of the relativity between grain samples in the amount eaten when incorporated into a diet. Values are shown for the first calibration established following experiments with 109 grains and the last calibration with 309 grains.

Table 1 - NIR calibration statistics for AME content and AME Intake Index following whole grain and milled grain scans for the initial and final calibrations

Calibration	N[1]	Mean[1]	SD[1]	RSQ_{CAL}[1]	SEC[1]	RSQ_{CV}[1]	SECV[1]	RPD[1]
AME (MJ/kg as fed)								
Whole grain scans								
Calibration 1	102	12.40	1.15	0.88	0.40	0.82	0.48	2.40
Calibration 8	295	13.01	1.28	0.93	0.35	0.90	0.40	3.19
Milled grain scans								
Calibration 1	106	12.37	1.20	0.91	0.35	0.86	0.45	2.67
Calibration 8	293	12.99	1.31	0.93	0.36	0.90	0.42	3.10
AME Intake Index (0-100)								
Whole grain scans								
Calibration 1	104	64.8	8.84	0.84	3.57	0.70	4.85	1.82
Calibration 8	298	76.4	9.83	0.87	3.55	0.93	4.31	2.38
Milled grain scans								
Calibration 1	105	65.0	8.64	0.81	3.79	0.76	4.25	2.03
Calibration 8	296	76.3	9.82	0.85	3.83	0.81	4.24	2.31

[1]N, observations used in calibration; Mean of observations; SD, standard deviation of observations; RSQ_{CAL}, R^2 for all samples used in calibration; SEC, standard error of calibration; RSQ_{CV}, R^2 in cross-validation; SECV, standard error of cross-validation, RPD, ratio of Prediction to Deviation = SD/SECV an indication of the value of the calibration, < 1.5 calibration unsatisfactory; 1.5–2.0: calibration can distinguish between high & low values; 2.0-2.5: calibration is quantitative; 2.5-3.0: calibration predictions good; > 3.0: calibration predictions excellent.

Inclusion of an additional 200 grains improved substantially both the accuracy and robustness of the calibrations for predicting the AME content and AME Intake Index of cereal grains for broiler chickens compared to the initial calibrations. The accuracy of the calibration (SECV) for AME content based on whole grain scans improved from ± 0.48 for the initial calibration to ± 0.40 (MJ/kg as fed) for the final calibration. This result means that values are predicted with 95% confidence to be within ± 0.80 MJ/kg as fed of the actual value. Similar improvement from ± 4.85 for the initial calibration to 4.31 (% units) for the final calibration for AME Intake Index was found. The robustness of the calibrations as assessed by the Ratio of Prediction to Deviation (RPD) was also substantially improved and means that fewer grains will be seen by the calibrations as outliers. RPD for the NIR calibration predicting AME content increased substantially from 2.40 for the initial calibration to 3.19 for the latest calibration. Values over 3.0 for RPD are generally considered excellent by NIR specialists for such applications. The RPD value for AME Intake Index increased from 1.82 for the initial calibration to 2.38 for the latest calibration. The NIR calibrations predicting AME content are more accurate and robust than for those predicting AME intake, but the latter still provides useful information to separating cereal grains on the basis of likely feed intake.

The R^2_{CAL}values shown in Table 1 of 0.93 for the regression between NIR predicted and observed AME contents of cereal grains are considerably higher than 0.35 for an AME calibration derived from 94 whole grain scanned wheat samples developed by Owens et al. (2009) or the value of 0.45 for predicting AME of wheat from a calibration based on milled grain scans of 160 samples by Garnsworthy et al. (2000). However, Hughes et al. (2014) has shown considerably greater accuracy of prediction when a 'global' calibration including several grain species is used than when within grain calibrations alone are developed. The within grain R^2 values ranged from 0.23-0.36 for wheat, barley and triticale, but the exception was sorghum where the within grain R^2 was 0.90.

The accuracy of prediction from the NIR calibration established to predict the AME content of cereal grains for broilers as shown by the SECV of ± 0.40 MJ/kg as fed is considerably less than when the same grains are fed to pigs where a value of ± 0.27MJ/kg for digestible energy (DE) content has been obtained. The number of grains used to establish the calibrations in both species of animals was similar, but the average standard error for measured AME values in broilers was 0.20 MJ/kg compared with 0.11 MJ/kg in pigs. This variation in measurement means that the accuracy of the multigrain NIR calibration for broilers cannot be greater than twice the standard error. Thus, the current accuracy of ± 0.40 MJ/kg as fed is unlikely to be improved unless the experimental variation can be reduced. The lower variation in measurements of DE in pigs compared with measurements of AME in poultry may be due to individual pigs being accounted for in the experimental design, but this is not possible with the broiler experiments where measurements of AME were for a cage of five birds. In addition, differences in gut microbial population between birds and variation in mean retention time of digesta in the gastrointestinal tract of broilers are known to have an effect on AME values (Torok et al., 2008; Hughes, 2008). There appear to be large differences between individual birds in the extent of antiperistaltic waves, which is likely to alter mean retention time of digesta.

b. Comparison of whole grain and milled grain scans

Comparison of the calibration statistics between whole grain and milled grain scans (Table 1) suggests there would be little difference in accuracy between the calibrations when used to predict either AME content or AME Intake Index. The accuracy of the final calibration for AME content based on whole grain scans was slightly better than that based on milled grain scans with values for standard error of cross validation being ± 0.40 and ± 0.42, respectively. Robustness of the calibration for AME using whole grain was also slightly better than when the calibration was based on milled grain (RPD, 3.19 compared with 3.10). Similar, slightly better results were found for the calibrations predicting AME Intake Index when using whole grain rather than milled grain.

The mean difference in AME content between whole grain and milled grain across all grains was minimal at 0.016 MJ/kg as fed. However, the regression equation relating predicted AME content (MJ/kg as fed) from calibrations based on milled grain (Y) or whole grain (X) was: Y=0.97+0.33. The R^2 for the regression was only 0.92, which means there are considerable differences in the predicted values for some individual grains. The greatest difference in predicted AME content between whole grain and milled grain was 1.19 MJ/kg as fed for one of the rice samples. Approximately 18% of all samples had differences in predicted values greater than the standard error of cross validation of ± 0.40 MJ/kg as fed for the whole grain calibration.

Similarly, the mean difference in AME Intake Index between whole grain and milled grain scans across all grains was minimal at 0.101 % units. However, the R^2 between the values predicted using whole grain or milled grain is only 0.88. The greatest difference in

predicted AME Intake Index between whole grain and milled grain was 11.01 % units and approximately 16% of all samples had differences in predicted values greater than the standard error of cross validation of ± 4.2 % units for the whole grain calibration. This comparison highlights one of the difficulties with NIR technology in statistically relating spectra obtained from different preparations of the same grain to measured values. It would be expected that the differences between predicted values from calibrations based on whole or milled grain scans would decrease as the number and range of samples was increased.

c. NIR calibrations for enzyme response

The response in AME content (MJ/kg) and AME intake (MJ/d) to the addition of xylanase and phytase enzymes varied greatly within and between grain types (Table 2). The greatest mean response in AME content of 0.74 MJ/kg as fed was for wheat samples, with a substantially lower mean response of 0.29 MJ/kg as fed for triticale and 0.22 MJ/kg as fed for barley. The mean response to enzymes included in sorghum or maize diets was low, with mean enzyme response values of 0.04 and 0.02 MJ/kg as fed, respectively. Six of the 35 barley samples, 20 of the 66 sorghum samples and four of the ten maize samples gave a negative response to enzymes. A similar pattern of response to enzymes was found for AME intake, except the response for maize was high in some samples.

Table 2 - Measured mean, minimum and maximum responses to xylanase and phytase enzymes for AME content (MJ/kg as fed) and AME intake (MJ/d) in broiler chickens fed diets with different grain types

Grain type	Mean	Minimum	Maximum	Number Total/Negative
	Grain AME enzyme response (MJ/kg as fed)			
Wheat	0.74	0.21	1.29	37/0
Barley	0.22	-0.13	0.63	35/6
Triticale	0.29	0.07	0.82	27/0
Sorghum	0.04	-0.39	0.31	66/20
Maize	0.02	-0.23	0.36	10/4
	Grain AME intake enzyme response (MJ/d)			
Wheat	0.086	-0.074	0.248	37/2
Barley	0.029	-0.114	0.192	35/7
Triticale	0.053	-0.039	0.129	27/3
Sorghum	0.012	-0.150	0.199	66/23
Maize	0.053	-0.059	0.242	10/5

Results from the responses to enzymes in AME content and AME intake were used to develop NIR calibrations for all grains including connectivity grains where enzymes were included in the diet. Statistics for calibrations developed using whole scans are shown in Table 3. A satisfactory NIR calibration could not be developed for the response in AME intake to enzymes, whereas the calibration for AME content was of marginal value. The response in AME content to enzymes could be predicted with a 95% probability to ± 0.38 MJ/kg as fed. The robustness of the calibration, as illustrated by the RPD of 1.81, was considered to be sufficient for distinguishing between high and low responders. However, the addition of more samples should strengthen the calibration.

Table 3 - NIR calibration statistics for AME content and AME intake using whole grain scans for the response to xylanase and phytase enzymes ([1]Refer to Table 1 for abbreviations)

Calibration	N[1]	Mean[1]	SD[1]	RSQ_{CAL}[1]	SEC[1]	RSQ_{CV}[1]	SECV[1]	RPD[1]
AME (MJ/kg as fed)								
Whole grain scan	200	0.29	0.34	0.73	0.18	0.70	0.19	1.81
AME intake (MJ/d)								
Whole grain scan	200	0.040	0.060	0.23	0.052	0.22	0.053	1.13

III. TOTAL AND AVAILABLE LYSINE IN OIL SEED MEALS

Variation in processing methods and temperature applied to oilseeds during the oil extraction process can markedly affect the availability for animals of essential amino acids, particularly lysine (Newkirk and Classen, 2002). Wide variation in amino acid availability between specific batches of oilseed meals can have marked effects on the efficiency of production, time taken for animals to reach market specifications and profitability of enterprises. The heating of proteins, particularly in the presence of reducing sugars, is known to induce the Maillard reaction, rendering the lysine incorporated into reaction products unavailable for metabolism by animals. However, a proportion of early Maillard or Amadori products can be reconverted to lysine during conventional amino acid analyses and is falsely assumed to be available to animals. True lysine can be converted to homoarginine through a guanidination reaction for the measurement of lysine available to animals for metabolism. Lysine measure by this process has been called reactive lysine (Moughan and Rutherfurd, 1996). Heat treatment of oilseed meals reduces the measured total lysine content, but reactive lysine as a proportion of total lysine can vary widely depending on the amount and time of heat treatment.

Two projects in Australia have been designed to develop NIR calibrations to predict the total and reactive lysine content of canola meal and soybean meal. Canola samples were collected from six commercial oilseed crushing plants using expeller and solvent oil extraction procedures (Spragg, 2011). The cooking temperatures and times for processing some of the samples were either increased or decreased compared with normal procedures. Total and reactive lysine content was measured on 129 samples, with total lysine ranging from 15.8 to 24.4 g/kg and reactive lysine as a proportion of total lysine ranging from 0.66 to near 1.0. NIR calibration statistics for the total and reactive lysine content of canola meal are shown in Table 4. Although these calibrations are promising, RPD values are relatively low at 2.14 for total lysine and 2.00 for reactive lysine. Consequently, an additional 60 samples, some of which have been autoclaved for different times, are being added to increase the range in values and to strengthen the NIR calibrations.

Soybean meal samples imported into Australia from major soybean meal producing countries (USA, Brazil, China, Argentina, India) have been collected with 216 being analysed for total and reactive lysine (Kim and Mullan, 2013). In addition, a further 68 soybean meal samples and 25 soy protein concentrate samples were subjected to autoclaving at 134°C and 217 Kpa for periods from 5 to 30 minutes prior to analysis for total lysine, reactive lysine and other amino acid contents. There were significant negative, linear effects of time of heat treatment on the measured total lysine, reactive lysine, arginine and cysteine concentrations of the meal. NIR statistics for total and reactive lysine content of combined soybean meal and soy protein concentrate are given in Table 4 and are highly positive. The standard error of cross validation was ± 1.02 g/kg and 0.96 g/kg (as fed) and R^2 values of 0.94 and 0.95,

respectively, for total and reactive lysine. These values mean that total and reactive lysine content of soybean meal and soy protein concentrate should be predicted with 95% confidence to be within 2.04 and 1.92 g/kg, respectively, of the actual value. The RPD values of 3.35 and 3.94, respectively, for total and reactive lysine, indicate that the calibrations have high robustness for predicting accurately values for unknown soybean meal and protein concentrate samples. Amino acid digestibility assays were also conducted during the experiments and NIR calibrations were developed for apparent, standardised and true ileal digestible total lysine and reactive lysine. RPD values for each calibration were between 3.4 and 4.9 (results not shown), indicating robustness in prediction.

Table 4 - NIR calibration statistics for the total and reactive lysine content (g/kg as fed) in canola meal and soybean meal - protein concentrate ([1]Refer to Table 1 for abbreviations)

Calibration	N[1]	Mean[1]	SD[1]	RSQ_{CAL}[1]	SEC[1]	RSQ_{CV}[1]	SECV[1]	RPD[1]
Canola meal								
Total lysine	126	19.4	1.63	0.90	0.51	0.78	0.76	2.14
Reactive lysine	124	16.6	1.85	0.84	0.73	0.75	0.93	2.00
Soybean meal and protein concentrate								
Total lysine	298	26.8	3.42	0.94	0.86	0.91	1.02	3.35
Reactive lysine	300	24.5	3.77	0.95	0.87	0.94	0.96	3.94

IV. OTHER RAPID ASSESSMENT OF INGREDIENT QUALITY

In addition to the energy content of cereal grains and available lysine content of oilseed meals, other information about feed ingredients is required to optimise diet formulation. Several commercial companies now provide rapid turnaround times for NIR analysis of many nutrients in feed ingredients including moisture, crude protein, essential amino acids, starch, ether extract, ash, acid detergent fibre, neutral detergent fibre, total and phytate bound phosphorus. The ingredients are scanned by the end-user and the scans uploaded via the world wide web where they are analysed using a range of NIR calibrations and the results returned promptly. Some companies also provide estimates of standardised digestible amino acid content. NIR calibrations also have been developed for several anti-nutritional factors including glucosinolates and sinapines in canola meal (Font et al., 1999; McFadden and Mailer, 2003). Image analysis or machine vision technology is now well advanced and is used in many industries for quality control. Image analysis software has been developed to identify cereal grain quality (Guevara-Hernandez and Gomez-Gil, 2011) and weed seed contamination (Pablo et al., 2002). Similarly, immunoassay and enzyme binding techniques with colorimetric analysis via test strips or image analysis have been developed to identify the presence of mycotoxins including ochratoxin A contamination in cereal grains (Bazin et al., 2010) and various of aflatoxins (Moscone et al., 2011).

The advent of many accurate and rapid methods for assessing the quality of feed ingredients before they are used by poultry producers greatly increases the opportunity to formulate diets that will optimise nutrient utilisation, reduce concentrations of anti-nutritional factors and decrease the risk of mycotoxin contamination to greatly improve productivity and enterprise profitability.

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FEED INGREDIENTS: ASSESSMENT AND ENHANCEMENT OF NUTRITIONAL VALUE

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Summary

The nutritional value of feed ingredients is primarily dependent on the contents of energy supplying nutrients (starch, fat and protein), amino acid supplying protein and the efficiency of the digestion and absorption in the gastro-intestinal tract. Although the chemical composition of feed ingredients can be analysed accurately by wet chemistry or NIR, nutrient digestibilities may vary between batches of the same feedstuffs and between poultry species. Parameters to predict nutrient digestibilities and/or to distinguish between high and low quality feed ingredients should be quick, accurate and cheap. The potential for *in vitro* models that simulate the rate of digestion of nutrients is discussed. Several possibilities to improve the nutritional value of feed ingredients and complete diets are indicated, based on conditions in the intestinal tract and on feed (ingredient) characteristics.

I. INTRODUCTION

The nutritional value of feed ingredients comprises all nutrients needed for maintenance and production of animals. This paper mainly focuses on the energy value of feed ingredients, which depends on the nutrient composition (starch, fat and protein), the efficiency of nutrient digestion and absorption in the intestinal tract, and the presence of anti-nutritional factors (ANFs). Although the composition of feed ingredients can be analysed by quick methods like NIR accurately, it does not take differences in the digestibility of nutrients in the intestinal tract into account, which can vary among different batches of the same feedstuff. Garnsworthy et al. (2000) indicated that NIR methods predicted the chemical and agronomic characteristics of wheat with high accuracy, whereas prediction of the nutritional value was much less accurate due to animal variation. Recently, Black et al. (2014) indicated NIR calibrations for nutritional value were improved substantially.

Processing of feedstuffs, like heat treatment to eliminate ANFs or for drying purposes and milling/pelleting, affects nutrient digestibility values. Good quality parameters for eg. soybean meal are still topic of debate, because of a questionable reliability of current parameters like urease activity, protein dispersibility index, KOH protein solubility and trypsin inhibitor. These techniques have low consistency and sensibility among laboratories or are very tedious (De Coca et al., 2008).

Feedstuff tables generally comprise one batch of single feed ingredients that can be used in feed formulation. In case of known variable nutritional values, more batches might be included, often still accepting a fixed ratio between digestible amino acids per unit of crude protein, and/or similar nutrient digestibility values for different qualities of a feedstuff and types of poultry. Of course tabulating nutritional values for several batches of a single feedstuff is only relevant if different qualities could be identified via quick, cheap and reproducible methods. It should be realized that only using variable nutrient contents, like eg. the different crude protein contents of soybean meal, are not be the best discriminating factors. Maize batches with a similar nutrient composition were shown to have distinctly different nutritional values (Gehring et al., 2012). Prediction of the quality of feed ingredients is therefore very important to be able to formulate diets the meet the nutrient requirements of the birds.

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II. ASSESSMENT OF NUTRITIONAL VALUES IN VIVO

Feedstuff databases are traditionally built on digestibility studies with adult roosters. These birds enable high through-put studies to evaluate the feeding value of single feedstuffs and to indicate variation in nutritional quality among different batches of the same feedstuff. Therefore, also many NIR calibrations are based on *in vivo* data with adult roosters. However, it is shown that feeding values vary between adult roosters and broilers (eg. Rodrigues et al. 2001, 2002), which justifies developing separate feeding tables for young broilers and adult birds. Apparent AMEn values for different types of poultry are summarized in Table 1, as modified from Cozannet et al. (2010).

Table 1 - The MEn[1] value in feeds for roosters, layers, broilers and turkeys (adapted from Cozannet et al., 2010)

	Rooster	Layer	Broiler	Turkey	
	Kcal/kg				
Feed	DM	relative to roosters (%)			Reference
Complete diets					
Diets (n=6)	3346		94.7	94.7	Barrier-Guillot and Métayer (2001)
Diets (n=7)	3250		95.2		Bourdillon at al. (1990)
Diets (n=10)	3250	96.2	94.6	93.2	Cozannet et al. (2010)
Diets (n=2)	3083	96.8			Lessire et al. (1995)
Diets (n=1)	3203	101.8			Parsons et al. (1982)
Cereals					
Wheat	3513		89.6	99.9	Barrier-Guillot and Métayer (2001)
Wheat (n=2)	3933		96.8	96.1	MacLeod et al. (2008)
Barley	3179		97.7	98.1	Barrier-Guillot and Métayer (2001)
Rye	3513		96.4	98.8	Barrier-Guillot and Métayer (2001)
Maize	3848		99.4	102.4	Barrier-Guillot and Métayer (2001)
Maize (n=3)	4098		100.9		Rodrigues et al. (2001)
Oats	4088		96.3	97.7	MacLeod et al. (2008)
Millet (n=3)	3919		94.6		Rodrigues et al. (2001)
Protein sources					
Soybean meal	2725	91.7			Askbrant (1988)
Soybean meal (n=4)	2826		95.2		Rodrigues et al. (2002)
Soybeans (full fat)	4045		91.0		Rodrigues et al. (2002)
Canola meal (n=3)	1793	102.9			Askbrant (1988)
Corn gluten meal	2291		98.8		Rodrigues et al. (2001)
Peas	2916	92.7			Askbrant (1988)
Peas	3011		96.6	96.5	Barrier-Guillot and Métayer (2001)
Wheat DDGS, light	2557	93.7	95.0	88.6	Cozannet et al. (2010)
Wheat DDGS, dark	2247	91.5	98.7	84.8	Cozannet et al. (2010)

[1]Values are AMEn values except for underlined data, which are TMEn values

It is indicated in Table 1 that the difference between roosters and broilers can be quite variable, eg. for wheat. Svihus and Gullord (2002) concluded from their study with twenty batches of wheat that the low-AME wheat phenomenon was observed in broilers but not in roosters, and was related to a high feed intake and low starch digestibility. This indicates that low quality feedstuffs might be evident in young broilers, but not in roosters.

For the purpose of building a more extensive dataset on AMEn values for laying hens, Schothorst Feed Research recently conducted two experiments evaluating sixteen feedstuffs in both broilers (at 21-24 days of age) and laying hens (at 24-26 weeks of age; minimum

laying rate 90%). These feedstuffs were added to a basal diet (which varied in composition for broilers and laying hens) to obtain the experimental diets. Diets were pelleted (pellet temperature < 65°C) and fed for *ad libitum* intake during ten to twelve days, followed by a three-day excreta collection period (collection once daily. Samples were immediately stored after collection at -20°C (assay similar to Bourdillon et al., 1990, except for using TiO_2 as an inert marker and omitting a feed withdrawal period)). The AMEn value of test feedstuffs were calculated from results of experimental and basal diets. All measurements were done with six replicates using twelve broilers or eight layers per replicate cage. Results are given in Figure 1.

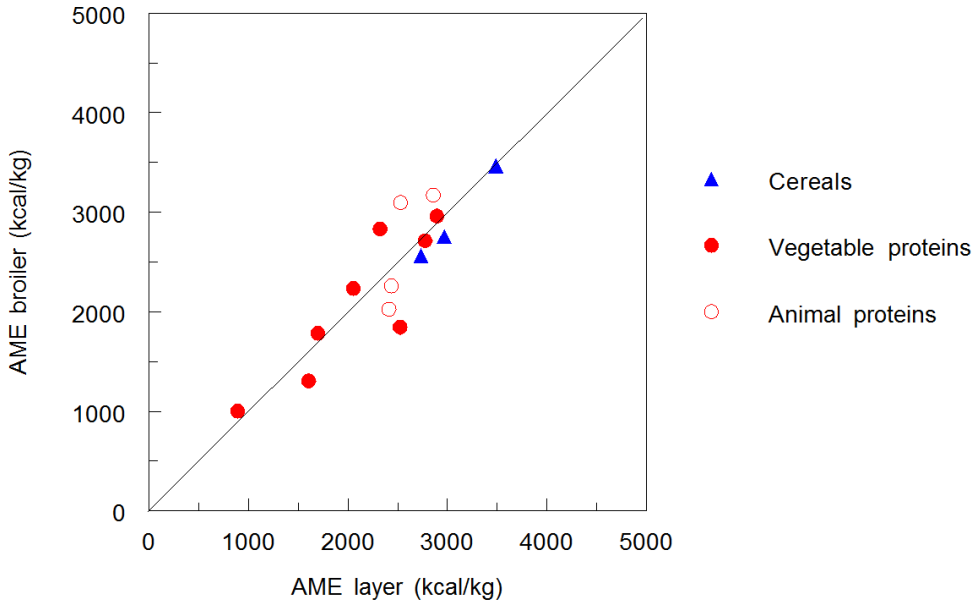

Figure 1 - The AMEn value in feedstuffs for laying hens and broilers (data Schothorst Feed Research). Line X=Y is indicated.

Based on Table 1 it was concluded that in general AMEn values in broilers are lower compared to roosters, whereas Figure 1 indicates that feedstuff AMEn values in laying hens can be both lower (min -20%) and higher (max +35%) than in broilers. Therefore, the validity of rooster data is not only questionable for broilers, but also for producing laying hens. This will have consequences for the type of bird that should be used for calibration of methods to predict the nutritional values of feed ingredients for poultry, as was also concluded by Yegani et al. (2013).

III. PREDICTION OF NUTRITIONAL VALUES OF FEED INGREDIENTS

The nutritional value of feed ingredients is dependent on the contents of energy delivering nutrients (starch, fat, protein) and their corresponding digestibility values. Starch is the main source of metabolizable energy in poultry diets. Although its digestibility is assumed to be almost complete at the lower ileum, potential variation has been reported as reviewed recently by Svihus (2011). Gutierrez del Alamo et al. (2009) showed that starch digestion rate in 30-d old broilers varied with wheat origin and cultivar, whereas Svihus (2011) indicated the following potential factors affecting starch digestibility: Cereal characteristics like starch granule size, interactions with the gluten matrix in wheat or sorghum, starch entrapment in the cell wall material, and the amylose/amylopectin ratio and high feed intakes of modern

broilers when fed pelleted diets. Amylopectin is due to its branched structure more easily degraded by amylase compared to the more linearly arranged amylose. Collins et al. (2003) reported an AMEn value of 2860 kcal/kg for waxy maize (3% of starch as amylose) and 2790 kcal/kg for normal maize (27% of starch as amylose) in 7-9 day old broilers, whereas such a difference was not evident for roosters (average AMEn of 3250 kJ/kg). Also Ertl and Dale (1997) did not observe differences between waxy and normal corn in roosters, despite a difference between maize varieties with a similar amylose/amylopectin ratio of 140 kcal/kg DM. Ravindran et al. (2007) observed lower AMEn values in waxy hull-less barley compared to normal hull-less barley (being 2410 and 3015 kcal/kg DM, respectively), which was in their study related to the higher soluble β-glucan content in waxy barley cultivars.

Protein-starch interactions were not only reported in wheat (Svihus, 2011), but also in maize (Gehring et al., 2012) and sorghum (Selle, 2010). Gehring et al. (2012) showed that salt-soluble protein in maize (ranging from 25 to 50%), indicating differences in the starch accessibility related to the starch-protein interface, was correlated with dietary AMEn (ranging from 3262 to 3342 kcal/kg) in 30-d old broilers. However, the AMEn value was not correlated with starch digestibility, and therefore needs further validation.

In vitro techniques have been developed to estimate the potential digestibility of nutrients enabling a more accurate calculation of the energy value of feed ingredients. Weurding et al. (2001) showed a good correlation between *in vitro* starch digestion during 2h and 4h incubation and the rate of *in vivo* starch digestion in the small intestine of broilers for twelve feed ingredients, whereas Yegani et al. (2013) showed a high correlation between *in vitro* digestibility of gross energy and *in vivo* AMEn values in 14-day old broilers using six batches of wheat and two batches of triticale. However, as this *in vitro* method that was optimized for wheat and triticale samples, it needs to be validated for other feed ingredients. In general, techniques that estimate the rate of nutrient digestion predict the actual feeding value more accurately than end point titrations, as was summarized by Van der Klis and Kwakernaak (2013). Black et al. (2014) highlight the value of NIR as rapid assessment of feed ingredient quality in their Australian Poultry Science Symposium paper and will therefore not be discussed here.

IV. IMPROVEMENT OF NUTRITIONAL VALUES

Tabulated data on nutritional values of feed ingredients are generally based fecal apparent nutrient digestibility values, i.e. not corrected for endogenous losses and for fermentation. Therefore, ANFs that normally induce endogenous losses will directly impact such nutrient digestibility values (especially of amino acids) and to a lesser extend AMEn values. Moreover, nutritional values of feed ingredients are supposed to be additive, without interactions. Absence of interactions between feed ingredients, however, is unlikely. Nutritionists try to limit those via constraints in diet formulation with respect to e.g. the ratio between unsaturated and saturated fatty acids, a minimum starch inclusion level and/or maximizing inclusion levels of ANF containing feed ingredients. As said interactions between feed ingredients do exists, like effects of low AMEn wheat varieties becoming more pronounced with increasing inclusion levels of animal blended fat, resulting in decreasing AMEn values of wheat (Van der Klis et al., 1995).

This example indicates that the nutritional value of feed ingredients should not be regarded separately from the intestinal physiology of the animal. For example, feed retention time in different intestinal segments, viscosity, reflux of chyme and microbial activity in the proximal parts of the small intestine clearly affect the efficiency of nutrient digestion and absorption. Increasing gastro-intestinal development and reflux by structural components in poultry diets (like adding inert fiber and/or increasing feed particle size) improves AMEn

conversion (MJ/kg BWG) in case of suboptimal conditions in the intestinal tract (Figure 2). Highly methylated citrus pectin increases chyme viscosity and microbial growth in the small intestine and thereby reduces nutrient absorption and production performance.

Coarse grinding of cereals stimulates the development of the "proventriculus plus gizzard" in broilers, potentially improving the digestibility of the feed. Indeed coarse grinding was shown repeatedly to stimulate production performance (Amerah et al., 2007). However, milling for small particle sizes will increase the surface area of the substrate for enzymes. Fine grinding of maize (milled to pass a 2 mm sieve) was shown to have a higher ileal digestibility of crude protein and energy than more coarse grinding (milled to pass a 4 mm sieve): 0.82 vs 0.79 and 0.76 and 0.73 respectively), whereas starch digestibility was not affected (0.96 on average) (Bhuiyan et al, 2013). Péron et al. (2005) indicated that finely ground hard wheat improved the digestibility of starch (fine was milled to pass a 2 mm sieve (GMD 380 µm) and coarse to pass a 6 mm sieve (GMD 955 µm). This might indicate that starch granules are entrapped in cell walls and/or the protein matrix in cereals, limiting digestibility (Svihus, 2011).

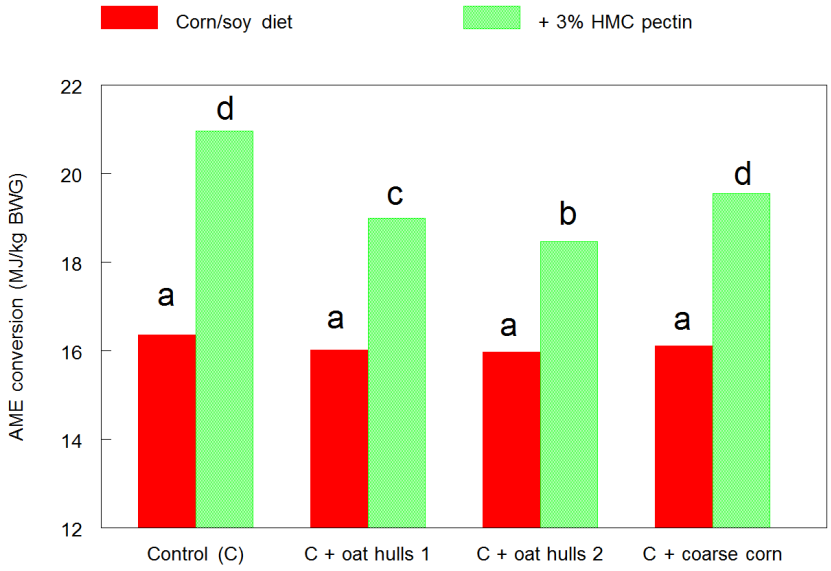

Figure 2 - The AMEn conversion (MJ/kg BWG) in 21-day old broilers fed a corn/soy diet with and without 3% high methylated citrus (HMC) pectin. Pelleted diets were fed with finely ground corn (milled to pass a 3 mm sieve), coarsely ground corn (milled to pass a 8 mm sieve) with 5% added oat hulls on isocaloric basis (1) or added on top (2). (LSD 0.432; P<0.01), van der Klis (2012).

Nutritional values of feed ingredients can also be improved by dietary supplementation of exogenous enzymes. The use of endoxylanases and β-glucanases in cereal-based poultry diets containing high levels of soluble non starch polysaccharides has become common practice, as it improves the nutritional value of the diet. In contrast, effects of carbohydrases, amylases and proteases to corn/soy diets can be inconsistent, being small to negligible. Meng et al. (2005) screened combinations of carbohydrases (cellulase, pectinase, xylanase, glucanase, and mannanase) *in vitro* on wheat, soybean meal, canola meal and peas, showing positive effects of these enzymes on all feed ingredients, achieving more pronounced effects when these carbohydrases were used in concert. Effective enzyme combinations were evaluated *in vivo* using 5 to 18 day old broilers fed mash diets, which improved dietary AMEn and ileal digestibility values of starch and protein (only the full combination of enzymes is shown in Table 2). They concluded that these carbohydrases were

effective in degrading cell wall polysaccharides and nutrient utilization, which probably has eliminated nutrient encapsulating affects of cell wall polysaccharides.

Table 2 - The effect of a combination of carbohydrases in the AMEn value and ileal digestibility of protein and starch in 18 day old broilers.

Enzyme	AMEn (kcal/kg)	Starch (%)	Protein (%)	NSP (%)
None	2902[b]	92.6 [b]	73.2[b]	6.3[b]
Full combination	3046[a]	96.7 [a]	79.8[a]	14.9[a]
SEM	21.0	0.49	0.72	1.37

Full combination: cellulase, pectinase, xylanase, glucanase, and mannanase

Yegani and Korver (2013) discussed potential reasons for inconsistent response of broilers when supplementing corn/soy diets with exogenous enzymes, being differences in the types and activities of the enzymes, using single enzymes or mixtures of enzyme activities, the nutritional quality of the dietary ingredients, form of the diet and age of the birds. They showed the largest effects in the grower phase from 12 to 28 days of age, but only in one out of the three corn batches tested, despite similar analyzed nutrient contents. Unfortunately, details on nutrient composition of diets and characteristics of feed ingredients in such experiments is too limited to start a proper evaluation of the reason for these inconsistent responses.

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SEPARATE CEREAL GRAIN CALIBRATIONS FOR PREDICTION OF APPARENT METABOLISABLE ENERGY BY NEAR INFRARED REFLECTANCE ANALYSIS

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Summary

Separate cereal grain calibrations for prediction of apparent metabolisable energy (AME) by near infrared reflectance (NIR) analysis were developed from the AusScan database containing all cereal grain types commonly used in Australia. Single-grain calibrations for wheat (82 samples), sorghum (80), barley (71) and triticale (54) were inferior to the global calibration involving all grain types (293 samples), however, the calibration involving sorghum has usefulness as a predictor of AME. The poor predictive ability of calibrations for wheat, barley and triticale appeared to be related to relatively few samples of grain with lower AME values often seen in Australia, particularly among freshly-harvested grains. The predictive power and robustness of both global and single-grain calibrations are expected to improve when more grains samples are added to the database.

I. INTRODUCTION

Cereal grains used by the poultry industry in Australia vary widely in available energy and protein content which is often reflected as variation in bird performance because standardised ingredient values are used for diet formulation. Rapid or real-time techniques for measuring the AME content of grains for birds will assist the purchase of grains at appropriate prices and the accuracy of feed formulation that should result in a marked reduction in variability in nutrients supply across formulated batches of feed.

Calibrations based on NIR spectroscopy for estimating the AME content and AME Intake Index of cereal grains for broiler chickens have been developed in Australia (Black et al., 2009). The calibrations were developed from results obtained in the Premium Grains for Livestock Program. Updated NIR calibrations were reported by Black et al. (2010). AusScan, a business arm of the Pork CRC Ltd, released the latest version of NIR calibrations for broiler chickens in 2012. To date, all AusScan NIR calibrations licensed for industry usage have been of a global nature involving several cereal grain types. Australian users of the calibrations for predicting AME for broilers have expressed interest in the development of single-grain calibrations from existing information in the global database.

This paper compares separate NIR calibrations for predicting AME of wheat, sorghum, barley and triticale with a global calibration involving all cereal grain types.

II. METHOD

The 2012 AusScan calibration for broiler AME is based on 293 grain samples. Single-grain calibrations developed from this database contained 82, 71, 54 and 80 samples for wheat, barley, triticale and sorghum, respectively. The statistical characteristics of the calibrations are summarised in Table 1.

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III. RESULTS

Table 1 - Statistical characteristics of the single-grain and global calibrations for broiler AME (MJ/kg as fed basis). Whole grains were scanned in a Foss 6500 spectrophotometer. (Calibrations were developed with WinISI software)

Calibration	N	Mean	SD	RSQ	SEC	1-VR	SECV	RPD
Wheat	82	12.50	0.64	0.27	0.55	0.07	0.62	1.03
Barley	71	11.43	0.53	0.23	0.47	0.18	0.48	1.11
Triticale	54	13.07	0.37	0.36	0.30	0.31	0.31	1.19
Sorghum	80	14.44	0.39	0.90	0.12	0.79	0.18	2.20
Global	293	12.99	1.31	0.93	0.36	0.90	0.42	3.10

Statistic	Meaning
N	Number of observations used in final calibration – excluding outliers
Mean	Mean of experimental observations
SD	Standard deviation of experimental observations
RSQ	R^2 values - fraction of the variance accounted for by the NIR calibration when all accepted observations are included in the relationship
SEC	Standard error of the calibration – when all accepted observations are included in the relationship
1-VR	1-Variance Ratio – Fraction of variance accounted for in NIR prediction when some observations are used for 'cross validation' of the calibration as determined by the NIR software
SECV	Standard error of cross validation indicates the accuracy expected for a predicted value (the true value with a probability of 95%
RPD	Ratio of prediction to deviation indicates the robustness and reliability of the calibration for predicting values for unknown samples. Values < 1.5: unsatisfactory, 2.0 - 2.5: approximately quantitative, > 3.0: excellent

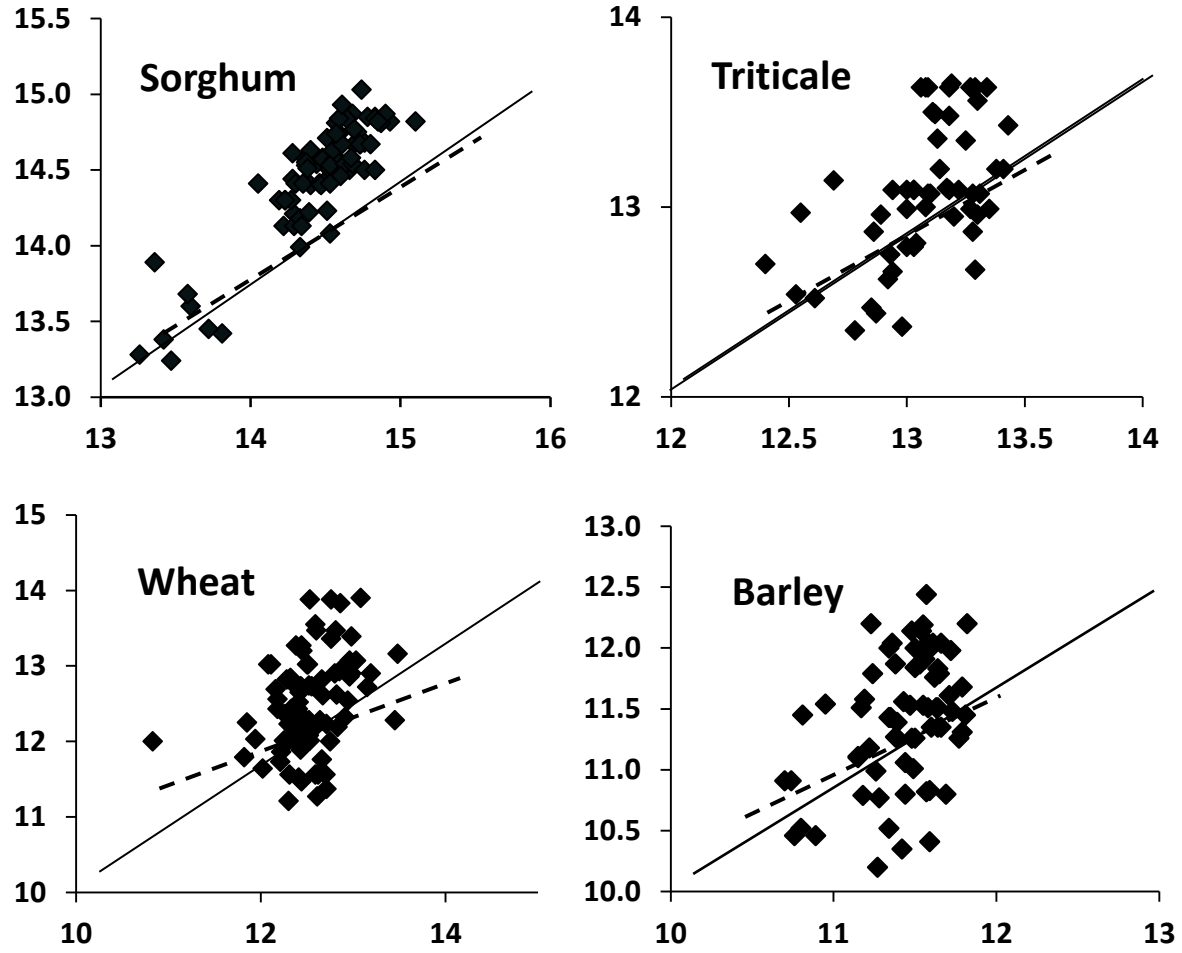

Figure 1 - Measured versus predicted values for AME (MK/kg as fed) for sorghum, triticale, wheat, and barley.

III. DISCUSSION

Measured versus predicted plots of AME (MJ/kg as fed) for wheat, barley, triticale and sorghum (Figure 1) indicate why single-grain calibrations had relatively poor statistical qualities. Sorghum has a reasonable range of values, as do triticale and barley, but the bulk of wheat samples appear in a narrow band (12-13 MJ/kg as fed). Other studies of AME values for 58 samples of wheat from widely separated growing regions in Australia in 2010, 2011 and 2012, showed a large proportion of samples in the range 10.5–12.0 MJ/kg as fed NIR predicted values were overestimates of the measured AME values for these wheats which were regarded as acceptable quality for inclusion in commercial diets. Therefore, it appears that wheats with lower AME values are poorly represented in the AusScan database, and that future work is needed, particularly with the inclusion of new season grains.

In conclusion, the results show that separate cereal grain calibrations were inferior to a global calibration involving all grain types. The usefulness of single-grain calibrations may improve when more grain samples, particularly those with AME values in the upper and lower ends of the ranges, are added to the database.

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PHYTASE SUPPLEMENTATION OF MAIZE-, SORGHUM- OR WHEAT-BASED BROILER DIETS

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Summary

In this preliminary report, equivalent diets based on maize, sorghum or wheat were offered to male Ross 308 chicks from 7 to 27 days post-hatch and treatment effects on growth performance and nutrient utilisation were determined. Phytase significantly increased weight gain, feed intake and FCR but, overall, maize-based diets were the most responsive to phytase as significant interactions between grain type and phytase supplementation were observed for nutrient utilisation parameters. Maize and sorghum outperformed wheat and relative gizzard weights were significantly lighter in chicks offered wheat-based diets. Relative gizzard weights were significantly correlated with gizzard pH, pancreas weight, FCR, AME, N retention and AMEn. Interestingly, phytase significantly reduced relative pancreas weights. The intention is to publish a complete report of this feeding study in the near future.

I. INTRODUCTION

Relatively few feeding studies have compared the effects of exogenous phytase in conventional broiler diets based on different cereal grains. However, Ravindran *et al.* (1999) compared the effects of a fungal phytase (1200 FTU/kg) on ileal amino acid digestibility coefficients in 'all-grain' diets (918 g/kg). Phytase increased ($P < 0.01$) average digestibility coefficients of 14 amino acids by 3.36% (0.800 versus 0.774) in maize, by 6.46% (0.791 versus 0.743) in sorghum and by 9.04% (0.844 versus 0.774) in wheat, which indicates that responses to phytase are variable across different cereals. This paper is a preliminary report of a study in which conventional, equivalent diets, based on maize, sorghum or wheat, were supplemented with a bacterial phytase at an inclusion level of 1000 FTU/kg. This paper takes into consideration the effects of cereal type and phytase addition in a 3 x 2 factorial array of dietary treatments. The parameters assessed include growth performance, relative gizzard and pancreas weights, pH of gizzard digesta, and nutrient utilisation.

II. MATERIALS & METHODS

The three cereals were mediumly-ground (3.2 mm hammer-mill screen) prior to incorporation into diets that were steam-pelleted at a conditioning temperature of 80°C. The phosphorus adequate experimental diets were based 560 g/kg maize, sorghum or wheat and were formulated to be equivalent for energy density (12.54 MJ/kg) protein (205 g/kg) and amino acids. In a 3 x 2 factorial array, diets were non-supplemented or supplemented with phytase (Axtra® PHY; Danisco Animal Nutrition) at 1000 FTU/kg. Each of the six dietary treatments was offered to 8 replicates (6 birds per cage) of male Ross 308 chicks from 7 to 27 days post-hatch. The parameters assessed by standard procedures included weight gain, feed intake, feed conversion ratio (FCR), relative gizzard and pancreas weights, gizzard pH, apparent metabolisable energy (AME: expressed as MJ/kg and MJ/day), nitrogen (N) retention, and N-corrected AME (AMEn). The experimental data were analysed by IBM® SPSS® statistical

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programs (Version 20). The conduct of the feeding study complied with specific guidelines (N00/6-2013/1/5981) of the Animal Ethics Committee of the University of Sydney.

III. RESULTS

Growth performance results are shown in Table 1 where the 4.2% mortality rate was not related ($P > 0.45$) to treatment. Broilers offered maize- and sorghum-based diets had significantly ($P < 0.01$) better weight gains and feed conversion ratios (FCR) than those offered wheat-based diets by averages of 5.6 and 3.1%, respectively. Phytase significantly increased ($P < 0.001$) weight gain (6.59%) and feed intake (6.23%) and improved FCR ($P < 0.05$) by 1.79%. The treatment interaction for weight gain closely approached significance ($P = 0.058$) because the phytase response with maize (12.3%) was more pronounced than with sorghum (3.27%) or wheat (4.49%). Relative gizzard weights were significantly heavier with maize and sorghum by an average of 20.6% (19.26 versus 15.97 g/kg; $P < 0.001$) compared to wheat-based diets and phytase reduced pancreatic weights by 9.2% (2.65 versus 2.92 g/kg; $P < 0.001$). Gizzard weights were negatively correlated with gizzard pH ($r = -0.451$; $P < 0.01$) and positively correlated with pancreas weights ($r = 0.438$; $P < 0.01$).

There were significant grain x phytase interactions ($P > 0.01$) for all four parameters of nutrient utilisation. In maize-based diets, phytase significantly increased AME by 0.58 MJ (12.82 versus 12.24 MJ/kg), energy intake by 12.2% (1.29 versus 1.15 MJ/day), N retention by 5.5% (65.4 versus 62.0%) and AMEn by 0.46 MJ (11.49 versus 11.03 MJ/kg). In contrast, phytase did not significantly influence nutrient utilisation in sorghum-based diets. In wheat-based diets, phytase significantly depressed N retention by 5.1% (57.40 versus 60.46%) without significantly altering the remaining parameters. As main effects, grain type influenced all parameters ($P < 0.05 – 0.001$) and phytase increased energy intake by 6.0% (1.23 versus 1.16 MJ/day; $P < 0.001$). Relative gizzard weights were negatively correlated with FCR ($r = -0.484$; $P < 0.01$) and positively correlated with AME ($r = 0.389$; $P < 0.05$), N retention ($r = 0.470$; $P < 0.01$) and AMEn ($r = 0.470$; $P < 0.01$).

IV. DISCUSSION

Both maize and sorghum supported similar weight gains and FCR that were superior to wheat-based diets. The sorghum performance was not anticipated as it is often associated with sub-standard broiler performance (Selle *et al.*, 2010) and, equally, the relatively poor performance of wheat was not anticipated. However, the likelihood is that the wheat in question contained high levels of soluble non-starch polysaccharides because it responded well to an exogenous carbohydrase enzyme (data not shown). Nevertheless, that wheat-based diets generated lighter relative gizzard weights is noteworthy because gizzard weights were significantly correlated to several important parameters. Notionally, the particle sizes of the three grains were similar (3.2 mm hammer-mill screen) but the particle size mean and distribution may have been quite different dependent on grain texture, which is under investigation. The likelihood is that maize and sorghum had harder grain textures and/or coarser particle sizes than wheat, which stimulated gizzard development.

In this study, relative gizzard weights were positively correlated with AME, N retention, AMEn and negatively correlated with FCR to significant extents while pancreas weights were correlated with AMEn ($r = 0.331$; $P < 0.05$). Similarly, Wu *et al.* (2004) found that heavier relative gizzard and pancreas weights were associated with improvements in FCR and AME in a whole grain feeding study. Heavier, more acidic, gizzards would be expected to promote the initiation of protein digestion by pepsin and HCl and, in addition, peptide end-products of pepsin digestion stimulate pancreatic function and secretion of proteolytic enzymes via the release of enteric hormones including CCK and gastrin (Guan

and Green, 1996). However, it appears that performance responses to heavier, more functional, gizzards in broilers may involve mechanisms other than enhanced protein utilisation.

The phytase responses in maize-based diets were unequivocal in this study; this was not the case with sorghum or, somewhat surprisingly, wheat. However, sorghum appears to be curiously recalcitrant to feed enzymes in general (Selle *et al.,* 2010) although robust responses were recorded in a sorghum-based broiler diet with reduced nutrient specifications that contained high phytate levels of 12.06 g/kg (Selle *et al.,* 2003). Given the phytase amino acid responses in wheat reported by Ravindran *et al.* (1999) the wheat results in this study were not anticipated. However, Leske and Coon (1999) determined the extent of phytate hydrolysis generated by a fungal phytase (600 FTU/kg) in atypical broiler diets based on maize or wheat (600 g//kg) that contained low calcium levels (5.0 g/kg). Phytate concentrations in maize and wheat were 8.48 and 11.77 g/kg, respectively, on a dry matter basis. Interestingly, phytase increased total tract hydrolysis of phytate (IP_6) in maize by 28.2 percentage units (59.0 versus 30.8%), which was noticeably greater than the increase of 16.1 percentage units (46.8 versus 30.7%) in wheat. This report suggests that maize phytate is more susceptible to phytase activity than wheat phytate, which could explain the outcomes observed herein. However, it is planned to determine the extent of ileal phytate degradation in the present study, which could prove highly instructive.

The 9.2% reduction in pancreatic weights generated by phytase is of interest and the reduction with wheat-based diets was, numerically, the least pronounced. The implication is that phytate was inhibiting one or more endogenous pancreatic enzymes and triggering compensatory hypersecretions. Perhaps the most likely candidate is amylase as phytate is recognized as a potent amylase inhibitor (Desphande and Cheryan, 1984). While compensatory enzyme secretions may accommodate phytate inhibition of digestive enzymes in terms of nutrient digestibility they would still result in increased endogenous amino acid flows.

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Table 1 - Effect of grain type and phytase supplementation on growth performance, relative organ weights and nutrient utilisation in broiler chickens at 27 days post-hatch

Treatment		Growth performance			Relative organ weights		Nutrient utilisation			
Grain	Phytase (FTU/kg)	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Gizzard (g/kg)	Pancreas (g/kg)	AME (MJ/kg)	AME (MJ/day)	N retent-ion (%)	AMEn (MJ/kg)
Maize	0	1235	1871	1.518	19.61	3.07	12.235[a]	1.145[a]	61.97[b]	11.026[a]
	1000	1387	2018	1.457	19.45	2.80	12.820[b]	1.294[c]	65.40[c]	11.488[b]
Sorghum	0	1316	1939	1.475	19.37	3.02	12.304[a]	1.193[ab]	60.99[b]	11.001[a]
	1000	1359	1993	1.466	18.62	2.58	12.164[a]	1.212[b]	61.67[b]	10.842[a]
Wheat	0	1226	1879	1.532	15.86	2.68	12.185[a]	1.144[a]	60.46[b]	10.846[a]
	1000	1281	1946	1.520	16.08	2.58	12.052[a]	1.173[ab]	57.40[a]	10.791[a]
SEM		24.529	28.405	0.0158	0.3603	0.0806	0.1124	0.0194	0.9572	0.1000
Main effect: Grain										
Maize		1311[a]	1945	1.487[b]	19.53[a]	2.94[a]	12.528	1.219	63.68	11.257
Sorghum		1338[a]	1966	1.471[b]	18.99[a]	2.80[a]	12.234	1.202	61.33	10.922
Wheat		1254[b]	1912	1.526[a]	15.97[b]	2.63[b]	12.119	1.159	58.93	10.818
Main effect: Phytase										
0 FTU/kg		1259[b]	1896[b]	1.508[a]	18.28	2.92[a]	12.241	1.161	61.14	10.958
1000 FTU/kg		1342[a]	1986[a]	1.481[b]	18.05	2.65[b]	12.345	1.226	61.49	11.040
Significance (P =)										
Grain type (G)		0.004	0.181	0.002	< 0.001	0.003	0.002	0.013	< 0.001	< 0.001
Enzyme (E)		< 0.001	< 0.001	0.034	0.440	< 0.001	0.263	< 0.001	0.658	0.318
G x E interaction		0.058	0.226	0.174	0.415	0.114	0.003	0.004	0.006	0.007

[a,b,c] Means within columns not sharing common superscripts are significantly different (P < 0.05)

PHENOLIC COMPOUNDS AND PHYTATE INFLUENCE STARCH AND PROTEIN DIGESTION RATES IN SORGHUM-BASED BROILER DIETS

S.Y. LIU[1], A. KHODDAMI[2], T.H. ROBERTS[2] and P.H. SELLE[1]

Summary

A study was conducted to examine the effect of anti-nutritive factors on starch and nitrogen digestion rates in broiler diets based on three different sorghums (red, white and yellow pericarps). Mash and steam-pelleted dietary treatments were offered to chickens from 6 to 27 days post-hatch. Parameters of starch and nitrogen digestive dynamics were determined by an exponential mathematical model to relate digestibility coefficients in proximal jejunum, proximal ileum and distal ileum with mean retention times in each segment and these parameters were compared with sorghum characteristics. In mash diets, starch digestion rates were negatively correlated with phenolic compounds ($r = -0.600$, $P < 0.01$) but there was no such relationship in steam-pelleted diets ($r = 0.040$; $P > 0.85$). Protein digestion rates were negatively correlated with phytate in both mash ($r = -0.599$; $P < 0.01$) and steam-pelleted ($r = -0.503$; $P < 0.05$) diets. These results suggest that phenolic compounds and phytate levels in sorghum-based diets influence starch and protein digestive rates but the influence of phenolics is impacted by steam-pelleting diets.

I. INTRODUCTION

The present study is an extension of earlier experiments (Selle *et al.*, 2012, Liu *et al.*, 2013), pursuant to analyses of phenolic compounds and the detection of pigmented or non-pigmented testas in the three sorghums. Liu *et al.* (2013) indicated that digestive dynamics of starch and protein are more important performance determinants than static apparent digestibility coefficients. This is because glucose and amino acids are both required for protein accretion in breast muscle (Yaman *et al.*, 2000) and starch is usually digested more rapidly and completely than protein. Thus, the relative availability of glucose and amino acids, stemming from different starch and protein digestion rates, influences both nutrient absorption and protein deposition. However, anti-nutritive factors in sorghum, including phenolics and phytate, may impede starch and protein digestion in broilers. Therefore, this study investigated the impact of phenolics and phytate on starch and nitrogen digestion rates.

II. MATERIALS AND METHODS

The general methodology used in this experiment has been previously outlined (Selle *et al.*, 2012) and is not repeated herein. Sorghums with three seed colours were selected to ensure genetic diversity. Total phenolic compounds were measured using the modified Folin-Ciocalteu method of Kaluza *et al.* (1980). Sorghum grains were ground using a Cyclone sample mill prior to extraction in 25 ml of methanol acidified with 1% HCl for 2 h with shaking at low speed. The extract was then centrifuged, decanted and immediately analysed. The presence or absence of a pigmented testa was detected by the Clorox bleach test (Waniska *et al.*, 1992). Phytate was determined by a standard ferric chloride-precipitation method. At day 27, digesta samples were collected in their entirety from the proximal jejunum, proximal ileum and distal ileum, freeze-dried and weighed to determine mean retention time (MRT) and apparent digestibility coefficients of starch and nitrogen (N) using

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acid insoluble ash (AIA) as the inert dietary marker. Digestion rates, potential digestible starch and nitrogen and MRT were determined by the method described by Liu *et al.* (2013). The experimental units were cage means and statistical procedures including Pearson correlations and single linear regressions were performed by using JMP® 9.0.0. This feeding study complied with specific guidelines approved by the Animal Ethics Committee of the University of Sydney.

III. RESULTS

The previously reported dynamics parameters of starch and protein (N) are shown in Table 1. (Liu *et al.*, 2013) The concentrations of phenolic compounds, phytate, and the kafirin index of the three sorghums are shown in Table 2 (Selle *et al.*, 2012). In mash diets, phenolic compounds were negatively correlated with starch digestion rates ($r = -0.600$, $P < 0.01$); whereas, in steam-pelleted diets, this correlation was not significant ($r = 0.040$; $P > 0.85$) as shown in Figure 1. Phytate contents in sorghum were negatively correlated with N digestion rates in both mash ($r = -0.599$; $P < 0.01$) and steam-pelleted ($r = -0.503$; $P < 0.05$) diets (Figure 2). Phenolic compounds were not significantly correlated with N digestion rates and phytate was not correlated with starch digestion rates, irrespective of feed form.

IV. DISCUSSION

Black *et al.* (2005) suggested that one reason for the inferiority of sorghum relative to wheat was a lack of synchrony in starch and protein digestion. Liu *et al.* (2013) demonstrated that starch and protein digestive dynamics are more important performance determinants than static apparent digestibility coefficients. However, anti-nutritive factors including phenolic compounds and phytate, plus kafirin, may influence starch and protein digestion in sorghum (Selle *et al.*, 2012). In sorghum endosperm, starch granules are embedded with kafirin protein bodies in a glutelin protein matrix and these protein fractions may impede starch enzymatic hydrolysis. The kafirin index is only a guide to the amount of kafirin in sorghum. Nevertheless, it is interesting that kafirin indices were negatively correlated to N digestion rates in mash ($r = -0.598$; $P < 0.01$) and steam-pelleted ($r = -0.551$; $P < 0.05$) diets. Kafirin indices were negatively correlated to starch digestion rates in steam-pelleted diets ($r = -0.505$; $P < 0.05$) but not in mash diets ($P > 0.40$). This suggests that kafirin has a greater negative influence on protein than starch digestive dynamics in sorghum-based broiler diets.

Sorghum contains more phenolic compounds and phytate than other cereal grains, and both anti-nutritive factors may impede digestion by directly or indirectly binding with protein and starch. The negative impact of phytate on protein digestion is recognised and this is consistent with the negative correlations between nitrogen digestion rates and phytate contents in both diets types. However, after steam-pelleting, the negative impact of phytate was somewhat less pronounced. Selle *et al.* (2007) suggested that pre-pelleting wheat may reduce the solubility and ameliorate the anti-nutritive properties of phytate and this may have been a factor in the present study.

Importantly, in mash diets, increasing phenolic compounds depressed starch digestion rates in sorghum-based diets, but this correlation was not established in diets after steam-pelleting. A possible explanation is that extrusion has been shown to reduce molecular weights of phenolic polymers (Awika *et al.*, 2003) and to reduce total phenolic concentrations substantially (Dlamini *et al.*, 2007). Thus steam-pelleting sorghum-based diets at 90°C may have had a similar, but lesser, impact on phenolic compounds. It appears that steam-pelleting sorghum-based diets profoundly influenced subsequent interactions between phenolics and starch in the avian gut and this merits further investigation.

Table 1 - Effects of sorghum variety and diet type on starch and protein digestion dynamics [potential digestible starch (PDS), starch digestion rate (K_{starch}), potential digestible nitrogen (PDN), nitrogen digestion rate ($K_{nitrogen}$)]

Sorghum	Diet	PDS (g/100g)	K_{starch} ($\times 10^{-2}$ min^{-1})	PDN (g/100g)	$K_{nitrogen}$ ($\times 10^{-2}$ min^{-1})
Red	Mash	99.3	1.39^{b}	99.3^{a}	0.95^{b}
	Pellet	97.6	2.57^{a}	83.3^{b}	1.82^{ab}
White	Mash	99.4	1.78^{ab}	98.1^{a}	1.09^{b}
	Pellet	96.1	2.18^{ab}	83.8^{b}	2.27^{a}
Yellow	Mash	99.4	1.73^{b}	99.4^{a}	0.92^{b}
	Pellet	99.4	1.94^{b}	99.4^{a}	0.98^{b}
SEM		1.13	0.19	3.03	0.223
Main effect					
Sorghum	Red	98.4	1.98	91.3	1.39
	White	97.7	1.98	91	1.68
	Yellow	99.4	1.84	99.4	0.95
Diet type	Mash	99.4	1.63	99	0.99
	Pellet	97.7	2.23	88.8	1.69
P-value	Sorghum	0.354	0.712	0.014	0.01
	Diet type	0.079	<0.001	<0.001	<0.001
	Interaction	0.381	0.038	0.025	0.048

*Means within columns followed by different letters are significantly different at $P = 0.05$; derived from Liu *et al.* (2013)

Table 2 - The concentrations of phenolic compounds, phytate and karifin index of three sorghums (Selle *et al.*, 2012)

Sorghum	Phenolic compounds (mg GAE/g)	Phytate (g/kg)	Kafirin index*
Red	3.82	9.57	6.8
White	2.02	6.74	4.1
Yellow	2.19	9.93	7.8

*kafirin index = Leu – (Arg + His + Lys); amino acids values were estimated by NIR

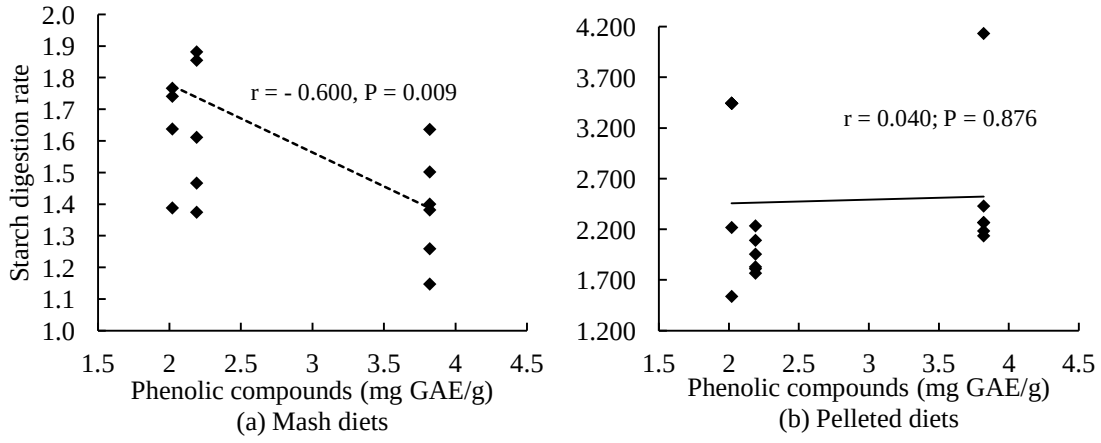

Figure 1 - The relationship between phenolic compounds and starch digestion rate in mash and pelleted sorghum-based broiler diets.

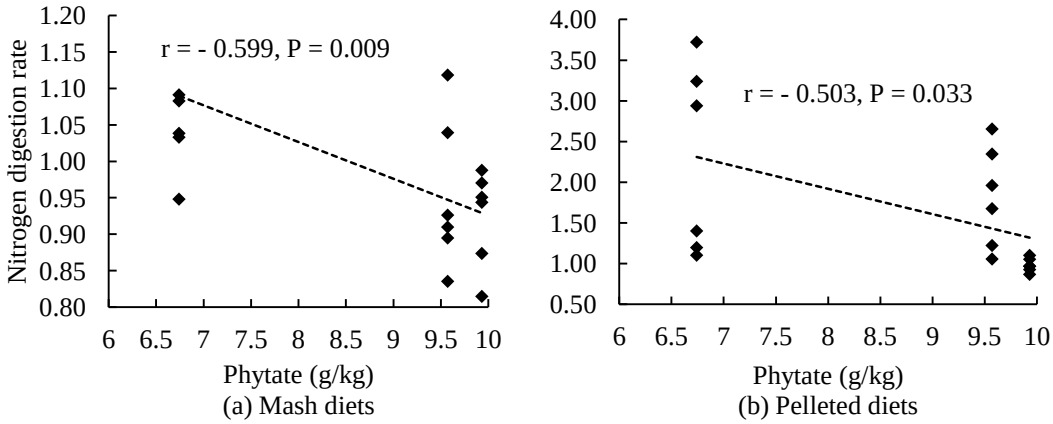

Figure 2 - The relationship between phytate and nitrogen digestion rate in mash and pelleted sorghum-based broiler diets.

It may be concluded that phenolic compounds and phytate are both factors causing slow and incomplete starch and protein digestion in sorghum-based broiler diets. Given the importance of digestive dynamics as demonstrated by Liu *et al.* (2013), further investigations into phenolic compounds and phytate in various sorghums are required. This equally applies to kafirin levels in sorghum, particulary if they can be quantified.

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IMPACT OF PROCESSING CONDITIONS AND CHEMICAL COMPOSITION ON ENERGY UTILIZATION OF EXPELLER-EXTRACTED CANOLA MEAL FOR BROILER CHICKENS

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Low metabolizable energy content is regarded as one of the factors restricting high inclusion level of canola meal (CM) in broiler diets. It is known that expeller-extracted CM has higher energy content than solvent-extracted CM because the former contains higher residual oil (Woyengo et al., 2010). However, the impact of other chemical constituents is usually overlooked. The present study was designed to investigate the effect of processing conditions and chemical composition on AME and AME_n of six expeller-extracted canola meal samples (ECM) subjected to 90, 95 or 100^o C and high or low screw force during oil extraction. The ECM samples were incorporated into a corn-soybean meal reference diet at 300 g/kg by replacing the energy yielding ingredients. A total of 210 day-old male broiler chicks (Ross 308) were fed a common starter and grower diet until 18 d of age, and then were assigned to each of seven experimental diets replicated six times with five chicks per cage. After a 5-d diet acclimation period from 18 to 22 d of age, a 72-h excreta collection period from 22 to 25 d was conducted. The difference method was used to determine AME which was corrected to zero N balance to obtain AME_n.

The AME and AME_n values differed significantly ($P < 0.05$) between the samples (Table 1). The average AME and AME_n across the samples were determined to be 10.12 and 9.45 kJ/kg DM, respectively. Crude fibre and neutral detergent insoluble nitrogen (NDIN) were inversely correlated with screw force ($r = -0.71$, $P < 0.001$; $r = -0.56$, $P < 0.003$, respectively). Crude protein was positively correlated with temperature ($r = 0.59$; $P < 0.001$) and screw force ($r = 0.61$; $P < 0.001$). The AME_n values exhibited the strongest inverse correlation with NDF ($r = -0.91$; $P < 0.001$), followed by NDIN and then hemicellulose ($r = -0.87$; -0.86, respectively, $P < 0.001$). In conclusion, the current results show that AME value of expeller-extracted canola meal can vary significantly between different samples based on the chemical composition of meals. Processing conditions of canola meal may also affect digestibility of energy, likely because of alteration to the chemical constituents of the meal.

Table 1 - Apparent metabolisable energy (AME), apparent metabolisable energy corrected for nitrogen (AME_n) and gross energy (GE) retention of expeller canola meal samples fed to broiler chicks

Item	Experimental diets[1]						SEM	*P*-value
	ECM 1	ECM 2	ECM 3	ECM 4	ECM 5	ECM 6		
AME (kJ/kg)	10.75^a	10.93^a	9.41^b	8.74^c	11.11^a	9.75^b	46	<0.0001
AME_n (kJ/kg)	10.03^a	10.10^a	8.83^b	8.27^c	10.39^a	9.09^b	42	<0.0001
GE retention %	50.6^a	51.2^a	44.3^b	41.4^c	52.4^a	45.8^b	0.94	<0.0001

$^{a-c}$ Means in the same row followed by different letters differ significantly (Tukey test; $P < 0.05$)
[1] = Expeller-extracted canola meal (samples 1 to 6)

Woyengo TA, Kiarie E & Nyachoti CM (2010) *Poult. Sci.* **89:** 1182–1189.

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IMPROVING THE NUTRITIVE VALUE OF LUPIN USING A COMBINATION OF PECTINASE AND XYLANASE

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Summary

The synergistic effect of two non-starch polysaccharide-degrading enzymes was tested *in vitro* with the aim of breaking down the cell wall content to improve the nutritive value of lupin for poultry. Lupin kernels were incubated without (control) and with enzymes (pectinase, xylanase or a combination of pectinase and xylanase) for 1 hour at 38ºC. The combination of pectinase and xylanase greatly reduced water-holding capacity, viscosity and cell wall content, compared to pectinase or xylanase alone. In addition, the pectin content, chain length of pectin and galacturonic acid concentrations were reduced by the combination of pectinase and xylanase more than the individual enzymes. It was concluded that pectinase and xylanase act synergistically to break down the non-starch polysaccharides in the lupin kernel, and thus may improve the nutritive value of lupins for poultry.

I. INTRODUCTION

Australia is the world leading producer and exporter of lupin (White et al., 2007). The Australian lupin varieties have very low alkaloid content of 0.01% and sweet lupins are currently used as a source of protein and energy in a range of monogastric diets in poultry, pigs, fish and rabbits (Inborr, 1990; Glencross et al., 2004). Sweet lupins (*Lupinus angustifolius*) are locally available and relatively inexpensive so are a potential alternative to high-priced feedstuffs such as soybean meal. Lupins have high content of protein (34%) and energy (18 MJ/kg) so can be used as the main source of protein and energy in poultry diets (Petterson and Mackintosh, 1994). However, the use of lupins in poultry diets is still limited to 5% by feed manufacturers and poultry producers because they contain high amounts of non-starch polysaccharides (NSPs, 35%) that cannot be digested by monogastrics due to the absence of endogenous enzymes that can break them down into simple sugars. The main problematic NSPs are pectins, galactans, rhamno-galacturonans, arabinans and xylans, (Cheetham et al., 1993; Choct, 2006), with structures shown in Figure 1. These NSPs increase the viscosity of digesta in the intestinal tract and inhibit the digestion of nutrients. This results in poor growth and low feed conversion efficiency (Kocher et al., 2000; Steenfeldt et al., 2003).

Supplementing legume grains with exogenous enzymes can break down the NSPs (Perez-Maldonado et al., 1999; Jia et al., 2008), releasing cell contents and overcoming the negative effects (Ali et al., 2009). Since lupins contain xylans that are attached to the main pectin chain, it seems likely that some enzymes will work synergistically (Ravindran et al., 1999; Wu et al., 2004) to break down pectins and xylans. For example, when pectinase breaks down the main chain of pectin, it gives xylanase access to the xylan side branches attached to main pectin backbone.

In the present research we tested whether the combination of pectinase and xylanase will break down the NSP in lupin kernel more effectively than pectinase or xylanase alone.

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This hypothesis was tested *in vitro* by incubating lupin kernel with enzymes and measuring the degradation of pectins and xylans.

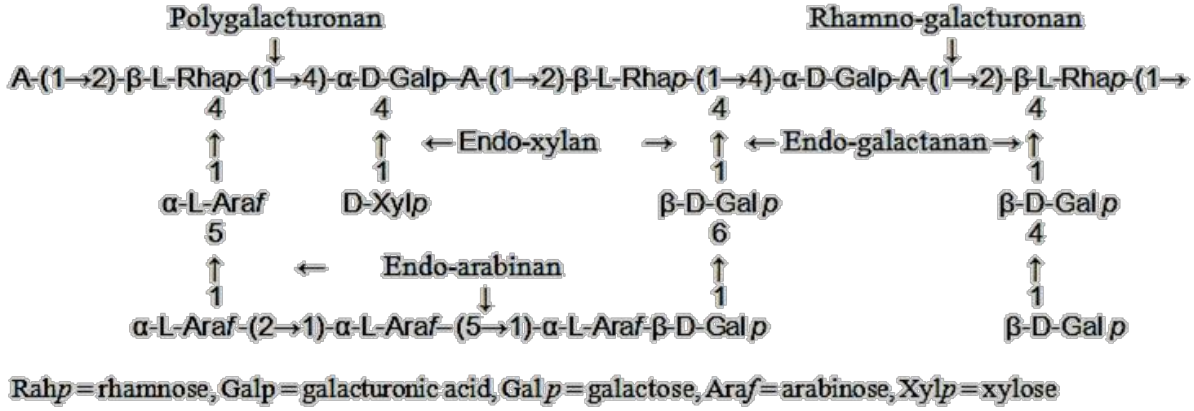

Figure 1 - Complex structure of pectin.

II. MATERIALS AND METHODS

Lupin kernels were incubated with three NSP degrading enzymes; pectinase (1.4 U/g polygalacturnose & 0.2 U pectinestrase, Novozymes Australia), xylanase (0.38 U/g, Rovabio) and a combination of mentioned pectinase and xylanase with the same doses.

Twelve lupin samples per treatment were dissolved in 25 ml deionized water and incubated without enzymes (control) and with enzymes in an incubator-shaker (150 rpm) for 1 hour at 38ºC. The samples were centrifuged at 15,000g for 15 min at 20ºC. The residues were then freeze-dried and water-holding capacity (WHC) was measured as gram of water per gram of organic matter (g:g). The filtration rate was calculated by measuring the volume of supernatant filtered through filter paper (8 µg, no. 41, Whatman) divided by the filtration time (µl/sec). Following this, the viscosity was measured using a Viscotester (HAAKE, PK 100, VT 550) by placing 0.50 ml supernatant on a cone plate PK5 at a shear rate of 3000/sec and speed rate of 500/min at a temperature of 23°C.

A half gram of each sample was placed in a filter bag in duplicate and digested in an ANKOM200 fibre analyzer (ANKOM Corporation Technology Fairport, NY). The xylan content was measured using the fibre analysis method (Van Soest et al., 1991). The galacturonic acid (GA) content was measured by spectrophotometer according to the method described by (El-Rayah and Labavitch, 1977).

III. RESULTS

The combination of pectinase and xylanase reduced viscosity by 21% ($P < 0.05$), WHC by 4% and the filtration rate by 50% (Table 1) as compared to control. This breakdown was manifested by reduction in cell wall (13%) and pectin (35%), but xylan breakdown was not statistically significant. In addition, the combination reduced the pectin chain length by 51% and galacturonic acid content by 40%. When pectinase and xylanase were used alone, the changes in physico-chemical properties of lupin kernel were less than that achieved by combination of two enzymes.

IV. DISCUSSION

The combination of pectinase and xylanase reduced the physico-chemical properties of lupin kernel more than the individual enzymes, more effectively reducing the content of galacturonic acid and cell walls. The superior effect of the enzyme combination supports the hypothesis of a synergistic interaction between pectinase and xylanase, since xylan is attached to the main chain of pectin.

Table 1 - Effect of enzymes on physical-chemical properties of lupin kernel *in vitro* (mean ± sem)

Parameters	Control	Pectinase	Xylanase	Combination
WHC (g:g)	$3.55^{a} \pm 0.01$	$3.50^{a} \pm 0.02$	$3.51^{a} \pm 0.09$	$3.42^{b} \pm 0.03$
Viscosity (mPas/sec)	$1.46^{a} \pm 0.01$	$1.35^{b} \pm 0.01$	$1.25^{c} \pm 0.01$	$1.15^{d} \pm 0.01$
Filtration rate (µl/sec)	$31.2^{a} \pm 1.5$	$26.7^{b} \pm 1.8$	$20.6^{c} \pm 1.2$	$15.4^{d} \pm 0.9$
Pectin (%)	$10.8^{a} \pm 0.3$	$9.02^{b} \pm 0.2$	$9.25^{b} \pm 0.1$	$6.92^{c} \pm 0.1$
Xylan (%)	$6.17^{a} \pm 0.1$	$6.09^{a} \pm 0.1$	$5.97^{a} \pm 0.1$	$6.01^{a} \pm 0.1$
Cell wall content (%)	$23.3^{a} \pm 0.2$	$22.1^{b} \pm 0.2$	$21.8^{b} \pm 0.2$	$20.4^{c} \pm 0.2$
Chain length of pectin (%)	$76.5^{a} \pm 3.5$	$58.9^{b} \pm 1.2$	$60.2^{b} \pm 2.9$	$37.3^{c} \pm 1.4$
GA concentration (µg/g)	$32.8^{a} \pm 1.4$	$26.9^{b} \pm 0.4$	$29.4^{ab} \pm 1.5$	$19.5^{c} \pm 0.7$

[abc] Means within rows with different superscripts differ (P<0.05).

These reductions in content of NSPs should improve nutritive value and digestibility of lupin kernel for monogastrics. For example, supplementing lupins with multi-enzyme preparation significantly improved feed consumption by 5% and chicken growth by 10% (Brenes et al., 1993; Ali et al., 2009; Olkowski et al., 2010). Similarly, using NSP-degrading enzymes such as xylanase or cellulase in lupin-based diets improves the production performance of chickens (Naveed et al., 1999).

In conclusion, the combination of pectinase and xylanase is more effective than the individual enzymes *in vitro*, so we will now test whether this outcome can be reproduced *in vivo*, testing for potential benefits in feed conversion efficiency and growth rate in quail. The synergistic interaction between the two enzymes could make inroads towards greater inclusion of up to 20% lupin kernel into diets for all poultry species without compromising production performance or increasing wet droppings.

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THE EFFECT OF NSP ENZYME AND PARTICLE SIZE ON THE APPARENT METABOLIZABLE ENERGY OF WHEAT BASED BROILER DIET

J. HAYAT[1], A. PREYNAT[2] and P. DALIBARD[2]

Summary

Exogenous enzymes exhibit improvements in growth performance and apparent metabolisable energy (AME) depending on experimental conditions. This trial examined the interaction between a commercially available enzyme and the grinding conditions of raw materials on apparent metabolizable energy of wheat and soybean meal based broiler diet. The effect of particle size and enzyme supplementation was evaluated by determining the energy digestibility of diets formulated with wheat, soybean meal and extruded soybeans grounded at different screens and speeds in order to reach 650, 850 and 1450 µm. Enzyme addition improved metabolizable energy by 67, 107 and 160 kcal/kg DM for 650, 850 and 1450 µm, respectively ($P < 0.001$). In summary, the trial results showed that enzyme addition and particle size of raw material had a greater influence in improving the nutritive value of that ingredient and the better enzyme efficacy was associated with coarser particles in the diet.

I. INTRODUCTION

The efficacy of exogenous enzyme for poultry can be affected by the physical form of the feed, feed processing method and the ingredients used in the diet (Acamovic, 2001). Feed particle size is especially important in feed processing because of being second largest energy cost after that of pelleting for the broiler industry (Reece et al., 1985) and any reduction in energy consumption from grinding could significantly lower the feed cost. Poultry feed particle size and shape can influence the bird performance (Axe, 1995, Amerah et al., 2007). Chicken are known to have preference for larger feed particle size (Schiffman, 1968). Variability exists in apparent metabolisable energy (AME) of wheat for poultry due to soluble non-starch polysaccharides (NSP) in wheat (Annison and Choct, 1991) which have negative impact on digestion and absorption of nutrients and bird performance. These negative impacts can be overcome by exogenous enzyme supplementation (Bedford and Schulze, 1998) which can be attributed to improved nutrient utilisation through NSP degradation. The beneficial effect of enzyme on coarser feed was more evident when Wu and Ravindran (2004) added whole wheat in the diet. This relationship of enzyme efficacy and degree of grinding of wheat-based diet was also noted for other species for instance, pigs (Kim et al., 2003).

The objective of this study was to determine whether ingredients particle size in the diets can be a factor in enzyme response variation for broilers.

II. MATERIALS AND METHODS

The trial was conducted at an experimental farm in Commentry (France). One hundred and twenty Ross PM3 male broilers were used in this trial. Diet formulation was based on the recommendations of the Rhodimet® Nutrition Guide 2013 and birds received a starter diet from 0 to 12 days followed by a grower diet up to 23 days. The pelleted diets were offered *ad libitum* and water was freely available at all times. Three particle sizes were obtained by

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changing grinding conditions such as screen size and rotation speed for the 3 main ingredients namely wheat, soybean meal and full-fat soybeans, already premixed. The materials were ground in a hammer mill (Sogem, 60 hp, Euronutrition, Saint-Symphorien, France) to pass through a screen size of 2, 8 or 12 mm for fine, medium and coarse grades, respectively. The particle size spectrum of each grade was subsequently characterized by dry sieving (Rotachoc) at 0.315, 500, 1, 2, 3.15 and 5 mm sieve during 5 minutes. The D_{50} of particle size had been calculated after weighing the remaining feed in each sieve. Six dietary treatments, T1 – T6, containing different particle sizes defined as fine, medium and coarse with and without enzyme supplementation (5500 visco units of xylanase/mL, 7500 visco units of beta-glucanase/mL; Rovabio® Excel LC) were randomly assigned to 20 replicates of one broiler per pen. The dietary treatments were:

Treatments	T1	T2	T3	T4	T5	T6
D_{50} particle size (target)	Fine (300 µm)		Medium (700 µm)		Coarse (1 000 µm)	
Rovabio® Excel LC (200 mL/t)	-	+	-	+	-	+

The feed intake and total excreta output of each pen were measured on day 20 and 23 post hatching. The apparent metabolizable energy (AME) was determined using the European Reference Method with *ad libitum* feeding and total excreta collection between 20 and 23 days of age (Bourdillon et al., 1990). All variables were analyzed by using the GLM procedure of SAS (SAS Institute, 2001) according to a complete block design with treatments as fixed effects and block as random effect and means were compared by using a student t test.

III. RESULTS AND DISCUSSION

The visual examining of pelleted diets indicated that the three "premixed" feeds were different in terms of particle size distribution with more fine particles for the feeds grinded at low screen numbers (Figure 1).

Figure 1 - Visual comparison of the feeds

P1 and P2 were more homogeneous compared to P3. Visually, pellets from P3 were more crumbled than usual (Figure 1). Dry sieving of pelleted feed revealed that a difference between expected and actual particle size existed after pelleting. The measured average particle sizes were higher than those expected (Table 1).

Table 1 - Comparison of average particle size (dry sieving).

Premixed feeds	Diets	Expected D_{50} (µm)	Measured D_{50} (µm)
P1	T1/T2	300	650
P2	T3/T4	700	850
P3	T5/T6	1000	1450

The influence of dietary treatments on the performance of 12 - 23 days old broilers is presented in Table 2. There was no significant difference in performance parameters between the treatment groups however the broiler chicken fed feed with medium to coarser particle size supplemented with enzymes were slightly heavier than their un-supplemented counterparts. This response is similar to earlier reported by Svihus et al. (2004) where no effect of wheat particle on broiler performance was observed. There was no significant difference in AME between treatments without enzyme addition. However, a significant interaction between enzyme and particle size effects was observed on energy digestibility (Table 2).

Table 2 - Effect of NSP[#] enzyme and particle size on metabolizable energy.

Treatment	Fine particles T1	T2	Medium particles T3	T4	Coarse particles T5	T6	Grinding effect	Enzyme effect	Inter
Enzyme	-	+	-	+	-	+			
Performance 12-23 days									
Weight gain, g	526	526	507	511	490	517			
Feed intake, g	911	896	897	873	871	875			
Feed conversion	1.733	1.708	1.775	1.777	1.696	1.691			
Digestibility results									
DM digestibility, %	67.1^{b}	68.8^{ab}	67^{b}	69.5^{a}	66.9^{b}	69.9^{a}	***	***	NS
AME (kcal/kg)	3012^{bc}	3078^{abc}	3005^{bc}	3105^{ab}	2976^{c}	3120^{a}	**	***	*
AME (kcal/kg DM)	3323^{bc}	3395^{abc}	3308^{bc}	3421^{ab}	3280^{c}	3447^{a}	***	***	*
AME_N (kcal/kg)	3160^{bc}	3227^{abc}	3148^{bc}	3255^{ab}	3118^{c}	3278^{a}	**	***	NS
AME_N (kcal/kg DM)	2868^{bc}	2931^{abc}	2864^{bc}	2959^{abc}	2835^{c}	2972^{a}	***	***	*

NS: Non significant, *: P < 0.05, **: P < 0.01, ***: P < 0.001

Enzyme effect tended to increase with particle size (Figure 2). This beneficial response might be due to enhanced substrate mixing in the gizzard which was attributed to higher grinding activity in the gizzard when birds were fed higher particle size (Wu and Ravindran, 2004). From 650 to 1450 µm, AME was increased by 66 to 144 kcal/kg respectively with an average increase in AME of 103 kcal/kg. This study provides an indirect solution of optimising feed utilisation and improving production efficiency.

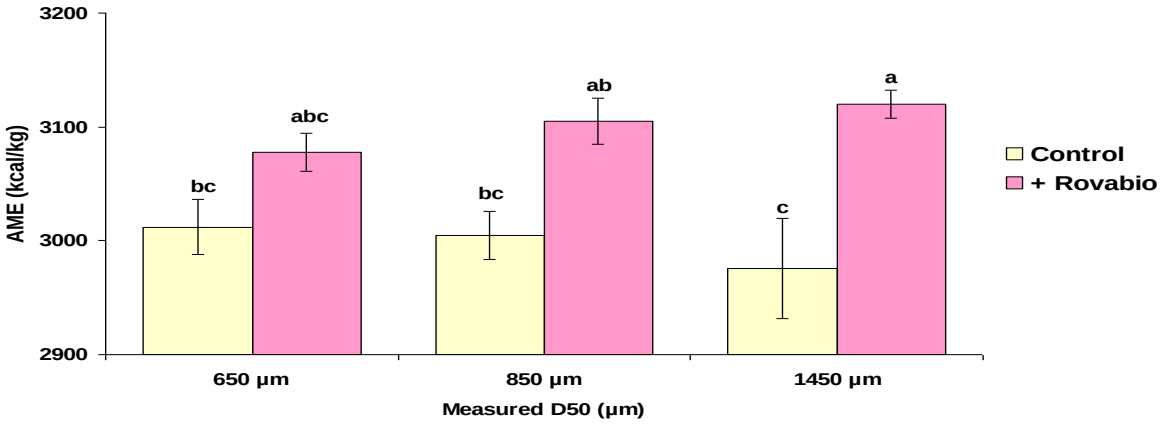

Figure 2 - Effect of NSP[#] enzyme on AME depending on average particle size.

[#] Rovabio® Excel LC

Enzyme supplementation significantly enhanced AME value (+144 kcal/kg) for D_{50} of 1450 µm compared to unsupplemted group, whereas only numerical improvements (+66 and 100 kcal/kg) had been obtained for D_{50} of 650 and 850 µm respectively.
In summary, the trial results showed that enzyme addition and particle size of raw material had a greater influence in improving the nutritive value of that ingredient and the efficacy of the enzyme was better when there was a greater proportion of coarser particle in the diet.

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BROILER STUDY SHOWS FULL EFFICACY OF METHIONINE HYDROXY ANALOGUE

Y.G. LIU[1] and J. HAYAT[1]

Summary

The biological value of the two main sources of methionine, namely DL-Methionine (DLM) and hydroxy-analogue of methionine (2-hydroxy-4-methyl thio-butanoic acid or HMTBA), has been debated for nearly three decades around the world, without reaching a definite conclusion. A broiler study was conducted to generate new results under practical raising condition. This study employed 900 day old Ross 308 chicks on a 3 x 2 factorial design: 3 levels of nutrition (ME/DAA) and 2 sources of methionine (DLM and HMTBA on equimolar basis). The results showed that the nutrition levels significantly affected broiler performance but both methionine sources performed equally well across all the three nutrition schemes.

I. INTRODUCTION

For the biological efficacy of HMTBA, one opinion was made up by using highly methionine deficient diets, supplemented with different levels of methionine, the results were evaluated through exponential model that derives biological value 65% of DLM on product weight basis. A typical analysis by Jansman et al. (2003) on a number of trials using such mathmatical model seemed to support the said value. In contrast, by using the same set of data, another group of scientists determined "true methionine efficacy", by matching extra weight gain to each gram of added methionine sources and revealed that the biological value of HMTBA relative to DLM varied with dose of the addition (Geraert and Mercier, 2005) and HMTBA appeared to value between 95 to 105 % of DLM on a molecular basis over a practical range of supplementation. This analysis indicates its biopotency slightly lower in methionine deficient diets, but higher on practical application doses. This range of relative efficiency between sources has later been confirmed by gathering 11 studies, over 23 rearing periods, in practical diets comparing methionine sources on isomolecular basis (Mercier, 2011).

The current study was conducted to compare performance of broiler birds fed on practical diets of three nutrition schemes supplemented with either DLM or HMTBA on equimolar basis.

II. MATERIALS AND METHODS

The current study was conducted in the Bangkok Animal Research Centre (BARC) Thailand. Nine hundred newly hatched male broiler chicks of commercial strain (Ross 308) were randomly allocated to 6 dietary treatments using a 3x2 factorial design. There were 6 replicates per treatment, each replicate containing 25 male birds housed in a floor pen as an experimental unit. The nutrition schemes and methionine sources are shown in Table 1.

Diets were formulated based on standardized ileal digestible amino acids (SID) following the ideal protein profile. HMTBA (Rhodimet® AT88, 88%) was valued and priced as 88% of DLM (equimolar basis). The birds were raised in a closed house with solid-concrete floor pens using rice hulls as bedding, with tunnel ventilation and evaporative cooling system. Feed and water were provided *ad libitum*. Diets were provided in to birds in crumble form to 12 days and in pellet form thereafter until finishing the 36-day period. All birds were vaccinated for

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Newcastle and Infectious Bronchitis diseases at 7 days of age and Gumboro disease at 14 days of age. Total pen feed consumption was recorded weekly. Body weight and feed intake as pen basis were measured at 17 and 36 days of age. Mortality was recorded daily, including the cause of death. Flock uniformity (individual weighing) was done at the end of the trial. After weighing at the end of the trial, one bird of average live weight of each replicate pen was slaughtered for carcass measurement (dressed carcass percentage, breast meat and abdominal fat). Final body weight, body weight gain, feed intake, feed conversion ratio, livability, feed cost per gain (FCR x feed price) flock uniformity, carcass, breast meat and abdominal fat were subjected to analysis of variance as a randomized complete block design.

Table 1 - Experimental design.

Treatments	Nutrient level	Nutrients specs: ME (kcal/kg) /dig. lys.%		Methionine source*, %	
		Starter	Grower	DLM	HMTBA
1	High	3000/1.25	3100 /1.15	0.25/0.24	-
2				-	0.28/0.27
3	Medium	2900/1.15	3000/1.05	0.18/0.18	-
4					0.20/0.20
5	Low	2800/1.05	2900/0.95	0.12/0.12	-
6				-	0.14/0.14

* Methionine supplementation in starter and grower diets.

III. RESULTS AND DISCUSSION

Broiler performance for each phase and statistical analyses are presented in Tables 2 and 3. The effect of nutrient density and methionine sources on overall performance are presented in Figures 1-4. The diet formulations supported satisfactory growth performance in general with very low mortality. The nutritional specifications significantly affected weight gain, feed intake and feed conversion, but no differences were observed between the two sources of methionine in each nutritional scheme. There were no statistical differences in dressing percentage and flock uniformity, nor interaction between nutrition specification and methionine sources.

In conclusion, the levels of dietary nutrition caused clear responses on broiler weight gain, feed intake and feed conversion ratio; both DL-methionine and HMTBA supported satisfactory performance and HMTBA exerts full efficacy as DLM on equimolar basis across all three nutrition schemes.

Table 2 - Effect of the nutritional levels and methionine sources on broiler performance.

Nutrient	High		Medium		Low	
densitiy	DLM	HMTBA	DLM	HMTBA	DLM	HMTBA
Days 1-17						
Start weight, g	39	39	39	39	39	39
Weight gain, g	697 [a]	700 [a]	668 [b]	659 [bc]	632 [d]	635 [cd]
Feed intake, g	907	917	910	909	912	915
Feed conversion	1.33 [c]	1.32 [c]	1.37 [b]	1.40 [b]	1.45 [a]	1.44 [a]
Days 18-35						
Weight gain, g	1 775	1 749	1 752	1 742	1 731	1 691
Feed intake, g	2 936 [d]	2 926 [d]	3 028 [ab]	3 008 [bcd]	3 097 [a]	3 047 [ab]
Feed conversion	1.66 [c]	1.67 [c]	1.74 [b]	1.74 [b]	1.82 [a]	1.80 [a]
Days 1-36						
Final weight, g	2 510 [a]	2 488 [a]	2 459 [ab]	2 440 [ab]	2 402 [bc]	2 365 [c]
Weight gain, g	2 471 [a]	2 449 [a]	2 420 [ab]	2 400 [ab]	2 363 [ab]	2 326 [b]
Feed intake, g	3 840 [d]	3 841 [d]	3 935 [abc]	3 913 [bcd]	4007 [a]	3 962 [ab]
Feed conversion	1.56 [c]	1.57 [c]	1.64 [b]	1.65 [b]	1.73 [a]	1.70 [a]
Livability, %	100.0 [a]	99.3 [ab]	98.7 [ab]	98.7 [ab]	97.3 [b]	100.0 [a]
Uniformity, %	92.45	91.48	92.59	93.79	92.99	93.60

*Feed conversion ratio was corrected for mortality and culls. Means in the same row not bearing the same alphabet differ significantly ($P < 0.05$).

Table 3 - Statistical analysis.

	Factor	Weight gain 0-36 d (g)	Feed intake 0-36 d (g)	Feed conversion *	Mortality (%)	Dressing, %
Nutrition specification	High	2 463 ±78	3 845 ±111	1.57 ± 0.03	1.78 ± 2.46	75.7 ± 1.1
	Medium	2 416 ± 81	3 930 ± 94	1.65 ± 0.03	2.67 ± 1.94	75.6 ±0.9
	Low	2 344 ± 71	3 984 ±111	1.72 ± 0.04	1.67 ± 2.67	75.7 ±1.3
	p value	0.002	0.005	<0.001	0.256	0.873
Methionine source	DLM	2 418 ± 90	3 916 ±126	1.64 ± 0.06	1.56 ± 2.01	75.8 ±1.0
	HMTBA	2 392 ± 85	3 905 ±105	1.63 ± 0.07	2.40 ±2.49	75.5±1.2
	p value	0.269	0.421	0.641	0.146	0.466
Interaction	*p value*	0.961	0.899	0.287	0.047	0.882

* Feed conversion ratio was corrected for mortality and culls.

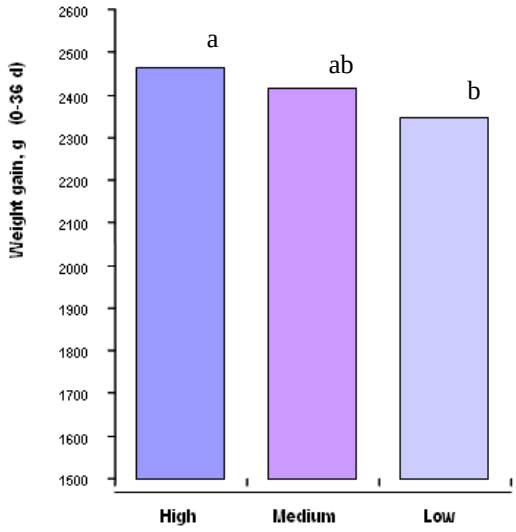

Figure 1 - Effect of nutrients' density on weight gain.

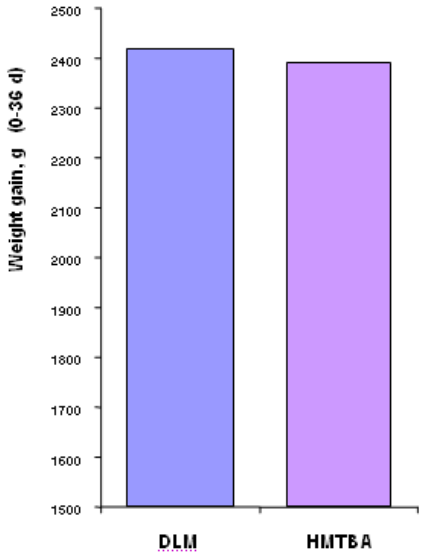

Figure 2 - Effect of methionine sources on weight gain.

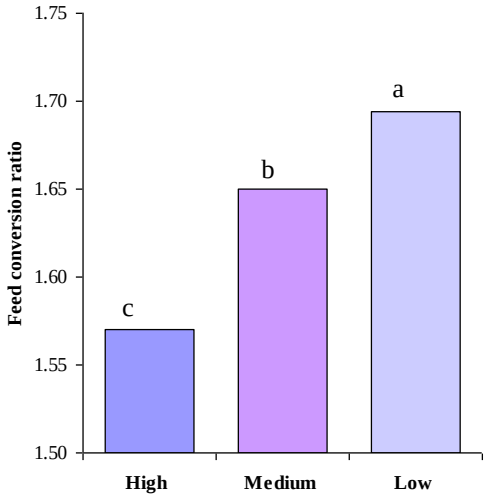

Figure 3 - Effect of nutrients' density on FCR

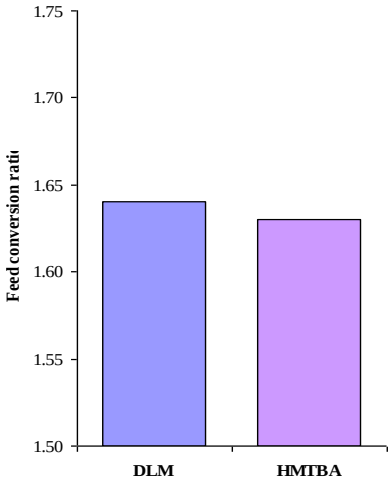

Figure 4 - Effect of methionine sources on FCR.

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A NEW PURE ORGANIC SELENIUM SOURCE BASED ON SELENO-HYDROXY-METHIONINE: A NEW EFFICIENT ALTERNATIVE FOR BROILERS

M. BRIENS[1,2], M. JLALI[1], P.A. GERAERT[3], F. ROUFFINEAU[1] and Y. MERCIER[1]

Summary

This work aimed investigating the relative selenium deposition in breast muscles of broilers depending on the selenium sources and dietary levels. A total of 816 day-old chicks were allocated to eight treatments with six pen replicates of 17 birds for 21 days. The diets used in the experiment were supplemented with different Se sources and levels as followed: negative control (NC) (not supplemented in Se); SS-0.1, SS-0.3 supplemented with Sodium Selenite at 0.1 and 0.3 mg Se/kg feed, respectively; SY-0.1, SY-0.3 supplemented with Selenized yeasts at 0.1 and 0.3 mg Se/kg feed, respectively; SO-0.1, SO-0.2 and SO-0.3 supplemented with a new organic selenium source : Se-OH-methionine or HMSeBA (SO). Total selenium was measured in pectoralis muscle for all treatments and seleno amino acid speciation was done on NC, SY-0.3 and SO-0.3. The results obtained after the 21-d period of dietary supplementation showed that all selenium sources increased selenium deposition compared to negative control ($P<0.05$) in a dose dependent manner. The selenium deposition appeared higher ($P<0.05$) with organic selenium sources compared to inorganic source but differences appeared also between organic sources with higher selenium deposition with SO treatments compared with SY ones. The relative muscle selenium deposition with HMSeBA compared to seleno-yeast appeared to be 39% higher ($P<0.05$). HMSeBA was not detectable in the muscle demonstrating its complete metabolisation and transformation into Se-Met and Se-Cys. Moreover, the comparison of seleno amino acid level in muscle showed that SO treatment led to obtain higher SeCys level than SY suggesting more selenoproteins or active selenium forms with SO. This study allowed to conclude that the new pure organic selenium source HMSeBA is highly effective for dietary selenium supplementation in broilers.

I. INTRODUCTION

Selenium is certainly the most important micro-nutrient in nutrition since it is involved in many functions from antioxidant defence, immune system or thyroid hormone metabolism through about 25 selenoproteins. In animal, selenium is mainly found under seleno-amino acid forms (e.g. seleno-methionine and selenocysteine) and presents some similarity with sulphur amino acids. For instance, selenomethionine can be incorporated in an unspecific manner in body protein instead of methionine that constitutes a storage form of selenium. Selenium supply in animal diets is partially covered by raw material selenium but selenium supplementation is frequently needed to cover the requirement estimated at 0.2-0.3 mg of Se/kg of feed depending on species and physiological state considered. This dietary selenium level is generally satisfied by mineral selenium supplementation (e.g. sodium selenite) or during the last decade organic selenium sources such as selenium enriched yeast. Recently, a new organic selenium form had been developed: the hydroxy-selenomethionine (2-hydroxy-4-methyl selenobutanoic acid; HMSeBA). The purpose of this study was to compare selenium source efficacy for tissue selenium deposition in young broilers.

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II. MATERIALS AND METHODS

A total of 816 day-old chicks (average BW: 41 g) obtained from a commercial hatchery were allocated to eight treatments with six pen replicates of 17 birds. The eight starter diets used in the experiment were supplemented with different Se sources and levels as follow: negative control (NC) (not supplemented in Se); SS-0.1, SS-0.3 supplemented with Sodium Selenite (Microgan Se 1% BPM, DSM) at 0.1 and 0.3 mg Se/kg feed, respectively; SY-0.1, SY-0.3 supplemented with Selenized yeast (Sel-Plex 2000, Alltech) at 0.1 and 0.3 mg Se/kg feed, respectively; SO-0.1, SO-0.2 and SO-0.3 supplemented with Se-OH-methionine, HMSeBA, (Selisseo® 2%, Adisseo) at 0.1, 0.2 and 0.3 mg Se/kg feed, respectively. Feed and water were provided *ad libitum* throughout the experiment. Feed intake (FI) and feed conversion ratio (FCR) were recorded once for the period 0-21 days. At the end of the experiment (d21), 12 birds (two per pen replicate, close to average pen weight) were weighed and euthanized by carbon dioxide inhalation after overnight fasting. Muscle samples (*pectoralis major*) were collected and stored at -20°C until analysis. Total Se measurements were realized according to the method of Mester *et al.* (2006) with ICP-MS detection. For tissue samples, the mass uptake was reduced to 250 mg digested by 2 ml of HNO3 and 1 ml of H_2O_2. The solution was further diluted with water and total Se content subsequently determined by ICP MS (Agilent 7500cx, Tokyo, Japan). Isotopes 76, 77 and 78 were used for quantification. The method of the standard addition was used. Tissue selenium speciation of SeMet and SeCys was carried out according to the method described by Bierla *et al.* (2008). Both amino acids were then quantified by RP HPLC – ICP MS. In order to assess the conversion of the HMSeBA into SeMet and further SeCys, a method was implemented to detect HMSeBA in muscle samples and analyzed by RP HPLC – ICP MS according to the method described in detail by Vacchina *et al* (2010). All results (growth performance and Se content) were analyzed using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). The Se dose response for total muscle Se content was tested for linearity for the three Se sources with F test. The growth performance data were analyzed with PROC GLM and LS means were grouped using the Adjust=Tukey option. The relative bioavailability of SO versus SY was evaluated using a five points slope ratio design (NC, SY-0.1, SY-0.3, SO-0.1 and SO-0.3), according to Finney (1971). As stated by Littell *et al.* (1997) a non linear model (in the parameters) was fitted to the data using the PROC NLIN from SAS. The model was:

$$Se\_dry\_muscle = a + a0*X0 + bS*(bTS*DoseSO + DoseSY)$$

Where Se_dry_muscle is the content of Se in the muscle (in mg/kg of dry product), a is the intercept (a0*X0 is a correction for the NC), dose SO and dose SY are the Se amount from SO and SY sources, bS is the slope for the effect of SY on the response and bTS is the ratio between bT (the slope for the effect of SO) and bS. This allows obtaining directly an estimate of the relative biological value (BV) (i.e. the ratio between slopes bS and bT) and its confidence interval (CI).

III. RESULTS AND DISCUSSION

The analysis of total Se content in each diet showed that the expected Se levels were obtained in control and in the different experimental treatments (data not shown).

Birds performance during the 21-d rearing period were not modified by the treatments whatever the source or level of selenium considered. This finding is consistent with other works that did not show performance differences related to selenium supplementation in broilers under standard conditions (Payne & Southern, 2005; Yoon et al., 2007). As expected, all selenium supplemented treatments allowed to increase ($P<0.05$) Se *Pectoralis* muscle content compared to non supplemented group (NC) (Figure 1). Moreover, the muscle

selenium content increases according to dose and for each source the 0.3 mg/kg of dietary Se supplementation resulted in significantly higher selenium deposition in the muscle tissue compared to 0.1 mg/kg of feed. However, the dose effect on selenium deposition with organic selenium sources (e.g. Seleno yeasts (SY) and HMSeBA (SO)) appeared tremendously higher ($p<0.05$) compared to that obtained with inorganic form SS. Indeed, the selenium deposition with 0.3 mg/kg of feed with SY and SO allowed respectively three and four times more selenium than 0.3 mg/kg of sodium selenite. Also, a significant difference appeared between organic sources: the addition 0.2 mg/kg of Se with HMSeBA (SO-0.2) allowed similar selenium deposition than 0.3 mg /kg of feed with seleno yeast. The relative bioavailability between organic selenium sources was tested using slope ratio (Figure 1, insert) and demonstrated that SO treatment allowed 1.39 times (95% CI 1.28, 1.49) more selenium deposition in pectoralis muscle. The higher selenium deposition in tissues with organic selenium forms have been reported by other authors in broiler muscles (Surai, 2002; Payne and Southern, 2005, Wang and Xu, 2008; Markovic *et al.* 2008). This higher selenium content is linked to the ability of selenomethionine to replace methionine in protein synthesis. This accumulation of seleno-methionine is considered as a storage form of selenium (Surai, 2002) that could be mobilized under stress condition to form selenoproteins that would help for body defence.

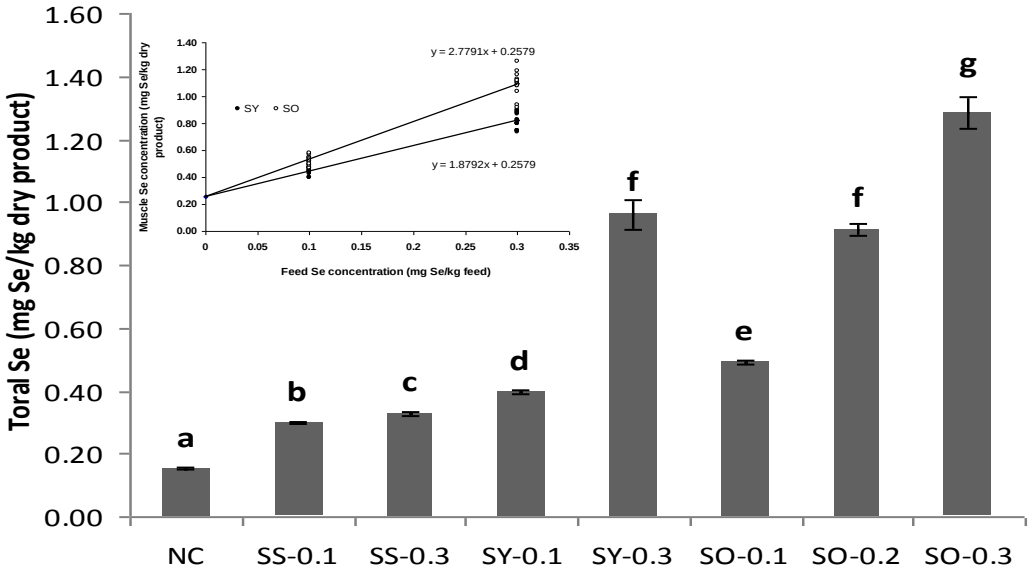

Figure 1 - Total selenium deposition in broiler *pectoralis* muscle depending on dietary selenium levels and forms. Results are expressed as mean ± SEM. (Insert: slope ratio model for relative bioavailability between SY and SO treatments).

Total selenium is a good indicator for selenium deposition but do not reveal under which form this deposition is done. The analysis of residual HMSeBA in muscle showed that this molecule has been totally converted as no remaining HMSeBA was measurable in muscle tissues (data not shown). The selenium species analysis in the muscle of NC; SY-0.3 and SO-0.3 treatments revealed that, in muscle, the main forms of selenium deposited are selenomethionine (SeMet) and selenocysteine (SeCys) whatever the source considered and that the sum of SeMet+SeCys allowed 100 % recovery of the total selenium value in all treatments (Figure 2). In both sources SeMet and SeCys appeared well balanced but it appeared also that in the SO-0.3 group the SeCys value was higher (0.76 vs 0.41) compared to that found in the SY-0.3 group, in the muscle. Selenocysteine, conversely to selenomethionine, is specifically incorporated into selenoprotein with a specific codon

(UGA) through specific selenium transfer as seleno-phosphate to Ser-tRNA (Suzuki, 2001). It could thus be suggested that the better selenium deposition obtained with SO-0.3 treatment will give more functional selenoproteins and further support a different metabolic pathway from HMSeBA and seleno-yeasts. Indeed, it has been shown that hydroxy-methionine (HMTBA) is more transsulfurated than methionine, giving more cysteine than L or DL-Methionine (Martin-Venegas *et al.* 2006). It could thus be hypothesized that HMSeBA would have similar metabolic fate than HMTBA. More studies are needed to investigate the involved metabolic pathways of this new molecule.

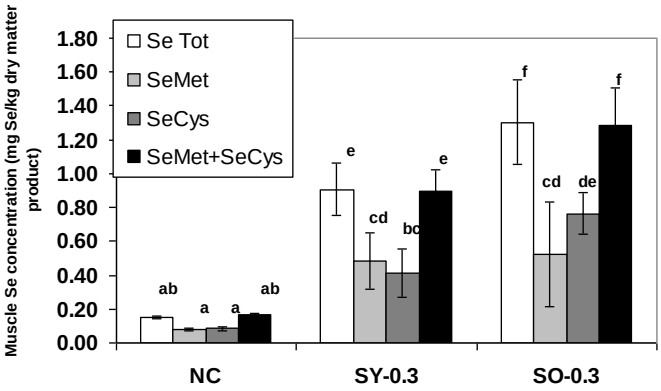

Figure 2 - Muscle total selenium and selenium species depending on dietary selenium supply.

This study allows to conclude that the new pure organic selenium source, Se-OH-methionine or HMSeBA (S0) is highly effective for tissue selenium deposition in broilers. The higher muscle selenocysteine level found has to be further elucidated in term of higher functional selenoproteins.

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MICROBIAL PHYTASE SUPPLEMENTATION INCREASES NET ENERGY OF A WHEAT-SOYBEAN MEAL DIET

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Reports about the impact of supplemental phytase on metabolisable energy (ME) have been inconsistent. However, ME does not consider partitioning of energy for maintenance, product, or heat increment. Net energy (NE) is a more meaningful measure to evaluate the response of broilers to phytase application in this regard. To date, few data can be found on the effect of microbial phytase on dietary net energy. The current study assessed the impact of three phytase products supplemented at an unconventional high level (all at the inclusion rate of 1000 FTU / kg feed) on net energy of broilers using the indirect calorimetric method (IC). The four treatments were: control, which is a wheat-soybean meal (SBM) based diet with reduced available phosphorus (aP) (2.0 g/kg) and calcium (Ca) (6.0 g/kg); control + intrinsically thermostable *E. coli* phytase A (phytase A); control + intrinsically thermostable *E. coli* phytase B (phytase B); control + coated *E. coli* phytase (phytase C). On d 21, 32 male broilers (Ross 308) were allocated to 16 closed-circuit chambers. The adaptation period was four days and then heat production was calculated by applying the Brouwer equation to measurements of O_2 consumption and CO_2 production from 25 d to 27 d. ME was determined by the total collection method and NE was calculated as fasting heat production (450 kJ/$BW^{0.70}$) + energy gain (Noblet et al., 2010).

Dietary inclusion of either phytase had no impact on average body weight (BW), average daily gain (ADG), feed conversion ratio (FCR), heat production (HP) or respiratory quotient (RQ). However, there was a tendency for certain phytase(s) to increase MEI: B>A>C=control ($P < 0.07$). Both phytase A and phytase B improved NE over birds fed the control diet ($P < 0.05$). It is likely that some of the phytases tested reduced energy demands of tissues involved in digestion of feeds. The results indicate that some phytase supplementation can increase NE of broiler chickens fed a wheat SBM-based diet with reduced aP and Ca levels.

Table 1 - Energy determination by IC in broilers (25-27d)

	Control no phytase	Phytase A	Phytase B	Phytase C	SEM	P > F
BW, g	1402	1404	1391	1386	13.9	0.972
ADG, g	88.0	92.8	97.9	86.4	2.4	0.407
FCR	1.595	1.560	1.510	1.618	0.023	0.456
MEI, kJ/kg $BW^{0.70}$	1368	1460	1503	1354	23.7	0.068
HP, kJ/kg $BW^{0.70}$	851	851	866	825	7.2	0.267
RQ	1.02	1.04	1.02	1.02	0.004	0.354
ME, kJ/g feed DM	13.87	14.26	14.23	13.65	0.103	0.086
NE, kJ/g feed DM	9.80^{b}	10.35^{a}	10.30^{a}	9.86^{b}	0.088	0.024
NE: ME (%)	70.65	72.56	72.34	72.32	0.397	0.288

[a,b] means within a row with different superscripts are significantly different ($P < 0.05$)

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FEED STRUCTURE: HOW MUCH DOES THE BROILER REQUIRE?

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Summary

A broiler trial was performed in order to determine the level of structural material required to optimise gizzard function and therefore bird performance. Oat Hulls were used as a model structural component (SC) at two particle sizes, whole and ground with different levels of inclusion to give ten treatment groups: 0%, ground SC at 0.5 %, 1 %, 2 %, 4%, 8% and whole SC at 0.5 %, 1 %, 2 %, and 4%.

The results illustrated that even with a low level of SC feed conversion is positively influenced without negatively impacting growth. The highest inclusion of ground SC, 8%, resulted in the least desirable FCR of all treatments, greater than that of the control. The quadratic model suggested that for whole SC, 2.5% is optimal, however the data clearly shows that between 1 and 4 % differences were not significant, and an inclusion of SC within this range should result in 2 to 3 points improvement in FCR. Ground SC responded less predictably, the quadratic model suggesting optimal inclusion for ground structural sources should be between 2.5 and 3 %, slightly higher than for whole SC.

I. INTRODUCTION

In the last ten years, the inclusion of coarse structural particles in poultry rations has received renewed interest in the scientific community and within the industry. In particular the use of whole wheat in feed has become common practice in numerous European countries, supported by studies reporting the beneficial effects of whole grain on performance (Ravindran, et al., 2006; Svihus, et al., 2010). The gizzard plays a principle role in the observed improvements therefore attention has turned to structural components (SC) other than whole grain that may stimulate development and function of this organ.

Structure can be defined as the size and internal binding strength or hardness of feed particles. Structure in the diet can come from a variety of sources for example, cereal hulls, wood shavings, and whole grains. The driving force behind the reported gains in feed conversion is enhanced gizzard function indicated by significant increases in gizzard weights in birds exposed to SC as a result of increased grinding activity (Rogel, et al., 1987; Hetland and Svihus, 2001; Sacranie, et al., 2012; Sacranie, et al., 2013). A well-developed gizzard results in a cascade of positive responses in the bird, principally: reduced gizzard pH, heightened pepsin activity, a longer retention of feed particles in the foregut therefore increasing exposure to digestive processes and controlling digesta flow into the lumen (Hetland, et al., 2002; Sacranie, et al., 2012). Modulation of flow into the duodenum reduces the starch concentration in the intestines optimising enzyme/substrate interaction, resulting in improved digestion and absorption.

Although the benefits of SC in broiler diets is widely accepted it is not clear how much is required and in what form to insure benefits are observed in the bird. The following study was conducted, using oat hulls as a model for SC, to determine optimal inclusion levels.

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II. MATERIALS AND METHODS

The study was conducted with 1530 Ross 308 male broilers. Day old chicks were randomly distributed amongst 90 pens, 17 birds per pen to give a total of 10 treatments. The design of the trial incorporated 2 points of study. Firstly, a 2 structural size x 4 inclusion level factorial arrangement where the size of SC was either whole or ground (2.5mm) oat hulls, added to wheat/soya diets substituting wheat at 0.5, 1, 2 or 4 %. Secondly, a control without SCs and a treatment with 8% SCs was included. A 3 phase feeding program of pelleted diets was employed with decreasing levels of CP, and increasing levels of energy from starter to finisher. Birds and feed were weighed on days 0, 8, and 35. At 9 and 35 days of age, 1 animal/replicate, with a body weight within 5% of the average pen BW, were slaughtered, and the gizzards collected, cleaned, and weighed before scoring for differentiation between proventriculus and gizzard where 1 = no distinction and 10 = two discrete organs.

Data was analysed using the GLM procedure of SAS, in a one way analysis for all treatments and factorially for treatments 2-9. Significant differences among treatment means were separated by LSD with 5% level of probability. To best determine optimal levels in terms of FCR a quadratic response was modelled for whole and ground treatments.

III. RESULTS

Table 1 displays the performance and physiological outcomes of the factorial aspect of the study over the entire experimental period. Weight gain (WG) was unaffected by SC inclusion level, however whole structure did result in a tendency for loss in growth (p=0.075). This tendency was a result of the reduced daily feed intake (DFI) in whole SC treatments (p=0.002), leading to the observed reduction in FCR in those treatments.

Table 1 - Performance 0-36 days of age and physiological observations, treatments 2-9

Inclusion Level (%)	DFI, g/d	WG, g/d	FCR	9d, Rel. Gizz. Wt. (%)	9d, Differ. Gizz/Provtr.	35d, Rel. Gizz. Wt. (%)	35d, Differ. Gizz/Provtr.
0.5	102.9	71.22	1.445^{a}	3.687^{b}	6.778^{b}	1.351^{a}	5.778^{bc}
1	102.8	71.97	1.429^{b}	3.691^{b}	6.444^{b}	1.156^{b}	5.222^{c}
2	102.9	71.69	1.436^{ab}	3.799^{b}	8.000^{a}	1.355^{a}	6.778^{b}
4	102.9	71.73	1.434^{ab}	4.065^{a}	8.778^{a}	1.432^{a}	9.000^{a}
Ground	103.8^{a}	*72.05^{a}*	1.441^{a}	3.819	7.056^{b}	1.333	6.333
Whole	102.0^{b}	*71.26^{b}*	1.431^{b}	3.803	7.944^{a}	1.313	7.056
Inc.*Form							
0.5 Ground	104.0	71.87	1.447	3.694	5.333^{b}	4.889	4.889
0.5 Whole	101.9	70.57	1.444	3.679	8.222^{a}	6.667	6.667
1 Ground	103.1	72.10	1.431	3.689	6.222^{b}	4.889	4.889
1 Whole	102.6	71.85	1.427	3.694	6.667^{b}	5.556	5.556
2 Ground	103.8	71.73	1.447	3.826	8.222^{a}	6.222	6.222
2 Whole	102.0	71.66	1.424	3.772	7.778^{ab}	7.333	7.333
4 Ground	104.3	72.51	1.438	4.065	8.444^{a}	9.333	9.333
4 Whole	101.5	70.95	1.431	4.065	9.111^{a}	8.667	8.667
SEM (n=9)	0.794	0.613	0.005	0.095	0.549	0.077	0.759
LSD	2.588	2.000	0.018	0.311	1.789	0.250	2.474
P-inc.			*	***	***	**	***
P-Form.	**	*0.075*	*		*		
P-inc. x str.					*		

$^{a-c}$Means within a column with unlike superscripts differ significantly at P < 0.05. *=P < 0.05, **=P < 0.01, ***=P < 0.001.

FCR was significantly improved when birds were exposed to 1% SC, compared to 0.5% however this was not an additive affect, as birds from the 2 and 4% treatments did not

exhibit an improvement in FCR compared to birds from the 0.5% treatments. Mortality for the total trial period was not affected by either SC form or inclusion level.

At 9 days, the gizzard of birds consuming diets with 4% SC were larger ($p<0.001$). The differentiation score for the 2 and 4 % levels was higher ($p<0.001$), while a significant effect of Structural Form was observed with whole SC birds yielding gizzards with a more clearly defined proventriculus and gizzard ($p<0.05$) than with ground SC. The individual treatment means however reveal that within inclusion levels, an increase in differentiation was not always observed moving from ground to whole SC resulting in the observed interaction ($p<0.05$). At 36 days, relative empty gizzard weights were significantly impacted by inclusion level, with the 1% treatments revealing lighter gizzards ($p<0.05$). The highest score for differentiation was recorded in the 4% treatments, and the lowest in the 1 % ($p<0.001$).

Table 2 - Performance 0-36 days of age and physiological observations, all 10 treatments

Treatment	Inclusion Level (%)	Structure Form	DFI (g/d)	WG, g/d	FCR	35d, Rel. Gizz. Wt. (%)	35d, Differ. Gizz/Provtr.
1	0	NA	103.4^{bcd}	71.2	1.451^{b}	1.0^{e}	4.7^{d}
2	0.5	ground	104.0^{abc}	71.9	1.447^{bc}	1.5^{ab}	4.9^{d}
3	1	ground	103.1^{bcd}	72.1	1.431^{cd}	1.1^{de}	4.9^{d}
4	2	ground	103.8^{abc}	71.7	1.447^{b}	1.4^{bc}	6.2^{cd}
5	4	ground	104.3^{ab}	72.5	1.438^{bcd}	1.4^{ab}	9.3^{a}
6	8	ground	105.7^{a}	71.7	1.474^{a}	1.6^{a}	8.7^{ab}
7	0.5	whole	101.9^{cd}	70.6	1.444^{bc}	1.3^{bcd}	6.7^{bcd}
8	1	whole	102.6^{bcd}	71.9	1.427^{d}	1.2^{cde}	5.6^{cd}
9	2	whole	102.0^{cd}	71.7	1.424^{d}	1.4^{abc}	7.3^{abc}
10	4	whole	101.5^{d}	71.0	1.431^{cd}	1.5^{ab}	8.7^{ab}
SEM (n=9)			0.783	0.602	0.006	0.075	0.749
LSD			2.554	1.964	0.019	0.243	2.442
P-value			**		***	***	***

[a-e]Means within a column with unlike superscripts differ significantly at $P < 0.05$. *=$P < 0.05$, **=$P < 0.01$, ***=$P < 0.001$.

The one way ANOVA for the 10 treatments, over the entire period studied, revealed no differences in body weight at 35 days of age between treatments (Table 3). The 4% whole SC treatment displayed the lowest figure for feed intake however this was not significantly lower than the control, while birds from the 8% SC (treatment 6) had a feed intake higher than the control and treatments 3, 7, 8, 9, and 10 ($p<0.05$). The high intake observed in birds from treatment 6 resulted in a poor FCR, higher than the control group ($p<0.001$). An improvement in FCR compared to the control was observed in birds from treatments 3, 8, 9 and 10, with treatment 9 yielding the lowest numerical values for FCR. At 36 days, the heaviest gizzards were recorded in treatments 2, 5, 6, 9 and 10, while the most defined were observed in only treatments 8, 9 and 10 ($p<0.001$).

The quadratic response in FCR to ground and whole SC, at increasing levels of inclusion revealed optimal inclusion levels for whole SC to be 2.54% while for ground was slightly higher at 2.77% (data not shown).

IV. DISCUSSION

In general, the observed improvements with structural addition in gizzard development and FCR, without impeding growth, are in line with previous investigations into the effects of

coarse fibre and structural feed components (Hetland, et al., 2003; Svihus, 2011; Sacranie, et al., 2012; Sacranie, et al., 2013).

It has been suggested in the literature that the broiler chick must adapt to structure, during which gizzard function develops in terms of grinding mechanics, leading to increased gastric secretions and holding capacity resulting in enhanced nutrient utilisation (Svihus, 2011; Sacranie, et al., 2012). However the data from this trial suggests that at day 9 chicks exposed to structure already possessed improved gizzard development. In addition, although not shown in this paper, the results from the initial feeding phases revealed no differences in feed intake in chicks exposed to increasing levels of structure. The real gains in performance are more closely related to subsequent feeding phases due to the exponential increase in feed intake the commercial broiler exhibits with age. A developed gizzard will limit flow of feed in the lumen thereby prolonging satiety and reducing intake slightly while the enhanced digestive function will effectively improve the feeding value of the diet (Sacranie, et al., 2013). The gizzard data supports the theory of organ development being directly related to performance over the entire growth cycle (Svihus, 2011). There are limits, as illustrated by the treatment with an 8% inclusion of structure. Diluting the nutrient density of the diet beyond the potential of the gizzard to improve nutrient utilisation, may still improve gizzard development but will compromise FCR due to compensatory feed intake and as observed by other researches in diets with high levels of coarse structure, losses in growth (Sacranie, et al., 2012).

Over the entire experimental period the best performing treatment was the 2% whole hull treatment although it is important to highlight that FCR was comparable to ground 1 and 4%, and whole 1 and 4 % treatments.

The answer to the question put forth in the title of this study is a fluid one. The results illustrate that even with a low inclusion of structure, for example 1%, feed conversion is positively influenced without negatively impacting growth. The quadratic models suggests that for whole hulls 2.5% is optimal, however as stated already there remains flexibility in responses between 1 and 4 %, within this range 2 to 3 points improvement in FCR can be expected. Ground hulls responded less predictably, most likely due to less uniformity in particle size after grinding. The results of the quadratic model suggest a slightly higher optimal inclusion for ground structural sources between 2.5 and 3 %.

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PERFORMANCE OF BROILERS FED DIETS ADDED WITH TWO LEVELS OF PORCINE OR BOVINE SPRAY-DRIED PLASMA

M.M. BHUIYAN[1], D. CADOGAN[2] and P.A. IJI[1]

Spray dried plasma (SDP) is a highly digestible protein source containing immunoglobulins, growth factors, biologically active peptides, enzymes and other factors that are biologically active in the gut (Borg et al., 2002). Bovine (B) SDP has been shown to produce improved growth rate, feed intake, feed efficiency and produce superior breast yield in broilers (Bregendahl et. al., 2003). Porcine (P) SDP has been used in piglet starter diets to improve feed intake, growth rate and feed efficiency (Coffey and Cromwell, 2001). There is very limited information on the use of PSDP in broiler feed. This experiment was conducted to compare performance of broilers fed diets supplemented with two levels of BSDP and PSDP. Two hundred and forty d-old male Ross-308 broiler chicks were divided equally among five treatments with six replications consisting of eight birds in each cage as an experimental unit. A wheat-soy based basal diet with an AME (12.04 MJ/kg or 2880 kcal/kg), dlys (12.5, g/kg) and dM+C (9.4 g/kg) was prepared and supplemented with 5g/kg and 10g/kg of each BSDP and PSDP to make five treatment diets as shown in Table 1. The treatment diets were offered for the first 10 d, after which the birds were transferred on to commercial grower (11-24 d) and finisher (25-35 d) diets. The diets were supplemented with an indigestible marker (TiO2) to measure nutrient and energy digestibility. Feed intake (FI) and body weight (BWG) were measured at 10, 24 and 35 d. Mortality rate was recorded on each cage to calculate the corrected feed conversion ratio (FCR).

Table 1- Performance of broilers fed two levels of Bovine and Porcine SDP

Treatments		FI, g/b		BWG, g/b		FCR		Weight-Corr. FCR
Plasma type	Dosage (g/kg)	*0-10d*	*0-35d*	*10d*	*35d*	*0-10d*	*0-35d*	*35d*
Nil	0	357.1	3806.1	304.7	2418.8	1.17[a]	1.58	1.58
PSDP	5	340.9	3815.2	303.4	2542.5	1.12[b]	1.50	1.48
PSDP	10	357.6	3732.9	315.0	2513.7	1.14[b]	1.48	1.47
BSDP	5	350.6	3578.5	304.9	2480.3	1.15[ab]	1.44	1.44
BSDP	10	344.0	3618.7	297.9	2456.2	1.16[ab]	1.47	1.47
SEM		*4.990*	*41.18*	*4.211*	*21.72*	*0.005*	*0.015*	*0.017*
P value		*0.784*	*0.268*	*0.770*	*0.460*	*<0.032*	*0.088*	*0.098*

[a,b] Means within column with no common superscript differ significantly (P < 0.05).

Inclusion of PSDP or BSDP at both levels up to d 10 had no significant impact on feed intake and body weight gain in broilers at various stages of growth (P > 0.05). However, PSDP supplementation at both levels during the starter phase improved FCR (P < 0.05) by 5 and 3 points, respectively and by 8-10 points at d 35. Inclusion of BSDP at both levels decreased FCR by 11-14 points at d 35 but no significant improvement was seen during the starter phase. This study showed that it is possible to gain 8-14 points FCR in finishing birds through inclusion of both porcine and bovine SDP in broiler starter diets.

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GUT HEALTH, INTESTINAL INNATE IMMUNITY AND PERFORMANCE

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Summary

Gut health is a very important determinant for health and performance in particular in production animals. The intestinal innate immune system is pivotal in maintaining gut health. Each meal leads to postprandial (low-grade) inflammation response in the (small) intestines, the magnitude of which is related to the caloric value, the glyceamic index and specific components. If not properly regulated, postprandial inflammation could lead to unwanted consequences such as muscle catabolism, inappetite, and predisposition to infections. Gut health can be monitored *in vivo* using markers of gut inflammation. Whereas many are available for mammals, for poultry the number is very limited. Because of the importance of intestinal inflammation, the body has evolved a system to control inflammation by the so-called nervous anti-inflammatory reflex. The gut of production animals is exposed to large amounts of (high) energy feed which is a risk factor for overwhelming the anti-inflammatory reflex, leading to production losses. In the past, this was remedied by adding anti-inflammatory compounds to feed such as the antimicrobial growth promoters (AGP). With the increasing restrictions on the use of antibiotics either as AGP or as therapeutics, there is a great need for alternative compounds and approaches.
Looking at feed composition, some compounds present are potentially pro-inflammatory, and should be removed, whereas others could be anti-inflammatory and therefore maintained and even increased. Another possibility is to use (preferably natural) non-antibiotic anti-inflammatory additives. Compounds can be easily analysed *in vitro* for their pro- or anti-inflammatory properties, and subsequently tested *in vivo* for performance characteristics.

I. INTRODUCTION

The gut is a very crucial organ for maintaining health. Apart from absorbing nutrients, it is also the barrier against unwanted compounds and germs. The immune system in the intestines plays an important role in this. Immune cells such as inflammatory cells were thought to be central, and until recently, the enterocyte layer was considered a simple physical barrier. Now, it is known that enterocytes are immunocompetent cells as well, in particular in the innate part of the immune system. Enterocytes play an important part in the crosstalk with intestinal microbiota. It is important to realise that as opposed to the systemic counterpart, which is geared towards reaction, the larger mucosal immune system is geared towards tolerance. This is particularly relevant concerning inflammation. Inflammation if not tightly controlled can cause great damage to the intestine itself. On the local level, enterocytes, dendritic cells, macrophages are involved in achieving a balanced response (Wells et al., 2011). Furthermore, the presence of numerous nerve endings suggests involvement of the central nervous system. This was confirmed in the case of post-prandial inflammation. The latter is a low-grade inflammatory response in the intestines after each meal. It is the normal physiological response of the body to a meal, and the degree of inflammation is related to the caloric value, the glyceamic index and specific constituents such as fatty acids and others (Margioris, 2009). If not contained, postprandial inflammation could ultimately lead to unfavourable phenomena such as muscle catabolism, inappetite, and intestinal tissue damage

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which predisposes for certain pathogens. Because the intestines are constantly exposed to foreign substances such as feed, it is not surprising that the body has evolved an intestinal system to control inflammation and immunity. This is the nervous anti-inflammatory reflex through the nervus vagus which plays a pivotal role in the control and containment of the intestinal inflammatory system, and is therefore essential for health and survival of the animal (Tracey, 2002) (Figure 1). However, this mechanism can be overwhelmed by risk factors such as large amounts of (high) energy feed. This means that gut health in production animals is at risk, and this should be monitored. The determination of the status of intestinal health in general is not that easy, and in particular not in poultry for the absence of suitable biomarkers and methods.

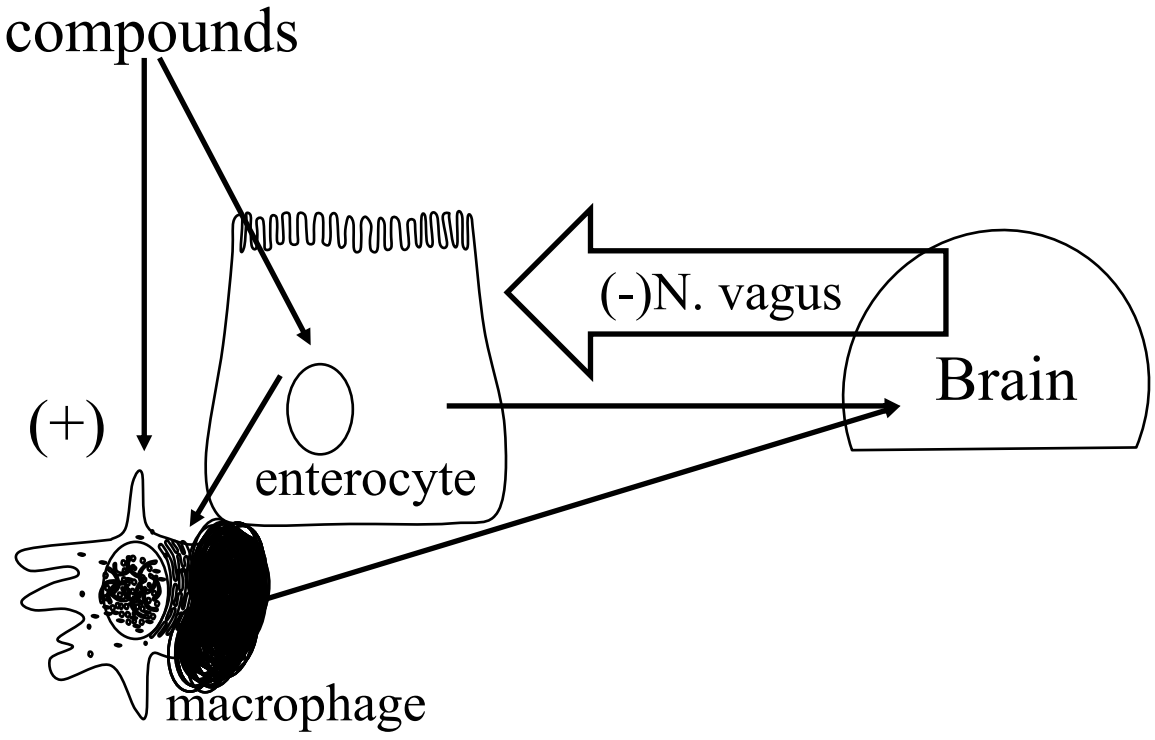

Figure 1 - Schematic representation of the intestinal anti-inflammatory reflex. Feed compounds can give a pro-inflammatory (+) stimulus to enterocytes and macrophages. This leads to the production of pro-inflammatory interleukins (ILs), which also reach the brain. A down (-) regulatory signal is returned to the intestine through the nervus (N.) vagus. Adapted after Niewold, 2013.

II. BIOMARKERS FOR GUT HEALTH IN POULTRY

A major problem in trying to determine intestinal health *in vivo* is the relative inaccessibility of large parts of the GI-tract. There is a great need for biomarkers for intestinal health, which can be determined in samples obtained in a non-invasive or minimally invasive way, meaning from blood, excreta, saliva, urine, or other bodily fluids. Good candidates should be compounds of the GI-tract itself or related to it. They should be validated as indicators of intestinal health, and reagents and assays should be available. Many biomarkers of (intestinal) inflammation have been validated in humans and experimental animals. Unfortunately, the great evolutionary distance with birds makes that knowledge not directly applicable in poultry. For instance, chickens use a totally unrelated protein (PIT54) for haemoglobin binding instead of the acute phase protein haptoglobin (Wicher and Fries, 2006). Even if a homologous protein is found, immunological reagents often do not cross react. Another complication of chickens is the composition of the excretions, containing a lot of uric acid,

and the fact that biomarkers could derive from the kidneys rather than the intestines. Intestinal markers are related to the physical and immunological intestinal barrier function. Enterocytes and tight junctions between enterocytes are crucial in this respect. Inflammation in the intestinal mucosa is tightly controlled too, because it may cause damage and enhanced permeability. As a consequence, biomarkers for damage to intestinal health could be either constituents of enterocytes such as tight junctions proteins, and intracellular products and proteins, either constitutive or induced. Also, products from inflammatory cells should be useful indicators (Kosek et al., 2013). Concerning the latter, myeloperoxidase (MPO) from neutrophiles has been used extensively in mammals. Faecal MPO is used to establish the degree of intestinal inflammation in humans. Unfortunately, chicken heterophiles do not contain MPO or equivalent activity (Brune et al., 1972). For other faecal inflammatory cell biomarkers such as calmodulin also designated S100, lipocalin 2 and HMGB1, it is not clear if these biomarkers are at all present in chicken. Faecal neopterin is a marker of intestinal inflammation. The advantage of neopterin is that is identical in all species, not requiring species specific reagents. Unfortunately, in chicken excreta neopterin excreted by the kidneys can be derived from inflammatory processes other than the intestines. There are several markers for enterocytes described. Intestinal fatty acid binding protein (I-FABP) is a constitutive cytosolic enterocyte protein of the small intestine, and is a useful marker of enterocyte damage in blood and faeces. The chicken I-FABP gene is present and was expressed only in intestinal tissues (Wang et al., 2005); however, reagents are not available as yet. The same is the case for claudin-3. Finally, the level of circulating citrulline is a parameter for functional enterocyte mass in mammals, but not in chicken (Wu et al., 1995) (Table 1).

Table 1 - Intestinal health biomarkers in mammals, their presence in chicken (Ch), sampling method, and the availability of reagents. *In italics: proposed but not proven*.

Marker	Specificity	Sample	Imm(unoassay) Biochem(ical assay)
intestinal fatty acid binding protein (I-FABP)	small intestinal (SI) enterocyte damage	Blood, faeces	Imm: *Ch*
claudin 3	tight junction loss, intestinal permeability	Blood	Imm: *Ch*
citrulline	SI epithelial loss	absent in Ch	Imm:
myeloperoxidase (MPO)	intestinal inflammation	absent in Ch	Imm:
S100 calmodulin	intestinal inflammation	Faeces	Imm: *Ch*
HMGB1	intestinal inflammation	Faeces	Imm: *Ch*
neopterin	intestinal inflammation	Faeces	Imm: Ch, Biochem: Ch

In the case of chronic intestinal diseases in humans, plasma acute phase proteins (APP) such as C-reactive protein (CRP) and haptoglobin are used as markers of intestinal inflammation. It is evident that APP are only good markers for intestinal inflammation in the absence of other inflammatory processes in the body (Margioris, 2009). Several APP, such as haptoglobin can be measured in chicken, using a biochemical method. In any case, it is expected that the level of APP is reciprocal to growth, and that is indeed what is generally observed (Korver et al., 1998).

III. HOW TO REDUCE INTESTINAL INFLAMMATION AND PROMOTE GROWTH

It is clear that increased intestinal inflammation poses a risk to high producing poultry such as broilers. Apart from growth retardation by reduced appetite and muscle catabolism, there is also an increased risk of infections because many pathogens such as *Clostridiae* benefit from inflammation (Ng et al., 2010). Whereas it is economically hardly feasible to reduce the energy content to reduce the inflammatory stimulus, there are other possibilities. In the past, anti-inflammatory components have been added to feed, and the most prevalent of those were the antimicrobial growth promoters (AGP). Earlier, the beneficial effects of AGP were attributed to their antibiotic character (e.g. Dibner and Richards, 2005), but this is unlikely for a variety of reasons, the main one being the sub therapeutic concentrations used. AGP such as oxytetracycline (OTC) work by inhibition of the intestinal inflammatory response by direct inhibition of inflammatory cells (Niewold, 2007), and indeed there proved to be a perfect correlation between (past) use as AGP and the direct anti-inflammatory properties of antibiotics (Table 2).

Table 2 - The relationship between the direct anti-inflammatory properties of antibiotics and their use as antimicrobial growth promoters (AGP). Adapted after Niewold, 2007

Type of antibiotic	Anti-inflammatory	use as AGP
Beta-lactams	no	no
Cyclines	yes	yes
Quinolones	no	no
Macrolides	yes	yes
Peptides (e.g. Zn-Bacitracin)	yes	yes

With the increasing pressures to restrict the use of antibiotics either as AGP or as therapeutics, there is a great need for effective alternatives. These alternative additives could be selected on the basis of known anti-inflammatory activity, and tested directly *in vivo* for their effect on intestinal health and growth. In addition, one could look at the pro- and anti-inflammatory properties of existing normal feed constituents. Intestinal inflammation could be alleviated or prevented by an anti-inflammatory feed composition by for instance removing the pro-inflammatory constituents and maintaining or increasing the levels of anti-inflammatory constituents. New compounds could be selected for anti- and pro-inflammatory properties (and toxicity) using simple *in vitro* assays available (Wu et al., 2003, Niewold and de Backer, 2010). If found to be effective (and non-toxic) these compounds can be tested *in vivo*. This approach makes it possible to select effective compounds from large amounts of candidates. Several promising compounds have been successfully selected using these techniques. The preferable sources of compounds are plants (extracts). Plants are seen as natural, and do contain a host of candidate substances. Alternatives should preferably not be (registered) drugs, because regulatory action can be foreseen. As reviewed earlier (Niewold, 2009), there is a long list of feed components with proposed immunomodulatory properties, including macronutrients, polyphenols, essential oils, herbal compounds etc. For most of these compounds, the results reported are quite variable, and are from empirical trials often built on an inadequate theoretical basis. Of only a few compounds the anti-inflammatory activity and associated growth promotion are fairly well established and three examples from different origin are given. Concerning herbal extracts, sanguinarine containing extracts were previously shown to have growth promoting activity in broilers (Vieira et al., 2008). It also

had *in vitro* anti-inflammatory activity (Niewold and De Backer, 2010), which is consistent with the *in vivo* claim. Furthermore, polyphenols were described as anti-inflammatory (Chen et al., 2006), and indeed anti-inflammatory and growth promoting effects in piglets were demonstrated (Deng et al., 2010), whereas the evidence in chicken is much less clear, which is likely related to the anti-nutritional properties of some polyphenol extracts used. Fat and fatty acids as normal or added feed components are most interesting concerning inflammation. In the first place, the n-3 and n-6 polyunsaturated fatty acids (PUFA), and in particular the ratio between the two are very important (Margioris, 2009). The n-3 PUFA are anti-inflammatory, whereas the n-6 PUFA are pro-inflammatory. A higher 3 to 6 ratio leads to better performance in chicken, and to attenuated growth retardation after inflammatory challenge at the optimum dosage (e.g. Korver et al., 1998). It should be realised, however, that fat is largely responsible for the high caloric value of a meal. This means that when applied in higher concentrations the pro-inflammatory effect may override the anti-inflammatory effect.

IV. CONCLUSION

Inflammation is inversely related to growth (Korver et al., 1998, Niewold, 2007). As discussed here, post-prandial inflammation is a major factor in growth in broilers. Therefore, the research in feed should focus on anti-inflammatory compounds and anti-inflammatory feed composition. Large amounts of new components can easily be screened in the *in vitro* anti-inflammatory assay before costly feed experiments are performed. *In vivo*, the anti-inflammatory theory predicts effective compounds to promote growth to coincide with lower levels of markers of inflammation such as the plasma levels of acute phase proteins.

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THE GUT IMMUNE SYSTEM: A NEW FRONTIER IN GUT HEALTH RESEARCH

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Summary

As the world population grows and developing countries become more affluent, the global consumption of meat will increase by more than 50% within the next 10 years. Confronting the increasing demand for poultry food products are emerging field diseases, increasing regulatory ban of antibiotics growth promoters (AGPs), high-density growth conditions, and waste management. A new paradigm is needed to develop a sustainable poultry production system in view of growing challenges in managing complex ecosystem that control poultry production. There is also increasing scientific evidence that implicates negative consequences of dietary antibiotics on gut microflora, local innate immunity, disease resistance, and overall animal well being. As we move into the 21st Century and the demands for animal food products increase to meet the nutritional needs of a growing world population, developing drug-free alternative strategies to prevent and control animal diseases is a timely global issue and a critical component of our long-term efforts to alleviate poverty and world hunger. In this paper, new understanding on the role of host immunity in controlling cross talks among nutrition, gut microbiota, neuroendocrine and epigenetic systems will be discussed. This paper will also highlight some emerging strategies to enhance gut immunity and to decrease economic losses due to poultry diseases such as coccidiosis and necrotic enteritis. Such information will enhance our understanding of host-parasite biology, mucosal immunology, and facilitate the design of future dietary interventions and vaccination strategies to reduce economic losses due to coccidiosis and necrotic enteritis.

I. INTRODUCTION

Coccidiosis is an ubiquitous intestinal protozoan infection of poultry which seriously impairs the growth and feed utilization of infected animals (Shirley and Lillehoj, 2012; Lillehoj and Lillehoj, 2000). Conventional disease control strategies rely heavily on chemoprophylaxis costing the industry large amounts of money. The existing vaccines comprise live virulent or attenuated *Eimeria* strains with limited scope of protection against an ever evolving and widespread pathogen. The continual emergence of drug resistant strains of *Eimeria*, coupled with the increasing regulations and bans on the use of anticoccidial drugs in commercial poultry production, urges the need for novel approaches and alternative control strategies. Due to the complexity of the host immunity and the parasite life cycle, a comprehensive understanding of the host-parasite interactions and protective immune mechanisms becomes necessary for successful prevention and control practices. Recent progress in functional genomics technology has facilitated the identification and characterization of host genes involved in immune responses as well as parasite genes and proteins that eliciting protective host response (Kim et al., 2008; Kim et al., 2010; Kim et al., 2011).

While natural infection with *Eimeria* spp. induces immunity, vaccination procedures on a commercial scale have shown limited effectiveness and disease control remains largely dependent on routine use of anti-coccidial drugs. Available live vaccines are composed of either virulent or attenuated strains with the major disadvantage which consists of the large number of live parasites making them laborious and costly to produce. Although live oocyst vaccines represent a limited but useful alternative to anticoccidial drugs, a vaccine composed

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of parasite antigens/antigen-encoding genes that elicit specific immunity is eminently preferable. While it would be cost-effective to produce recombinant vaccines (proteins or DNA), the difficulty remains to identify which antigens or genes are responsible for eliciting protective immunity and how these recombinant vaccines should be delivered and presented to the bird's immune system. Also, such subunit vaccines would eliminate the danger of emerging resistant strains which encounter the live vaccines but until efficient vaccines become commercially available, the poultry industry is forced to rely upon prophylactic chemotherapy to control the disease. Further, the introduction of alternative prevention/treatment measures, such as non-chemical feed supplements that effectively enhance productivity and non-specific immunity, may help limit the use of anticoccidials. However, the lack of efficient vaccines, the increasing incidence of drug resistant strains, and the escalating public anxiety over chemical residues in meat and eggs mandate the development of alternative control methods.

II. INNATE AND ACQUIRED IMMUNE RESPONSES TO *EIMERIA*

A comprehensive understanding of protective immunity to coccidiosis is required before we can develop alternative strategies to control coccidiosis. Although both circulating and secretory antibodies, specific for coccidia parasites, have been detected in serum, bile and intestine (Lillehoj and Ruff, 1987; Yun et al., 2000), the antibody titers in serum and intestine do not correlate with the level of protection after oral infection with coccidia (Lillehoj and Ruff, 1987). In general, antibodies are the hallmark of host immune response to *Eimeria* parasites, but do not seem to be involved in protection against coccidiosis (Lillehoj and Lillehoj, 2000). Extensive experimental evidence supports the notion that immunity mediated by lymphocytes and their secreted products, such as cytokines, mediates antigen-specific protection against challenge infection with *Eimeria* (Lillehoj and Lillehoj, 2000; Lillehoj et al., 2004; Lillehoj et al., 2012). In contrast to the plethora of mammalian cytokines, only a few chicken homologs have been described; the major ones including IFN-γ, IL-1, 2, 6 (Schneider et al., 2001), 8, and 15 (Lillehoj et al., 2004; Staeheli et al., 2001). More recently, a series of new chicken cytokines and their receptors (Min et al., 2002; Jeong et al., 2011; Jeong et al., 2012) have been described, including IL-17 (Min and Lillehoj, 2002; Yoo et al., 2009), 18 (Schneider et al., 2000), 16 (Min and Lillehoj, 2004), 12 (Degen et al., 2004), and Th2-type cytokines, such as IL-4, 5 (Koskela et al., 2004), IL-10 (Rothwell et al., 2004), 13 and the granulocyte-macrophage colony-stimulatory factor (GM-CSF) (Avery et al., 2004). The IL-17 family cytokines are the newest cytokines described recently and have been associated with Th17 CD4+ T cell population which is distinct from the classical Th1 and Th2 lymphocyte lineages (Iwakura, 2011). Although traditionally thought of as a component of adaptive immunity, Th17-related cytokines are now recognized as part of the rapid response that develops during the initial phases of immune system activation. Once secreted, these cytokines, and others, regulate the interactions between mucosal epithelia and their associated lymphocytes to eradicate invading pathogens, and to restore immune homeostasis. In the case of avian coccidiosis, the immunoregulatory roles of the newly described proinflammatory IL-17 cytokine family in the host response to parasite infection deserves continued study (Min et al., 2013). Involvement of multiple cytokines in different stages of coccidia infection supports a notion that host immune response to coccidiosis is cell-mediated and complex (Hong et al., 2006). The complexity of cytokine response associated with different species of *Eimeria* infections was investigated using a genome-wide transcriptional microarray (Kim et al., 2011).

III. NEW CONTROL STRATEGIES AGAINST COCCIDIOSIS

Recent studies documented that the dietary immunomodulation of gut immunity in broiler chickens using natural dietary supplements, such as TLR ligands, DFMs and plant-derived phytochemicals that interact with innate sensing molecules to stimulate innate immunity, is a promising alternative strategy that can be applied to many infectious diseases besides coccidiosis where traditional prevention methods show limitations (Lillehoj and Lee, 2012). Furthermore, the underlying immune mechanisms involved in various dietary strategies using TLR ligand-, DFM- and plant phytochemical-mediated immune enhancement of innate immunity should be investigated in order to maximize its effect and to develop a rational synergistic approach for disease control. Some examples of the immune modulation strategies which we have been developing to increase host protective immunity to coccidiosis and to mitigate the use of antibiotics in poultry production include molecular vaccine (Jang et al., 2010; Jang et al., 2011a, 2011b, 2011c), probiotics (Lee, et al., 2010a, 2010b), passive immunization using hyperimmune IgY antibodies (Lee et al., 2009a, 2009b) and dietary immune modulation using plant-derived phytonutrients (Lillehoj et al., 2011; Lee et al., 2007, 2008, 2009, 2010a, 2010b, 2011a, 2011b).

One of the initial steps triggering the innate immune response involves germ-line encoded, highly conserved innate immune sensing molecules of PRRs which include TLRs, nucleotide-binding oligomerization domain proteins (NODs), retinoid-inducible gene 1 (RIG-1) and C-lectin binding receptors. *Eimeria* parasites, the causative pathogens of coccidiosis, contain several components which are stimulatory for immune cells, and they activate innate immunity and inflammatory response. In 2005, our laboratory (Lillehoj, 2004) showed that a conserved antigen of sporozoites of *Eimeria*, profilin, is a parasite PAMP which stimulates T-lymphocytes and induces IFN-γ production. Recently, a significant effect of the oil-based ISA 71 VG, or aqueous nanoparticle-based Montanide IMS 1313 N VG (IMS 1313) adjuvant on recombinant vaccination against coccidiosis was demonstrated (Jang et al., 2011a). These latest studies have opened other doors for the development of recombinant vaccines against coccidiosis and illustrate the importance of elucidating the underlying molecular mechanism of vaccination.

Commensal bacteria on the intestinal mucosa contain many probiotics ligands (such as long surface appendages, polysaccharides and lipoteichoic acids) which can communicate with PRRs inducing downstream signaling pathways that lead eventually to probiotic (health-promoting) effects. Direct-fed microbials (DFM) and their associated ligands can modulate host innate immune response. We have recently evaluated several field isolates of *B. subtilis* strains by the continuous feeding of young broiler chickens with the spore-supplemented standard poultry diet to investigate the probiotic effects of *Bacillus* strains. Depending on the *B. subtilis* strain, feeding diets supplemented with *B. subtilis* spores increased the various intestinal intraepithelial T cell subpopulations, cytokine mRNA levels, and macrophage function. Following an *E. maxima* challenge infection, DFM-fed chickens showed an enhanced disease resistance with higher body weight gain and decreased intestinal lesions as compared with the uninfected control birds (Lee et al., 2010a, 2010b). Detailed immune pathways that were affected by *Bacillus* treatment were further examined using a high-throughput gene expression analysis. Various immune-related genes, especially ones associated with the inflammatory response, were up-regulated in the gut of probiotic-treated chickens.

One promising new avenue to achieve this goal is the use of natural foods and herbal products to enhance host defense against microbial infections and tumors. A growing body of scientific evidence demonstrates the health-promoting effects of plant-derived phytochemicals, chemical compounds derived from plants or fruits. "Phytonutrients" refer to

phytochemicals or compounds from edible plants that have been used as health-promoting agents by many cultures for several millennia. In 400 B.C.E., Hippocrates prescribed willow tree leaves (containing salicylic acid) to abate fever. There is abundant evidence from epidemiological studies that phytochemicals can significantly reduce the risk of cancer and may reduce high blood pressure, pain, and asthma. The most popularly used drug for cancer chemotherapy worldwide is Taxol (paclitaxel), a phytochemical initially extracted and purified from the Pacific Yew tree. Taxol possesses anti-viral, anti-bacterial, and anti-cancer properties. Many other phytochemicals with potent medicinal properties are currently in clinical trials for treatment of a variety of diseases. Lycopene, for example, from tomatoes is in clinical trials for cardiovascular diseases and prostate cancer. Its beneficial effects may be due to its anti-oxidant and anti-inflammatory effects. While numerous studies have showed disease prevention or immune enhancing effects resulting from oral feeding of plants, only a few reports have examined the specific effects of plant-derived phytochemicals on gut defenses. The intestinal mucosal system plays a central role in the exclusion and elimination of harmful dietary substances in humans and animals. Part of the intrinsic gut defense mechanisms are mediated by the lymphoid system and the intestine contains a relatively large component of lymphatic tissues.

Recent studies from our laboratory (Lillehoj et al., 2011) provided clear evidence that the dietary supplements of natural phytochemicals activate innate immunity in poultry and, in particular, enhanced protective immune responses against avian coccidiosis. Phytochemicals are plant- or fruit-derived chemical compounds which possess the health benefits including promotion of tumor killing and the increased resistance to infectious diseases caused by bacteria, virus and parasites. However, very limited information is available on the mode of action of most of health-promoting plant phytochemicals. Therefore, in order to obtain a basic understanding of how dietary supplements, such as plant and fruit extracts, exert immunostimulatory effects in poultry, we carried out *in vitro* and *in vivo* feeding trials using an intestinal protozoan disease model, avian coccidiosis. In various *in vitro* studies, culture of chicken spleen lymphocytes with crude extracts from milk thistle, turmeric, shiitake and reishi mushrooms, persimmon, tomato, safflower leaf, plum fruit, and cinnamaldehyde induced significantly higher cell proliferation compared with the untreated control cells. Stimulation of chicken macrophages with crude extracts of milk thistle, shiitake and reishi mushrooms, persimmon, raspberry, safflower leaf, plum fruit, and cinnamaldehyde resulted in a robust nitric oxide production to the levels that were similar with those induced by recombinant chicken IFN-γ. Most of the phytochemical extracts and cinnamaldehyde inhibited the growth of chicken tumor cells *in vitro*. The levels of mRNAs encoding IL-1β, IL-6, IL-12, IL-18, and tumor necrosis factor superfamily member 15 (TNFSF15) were enhanced in macrophages that were treated with extracts of turmeric or shiitake mushroom as compared with the untreated control (Lee et al., 2009, 2010b, 2011a).

Cinnamaldehyde also directly reduced the viability of *Eimeria tenella* parasites at 10 and 100 μg/ml ($P<0.05$ and $P<0.001$, respectively), as compared with the media controls (Lee et al., 2011a). The effects of plant extracts on enhancing the various *in vitro* parameters of protective immunity have been positively correlated with their ability to protect against microbial infections. In several *in vivo* trials, the feeding of broiler chickens with diets supplemented with extracts of mushroom, safflower, plum, and cinnamaldehyde consistently enhanced innate immunity and provided enhanced protection against live, oral parasite challenge infections. For example, mushroom extracts and cinnamaldehyde significantly protected chickens against weight loss characteristically seen during coccidiosis and promoted parasite killing as indicated by reduced fecal oocyst shedding. Dietary supplementation of young broiler chickens infected with *Eimeria acervulina* using plum, safflower leaf extracts, c*urcuma*, c*apsicum*, and cinnamaldehyde increased body weight gain,

reduced fecal oocyst shedding, and increased cell-mediated immunity as measured by the transcriptional changes in key cytokines such IL-1β, IL-6, IL-15, and IFN-γ (Lee et al., 2006, 2008, 2009, 2010a, 2011a).

Furthermore, combination of two phytonutrient mixtures, VAC (carvacrol, cinnamaldehyde, and capsicum oleoresin), and MC (capsicum oleoresin and turmeric oleoresin), were evaluated for their effects on chicken immune responses following immunization with a recombinant *Eimeria* profilin protein. Following immunization and infection, chickens fed the VAC- or MC-supplemented diets showed increased body weights, greater profilin antibody levels, and/or greater lymphocyte proliferation as compared with non-supplemented controls. Immunized chickens fed the MC-supplemented diet exhibited increased MHC class II^+, $CD4^+$, $CD8^+$, $TCR1^+$, or $TCR2^+$ T cells as compared with nonsupplemented controls while chickens on the VAC-containing diet displayed an increase in $K1^+$ macrophages. Finally, the dietary supplementation with VAC or MC alters immune parameters following recombinant protein vaccination and shows vaccine-stimulated immunity against avian coccidiosis (Lee et al., 2011 b).

IV. CONCLUSIONS

In view of increasing consumers' concerns about drug residues in the food chain, the poultry industry will eventually discover alternative methods to control economically important avian diseases. Chickens will continue to provide a major and increasing supply of the world's animal protein. It is hard to imagine disease control in the field without the use of anticoccidial drugs, but it is probable that the current methods of control will continue unabated and supplemented with drug-free alternatives. There are, however, increasingly negative political views towards in-feed medication of livestock (especially within Europe) and an overall increasing negative view on the use of prophylactic chemotherapy provides a significant spur for work on the immunological control of avian coccidiosis. Application of the recently described innovative technology in immunomodulation and vaccination may lead to the development of alternatives to prophylactic medication. With rapidly developing technologies in functional genomics and computational biology, it is anticipated that new paradigms for coccidiosis control will be formulated. It is possible that the use of genetic tools could become one way of combating parasites, in synergy with other strategies of coccidiosis control such as vaccination, nutrition, and management.

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THE HISTOMORPHOMETRY CHANGES PRODUCED BY EIMERIA AND FISHMEAL IN A BROILER NECROTIC ENTERITIS CHALLENGE MODEL

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Summary

Factors predisposing necrotic enteritis in broiler chickens are complex and their respective influences on the morphological changes of gut epithelial layer have not been widely investigated. A 2 x 2 x 2 factorial experiment with or without dietary fishmeal, *Eimeria* inoculation and *Clostridium perfringens* challenges was performed with each treatment having six replicates and each replicate containing 25 birds to investigate the influence of these factors on gut morphology. Challenge of the birds by *C. perfringens* was carried out on d 14 and 15. Duodenal histomorphometrics were conducted using standard paraffin sectioning procedure and the villus height and crypt depth were measured using optical microscopy. The results showed no effects of dietary fishmeal inclusion or *Eimeria* inoculation or their interaction prior to the *C. perfringens* challenge, i.e., at d 13. At d 16, *Eimeria* inoculation significantly reduced villus height ($P < 0.001$), while 250 g/kg dietary fishmeal significantly increased the crypt depth ($P < 0.05$). Furthermore, *Eimeria* inoculation significantly reduced villus height to crypt depth ratio. *C. perfringens* challenge on d 14 and 15 did not alter the morphometric characteristics. The morphometric data presented herein confirms previous suggestions that *Eimeria* inoculation is more important than dietary fishmeal inclusion in predisposing birds to necrotic enteritis under experimental conditions.

I. INTRODUCTION

Factors predisposing necrotic enteritis (NE) in broiler chickens are complex. The roles these factors play in predisposing the birds to the disease have been considered to be related to the gut status including the morphology of epithelial layer. *Eimeria* and fishmeal are two factors that have been used in NE challenge models to predispose birds to the disease. However, their respective influences on the morphological changes of gut epithelial layer and possible interactions between them have not been widely investigated. In the present study, we aimed to examine the roles of dietary fishmeal, *Eimeria* inoculation and the pathogenic bacteria *Clostridium perfringens* challenge on the histomorphometric changes in a NE challenge model used at the University of New England.

II. MATERIALS AND METHODS

Ross 308 male day old broilers (n=1200) were allocated to a 2 x 2 x 2 factorial design with or without 250 g/kg dietary fishmeal inclusion between d 8 and 14, *Eimeria* inoculation on d 9, and *C. perfringens* inoculations on d 14 and 15. Each treatment was replicated six times, each with 25 birds. All the birds were raised and handled humanely, and the animal trial was approved by the Animal Ethics Committee of the University of New England. Nutrient and dietary composition of the starter and finisher diets were the same as reported previously (Wu, *et al*., 2012). The NE challenge was performed based on previous experiments conducted at the University of New England, Australia (Wu *et al*, 2010). Birds had *ad libitum* access to feed and water throughout the experiment. A primary poultry isolate of *C.*

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perfringens type A strain EHE-NE18 (CSIRO Animal, Food and Health Sciences, Geelong, Australia) was applied to birds in appropriate pens by oral gavage. Two birds from each replicate were killed and 1 cm of the mid section of duodenum was collected and fixed in 10% buffered formalin (3.7 % formaldehyde, pH 7.5) at d 13 and 16. Formalin-fixed duodenum samples were processed in consecutive steps of dehydration by serial ethanol solutions (30% to 100%), clearing by xylene, and infiltration by paraffin. The tissue was embedded in paraffin and subsequently sectioned at a thickness of 8 µ with a Reichert-Jung 820 Histocut Microtome (Cambridge Instruments GmbH, Germany). The tissue sections on the slides were stained using Harris's haematoxylin (George Gurr Ltd., London, UK), and eosin (Gur Certistain, VWR International Ltd., Poole, UK), and mounted with DPX (distrene polystyrene xylene) mountant. The sections were viewed under an Olympus Vanox microscope (Olympus Optical Co. Ltd., Tokyo, Japan) and the images captured with a colour video camera LY-MN-HP SUPER CCD (Chengdu Liyang Precision Machinery Co. Ltd, Chengdu, China). Morphometric indices were determined using computer-aided light microscope image processing analysis software VideoPro 32 package (Leading. Edge Pty Ltd, Adelaide, Australia). Villus height and crypt depth were measured in 20 vertically, well-oriented, intact villi and crypts. All measurements were calibrated with a micrometer. The data were analysed using the GLM model of the statistical package IBM® SPSS® Statistics package version 19 (IBM Corporation).

RESULTS

At d 13, morphometric measurements were not different indicating no effect of fishmeal inclusion in the diet from dd 8 to 14 or *Eimeria* inoculation on d 9 ($P > 0.05$). No interactions between these two treatments were observed at d 13 ($P > 0.05$) as shown in Fig. 1.

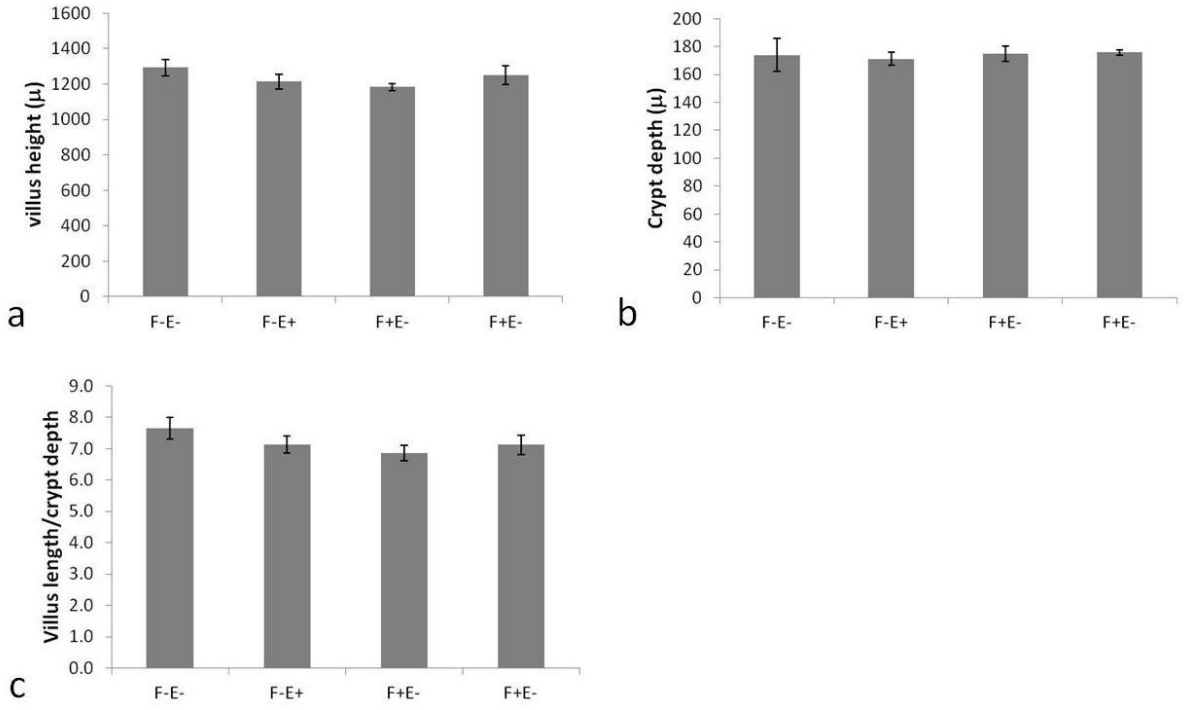

Figure 1 - Duodenal villus height, crypt depth, and the ratio of villus height to crypt depth of the birds treated with fishmeal and *Eimeria* at d 13. No significant main effects were observed in the respective measurements ($P > 0.05$), and no interactions between fishmeal and *Eimeria* were detected ($P > 0.05$).

At d 16, i.e., following two *C. perfringens* inoculations on ds 14 and 15, the *Eimeria* inoculation at d 9 significantly reduced villus height ($P < 0.001$), whereas 250 g/kg fishmeal addition in the starter diet from d 8 to d 14 significantly increased the crypt depth ($P < 0.05$). Consequently, *Eimeria* inoculation also significantly reduced villus height to crypt depth ratio. No significant effect of *C. perfringens* challenge on the villus-crypt architecture was observed at the 0.05 probability level. However, its effect on reduction of villus height tended to be significant ($P = 0.077$). Furthermore, no significant interactions between the treatments were detected for the parameters measured (Table 1).

Table 1 - Morphometric responses upon the addition of fishmeal, *Eimeria* innoculation and the challenge by *C. perfringens* in the birds at d 16 of age.

Treatment		Villus height		Crypt depth		Villus height : Crypt depth	
		Mean	SE	Mean	SE	Mean	SE
F-E-C-		1510a	86	186	7	8.1ab	0.4
F-E-C+		1454ab	136	171	11	8.7b	1.1
F-E+C-		1313ab	85	194	12	6.8ab	3.2
F-E+C+		1083b	72	188	11	5.8a	3.6
F+E-C-		1590a	61	207	16	7.9ab	0.8
F+E-C+		1525a	31	207	13	7.5ab	0.4
F+E+C-		1350ab	69	185	9	7.4ab	0.6
F+E+C+		1234ab	59	215	9	5.7a	0.5
Main Effect							
F	-	1340	57	185	5	7.4	0.8
	+	1425	51	204	6	7.1	0.6
E	-	1520	45	193	7	8.1	0.7
	+	1245	49	196	5	6.4	0.5
C	-	1441	48	193	6	7.6	0.6
	+	1324	59	195	6	6.9	0.8
GLM ANOVA (P)							
F		NS		< 0.05		NS	
E		< 0.001		NS		< 0.001	
C		0.077		NS		NS	
F x E		NS		NS		NS	
F x C		NS		NS		NS	
E x C		NS		NS		NS	
F x E x C		NS		NS		NS	

DISCUSSION

In general, the NE challenge model reduces the villus height and increases the crypt depth as reported in previous studies (Bains, 1968; Golder, *et al*., 2011; Helmboldt and Bryant, 1971). However, detailed descriptions of how different predisposing factors act on these features are scarce. In this study, we employed an 2 x 2 x 2 experimental design to desect the roles of fishmeal, *Eimeria* and the pathogenic bacteria *C. perfringens* on the cause of intestinal epithelial damage. It was revealed that *Eimeria* inoculation at d 9 of the trial reduced the villus height and villus height to crypt depth ratio whereas 250 g/kg fishmeal feeding in the

diet did not significantly affect the development of villus. This indicated a more important role of *Eimeria* than fishmeal in predisposing birds to sucumb to NE, as fishmeal only increased crypt depth and did not reduce the villus height. This histomorphometric evdence is consistent with our previous report on mortbility and mortality data that indicated less importance of fishmeal in predisposing birds to NE at least when *Eimeria* inoculation is applied in the challenge process (Wu, *et al.*, 2012). Interestingly, *C. perfringens* did not significantly affect the villus height nor crypt depth. This finding contrasts with the results of Golder *et. al.* (2011) who showed that the feeding of fishmeal diets and Eimeria challenge prior to challenge with *C. perfringens* as per the UNE model was disruptive to villus-crypt architecture (Golder *et al*., 2011). As the infection of birds by *C. perfringens* alone does not usually cause NE in the UNE challenge model, it is not surprising that the level of damage to the gut epithelial layer it produces is limited if at all. However, further investigation to which extent the bacteria can lead to the disruption of mucosa should be conducted.

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SODIUM METABISULPHITE INFLUENCES SITES OF STARCH DIGESTION, ENERGY UTILISATION AND FEED CONVERSION IN SORGHUM-BASED BROILER DIETS

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Summary

Graded inclusions of sodium metabisulphite (SMBS) in sorghum-based broiler diets reduced disulphide bonds; this was anticipated. However, SMBS also profoundly modified RVA properties of extracted starch, which was probably due to oxidative-reductive depolymerisation of starch polysaccharides. SMBS significantly improved energy utilisation and feed conversion efficiency in broiler chickens. This appeared to be associated with changes in the site of starch digestion with more starch ('gradually' digestible starch) being absorbed from the distal jejunum, proximal ileum and distal ileum. Consideration is given to the underlying mechanisms whereby SMBS generated more gradually digestible starch and this, in turn, enhanced energy utilisation and feed conversion efficiency in broiler chickens offered sorghum-based diets.

I. INTRODUCTION

There is evidence that the site of starch digestion and the provision of slowly digestible starch enhance feed conversion efficiency in broiler chickens (Weurding *et al.,* 2003b). The *in vitro* rate of sorghum starch digestion is considerably slower than that of wheat and the potential digestibility of sorghum starch is less than that of barley, maize and wheat (Giuberti *et al.*, 2012). The poor utilisation of energy derived from sorghum starch compromises the nutritive value of sorghum as a feedstuff for broilers (Black *et al.,* 2005; Selle *et al.,* 2013). The inclusion of graded levels of sodium metabisulphite (SMBS) in sorghum-based diets improved energy utilisation (AME and AMEn) and feed conversion ratios to significant extents in as yet unpublished data. Moreover, SMBS generated more gradually, or slowly, digestible starch. This paper is a specific consideration of the possible mechanisms whereby SMBS generated more gradually digestible starch and, in turn, positively influenced the efficiency of energy utilisation and feed conversion in sorghum-based broiler diets.

II. MATERIALS AND METHODS

Seven graded inclusions of SMBS (0 to 5.25 g/kg) was included in steam-pelleted sorghum based-broiler diets that were offered to 294 male, Ross 308 chicks from 10 to 24 days post-hatch. Disulphide bond concentrations in the diets were determined and the properties of extracted starch were assessed by rapid visco-analysis (RVA). Growth performance and nutrient utilisation parameters were determined by standard procedures. Apparent starch digestibility coefficients were determined in four small intestinal segments from which 'abruptly and 'gradually' digestible starch was calculated. Abruptly digestible starch is defined as the quantity of starch (g/bird) absorbed from the proximal jejunum; gradually digestible starch is the quantity absorbed from the distal jejunum, proximal and distal ileum. The IBM® SPSS® statistics program (Version 20) was used for analysis of the experimental

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data and the feeding study complied with guidelines of the Animal Ethics committee of the University of Sydney.

III. RESULTS AND DISCUSSION

As shown in Table 1, the sulphite reducing agent SMBS linearly reduced ($r = -0.775$; $P < 0.001$) disulphide cross-linkages in sorghum-based diets steam-pelleted at 85°C, which was associated with significant increases in free sulphydryl groups and protein solubility of the diets. Moreover, SMBS profoundly influenced RVA properties of starch extracted from the diets, which included a marked reduction in final starch viscosity ($r = -0.986$; $P < 0.001$). However, sulphite reducing agents are capable of depolymerising starch polysaccharides by oxidative-reductive reactions (Paterson *et al.*, 1996, 1997). In broilers, SMBS linearly reduced abruptly digestible starch ($r = -0.413$; $P < 0.005$) but, reciprocally, linearly increased gradually digestible starch ($r = 0.483$; $P < 0.001$), so that more starch was being absorbed in caudal small intestinal segments. Furthermore, SMBS linearly reduced FCR ($r = -0.356$; $P < 0.02$) and linearly increased AMEn ($r = 0.437$; $P < 0.01$).

Table 1 - Linear effects of sodium metabisulphite inclusion levels in sorghum-based broiler diets on disulphide bonds, final viscosity of starch extracted from diets, abruptly and gradually digestible starch, feed conversion ratio and N-corrected apparent metabolisable energy

Sodium meta-bisulphite (g/kg)	Disulphide bonds (μmol/g protein)	Final viscosity, extracted starch (cP)	Abruptly digestible starch (g/bird)	Gradually digestible starch (g/bird)	Feed conversion ratio (FCR g/g)	N-corrected AME (AMEn MJ/kg DM)
0.00	41.67	1035	508	155	1.458	11.85
1.50	37.63	886	410	217	1.422	12.30
2.25	37.80	789	454	175	1.434	12.23
3.00	37.00	630	490	160	1.425	12.16
3.75	39.07	628	486	183	1.414	12.15
4.50	36.77	457	387	246	1.424	12.36
5.25	34.77	441	394	256	1.422	12.28
Linear effect	r = -0.775 P < 0.001	r = -0.986 P < 0.001	r = -0.413 P = 0.003	r = 0.483 P < 0.001	r = -0.356 P = 0.015	r = 0.437 P = 0.008

As shown in Table 2, gradually digestible starch was correlated with improved FCR ($r = -0.291$; $P < 0.05$) and abruptly digestible starch was correlated with depressed AMEn ($r = -0.282$; $P < 0.02$) but the other two single regressions were not significant. However, when gradually and abruptly digestible starch are considered in multiple linear regressions there are significant relationships with both FCR ($r = 0.382$; $P < 0.04$) and AMEn ($r = 0.385$; $P < 0.03$). These relationships indicate that sites of starch digestion influence efficiency of energy utilisation and feed conversion in sorghum-based broiler diets. The respective correlation coefficient squared values ($r^2 = 0.146$; $r^2 = 0.148$) indicate that nearly 15% of variation in AMEn and FCR may be attributed to sites of starch digestion. Thus, the site of starch digestion is clearly an influential factor where some gradually digestible starch, as defined, is beneficial.

Table 2 - Single and multiple linear regressions between abruptly and gradually digestible starch with feed conversion ratios and N-corrected AME

Linear regression	Correlation coefficient (r)	Significance (P)
Abruptly digestible starch with FCR	r = 0.128	P = 0.393
Gradually digestible starch with FCR	r = -0.291	P = 0.047
Abruptly and gradually digestible starch with FCR	r = 0.382	P = 0.031
Abruptly digestible starch with AMEn	r = -0.282	P = 0.019
Gradually digestible starch with AMEn	r = 0.112	P = 0.486
Abruptly and gradually digestible starch with AMEn	r = 0.385	P = 0.025

This outcome raises two issues. The first is the mechanisms whereby SMBS alters sites of starch digestion and the second is the mechanisms whereby the generation of more gradually digestible starch enhances FCR and AMEn. SMBS profoundly reduced the final viscosity of starch extracted from sorghum-based diets so the fact that SMBS is capable of reducing disulphide linkages is irrelevant in this 'protein-free' situation. Thus, the likelihood is that SMBS directly modified starch by oxidative-reductive depolymerisation and, as a consequence, this depolymerised starch partially escaped proximal jejunal digestion. While speculative, depolymerised starch may be less readily digested and/or transit the proximal jejunum more rapidly. Chemically modified and depolymerised starch has been shown to be less readily digested (Wolf *et al.,* 1999) and, being more soluble, may have more rapid gut transit rates (Amerah *et al.,* 2007) A further possibility is that SMBS impedes endogenous amylase activity as demonstrated by Chen *et al.* (1972) by altering the enzyme's structure and function via disulphide bond reduction. SMBS is absorbed quite rapidly from the gastrointestinal tract in rats (Wever, 1985) so SMBS may initially retard starch digestion by pancreatic amylase but that this would diminish as SMBS is absorbed from the gut. These related factors may also contribute to the generation of more gradually digestible starch by SMBS.

Gradually, or slowly, digestible starch has been linked to improvements in FCR in broiler chickens. Weurding *et al.* (2003a) made several proposals as to how slowly digestible starch improves feed conversion efficiency. Glucose is converted to lactate in the gut wall in response to rapid glucose absorption and hepatic reconversion of lactate to glucose expends energy. Slowly digestible starch may prompt a lower but more sustained insulin response resulting in more efficient protein deposition. Synchronicity of starch/protein digestion and glucose/amino acid absorption may be important as imbalances could compromise protein accretion and waste energy. There is also the distinct possibility that slowly digestible starch has a 'protein-sparing' effect. Weurding *et al*. (2003b) reported a significant interaction between dietary lysine levels and slowly versus rapidly digestible starch where improvements in feed conversion from slowly digestible starch were more pronounced with lower lysine levels in broilers after nine days. The gastrointestinal tract consumes about 20% of incoming energy and Fleming *et al.* (1997) found that glucose and glutamine are approximately equally energy substrates for small intestinal mucosal cells in the rat. However, net adenosine triphopshate (ATP) production from glucose (120.0 μmol/g/minute) was greater than from glutamine (57.1 μmol/g/minute) and net ATP production with both substrates present was 155.5 μmol/g/minute. It follows that glucose from rapidly digested starch sources would be depleted in the upper small intestine, effectively forcing mucosal cells in the lower small

intestine to oxidise amino acids to provide energy. Thus slowly digested starch sources would provide more glucose to the lower small intestine thereby sparing protein from oxidation and energy generation from glucose would be substantially more efficient than from amino acids. A pivotal study was completed by van der Meulen *et al*. (2007) with purified starch sources in pigs. Less rapidly digested native pea starch significantly increased the net portal flux of essential amino acids by 22.9%, including a 28.6% increase in lysine, in comparison to more rapidly digestible maize starch. However, the net portal flux of glutamic acid was negative indicating that they were extensively oxidised to provide energy to the gut mucosa. The more sustained availability of glucose along the small intestine as an energy substrate for gut function probably spares amino acids from oxidation, increases their entry into the systemic circulation and meets the gut's energy requirements more efficiently .

Finally, it is clear that SMBS improved energy utilisation and FCR of sorghum-based broiler diets. The underlying mechanisms are not equally clear but the likelihood is that these improvements stemmed from SMBS-induced oxidative-reductive depolymerisation of starch rather that the reduction of disulphide cross-linkages in protein. It appears that the pivotal factor was the generation of more gradually digestible starch, which enhanced both AMEn and FCR.

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THE RELATIONSHIP BETWEEN FEED ENZYME EFFICACY AND STARCH DIGESTION RATE IN BROILER CHICKENS

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Summary

An *in vitro* starch experiment was conducted to determine the correlation between *in vitro* starch digestion rate and feed enzyme efficacy for *in vivo* ileal protein, starch and fat digestibility in broiler diets. The magnitude of effects of a commercial enzyme admixture containing endo-xylanase from *T. ressei*, α-amylase from *B. licheniformis*, and protease from *B. subtilis* on nutrient digestibility, was partially dependent on *in vitro* starch digestion rate and may be significantly improved in broiler chicken diets containing slowly digested starch or incompletely digested protein and fat concentrations.

I. INTRODUCTION

It is known that the inclusion level of specific feed ingredients or substrates contributes to the magnitude of the exogenous feed enzyme response (Rosen, 2010). The so-called 'viscous' feed ingredients such as wheat, barley, rye, triticale and oats have been well-documented to respond to exogenous xylanase due to the higher concentration of soluble non starch polysaccharides (SNSP) (Choct, 2006), since high concentrations of SNSP reduce nutrient digestibility. Furthermore, feed ingredients with high ileal digestibility respond less readily to feed enzymes compared with poor digestibility (Cowieson, 2010). As more than half of the AME content of common diets for broiler chickens is provided by dietary starch, the kinetics of the digestion of starch may be influential and it has been suggested that starch slowly digested in the lower part of the small intestine may improve broiler chicken performance (Weurding, et al., 2003). Thus, the efficacy of a commercially available enzyme admixture containing endoxylanase, α-amylase and subtilisin protease (XAP) may be influenced by the inherent rate and the extent of nutrient digestion of the diet. The objective of this study was to investigate the correlation between *in vitro* starch digestion rate and the magnitude of XAP effects on ileal protein, starch and fat digestibility.

II. MATERIALS AND METHODS

The enzyme admixture (XAP; DuPont Industrial Biosciences - Danisco Animal Nutrition, Marlborough, UK) provided a guaranteed minimum of 2000 U xylanase, 200 U amylase and 4000 U protease per kg of feed. A total of 15 control diets were formulated using 7 commonly used feed ingredients including maize, soybean meal, canola meal, wheat, wheat bran (millrun), full fat rice bran and sorghum, which were systematically titrated at varying inclusion concentrations (Bao et al., 2012). These 15 control diets were divided into two equal batches and XAP was added to one of the batches. The resulting 30 diets were cold pelleted and fed to a total of 1080 (6 replicate cages of 6 birds per cage) 17 d male broiler chickens for a period of 7 days. Distal ileal contents were collected for digestibility of protein, starch, fat and dry matter (DM). The XAP efficacy for protein, starch and fat digestibility was calculated as a proportion of the undigested nutrients by the following formula:

$$XAP\ response = \frac{UNTc - UNTe}{UNTc}$$

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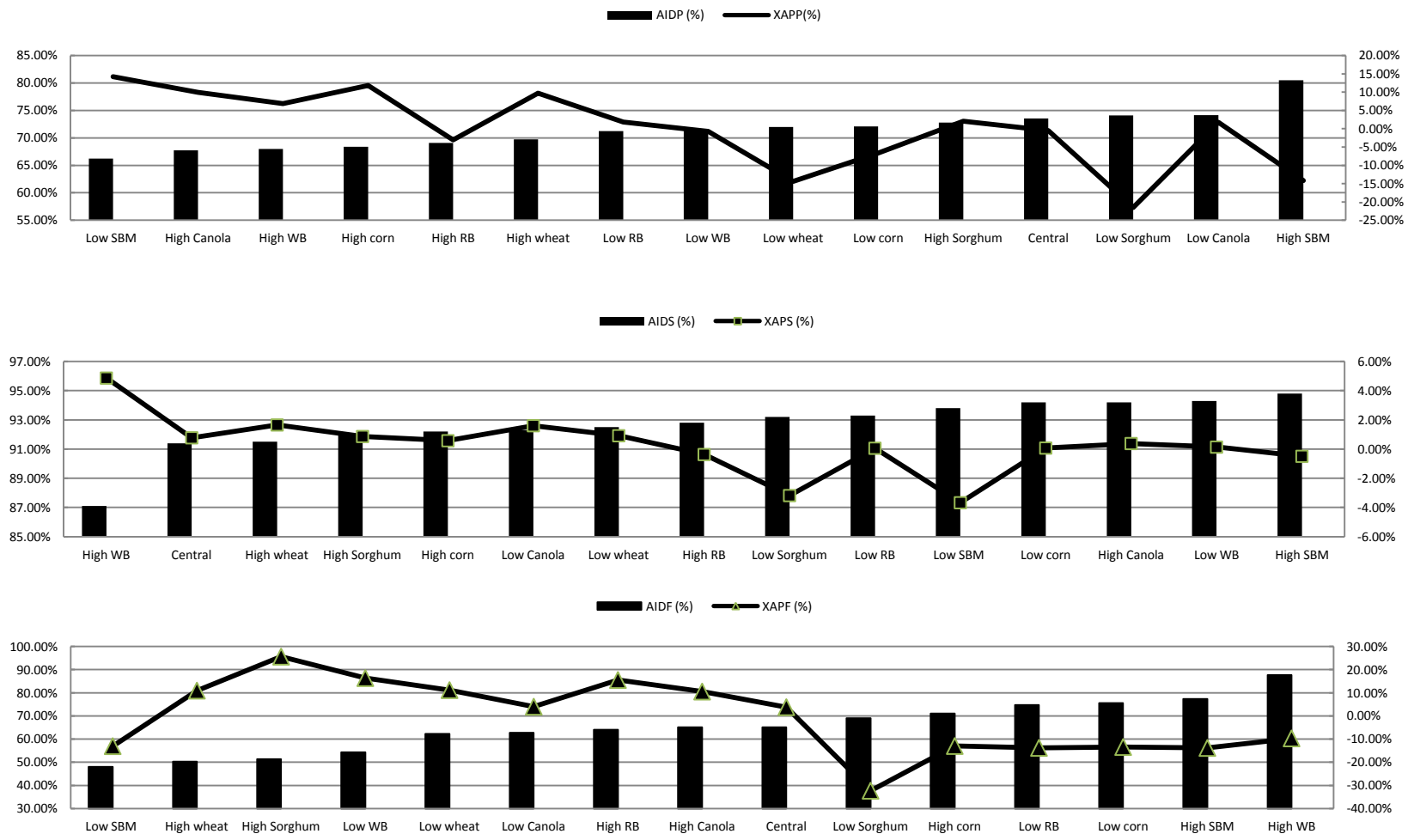
Where UNTc = Nutrients concentration × (1− Nutrient digestibility in control diets) and UNTe = Nutrients concentration × (1− Nutrient digestibility in diets with XAP addition)

Each control diet was analyzed for starch, protein, fat, soluble and insoluble non-starch-polysaccharides (NSP), slowly digested starch, rapidly digested starch and free sugar. *In vitro* starch digestibility was determined by modifying the method described by Sopade and Gidley (2009). The *in vitro* starch digestion rate (K_s) was calculated following Michallis-Menten kinetics: $K_s = V_{max} / K_t$; where V_{max} was the highest digestibility and constant K_t was considered the time (min) needed to approach the half maximal starch digestibility (Broderick and Clayton, 1992).

The *in vitro* starch data were analyzed using JMP 9.0 software with non-linear Michallis-Menten model. The relationships between XAP efficacy and Ks and other dietary characteristics were assessed with a principle components analysis. To determine significant differences, the multivariate pairwise program in JMP 9.0 was used at $P < 0.05$.

III. RESULTS AND DISCUSSION

The overall effect of proportional changes in different feed ingredients and XAP addition on apparent ileal nutrient digestibility (AID) of 24 day old broiler chickens is shown in Figure 1. The correlation between AID or XAP response and dietary characteristic parameters is shown in Table 1. Even though it does not represent commercially feasible diets, the current geometric mixture design successfully generated considerable variance in ileal protein, starch and fat digestibility (AIDP, AIDS and AIDF, respectively) ($P < 0.01$), which is desirable to analyze relationships between diet characteristics and enzyme responses. The magnitude of XAP response was partially explained by the inherent ileal protein, starch and fat digestibility in the respective control diet, confirming that broiler chickens fed diets with poorer ileal nutrient digestibility more readily responded to XAP addition (Table 1). Thus, understanding the factors that contribute to the inherent digestibility of the diet, including *in vitro* starch digestion rate, may maximize XAP efficacy. A high value of K_s is considered a high affinity of the amylase for available starch at the beginning of the digestion or a relatively faster starch digestion rate. This term was positively correlated with ileal protein ($r = 0.348$) and fat digestibility ($r = 0.480$). Accordingly, the effect of XAP addition on protein and fat digestibility was negatively correlated with K_s, suggesting that XAP addition may improve protein digestibility (Romero and Plumstead, 2013) and fat digestibility in diets with slowly digested starch. In other words, incompletely digested or insufficient protein and fat concentrations in chicken diets was related to slow or depressed starch digestion. Surprisingly, although K_s was strongly correlated with slow digested starch (SDS) concentration ($r = -0.627$), neither K_s nor SDS had significant relationship with ileal starch digestibility ($r = 0.021, -0.086$, respectively). However, the free sugar concentration had a weak but significant relationship with ileal starch digestibility (0.389, $P < 0.01$), which negatively correlated with SDS and rapid digested starch (RDS) concentrations. In chicken diets with essentially complete starch digestibility by the terminal ileum, a slower *in vitro* starch digestion rate might be related to increased potential for XAP to improve starch digestibility. In contrast, a relatively faster starch digestion rate in broiler chicken diets may correlate with a poorer XAP response magnitude due to relatively high digestible protein, and fat concentrations. Weurding et al (2003) indicated that slowly digested starch in the lower part of small intestine improved the feed conversion ratio of broiler chickens. However, in their experiment, the dietary treatment with the slowly digested starch contained a higher and more completely digested total starch concentration. In general, a slowly starch digestion rate may provide a longer dietary transit time for exogenous enzymes to sufficiently get access to substrates (Choct, 1996). It can be concluded that greater efficacy of XAP in broiler chickens



(SBM: soybean meal; RB: rice bran; WB: wheat bran; AIDP, AIDS and AIDF: apparent ileal protein, starch and fat digestibility; XAPP, XAPS and XAPF: the efficacy of XAP for protein, starch and fat digestibility)

Figure 1 - The effect of different feed ingredients and the XAP addition on the ileal nutrients digestibility.

might be obtained in diets containing slowly and incompletely digested starch, which appears to be mediated via correlations with incompletely digested fat and protein concentrations at the terminal ileum.

Table 1 - The correlation between the *in vitro* starch digestion rate and XAP efficacy for ileal nutrients digestibility *(r)*.

	AIDP (%)	AIDS (%)	AIDF (%)	XAPP (%)	XAPS	XAPF (%)	Ks (Min^{-1})	FS (%)	SDS (%)
AIDS (%)	−0.160 *(0.133)*	/	/	/	/	/	/	/	/
AIDF (%)	−0.089 *(0.402)*	0.100 *(0.347)*	/	/	/	/	/	/	/
XAPP	−0.679 *(<0.00*	−0.010 *(0.922)*	−0.118 *(0.270)*	/	/	/	/	/	/
XAPS	−0.118 *(0.270)*	−0.326 *(0.002)*	0.126 *(0.270)*	0.098 *(0.360)*	/	/	/	/	/
XAPF	−0.165 *(0.121)*	0.042 *(0.698)*	−0.639 *(<0.00*	0.198 *(0.062)*	0.285 *(0.00*	/	/	/	/
Ks (Min)	0.348 *(<0.00*	0.021 *(0.844)*	0.480 *(<0.00*	−0.362 *(<0.00*	−0.172	−0.521 *(<0.00*	/	/	/
FS (%)	0.141 *(0.186)*	0.389 *(<0.00*	0.224 *(0.034)*	−0.291 *(0.006)*	−0.231	−0.151 *(0.154)*	0.433 *(<0.00*	/	/
SDS (%)	−0.111 *(0.299)*	−0.086 *(0.419)*	−0.567 *(<0.00*	0.438 *(<0.00*	0.154 *(0.14*	0.408 *(<0.00*	−0.627 *(<0.00*	−0.646 *(<0.00*	/
RDS (%)	−0.270 *(0.010)*	−0.088 *(0.408)*	−0.428 *(<0.00*	0.474 *(<0.00*	−0.196	0.045 *(0.675)*	−0.374 *(<0.00*	−0.538 *(<0.00*	0.760 *(<0.00*
INSP (%)	−0.084 *(0.429)*	−0.158 *(0.136)*	0.357 *(<0.00*	−0.321 *(0.002)*	−0.327	−0.541 *(<0.00*	0.440 *(<0.00*	0.124 *(0.245)*	0.676 *(<0.00*

Note: SDS, slowly digested starch; RDS, rapidly digested starch; INSP, insoluble NSP; SNSP, soluble NSP. Protein%, starch%, PJ%, Fat% and DM% mean the basal nutrients digestibility; XAPP, XAPS, XAPPJ, XAPF and XAPD mean the effect of XAP on protein, starch, proximal jejunum starch, fat and DM digestibility; Values in italics underneath the correlations are P values.

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EFFECTS OF ELECTROLYTES AND VITAMIN C ON GROWTH PERFORMANCES AND ANTIOXIDANT ENZYME ACTIVITY OF BROILERS DURING SUMMER PERIOD

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Summary

Heat stress is one of broilers' common problems found in high temperature and humidity area. Supplementation of electrolytes or vitamin C in drinking water helped to alleviate the adverse effects of heat stress. This study aimed to compare the effects of vitamin C and mixed electrolytes on growth performances and antioxidant enzyme activity in 210, male and female Arbor Acre broilers during grower-finisher period (22-42 d-old). The broilers were allocated into 3 groups with different additives in drinking water: T1 (1,000 ppm vitamin C), T2 (2,000 ppm mixed electrolytes containing only Na^+, K^+ and HCO_3^-) and T3 (control group). The experiment was conducted during summer period between April and May. The average temperature was 34.0±0.7 °C and the relative humidity was 51.0±3.9%. Blood samples were collected from wing vein or jugular vein for analyses of osmolarity, glutathione peroxidase (GPx) enzyme activity, malondialdehyde (MDA) concentrations. The result indicated that there were no significant differences in broiler performances. The control group (T3) had the highest mortality rate (30%). Broilers in T1 (vitamin C) had significantly higher glutathione peroxidase enzyme and lower MDA levels ($p<0.05$) compared to other groups. Osmolarity values of broilers at 28 d-old decreased in all groups and broilers in T2 (mixed electrolytes) had increased serum osmolarity to normal level compared to control ($p<0.05$) at 35 d-old. In conclusion, heat stress increased mortality rate in broilers and exacerbated FCR. Vitamin C supplement helped to increase GPx enzyme and decreased MDA without improving all performances. Supplementation of mixed electrolytes numerically reduced mortality rate and tended to improve performances of heat stressed broilers.

I. INTRODUCTION

Heat stress is one of the most common problems found in high temperature and humidity area as in Thailand. It can interfere with the broilers comfort and suppresses productive efficiency. Chickens respond to heat stress by panting to evaporate metabolic heat increment. Panting is also accompanied with increased respiratory rates, thus causing higher losses of CO_2 that result in increased blood pH and interruption of acid-base balance (Toyomizu et al., 2005). The balance is changed towards either alkalosis or acidosis, and then metabolic pathways are diverted to thermo-regulatory adaption rather than used for growth reinforcement. Chickens increasingly consume water to compensate for water loss and to enhance the capacity of heat dissipation. However, water retention is reduced due to the increase in electrolyte excretion via urine and faeces (Belay and Teeter, 1996). Heat stress diminishes potassium and other minerals in chickens, altering the electrolyte balance in the body.

In addition, heat stress increases lipid peroxidation in broilers (Altan et al., 2000). Under acute heat stress, the production of mitochondrial superoxide radical was increased in chicken skeletal muscle (Mujahid et al., 2005) Chronic heat stress could minimize metabolic

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oxidation capacity because of a self-propagating scavenging system (Azad et al., 2010). Electrolytes play a significant role in maintaining acid-base balance, osmotic pressure and electrical potential of cell membranes; and are also essential for intracellular-extracellular homeostasis. Monovalent ions (Na^+, K^+ and Cl^-) are the vital minerals involved in acid-base balance of the body fluids, because they have a higher permeability and have greater absorption than divalent ions (Ca^{2+} and Mg^{2+}) (Borges et al., 2004). Moreover, Roussan et al., (2008) reported that different substances have been used to reduce the harmful effects of heat stress on the broiler performance, such as ascorbic acid (vitamin C), acetylsalicylic acid (ASA), potassium chloride (KCl), and sodium bicarbonate ($NaHCO_3$). However, the comparison of effect of vitamin C and mixed electrolytes addition in water on antioxidant enzyme activity of heat-exposed broilers is scarcely examined. Therefore, the objectives of this study are to determine effects of these additives on osmolarity, antioxidant enzyme activity and on broiler performance during heat stress period in Thailand.

II. MATERIALS AND METHODS

A total of 210 d-old male and female Arbor Acre broilers were used in this study. The broilers were randomly allocated into 3 groups with different additives in drinking water from 22-42 d-old: treatment 1 (T1) 1,000 ppm vitamin C, treatment 2 (T2) 2,000 ppm electrolytes* containing Na^+, K^+ and HCO_3^- and treatment 3 (T3) control group. Each group was composed of 7 replicates of 10 birds. The experiment was conducted during summer period between April and May. The average temperature and relative humidity were 34.0±0.7 °C and 51.0±3.9% respectively. All groups were raised under the same condition in the opened house with 0.12 m^2/bird. The floor was covered with 10 cm of rice hulls. All chicks were received clean water and fed *ad-libitum* with commercial broiler feed#. This feed was formulated according to the requirements suggested by the National Research Council (1994). They were also vaccinated with the Newcastle plus Infectious Bronchitis vaccine (sprayed at the hatchery at 1 d-old).

The growth performances, i.e., body weight (BW) gain, feed conversion ratio (FCR) and mortality rate were determined in all groups at 28 and 42 d-old. BW gain was determined by the difference between weights at the beginning and end of trial. Feed intake was measured as the difference between feed supplied and remaining feed in each feeder. At 28, 35 and 42 d-old, 2 chicks per replicate were randomly selected and measured body temperature using thermometer into the cloaca. Blood was collected from wing vein or jugular vein using sterile needles and centrifuged to separate serum for determining osmolarity using Osmometer model 3D3 (Advanced Instrument Inc, USA), GPx enzyme activity (Bolcal et al., 2007) and MDA concentration (Ohkawa et al., 1979). For statistical analyses, all means of experimental treatments were analyzed using ANOVA. When a significant ($P < 0.05$) F statistic was noted, treatment means were separated by Duncan's multiple range test using the Sigmastat. Data presented are arithmetic means ±standard errors of means (SEM).

III. RESULTS AND DISCUSSION

There were no significant differences among all groups for growth performances at the starter and the grower-finisher periods (Table 1). The temperature at the grower-finisher period had

* Selectolyte, RCI, Australia

Feed analyses (starter/grower/finisher): Gross energy (kcal/kg) using bomb calorimeter = 3583.6/3752.1/3744.4; Crude protein concentration (%) using Lowry et al. (1951) = 22.7/20.1/17.9; dietary electrolyte balance (mEq/kg) using Mongin (1981) = 264.3/223.7/194.0

a negative impact on growth performances, specifically in the control group (T3) that showed the worst performances. This range temperature (31.0-34.0°C) was higher than thermoneutral zone (i.e.26.0± 2.0°C) of 4-6 wk-broilers and the recommended temperature (i.e. 21.0-23.0°C) of 3-6 wk Arbor Acre broilers. Consequently, the broilers faced on heat stress. This result is consistent with their high body temperatures (above 42.0°C). In addition to high temperature, there were 3 heat waves at the 22, 30 and 38 d-old and many fluctuations of relative humidity, resulting in the increased dead chicks.

Table 1 - Growth performances of broilers at the starter and the grower-finisher periods

	Starter (1-28 d-old)			Grower-Finisher (29-42 d-old)		
	T1	T2	T3	T1	T2	T3
Body weight gain (kg/bird)	0.77±0.007	0.77±0.006	0.78±0.007	1.21±0.08	1.21±0.08	1.15±0.07
Feed Intake (kg/bird)	0.99±0.006	0.97±0.009	0.98±0.010	2.94±0.10	2.70±0.16	3.01±0.08
FCR	1.29±0.02	1.26±0.01	1.25±0.01	2.57±0.33	2.33±0.22	2.70±0.18
Mortality (%)	1.43±1.43	0	0	27.5± 9.2	24.3±5.7	30.0±6.2

Table 2 - Osmolarity, MDA concentration and GPx activities at 28, 35, 42 d-old

	T1	T2	T3
28 d-old			
Osmolarity (mOsm/L)	309.7 ±1.4	309.9 ±1.4	309.3 ±1.3
MDA concentration (nmol/ml)	0.035±0.002	0.044±0.005	0.051±0.009
GPx activity (unit/mg protein)	2,376.2±253.8^{a}	1,189.8±293.8^{b}	1,174.6±346.2^{b}
Body temperature (°C)	42.5 ±0.3	42.7 ±0.2	42.3 ±0.2
35 d-old			
Osmolarity (mOsm/L)	316.0 ±2.0^{ab}	318.9 ±3.7^{a}	310.7 ±2.5^{b}
MDA concentration (nmol/ml)	0.063±0.022	0.050±0.004	0.099±0.041
GPx activity (unit/mg protein)	1,151.6±214.7	1,337.4±237.9	821.2±138.5
Body temperature (°C)	41.8 ±0.2	42.1± 0.2	42.2 ±0.1
42 d-old			
Osmolarity (mOsm/L)	321.4 ±3.2	317.0 ±2.9	319.3± 2.2
MDA concentration (nmol/ml)	0.044±0.003^{b}	0.065±0.005^{a}	0.075±0.006^{a}
GPx activity (unit/mg protein)	4,335.7±720.7	2,986.2±570.9	2,868.6±323.0
Body temperature (°C)	43.0 ±0.4	43.0 ±0.3	43.0 ±0.2

*Means within rows followed by different letters are significantly different at $p < 0.05$

In terms of osmolarity, there was significant difference between treatments 2 and 3 at 35 d-old. The supplementation of mixed electrolytes could maintain the normal level of plasma osmolarity (i.e.approximately 320 mOsm/L). Belay and Teeter (1996) reported that plasma Na^+, K^+ concentrations decreased significantly during heat stress in broilers. The water intake increases in heat-exposed broilers and then the water consumed enters into the blood circulation, inducing an elevated urine production. It is consistent with the report by Zhou et al. (1999) in that plasma osmolality was decreased by the increased water consumption. The GPx activities in treatment 1 supplemented with vitamin C were highest at 28, 35, 42 d-old. There was significantly different between treatment 1 and other groups. Vitamin C probably plays a vital role in suppression of adrenocortical steroidogenesis thus ameliorating the negative effects of stress. The reaction of hydroperoxides is catalysed by GPx with lessened glutathione to form glutathione disulphide (GSSG). Hence, elevated GPx activity may enhance the steady state of antioxidant system of broilers (Satterlee et al., 1989). Moreover, vitamin C may stimulate interferon activity and accelerate differentiation of

lymphoid organs. It is known that heat stress can be attributable to an increase in MDA production in serum and liver. However, MDA concentration in treatment 1 was significantly decreased at 42 d-old compared with that in treatments 2 and 3. The result is in agreement with Sahin et al. (2002) who reported that the addition of either vitamin C or vitamin E alone or the addition of both vitamins could decrease serum MDA concentration ($p < 0.05$). It is due to antioxidants, vitamins C and E inhibit free radical production by blocking lipid peroxidation (Amakye-Anim et al, 2000)

In conclusion, heat stress increased mortality rate in broilers and exacerbated FCR. Vitamin C supplement helped to increase GPx enzyme and decreased MDA without improving mortality rate and other performances. Addition of mixed electrolytes numerically reduced mortality rate and improved performances of heat stressed broilers.

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SEQUENTIAL DILUTION OF STARTER DIET WITH WHOLE GRAINS: EFFECT ON PERFORMANCE OF BROILER CHICKENS

N.K. SHARMA[1], D. CRESWELL[2] and R.A. SWICK[1]

Several investigations have been carried out with feeding programs using whole grains that have generally shown improved FCR, decreased feed intake and lower mortality (Svihus, 2010; Jones and Taylor, 2001). Published data on feeding whole grains by incremental dilution of starter diets are scant. This study summarizes performance results of two such experiments using broilers fed whole wheat (WW), cracked maize (CM) or whole pearl millet (WPM). In experiment one, 360 Cobb 500 unsexed d old chicks were allocated to three treatments employing a completely randomized design (CRD) with six replications of 20 birds each. Treatment 1 (T1) received a pelleted maize-soy based starter (S) diet (ME 12.48 MJ/kg, dlys. 12.0 g/kg, dM+C 8.6 g/kg) up to 15 d and grower (G) diet (ME 12.48 MJ/kg, dlys. 11.0 g/kg, dM+C 8.2 g/kg) thereafter. T2 and T3 received the same S diet up to d 5 but diluted from d 6 with CM in T2 and WPM in T3 at levels increasing 50 g/kg diet every 5 d up to 37 d. In experiment two, 312 newly hatched male Arbor Acres broiler chicks were allocated to four treatments employing a CRD with six replications of 13 birds each. Three pelleted maize-soy basal feeds were prepared: S1 - ME 12.56 MJ/kg, dlys. 13.0 g/kg, dM+C 9.10 g/kg; S2 - ME 12.56 MJ/kg, dlys. 13.5 g/kg, dM+C 9.50 g/kg; G- ME 12.98 MJ/kg, dlys. 11.0 g/kg, dM+C 8.00 g/kg. Whole grains were added at levels increasing 40 g/kg diet every 4 d up to 40 d. T1 diet was changed from S to G on d 16. T2 used WW with S1; T3 used CM with S1 and T4 used CM with S2.

Table 1 - The effect of whole grain feeding on performance of broiler chickens

	Treatment			Feed intake, g	Body weight, g	Corrected FCR	Mortality, n
	Group	Diet	Whole grain				
Exp. 1	1	Starter/grower	-	3442[a]	2031[a]	1.694	3
(0-37 d)	2	Starter	Cracked maize	3272[b]	1926[b]	1.698	3
	3	Starter	Pearl millet	3240[b]	1878[b]	1.725	3
P-value				0.0012	0.0001	0.5356	
CV, %				2.42	2.37	2.914	
	1	Starter/grower	-	4968	3108	1.598[b]	1
Exp. 2	2	Starter 1	Whole wheat	5101	3056	1.669[a]	3
(0-40 d)	3	Starter 1	Cracked maize	4999	2993	1.670[a]	3
	4	Starter 2	Cracked maize	5037	3043	1.655[a]	3
P-value				0.0832	0.2324	0.0070	
CV, %				1.70	2.99	2.07	

[a,b] Means within column with no common superscript differ significantly ($P < 0.01$)

In experiment 1, whole grains decreased feed intake ($P < 0.01$) and final body weight ($P < 0.01$) but treatments had no impact on FCR ($P > 0.05$). In experiment 2, no differences in feed intake ($P > 0.05$) or body weight ($P > 0.05$) were observed between treatments. FCR however was favored in birds fed the T1 control over whole grain diets by 6-7 points ($P < 0.01$). This study clearly showed that whole grains can be successfully fed to broilers by sequentially diluting a starter diet without larger variation in broiler performance.

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HIGH LIPID, LOW PROTEIN DIET INCREASES NET ENERGY IN BROILERS

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Poultry diets are typically formulated to meet metabolisable energy (ME) and other nutritional needs. ME does not account for the heat increment (HI) of birds fed diets of differing chemical composition. Formulating for energy needs based on the net energy (NE) system (AME minus HI) accounts for HI differences between diets. HI comprises heat associated with prehension, digestion and metabolism of ingested feed. Minimising the feed HI may therefore improve conversion efficiency of feed energy to energy used for production. Classen (2013), amongst others, has reported that the relationship between ME and digestible amino acids is variable depending on the source of energy in the diet (lipid, carbohydrate, protein) among other factors. De Groote (1974) determined the relative efficiency of energy utilisation from carbohydrate (100%), protein (78%) and lipid (133%). Thus, birds fed diets containing high lipid and low protein may have a lower HI than birds fed high protein, low lipid diets. A study was conducted to determine if low lipid, high protein and high lipid, low protein diets had different HI, NE, NE:AME (to account for different dietary AME values) and retained energy (RE) in broilers.

Two wheat-based diets were formulated to contain (.kg^{-1}): Diet X (ME, 11.72MJ; CP, 241.8 g; EE, 18.1 g; starch+sugars, 358.9 g); Diet Y (ME, 13.60 MJ; CP, 200.0 g; EE, 63.9 g; starch+sugars, 425.9 g). Essential amino acid requirements were met according to Ross 308 nutrition specification guidelines. Following diet adaptation from 18 to 25 d, calorimetry on eight, two male broiler (Ross 308) replicates of each diet was conducted from 25 to 28 d. Calorimetry was conducted according to the methods of Swick *et al*. (2013).

The HI tended to be lower ($P = 0.099$) for Diet Y than Diet X. The NE ($P < 0.001$) and NE:AME ($P < 0.05$) was higher for Diet Y, however, retained energy was not different between treatments. Gain and FCR were not different between treatments during the three d measurement period ($P > 0.05$).

This study demonstrated that a diet high in lipid and low in protein tended to reduce HI, compared to a low lipid, high protein diet. A NE system may allow more accurate formulation for energy in broiler diets differing in chemical composition as HI of ingested lipid, protein and carbohydrate is accounted for. Further work is warranted to determine if formulation on a NE basis yields improved conversion efficiency of dietary energy provision to broiler productivity.

Table 1 - Net energy and performance comparison of the diets X and Y

Measured parameter	Diet X	Diet Y	SEM	P value
HI (kJ/kg LW$^{0.7}$)	591	534	17	0.0990
NE_{feed} (MJ/kg DM)	9.26	10.98	0.25	<0.0001
NE:AME	0.714	0.746	0.007	0.0118
RE (kJ/kg LW$^{0.7}$)	802	893	29	0.1234

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EVALUATING RANGE USAGE OF COMMERCIAL FREE RANGE BROILERS AND ITS EFFECT ON BIRD PERFORMANCE USING RADIO FREQUENCY IDENTIFICATION (RFID) TECHNOCLOGY

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Summary

Commercial free range broiler birds were used to evaluate range usage by using RFID technology specifically designed for chickens. Two trials using Cobb 500 as hatched broiler chickens (260 trial 1 and 270 trial 2) were conducted. In the first trail body weight gain and its interaction with range usage was assessed. The second trial used an RFID system to evaluate range usage and its effect on bird organ (bursa fabricius, spleen and gizzard) weights and leg health (latency-to-lie, foot pad and hock scores). Range usage had a significant negative effect on body weight gain in both trials ($p<0.05$). Birds which used range had significantly heavier gizzard weights($p<0.05$) but bursa and spleen weights were not affected. Latency-to-lie times for birds using the range were not significantly different to that of birds which did not visit the range area. Further work is required to explore the nutritional and health consequences of range access for free-range broilers, particularly considering the effect on gastrointestinal physiology as well as diet energy, amino acid and mineral balance.

I. INTRODUCTION

Alternative farm animal production systems which require higher animal welfare standards are becoming more popular around the globe. Harper and Makatouni (2002) reported that the two main reasons behind consumer choice of free range products were animal welfare and a perceived health benefit. Consumers identify free range products as more beneficial than conventional products in terms of human health. In Victoria commercial free range broiler production was less than 1% of total production in mid 1990s (Dixon, 2002). According to Australian Chicken Meat Federation (2011) free-range broiler production accounted for 4% of total broiler production in 2006 and today it is around 15-20 %. Weeks et al., (1994) demonstrated that free range broilers had significantly lighter body weight (4.08 ± 0.08kg) than conventionally reared broilers (4.49 ± 0.08kg) at ten weeks of age. This performance gap has been observed in a long term commercial comparison study as a 2-3% increase in mortality, 0.10-0.15 increase in feed conversion ratio and a retardation in growth rate in that birds took an extra 2.5 days to reach a 2.45 kg target body weight (Durali et al., 2012). This 'performance gap' is not well understood but is thought to be as a result of range access, variable pasture consumption, nutritional inadequacy and poorer digestive health including more dysbacteriosis challenge. These performance challenges contribute to poor economic sustainability in the industry. It is the purpose of this paper to describe effect of range usage on performance of free-range broilers under commercial production constraints. A specifically designed Radio Frequency Identification (RFID) system was used to assess birds range usage and evaluate the correlation between range usage and birds' performance.

II. MATERIALS AND METHODS

A commercial free range broiler farm and diet were used for this study. The farm used in this study is located Central Coast of NSW and accredited by the Free Range Egg and Poultry Association (FREPA). The farm followed FREPA's Free Range Meat Chicken Standards,

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which requiring 8 hours range access every day unless weather is extreme. The free range shed used had eight regularly distributed doors (pop holes) 3.4 meters wide for access to a range area at the side of the shed. The end pop hole section of the shed was separated from rest a fence inside and outside to restrict a group of broiler chickens to that area. The RFID system was placed across this pop hole. The bird number in the RFID section was calculated based on drinker and feeder space to have the same bird density inside the shed and in the range area as the rest of the shed. The RFID system consists of a computer with specific software designed for analysing bird movement, two readers and six antennae (three for inside and three for outside).

In the first study 260, and in the second study 270, randomly selected 20 day-old Cobb 500 as hatched broilers within the separated section of the shed were fitted with RFID leg rings, each with a specific ID number. In both trials these birds were weighed individually on day 21 before they were allowed access to range area. In the first trial (birds hatched 21 January 2013)birds were given range access at 21 days of age. Due to rainfall during the grow out of these birds, only 11 days of range access were possible. In the second trial (hatched 18 March 2013) weather conditions allowed a total 17 days access to the range area. All leaving and entering movements and time spent inside and outside of the shed were recorded by the RFID system in both trials. At the end of the both trials all birds were weighed individually. In the second trial during the final individual weighing, 30 birds that used range area most and 30 birds that used the range area least were selected for closer study.foot pad lesion and hock burn scoring. These 60 birds were subjected to a modified Latency-to-Lie test (LTL; Berg and Sanotra, 2003) to evaluate their ability to stand for up to 5 minutes.. After the LTL test these 60 birds were scored for foot pad and hock lesions and were then euthanized. These birds were then evaluated for intestinal health score, weights of gizzard, spleen and bursa Fabricius and ceacal contents were collected and frozen. Body weight gain, foot pad and hock score and organ weights were analysed using Standard Least Square ANOVA. LTL tests were compared using Kaplan-Meier Survival Analysis. STATISTICA ver 6 was used forthe statistical analyses. Significance was set at $P<0.05$ in all cases.

III. RESULTS AND DISCUSSION

In first trial, 257 of the selected birds survived to day 40. Of these 13 birds never ventured into the outside range area over the 11 days of range availability. Figure 1 depicts the distribution of total hours of range usage by individual birds in trial 1. This distribution differed significantly ($P<0.01$) from normal and hence this data was not suitable for use in a linear regression analysis. Hence, total hours of range use was split into quartiles. Weight gain was compared across the birds represented in each quartile (Table 1).

Table 1 - Trial 1 - Weight gain (gm) by quartile of time spent in range area.

Quartile of Total Hours Spent Outside	Weight Gain (gm) (Mean)	Bird No.	Weight Gain (Std. Dev.)
>8.68	1465^{B}	101	280.56
6.00 to 8.68	1549^{AB}	27	248.70
1.08 to 6.00	1573^{AB}	65	237.30
<1.08	1613^{A}	64	276.34
All Groups	1538	257	271.52

A,B – means without common superscripts differ significantly ($P<0.05$)

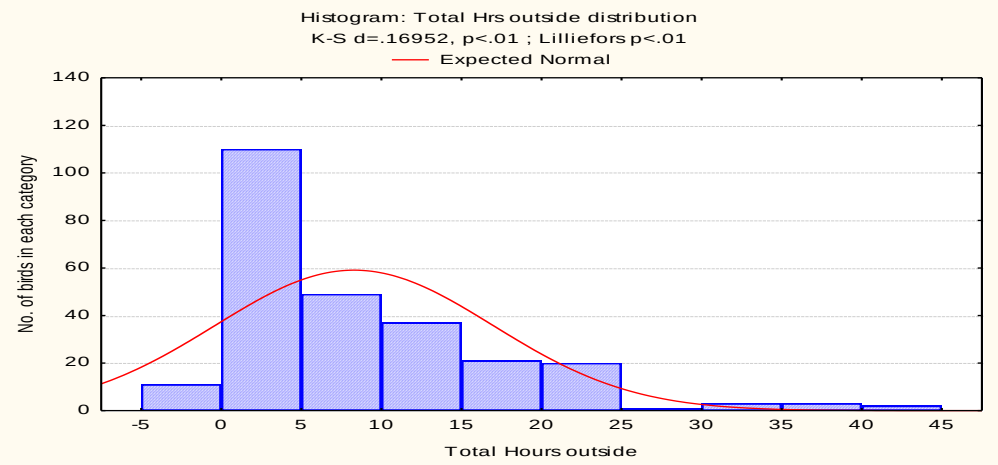

Figure 1 - Distribution of total hours spent outside by bird number

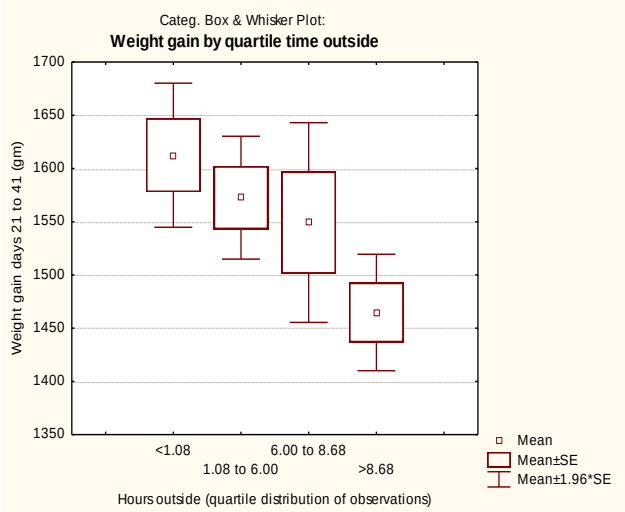

Figure 2 - Weight gain by quartile time spent outside.

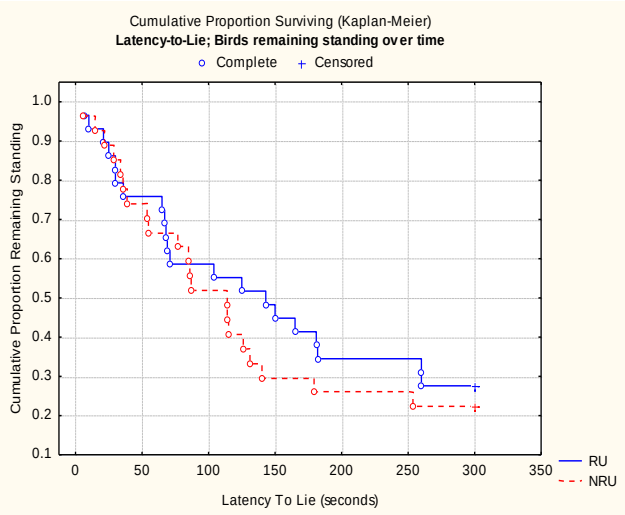

Figure 3 - Latency-To-Lie test at day 42 (p = 0.57698)

Table 2 - Range usage, sex and gizzard weight % of body weight.

Range Use-Sex	Gizzard weight as % of body weight	95% Confidence limits of mean % Gizzard wt	No. of birds
Range Users	2.08^{A}	1.94 - 2.23	29
Non-Range Users	1.85^{B}	1.68 - 2.02	27
Male	1.83^{B}	1.64 - 2.02	18
Female	2.13^{A}	1.98 - 2.23	38

A,B – means without common superscripts differ significantly (P<0.05)

Birds in the quartile which spent the longest total time outside (8.68 hours or more) had significantly lower weight gains over days 21 to 40 than birds from the lowest range usage quartile (less than 1.08 hours) (Table 1). The intermediate quartiles displayed a decreasing pattern of weight gain with longer time spent outside (Figure 2).

In the second trial there was no significant difference in ability to stand for up to 5 minutes in the LTL test between birds which had spent the longest and least times in the range area (Figure 3). Range usage had no significant effect on intestinal health scores, foot pad lesions or and hock score.

Range usage had no effect on bursa of Fabricius or spleen weights as percentage of body weight however gizzard weight as percentage of body weight was significantly affected by outdoor range usage (Table 2). Birds which used the range most had significantly heavier gizzards than those which used it least. This could be due to consumption of forage, stones and other objects available in the range area. Females also appeared to have higher percentage gizzard weights than males (Table 2).

Range usage of commercial free range broilers has previously been shown to have a significant effect on body weight gain (Weeks et al., 1994, Durali et al., 2012). Commercial free range broiler diets are not customarily formulated to accommodate for the effects of range access and unknown vegetation and insect consumption on digestible nutrient intake. It has been reported that range access improved ileal dry matter and energy digestibility (Durali et al., 2013). In the second trial outdoor usage was associated with increased gizzard weight which could improve physical feed digestion. Further work is required to explore the nutritional and health consequences of range access for free-range broilers, particularly considering the effect on gastrointestinal physiology as well as diet energy, amino acid and mineral balances.

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BETAINE SUPPLEMENTATION AFFECTS ENERGY PARTITIONING IN BROILERS

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The gastrointestinal tract consumes up to 25% of the total body oxygen consumption, thereby utilizing a large part of total ingested energy. Furthermore, around 35% of this energy is associated with the maintenance of ionic homeostasis (Jessop, 2000). Betaine is believed to reduce energy costs of the ion pump when acting as an organic osmolyte. However, the consequences of such an effect on energy utilization in broiler chickens are not well known. This study examined the effect of betaine supplementation on energy partitioning in broilers using indirect calorimetry. Male broilers (Ross 308 strain, N=36) were randomly assigned into three treatment groups in closed–circuit respiratory chambers (Swick et al., 2012). Each treatment consisted of six replicates of two birds per chamber. A basal diet based on wheat, soybean meal, meat bone meal and canola meal met the Ross 308 nutrient specifications. Betaine was added to the basal diet at 0, 0.75 and 1.50 g/kg of feed. The heat production (HP), apparent metabolisable energy (AME) and net energy (NE) of feed were estimated from 25 d to 27 d. The Brouwer equation was used to calculate the HP via the oxygen consumption and carbon dioxide production in the chambers and then estimate the NE (Noblet et al., 2010).

The inclusion of betaine had a significant impact on HP and heat increment (HI) but not the other energy measures. Particularly, betaine inclusion at 1.50 g/kg of feed increased HP and HI by 5.3 and 12.5% ($P < 0.05$), respectively (Table 1). An increase in HP did not lower the NE value or the NE:ME ratio of feed. Therefore, this increased HP value likely reflects the higher growth performance and/or a change in the ratio of lipid:protein deposition of broilers in this treatment. To clearly support the energetic efficiency of betaine supplementation, further studies should elucidate the role of betaine as an organic osmolyte at the digestibility and absorption stages in broilers.

Table 1 – The effect of betaine supplementation on energy utilisation in broiler chickens

	Levels of betaine inclusion in the diets				
Measure	0 g/kg	0.75g/kg	1.5 g/kg	SEM	P values
Mean BW, g/b(d 25-27)	1646	1675	1689	18	0.247
FCR (d 25-27)	1.540	1.557	1.553	0.037	0.942
ME intake, kJ/kgBW$^{0.70}$	1395	1426	1476	28	0.155
HP, kJ/kg BW$^{0.70}$	782.8^{b}	799.9^{ab}	823.9^{a}	8.9	0.017
HI, kJ/kg BW$^{0.70}$	332.8^{b}	349.9^{ab}	373.9^{a}	8.9	0.017
RQ	1.003	1.014	1.006	0.006	0.470
ME, kJ/g feed	13.41	13.68	13.36	0.289	0.714
NE, kJ/g feed	10.22	10.32	9.98	0.22	0.548
NE:ME, %	76.15	75.42	74.66	0.005	0.123

[a,b] Means having common superscripts in a row do not differ significantly ($P < 0.05$)
BW: Body weight; FCR: Feed conversion ratio; ME: Apparent metabolisable energy; HP: Heat production; HI: Heat increment; RQ: Respiratory quotient; SEM: Standard error of mean.

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THE EFFECT OF THREONINE AND ARGININE SUPPLEMENTATION IN LOW CRUDE PROTEIN DIETS ON PERFORMANCE AND INTESTINAL MORPHOLOGY IN BROILER CHICKENS

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Feeding high crude protein diets are resulted in excess of amino acid and elevated nitrogen excretion in broiler chickens. Furthermore, supplementing the crystalline amino acid into broilers diet might increase the nitrogen retention efficiency through making balance between maintenance and tissue accretion needs. Threonine is an essential amino acid for poultry and they are also unable to synthesize arginine (Kidd, 2002; Khajali and Wideman, 2010). Additionally, it is possible that antioxidative effect of nitric oxide which produced by dietary supplementation of arginine increases the growth of epithelial cells in the intestine and improves nutrient assimilation (Foye et al., 2007). Therefore, the objective of this study was to evaluate the effect of supplementing different levels of threonine and arginine in low crude protein diets on broilers performance and their intestinal morphology.

In this study, five hundred and twenty day-old broiler chicks (Ross 308) were used base on a factorial arrangement (2 × 2 × 2) in completely randomized design with 8 treatments and 5 replicates. The dietary treatments included: two levels of protein (100 and 88% of standard requirements), two levels of threonine (100 and 110% of standard requirements) and 2 arginine supplementation (0 and 0.1% of diet). The amino acid profiles of corn and soybean meal were analyzed by NIRS. Broilers received the dietary treatments in three phases: starter from day 0 to14 (ME: 2900 kcal/kg, CP: 22.2%), grower from day 14 to 28 (ME: 2980 kcal/kg, CP: 20.7%) and finisher from day 28 to 42 (ME: 3000 kcal/kg, CP: 19.1%). Performance parameters include of body weight, daily feed intake, daily weight gain and feed conversion ratio (FCR) were measured at different periods and intestinal morphology (villus height, crypt depth and villus height to crypt depth ratio) were evaluated on day 30. The data were analyzed by SAS (2008) and differences were considered significant at $P< 0.05$.

The result showed that decreasing dietary crude protein (CP) and maintaining the recommended levels of threonine and arginine had no significant effect on performance and intestinal morphology. Similar response was obtained by Saki et al. (2007) who showed that 13% decreasing dietary CP for starter and 10% for grower periods were not significantly different from control group. Increasing threonine and arginine at recommended CP levels significantly improved FCR ($P< 0.05$). Increasing threonine in both CP levels and also arginine in recommended CP level increased villus height to crypt depth ratio. Threonine may participate in synthesis of protein and materials from its metabolism such as glycine, acetyl CoA and pyruvate that are important for growth (Kidd and Kerr, 1997). The 0.1% addition of arginine suplementation improved FCR ($P < 0.05$). In conclusion, increasing dietary threonine and arginine levels could improve performance of broiler chicks and intestinal morphology was affected by dietary threonine.

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EFFECT OF LOW NUTRIENT-LOW DENSITY DIET ON GUT ENVIRONMENT AND PRODUCTION OF BROILER CHICKEN

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Summary

The experiment was conducted to make the diet of low density and of low nutrition incorporating softwood sawdust and wood shaving particles and to know their effect on gut organ development in broiler. The experimental diets contained 216g/kg CP (isonitrogenous) and 12.5 MJ energy (isocaloric), which was adjusted by replacement of rice polish and inclusion of protein concentrate to the formulation of control diet. Liver weight was significantly highest in basal diet 19.05gm and 24.30gm during 4th and 5th respectively. Gizzard weight was significantly highest in basal diet 26.26gm and 36gm during 4th and 5th respectively. Significantly highest body weight (1190.56g) and better FCR (1.86) was obtained in control diet. Intestinal examination showed that the highest PH-7.6 was found in the caecum in wood saving diet and PH-7.3 in the colon in sawdust diet. However, caecum contained highest (1054) colonies in control diet followed by 356 and 219 in sawdust and wood saving diet, respectively. Mortality was found insignificantly different among the treatments, except no mortality in basal (control) diet. It may be concluded that although the better result was obtained from control diet, untreated sawdust and wood shavings are abundant in the country and can be used as stimulator in the diet of poultry ration to make it cheaper.

I. INTRODUCTION

Broilers housed in a litter floor system consume wood shavings, possibly to compensate for the low levels of coarse fibrous materials in their diet. About 40% of wood is cellulose. Both of the sawdust and wood savings are of wood by products and are different from it's structure. Sawdust in the form of wood polish obtained from sawmills and wood shavings from the furniture and timber chipping consisting mostly of cellulose and lignin (coarse fibre). This type of materials in poultry ration can make it voluminous and low density, which is preferred by the chicken and increase in feed intake in tropics. On the other hand high density diets have adverse effect on the performance of broiler under tropical environment (Meremikwu et al., 2013) and can result in obese birds and a general loss of vigour in tropics because of the harsh climate conditions of this region (Cheema et al., 2003). Due to poorer quality of feedstuffs in ration formulation makes it low density diet and may be the most economical option (Farrell, 2005). Diets incorporated with wood savings can improve gizzard function. Wood shavings improved starch digestion and the performance of broiler chickens (Amerah A.M. et al., 2007) by stimulation of gizzard. Poultry meat is an excellent source of protein and other nutrients. The most possible ways of improving the profits are increasing the output or reduce the inputs. Feed is the single; largest input in poultry farming. The ideal approach will be to derive maximum benefit out of this single input. While predicting future needs is risky, it is very likely that the human population will increase substantially in the next twenty years. Poultry and livestock will be competing with humans. Producers may be forced to use poorer quality feed ingredients for animals than are currently in use. Research on the practical use of ingredients such as cellulose, uric acid, and chitin should be undertaken (Gary E. Duke, 1996). Hunter et al. (1981) stated that the main value of sawdust appears to be as a source of dietary fibre. Wood and wood by products are abundant in Bangladesh. Thus, this study was considered to investigate the use of sawdust and wood shavings as dietary coarse fibre in broiler diets in relation to production performance and cost effectiveness.

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II. OBJECTIVES

i)To find the effects of low density diet on development of gut organ of broiler chicken.
ii)To measure the production performance(body weight gain, FCR and mortality of broiler).
iii)To know the change of PH and micro flora in gut.

III. MATERIALS AND METHODS

The experiment was conducted under a contact grower farm. Two hundred and seventy day old chicks of Hubbard Strain were purchased from local market and were divided randomly into nine pens; 30 chicks/ pen. A total of three treatments and three replicate / treatment was considered for chick distribution. Each experimental unit was separated by fencing. The floor of the house was washed thoroughly with tape water then by using washed and dried. After drying the house was kept empty for five (5) days for killing the microorganisms after spraying with disinfectants. Experimental feed formulated with available ingredients e.g. maize , rice polish , soybean meal, protein concentrate, soybean oil, DCP , common salt, vitamin-mineral premix , methionine, lysine. Sawdust and wood shavings were collected from sawmills and carpenter's house and incorporated in feed with 6% replacement of rice polish and 01% inclusion of protein concentrate to the formulation of control ration to meet calculated chemical composition of CP-216.88g (isonitrogenous) and ME- 12.62MJ energy per Kg(isocaloric) of the experimental diets. The experimental ration was prepared to meet the nutrient requirement of broiler according to National Research Council, 1994. Nutrient Requirements of Poultry, 9th Ed. National Academy Press, Washington, DC. Individual body weight of broiler was measured weekly by using top loaded scale balance. During 4th and 5th week nine chicken per treatment (total 27 birds) were sacrificed and slaughtered for measurement of organ weight. Feed intake was determined by subtracting the residue from supply and FCR was calculated by dividing the total feed intake and live weight of an individual bird. Staining and by direct microscopic study, bacterial colony was observed and fluid from caecum and colon was collected by siring and PH was determined by using PH meter. All the recorded and calculated data were analyzed for ANOVA (Steel and Torie, 1980) using a completely randomized design (CRD) with the help of a computer package, SPSS. Significant difference of all recorded and calculated parameters among the treatments were detected to compare mean by using Least Significant Differences (LSD).

IV. RESULTS

The 4th week liver weight was found highest (19.05gm) in T1 group followed by 15.80 gm and 15.05 gm in T2 and T3 respectively. During 5th week liver weight was found highest (24.30gm) in T1 group followed by 20.40 gm and 20.20 gm in T3 and T2 respectively. The 4th week gizzard weight was found highest (26.60gm) in T1 group followed by 23.65 gm and 23.55 gm in T3 and T2 respectively. During 5th week liver weight was found highest (36.00gm) in T1 group followed by 31.85 gm and 31.70 gm in T2 and T3 respectively. Setia A. and Mikkelsen L.L. (2008) found that increased weight of gizzard was obtained at 6% and 12% of hardwood litter diet.

The initial body weight was found highest (39.82gm) in T3 group followed by 39.79 gm and 39.5 gm in T2 and T1 respectively. The 1st week body weight was found highest (91.28gm) in T1 group followed by 77.88 gm and 74.03 gm in T2 and T3. The 2nd week body weight was found highest (252.11gm) in T1 group followed by 210.25 gm and 206.15 gm in T2 and T3.respectively. The 3rd week body weight was found highest (517.49gm) in T1 group followed by 475.92 gm and 469.02 gm in T2 and T3. The 4th week body weight was found highest (956.01gm) in T1 group followed by 782.49 gm and 778.19 gm in T2 and T3. The 5th week body weight was found highest (1190.56gm) in T1 group followed by 1031.28 gm and 1007.44 gm in T2 and T3.

Table 1 - Weight of gut organs (g) fed with different density of diet of broiler

Organ weight / age	Density diet (T1)	Low density diet with sawdust (T2)	Low density diet with wood shaving (T3)	Statistical Significance
Liver weight :				
4th week	19.05±.25a	15.80±.30b	15.05±.45b	*
5th week	24.30±.28a	20.20±.28b	20.40±.14b	*
Gizzard weight:				
4th week	26.60±0.20a	23.55±.150b	23.65±.450b	*
5th week	36.00±.424a	30.85±.495b	31.70±.282b	*
Visceral weight with organs	862.05 ±14.75a	719.35 ±5.15b	767.85 ±10.15c	*

*Means that do not share common superscripts in the same row differ significantly ($p < 0.05$)

Table 2 - Body weight gain fed with different density of diet

Age	Body weight			
	Density diet (T1)	low density diet with sawdust (T2)	low density diet with wood shaving (T3)	Statistical significance
Day 0	39.52±0.05	39.79±.03	39.82±.04	*
1st wk	91.28±.80a	77.88±.21b	74.03±.26b	*
2nd wk	252.11±2.96a	210.25±.56b	206.15±.71b	*
3rd wk	517.49±2.74a	475.92±.63b	469.02±.76b	*
4th wk	956.01±11.08a	782.49±1.26b	778.19±1.23b	*
5th wk	1190.56±22.20a	1031.28±10.36b	1007.44±15.62b	*

*Means that do not share common superscripts in the same row differ significantly ($p < 0.05$)

Table 3 - Feed intake of experimental birds at different ages by different treatments

Age	Feed intake (g)			
	Density diet (T1)	Low density diet with sawdust (T2)	Low density diet with wood saving (T3)	Statistical significance
1st wk	79.10±.26a	67.00±.20b	66.96±.26b	*
2nd wk	292.07±1.19a	251.03±.24b	246.03±.53b	*
3rd wk	767.03±2.58a	722.10±.46b	712.97±.27b	*
4th wk	1485.10±.50a	1356.03±.29b	1342.00±.47b	*
5th wk	2249.20±13.07a	2035.13±12.32b	1985.17±3.33c	*

*Means that do not share common superscripts in the same row differ significantly ($p < 0.05$)

Table 4 - FCR of experimental birds at different ages by different treatments

Age	Feed intake (g)			
	Density diet (T1)	Low density diet with sawdust (T2)	Low density diet with wood shaving (T3)	Statistical significance
1st wk	0.86±.00a	0.86±.00a	0.91±.01c	*
2nd wk	1.15±.00a	1.19±.00b	1.19±.00b	*
3rd wk	1.47±.00a	1.52±.00b	1.52±.00b	*
4th wk	1.53±.00a	1.73±.00b	1.72±.00b	*
5th wk	1.86±.01a	1.99±.01b	1.97±.01b	*

*Means that do not share common superscripts in the same row differ significantly ($p<0.05$)

The 1st week feed intake was found highest (79.10gm) in T1 group followed by 67 gm and 66.96 gm in T2 and T3 respectively. The 2nd week feed intake was found highest (292.07gm) in T1 group followed by 251.03 gm and 246.03 gm in T2 and T3 respectively. The 3rd week feed intake was found highest (767.03gm) in T1 group followed by 722.10 gm and 712.97 gm in T2 and T3 respectively. The 4th week feed intake was found highest (1485.10gm) in T1 group followed by 1356.03 gm and 1342.0 gm in T2 and T3 respectively. The 5th week feed intake was found highest (2249.20gm) in T1 group followed by 2035.13 gm and 1985.17 gm in T2 and T3 respectively.

The FCR was found best 0.86, 1.47, 1.47 1.53 and 1.86 in T1 at 1st, 2nd, 3rd, 4th and 5th week of age, respectively ($p < 0.05$). Further, intestinal content was examined and caecal digesta was found sticky and black in color with highest PH-7.6 in the caecum in wood saving diet followed by 6.6 both in control and wood saving diet. The highest PH-7.3 was in the colon in sawdust diet followed by 6.7 and 6.3 in wood saving and control diet, respectively. Staining and by direct microscopic study, gram positive bacteria (rod and round shape) was observed in all of the diet groups of study. However, caecum contained highest (1054) colonies in control diet followed by 356 and 219 in sawdust and wood saving diet, respectively. There were no significant differences in mortality of experimental birds during the study period (1st week to 5th week). It was varied from 0-0.67%.

V. DISCUSSION

The present study showed inferior growth of broiler in saw dust and wood saving diets than that of the control diet, which contradicts with the result of Oke, D.B. *et al.*, (2007), who investigated that broiler birds fed on diets with sawdust had the highest daily weight gain than the control diet. Also, Hetl and H. et al., (2003), Davis F. et al., (1947), Saito M. et al.,(1959) in chicks fed with diet of crude fibre. There may age differences in the variation of result in growth. Oke, D.B. *et al.*, (2007) also have investigated that broiler birds on diets of sawdust had the highest daily weight gain than the control diet. There was less feed intake in low density/fibre diets (saw dust and wood saving diets), which also contradicts with Emmans (1994), who suggested that increase in fibre content in broiler diet increase in feed intake. Weight of gizzard was not increased in saw dust and wood saving diets, which is not in agreement with the result of Amerah, A.M. et al., (2007), who found improvement in development of the gizzard fed with sawdust diet. Insoluble fibre itself has shown beneficial effects on nutrient digestion and gizzard activities (Hetl and et al., 2003). Trowell H. (1972) reported that dietary fibre may have protective effects against accumulation and lipid metabolism in the certain diseases in growing chicks. Highest bacterial colonies in the present study contradicts with Wyatt et.al.(1988), who reported adaptation of intestinal microflora to polysaccharides.

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GO OUTSIDE AND PLAY? BEHAVIOURAL TIME BUDGET OF FREE-RANGE LAYING HENS IN A NATURAL SHRUB STRUCTURE

H. LARSEN[1] and J-L. RAULT[1]

Summary

The main feature of free-range production systems is the provision of an outdoor area. However, hens natural behaviour outdoors, remains poorly understood. Some free-range farms see low numbers of birds outside, and/or uneven distribution across the range. Environmental enrichment such as the provision of trees, bushes or other types of cover could help solve these problems. However there is little scientific evidence about what enrichment strategy will work and most importantly why. We investigated the behaviours performed by free-range laying hens in a commercial setting with a range area consisting of naturally occurring Kangaroo Apple trees. We found that the hens performed a variety of behaviours (predominantly foraging, preening and perching) in these shrub-like structures, and that the primary use of these structures changed throughout the day. The search for structures that allow the hens to perform similar behaviours could ultimately optimise range use.

I. INTRODUCTION

Although it is assumed that free-range production systems provide greater opportunities for laying hens to perform more "natural behaviours", there is little scientific evidence about what those behaviours are and when and where the hens are likely to perform them.

Use of the outdoor range by commercial laying hens in free-range systems is often limited to a small proportion of the flock at any one time (Hegelund et al., 2006). It is also apparent that the hens' distribution over the range is not uniform, with the hens usually staying close to the shed (Hegelund et al., 2006). Enrichment of the outdoor range as a means to encourage more hens outside and a more uniform distribution across the range has become an increasingly popular and necessary topic of interest. Ranges that contain natural structures such as trees and shrubs can increase the number of chickens in the range, and with the right placement, could improve the distribution of the flock (Dawkins et al., 2003). However, in many commercial settings there is not a sufficient established natural biota that will encourage range use. An alternative may be in the form of artificial structures that mimic the important principles of natural structures to the hens and therefore increase range use and distribution. Items such as hay bales, shelterbelts, shade cloth and sand boxes have been investigated and showed marginal to significant improvements in either range use or distribution (Hegelund et al., 2005; Nagle and Glatz, 2012; Zeltner and Hirt, 2003; Rault et al., 2013). However, the way hens perceive and utilise these structures is still poorly understood. Elucidating the behaviours performed by hens utilising the outdoor range will help designing reliable artificial enrichment in commercial settings that fulfil the hens' needs.

The Kangaroo Apple (*Solanum laciniatum*) is a native shrub that occurs in temperate regions of South Eastern Australia and New Zealand. Kangaroo Apple is a soft wooded, tolerant, fast growing (>2m in approx. 6 months) and short lived (5-6 years) plant. On a commercial free-range farm in Victoria, Kangaroo Apple shrubs have been allowed to self cultivate and grow randomly throughout the ranges to form large (4 m high x 5 m wide) complex shrubs, that grow in tight groups forming larger shrubberies of up to 200m^2. After onsite visits and conversations with the farm managers, it became apparent that the laying hens utilise the areas in and around the shrubs more than any other location on the range. An

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observational pilot study on this farm was designed with the overall goal to use the information gained from observing the hens' behaviour to apply to further research hypotheses on outdoor range design. We had three objectives: 1) Establish the baseline behavioural time budget in an attractive naturally occurring structure, 2) Derive principles from the behaviours observed during this study to aid the design of artificial enrichment structures, and 3) Generate hypotheses on the behavioural implications of outdoor range use based on the principles identified in this study for further research.

II. METHODS

We observed two flocks of ISA Brown hens on one commercial free-range farm in Victoria with naturally occurring Kangaroo Apple shrubs in the range. Flock one contained 120 pullets 25 weeks of age that had arrived approximately one month before the start of the study. Flock two contained 200 hens at 45 weeks of age, arriving approximately four months before the study, and 180 pullets at 25 weeks of age that had arrived one month before the study. The hens were not beak trimmed and had 24 h access to the range from mobile sheds that contained nesting boxes, feeding and watering areas and perches. Each flock were protected from ground predators by Maremma sheep dogs.

Observations were carried out using a Canon1000d SLR camera equipped with an intervalometer. The camera, tripod and protective case was placed in the Kangaroo Apple shrubs in an area that allowed for maximum field of view without obstructions from branches or leaves. Each field of view contained an approximate area of $15m^2$. Scan samples were taken for three two-hour periods daily: 0730-0930 h ('morning'), 1130-1330 h ('midday') and 1530-1730 h ('evening'). During these periods, the camera was programmed to take continuous photos at two minute intervals for one second (average of three shots). A total of six days worth of photographs were obtained for each flock, equivalent to 1080 scans per flock.

Photographs were analysed by one observer to identify the number and behaviour of the hens present in the Kangaroo Apple area in accordance to a behavioural ethogram designed for this study. The main behaviours recorded were foraging, preening, perching, dust bathing, walking, standing upright (head erect and alert, eyes open), lying (body and head on ground or head tucked under wing, not moving) and standing (in non alert position, neck not outstretched, eyes may be open or closed). Behaviours were recorded only when they could be positively identified; hens that were obscured by conspecifics, branches, partially in the shot or where the head was not visible were counted but their behaviour was listed as 'unidentifiable'. Behavioural time budgets were constructed for each flock. Daily weather observations of wind speed, min and max temperature and rainfall were collected from the nearest Australian Bureau of Meteorology weather station. Results are presented as means ± standard error and results are considered significant at $P < 0.05$.

III. RESULTS

A combined total of 9517 behaviours were recorded from the two flocks over the study period. Flock one and two had much higher numbers of hens present in the Kangaroo Apple area in the midday session (58.4 ± 0.69 and 58.1 ± 0.75 respectively) compared to morning (18.9 ± 0.55 and 22.0 ± 0.63 respectively; both $P < 0.05$) and evening (22.7 ± 0.58 and 19.9 ± 0.60 respectively; both $P < 0.05$). Flock one also had higher number of hens in the evening compared to morning sessions ($P < 0.05$).

Foraging patterns were similar for both flocks, where foraging behaviours significantly increased in the evening session compared to morning and midday sessions ($P < 0.05$; Figure 1). Preening behaviours showed a decline throughout the day for both flocks (P

< 0.05). Differences between the two flocks were observed in their perching and lying behaviours. Perching behaviours for flock one was highest at midday than in the morning, and finally, in the evening (P < 0.05). Flock two did not differ significantly in perching behaviour by time period, and had lower proportions of this behaviour overall. However, flock two did show a significant increase in lying behaviour for the midday session over both morning and evening sessions (P < 0.05).

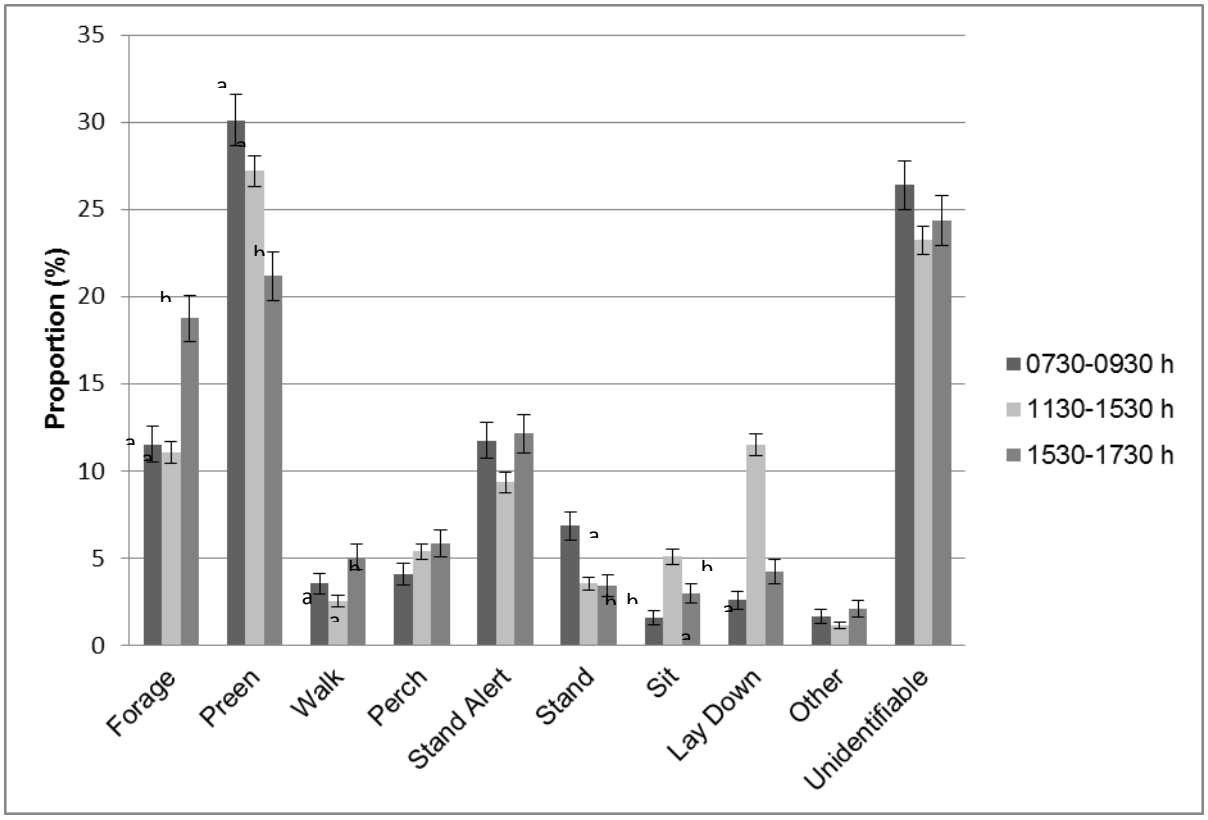

Figure 1 - Proportion of hens' behaviours (%) in flock two for each sampling time period. Behaviours with different superscript (a-c) differ (P < 0.05).

The overall proportion of unidentifiable behaviours was greater in flock one than in flock two, likely due to the placement of the camera in the field. The visible area in flock one was more complex in the front than that of flock two, therefore more hens were obscured in photos by branches and leaves. This may also explain other differences in proportions of behaviours observed, in particular preening. Hens that were categorised as unidentifiable were often stationary, in groups and had often their head tucked.

Observations were conducted during early spring, temperatures were average for the season (min av. 10.5 ± 0.4°C, max av. 17.5 ± 0.7°C) and rainfall low during the study period (1 day at 12mm, 1 day at 8mm 4 days at 1.6-2.2mm and the rest dry days). Regression analyses revealed that weather conditions had no significant influence on the number of hens using the structure, but we deliberately chose to avoid particularly poor weather days.

IV. DISCUSSION

More laying hens utilised the Kangaroo Apple areas at midday. This was somewhat contradictory to previous studies of range use, which have indicated that there is a peak in range use in mornings and evenings (Hegelund et al., 2006; Rault et al., 2013). As numbers were recorded only for the proportion of Kangaroo Apple area, we cannot judge whether the

natural structures alter the diurnal pattern of range use. The hens may have been more attracted to other areas of the range during the morning and evening periods.

Analysis of the behaviours performed by each flock in each of the time periods indicated that the principal uses of the natural structure changed throughout the day. This is particularly evident in flock one, where the morning was dominated by preening, midday by perching and evening by foraging behaviours. Flock two also demonstrated changes, where preening decreased from morning to evening, foraging increased in the evening, lying behaviour increased midday as well as a decrease in alert vigilance behaviour. This pattern of change suggested that the laying hens may utilise the Kangaroo Apple area more for grooming and comfort behaviours in the morning, resting and shelter in the warm midday periods, and more actively as a secondary foraging source in the evenings.

The overall complexity of the Kangaroo Apple is a favourable feature to the hens and our study showed that a large number of behaviours can be performed in this structure. Dust bathing is a behaviour that was conspicuously not observed in this study, possibly because the ground was moist at the time of the study, and may not be ideal for dust bathing. On site visits prior to the study, dust bathing was observed, in addition to the presence of many dust bathing divots in the ground. Our observations also suggest that social facilitation is important in laying hen behaviour: in cases where there were multiple 'groups' of hens that were dispersed throughout the observation area, most of the individuals of each group would be performing the same behaviour, e.g. preening simultaneously, foraging in the same spot, or perching together etc.

Considerable variation exists between the two flocks, in particular age, group size, and range configuration. However, despite this variation, some important similarities between the two flocks' behaviours indicated that the use of natural structures may follow predictable patterns. The particular behaviour performed in this natural structure provides valuable information for further research in the field of artificial enrichment of the range.

V. CONCLUSION

Kangaroo Apple trees are a complex natural structure that facilitate a large number of behaviours for free-range laying hens. The structure was utilised mostly in the middle of the day as an area for shelter and rest when the hens were most inactive, however primary use of the structure changed in the mornings and evenings. This indicates that the most effective artificial enrichment items could be complex enough to allow for changes in key use, as well as large enough to accommodate and shelter large number of hens throughout the day.

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THE ATTRACTIVENESS OF LOOSE FEATHERS TO FREE-RANGE LAYING HENS EXHIBITING SEVERE FEATHER-PECKING

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Many contributing factors are thought to be involved in the development of severe feather-pecking behaviours in laying hens (Rodenburg et al., 2008). In particular, feather-eating was reportedly correlated with feather-pecking (McKeegan and Savory, 1999). However, few studies have investigated the attractiveness of loose feathers to populations of birds exhibiting severe feather-pecking. This experiment aimed to investigate the level of interest shown by feather-pecking birds in loose feathers from "unfamiliar" hens of the same age, presented on the floor of their home pens. Six pens of 50 free-range ISA Brown laying hens aged 42 weeks were used. The birds in three of the pens had been documented to perform severe feather-pecking behaviour. Birds in the other three pens did not exhibit signs of severe feather-pecking as indicated by plumage condition. In order to examine the birds' level of interest in unfamiliar feathers, loose individual feathers were presented to each pen of birds. Feathers were collected from ISA Brown laying hens of similar age to the tested hens, although the hens were housed in a different shed and had not been in contact with the tested birds. Individual feathers were then introduced to the home pens and placed in the centre of the pen. This was repeated 15 times per pen in a random order each time. When the feather was placed in the test situation, two stopwatches were started concurrently. One stopwatch measured the latency for the first peck administered to the feather and the other measured time to feather ingestion. The test was ended if the birds did not interact with the feather after 30 s. Data were analysed using logistic generalized linear mixed models for binomial data and survival analysis using Cox's proportional hazard test for the latency to peck and latency to ingest feathers.

The birds in the pens that exhibited severe feather-pecking had shorter mean latency to peck at the individual feathers presented to them ($P < 0.001$) and shorter mean latency to ingest the feathers ($P = 0.001$). They were not more likely to ingest feathers than pens of birds not exhibiting severe feather-pecking ($P = 0.20$). When comparing the response to feathers from different birds, there was no difference in latency to peck ($P = 0.13$) or ingest ($P = 0.25$) feathers.

As feather-pecking is thought to be a multi-factorial problem, different causative factors should be investigated to determine the underlying motivation behind the behaviour. Nutritive and physiological factors should be considered in their ability to affect the expression of severe feather-pecking. The results of this experiment support previous studies that found feather-eating was positively correlated with severe feather-pecking and indicate that the attractiveness of the feathers may play a part in feather-pecking. The findings may aid diet formulation and inclusion of additional forages to prevent feather-pecking.

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BODY WEIGHT, FLOCK UNIFORMITY AND EGG QUALITY OF FLOCKS REARED IN TWO DIFFERENT REARING SHEDS

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Summary

Birds from two flocks of commercial caged hens, of the same age but reared in different sheds, were weighed at the ages of 6, 15, 19, 26, 37, 50 and 60 weeks. Body weight increased with increasing hen age for both groups. Eggs were collected from the flocks at the ages of 19, 26, 37, 50 and 60 weeks. Cuticle cover was measured using MST cuticle stain and a hand-held Konica Minolta spectrophotometer. Cuticle cover increased as hens aged and was higher for rearing shed A. There were significant effects of hen age and shed for ΔE^*_{ab} which was higher for shed A than shed B indicating better cuticle cover for birds originating from shed A. Traditional egg quality measurements were determined using specialized equipment supplied by TSS UK. A significant effect was recorded for flock age for all egg quality measurements. With advancing hen age, egg weight, shell weight, yolk colour and shell thickness increased, whereas shell breaking strength, shell deformation decreased. Albumen height, Haugh Units and percentage shell decreased then increased at late lay. Egg shell quality and egg internal quality were better, overall, for birds reared in shed A than for birds reared in shed B, an indication that initial rearing conditions may have a persistent effect on bird performance.

I. INTRODUCTION

Deteriorating shell quality is still a big concern in commercial egg production. There are many factors that affect the overall quality of the egg. The age of hens is reported to influence egg weight and eggshell quality (Silversides and Scott, 2001; Van den Brand et al., 2004). Body weight uniformity is another factor that can influence overall egg quality. Parkinson et al. (2007) studied the influence of flock uniformity in several commercial layer farms and found that flocks studied had an average body weight 100-300 grams above the breed standard, which indicated obesity. These obese birds produced excessively large eggs which resulted in lower egg shell quality (Parkinson et al., 2007). The goal for flock uniformity is to have 80 per cent of the pullets within plus or minus 10 per cent of the average flock body weight. Flocks with high uniformity have been reported to reach peak egg production earlier and have higher peak production than flocks of low uniformity (Hudson et al., 2001; Kosbah et al., 2009). On the other hand, poor uniformity is associated with variation in the degree of sexual maturity of hens, where underweight pullets have delayed onset of egg production (Yuan et al., 1994). Productive and profitable layers begin with good quality pullets. Having the correct body weight at the start of egg production will enable pullets to achieve their genetic potential. Problems that develop during the growing period cannot be corrected after egg production begins.

II. MATERIALS AND METHODS

A total of 100 birds were weighed from each flock at different ages: 6, 9, 12, 15, 19, 26, 36, 50 and 60 weeks of age. Body weight uniformity was calculated.

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A total of 90 eggs were collected directly from the cage fronts of each flock at 26, 35, 50 and 60 weeks of age. Thirty eggs were processed for the amount of cuticle with MST cuticle blue stain. A hand-held Konica Minolta spectrophotometer (CM-2600d) was used to measure the cuticle colour. The colour of the eggshell cuticle, stained with MST cuticle blue dye was measured using the L*a*b colour space. L* has a maximum of 100 (white) and a minimum of 0 (black). Green is indicated by –a* and red by +a*. Blue is indicated by –b* and yellow by +b*. ΔE^*_{ab} was calculated as described by Leleu et al. (2011). Sixty eggs were used for determination of traditional egg shell quality measurements: shell reflectivity, egg weight, eggshell breaking strength, shell deformation, shell weight and shell thickness, using specialized equipment (Technical Services and Supply, TSS, UK). Egg internal was also measured in the form of albumen height, Haugh Units and yolk colour.

Data were analyzed using Statview Software (SAS Institute Inc., Version 5.0.1.0). A two way analysis of variance was conducted taking flock age and shed/flock as the independent variables and body weight, egg quality measurements, SCI a* after staining and single score (ΔE^*_{ab}) as dependent variables. Level of significance was indicated by probability of less than 5%. The Fishers PLSD test was used to differentiate between mean values

III. RESULTS

Between the flocks/sheds, body weight was not significantly different for all ages combined although body weight was lower for shed A until 26 weeks of age. However, body weight was significantly affected (P<0.0001) by hen age and there was a significant interaction between hen age and shed. Body weight increased with hen age. Body weight uniformity ranged from 70% to 87 % for shed A, and from 71% to 89% for shed B (Table 1).

Table 1 - Body weight and flocks uniformity

Flock Age	Body Weight (kg)		Flock Uniformity (%)	
(weeks)	Shed A	Shed B	Shed A	Shed B
6	$^{f}0.55 \pm 0.004$	$^{g}0.57 \pm 0.005$	75	76
15	$^{e}1.31 \pm 0.009$	$^{f}1.37 \pm 0.010$	87	84
19	$^{d}1.69 \pm 0.014$	$^{e}1.78 \pm 0.011$	80	89
26	$^{c}1.92 \pm 0.014$	$^{d}1.91 \pm 0.014$	82	86
37	$^{b}2.09 \pm 0.017$	$^{c}1.99 \pm 0.016$	78	78
50	$^{b}2.07 \pm 0.023$	$^{b}2.04 \pm 0.019$	70	79
60	$^{a}2.12 \pm 0.019$	$^{a}2.11 \pm 0.021$	72	71
PValue				
Age (A)	<0.0001		<0.0001	
Shed (S)	NS		NS	
A*S	<0.0001		<0.0001	

^{a,b,c,d,e,f,g} Across a column, values with different superscripts are significantly different from each other. Values are Mean ± SE

There was a significant effect of hen age for L* which fluctuated with hen age. There was also a significant difference among age categories for a* after staining. Means values for a* increased, with the most negative values for shed A at 50 weeks and 37 weeks for shed B. A similar pattern was recorded for the ΔE^*_{ab} value (Table 2).

There was a significant main effect (P<0.0001) of hen age for all eggshell quality measurements. As hen age increased, shell reflectivity increased (varied between the sheds), egg weight increased, shell breaking strength and shell deformation decreased, shell weight

increased, percentage shell decreased, shell thickness fluctuated, albumen height slightly decreased, Haugh Units fluctuated and yolk colour increased (Table 3).

Table 2 - Spectrophotometric measurements of stained cuticle

Measurement		19 wk	26 wk	37 wk	50 wk	60 wk	P Value
L	Shed A	^{a}54.9 ± 0.8	^{c}51.0 ± 0.6	^{bc}52.8 ± 0.5	^{a}54.8 ± 0.8	^{ab}54.6 ± 0.7	0.0002
	Shed B	^{b}52.9 ± 1.0	^{b}55.0 ± 0.6	^{b}52.96 ± 0.8	^{a}57.7 ± 1.1	^{b}54.2 ± 0.8	0.001
a	Shed A	^{a}4.4 ± 1.2	^{b}-0.8 ± 0.9	^{bc}-1.3 ± 0.9	^{c}-3.8 ± 1.1	^{bc}-1.9 ± 0.9	<0.0001
	Shed B	^{a}3.3 ± 1.1	^{ab}1.4 ± 1.0	^{c} -1.6 ± 0.8	^{bc}-0.8 ± 1.1	^{bc}-0.3 ± 0.8	0.003
ΔE^*_{ab}	Shed A	^{b}16.9 ± 1.2	^{a}21.6 ± 1.0	^{a}21.5 ± 1.0	^{a}22.6 ± 1.2	^{a}21.0 ± 1.0	0.003
	Shed B	^{b}17.6 ± 1.2	^{b}17.8 ± 1.0	^{a}21.9 ± 1.0	^{b}18.5 ± 1.3	^{ab}19.8 ± 0.9	0.038

$^{a, b, c}$ Across a row, values with different superscripts are significantly different from each other. Values are Mean ± SE

Table 3 - Traditional measures of eggshell quality

Measurement		19 wk	26 wk	37 wk	50 wk	60 wk	P Value		
							Age	Shed	A*S
Translucency score	A	2.6 ±0.12	2.6 ± 0.08	2.7 ± 0.09	2.1 ± 0.11	2.7 ± 0.11	<.0001	NS	NS
	B	2.7 ± 0.11	2.7 ± 0.08	2.9 ± 0.10	2.1 ± 0.09	2.8 ± 0.13			
Reflect (%)	A	29.1 ± 0.5	26.5 ± 0.3	27.1 ± 0.4	29.0 ±0.5	27.9 ± 0.4	<.0001	.0025	<.0001
	B	27.7 ± 0.4	27.3 ± 0.4	30.8 ± 0.6	29.7 ± 0.5	28.6 ± 0.5			
Egg wt (g)	A	49.5 ±0.5	59.5 ± 0.4	62.9 ±0.5	63.4 ±0.4	65.9 ±0.5	<.0001	.0001	<.0001
	B	51.4 ±0.5	57.8 ±0.5	59.4 ± 0.5	62.7 ± 0.5	63.8 ± 0.7			
BSN (N)	A	45.5 ± 0.9	43.7 ± 0.7	41.7 ±0.8	40.8 ±0.9	41.6 ±1.0	<.0001	NS	NS
	B	45.6 ±0.7	44.1 ±0.7	40.0 ± 0.9	39.7 ± 0.8	40.7 ± 1.0			
Def (µm)	A	311.2±2.97	280.8±3.5	287.3±4.7	259.3±4.2	258.0±4.0	<.0001	NS	NS
	B	311.2±2.97	288.7±3.4	280.5±4.6	248.5±4.3	255.3±4.3			
Shell wt (g)	A	4.94±0.05	5.78±0.04	6.07±0.06	6.09±0.06	6.17±0.08	<.0001	<.0001	.0050
	B	4.97±0.06	5.60±0.06	5.64±0.06	6.01±0.06	5.99±0.07			
Percentage shell (%)	A	10.0±0.10	9.7±0.07	9.6±0.06	9.6±0.08	9.4±0.09	<.0001	NS (.0668)	NS
	B	9.7±0.08	9.7±0.07	9.5±0.08	9.6±0.07	9.4±0.10			
Shell thick (µm)	A	398.2±3.1	413.0±2.6	398.2±2.8	409.9±3.7	409.2±3.6	<.0001	.0010	NS
	B	392.8±3.0	406.2±2.9	381.3±3.2	409.1±2.9	404.9±4.5			
Albumen Ht (mm)	A	10.9±0.1	9.6±0.1	9.2±0.2	9.2±0.2	9.4±0.2	<.0001	.0001	NS
	B	10.8±0.1	9.2±0.1	8.8±0.2	8.8±0.1	9.1± 0.1			
Haugh Unit	A	105.2±0.4	97.5±0.6	94.3±0.8	95.0±0.9	95.4±0.8	<.0001	.0059	NS
	B	104.1±0.5	96.1±0.5	93.4±0.9	93.0±0.7	94.6±0.7			
Yolk colour	A	10.5±0.13	11.2±0.08	11.4±0.08	11.7±0.10	11.7±0.10	<.0001	.0033	NS
	B	10.2±0.10	11.0±0.07	11.2±0.11	11.6±0.06	11.6±0.10			

There was a significant difference between the two shed groups for a number of egg quality measurements with birds reared in shed A having higher shell reflectivity, egg weight, shell weight, shell thickness and a tendency to higher percentage shell than birds reared in shed B. In addition, egg internal quality measures (albumen height, Haugh Units and yolk colour) were significantly higher for birds reared in shed A than for those reared in shed B.

IV. DISCUSSION

Body weight of all hens was close to the target body weight for HyLine Brown laying hens reared in an intensive production system. The highest uniformity was recorded at 15 week of age for shed A (87%) and at 19 week of age for shed B, (89%). The quality of a flock is judged by its uniformity. Haider and Chowdhury (2010) reported that the uniformity of

commercial brown layer chicks (Shaver 579) at 8-17 weeks of age achieved an average of 84% which was higher than minimum standards (80%) provided by the Shaver 570 Management guide. Having the correct body weight at the start of egg production will enable pullets to achieve their genetic potential. Uniform flocks with the correct body weight give several benefits: birds are managed in a large group and management changes (lighting, feeding and housing) can be more easily instituted (Kosbah et al., 2009).

For the cuticle colour, the L* value increased with the age of flock indicating a lightening of shell colour with age. However, it decreased slightly when hens reached 60 week of age for reasons that are not clear. For the a* value, the more negative value at 37 and 50 weeks of age for shed B and shed A, respectively, was due to a greener colour which indicated more cuticle on the shell. There was a similar pattern for the ΔE^*_{ab} value which has been shown to be a reliable indicator of the extent of staining of the cuticle (Leleu et al., 2011). The cuticle is in direct contact with the outside environment, therefore represents the first line of defense against a harsh environment, including bacteria.

Age has an important effect on egg shell and internal quality. With increasing hen age, egg weight, shell weight and yolk colour increased. On the other hand, shell breaking strength, shell deformation, percentage shell and albumen height decreased, which is in agreement with previous studies (Robert and Chousalkar, 2012; Van Den Brand et al., 2004). A particularly interesting finding of the present study was that shell quality and egg internal quality of birds reared in shed A was consistently higher by most indicators than for birds reared in shed B. The notable exceptions were shell breaking strength and shell deformation, which were not different between birds from the two rearing sheds.

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MAINTENANCE OF SHELL COLOUR IN FREE RANGE LAYING HENS

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Summary

Loss of shell colour in brown-egg layers is a shell quality issue in Australia and is periodically associated with free range egg production. It has been suggested that exposure to sunlight on the range may affect shell colour. Birds from the same free range flock, exhibiting shell colour deterioration, were either maintained in the free range or transferred to cages in an experimental facility. Egg quality was measured immediately following transfer to cages and then up to five weeks following transfer. Shell colour was measured by shell reflectivity, protoporphyrin IX levels and staining of the cuticle with MST cuticle blue dye. Transfer of birds to cages improved shell colour compared with birds remaining *in situ*. The lighter coloured shells of the birds that remained in free range were due to lower cuticle cover and less protoporphyrin in the eggshell of that group. The hypothesis that loss of shell colour was related to sunlight and levels of vitamin D was tested experimentally by supplementing diets with different levels of vitamin D and its metabolites. When birds received doses of 526, 666, 993 and 1926 IU per day of vitamin D, there was a steady linear numeral decrease in shell reflectivity with increasing vitamin D dose. However, this change was not statistically significant. The a* spectrum following staining with cuticle blue dye was higher for the groups receiving 666 and 1926 IU vitamin D per day than for the control group, with the 993 IU vitamin D per day group intermediate. Therefore, it appears that the loss of brown egg shell colour observed in free range flocks cannot be attributed to exposure to light resulting in increased levels of vitamin D in the birds as has been suggested previously.

I. INTRODUCTION

As hens age, brown egg shell colour deteriorates in strains of laying hen that lay brown-shelled eggs. In addition, eggs laid even by young flocks are sometimes lighter in colour and anecdotal evidence suggests that this may be a particular problem with some free range flocks. The egg shell cuticle is a protein and carbohydrate complex. The protoporphyrin pigment of egg shells appears to be contained in both the cuticle and the calcite matrix but, for many years, it was thought to be mainly in the cuticle, as evidenced by the occasionally very dark shelled egg which has been retained in the oviduct (Hutt & Sumner,1952). However, more recent studies suggest that the bulk of protoporphyrin may be contained in the outer layers of the egg shell proper (Samiullah & Roberts, 2013). Shell colour is an egg shell quality trait that is important to the consumer in countries where brown-shelled eggs are preferred.

The current study utilized birds obtained from a commercial free range flocks which were relocated to an experimental cage facility at various stages in their production life. In addition, the hypothesis that loss of shell colour in free range flocks is associated with exposure to sunlight and effects on vitamin D levels was investigated by supplementation of the diet with different levels of vitamin D.

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II. MATERIALS AND METHODS

In Experiment 1, a sample of birds (172) from a commercial free range flock was moved into cages at an experimental facility when the birds were 25 weeks of age (Group 1). Subsequently, the commercial free range flock began to have problems with loss of shell colour. A sample of these birds (50) was transferred to the experimental cage facility when birds were 59 weeks of age (Group 2). Group 3 consisted of birds that remained housed at the commercial free range facility. Eggs were collected from Group 2 birds 11 days following transfer of birds from free range to cage and then two, three and four weeks later, from all three treatment groups. At each of these sampling times, 24 eggs were collected from each treatment group for analysis as described below, 12 eggs for traditional egg quality measurements and 12 eggs for measurement of protoporphyrin in the shells.

Experiment 2 tested the hypothesis that loss of shell colour in free range flocks is associated with the effects of sunlight on production of vitamin D. Birds (50) from the free range farm were transported to the research cage facility and divided into four groups, A, B, C and D containing an average of 12 birds (any reason for different numbers in each treatment group?). The birds were 71 weeks of age at the commencement of the experiment. Group A received the control diet containing 526 IU vitamin D per hen per day (assuming 100g intake per day); Groups B, C and D received the control diet with supplemental vitamin D sufficient to achieve 666, 993 and 1926 IU per day, respectively (assuming 100g intake per day). Eggs (12 from each group) (not sure how 12 eggs can come from 10 birds in a single day?) were collected over two days at the commencement of the experiment and then at weekly intervals for 4 weeks.

Eggs for traditional quality and cuticle cover analysis were initially measured for shell reflectivity. Eggs were then stained with MST cuticle blue stain and the cuticle colour measured using a Konica Minolta hand-held spectrophotometer (CM-2600d). Eggs were immersed in cuticle blue dye (MS Technologies, Europe Ltd), made up according to the manufacturer's recommendation, for one minute. They were then rinsed in water for 3 seconds, placed on a plastic egg filler and allowed to dry. The colour of the egg shell cuticle, stained with MST cuticle blue dye was measured using the "L*a*b*" colour system. "L*" has a maximum of 100 (white) and a minimum of 0 (black). For "a*", green is towards the negative end of the scale and red towards the positive end. For "b*", blue is towards the negative end and yellow towards the positive end of the scale. The difference between the reading before and after staining was calculated. A single score was calculated after the method of Leleu *et al.* (2011) as: $\Delta E^*_{ab} = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$.

Eggs were analysed for traditional egg shell quality measurements: shell colour by reflectivity, egg weight, egg shell breaking strength by quasi-static compression, shell deformation to breaking point and shell weight (egg quality equipment, Technical Services and Supplies, U.K.). Shell thickness was measured using a custom-made gauge based on a Mitutoyo Dial Comparator gauge Model 2109-10. Percentage shell was calculated from shell weight and egg weight. Egg internal quality was measured as albumen height, Haugh Units and yolk colour (TSS equipment).

Eggs were analysed for protoporphyrin IX in the whole eggshell (including the cuticle) and in shell with cuticle removed, using the method described by Poole (1965) and used by Ito et al. (1993) and Wang et al. (2007, 2009) with some modification (see Samiullah and Roberts, 2013).

Data were analysed by ANOVA and differences between means separated using Fisher's LSD test. Significance was assumed at $P<0.05$.

III. RESULTS

In Experiment 1, production at 62 weeks of age was 88%, 86% and 85% for Groups 1, 2 and 3, respectively. For the birds transferred from free range to cage, shell reflectivity decreased significantly within the first two weeks from a mean of 34.1% at day 1 of transfer to 29.3% at day 14. The shell reflectivity of this group increased slightly at 21-28 days. Egg shell reflectivity was significantly different among the groups, being higher for Group 3 than for the other two groups, indicating that shell colour was lightest for the birds that remained in the free range production system (Figure 1). The SCI a* values following staining of the cuticle with cuticle blue dye, as well as the single score calculated after Leleu *et al*. (2011), were not significantly affected by treatment group or day of sampling. However, the difference in a* before and after staining was lowest for Group 3, suggesting that there was less cuticle present in the shells of the birds that remained in the commercial free range production system. This difference declined with time in all three groups.

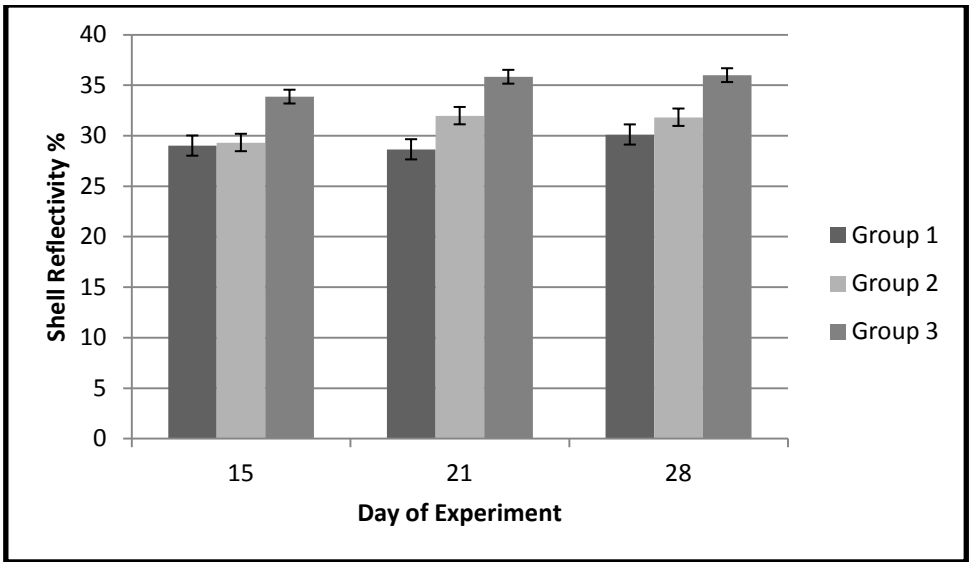

Figure 1 - Experiment 1: Shell reflectivity of each treatment group of each collection day.

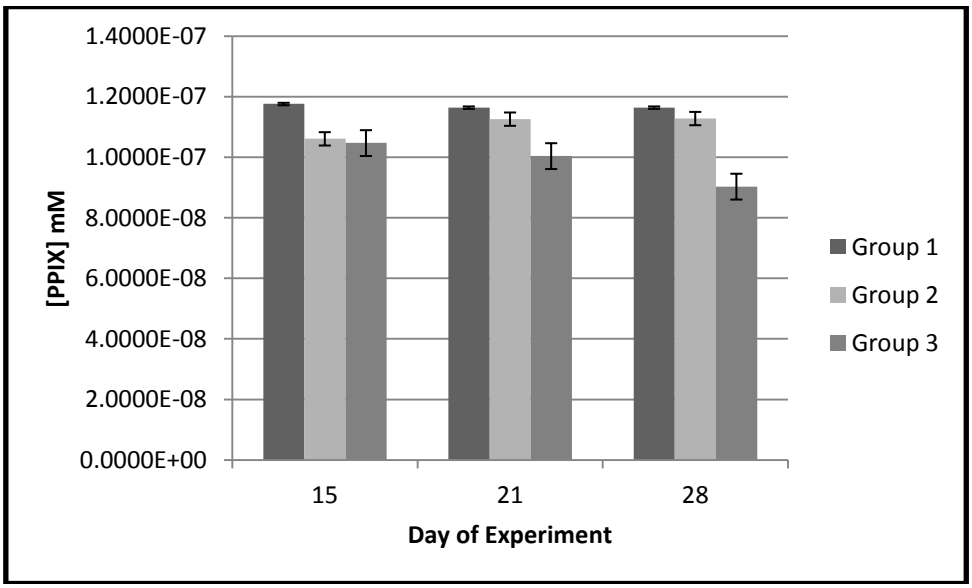

Figure 2 - Experiment 1: Protoporphyrin in 1 g of whole eggshell of each treatment group on each collection day.

The levels of protoporphyrin in the eggshells (with cuticle) were significantly different among the treatment groups, being highest in Group 1, lowest in Group 3, with Group 2 intermediate but all significantly different from one another (Figure 2). The level was stable across the sampling times for Group 1, increased with time in Group 2 and declined with time in Group 3. Most of the protoporphyrin was found in the shell rather than the cuticle itself.

For Experiment 2, there was no effect of treatment group on shell reflectivity or the levels of protoporphyrin in the shell. For the measures of cuticle cover, there was a significant main effect of day of experiment on cuticle cover but no main effect of treatment group and no significant interaction between treatment group and day of experiment.

Table 1 - Experiment 2: Cuticle cover for all treatment groups combined

Day of Expt	a* value after staining	Δa* value before & after staining	Single Score Leleu et al., 2011
1	^{a}6.20 ± 1.06	^{c}8.51 ± 0.88	^{b}9.64 ± 1.03
9	^{ab}5.35 ± 1.21	^{bc}10.37 ± 1.21	^{b}11.82 ± 1.50
14	^{bc}1.58 ± 1.05	^{a}14.29 ± 1.06	^{ab}15.88 ± 1.23
21	^{c}1.42 ± 1.94	^{ab}13.68 ± 1.84	^{ab}15.06 ± 2.12
28	^{c}-0.22 ± 1.33	^{a}16.91 ± 1.46	^{a}19.31 ± 1.43

Values are Mean ± SE. Values within a column with different superscripts are significantly different (P<0.05)

IV. DISCUSSION

For birds originating from a commercial free range flock, some of which were relocated to cages in an experimental facility early in lay, loss of shell colour occurred only in the birds remaining in the free range production system. Relocation of these birds to cages in the experimental facility resulted in rapid improvement of shell colour. However, when different levels of vitamin D were added to the diets of birds maintained in cages, there were no significant differences in shell colour and protoporphyrin levels among the experimental groups although cuticle cover improved with time in all groups. In addition, there were no significant differences among the groups for eggshell cuticle cover. The increased cuticle cover in all groups over the course of the experiment suggests that, although shell colour had stabilised at the commencement of the experiment, the amount of cuticle being deposited continued to increase. The loss of shell colour in the commercial free range flock appears to have been due to factors other than vitamin D levels in the diet.

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EFFECTS OF DIETARY CORTICOSTERONE ON EGG PRODUCTION AND QUALITY IN LAYING HENS

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Although corticosterone treatment induces reduction in hens' performance, fertility and hatchability of eggs, and increase in the number of early dead embryos in eggs (Schmidt et al., 2009; Shini et al., 2009), little is known as to the effects of corticosterone treatment on egg quality. The objective of this study was to determine the effects of dietary corticosterone on egg production, egg quality, and plasma biochemical concentrations in laying hens.

Twenty, forty seven-week-old Single Comb Brown Hy-Line Leghorn laying hens were housed into individual cages in a room with 15h lights (on at 06:00h) and at 20 °C ambient temperature throughout the study. Feed intake and egg production were monitored daily in the morning and body weight measured weekly throughout the experimental period. After a two-week adaptation, hens were divided into two groups and fed for two weeks either a control diet or the experimental diet containing corticosterone at 30 mg/kg diet. Eggs collected on days 0, 1, 5, 10, and 14 were tested within 24h after the collection for egg weight, albumen height, Haugh units, and yolk color using an Egg Multi Tester (QCM+), and eggshell thickness using a micrometer. Plasma biochemical concentrations were determined using a VetTest® Chemistry Analyzer (IDEXX). A t-test was performed to assess differences between both groups at $P \leq 0.05$.

Dietary corticosterone for 2 weeks resulted in significant increase in food intake and body weight ($P < 0.05$), but drastic reduction in egg production. Egg weight, egg shell strength, and shell color were not significantly different between both groups. Corticosterone significantly increased shell thickness and yolk redness but decreased albumin height and Haugh unit on day 10 ($P < 0.05$). Decreased calcium and triglyceride, but increased albumin, amylase, creatine kinase, glucose, lactate dehydrogenase, lipase, total protein cholesterol, and uric acid concentrations were found in plasma of hens treated with dietary corticosterone ($P < 0.05$).

Table 1: Effects of dietary corticosterone on egg production and quality on day 10

Parameters	Control	Corticosterone	P-value
Egg production, %	92.9	10	-
Egg weight, g	58.8 ± 1.45	59.1 ± 2.19	0.35
Egg shell strength, kg/cm^2	5.03 ± 0.39	5.69 ± 0.15	0.1
Shell thickness, mm	0.41 ± 0.02	0.47 ± 0.01	0.01
Yolk redness	-3.36 ± 0.23	-0.74 ± 0.67	0.0004
Albumin height, mm	7.76 ± 0.55	5.51 ± 0.78	0.01
Haugh unit	87.5 ± 3.2	69.5 ± 7.8	0.01

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EFFECTS OF SELENIUM SOURCES ON EGG SELENIUM ENRICHMENT: A NEW ALTERNATIVE

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Summary

The aim of this study was to investigate the effects of a new organic selenium source in comparison with other organic and inorganic sources of Se on selenium deposition in eggs and breast muscle of laying hens. A total of 240 ISA-brown laying hens (40 wk old) were used over a 56-d experimental period. Layers were randomly allocated to the following groups: the control group (CG) received a basal diet without Se supplementation; a second group received the basal diet enriched with 0.2 ppm of sodium selenite (SS-0.2) third and fourth groups received selenium enriched yeast at respectively 0.1 and 0.2 ppm (SY-0.1 and SY-0.2) and the last two groups were supplemented with Seleno-OH-methionine (HMSeBA), the new pure organic selenium source, at 0.1 and 0.2 ppm respectively (SO-0.1 and SO-0.2). All selenium supplementations tested increased whole egg selenium content compared to the control group ($P<0.01$) and both organic sources (SY and SO) allowed higher ($P<0.05$) Se concentration in eggs than inorganic source (SS) at 0.2 ppm total Se dietary inclusion level. Moreover, SO-0.2 significantly improved ($P<0.05$) total egg Se content compared with SY-0.2 treatment. A regression analysis allowed to calculate that selenium deposition with HMSeBA was significantly higher than those obtained with selenium enriched yeast. Moreover, HMSeBA fed layers exhibited significantly higher Se deposition in breast muscle ($P<0.05$) than Se-yeast. These results clearly demonstrated that the new organic selenium source (Se-OH-methionine) is efficient to sustain both egg and muscle selenium enrichment in laying hens and has to be considered for functional food strategy.

I. INTRODUCTION

Selenium (Se) is an essential micronutrient which plays an important role in the antioxidant, endocrine, reproductive and immune systems in humans and animals through about 25 selenoproteins. During the last decade, remarkable studies compared the bioavailability and benefits of several Se sources in animal species. In poultry, as in mammals, organic Se has been shown to increase more efficiently tissue selenium deposition than mineral or inorganic sources. Recently, a new organic Se source based on 2-hydroxy-4-methylselenobutanoic acid or Se-OH-methionine (HMSeBA) as active component was developed and its efficacy for Se deposition in tissues was proven both in poultry (Briens et al., 2013; Jlali et al., 2013a) and swine (Jlali et al., 2013b). Therefore, the objective of this study was to investigate the effects of this Se source in comparison with usual selenium sources on egg and muscle selenium deposition for laying hens.

II. MATERIALS AND METHODS

A total of 240 laying hens (40 wk, ISA Brown) were used in this experiment. Layers were randomly allocated to 1 of 6 treatments with 8 replicate collective cages with 5 birds per cage (660 cm^2/ bird) equipped with a feed trough and nipple drinkers. Six treatments were supplied for 56 days as follows: control group (CG) received basal diet without Se

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supplementation; a second group received the basal diet enriched with 0.2 ppm of sodium selenite (SS-0.2, Microgan Se 1% BPM; DSM), third and fourth groups received selenium enriched yeast at respectively 0.1 and 0.2 ppm (SY-0.1 and SY-0.2, Sel-Plex 2000; Alltech), the last two groups were supplemented with HMSeBA selenium source at 0.1 and 0.2 ppm respectively (SO-0.1 and SO-0.2; Selisseo 2% Se; Adisseo). All hens were given ad libitum access to water and feed. The temperature was maintained at 22°C and lighting program was fixed to 16 h light/8 h dark throughout the experimental period. Egg production was recorded during the whole trial and 8 eggs per treatment (1 per replicate) were collected at d0; d8, d14 and d56 for selenium determination and egg shell quality. At the end of the 56-d period, 8 hens per group, for SY-0.2 and SO-0.2 treatments were slaughtered for breast muscle selenium analysis. Total Se concentrations in feed, egg, and muscle samples were determined according to the method adapted from Vacchina et al. (2010). Approximately 1 g of feed sample was mineralized in a mixture (2:1,vol/vol) of HNO_3 (69 to 70%) and H_2O_2 (35%) at 85°C for 4 h within a closed vessel heating block system (DigiPrep; SCP Science, Courtaboeuf, France). Se content was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500cx, Tokyo, Japan). All values were calculated on a dry matter basis. All data were analyzed using SAS. The accepted type I error was 5%. The effects of treatment, period, and their possible interactions were analyzed in relation to feed intake, egg weight, egg mass, laying rate, feed efficiency and eggshell strength using repeated-measures 2-way ANOVA (GLIMMIX procedure). The period was added to the model as a repeated factor with the cage as the subject. For Se variables, the treatment effect, which is a combination of Se sources and levels, were analyzed using GLM procedure. Comparisons of means for each significant effect were performed by Tukey's test using the least square mean statement. Data are presented as means ± SEM or SD. The bioavailability of Se from HMSeBA relative to SY was calculated according to Finney (1971) by using 5-point slope ratio design: control, SY-0.1, SY-0.2, SO-0.1 and SO-0.2. As suggested by Littell et al. (1997) a nonlinear model was fitted to the data using the NLIN procedure of SAS. The model was as follows:

$$\text{Egg Se concentration} = a + a_0 \times X_0 + bS \times (bTS \times \text{SO dose} + \text{SY dose}).$$

in which egg Se concentration is the content of Se in egg (in mg/kg of dry product), a is the intercept, $a_0 \times X_0$ is a correction for the control diet, SO dose and SY dose are the Se amounts added to hen diets from HMSeBA and SY, respectively, bS is the slope for the effect of SY on the response, and bTS is the ratio between bT (the slope for the effect of HMSeBA) and bS. This allows an estimate of the relative biological value (i.e., the ratio between slopes bS and bT) and its confidence interval (CI) to be obtained directly.

III. RESULTS AND DISCUSSION

The analyses of total Se content in each diet showed no difference with the expected Se levels for control and experimental treatments (data not shown).

Feed intake, egg weight, egg mass, and laying rate were not affected by dietary treatments. In those particular breeding conditions, the laying hen performance was not influenced neither by the Se source (inorganic vs. organic forms) or the supplementation levels (0.1 vs. 0.2 mg Se/kg) (Table 1). This finding is also in line with other studies on broilers or pigs that did not show performance improvement in standardized rearing condition with the dietary selenium supplementation (Payne & Southern, 2005; Li et al., 2011; Briens *et al.* 2013). No treatment effects (source and dose of Se) were observed on the eggshell breaking strength ($P = 0.10$). The lack of effect of the selenium sources and level used in this study is in agreement with Pavlovic et al. (2010).

Table 1 - Effects of Se sources on performance traits and eggshell strength in laying hens

Item	Treatment						SEM	*P*-value
	Control	SS-0.2	SY-0.1	SY-0.2	HMSeBA-0.1	HMSeBA-0.2		
Feed intake, g/d	113.0	117.0	115.4	112.4	111.6	115.4	2.1	0.42
Egg weight, g	66.2	65.7	65.4	65.5	65.1	66.6	0.6	0.47
Egg mass, g/d	61.0	62.3	63.1	60.2	60.2	61.9	1.3	0.53
Laying rate, %	92.2	94.7	96.6	92.8	92.9	92.9	1.6	0.54
Feed efficiency, g/g	0.54	0.53	0.55	0.54	0.54	0.54	0.01	0.93
Eggshell strength, N	38.0	36.3	35.9	35.4	34.9	35.1	0.8	0.10

No interaction was detected on the average total Se content between dietary treatments and sampling day during the last 3 days of experiment. Total Se concentrations measured in eggs (Fig. 1) from the hens supplemented with Se were greater than those without supplementation ($P < 0.01$). At the level of 0.2 mg Se/kg of diet, Se was more efficiently deposited in eggs from hens supplemented with SO ($P < 0.01$) and SY ($P < 0.05$) compared with those supplemented with SS. The better selenium deposition in eggs with dietary organic selenium sources compared to inorganic have frequently been reported (Payne *et al.*, 2005; Utterback *et al.*, 2005; Kralik *et al.*, 2009; Bennett and Cheng, 2010). It is indeed interesting to notice that both organic sources at 0.1 ppm are able to deposit selenium in eggs as efficiently as 0.2 ppm of sodium selenite. Moreover, comparing only organic Se treatments, hens fed the SO-0.2 diet exhibited greater ($P < 0.01$) egg Se concentrations compared with those fed the SY-0.2 diet. The better selenium deposition in tissues with HMSeBA molecule compared to selenium enriched yeast at same dosage has been reported previously in broiler breast muscle at 21-d (Briens *et al.* 2013).

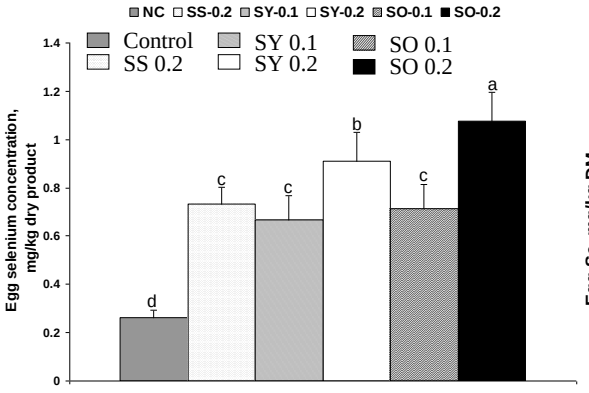

Figure 1 - Effects of different Se sources and levels on egg Se concentrations (mg/kg DM) in laying hens during last 3 d of the experimental period. Data are expressed as means ± SD; different letters indicate significant difference (P<0.05).

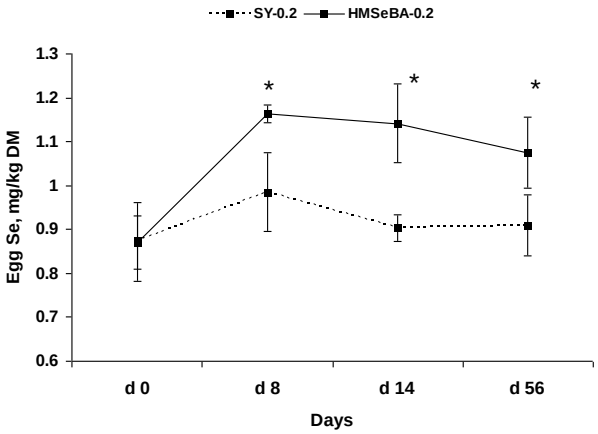

Figure 2 - Kinetic of egg Se deposition depending on the organic sources at 0.2 ppm (mg/kg DM). Data are expressed as means ± SD: * = P<0.05.

Morover, it appears that beyond the egg selenium enrichment by SO-0.2 treatment compared to SY-0.2, significant higher selenium deposition was observed in breast muscle after the 56 day period (not shown).

Selenium content was greater ($P < 0.05$) in eggs from hens supplemented with SO than with SY (Fig. 1). Results of the kinetic study showed that HMSeBA at the dose of 0.2 mg Se/kg of diet could more efficiently increase the Se content of eggs ($P < 0.05$) compared with the equivalent amount of SY from 8 days of supplementation (figure 2). The Se transfer efficiency values determined in relation to Se egg output and daily Se intake showed that the Se transfer efficiency was greater ($P < 0.01$) in birds supplemented with Se as HMSeBA at

0.2 mg/kg of diet (76.26%) than those provided the same amount of SY (56%). The relative bioavailability of egg Se concentration between SO and SY sources was tested with a slope ratio model. The results obtained with the model indicated that the relative bioavailability of SO was 28.78% [95% CI: 16.99, 40.57%] higher ($P < 0.01$) than that of SY.

These results suggest that the new organic selenium source, Seleno-hydroxy-methionine (HMSeBA) is more effective than seleno-yeast to improve egg selenium deposition and could be a real opportunity for functional food strategy.

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EFFECT OF 25-HYDROXYCHOLECALCIFEROL SUPPLEMENTATION ON EGG QUALITY, BODY WEIGHT AND FEED INTAKE IN COMMERCIAL LAYING HENS

K. AL ZAHRANI [1] and J.R. ROBERTS [2]

Summary

HyLine Brown layer hens from 19 to 50 weeks of age were used to determine the effects of supplemental 25-hydroxycholecalciferol ($25(OH)D_3$) on egg internal quality, eggshell quality, amount of cuticle present on the eggshells, body weight, and feed intake. Birds were housed individually in cages and divided into three groups (A, B, and C), 30 birds per group, where group A was control fed with normal commercial layer mash feed, group B was fed with normal layer mash feed plus 0.5 g of $25(OH)D_3$ premix (68.9 µg $25(OH)D_3$) per kg of feed, and group C fed with normal layer mash feed plus 1 g of $25(OH)D_3$ premix (137.8 µg $25(OH)D_3$) per kg of feed. Our results indicated there was a significant main effect ($P \leq 0.05$) of hen age and treatment group on albumen height, Haugh unit, and yolk color score, whereas a significant interaction between these two factors was recorded only for yolk score. Shell quality results indicated that there was a significant main effect of both hen age and treatment group on shell reflectivity, egg weight, shell deformation, shell weight, percentage shell, shell thickness, translucency score, and cuticle single score value. There was a significant effect of hen age on shell breaking strength and shell reflectivity prior to staining. The results showed that the highest shell weight and percentage shell and lowest shell deformation were found in group C. The lowest shell thickness and highest albumen height, Haugh unit, yolk color score, difference in shell reflectivity, translucency score, and cuticle single score value were found in group A. Egg weight was higher in groups A and C than group B. Body weight and feed intake were not significantly different among the groups.

I. INTRODUCTION

Diets fed to laying hens are based on cereals and oilseeds and must be supplemented with either vitamin D_3 or 25-hydroxycholecalciferol (25-OH-D_3) for optimal performance (Kidd, 2009). More than 60 years ago, breeding companies focused on increasing egg number and the efficiency of feed utilization. However, in recent years, there has been a focus on egg quality to minimize yolk content and eggshell quality deterioration (Rossi et al., 2013). A major factor that affects the entire poultry industry is egg quality over the laying life of the hen (Dunn et al., 2011). The thickness of an eggshell exceeds 0.3 mm which makes it very strong with mechanical pressure resistance up to more than 3 kg (Hincke et al., 2011). The decline of internal and external egg quality characteristics results mostly from increasing hen age (Bell, 2003). Furthermore, studies have found that there is no effect on shell quality of adding vitamin D_3 to the feed of older hens (Jones, 2006). Accordingly, the purpose of this study was to attempt to enhance egg quality by supplementing the diet with a vitamin D metabolite from the point of lay.

II. MATERIALS AND METHODS

Ninety HyLine Brown layer hens from 19 to 50 weeks of age were housed in individual cages in shed B on the University of New England (UNE) campus. Birds were divided into three groups (A,B, and C), 30 birds per group, where group (A) was the control, fed with normal

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commercial layer mash, group (B) was fed the layer mash + 0.5 g of 25(OH)D_3 premix (68.9 µg 25(OH)D_3) per kg of feed, group (C) was fed layer mash + 1 g of 25(OH)D_3 premix (137.8 µg 25(OH)D_3) per kg of feed. Layer mash feed was formulated by a nutritional consultant and mixed by a commercial feed company, then Hy-D® premix was added to the layer mash for the B and C groups in the feed mixing room at UNE. Treatments were applied from 19 weeks of age (when birds reached to 5% of production) to 50 weeks of age.

Feed intake was measured weekly and body weight was measured in 19, 26, 37 and 50 weeks of age. Egg quality analysis and measurements were applied weekly for egg internal quality, eggshell quality, and the amount of cuticle present on the eggshells. Specialised equipment (Technical Services and Supply U.K. egg quality equipment and Konica Minolta Spectrophotometer, shell thickness gauge based on a Mitutoyo Dial Comparator gauge), available in the Egg Quality Laboratory at UNE, was used for these analyses. Cuticle cover was estimated using cuticle blue dye and a single score value was calculated after the method of Leleu et al. (2011).

Data were analysed by ANOVA using StatView software. Differences between means were established using Fisher's Least Protected Difference test.

III. RESULTS

There was a significant main effect of both hen age and treatment group on albumen height, Haugh unit, and yolk color score, and a significant interaction (P>0.05) between hen age and treatment group for yolk colour score. Albumen height and yolk color score were highest for A and lowest for B and C which were not different from each other, whereas Haugh unit was highest for A, lowest for C, with B intermediate (Table 1).

Table 1 - Egg quality of HyLine Brown layer hens from 19 to 50 weeks of age.

Variable	Treatment Group		
	A	B	C
Albumen height (mm)	8.4[a]	8.28[b]	8.26[b]
Haugh unit	91.29[a]	90.82[ab]	90.51[b]
Yolk colour score	9.5[a]	9.37[b]	9.35[b]
Difference shell reflectivity (%)	4.72[a]	4.2[b]	4.07[b]
Egg weight (g)	60.21[a]	59.56[b]	60.54[a]
Shell weight (g)	5.8[b]	5.77[b]	5.91[a]
Percent shell (%)	9.64[b]	9.68[b]	9.77[a]
Shell thickness (µm)	408.86[b]	414.42[a]	416.21[a]
Shell deformation (µm)	275.16[a]	277.59[a]	270.5[b]
Translucency score	1.88[a]	1.83[ab]	1.8[b]
Cuticle single score value	18.59[a]	17.3[b]	17.04[b]
Body weight (kg)	1.84	1.86	1.83
Feed intake (g/bird/day)	120.4	118.1	119.2

A is control diet; B is control diet supplemented with 68.9 µg 25(OH)D_3/kg feed; C is control diet supplemented with 137.8 µg 25(OH)D_3/kg feed.
[a-b]Means within a row with no common superscripts are significantly different (P≤0.05).

Shell quality results indicated that there was a significant main effect of both hen age and treatment group on reflectivity, egg weight, shell deformation, shell weight, percentage shell, shell thickness, and translucency score (Table 1). There was a significant effect only of hen age on shell breaking strength and shell reflectivity prior to staining with cuticle blue dye. For shell reflectivity after staining and shell thickness, there was no significant difference between treatments B and C which were higher than treatment A. The difference

between the two reflectivity measurements showed the same pattern as the reflectivity after staining. Egg weight was lower for B than for groups A and C which were not different from each other. There were no differences in shell breaking strength among the treatments, whereas shell deformation was the same for A and B which were higher than C. The highest shell weight and percentage shell were found in group C eggshells. There was no significant interaction between treatment group and hen age for any eggshell quality variable. For the amount of cuticle estimation, there was a significant effect of group and hen age, but no significant interaction between the two for the single score value (ΔE*ab).
Body weight and feed intake varied with hen age but were not different among the treatment groups (Table 1).

IV. DISCUSSION

Our results indicated that supplementation of the diet with 25-hydroxycholecalciferol reduced the internal quality characteristics of eggs (albumen height, Haugh unit, and yolk color score) but improved shell quality, particularly at the higher dose. This improvement was for shell thickness for both groups B and C, and in egg weight, shell weight, and percent shell for group C. These findings differ from those of Keshavarz (2003) who reported no improvement in shell quality from inclusion of 25-OH-D_3 at 69 μg of 25-OH-D_3/kg diet. On the other hand, our results concur with those of McLoughlin and Soares (1976) who found that supplementation of 25-OH-D_3 with oyster shells improved shell quality for eggs laid by older hens (74 weeks of age) and eggs laid from hens reared for the second year of production. Difference in shell reflectivity following staining and cuticle single score value (ΔE*ab value) results were lower for eggs laid by the group B and C hens than for the control group (group A), indicating that cuticle cover was better for the control group than for the vitamin D supplemented groups. Translucency score was highest for group A and lowest for group C with group B intermediate. This suggests that supplementation with 25-OH-D_3 has the potential to improve shell ultra structure.

In conclusion, supplementation of 25-hydroxycholecalciferol to the diet of laying hens decreased internal egg quality but improved shell quality, particularly at the higher level of inclusion. However, there was no effect on hen bodyweight or feed intake resulting from 25-hydroxycholecalciferol supplementation..

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EVALUATION OF DIFFERENT PROTECTIONS OF BUTYRIC ACID ON PERFORMANCE AND EGG CHARACTERISTICS OF WHITE LEGHORN LAYER HENS

M. PUYALTO[1], S.V. RAMA RAO[2] and J.J. MALLO[1]

Summary

The present study was conducted to determine the effect of supplementing the diet of hens with different forms of butyric acid on performance (feed efficiency (FE), egg production (EP)), egg quality (egg weight (EW), egg mass (EM), shell weight (SW), shell thickness (ST) and egg density (ED)) and digestibility of energy and protein. The treatments were: sodium butyrate protected with palm stearine (SBP) and calcium butyrate (CB). Two thousand four hundred ninety 38-week old White Leghorn (BV 300) laying hens were use in this experiment. They were housed in 30 identical pens, each containing 83 birds, and 10 pens were used per treatment. The hens were fed with diets (168 g CP and 10.88 MJ ME / kg). Both forms of butyrate were included at same dosage of butyric acid (280 g / mT) for a period of 16 weeks. Responses were compared with an unsupplemented treatment. The pooled data of 4 periods (16 weeks) indicated that incorporation of butyrate in both forms in layer diet did not influence ($P > 0.05$) EP, FI, FE, EW, EM, SW or ST. However, ED improved significantly ($P < 0.05$) in groups fed both forms of butyrate compared to those fed control diet without butyrate. The improved ED might be due to non-significant ($P > 0.05$) increase in shell thickness in layers fed both forms of butyrate. Supplementation of SBP significantly improved the digestibility of energy ($P < 0.001$) and protein ($P < 0.005$) compared to those fed the control diet or diet supplemented with CB. The percentage of eggs produced by SBP (84.04%) was higher than control (83.43%) and CB (83.14%) groups without significant differences. Similarly, feed intake to produce a unit EM in SBP (2.191 g) was lower than those fed other two diets (2.236 and 2.247 g). Livability in SBP (96.2%) was higher than control and CB (95.8 and 94.8%, respectively). Haugh unit score was higher for SBP group compared with Control and Calcium butyrate (73.58, 72.25, 71.83) with non significant effect ($P > 0.05$). Digestibility of energy and protein improved significantly in SBP compared to those fed the other two diets. From the obtained data it can be concluded that supplementation of the diet with sodium butyrate protected with fat increases eggshell quality, nutrient digestibility and layer production when compared to the control.

I. INTRODUCTION

Conventionally, antibiotic or chemical growth promoters are used as a tool to reduce pathogen count in the gut in commercial poultry, which also enhances feed efficiency (Jin et al., 1997). But, the use of antibiotics as routine feed additives are being discouraged due to consumer concerns about the safety and possible threat of developing drug resistant pathogenic bacteria (Leeson, 2007) by consuming poultry products from such practices. Several alternate approaches like the use of essential oils (Lee et al., 2004), probiotics (Panda et al., 2003; Rama Rao et al., 2004), and organic acids (Panda et al., 2009), are suggested to maintain optimum gut health *in lieu* of chemical or antibiotic growth promoters in avian diets. Butyric acid, an organic acid, is known to play a vital role in the development of the gut membrane (Mallo et al., 2012) and reduce the population of harmful bacteria in the chicken gut. Butyric acid is, however, a corrosive liquid, so, in order to facilitate its use in animal

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nutrition, it is transformed into a salt (amongst other transformations and mixes). The efficiency of butyric acid predominantly depends on the salt into which it has been transformed. Butyrate salts have very different solubilities, being much higher for the sodium butyrate, 115 g / 1000 ml, than for the calcium butyrate, 6 g / 1000 ml (Mallo *et al.*, 2012). The liberation of the butyrate anion into the intestinal lumen, and the transformation of that anion into butyric acid or its direct absorption by the enterocytes, can be controlled with adequate protection. As calcium butyrate solubility is very low, it allows a slow release of the butyrate anion into the intestinal lumen; while sodium butyrate requires a physical protection(given by vegetable fat) in order to ensure the slow release of active principle. This experiment was conducted to compare the addition of two presentations of butyric acid (sodium butyrate protected with palm stearine, SBP and calcium butyrate, CB) in layer diets on egg production (EP), feed efficiency (FE), egg mass (EM), egg weight, egg shell quality, Haugh unit score and digestibility of energy and protein.

II. MATERIALS AND METHODS

A total of 2490 White Leghorn (BV 300, Babcock) laying hens (38 weeks of age) were distributed equally among 30 replicates (four bird colony cages – 83 birds per replicate). The cages were on an elevated platform in an open-sided poultry house (ambient temperature range 23-39.2°C). A control diet (CD) containing 10.88 MJ ME and 168 g CP/kg was prepared. The CD was supplemented with two forms of butyric acid (sodium butyrate protected with palm stearine, SBP and calcium butyrate, CB) independently to provide a uniform dose of butyric acid (280 g/T). The CD without butyric acid supplementation was fed to another group to compare the performance of layers fed diets with and without butyric acid. Each diet was offered *ad libitum* to ten replicates from 38 - 54 weeks of age.

Eggs were collected twice daily to record daily egg production (EP) and quantity of feed consumed to produce an unit egg mass (feed efficiency – FE). Average egg weight (EW), egg density (ED), egg breaking strength, shell weight and shell thickness were recorded on all the eggs produced during the last 3 days of each period (28 d interval). Egg mass (EM) was calculated by multiplying the average EW and EP percent and expressed as g per bird per day. During period 4, a 3-day metabolic trial was conducted (six replicates per treatment) after 7 day adaptation period to study the apparent digestibility of energy and protein by the total excreta collection method. The data of all 4 periods were pooled and subjected to a one way analysis of variance (Snedecor and Cochran, 1980).

III. RESULTS AND DISCUSSION

The pooled data of 4 periods (16 weeks) indicated that incorporation of butyrate in both forms in diet did not influence ($P > 0.05$) EP, FI, FE, EW, EM, SW or ST. However, ED improved significantly ($P < 0.05$) in groups fed both forms of butyrate compared to those fed the CD without butyrate. The improved ED might be due to non-significant ($P > 0.05$) increase in shell thickness in layers fed both forms of butyrate. Haugh unit score was not affected ($P > 0.05$) by treatments in the present study. The percentage of eggs produced by SBP (84.04) was higher than control (83.43) or CB (83.14) groups without significant differences. Similarly, feed intake to produce a unit EM in SBP (2.191 g) was lower than those fed other two diets (2.236 and 2.247 g). Livability in SBP (96.2 %) was higher than CD and CB (95.8 % and 94.8 %, respectively) fed groups.

Supplementation of SBP significantly improved the digestibility of energy ($P < 0.001$) and protein ($P < 0.005$) compared to those fed the CD or CB supplemented diet. The improvement in layer performance observed in this study could be due the beneficial role of protected sodium butyrate. The calcium butyrate (CB) was found to be ineffective in

improving these parameters. Supplementation of sodium butyrate was reported to enhance the development of intestinal epithelium (Mallo et al., 2010) and reduce pathogenic bacteria count in chicken intestine (Van Immerseel et al., 2005; Fernandez-Rubio et al., 2009). Though the majority of layer performance parameters showed improvements with SBP supplementation compared to the control group, the difference did not reach significance, which may be due to dose of the organic acid included in the current study. The non-significant improvement in layer production parameters observed in SBP fed groups in the present study may partly be due to the beneficial effects associated with optimum gut development (Mallo et al., 2012) and possible reduction in pathogen count in the intestine (Rama Rao et al., 2004). From the obtained data it can be concluded that sodium butyrate protected with fat is more effective than calcium butyrate, as it increases eggshell quality, nutrient digestibility and layer production.

Table 1 - Performance of WL layers fed different forms of butyric acid

Treat	EP	FI	FE	EW	EM	SW	ST
	%	g/b/d	FI/EW	G	g/d	%	Mm
Control	83.43	101.3	2.236	54.38	45.37	8.984	0.379
SBP	84.04	100.2	2.191	54.47	45.78	8.985	0.385
CB	83.14	101.4	2.247	54.38	45.22	9.039	0.384
P	0.734	0.135	0.161	0.870	0.651	0.956	0.685
N	10		10	10	10	10	10
SEM	0.466	0.274	0.0127	0.078	0.248	0.0828	0.0030

$^{A\,B}$ means having common superscript in a column do not vary significantly (P < 0.05)

Table 2 - Performance of WL layers fed different forms of butyric acid

Treat	ED	BS	HU	Energy	Protein
		N		%	
Control	1.047^{B}	13.32	72.25	71.76^{B}	71.24^{B}
SBP	1.052^{A}	13.50	73.58	80.64^{A}	75.52^{A}
CB	1.052^{A}	11.75	71.83	73.24^{B}	71.69^{B}
P	0.017	0.062	0.653	0.001	0.005
N	10	10	10	6	6
SEM	0.0009	0.338	0.787	1.056	0.656

$^{A\,B}$ means having common superscript in a column do not vary significantly (P < 0.05)

SBP sodium butyrate protected with palm stearine, CB calcium butyrate, EP egg production, FI feed intake, FE feed efficiency, EW egg weight, EM egg mass, SW shell weight, ST shell thickness, ED egg density, BS egg breaking strength, HU Haugh unit score

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EFFECTS OF DIETARY AVAILABLE PHOSPHORUS AND CALCIUM LEVELS WITH OR WITHOUT PHYTASE ON PERFORMANCE OF HY-LINE LAYING HENS

T.Y. YANG[1], X. LI[1], D. ZHANG[1] and W.L. BRYDEN[1]

In our previous study, it was found that the requirement of available P (AP) was much lower than the recommendation of NRC (1994) and the values currently used by industry (Zhang et al., 2013) when Hy-line brown egg laying hens were fed diets containing graded levels of AP (1.5, 2.0, 2.5, 3.0, 3.5 and 4.5g/kg diet) and the same Ca level of 42 g/kg diet from 20 to 80 weeks of age. Dietary AP of 1.5g/kg had no significant detrimental effect on egg production, egg mass or eggshell quality. The objective of this study was to investigate the effect of different dietary Ca levels on AP requirement with or without phytase supplementation in laying hens from 16 to 80 weeks of age.

A total of 720 Hy-Line brown egg laying hens (Hy-line Australia) were randomly allocated into 120 cages with 6 layers per cage in a controlled environmental (24-26°C) shed with 16 hours light/day. The experimental diets based on sorghum and wheat contained AP levels of 1.5 and 2.5 g/kg diet and each with three levels of Ca (32, 40 and 48 g/kg diet). The diets were prepared with or without phytase (Phyzyme XP, 10000 FTU/g) supplementation. Each diet was fed to 10 replicate cages. Egg production and egg shell defects were recorded daily. Body weight, feed intake, egg weight, egg and eggshell quality (specific gravity, albumin height, Haugh Unit, yolk colour, egg shell colour, breaking strength, weight and thickness) parameters were measured every four weeks.

The results up to 52 weeks of age indicate that there were no significant differences between treatments in hen day egg production, egg shell defect percentage, feed intake and feed egg conversion ratio. However, overall there was an indication that as the AP to Ca ratio became greater bird performance decreased. Egg mass was significantly lower in layers fed on diets contained AP level of 2.5 and Ca level of 32 g/kg without phyatse supplementation than most of other treatments from 17 to 52 weeks of age. There were no significant differences between treatments in egg and egg shell quality parameters measured from 17 to 52 weeks of age. However, the yolk colour was lighter in layers fed on diets contained lower Ca levels than those fed higher Ca levels.

The results from current study indicate that high AP and low Ca levels in layer diets appear to have a detrimental effect on layer production performance up to 52 weeks of age although the differences were not statistical significant. This observation may become important for bird production and welfare in the later period of the laying cycle. It is also confirmed that AP requirement of layers is much lower than NRC (1994) recommendations.

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EXPLORING THE CALCIUM APPETITE OF LAYERS WITH CHOICE FEEDING

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Summary

Previous studies have demonstrated the ability of poultry to self-select their own diets in order to meet their nutritional requirements. Calcium (Ca) is essential for biological processes, skeletal integrity and eggshell production. Poultry are known to possess a specific appetite for Ca, and will seek feedstuffs with a high Ca concentration when the diet is deficient. To confirm that contemporary layers maintain a Ca specific appetite, 80 Isa Brown layers were fed one of four dietary treatments in conjunction with a separate Ca source. Dietary treatments were formulated to one of four levels of total dietary Ca (1, 2, 3 and 4% Ca). Weekly separate Ca intake was significantly influenced by dietary Ca concentration ($P < 0.05$) where birds that were fed high dietary Ca ate significantly less Ca than those on low Ca diets. Egg production was also affected by dietary Ca concentration, with birds receiving 1% dietary Ca laying significantly less eggs than birds receiving 4% dietary Ca. Dry egg shell weight and egg shell thickness were significantly lowest in birds receiving 1% dietary Ca than birds that received 3% dietary Ca.

I. INTRODUCTION

Calcium (Ca) is an essential nutrient for poultry for biological processes such as bone development and egg shell formation (De Vries *et al.*, 2010). Laying hens must be provided with Ca at approximately 4% of the total feed volume. Very little Ca is provided within the cereal grain component of the diet, therefore diets must be supplemented with sources of Ca. Dietary Ca is provided to layers as either a calcium carbonate grit or flour, such as limestone, with a small proportion being provided by the inorganic P source and also from meat and bone meal, if used in the diet. Although Ca is a necessary dietary component, the addition of limestone can result in a reduction of the digestibility of key nutrients in the diet, in particular phytate-P (Selle *et al.*, 2009).

Commercial poultry diets are typically corn- and/or wheat-soy based and contain relatively high levels of phytate-P which has limited availability for poultry (Cowieson *et al.*, 2004; Selle *et al.*, 2009). Phytate carries a strong negative charge, and thus has a high affinity for divalent cations (Angel *et al.*, 2002). Due to the high inclusion levels of Ca in the diet, Ca^{2+} is the dominant cation in the diet and chelates with phytate, forming Ca-phytate complexes (Selle *et al.*, 2009). The formation of Ca-phytate complex reduces the bioavailability of both Ca and P, effecting skeletal health and egg quality. To improve the digestibility of phytate-P and other nutrients as well as to maintain skeletal integrity, physical and temporal separation of Ca from phytate may be necessary.

Laying hens are known to possess a specific appetite for Ca (Wood-Gush and Kare 1966; Hughes and Wood-Gush 1971; Joshua and Mueller 1979). This specific appetite is thought to originate from the ancestral Red Jungle Fowl, whereby the bird would have foraged consuming seeds, roots, insects and soil/silicates to satisfy nutritional requirements. This study aims to exploit the putative Ca specific appetite of layers to investigate the effect of

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diets with reduced dietary Ca concentration in conjunction with a separate source of Ca on bird performance and egg quality.

II. MATERIALS AND METHODS

All experimental procedures conducted had approval from The University of Sydney Animal Ethics Committee. A total of 80 Isa Brown laying hens (approximately 35-42 weeks of age) were randomly allocated to one of four dietary treatments, with 20 replicate birds per treatment. Dietary treatments were formulated to four different Ca concentrations (1, 2, 3 or 4%.), were wheat-soy based and formulated to an AME of 11.72 MJ/kg. Birds had access to a separate Ca source, approximately 95% $CaCO_3$ with an average particle size of 1.4 mm (AB Grit, Omya Australia). Birds had ad *libitum* access to feed, water and the separate Ca source throughout the study. Birds were housed in individual cages (23cm W x 45cm D x 45cm H) in an environmentally controlled shed and were exposed to a lighting regime of 16h:8h (light:dark). Prior to the commencement of the study, birds were placed into cages two weeks beforehand and fed the 4% Ca. This was to ensure birds were habituated to their environment and reduce stress which could have affected the study's results.

Individual bird body weight and plasma Ca an P concentrations was measured at the start and end of the trial. Approximately 3 ml of blood was collected from the brachial vein from each bird. Samples were refrigerated overnight before being spun to separate the plasma that were analysed for Ca an P. Feed intake, separate Ca source intake and egg production were recorded daily. Egg quality (egg weight, albumin height, dry eggshell weight, eggshell thickness and Haugh units) was measured three times per week. All data were analysed using REML including random effects in GenStat (14th Edition, VSN International).

III. RESULTS AND DISCUSSION

Feed and separate Ca intake were analysed using weekly values while egg quality parameters were analysed using treatment averages. Feed intake increased over time (Figure 1) and overall was significantly higher in birds fed 3% Ca and lowest in birds fed 1% Ca (Table 1). Birds fed 2 or 4% Ca diets were intermediary. Separate Ca intake (Figure 2) decreased with increasing dietary Ca concentration ($P < 0.05$). Body weight was not influenced by diet but birds were significantly lighter at the conclusion of the study ($P = 0.003$) with birds fed 1% Ca losing approximately 172g over the study. Birds fed 1% Ca had numerically lower plasma Ca and P concentrations at the conclusion of the study when compared to the start of the study while birds from the other groups had increased plasma Ca concentrations. Birds fed the 2% Ca diet had significantly lower plasma Ca concentration than birds fed 4% Ca.

Table 1 - Effect of dietary calcium concentrations on feed intake, egg quality

Ca Level (%)	Feed intake (g/b/d)	Egg production (n)	Egg weight (g)	Albumin height (mm)	Haugh units	Eggshell thickness (mm)	Eggshell weight (g)
1	106.5^{c}	30^{b}	64.2	7.67	86.3	0.35^{c}	5.41^{b}
2	112.6^{b}	38^{a}	62.8	7.35	84.4	0.37^{bc}	5.84^{a}
3	116.8^{a}	40^{a}	63.4	6.85	79.7	0.40^{a}	6.30^{a}
4	110.9^{b}	41^{a}	62.3	7.25	83.5	0.39^{ab}	6.13^{a}
SEM	1.13	0.8	0.75	0.132	0.96	0.004	0.094
P	< 0.001	< 0.001	0.838	0.187	0.115	< 0.001	0.004

^{abc}Data that have different superscripts are statistically different ($P < 0.05$)

The influence of dietary Ca on egg quality results are presented in Table 1. Egg production increased with increasing dietary Ca and birds fed 1% Ca diets laid significantly less eggs than birds fed the other diets. Eggshells from birds fed 1% Ca diets were significantly thinner than those from birds fed diets containing 3 and 4% Ca. Dry egg shell weight was lowest in birds fed diets containing 1% Ca when compared to the other groups ($P < 0.05$). No significant difference between groups was observed for egg weight, albumin height or Haugh units.

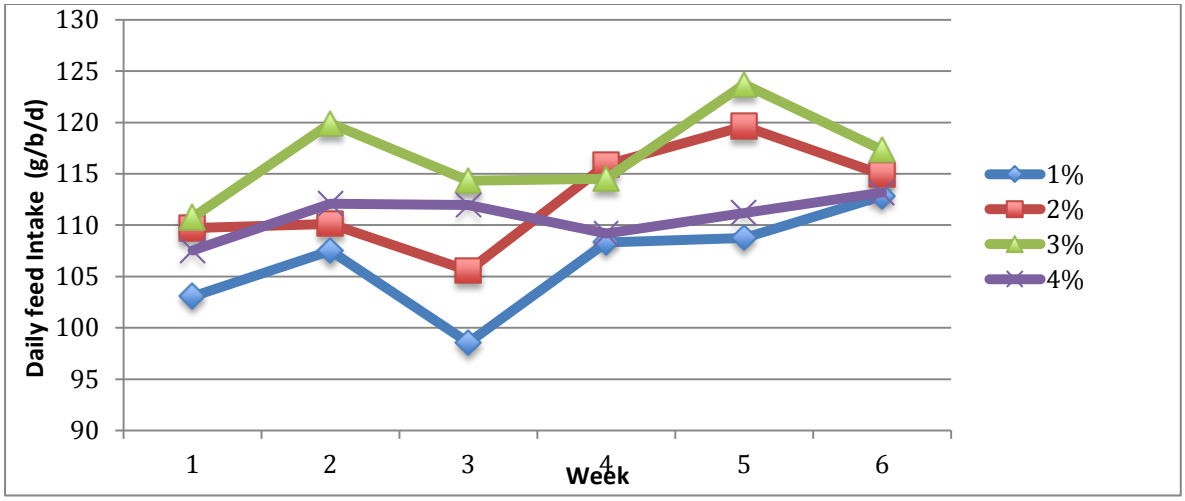

Figure 1 - Average weekly feed intake of diets over the experimental period. Diet $P < 0.001$, Week $P = 0.004$, SEM 1.132.

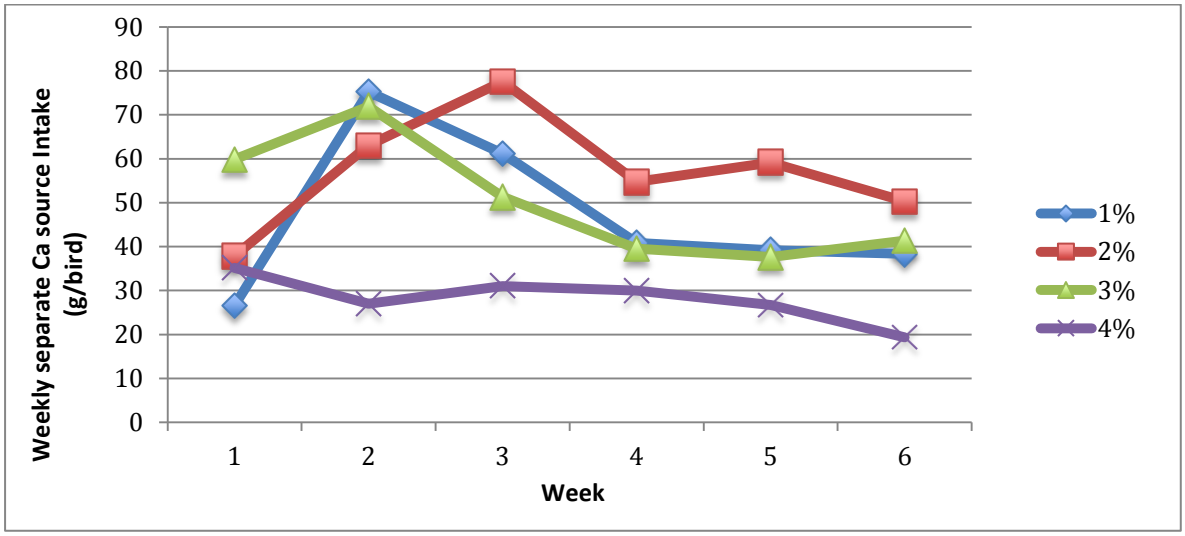

Figure 2 - Average weekly separate calcium source intake over the experimental period. Diet $P = 0.007$, Week $P = 0.142$, SEM 3.371.

Poultry have been shown to possess a specific appetite for Ca (Wood-Gush and Kare 1966; Hughes and Wood-Gush 1971; Joshua and Mueller 1979) and the results of the present study are in general agreement with this. Birds decreased their consumption of the separate Ca source with increasing dietary Ca. This is consistent with the findings of Griminger and Lutz (1964) who showed birds consumed less supplemental Ca when fed high Ca diets, while

birds had a high intake of supplemental Ca when fed low Ca diets. The results of the present study also show that some birds fed low Ca diets did not adapt to separate Ca choice feeding, particularly those fed 1% Ca diets and it is possible that 1% dietary Ca was too low in the context of this study. Similarly, Salim (1981) showed that some hens refused to eat a Ca supplement when dietary Ca was as low as 0.5%. The results did show that feeding diets with 2% Ca is feasible, however, birds may take time to find an equilibrium. It appears that birds fed diets with 1-3% Ca were able to recognise the Ca deficiency in week 1 and over compensated for this in weeks 2 and 3 before reaching an equilibrium point in week 4. If the 1% dietary Ca groups is excluded from the results from week three onwards, the consumption of the separate Ca source follows the assumption that birds on a low Ca diet will increase their separate Ca intake accordingly.

Birds fed 1% Ca diets produced significantly less eggs, with thinner and lighter eggshell weights and may be described as being Ca deficient. Ca deficiency decreases egg production and a reduces the thickness of the eggshell (Scott *et al.*, 1971). Several birds in this study fed the 1% Ca diet ceased egg production and one bird in particular regularly ate its own egg shell, possibly in an attempt to obtain Ca.

It can be concluded that the Ca-specific appetite is not universally or uniformly expressed in laying hens. Though some birds were able to regulate to a common Ca intake and maintain production when fed reduced dietary Ca, others were unable to adapt to this new feeding regime. Further work is required to explore the optimum time, ontogenetically, to introduce a separate Ca source and to consider groups of birds where birds with reduced ability to self-regulate may learn from the early adopters. The effect of separating Ca from phytate on nutrient digestibility in layers is currently being assessed. In the future it may be possible to spatially separate Ca from the basal diet and exploit the Ca-specific appetite of layer hens to specifically improve the digestibility of phytate-P and amino acids. This would enhance the profitability and sustainability of the egg industry.

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EGGSHELL UNIFORMITY AND THE RELATIONSHIP BETWEEN SHELL STRUCTURES AND SHELL TRANSLUCENCY, EXAMINED BY COMPUTATED TOMOGRAPHY (CT) AND SCANNING ELECTRON MICROSCOPY (SEM)

A. RAY[1], J.R. ROBERTS[1], K. CHOUSALKAR[2] and R. HALING[1]

While it has been determined that eggshell translucency is caused by the accumulation of moisture within the structures of the shell, it is still unclear precisely which shell structures are related to translucency. As part of a larger study, initial work aimed to determine how many shell samples from a single egg were required to adequately identify any features or abnormalities of that shell. SEM and CT analysis of shell samples is a time consuming process so avoiding unnecessary replication would allow for more eggs to be examined.

SEM has been used by many researchers to examine the mammillary ultrastructure of eggshells. Micro CT is a new way of investigating shell ultrastructure, allowing the construction of digital 3D models and viewing of transverse images of the shell. Micro CT also allows us to view transverse shell images from different angles, allowing for more accurate identification of pores and their structures. Micro CT is limited by long scans of ~10 minutes plus analysis time.

150 shell samples, 3 taken from the midline of 50 commercially laid table eggs were scanned in a GE Phoenix V-tomex CT Scanner at 70x magnification with a final resolution of 5.64µm. 80 of these same samples were processed and the mammillary layers were viewed at 100x and 200x magnification using a JEOL Neoscope SEM.

Results from the eggshell uniformity component showed that there was no significant difference between repeated same samples from an individual egg for all measures except the number of type B mammillary bodies. While there were some cases of variation between samples most were similar. Micro CT results comparing shell features to shell translucency found that there was a significantly increased rate of externally branching pores found in the high translucency score group, and more straight pores found in the low translucency score group. The average number of internally branching pores was the same for both groups at 0.3. Although a trend could be seen in some of the SEM results, there were no significant differences between the high and low translucency score groups.

Table 1 - Micro CT results showing average numbers of pores

	Average Number of Pores per sample (4.19mm^3)			
Translucency Score	Straight Pores	Internally Branching Pores	Externally Branching Pores	CT Determined Volume
High	1.92[a]	0.03	2.00[a]	1.11[a]
Low	2.73[b]	0.03	0.79[b]	0.99[b]

Table 2 - SEM results showing ultrastructural features of high and low translucency shells

Translucency Score	Mammillary Body Size (-3 to 3)	Fusion (-3 to 3)	Type A (0 to 3)	Type B (0 to 3)	Cubic Formations (0 to 3)
High	-0.96	0.61	0.91	0.87	0.09
Low	-1.11	0.61	0.33	0.17	0.00

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PROTOPORPHYRIN QUANTIFICATION FROM EGGSHELLS OF LAYING HENS CHALLENGED WITH INFECTIOUS BRONCHITIS VIRUS STRAINS (N1/88 AND T STRAINS)

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Summary

Eggshells were collected at days 1, 3 and 5 post infection from unvaccinated Isa Brown laying hens challenged with infectious bronchitis virus (IBV) strains N1/88 or T as well as from a control group of unvaccinated hens. Shell reflectivity (%) and shell color L* were measured on shells with and without cuticle and were further processed for the quantification of protoporphyrin IX (PP IX) from the same shell with and without the presence of cuticle. Shell reflectivity and shell color L* values for the control eggs were significantly lower ($P < 0.0001$) compared to those from hens challenged with both strains of IBV and were also significantly lower on day 1 compared to days 3 and 5 p.i. The PP IX was quantified by spectrophotometric analysis of digested eggshell solutions. The mean PP IX in 1g of shell with and without cuticle was significantly higher on day 1 compared with days 3 and 5. The amount of PP IX in the shell decreased with time post infection for the IBV groups. The amount of PP IX in whole egg shell was highest for the control group and lowest for the T strain group, with the N1/88 group intermediate.

I. INTRODUCTION

Infectious bronchitis virus (IBV) is one of the factors responsible for eggshell deterioration and lighter shell color (Chousalkar and Roberts, 2009). The Australian strains of IBV have the ability to multiply in the shell forming region of oviduct of unvaccinated Isa Brown hens (Chousalkar and Roberts, 2007). The T strain of IBV is mainly nephropathogenic but has shown some effect on the oviduct of hen with the production of lighter color shells (Chousalkar and Roberts, 2009). The N1/88 strain infects the oviduct in laying hens causing inferior internal quality and shell quality (Chousalkar *et al.,* 2007). The eggshell color of brown eggs is a quality aspect for consumers (Curtis *et al.,* 1985). Shell color has been linked to egg quality parameters in brown eggs (Jones *et al.,* 2010) and with some known antimicrobial properties (Ishikawa *et al.,* 2010). Protoporphyrin IX, the main eggshell pigment (Kennedy and Vevers, 1976), is also used as a tool to assess the stress level and disease status of laying hens (Martinez-de la Puente, *et al.,* 2007). The objective of the present study was to quantify the amount of PP IX from the cuticle and true shell in brown shelled eggs of Isa Brown laying hens challenged with N1/88 and T strains of IBV, as compared to a control group.

II. MATERIALS AND METHODS

The amount of protoporphyrin was quantified from eggshells of unvaccinated Isa Brown laying hens challenged with T and N1/88 strains of IBV and from a control group flock.

Shell reflectivity (%) and shell color (L* component of L*a*b*) were measured on the shells using a reflectivity meter (Technical Services & Supply) and a Konica Minolta spectrophotometer (CM-2600d), respectively. Shell reflectivity, expressed as a percentage, is an indicator of shell color lightness – the higher the value, the lighter the color of the eggshell

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and vice versa. In the L*a*b* color space system, L* represents the grading between white (100) and black (0). The higher the value for L*, the lighter the shell color and vice versa. Each eggshell, individually, was soaked in an EDTA solution (0.34 M, pH 7.5) for 5 minutes and the cuticle was carefully scrubbed off in running tap water using a soft brush. Shell reflectivity and shell colour L* were measured as described earlier for the eggshells with cuticle intact.

Eggshells from the three groups on days 1, 3 and 5 post infection (p.i.) were analyzed for PP IX in whole eggshell including the cuticle as well as in eggshell from which the cuticle had been removed. The shells were soaked for 2 hr in water, the shell membranes removed manually and the shells were allowed to dry thoroughly. A 0.250 g sample from each shell was weighed into a 10 mL plastic centrifuge tube into which 4 mL of methanol- concentrated (36%) HCl (2:1) solvent was added. All tubes were wrapped in aluminum foil and placed in a refrigerator for 3 hr, avoiding exposure to light. The samples were centrifuged at 3000 rpm for 30 minutes. After centrifugation, the supernatant solution was decanted into spectrophotometer cuvettes and the absorbance of the supernatant was read at 412 nm (Shimadzu, UV-1201).

A standard curve was constructed from solutions of protoporphyrin (Sigma Aldrich Australia) ranging from 0 mM to 6.87 x 10^{-6} mM. The amount of protoporphyrin in 1g of eggshell (with and without cuticle present) was calculated. For determination of the amount of PP IX in the cuticle, the values of the eggshell samples without cuticle were subtracted from the values of the eggshell samples with the cuticle. Data were analyzed using Statview Software (SAS Institute Inc., Version 5.0.1.0). A two way analysis of variance (ANOVA) was conducted taking day (p.i.) and group as independent variables and reflectivity, shell color and amount of PP IX in shell with and without cuticle as dependent variables. Level of significance was indicated by probability of less than 5%. The Fishers PLSD test was used to differentiate between mean values.

III. RESULTS

There was a significant main effect ($P < 0.0001$) of day (p.i.) and group (i.e. control, N1/88, T strain) as well as a significant interaction between the two ($P < 0.005$) for shell reflectivity (%) and shell color L* measured on eggshells with and without cuticle. Egg shells from the T strain and N1/88 strain infected flocks had significantly higher reflectivity and shell color L* values when compared with the control group and the results for the two IBV strains were not different except for L* with cuticle intact which was lower for the N1/88 infected shells (Table 1). The reflectivity and L* values were significantly higher on days 3 and 5 compared to day 1 while days 3 and 5 were different only for L* with cuticle intact (Table 2). A similar pattern of results was observed when the shell reflectivity and shell color L* were measured on eggshells from which cuticle was artificially removed.

For a 1 g piece of eggshell, there was more PP IX in the shell with cuticle intact, as compared with a piece of shell from the same eggshell with cuticle removed. The total amount of PP IX in 1 g of shell both with and without cuticle was significantly higher in day 1 p.i. eggshells compared to days 3 and 5 p.i. which were not significantly different from each other (Table 3). Among the treatment groups, for shells with and without cuticle, control group eggshells had the most protoporphyrin, T strain the lowest, with the N1/88 group intermediate (Table 4).

Table 1 - Shell reflectivity and shell color L* values of eggshells from control, N1/88 and T strain challenged flocks

Group	Treatment group			P Value
	control	N1/88 Strain	T Strain	
Shell Reflectivity (%) of eggshell with cuticle intact	[b]30.06±0.33	[a]32.35±0.37	[a]33.23±0.35	<0.0001
Shell Reflectivity (%) eggshell with cuticle removed	[b]36.17±0.40	[a]38.29±0.37	[a]38.71±0.41	<0.0001
Shell color L* of eggshell with cuticle intact	[c]61.86±0.29	[b]63.86±0.30	[a]64.83±0.28	<0.0001
Shell color L* of eggshell with cuticle removed	[b]67.30±0.32	[a]68.96±0.28	[a]69.08±0.32	<0.0001

[a, b, c] Across a row, values with different superscripts are significantly different from each other. Values are Mean ± SE

Table 2 - Shell reflectivity and shell color L* values of eggshells processed post infection from control, N1/88 and T strain challenged flocks

Group	Day (p.i)			P Value
	1	3	5	
Shell Reflectivity (%) of eggshell with cuticle intact	[b]30.90±0.28	[a]32.35±0.44	[a]33.23±0.41	<0.0001
Shell Reflectivity (%) eggshell with cuticle removed	[b]35.68±0.29	[a]39.18±0.46	[a]40.03±0.43	<0.0001
Shell color L* of eggshell with cuticle intact	[b]62.59±0.23	[a]64.04±0.38	[a]64.82±0.28	<0.0001
Shell color L* of eggshell with cuticle removed	[c]66.63±0.22	[b]69.57±0.35	[a]70.58±0.30	<0.0001

[a, b, c] Across a row, values with different superscripts are significantly different from each other. Values are Mean ± SE

Table 3 - Protoporphyrin in mM in 1 g of shell collected on days 1, 3 and 5 (p.i.)

Group	Day (p.i)			P Value
	1	3	5	
Eggshell with cuticle intact	[a]9.953×10^{-8}	[b]8.320×10^{-8}	[b]8.311×10^{-8}	<0.0001
Eggshell with cuticle removed	[a]8.042×10^{-8}	[b]6.713×10^{-8}	[b]6.664×10^{-8}	<0.0001

[a, b] Across a row, values with different superscripts are significantly different from each other. Values are Mean

Table 4 - Protoporphyrin in mM in 1g of shell collected from the treatment groups

Group	Treatment group			P Value
	control	N1/88 Strain	T Strain	
Eggshell with cuticle intact	[a]9.415×10^{-8}	[ab]9.132×10^{-8}	[b]8.754×10^{-8}	<0.0001
Eggshell with cuticle removed	[a]7.538×10^{-8}	[ab]7.370×10^{-8}	[b]7.093×10^{-8}	0.0057

[a, b] Across a row, values with different superscripts are significantly different from each other. Values are Mean

IV. DISCUSSION

The reduction in shell colour resulting from challenge of unvaccinated hens with T strain and N1/88 strain IBV has been reported previously (Chousalkar and Roberts, 2009) and the current study utilized egg shells collected during that experimental trial. The results of protoporphyrin confirmed that the loss of shell colour resulting from IBV challenge correlates with a smaller amount of the pigment protoporphyrin being deposited into the egg shell. The current study is on-going and will investigate protoporphyrin levels in egg shells up to 10 weeks post infection.

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PERFORMANCE AND EGGSHELL QUALITY IN LAYING HENS FED TWO DIETS THROUGH THE DAY WITH DIFFERENT LEVELS OF CALCIUM OR PHOSPHOROUS

J. DE LOS MOZOS[1], A. SACRANIE[1] and T. VAN GERWE[1]

Summary

A trial with 6 treatments was designed in order to study the effect of different calcium and phosphorous levels in the morning and afternoon within the Split-Feeding system. Each treatment had 36 replicates of, individually caged hens. Animals were observed for 9 weeks, from 57 to 65 weeks of age. The control diet was a single diet formulated to meet the laying hens' requirements. Hens from treatments 2 to 6 were fed two diets. Morning and afternoon diets of treatments 2 and 3 varied in calcium levels whereas in treatments 4, 5 and 6 varied in available phosphorous levels. Animals of Treatment 2 exposed to low calcium level in the morning diet and higher in the afternoon showed lower daily calcium intake than CVB recommendations (4.32 g/d) without impairing performance and egg-shell quality. Treatment 3, in which morning calcium was also low but afternoon level was increased more, did not bring additional benefit and resulted in a higher calcium intake compared to the control. Reduction of the P level in the morning diets (Treatment 4) or in the morning and afternoon diets (Treatment 6) resulted in lower P intake than CVB recommendations (0.396 g avP/d) without affecting performance and eggshell quality.

I. INTRODUCTION

The protein, calcium and energy requirements of laying hens do not remain constant, but vary during the day depending on the hen's physiological requirements for the various stages of egg formation. The current method of feeding hens with a single or one diet with a constant level of nutrients may not result in optimal utilization of nutrients (Chah, 1985, Leeson and Summers, 1997). Nutreco has developed the Split-Feeding system based on the concept of feeding hens two diets with different nutrients levels during the morning and the afternoon to meet the different requirements through the day. The success of the Split-Feeding system relates to the fact that current layer hen lines lay the majority of eggs during the morning (Etches, 1987, Larbier and Leclercq, 1994, Lewis *et al*., 2004). This feature makes the use of different feeding systems as the Split-feeding easier to be applied as many of the animals in a flock are in the same egg formation phase with similar requirements. In a previous experiment, de los Mozos et al (2012a) tested the hypothesis of providing a high energy, high protein and low calcium feed during the morning and a low energy, low protein and high calcium diet during the afternoon. In this previous experiment, performance was maintained at a significantly lower daily energy and protein intake with the split feeding programme compared to the control group in which standard feeding was applied. A second trial (de los Mozos et al, 2012b) showed that using the Split-Feeding it is possible to reduce the AME content of the afternoon feed without losing performance, but also the crude protein content can be reduced at the same time without negative effects on performance.

Taking into account the principle of the system, calcium levels during the morning and afternoon can also be optimized (less calcium intake than with a single feed) resulting in equal or improved eggshell quality, as observed when a single feed is used. When Split-Feeding is applied, the phosphorus intake can clearly be reduced compared to a single feed as hens need the phosphorous during the morning to re-calcify the medullar bone used during the previous night. It is hypothesized that with Split-Feeding the requirement of calcium

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from the medullar bone is lower as this system more accurately meets the alleviated calcium requirements in the afternoon for shell formation than a single feed. Therefore, as an indirect effect, the phosphorus requirements might be lower with the Split-Feeding system.
The objective of this experiment was to study the effect of different levels of calcium and phosphorous in the morning and afternoon diets on egg weight, laying performance, feed consumption and eggshell quality.

II. MATERIALS AND METHODS

Two hundred and sixteen ISA Brown Classic laying hens, individually caged, at 57 weeks were housed in one facility of the Nutreco Poultry Research Centre. The experiment was performed for a period of 9 weeks. Water and feed were available ad libitum, and hens were exposed to 16 hours of continuous light per day. The experiment included 6 treatments (Table 1) with 36 replicates per treatment in the experiment. Treatment 1 was a single feed formulated to meet the laying hen requirements (CVB recommendations) the other treatments fed two feeds following the Split-Feeding system. The morning diet for treatments 2 to 6 had increased levels of energy and protein and the afternoon diet had lower energy and protein levels compared to the single feed (de los Mozos *et al*. 2012b). So, treatments under Split-feeding only varied in the level of calcium or available phosphorous, as shown in table 1. All feeds were wheat/maize /soya based. Morning feeds were provided from 07.30 to 14.30 and afternoon feeds from 14.30 to 07.30. The aim was to achieve a feed intake distribution of 40 % of the total feed intake during the morning and of 60 % during the afternoon. Morning and afternoon feed intake, egg production and egg weight were recorded weekly. Every three weeks of the 9 week trial one egg per hen was sampled (36 per treatment) to analyse egg-shell weight per egg surface area (SWUSA), egg-shell thickness, and egg-shell weight. The effect of treatment on feed intake, egg production, egg weight and egg-shell quality was analysed by using a linear mixed model with the Mixed procedure of SAS.

Table 1 - Description of treatments

Treatment	Morning	Afternoon	Morning	Afternoon
	Ca, g/kg		av. P, g/kg	
1	Nutreco recommendations		Nutreco recommendations	
2	-45%	+15%	Nutreco recommendations	Nutreco recommendations
3	-45%	+40%	Nutreco recommendations	Nutreco recommendations
4	Nutreco recommendations	Nutreco recommendations	-15%	Nutreco recommendations
5	Nutreco recommendations	Nutreco recommendations	Nutreco recommendations	-20%
6	Nutreco recommendations	Nutreco recommendations	-15%	-20%

III. RESULTS

Results are shown in Table 2 (performance and feed intake) and table 3 (eggshell quality and nutrients intake). Daily feed intake was unaffected by treatment diet, and consequently, Ca and av. P intake reflected the dietary composition with respect to Ca and av. P intakes (Table 2). No differences in egg weight, egg production and egg mass were observed between

treatments. However, treatment 3 had numerically lower egg mass values, resulting in higher FCR compared to treatment 1 and 4.

Table 2 - Performance parameters of laying hens from 57 to 65 weeks of age

Treatment	Feed intake, g/d	Calcium Intake, g/d	av. P Intake, g/d	Egg weight, g	Egg production, %	Egg Mass, g	FCR, g/g
1	115.4	4.417b	0.439a	65.43	87.26	56.90	2.060b
2	117.6	4.222d	0.433a	65.26	87.21	56.73	2.109ab
3	116.9	4.973a	0.428a	64.54	87.07	55.82	2.147a
4	117.3	4.457b	0.403b	63.83	91.17	58.23	2.044b
5	116.5	4.427b	0.384c	64.45	88.26	56.71	2.083ab
6	116.4	4.420b	0.359d	65.13	87.73	56.97	2.078ab
SEM (n=36)	1.192	0.049	0.004	0.708	1.395	0.896	0.026
P treatment	0.136	<.0001	<.0001	0.732	0.604	0.531	0.001
P week	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001
P week*treat	0.0006	0.0003	<.0001	0.310	0.048	0.163	0.103

The egg shell quality results (Table 3) revealed no differences between treatments indicating the different av. P levels and Ca levels tested did not impair egg shell quality parameters.

Table 3 - Egg-shell quality results

Treatments	Broken Eggs, %	Eggshell weight, g	Eggshell thickness, mm	SWUSA, mg/cm^2
1	3.188	6.194	0.376	81.44
2	2.670	6.313	0.383	83.26
3	2.806	6.231	0.381	83.18
4	3.265	6.236	0.383	83.13
5	3.243	6.208	0.378	82.22
6	3.487	6.307	0.383	83.09
SEM (n=36)	0.817	0.074	0.003	0.750
P treatment	0.990	0.766	0.256	0.197
P week	<.0001	0.041	<.0001	0.003
P week*treat	0.546	0.674	0.031	0.052

IV. DISCUSSION

The results of this study support the case for adopting the Split-Feeding system with the two diets optimizing nutrients intake without compromising performances and egg-shell quality (de los Mozos *et al.*, 2012a). The same author concluded that giving laying hens an energy and protein rich diet during the hours of albumen production and a calcium rich diet during the hours of shell calcification improved nutrient utilization without affecting egg shell quality, because of a more balanced nutrient intake in accordance with the needs for production (de los Mozos *et al.*, 2012b).

Calcium inclusion level in the morning feed can be reduced 45% the CVB recommendations increasing the afternoon level 15%. This change reduced the calcium intake compared to the CVB recommendations for a single diet (36 g/kg, with a feed intake of 120 g/d and an egg size higher than 60 g; 4.320 g/d for old hens) without any detrimental effect in performance and eggshell quality. Treatments in which morning calcium was reduced and afternoon calcium level increased did not give any added benefit compared to the previous one and increased FCR compared to the single diet. Although NRC (1994) recommends 36 g/kg of calcium to maximize performances higher levels could be needed to improve the eggshell quality. Safaa et al. (2008) indicated that Brown egg-laying hens late in the production cycle require more than 4.08 g of Ca/hen per day which agrees with the report of Lichovnikova (2007), who recommended 4.51 g of Ca/hen per day to ensure eggshell quality in the last third of the production cycle. In contrast, Rao et al. (2003) observed no benefit on eggshell weight or shell thickness when the Ca intake was increased from 3.51 to 4.82 g/hen (white strain) per day (3.25 to 4.50% of the diet).

Available phosphorous level in the morning diet can be reduced without affecting performance. Phosphorous level in the afternoon feed can also be reduced. Reducing the afternoon phosphorous level does not have a detrimental effect on performance and eggshell quality. CVB recommends a daily av. P intake of 0.396 g/d (3.3 g avP/kg feed). Treatments 5 and 6 had a lower phosphorous intake than recommended by CVB. Isa recommends 3.1 g/kg; 0.365 g/d considering a feed intake of 117.9 g/d) for a single feed. Only hens from treatment 6 consumed less available P than the genetic company recommendations.

It can be concluded that when calcium and phosphorous are fed in the moments the laying hens need them, total calcium and phosphorous intake can be reduced below established recommendations (CVB, NRC or genetic companies) without impairing performance and egg-shell quality.

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DURATION OF IMMUNITY FOLLOWING THE USE OF POULVAC® NEWCASTLE iK VACCINE IN PIGEONS

P.C. SCOTT[1], T.B. WILSON[1] and C. WALKER[2]

Summary

This report describes work which follows that reported by Scott *et al* (in press) by the same authors in which the initial serological response of pigeons to treatment with two doses of Poulvac Newcastle iK vaccine was examined, along with the relationship between ND vaccination in poultry and pigeons and serology and protection against Newcastle disease and Paramyxovirus disease in pigeons. Pigeons in the vaccinated group were kept in the same loft with unvaccinated controls and blood samples were taken at intervals up to a year after the second vaccination. Over 89% of vaccinated birds had a titre over 3 HI units a year after vaccination.

I. INTRODUCTION

Pigeon Paramyxovirus (PPMV) was identified in Australian domestic pigeons in 2011. No products are registered in Australia for the vaccination of pigeons against PPMV and an application to have the entry of Colombovac® PPMV vaccine permitted was refused by the Australian Quarantine and Inspection Service. Colombovac contains the La Sota strain of Newcastle Disease Virus (NDV) and is formulated for use in pigeons. In 1986 Duchatel and Vindevogel demonstrated the effectiveness of the La Sota strain of NDV in protecting pigeons against challenge with PPMV. Duchatel *et al* (1992) showed that one dose of Colombovac given to 3 week old pigeons from vaccinated parents was able to prevent mortality and greatly reduce clinical signs in the birds when challenged with virulent PPMV-1 a year later. The work in this study follows on from a study reported by Scott *et al* (in press) by the same authors which examined the initial results of vaccination of pigeons with Newcastle disease vaccines. In this study we have continued to collect blood from treated birds as well as from control birds on several occasions up to approximately one year after the second vaccination.

II. MATERIALS AND METHODS

Sixty racing pigeons ranging from 2 months to 10 years of age and tested negative for antibodies to Avian Paramyxovirus 1 APMV1 were used for the study. All birds were assessed as fit and healthy prior to inclusion. The study was conducted at the Melbourne Bird Veterinary Clinic, (1 George St, Scoresby, Victoria 3179) and the birds housed in the associated lofts. A group treated with live vaccine was housed separately from the control group and the group receiving killed vaccine only.

The pigeons were individually identified with leg bands and were ranked on weight and allocated to one of the three treatment groups randomly after blocking for weight. Random numbers were generated using the Microsoft© XL random number generator with an equal probability for selection in any treatment group. One of numbers 1 to 3 were each generated 81 times with numbers ignored after allocation of 20 of those to each group. The treatment groups are described below and tabulated in table 1. Three groups of twenty birds each were used in the original study and two in the duration of immunity study.

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Treatment group T1 received no vaccinations. Treatment group T2 which was included in the original protection study was not retained for the duration of immunity study. Treatment group T3 was injected subcutaneously with 0.5 mL of Poulvac® Newcastle iK vaccine (inactivated) (Pfizer Animal Health a division of Pfizer Australia Pty Ltd) (Batch number: 1782110A, Expiry: 25/11/2013). After 28 days group T3 birds were injected subcutaneously with 0.5 mL of Poulvac® Newcastle iK vaccine (inactivated). Birds in each treatment group were recorded on form 1. The 0.5 mL dose of Poulvac® Newcastle iK vaccine contains at least $10^{7.8}$ EID_{50} Newcastle Disease Virus La Sota strain prior to inactivation. This compares to the pigeon vaccine Colombovac® PMV which contains a minimum antigen dose of $10^{7.9}$ EID_{50} (La Sota strain) prior to inactivation.

Table 1 - Treatment groups

Treatment group		Initial treatment	Treatment 28 days after the initial treatment
T1	Control	No treatment	No treatment
T3	Killed/killed	Poulvac® Newcastle iK vaccine (inactivated)	Poulvac® Newcastle iK vaccine (inactivated)

Blood samples from treated birds were transported immediately to ACE Laboratories (Gildea Lane, East Bendigo) for the Haemagglutination Inhibition (HI) test for Newcastle Disease Virus antibody using NDV V4 as the antigen with chicken red cells. This assay has not been validated in pigeons. As antibody levels are indicative of protection in chickens and due to the similarity of the viruses and lack of validated serological tests in pigeons we used available and modified assays to give the best indication possible of the serological response of pigeons to vaccination. The inclusion of the Pigeon Paramyxovirus HI using the locally derived strain was designed to add confirmatory information. However as the HI test using NDV V4 with chicken blood cells had given consistent results in the initial study this was the test used for the duration of immunity study.

Two hypotheses were examined: that the mean HI NDV titres of group T3 were different to the mean HI NDV titre from the control group T1 and that the mean log 2 HI NDV titres from group T3 were equal to or greater than 3. Log 2 NDV HI values of 3 or more are accepted as evidence of efficacious vaccination in meat chickens (Newcastle Disease Vaccination Program Standard Operating Procedures 2008-2012 (NDV SOP) Animal Health Australia). Blood was sampled on day 28, 56, 120, 196, 280, 343 and 392[#] after the initial vaccination.

Analysis of Variance was performed Log 2 HI values of 0 were assigned the value of 1 for statistical evaluation. The alternate hypothesis was accepted when $P \leq 0.05$. The data analysis package of Microsoft® Office Excel® 2007 was used. As groups T01 and T03 were housed together there is no possibility of any block effect influencing the outcome.

The initial control group (T1) birds were removed at the end of the initial efficacy study. No further birds were introduced to the loft except by breeding and other birds in the same loft were bled as controls including some progeny.

This study was conducted as a veterinary intervention in the face of an outbreak of disease in the state of Victoria and all procedures were standard diagnostic activities conducted by a veterinarian (Dr Colin Walker) in his practice.

[#] The last blood sampling was split due to management requirements at the study site and approximately half the birds were sampled on the 23rd of April (385 days after the first treatment) and the other birds were sampled on the 7th of May 2013 392 days after the first treatment). The "average" of the two sampling times has been used to describe the time for the last sampling and the results combined for analysis.

III. RESULTS

Initial pre-treatment NDV HI titres were negative for all birds. There was no evidence of seroconversion in the control group (except in progeny of treated birds at the last time point where squabs under 7 days of age had titres of 2, 2 and 3). The geometric mean HI (NDV using chicken blood cells) titres at each time point are tabulated in Table 2 and the change in geometric mean titre over time is displayed in Figure 1 below.

Table 2 - Newcastle Disease titres in 20 pigeons vaccinated with Poulvac® Newcastle iK vaccine

Days after first vaccination[#]	Geometric mean NDV titre T3	Lowest titre	Highest titre	Variance	P value (vs control)[†]
0	0	0	0	0.0	Not done
28	3.4	0	7	5.2	<0.0001
56	7.3	6	9	0.5	<0.0001
120	5.7	4	8	1.3	<0.0001
196	5	3	7	1.3	<0.0001
280	4.9	3	7	0.7	<0.0001
343	4.7	3	7	0.8	<0.0001
392	3.8	2	6	1.2	<0.05

The pigeons were vaccinated with Poulvac Newcastle iK vaccine on days 0 and 28.

† Analysis of variance performed with titres of 0 transformed to 1 prior to analysis however the means shown utilize actual reported titres including 0

Maternal antibodies were noted in some of the progeny bled during the study. Up until the last time point tested (approximately one year after the second vaccination) all vaccinated birds had titres over 3. Two treated birds out of 20 had titres below the assay positive cut-off of 3 at the last time point.

One bird from group T3 died during the study on the 15th of July and autopsy examination revealed a mucopurulent airsaculitis with gram negative rods seen on cytology. The examining veterinarian considered the cause of death to be an *E. coli* airsacculitis. Otherwise all treated birds remained free from observable clinical signs throughout the study period.

IV. DISCUSSION

Given than no seroconversion occurred in the control group it can be concluded that no challenge occurred in the treated birds at least up until day 343 after the first vaccination. Even though no control birds were bled at the last time point and of the three progeny of treated birds tested one had a positive titre of 3 it can be concluded that no challenge occurred in the last two months of the study due to the steady decline in titres over that time. It is likely that the positive titre in the squab was due to maternal antibody transfer in yolk. This occurred in a one week old squab and no positives occurred in squabs over three weeks.

Both aspects of the alternate hypothesis were demonstrated after two doses of vaccine in the treatment group. That is the NDV titres were significantly higher in the treatment groups and the log 2 HI titres were above the cut off of 3 recognised as evidence of efficacious vaccination of meat chickens in the NDV SOP (which also requires at least 66% of the flock to be 3 or higher). Up until the last time point tested (approximately one year after the second vaccination) all vaccinated birds had titres over 3. Two treated birds out of

19 had titres below the assay positive cut-off of 3 at the last time point (ie 89.5% of the treated birds had titres over 2 at the end of the study period).

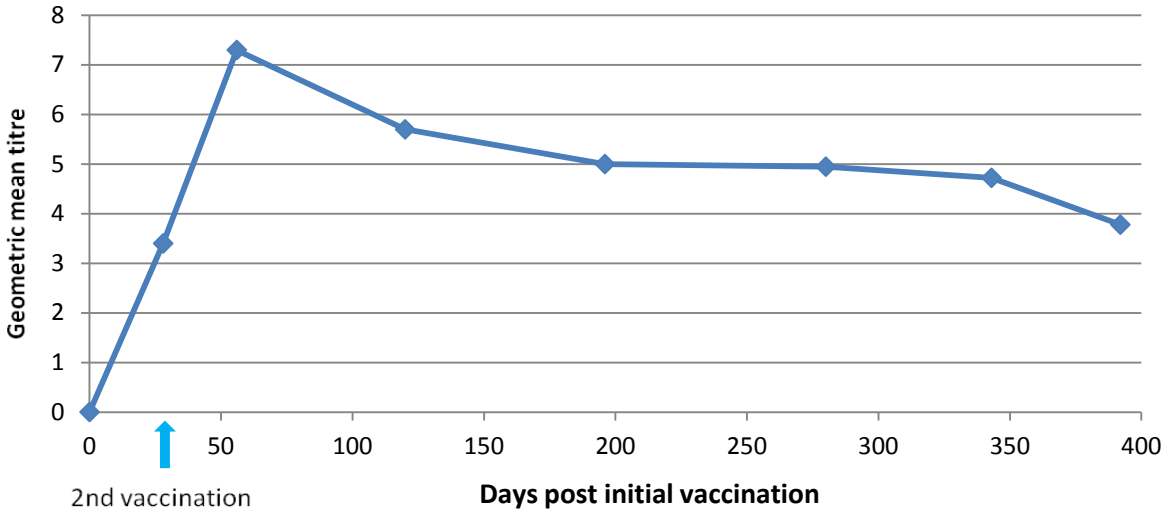

Figure 1 - NDV HI titres* in pigeons vaccinated with Poulvac® Newcastle iK vaccine

This study has clearly demonstrated that the use of two doses of the killed La Sota strain in Australian racing pigeons of various ages (2 months to 10 years old at the start of the study) induces a strong serological response four weeks after the second vaccination. This response is maintained with a gradual decline in geometric mean titre which is still above 3 one year after the second vaccination. Although two birds were below the selected cut off titre we noted in a previous report (Scott et al., in press) the association between serology and protection with some protection noted in vaccinated birds even in the presence of low titres. This has been reinforced anecdotally in client cases where birds vaccinated with just one dose remained disease free where completely unvaccinated birds in adjacent or the same loft succumbed to PPMV disease diagnosed by the veterinarian. We would therefore argue that this study demonstrates a serological response which is sustained for a year after the second vaccination and that given the decline in titres it also supports the necessity for an annual booster vaccination to be given to ensure good flock immunity.

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THE MOTIVATION OF JAPANESE QUAILS (*COTURNIX JAPONICA*) TO DUSTBATH IS INFLUENCED BY EARLY EXPERIENCE WITH PEAT

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Summary

In commercial production of eggs, laying quail do not have access to a substrate for dusthbathing. The inability of birds in battery cages to express normal dustbathing behaviour is the basis for welfare concern because the birds are thought to be highly motivated to dustbathe. However, it is not known if the absence of substrate during early life affects the motivation of adult quails to express dustbathing behavior when given access to a suitable substrate. We tested if quails reared on wire floor had a lower motivation to dustbath in peat than peat-experienced quails. A test was conducted to measure the birds' motivation to dustbath by increasing the resistance to open a door into an area containing peat. The naive birds stopped pushing the door at a lower resistance than experienced birds, but the latency to reach the peat and the latency to start dustbathing was not affected by early experience. Our results suggest that birds naive to peat are less motivated than experienced birds. However, as soon as they were given the opportunity to access dustbathing substrate the naive bird worked for it.

I. INTRODUCTION

In most production systems, Japanese quail (*Coturnix japonica*) are confined in cages in which they are not able to perform some behaviours such as dustbathing (Gerken and Mills, 1993). It has been argued that if a bird is highly motivated to express a behavior, such as dustbathing, and can not, the welfare of the animal is compromised because the deprivation leads to a negative mental state (Hughes and Duncan, 1988; Dawkins, 1990). The inability of birds in battery cages to express normal dustbathing behaviour in functional substrate is the basis for welfare concerns (Olsson and Keeling, 2005). However, it is not known if the absence of substrate during early life affects the motivation of adult quail to express dustbathing behavior when given access to a suitable substrate such as peat. We hypothesized that dustbathing motivation would not be influenced by early experience, whether reared with or without peat, because quail go through the motion of dustbathing on a wire floor in the complete absence of substrate (Gerken and Mills, 1993; Lindberg and Nicol, 1997). To test this hypothesis, we developed an operant conditioning method for quail to quantify their motivation to access substrate for dustbathing (Dawkins, 1988). The cost an animal pays for gaining access to a resource is often expressed in terms of how much work it does, for example, the number of key-pecks performed to gain access to a resource (Lagadic and Faure, 1987), or the amount of effort exerted to push a weighted door (Widowski and Duncan, 2000; Wichman and Keeling, 2008). Peat moss was selected as the dustbathing material because peat is a highly preferred dustbathing material for hens (de Jone et al., 2007). We used a weight-adjustable push-door apparatus to measure the motivation as it simulates the process of pushing through dense undergrowth which is a natural behavior of quail (Olsson et al., 2002a).

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II. MATERIAL AND METHODS

All procedures were conducted at the University Western Australia, Perth, Western Australia, and approved by the UWA's animal ethics committee. After hatching, the birds were kept in a quail rearing cage (S.V.302, Venturi Valter, Predappio, Italy; 2.4m x 1.06m x 1.85m; l x w x h). From 4 days of age, one group of hatchlings was given peat moss in two large trays (15 x 15 cm) to dustbathe (experienced group) and a second group was given access to two of the same but empty trays (naive group). Water and food (Commercial quail starter and grower diets, Specialty Feeds, Glen Forrest, WA) were supplied ad lib and the food trays were covered with wire to stop the birds from dusthbathing in the food. At 5 weeks of age birds were sexed. Females were selected, leg banded, and transferred to individual cages (Cimuka, Ankara, Turkey). The females from the experienced group received peat three times a day in an individual tray (10 x 20 cm) in their own cage, while the naive females were provided with empty trays.

The testing apparatus comprised of a wooden box (walls 250 mm high) divided into three sections: a starting box (200 x 250 mm), a runway (600 x 250 mm), and a goal box (400 x 400 mm). Each section was covered with a wire mesh lid. A sliding door (120mm x 250 mm) separated the start box from the runway. The push-door (120mm x 250 mm) at the entrance to the goal box was hinged at the top and was held closed by magnets located at the bottom of the door and the box floor. The magnetic resistance to be overcome by the bird in order to open the door (cost) was varied by increasing the number of PVC inserts (1mm thick) placed between the magnets. Six cost levels were tested, these being 0 g (R0), 25 g (R1), 55 g (R2), 120 g (R3), 250 g (R4) and 500g (R5).

Birds were trained to use the push-door before the tests to measure their motivation for peat were conducted. At the end of five daily training sessions, seven naïve birds and 8 experienced birds (68% of the birds in total) were able to push the door to access a small food reward located in the goal box within 3 minutes without showing any stress.

The evening before any motivation test, peat was removed from the experienced group. Beginning at R0, the birds' motivation to access peat was tested at consecutive levels of cost. For each cost, each bird was tested three times on one day, with about 3 hours between tests. A rest period of one day was given between test days. The goal box contained peat in half of its area. After settling in the start box for 3 second, the birds were released into the runway for a maximum of 3 min. Once in the goal box, the birds were given another 5 minutes to begin dustbathing. Data were collected on the number of birds that succeeded in opening the door, the latency to open the door, the latency to start dustbathing and the number of birds that engaged in dustbathing. The statistical analysis was carried out using SPSS (version 19). Pearson's Chi square was used to compare the proportion of birds succeeding in opening the door or expressing dustbathing behavior. ANOVA for repeated measures was used to compare the latency to express behaviour (only for cost where over 80% of the birds expressed the behavior).

III. RESULTS

Experience had no effect on the number of birds reaching the peat when the cost was low (R0-R3, Fig. 1). The proportion of naive birds failing to reach the peat was greater than that of the experienced birds at R4 (Fig. 1). Only one bird in each group reached the peat when the cost was at maximum (R5, Fig. 1). At low cost (R0-R3) the latency to pass through the door was similar between the two groups (Fig. 2, $P = 0.9$). At the higher cost of R4 the latency to reach the peat was greater for the naive birds than that for the experienced birds (Fig. 2, $P < 0.05$). The latency could not be tested at maximum cost since only one bird of each group reached the goal box.

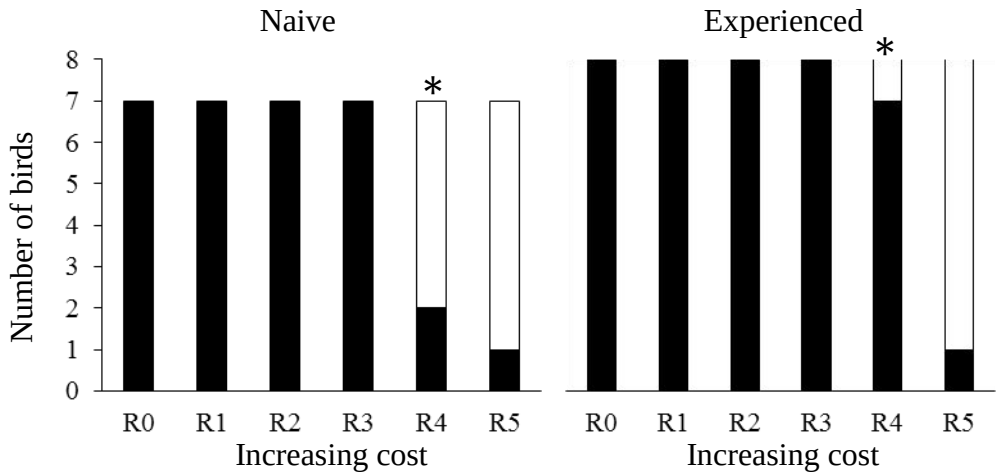

Figure 1 - Effect of increasing cost (resistance R0 to R5 - see text for details) on the number of peat-naive (left panel) or peat-experienced (right panel) adult female Japanese quail reaching (black bars) or failing to reach (open bars) the goal box containing peat.. * indicates difference between the two groups at the same resistance level.

All birds from the naive group that entered the goal box performed dustbathing but three birds from the experienced group did not dustbathe while in the goal box. There was no interaction between level of experience and cost to access the peat on the latency to start dustbathing (P = 0.72). There was no main effect of experience (P = 0.88) but there was a main effect of resistance on the latency to start duthbathing (P < 0.01).

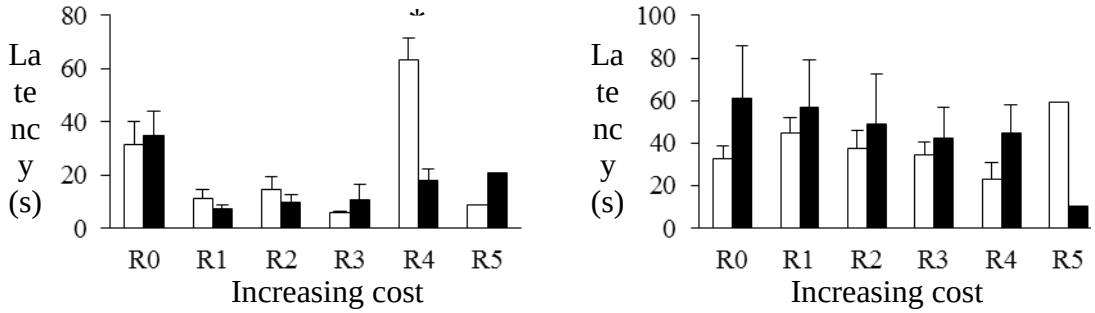

Figure 2 - Effect of increasing cost (resistance R0 to R5 - see text for details) on the latency to reach the goal box containing peat (left panel) and the latency to commence dustbathing (right panel) in adult female Japanese quail naive to peat (open bars) or experienced with peat (black bars).

IV. DISCUSSION

Our hypothesis was rejected because the motivation to dustbathe in peat was not as strong in the naive birds raised without access to peat as in experienced birds raised with peat. Both the number of birds failing to push the door and the latency to reach the substrate was greater in the naive group than in the experienced group. The differences in bodyweight between the two groups could explain these findings, but quails of low bodyweight in the experienced group worked at the same cost as naive group. It could be argued that the presence of peat during early life encourages muscle development through scratching and pushing substrate while dustabthing. However, except the absence of peat, both groups had access to the same space when in individual cages, and the tray provided was too small to facilitate scratching.

Motivation is often measured by the latency of an animal to express a behavior. In our study, the latency to reach the substrate was similar between the birds of the two groups at lower cost (R0-R3). However, when the cost was high (R4) the birds from the naive group

took longer to open the door, suggesting that their motivation was lower than that of the experienced birds. The number of attempts to open the door was not different between the two groups (data not presented). Once in contact with the substrate, the motivation to dustbathe was similar between the experienced group and the naive group since the latency to dustbathe was always numerically greater in the experienced group but never significantly different to that of naive birds.

Our study demonstrates that it is possible to train quails to operant condition by using a pushdoor apparatus. The level of work that quail can exert to access a dustbathing substrate can be greater than their own liveweight, suggesting that the motivation to dustbathe is quite high in birds that have experienced dustbathing during their life.

Although our study suggests that naive birds are not as motivated to dustbathe as experienced birds, the findings also suggest that the naive birds do have some natural drive to dustbathe, as they worked for access to peat when the opportunity was presented and they always engaged in dustbathing once in the peat. These findings support those in chickens, where adult hens that had never experienced peat before quickly began dustbath after only one previous experience (Wichman and Keeling 2008).

Our study partly supports the statement of Dawkins (1983): 'whether birds that have been in cages all their lives and have never experienced litter to scratch and dustbath in are still attracted to litter in the same way that litter-reared birds are known to be' in that quails seem to be equally attracted to litter once they have accessed it, however motivation to access the peat was lower in the naive birds than in the experienced birds. Thus early experience may lead to different needs and bird's welfare may need to be assessed differently. The impact and the practicality of providing dustbathing to Japanese quail raised by the egg and meat industry needs to be investigated in a commercial setting.

ACKNOWLEDGMENTS: This research was conducted within the Poultry CRC, established and supported under the Australian Government's Cooperative Research Centres Program.

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FERTILITY OF JAPANESE QUAILS IN MIXED AGE PAIR MATINGS

U. FAROOQ[1] and I.A. MALECKI[1]

Summary

We investigated the effect of aging on fertility in Japanese quails. Pair matings of 8, 16, 26, and 36-week old males and females were carried out and egg fertility, sperm, and sperm hole numbers in the perivitelline membrane estimated. Mating of young males with older females did not improve fertility and it appeared that mating same age birds led to better fertility outcome. Our experimental data did not confirm the age related decline in fertility observed in farmed quails after 26 weeks of age. While age-related decline in fertility is unavoidable, studies directed towards optimal flock management on a farm may have better fertility outcomes than spiking.

I. INTRODUCTION

In commercial Japanese quails, high egg fertility is maintained between 10 and 20 weeks of age with a peak at the age of 14-16 weeks (Woodard and Alplanalp, 1967; Narahari *et al*., 1988; Faroog et al. 2012). Loss of reproductive performance expressed by fertility and hatchability decline with age; older males reduce sperm production and libido (Woodward and Alplanalp, 1967; Otinger, 1991; Sefton and Seigel, 1973), while older females reduce ovulation rate. Similarly, in the domestic chicken, aging is associated with decrease in numbers of spermatozoa in an ejaculate, the volume of semen (Sexton et al., 1989), reduced retention of sperm in the sperm host glands of the oviduct, and increased rate of sperm loss from the glands (Bakst et al., 1994).

Spiking, although variably successful, appears feasible to tackle age-related decline in fertility in the broiler chicken (Casanovas, 2002). Whether spiking can be an effective management tool in the Japanese quail requires better understanding of fertility factors and testing mating of different age males and females. To obtain a preliminary understanding of the influence of age on fertility, this study was designed using pair-mating of differently aged males and females. We hypothesized that mating young (males and female) with old birds would improve fertility.

II. MATERIALS AND METHODS

Meat type quails (45 males and 360 females) provided by Game Farm Pty Ltd (Galston, NSW, Australia) were randomly selected and individually housed in commercial quail cages (Venturi Valter, Italy) of the University of Western Australia, Crawley, Western Australia. Quails were fed *ad-libitum* with a commercial quail breeder diet containing 20.0% CP and 11.5 MJ/kg ME. Environmental enrichment was achieved by the provision of a sand bath. Climate was maintained at 22-26°C and 14/10-h light/dark cycle with adequate ventilation. The Animal Ethics Committee of the University of Western Australia approved all procedures.

Four differently aged females (8, 16, 26 and 36) and 8-week-old males were used to begin the experiment that was carried out in 4 stages corresponding to male age (8, 16, 26 and 36 weeks), each subsequent stage beginning 8 weeks after the previous stage. After each stage, the oldest females were replaced by new 8-week-old females. The same males were

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retained. Each male was randomly assigned 4 females, one from each age group (8, 16, 26 and 36 weeks). Each male was with a female for 8 hours and every day was moved to a different female. After a 4-day mating round was completed the next round commenced. Mating was carried out for 2 weeks in each of 4 stages, followed by 2-week semen collection and 4-week rest periods.

Eggs laid during mating were opened to estimate fertility by viewing the germinal disc area and counting sperm and sperm hole numbers in the perivitelline layer. Both, males and females were weighed at the beginning of each stage. Duplicate semen samples were collected 2 times per week for 2 weeks using a teaser/manual massage method to estimate ejaculate volume and sperm concentration (Farooq et al 2012).

The data were analysed as 4 x 4 factorial design (age of male x age of female each with four age levels, 8, 16, 26 and 36 weeks) for egg fertility, $Sperm_{OPVL}$ and $Holes_{IPVL}$ with 9 replicates in each category. Male body weight, ejaculate volume and sperm concentration was analysed by repeated measures analysis of variance appropriate for randomized complete design, general linear model procedure of the statistics using PASW Statistics 18, Release Version 18.0.0 (SPSS©, Inc., 2009, Chicago, IL, www.spss.com). When the significant difference among treatments was found, means were separated using LSD test. Statistical significant was assessed at $P<0.05$.

III. RESULTS AND DISCUSSION

Male body weight did not change between 8 and 26 weeks of age, then increased and by 36 weeks it was higher ($P<0.05$) than at younger age (Fig. 1A). The female body weight increased between 8 and 16 weeks and did not change to 36 weeks of age.

Ejaculate volume increased between 8 and 16 weeks of age, then the volume did not change ($P>0.05$) between 16 and 36 weeks (Fig. 1B). The 8-week-old males had lower sperm concentration than when they were older. Sperm concentration increased ($P<0.05$) between 8 and 16 weeks of age but then reduced and remained lower ($P<0.05$) at 26 and 36 than at 16 weeks (Fig. 1B).

Egg fertility was lower at 8 weeks of age than in older birds (Fig. 1C). There was a linear increase in fertility between 8 and 26 weeks of age but egg fertility did not differ between 16, 26, and 36 weeks ($P>0.05$). The mean $Sperm_{OPVL}$ numbers varied between ages ($P<0.05$). At 8 weeks of age $Sperm_{OPVL}$ was higher than at 16 and 36 weeks but it did not differ from the mean at 26 weeks (Fig. 4). The mean number of $Holes_{IPVL}$ varied between ages, but showed a linear decline ($P< 0.05$), being the highest at 8 and the lowest at 36 weeks of age. The mean number of $Holes_{IPVL}$ did not differ between Week 16 and 26 (Fig. 1D).

When 8-week-old males were mated to 8, 16, 26 and 36-week-old females egg fertility did not differ between female age. The mean number of $Sperm_{OPVL}$ and $Holes_{IPVL}$ was higher from mating with 8-week-old than with older females (Table 1). Mating 16 week-old males, resulted in higher fertility with 8 and 16-week-old females than with older females. The mean number of $Holes_{IPVL}$ was higher for 8 and 16-week-old females as compared to 26 and 36-week-old females. When males were 26 weeks old, egg fertility with 26-week-old females was higher than with any other age female. The mean number of $Sperm_{OPVL}$ and $Holes_{IPVL}$ were significantly higher for 16 and 26-week-old females as compared to 36-week-old females. Egg fertility did not differ between different age females when they were mated with 36-week-old males; the numbers of $Sperm_{OPVL}$ and $Holes_{IPVL}$ did not differ either.

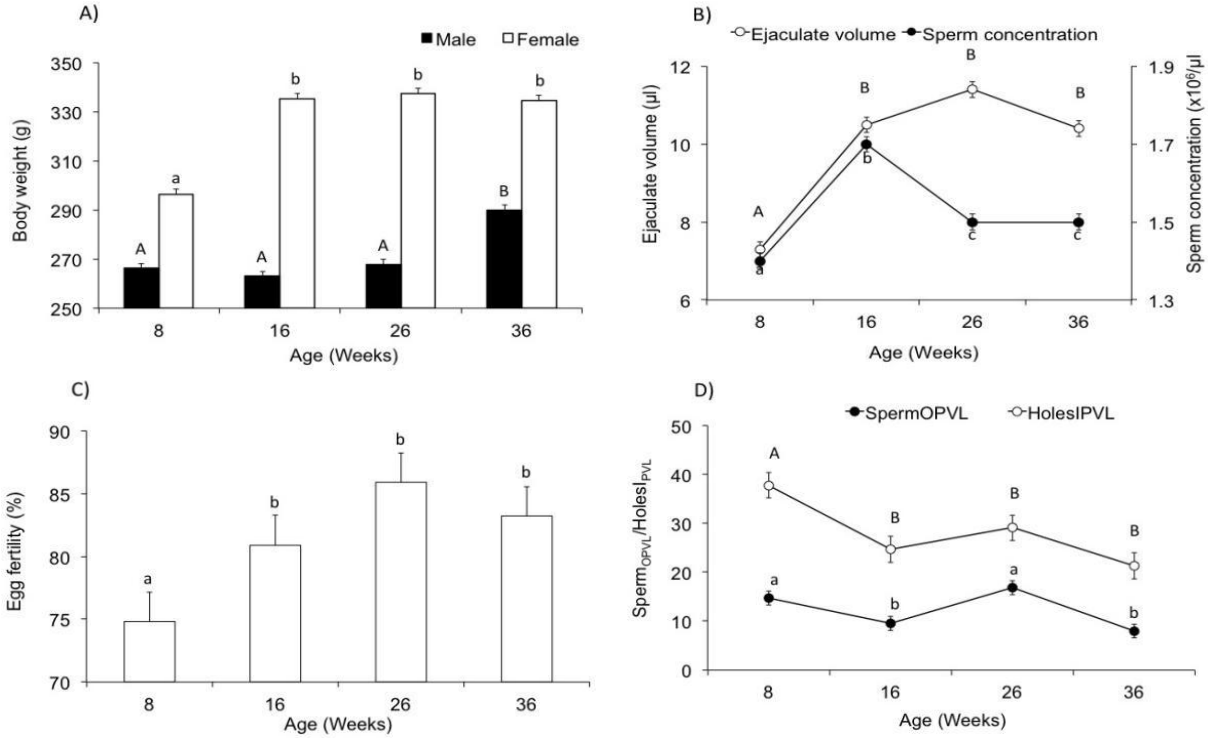

Figure 1 - Effect of age on male and female body weight (A); ejaculate volume and sperm concentration (B); egg fertility (C); and numbers of $Sperm_{OPVL}$ and $Holes_{IPVL}$ (D) in commercial Japanese quails. Means for the trait that have common superscript do not differ (P>0.05).

Table 1 - The effect of male and female age interaction on mean (±SEM) egg fertility, $Sperm_{OPVL}$ and $Holes_{IPVL}$.

Male age	Female age	Egg fertility (%)	$Sperm_{OPVL}$	$Holes_{IPVL}$
8	8	76.6^{aAB}±3.7	15.0^{aA}±1.4	38.5^{aA}±2.9
	16	70.0^{aA}±3.6	11.2^{bAB}±1.4	30.3^{bA}±2.8
	26	73.3^{aA}±3.6	9.6^{bA}±1.4	22.4^{bAB}±2.8
	36	69.4^{aA}±3.7	8.7^{bA}±1.4	23.1^{bA}±2.9
16	8	83.1^{aA}±3.7	10.7^{aA}±1.4	30.0^{aB}±2.9
	16	85.1^{aB}±3.8	9.9^{aA}±1.5	26.0^{abA}±3.0
	26	73.9^{bA}±3.6	7.5^{aA}±1.4	19.9^{bB}±2.8
	36	72.6^{bA}±3.6	8.6^{aA}±1.4	21.6^{bA}±2.8
26	8	70.1^{aB}±3.7	13.0^{abA}±1.4	25.0^{abB}±2.9
	16	76.7^{aA}±3.9	16.8^{aB}±1.5	31.5^{aA}±3.1
	26	85.9^{bB}±3.8	16.8^{aB}±1.4	29.1^{aA}±2.9
	36	72.0^{aA}±3.7	10.2^{bA}±1.5	19.1^{bA}±3.0
36	8	81.2^{aA}±3.6	11.5^{aA}±1.4	26.8^{aB}±2.8
	16	85.9^{aB}±3.8	10.8^{aA}±1.5	28.7^{aA}±2.9
	26	81.5^{aB}±3.8	9.0^{aA}±1.5	25.9^{aAB}±2.9
	36	87.4^{aB}±3.8	8.1^{aA}±1.5	21.4^{aA}±2.9

[a-b] Means for female age within same male age not having a common superscript differ significantly (P < 0.05).
[A-B] Means for same age females mated to different age males not having a common superscript differ significantly (P < 0.05)

Fertility decline previously observed in a farm flock after 26 weeks of age (Farooq at al. 2012) was not confirmed in this study. However, the data suggest a trend in age-related decline of fertility. Possibly holding animals individually and pair-mating account for this difference as normal farm practice for this species uses colony mating, characterised by intense mate competition.

The males retained their reproductive performance until 36 weeks of age. Their body weight increased, they produced high levels of fertility with different age females and had comparable sperm concentration in an ejaculate to that at younger age. A gradual decline in sperm hole numbers may be indicative of a reduction in the sperm retention rate of the sperm host glands; however, probability of egg fertilisation was not affected. Our results suggest that age related fertility decline may be delayed however appropriate management strategies needs to be developed. Given that males remain reproductively competent, a trend in fertility decline may be associated with female factors (Bakst et al., 1994). Spiking may be an effective strategy to delay decline in fertility however efforts may be compromised by female factors that, if understood and improved, could eliminate the need for spiking.

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GENE CLONING AND SEQUENCES ANALYSIS OF THE MC1R GENE IN LIANCHENG WHITE AND CHERRY VALLEY DUCKS

L.X.N. WANG[1,2], H. CHEN[2], D.B. YU[2], X. LI[1] and W.L. BRYDEN[1]

The colour of duck feathers is an important economic characteristic. The melanocortin receptor 1 (MC1R) plays an important role in melanin formation and is a candidate gene of fur and feather pigmentation. It has been shown that MC1R is a pivotal gene for feather colour in birds. The relationship between MC1R gene structure and regulation is poorly understood. Thus, it is important to determine the MC1R gene sequence from different avian genotypes and species to better understand the expression and regulation of the gene.

Three female and male ducks from 4 generations of both the Liancheng White duck and Cherry Valley duck were selected randomly. Blood sample was collected from each duck into heparinised tubes and stored at – 20^{o} C. DNA was extracted from red blood cells with phenol chloroform solution. The Primers were designed based on the MC1R gene sequence of chicken, *Gallus gallus, Anser caerulescens and Pavo cristatus* in Gene Bank. Oligo 6.0 and Primer 5.0 were used to design a pair of primers. The PCR procedure described by Newton and Graham (1997) was followed to amplify the DNA. The products from PCR were detected using 1% agarose gel electrophoresis. A gel extraction mini kit was used to retrieve the target fragment for cloning sequence detection. The fragment was sequenced by the Dalian Baosheng Biological Technology Company. Blastn and DNASTAR software on the NCBI website were used to analyse and compare the sequences.

Liancheng White ducks and Cherry Valley ducks both had 900bp nucleotide sequences with 299 amino acids in total. It was found that the two sequences have a high degree of similarity, with nine loci mutations at the nucleotide sequence: 144bp (A/G), 165bp (G/A), 183bp (C/T), 187bp (T/A), 232bp (G/A), 367bp (A/G), 402 (G/A), 607 (C/T) and 623 (G/A). The priority transformation is between G/A and A/G and the similarity is 99.7%. The amino acid sequence on the 41th (Q/L), 56th (E/G), 101th (R/H) and 181th (V/A) have a mutation, respectively, and the similarity is 98.9%.

The results show that the homology between Cherry Valley and Liancheng White duck genotypes is 98% and with other avian species greater than 90%. From the sequence of MC1R gene, there is an A/G substitution at the 623 locus in female duck of the F1 generation, but the change does not affect the order of the amino acid sequence. It is therefore, a nonsense mutation. This study illustrates that the MC1R gene is highly conserved in poultry. However, effect of mutation in amino acid sequence at 41 (Q/L), 56 (E/G), 101 (R/H) and 181 (V/A) on feather colours requires further research.

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EFFECT OF INTRAPERITONEAL ADMINISTRATION OF GHRELIN ON SERUM INSULIN AND GLUCOSE LEVELS IN NATIVE TURKEY

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Ghrelin is a multifunctional regulatory peptide that was discovered in the rat stomach by Kojima et al (1999). Chicken ghrelin includes 26 amino acids and is shorter than human or rat ghrelin with 28 amino acids (Kaiya et al., 2002). Mammalian ghrelin has a regulatory role in glucose homeostasis via the modulation of insulin secretion (Castańeda et al., 2010). The intraperitoneal injection of insulin lowered blood glucose levels in the ghrelin-administered and control mice (Dezaki et al., 2004), suggesting that the hyperglycaemic effect of ghrelin was due neither to the ability of ghrelin to release GH nor to the induction of insulin resistance, but it was primarily caused by a reduction of plasma insulin levels. The purpose of the present study was to investigate the effect of intraperitoneal injection ghrelin on insulin and glucose levels in turkey pullet.

In this experiment, seventy two 28 day-old domestic native male turkeys (BW: 730 g ± 50) were assigned in completely randomized design into three treatments by six birds in each treatment and four replicates. The lyophilized rat ghrelin was purchased from Sigma-Aldrich Co. (USA), dissolved in 1% acetic acid solvent and diluted with distilled water (in according to sigma brochure) to desired concentrations. Ghrelin was injected intraperitoneally (IP) on day 28 and before onset of experimental rearing period. The injected doses were as follows: treatment 1 (G0): intact without any injection; treatment 2 (G50): 50 ng ghrelin/kg body weight, and treatment 3 (G100): 100 ng ghrelin/kg BW. At the day 28 (12 h after ghrelin injection) two birds from each replicate of each treatment that had BW close to the mean replicate was selected. Blood samples were collected from wing veins using sterilized syringes.

The result suggested serum glucose concentration was greater in ghrelin administrated treatments, and it differed considerably compared with control group ($P < 0.01$) for 28 day-old or 68 day-old turkey by ghrelin injection. In the present study, results after injection similar were observed for the glucose level on day 68. It seems that ghrelin, similarly like leptin, has a considerable role in glucose homeostasis in neonatal chickens and this effect in mammals was reviewed by Castańeda et al. (2010). The serum insulin level was high in 100 ng ghrelin/kg BW group in comparison with control group. It has been shown that rat ghrelin, regardless to elevation of serum glucose concentration, had positive effect on insulin secretion from the pancreas. In chickens, insulin begins to be secreted from β-cells on day 4 of embryonic life, and the secretion rate could considerably increase from day 12 to hatching day (Bellairs and Osmond, 2005). It has been shown that insulin has a considerable role in chicken early embryonic morphogenesis with expression changes in the involved genes (Patwardhan et al., 2004). However ghrelin injection significantly increased glucose and insulin concentration compared to control group.

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METABOLIZABLE ENERGY VALUE OF GUAR MEAL FOR BROILER CHICKS CAN BE INFLUENCED BY METHOD OF DETERMINATION

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Guar meal (GM) is mostly used as a protein source in poultry diets. However it contains a broad range of galactomannan gum and some other anti-nutritional factors which decrease its metabolisable energy (ME) value. It is also showed that technique to measure metabolisable energy influences validity of results. In this regard, Sibbald (1987) showed that in comparison with the total collection method, the use of markers to determine ME and digestibility values avoids errors associated with inaccurate measurement of feed intake, excreta output, and contamination of excreta. Present study was carried out to determine ME of GM and soybean meal (SBM) using total collection and marker methods in 35 day-old broiler chickens. In the first experiment a total of thirty six 35 day-old broiler chickens (3 groups of 12 birds each) were housed individually in collection cages. The birds were fed three experimental diets including a corn-soybean meal reference diet and two test diets containing 30% GM and 30% SBM substituted in expense of corn and soybean of the reference diet. The excreta were collected daily (from 35 to 39 d) for each bird and stored at -20°C until further processing. Total feed intake of each bird was also measured during collection period. Chromic oxide (3g/kg) was added to the diets as an external marker. In the second experiment total of ten 35-day broiler chickens (2 groups of 5 birds each) were placed in individual metabolic cages to determine TME of guar meal according to McNab and Blair (1988).

Significant differences were found in AME and AME_n values between GM and SBM due to the applied method ($P<0.05$). Both AME and AME_n values for GM and SBM determined by total collection method were higher than marker method ($P<0.05$). In addition, the AME_n value for SBM was significantly ($P<0.05$) higher than GM in total collection assay (2496 vs. 2168 kcal/kg). On the other hand, in precision feeding experiment, TME value for GM was higher than SBM (2702 vs. 2551.5 kcal/kg). The findings of the current study indicate that high guar gum affect AME and AME_n values in broiler chickens. In addition, according to SEM of the means it seems than total collection method is a more reliable assay for metabolisable energy determination of GM and SBM.

Table 1 - Apparent metabolisable energy (AME), apparent metabolisable energy corrected for nitrogen (AME_n) and true metabolisable energy (TME) of guar and soybean meal fed to broiler chicks

Assay Method	Total Collection	Marker	Total Collection	Marker	Sibbal Method
Ingredient	AME	AME	AME_n	AME_n	TME
Soybean Meal	2495.6^{ax}	1842.9^{y}	2496^{ax}	1933.4^{y}	2551.5^{a}
Guar Meal	2168.3^{bx}	1851.6^{y}	2168.7^{bx}	1935.6^{y}	2702.8^{a}
SEM	72.67	161.05	72.67	168.79	98.18

[a-b] Means followed by the same letters in the same column are not different by Tukey test ($P < 0.05$)
[x-y] Means followed by the same letters in the same row are not different by Tukey test ($P < 0.05$)

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NUTRITIONAL IMPACT ON MOBILITY AND LEG ISSUES IN POULTRY

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Summary

Today's birds are completely different from their ancestors in growth, performance, and requirements for nutrients. They grow at a faster rate, produce more eggs, and utilize feed more efficiently. Poultry geneticists and breeders have been selecting for body weight gain, breast meat yield, high feed efficiency (broilers, turkeys, and ducks) and high egg production (laying hens and ducks). The traits selected for are in response to market demand for more poultry eggs and meat that is leaner, tender, and more affordable. This demand for more animal protein will continue to increase as the per capita income of developing and less developed countries continue to rise. Based on this trend, it is expected that efforts on further selection for rate of gain and feed efficiency would be intensified. One of the consequences of selecting for fast growth and heavier birds at market age is the pressure that this puts on the skeletal system. In most cases, mobility problems arise, in part, because of inadequacy of bone mineralization hence, the skeletal system is unable to adequately handle the enormous weight that the bird has been selected for. It is therefore imperative to select for skeletal integrity. Bone breakages and lameness which have been, in part, attributed to lack of proper bone mineralization due to inadequate level of available calcium (Ca) and phosphorus (P) in the diet. Secondly, the role of vitamin D_3 in Ca and P metabolism is also critical to proper bone mineralization and skeletal integrity. In addition to good genetic selection and management practices, some of the mobility issues in poultry could be addressed through adequate nutrition. Requirements for certain minerals such as Ca, P, and vitamins (D_3, A, B, C, and E) which are essential for bone growth and skeletal integrity, are not regularly updated to meet the need of today's birds. In addition to bird welfare as a result of mobility issues, bone fracture during meat processing is another challenge that makes our understanding of the relationship between bone pathology, dietary vitamin D_3, bone ash, and mineral composition such an important factor. Thus, some of the bone and mobility issues in poultry could be drastically reduced through proper and adequate nutrition that is timely and presented in a form that is readily available to the birds. Well mineralized and healthy bone in poultry is important bird welfare, economics, and environmental sustainability.

I. INTRODUCTION

A strong skeletal structure is important from the perspective of birds' welfare and economic considerations. Birds with well mineralized and healthy skeleton will outperform unhealthy birds and hence could significantly impact the bottom line of a poultry enterprise. Misirlioglu et al. (2001) reported that mortality arising from leg abnormality in broiler is up to 15%. In addition to an increase in mortality as a result of skeletal disorders, a weak/soft bone is fragile and could be problematic during processing by increasing the proportion of birds that are condemned. Lameness and bone breakage in turkeys affect up to 15% of the flock (Lilburn, 1994; Ferket et al., 2009). Incidence of lameness in poultry could be attributed to factors such as genetics, nutritional deficiencies, malabsorption as a result of gastrointestinal tract (GIT) health, gender, diseases (as it is the case with bacterial chondronecrosis), metabolic disorders, and bone deformities (Thorp and Waddington, 1997; Angel, 2007). Lameness in broilers has been reported to increase with age (d 28 vs. d 42 and 49) with males being more susceptible

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than females (Sorensen et al., 2000). Additionally, they (Sorensen et al., 2000) reported that higher stocking density may be associated with poorer walking ability.

Tibial dyschondroplasia (TD) and field rickets are the two common factors responsible for mobility problems in poultry. Incidence of leg abnormalities in ducks have also been reported. Wise (1975) reported that the occurrence of TD in ducks is more prevalent among the heavy strains than in light strains. The subclinical incidence of TD in ducks may be as high as 70% but this usually does not result into clinical signs of leg weakness (Wise, 1975). However, rapid rate of growth and high levels of some anions such as chloride could increase the incidence of TD in ducks.

In the study by Thorp and Waddington (1997), bacterial infection was found to be the most prevalent condition within the physis and cartilaginous epiphysis of birds with mobility issues. This problem was found to increase with P deficiency. Information on the important roles of nutrients on skeletal development and integrity have been extensively published (Riddell, 1975; Pierson and Hester 1982, Edwards and Veltmann, 1983; Sauveur, 1984, Leeson and Summers, 1988; Orth and Cook, 1994; Thorp and Waddington, 1997; Edwards, 2000). The effect of changes in acid-base and/or cation-anion balance in chicks fed purified and practical diets on the occurrence of TD has also been reported (Riddel, 1975; Sauver and Mongin, 1978). Advances in genetic improvement has resulted in birds reaching market weight in fewer number of days with higher proportion of breast meat yield, better feed efficiency, and changes in muscle mass (Angel, 2007). Laying hens have been selected to lay eggs almost throughout the year. This puts a lot of strain on bone mineralization with a drastic reduction in the rate of bone turnover (Fleming, 2008). Avian osteoporosis, which can be selected against, could also be reduced through increased level of activity, and proper nutrition. The forms and dietary levels of available Ca, P, and vitamin D_3 are critical to proper bone formation and mineralization. In laying hens for instance, Ca sources from coarse or particulate form have been reported (Fleming, 2008) to be more beneficial for stronger skeletal system and egg shell formation compared to Ca from fine textured sources. The role, importance, and adequate timing of Ca, P, vitamins K and D_3 in preventing leg problems in meat type poultry (broilers and turkeys) and osteoporosis in laying hens have also been discussed (Fleming, 2008).

For poultry production to continue to be profitable and sustainable there is the need to reduce skeletal problems through simultaneous selection for sound skeletal structure to keep pace with selection for rapid growth rate. Furthermore, a sound understanding of the role and timing of dietary intervention in minimizing skeletal problems in modern day birds is essential.

II. MINERALS AND SKELETAL STRUCTURE

Raising birds with healthy skeletal structure requires early establishment of strong skeletal system within the first few weeks of life. Proper nutrition is essential at this critical time because of rapid skeletal structure growth and most of the developmental abnormalities are established during the first few weeks of life (Huff et al., 2006; Dibner et al., 2007). Calcium and P are the most abundant minerals in the bone and the dietary adequacy and availability of these minerals in easily digestible and absorbable forms are critical for bone integrity. Furthermore, proper ratio of dietary Ca-to-P is very important as this could hamper intestinal absorption of minerals. This effect was demonstrated by Edwards and Veltmann (1983) with a diet that is high in P but low in Ca, which resulted in TD in the young broilers. Additionally, early growth restriction and feeding a diet that is high in bicarbonate mineral mix was shown to result in significant reduction in both the incidence and severity of TD (Wise and Nott, 1975). Phosphorus deficiency has been reported to increase the likelihood of

bacterial infections within the metaphyseal region of the bone growth plate of birds with mobility issues (Alderson et al., 1986, Alderson and Nade, 1987; Thorp et al., 1993; Thorp and Waddington, 1997). Lacey and Huffer (1982) had earlier reported a relationship between length of the metaphyseal vessels in hypertrophic chondrocytes in avian rickets and P deficiency. Results from the study conducted on three commercial broiler farms in Scotland, Northern Ireland, and Holland showed high prevalence of mineral deficiency-related pathologies with bones that can be attributed to P, Ca, or vitamin D_3 deficiency or Ca-P imbalance (Thorp and Waddington, 1997). The role of poly unsaturated fatty acids (PUFA) from the *n-6* and *n-3* families (derived from linoleic acid [n-6] or α-linolenic acid) in bone development and growth has also been reported (Fleming, 2008). The higher the ratio between *n-3* PUFA and *n-6* PUFA the higher the beneficial effect of the fatty acids on osteoblast functionality and bone formation (Liu et al., 2003; Fleming, 2008).

III. DYNAMICS OF CALCIUM, PHOSPHORUS, AND VITAMIN D_3 METABOLISM IN BONE MINERALIZATION

Vitamin D_3, its metabolites, and parathyroid hormone (PTH) play important roles in the regulation of Ca metabolism and bone mineralization (Hurwitz and Bar, 1972; Edwards et al., 1992; Marks, et al., 2010). The most potent and commonly used metabolite of vitamin D_3 in broilers is 25-OHD_3 (Norman and Wong, 1972; Boris et al., 1977; McNaughton et al., 1977; Edwards, 1989). In addition to Ca, the role of vitamin D_3 in intestinal phosphate absorption has been established in 14-d old rats (Xu et al., 2002). The influence of vitamin D_3 on Ca metabolism includes its effects on the GIT (transcellular and paracellular Ca absorption), bones (Ca deposition and resorption), and the kidney (Ca excretion and re-absorption) (Hattenhauer, et al., 1999). In order to optimize intestinal Ca and P absorption in poultry, particular attention must be paid to the vitamin D_3 status of the bird as well as dietary vitamin D_3 level. Additionally, it is important to have a good understanding of the interaction between Ca, P, and vitamin D_3. This according to Adedokun and Adeola (2013) should be tailored to different breeds/strains of poultry, ages, feed ingredients, and exogenous phytase inclusion levels (if supplemented in the diet).

Dietary Ca-to-P ratio could influence digestion and absorption of Ca and P in the GIT of poultry. These ratios could trigger different physiological responses such as the release of PTH and/or bone resorption or Ca deposition in bones. Hence, proper care must be taken to minimize the negative interactions between Ca and P that may be detrimental to their utilization. High dietary Ca could result in high digesta pH and may negatively impact the efficacy of phytase and the absorption of other minerals (Sebastian et al., 1996; Qian et al, 1997; Manangi and Coon, 2008). In order to effectively utilize minerals from plant-based feed ingredients and to minimize issues arising from Ca-to-P ratio on mineral utilization, there is a need to streamline terminologies (e.g. available P and non-phytate P) and methodologies employed in evaluating Ca and P digestibility and utilization in poultry.

Vitamin D_3 enhances paracellular Ca absorption through the upregulation of tight junction proteins in vitamin D_3 receptor knock-out mice and Caco-2 cells (Fujita et al., 2008). Adequate dietary vitamin D_3 level has been reported to ameliorate TD arising from low dietary Ca levels and to increase tibia ash (Edwards, 1989, 1990; Elliot, 1997; Khan et al., 2009; Tables 1 and 2). Because, the requirement for vitamin D_3 is a function of the criteria being evaluated, it is important to understand that although lower levels (275 ICU or 6.9µg) of vitamin D_3 is sufficient for growth, more than twice this amount (904 ICU or 22.6 µg/kg) is required for ricket prevention. The value for bone ash is slightly lower (552 ICU or 13.8 µg/kg) than for ricket prevention (Edwards, 2000). According to Ferket et al. (2009), supplementing turkey diets with orgainic minerals and 25-hydroxycholecalciferol improves

biomechnical properties of bones, and may improve performance and decrease incidence of leg abnormalities (Table 2).

Table 1 - Effect of dietary vitamin D_3 levels on tibia and toe ash, serum calcium and phosphorus concentration, and on the incidence and severity of tibial dyschondroplasia (TD) in 42-d-old broilers[1]

Vitamin D_3	Tibia ash	Toe ash	Incidence of TD	Severity of TD	Serun Ca	Serum P
IU/kg	----------Percentage point difference----------				--difference, mg/dL--	
200	0	0	29.85	17.25	0	0
1,500	2.65	1.15	14.61	9.67	2.25	2.69
2,500	3.98	2.74	10.94	4.07	4.91	4.69
3,500	4.56	2.90	0	0	6.90	5.94

[1]Values reported are the difference between the values for birds on 1,500, 2,500, or 3,500 IU of vitamin D_3/kg diet and those of birds on diet containing 200 or 3,500 IU of vitamin D_3/kg diet. Based on data presented by Khan et al. (2009).

Table 2 - Effect of dietary supplementation of 25-hydroxycholecalciferol (Hy-D) and organic trace minerals (MIN) on tibia biomechanical properties of 17-wk-old Nicholas turkeys at a 4-point bending test[1]

Hy-D	MIN	T[2] (mm)	Major axis diameter[3] (mm)	Minor axis diameter[4] (mm)	I_x[5] $(m^4)x10^{-9}$	M[6] (Nm)	σ_{max}[7] (Mpa)
No	Yes	0.20	0.30	-0.10	0.00	0.90	1.90
Yes	No	0.00	0.50	-0.10	0.00	-1.10	-5.20
Yes	Yes	0.40	0.20	-0.20	0.10	4.70	16.50

[1]Values are difference between the negative control (- HyD and - MIN) and the respective treatments. Based on data from Ferket et al. (2009); [2]Average thickness of each bone measure on the frontal, caudal, medial, and lateral sides of the bone above and below the break; [3]Bone diameter measured from the medial to lateral sides of the bones; [4]Bone diameter measured from the frontal to the caudal sides of the of the bone at the same point; [5]Areal moment of inertia of the bone; [6]Applied moment; [7]Maximum normal stress at breakage.

IV. OTHER FACTORS THAT MAY AFFECT BONE MINERALIZATION

In addition to the roles of Ca, P, and vitamin D_3 in bone integrity and health, Ferket et al. (2009) showed that increasing the dietary levels of micorminerals such as zinc, manganese, and copper may be equally important in ameliorating mobility issues in turkeys (Table 2). Although, the effects of over feeding dietary vitamin A on incidence of TD in broilers is inconclusive (Ballard and Edwards, 1988; Aburto et al., 1998; Whitehead et al., 2004; Waldenstedt, 2006), Li et al. (2008) showed that feeding very high level (65,512 IU/kg diet) of vitamin A could significantly increase the incidence of TD relative to thecontrol diet (5,512 IU/kg diet) in 35-d old broilers (Figure 1). Thus, overfeeding minerals and vitamins to poultry could be counterproductive. The role of dietary electrolyte balance, especially high chloride level, on the incidence of TD has also been reported (Sauveur and Mongin, 1974; Leeson and Summers, 1988).

Gut health is another factor that may influence the efficiency with which nutrients are utilized. Gut inflammation may alter both the transcellular and paracellular modes of nutrient absorption and tight junction functionality ultimately influencing growth performance and bone mineralization. This has been shown to affect expression on active sodium-dependent phosphate trasporters in broilers challenged with mild coccidial vaccine (Adedokun et al., (2012).

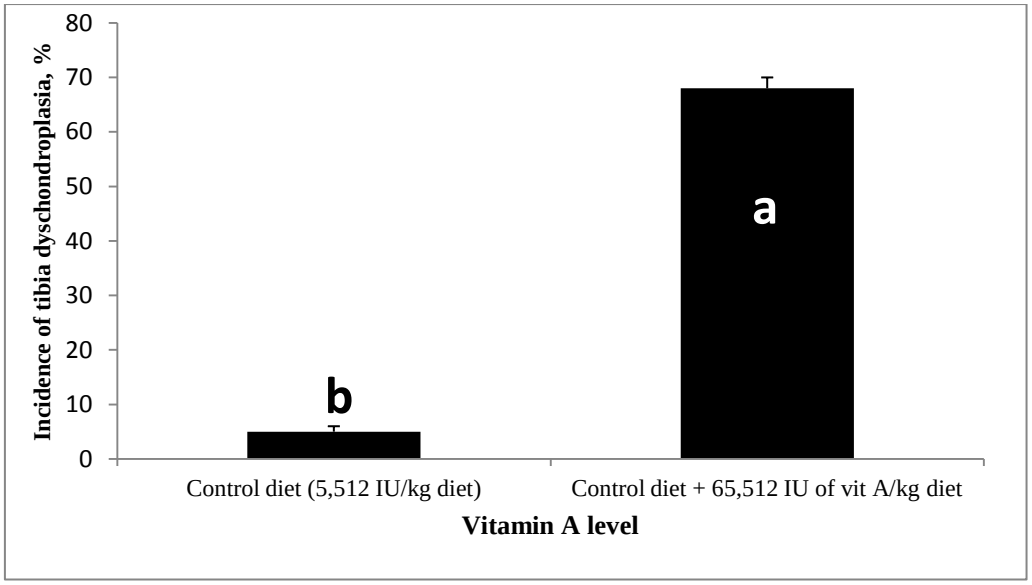

Figure 1 - Effect of high dietary vitamin A level on the incidence of tibia dyschondroplasia in 35-d-old broiler chickens. Data was taken from Li et al. (2008).

Passage rate varies and the resident time of digesta in the GIT could influence Ca and P digestion and absorption. Optimizing Ca and P utilization requires re-visitng NRC (1994) dietary vitamin D_3 recommendations for different classes of poultry in (Kasim et al., 2000; Whitehead et al., 2004; Adedokun and Adeola, 2013). The reasons for this are compelling, especially, if one considers the rate of growth and final mature weights of broilers, turkeys, and ducks.

Among several factors that may influence the efficacy of phytase in broiler diets are dietary phytate level, high Ca level, and Ca-to-P ratio. The consequence of high dietary Ca includes a reduction in birds' performance and an increase in GIT pH (Powell et al., 2011; Walk et al., 2012), as well as interference with the absorption of other minerals (Hurwitz et al., 1978). Several studies have shown that the efficacy of supplementing phytase in non-ruminant diet is affected by dietary Ca-to-P ratio (Qian et al., 1997). High dietary Ca has been shown to decrease the efficacy of phytase in releasing phytate-bound P (Manangi and Coon, 2008; Fisher, 1992; Lei, 1994; Sebastian et al, 1996). Based on this observation, a reduction in the amount of Ca in diets supplemented with exogenous phytase would enhance its efficacy. This will also reduce feed cost through a reduction in the quantity of inorganic sources of P and Ca added to the diet. Furthermore, an increase in phytate-bound P digestibility and P as well as Ca retention in the bones of broilers fed a P-deficient diet with reduced dietary Ca level but elevated cholecalciferol level compared to birds on P-deficient but adequate Ca and cholecalciferol levels has been reported (Mohammed et al, 1991). Details on the metabolites of vitamin D_3 in poultry nutrition and the important roles vitamin D_3 plays in Ca and P metabolism have been reviewed (DeLuca, 1979; Soares et al., 1995).

Phytase consistently liberates phytase-bound P and has the potential to significantly improve the utilization of Ca and other minerals as it is the case with new generation of phytase (Adedokun et al. 2013). Because more than 70% of ingredients used in formulating poultry diets comes from cereals and grains, the potential for further utilization of nutrients from these feed ingredients is huge. In order to optimize its use in poultry diet, it is essential to adequately adjust the diet matrix to reflect the effect of phytase on phytate-bound P and Ca. The efficacy of phytase in releasing phytate bound P in poultry has been extensively documented. Currently, there is no established Ca equivalency value of phytase and

supplementation of poultry diet with phytase may result in significant shifts in the Ca-to-P ratio which may negatively impact Ca utilization. The likelihood of the phytase increasing the Ca-to-P ratio may result in an increase in digesta pH, which may eventually result in mineral precipitation and could drastically reduce Ca absorption. It is important to make sure that diets are formulated to meet Ca requirements so as to avoid the need for Ca resorption from bones in an attempt to maintain blood Ca homeostasis. In order to achieve this, it has been suggested that diets should be formulated on digestible Ca and P basis as it has been done for amino acid (Adedokun and Adeola, 2013; Angel, 2013). It is important to note that factors such as birds' stocking density, lighting program, and genetics of the birds, may predispose birds to skeletal problems.

V. CONCLUSION

There is no doubt that there has been renewed efforts to address mobility and leg problems associated with bone integrity in poultry through adequate nutrition. Despite a reduction in bone breakages and lameness as a result of these efforts, a lot can still be done to nutritionally address this problem. It is important to review minerals and vitamins requirements of poultry to keep pace with selection for high-performing birds. The role of nutrition becomes critical because some of the mobility issues have been linked to inadequate or slow rate of bone mineralization in poultry. Because Ca, P, vitamins D_3, A, C, E, and K, and some fatty acids, have been shown to play important roles in bone development and skeletal integrity, there may be a need to generate more empirical data with the possibility of an upward revision of their requirements for modern high-performing birds. The sources and forms of these nutrients are also important and efforts should be made to ascertain that they are adequate in diets and correctly balanced to prevent deficiencies, excesses, and nutrient-nutrient interaction in the GIT. In addressing these, efforts should be made to avoid overfeeding as this could result in an increase in mobility problems. In order to effectively supply these nutrients, concerte efforts should be made to include Ca and P in diets on digestible basis as opposed to total or available basis. Finally, the supplementation of poultry diets with exogenous enzymes such as phytase have proven to be one of the most potents ways of increasing bone mineralization while simultaneously minimizing nutrient excretion into the environment. This will be good for birds welfare in terms of bone health and integrity, environmental stewardship as it relates to nutrient excretion reduction, and profitability of the enterprise due to a reduction in mortality associated with skeletal integrity as well as a reduction in the number of condemned birds due to bone breakages during processing.

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BROILER LAMENESS IN THE UNITED STATES: AN INDUSTRY PERSPECTIVE

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Summary

Lameness in broilers can result from both infectious and non-infectious sources. Some of the non-infectious sources maybe the result of genetic selection for fast growth and techniques such as reduced photoperiod can be effective in relieving. Bacterial Chondronecrosis (BOC), novel Reovirus infections and spondylolisthesis (kinky back) are three infectious diseases currently resulting in lameness issues in the US. While Reovirus infections and Kinky Back can be devastating, the incidence is sporadic. BOC is more chronic and is seen in most flocks at some level. Assessing lameness on a large scale broiler operation can be difficult and transecting walkthroughs may be a good alternative to gait scoring. Furthermore removing broilers prior to developing complete lameness is a critical practice for chicken growers and integrators must continue to encourage this practice.

I. INTRODUCTION

Broiler lameness is one of the top welfare issues facing the modern broiler industry. Despite the improvements in nutrition, health and genetics over the past decades, too many flocks end up with birds that are partially or completely immobile. Europe has focused more on the welfare aspect of lameness; however, the US industry is taking welfare more seriously as more major retailers, lead by food service companies, now require welfare audits. The incidence of birds with leg problems has been estimated to between 2 and 6%, but to my knowledge no large study of the US broiler industry has been completed in the last several years. A recent large study in the UK (Knowles et al., 2008) found that more than 27% of birds on average had poor walking ability and 3.3 % where considered clinical lame. Because of the many differences in management, genetics, nutrition and environment compared to North America, it is hard to infer that level of leg problems to the US broiler industry, but it does demonstrate that lameness is a real problem for broilers. This paper will look at current status of both infectious and non-infectious leg problems in the US. It will also explore how integrators have changed their perspective on leg problems from a production related issue to a welfare issue.

II. NON-INFECTIOUS LEG ISSUES

Rickets is the classic nutrition caused leg problem and can be separated into two distinct types of rickets: hypocalcaemic and hypophosphatemic. Clinical rickets is not very common in the US due to improvements in nutrition and feed manufacturing. Most cases of field rickets are due to one type or the other of feed manufacturing error. The incidence of sub-clinical rickets is more difficult to assess, but still periodically appears with little or no specific causes. Sub-Clinical rickets has been linked to other conditions such as femoral head necrosis, osteomyelitis, bone fractures and other lameness related problems (Dinev, 2012). My experience with sub-clinical rickets is it is often associated with “soft bones” normally observed around two to three weeks of age and detected on routine health surveys. These cases do seem to respond to water soluble vitamins, so I’ve hypothesized it is related to absorption of fat soluble vitamins. Some operations that are using coccidiosis vaccines, routinely run water soluble vitamin D as a preventative to rickets and soft bones. Phillips et

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al. (2012) has suggested that phosphorus requirements in current commercial strains to 10 days of age may be much higher than NRC or the industry is currently using.

Tibial Dsychondroplasia is a lesion characterized by a mass of avascular cartilage in the metaphysis of the proximal ends of the tibiotarsus and tarsalmetatarsis (Leach and Monsonego-Ornan, 2007). It has long been associated with a fast growing broiler. The condition was once considered the major cause of lameness in broilers. In recent years the condition has been greatly reduced by selection against the condition by utilization of a lixiscope. However, TD is commonly seen in broilers flocks and is frequently associated with other deformities such as valgus-varus and rotated tibias.

Valgus-Varus deformities (VVD) are characterized by angular deformity in the long bones (Bradshaw et al., 2002; Leterrier et al., 1992). It is frequently referred to by many common names such as "twisted legs" or "spraddle legs". Primary Breeders have also been selecting against it through various methods; some highly technical and other phenotypic. However, VVD is still common in many US broiler flocks.

VVD is detected in two specific stages. Varus has been found to occur very early (less than 2 weeks) usually due to the displacement of the gastrocnemius tendon (Bradshaw et al., 2002). It may be related to the incubation or early chick handling. Valgus is a more common condition and occurs progressively later in the growout cycle normally starting to be noticed around 5 to 6 weeks of age and the condition worsens through market age. Rotated tibia is frequently included in the VVD condition, but it has different pathology than VVD (Bradshaw, 2002). It is sometimes seen in conjunction with other leg problems such as TD and BOC. This connection points to some common links such as nutrition, malabsorption or genetics. Bone mineralization has been found to be low in severe VVD limbs (Leterrier et. al, 1992).

VVD including rotated tibia is the most common form of non-infectious causes of lameness in broilers based on my experience and is exacerbated by heavy processing weights of deboning plants. I find that visiting the thigh and/or drum deboning line of large bird processing plant is a good gaue to how much VVD is present in an area.

III. INFECTIOUS LAMENESS

Bacterial Chondronecrosis (BOC) frequently referred to as Femoral Head Necrosis is considered to be the most frequent form of lameness across the US broiler industry today. BOC is thought to be initiated by micro-trauma to poorly mineralized columns of cartilage cells in the proximal growth plates of the leg bones (Wideman and Prisby, 2013). The condition normally starts to develop clinical signs after 5 weeks of age and worsens as more weight is added. The incidence has elevated in importance given the increase in bird size of the US industry relative to the rest of the world. A major portion of the US broiler industry produces broilers for deboning plants that approach or exceed 4.0 kg live weight. Most of these flocks are grown as straight run, so many of the males will exceed 4.5 kg.

Research from Dr. Robert Wideman's group at the University of Arkansas has generated new interests in this disease that ranks as a constant in most broiler operations today (Wideman et al., 2012, 2013). Using the wire floor model, Dr. Wideman has developed theories on the role of stress and immunosuppression in the development of BOC. It is thought that bacteria of various species translocation from the gastrointestinal tract or respiratory system and settle into the areas of the growth plate with poor blood flow. This model has allowed new research into preventative strategies for the condition.

Kinky Back (spondylolisthesis) emerged as a disease of importance in the US in 2008 and continues to be seen broiler flocks today (Ginreich, 2009). Affected birds become completely lame and unable to access feed and water. The only treatment is culling. This

condition is associated with an abscess in the free thoracic vertebra (T4). It is distinct from the classical spondylolisthesis caused by a genetic condition, but carries the same common name. The abscess in the modern kinky back is normally associated with the isolation of the bacteria *Enterococcus cecorum*; but *Staph.* species can also frequently be isolated. Mortality is reported to be as high as 10-15% in flocks. Our experience shows that it frequently repeats on the same farms and houses. It has been combated by increased layout time, between flock cleanout or composting litter and disinfection.

Tenosynovitis or viral arthritis caused from Reovirus infections had been relatively rare in broiler flocks over the past 15 years and broilers are not frequently vaccinated with the common strains. Recently a novel strain emerged in the US broilers industry and available commercial strains of reovirus vaccine are not providing protection (Burleson, 2013; Rosenburger, 2013). I have personally had very little experience with Reovirus in broiler flocks until this past year. At of one our operation reports emerged about severe leg problems including soft bones and leg being completely stuck out to the side. The affected flocks were traced back to specific breeder flocks in one area. In the worst affected flocks up to 40% of the birds had to be culled. Other integrators in the areas had gone through a similar problem a few months earlier. In the affected breeder flocks, virus is shed for 6-8 weeks normally around peak production and no clinical symptoms are present in the hens. Economic losses in the affected broilers flocks are large as an increase in mortality and condemnations increase during the time the virus is present. Some integrators have started moving chicks from breeder flocks identified as positive into small bird programs to limit the losses.

IV. PERSPECTIVE OF A BROILER INTEGRATOR

The perspective on lameness from many broiler integrators has changed over the last 10 years from concern about how many birds were being lost to leg problems (economic loss) to concern about the impact on overall welfare and product quality (such as IP). My company has been on the forefront of animal welfare in the US and was the first broiler company to receive the Humanely Raised Process Verified Program from the USDA. We have learned a lot from acquisition of a smaller company that specialized in Organic and Antibiotic Free chicken production. They complied with some of the most rigid welfare practices in North America. These practices included growers keeping detailed records of birds culled from leg abnormalities and doing leg assessments at key times during the flock. Daily culling lame birds using proper euthanization techniques was not just encouraged; it was required as part of the contract and growers that did not comply risk losing it.

Most, if not all Integrators, encourage euthanization of lame and unthrifty birds. It is in their best interest since these birds will typically end up being condemned in the plant. The act of culling lame birds however can be tedious and difficult. The average age of poultry growers continues to rise and the physical demands of culling older birds can be taxing. Since lameness frequently develops in the last weeks prior to market, many growers may see culling as eating into their profit. This reluctance to cull is not limited to the US. Knowles et al. (2008) in a large scale assessment of lameness in UK broiler flocks noted that more than 3% of the birds classified as lame according to the UK gait method persisted despite strict protocols for culling birds above a gait score of 3.

IV. LAMENESS METRICS

One reason for the absence of large scale studies of lameness incidence in the US is the difficulty in measuring it. The 5 scale gait score has been widely used and reported on in studies in the EU. In this system a score above 3 is considered lame. The National Chicken

Council Welfare guideline (NCC. 2010) has a simpler 3 (0-2) point scale and considers that for broilers, even perfectly normal birds may appear ungainly. These methods may work well for a research program when looking at individual birds is desirable, but in a large scale operation these are not very good methods to assess the overall flock leg health. A recent publication from Spain (Marchewka et al., 2013) demonstrated that transect walks through the broiler house has good potential as a routine monitoring tool. Our company routinely uses a similar method to assess lameness incidence in production houses. It counts the numbers of birds affected by different category of lameness by walking through designated areas of the house. We realize that the system is not perfect with some intraobserver variation that will occur with most any system. However, we feel it is more accurate and practical than doing gait scores on a small sample set of bird.

V. CONTROL MEASURES

Lighting, including photoperiod, intensity and wavelength, is one of the most important, yet poorly understood aspects of poultry husbandry. Many top growers closely guard their own lighting program even locking the control box to protect their "secret". Recent work by Dr. Classen's group at University of Saskatchewan has demonstrated the affect of reduced photoperiod on performance and leg abnormalities. Reduced light periods of 14 and 17 hours was found to significantly improve gait scores versus 20 and 23 hours including infectious related issues (Schwean-Lardner et.al., 2013). The most basic light program for many growers is 23L:1D and many poorer performing farms actually perform better on this type of program. However, we have recently implemented programs with longer dark periods and feel we've seen benefits to both welfare and performance. Moreover, more growers are implementing the dark period at night to eliminate light from illuminating the house from the ventilation system.

Light Intensity has been shown to have lesser effects on welfare than photoperiod (Deep et al., 2010). However, the US industry as a whole at least believes in benefits of lower light intensity (1-5 lux) in the period after brooding. Much of the US industry has progressed to enclosed housing to provide improve environmental controls. Increases in Infectious Process in the processing plant and the resulting downgrades have driven growers to reduce intensity. While I have little evidence that this practice affects leg health either way, it does make it difficult for growers to interact with and assess the flock. One welfare program in which we participate requires light intensity bright enough to read a newspaper and this provides enough light to clearly obverse the flock.

Work from North Carolina State University has shown the importance of proper incubation temperature and chick transport (Oviedo-Rondón et al., 2009). These researchers showed that incubation and transport stress negatively affected the incidence of leg abnormalities such as twisted legs and crooked toes. Many hatchery professionals now routinely use chick rectal temperatures to monitor incubation parameters. The importance of proper chick transport is still an overlooked area. Chicks are frequently are kept in holding rooms for several hours prior to placement. Producers should routinely monitor rectal temperature throughout the transport vehicle at placement to determine whether overheating occurs is occurring.

As discussed above, the translocation of bacteria from the GI tract or respiratory system is thought to be a major contributor to BOC. Wideman et al., (2012) found that a probitoic regime significantly reduced the incidence of BOC in the wire-floor model. This highlights the importance of maintaining intestinal health to overall health. Many integrators in the US still routinely use antibiotic growth promoters; however, a few companies, including ourselves, have removed all growth promoting antibiotics from broilers and only

treat when a flock becomes ill. Preventative therapies such as probitotics, yeast cell walls and other "natural" products are now frequently used in feeds, but there is general confusion around their actual affects.

The goal of producing broilers is to produce meat for consumption and broilers have been developed for appetite to consume feed and fast growth. Lameness is frequently cited as having a direct link to fast growth. While it is true that many slow growing breeds have fewer leg problems, many faster growing flocks have few lameness issues and many slower growing flocks do have these issues. Many restrictive feeding techniques have been tried and early restriction has shown some success (Bradshaw, 2002). Feeding a low nutrient density diet may reduce overall leg problems, but the loss in productivity including key economic drivers like feed conversion and yield are greatly affected. A group from the Welfare Quality project (www.welfarequality.net) has published a feeding regime to eliminate lameness in broilers by feeding a rotating cycle of high protein/low energy and high energy/low protein diets. While I have not tried this approach in the field, we have tried other approaches with little or no evidence of lameness improvements unless the restriction is so severe to dramatically reduce bird performance. I feel that controlling photoperiod is a much more practical and effective approach than quantitative or qualitative feed restriction.

VI. CONCLUSION

Lameness from both infectious and non-infectious sources continues to be a productive loss to broilers producers and compromises the welfare of the animals. Improvements in nutrition, health, management and genetics have reduced the incidences of many types of leg problems and a comprehensive approach to include all areas is essential to reduce the incidence of broiler lameness. This comprehensive approach must include the poultry grower as removing broilers prior to a welfare problem developing due to lameness is critical.

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CHOICE FEEDING BROILERS: INVESTIGATING THE INTERACTION BETWEEN PHYTASE, CALCIUM AND PHOSPHORUS

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Calcium (Ca) and phosphorus (P) are essential minerals for poultry nutrition, however their relationship is multifactorial and highly complex. The majority of P present in corn-soy based diets is present as phytate-P, which due to poor solubility in the small intestine it is poorly digested by broilers. Calcium carbonate commonly used in poultry diets has a high acid binding capacity increasing the pH of the proximal small intestine, reducing the availability of phytate-P and amino acid digestibility (Selle *et al.*, 2009). Previously reported in the literature poultry posses a specific Ca appetite and are able to self-regulate their Ca intake. Wilkinson et al. (2013) reported broilers fed low dietary Ca in conjunction with a separate Ca source can increase phytate-P availability, improving bird performance. This study aimed to investigate the effects of digestible P (dP), Ca and phytase inclusion on broilers ability to self regulate Ca from a separate source and the effect on broiler performance and skeletal health.

A total of 1920 Ross-308 day old male chicks were allocated to one of 48 floor pens, 40 birds/pen. Birds were fed a starter diet from d0-6 before experimental diets were applied d7 onwards Eight dietary treatments (6 replicates/treatment) were arranged as a 2 x 2 x 2 factorial; two levels of phytase (0 or 1500 FTU/kg), two levels of Ca (0.15% or 0.64%) and two levels of dP (0.35% or 0.29%). In conjunction with access to a separate Ca source. Diets were corn-soy based formulated to an AME of 12 MJ/kg. Pen and feed weights were recorded on d0, 7, 21 and 28. Behavioural observations were conducted on d9, 18 and 25, eight focal birds/pen. All bird performance data were analysed factorially using the General Linear Model of SAS. Behavioural data were analysed in GenStat (14TH Edition) using a liner mixed model.

Body weight of birds receiving diets with 1500 FTU phytase, 0.29% dP had significantly reduced body weight than birds fed diets with 1500 FTU phytase, 0.35% dP ($P < 0.05$). Birds receiving diets with 1500 FTU phytase, 0.35%dP had significantly lower FCR (1.39) than birds feed 1500 FTU phytase, 0.29% dP (1.418, $P < 0.05$). Separate Ca intake was higher in birds fed diets with 0 phytase, 0.15% Ca, 0.29% dP and 0 phytase, 0.15% Ca, 0.35% dP when compared to birds receiving 1500 FTU phytase, 0.64% Ca, 0.29% dP ($P < 0.05$) resulting in a significant interaction between phytase*Ca*dP ($P = 0.02$). Behavioural observations showed no significant difference between dietary treatments ($P > 0.05$).

In conclusion the results show that when broilers are fed low Ca diets they are able to self regulate their Ca intake without decreasing performance. Further research is needed to evaluate choice feeding on phytate-P digestibility and mineral digestibility. The results show that it may be possible to spatially separate Ca from the basal diet, improving phytate-P digestibility and performance without any negative implications.

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EFFICACY OF A NOVEL BACTERIAL PHYTASE ON BROILER PERFORMANCE AND TIBIA ASH CONTENT WHEN OFFERED DIETS CONTAINING RICE BRAN

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Summary

A study was conducted to evaluate the efficacy of a novel bacterial 6-phytase for broilers offered corn soybean based diets over two phases starter (0-18 d), and grower (19-35 d) containing two rice bran (RB, Thailand origin) levels 0% and 5% (starter phase) and 10% (grower phase). A total of 624 male (Ross 308) chicks were placed in 48 floor pens at 13 birds per pen with six replicate pens per dietary treatment.

The treatments were arranged as a 2x2x2 factorial consisting of 2 levels of phytase (0, 100 g/t), 2 levels of RB (0, 5-10%) and two levels of AvP (0.45 - 0.36%, 0.30% - 0.21%). The positive control diets PC1 (without RB), PC2 (with RB) contained available phosphorous at 0.45% and 0.36% for starter and grower diets respectively. The negative control diets, NC1 (without RB), NC2 (with RB) were created by reducing DCP to achieve AvP levels of 0.30% and 0.21% for starter and grower respectively. The PC1, PC2 and NC1, NC2 diets were provided either with or without phytase (100 g/t).

On day 35 the birds that received the diet containing RB had significantly higher feed intake (FI) and weight gain (WG) compared with birds that received the standard corn/soy based diets. Birds fed the diets with low AvP concentration had lower FI and WG ($P<0.05$) than those that received the nutritionally adequate diets, irrespective of RB inclusion. Phytase supplementation to NC1 and NC2 diets significantly ($P<0.05$) increased FI and WG to levels that were similar ($P>0.05$) to that of PC1 PC2 diets containing adequate AvP. Phytase improved FCR only in diets that did not contain RB resulting in a phytase*RB interaction (P,0.05). The tibia ash of birds on the low AvP NC1 (without RB) diet was significantly ($P<0.05$) increased when supplemented with phytase (100g/t). It can be concluded that inclusion of novel bacterial 6-phytase is beneficial in improving FI, WG and FCR for broilers offered P adequate and P deficient diets.

I. INTRODUCTION

Over last 20 years the inclusion of microbial phytase enzyme in poultry diets (Selle et al., 2007) has become standard practice. Commercial phytase supplementation in poultry diets may liberate around 50-80% of phosphorus (P) from dietary phytate, improving P digestibility by 1-2 g/kg leading to significant reductions of inorganic P use (Selle et al., 2000, 2007). Though there is a plethora of published literature on phytase, the response of broilers to phytase is still not fully predictable (Selle et al., 2008; Karimi et al., 2011), this is also compounded by the non-linear dose response of phytase where different matrices needs to be used for different dose levels. Though phytase is capable of releasing P the efficacy could be variable due to pelleting temperature and storage conditions of feed, the source or concentration of phytate in the feed.

In-vivo phytate hydrolysis is complex and variable because of digesta transit time in the proximal GIT and the formation of nutrients-phytate complexes which leads to poor solubility which influences P availability (Cowieson et al., 2011). Phytate P hydrolysis and P retention is also dependent on feed ingredients and phytase efficacy is influenced by type of feed ingredient used in diets. For example, though rice bran (RB) contains high

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concentrations of phytate the accessibility or 'reactivity' of this phytate is considered to be low relative to phytate from soybean meal. Leske and Coon (1999) estimate that only around 20% of the phytate-P in RB is accessible to phytase under standard conditions within the intestines of poultry and pigs. Therefore an experiment was designed to study the effect of a novel heat stable form of 6-bacterial origin phytase in diets containing rice bran of Thai origin on broiler performance and tibia ash.

II. MATERIALS AND METHOD

A total of six hundred twenty four male Ross 308 broiler chicks were randomly allocated to 8 treatments. Each treatment was replicated with 6 pens of 13 birds per pen. All the birds were offered the test diets which were formulated to meet or exceed the requirements of all nutrients (NRC 1994) except for AvP and Ca and were based on corn and soyabean meal. The dietary treatments introduced two levels of RB (0 or 5%; starter and 0 or 10%; finisher), two levels of AvP (starter 0.45% and 0.30%, grower 0.36% and 0.21%) supplemented with or without phytase (100 g/t). Lime stone and DCP were used to obtain desired concentrations of Ca and AvP.

The positive control corn-soybean (PC1) and corn-soybean RB (PC2) treatments were calculated to contain Ca and AvP concentration of 0.9% and 0.45% respectively in the starter and 0.8%, 0.36% respectively in the grower phase. In the negative control diets NC1 and NC2 Ca and AvP concentrations were calculated to be 0.72%, 0.30% & 0.62%, 0.21% in the starter and grower phases respectively. The treatments were: (1) corn soybean positive control (T1), (2) T1 + phytase (T2), corn soybean negative control (T3), T3 + phytase (T4), corn soybean RB positive control (T5), T5 + phytase (T6), corn soybean negative control (T7), T7 + Phytase (T8). All the diets were steam pelleted at average conditioning temperature of 81^{o}C.

Growth performance and feed consumption were measured during the period of 0-18 and 18-35 days of age. After body weight measurement at 18 and 35 days of age, 2 birds from each pen were randomly selected for both right and left tibia collection and subsequent tibia ash analysis as per A.O.A.C (1984).

The data were subjected to analysis of variance as 2x2x2 factorial arrangement consisting of phytase, rice bran and AvP using the GLM procedures of SAS (SAS Institute, 1999). Pen was the experimental unit for body weight, body weight gain, feed intake, FCR, mortality and bone ash and the statements of significance among treatments were based on $P < 0.05$.

III. RESULTS AND DISCUSSION

The feed proximate composition were analysed and the results suggested all the diets had adequate nutrients concentration and were close to calculated values. Diets that contained RB had higher calculated phytate-P concentrations compared to corn soybean based diets. The interaction between RB and phytase on FCR and the interaction between RB and AvP on feed intake (FI) for the experimental period are presented in Table 1.

The phytase supplementation had no effect on FCR when were birds fed diets containing RB compared with birds fed corn soy based diets resulting in a significant interaction between RB*phytase for FCR. This might be due to the fact that though RB contains a high phytate concentration relative to most feed ingredients, phytate is not a readily available substrate for phytase. This is in agreement with Leske & Coon (1999) who indicated that the effect of phytase supplementation differs with type of feed ingredient, concluding that phytate from soybean meal is more readily hydrolysed by phytase than phytate from RB. The phytase supplementation improved feed intake of birds fed diets

containing RB with normal AvP concentration compared with birds fed RB diets containing low AvP concentration resulting in significant interaction between RB*AvP. The present study revealed that phytase supplementation had positive effect on the FCR, feed intake and weight gain when birds were fed corn soy diets which is further evidence of extra phosphoric effects of phytase, possibly because mediated via a reduction in the antinutritive effects of phytate in the digestive tract (Cowieson et al., 2009, Cowieson, 2011).

Table 1 - Growth performance of the chicks fed rice bran and phytase from 0-35 d

Treatments	Body weight, g	Weight gain, g	Feed Intake, kg	FCR[1]
Main effect RB[2] (A)				
0%	2.638[b]	2.593[b]	4.026[b]	1.553
5/10%	2.695[a]	2.650[a]	4.121[a]	1.555
P	*0.008*	*0.008*	*0.002*	*0.67*
Main effect AvP[3] (B)				
Normal (0.45/0.30%)	2.702[a]	2.657[a]	4.115[a]	1.549
Low (0.35/0.21%)	2.631[b]	2.586[b]	4.031[b]	1.559
P	*0.001*	*0.001*	*0.005*	*0.10*
Main effect Phytase (C)				
0	2.631[b]	2.586[b]	4.043[b]	1.564[a]
100 g/t	2.702[a]	2.657[a]	4.104[a]	1.545[b]
P	*0.001*	*0.001*	*0.034*	*0.002*
Main effects interaction				
RB x AvP	*0.12*	*0.12*	*0.019*	*0.19*
RB x phytase	*0.62*	*0.63*	*0.46*	*0.023*
AvP x phytase	*0.20*	*0.20*	*0.46*	*0.19*
A x B x C	*0.95*	*0.96*	*0.96*	*0.95*

[1]= Corrected FCR, [2] = Rice Bran [3]= Available phosphorus.

Effects of RB inclusion in broiler diets: The inclusion of 5-10% RB significantly ($P<0.05$) improved feed intake and weight gain, however RB had no effect on FCR. Based on the results presented herein it may be concluded that RB can be included in broiler diets up to 5% and 10% in starter and grower diets respectively. This positive effect is in line with the findings of Farrell, Martin (1998) who reported that RB could be safely included up to 20% in broiler diets, possibly as a source of functional fibre which may improve gizzard function or modulate the microbial flora in the distal gut.

Effect of available P levels: There was a consistent negative effect of low AvP level on growth performance and tibia ash content. This finding is consistent with many other studies (Broz et al., 1994; Dilger et al., 2004; Selle et al., 2007). It was observed that low body weight gain was primarily associated with low feed intake in low AvP diets and suggesting that P was the first limiting nutrient in the experiment and is essential element necessary for normal appetite.

Phytase effect: The results showed the positive effect of phytase supplementation on feed intake, weight gain, FCR and tibia ash. These observations are in agreement with previous work with this phytase (Aureli et al., 2011; Shaw et al., 2011; Favero et al., 2012; Rutherfurd et al., 2012). However the extra phosphoric effects observed in the present study requires further investigation with digestibility studies to measure the endogenous nutrient flow and sparing effect of this phytase on non P nutrients.

IV. CONCLUSION

It can be concluded that a novel bacterial 6-phytase, at standard dose of 100g/t, was effective in replacing 0.15% AvP from inorganic P in corn/soy-based diets for growing broiler chickens. Furthermore, though RB is a rich source of dietary phytate it may not be an entirely suitable substrate source for microbial phytase. Further work is required to delineate the reactivity phytate from different sources to avoid over-estimation of phytase matrices and/or under estimation of potential nutrient yield from phytase super-dosing strategies.

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EFFECT OF CHOICE FEEDING ON FOOTPAD DERMATITIS AND TONIC IMMOBILITY IN BROILER CHICKENS

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Nowadays, genetic improvements have provided chickens with faster growth in lower production periods. In this situation skeletal abnormalities and stress are the negative consequences of the chickens' modifications (Robinson et al., 1992). On the other hand, feeding planners have to exert several feeding programs due to the alternating changes of birds' requirements and also their welfare situation during the time (Shariatmadari, 2012). Therefore, with the ever increasing concern of consumers for food quality and animal welfare, it is necessary to use applicable welfare measurements. Choice feeding is a program which let chickens to feed base on their needs and so they can choice between diets offered to them (Munt et al., 1995). Some countries still using footpad scoring to evaluate the welfare situations of broiler chickens (Allain et al., 2009). Tonic immobility also is an indicator of chicken fearfulness (Jones and Faure, 1980). Therefore, the objective of the present experiment was to evaluate the impact of choice feeding with diets varied in energy and protein on footpad dermatitis and tonic immobility of chickens.

In this experiment, total of 195 day-old broiler chicks (Ross 308) were randomly allotted to 3 treatments and 5 replicates of 13 chickens in completely randomized design. The diets varied in energy (E+ = 3210 kcal/kg and E− = 2790 kcal/kg) and protein (P+ = 25.14 % CP and P− = 16.76 % CP) contents. Chickens were choice fed by dietary treatments included of control group (ME: 3000 kcal/kg, CP: 20.95 %) or either intermittently fed by HL (choice feeding of high energy diet and low protein diet followed by high energy diet and high protein diet); and LH (Choice feeding of low energy diet and high protein diet followed by high energy diet and high protein diet) in 48 h cycles, during growing period since day 8 until 28 of age. Pad dermatitis was evaluated by using a 4-point scale from 0 to 3 in which 0 shows no sign of damage and 3 is for extended burn and inflammation of 8 chickens per pen on day 35 of age (Sørensen et al., 1999). Tonic immobility also was tested on 3 male and 3 female chickens on day 39 from each pen considering Campo and Redondo (1996) method.

The results suggested that chickens fed by LH group had significantly greater values for footpad dermatitis scores ($P<0.05$) which might be attributed to the higher protein fed by chickens in this group and their subsequent excretion of ammonia to the litter which negatively compromise the footpad of chickens (Sirri et al., 2012). However LH group insignificantly showed the lower time duration in tonic immobility compared to HL but was not significantly changed compared to control group which might shows that choice feeding had no negative effect on chicken's fearfulness.

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