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MP00-75 The 2000-2001 Nebraska Poultry Report

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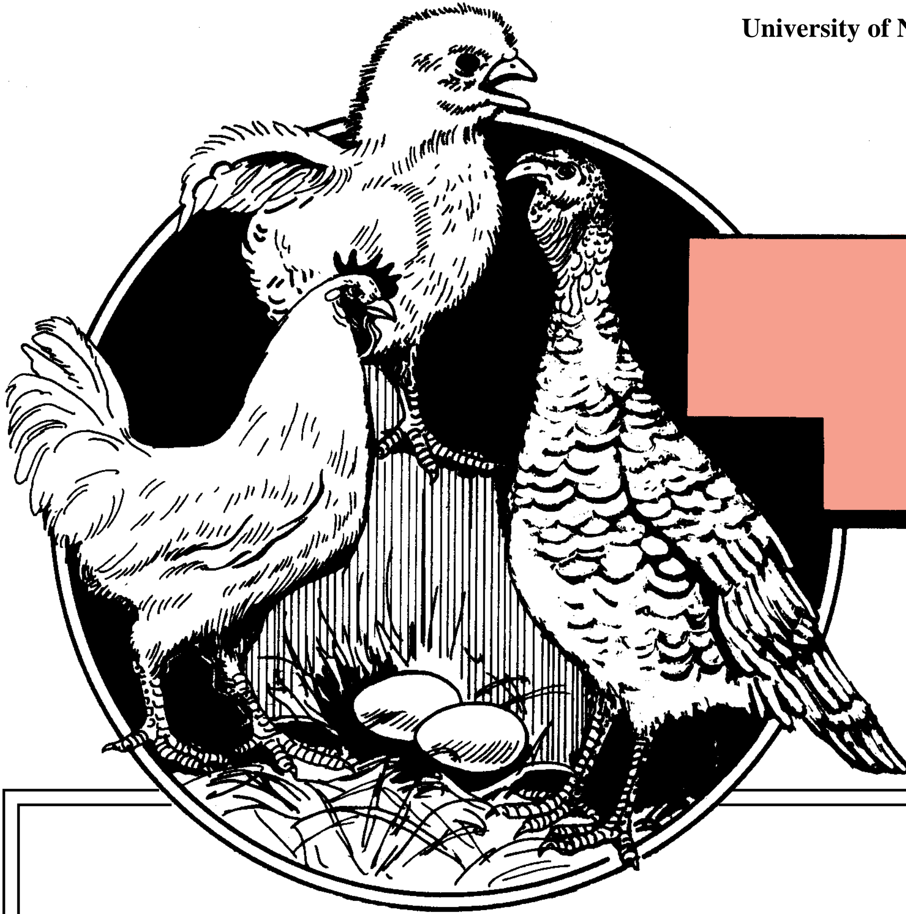

The Nebraska Poultry Report

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The 2000-2001 Nebraska Poultry Report

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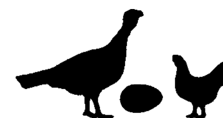

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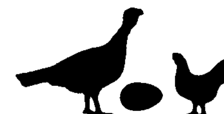
Introduction

The Nebraska Poultry Report is produced every two years by the Animal Science Department's poultry faculty with contributions from others in the University of Nebraska who work with avian species. The purpose of the report is to make our activities known to the poultry industries in Nebraska. The majority of articles are based on on-going research but are written in a relaxed style for ease of reading. If at any time an article or piece of information is of special interest to anyone in the industry, we hope you will contact us to discuss it.



Ratios of Linoleic to Linolenic Acid and Immune Function in Pullets

Uaichai Puthpongsiriporn
Sheila E. Scheideler[1]

It has been well established that immunologic reactions play a role in the development of infectious diseases. Young animals, including pullets, are susceptible to several endemic and epidemic diseases. In recent years, investigators have focused on the relationship of fatty acids to immune status and function in humans and animals. Several studies have reported that fatty acids have an immunomodulatory effect in various species. Since linoleic ($C_{18:2n-6}$; n-6) and linolenic ($C_{18:3n-3}$; n-3) acids are major essential fatty acids in poultry diets, poultry scientists have been interested in the effects of these fatty acids and their derivatives on immune function. Some researchers have reported enhanced lymphocyte proliferation by linoleic acid (Harbige *et al.,* 1995) and immunosuppression effects of linolenic acid (Calder and Newsholme,1993). In regard to these immunological effects, one might think that standard corn-soybean poultry diets, which are rich in linoleic acid, or diets supplemented by high levels of linoleic acid can enhance immune responses, while linolenic-enriched diets may suppress immune response in chickens. This is partially true; however, high levels of linoleic acid have also been demonstrated to show some immunosuppression action (Yaqoob *et al.,* 1994). Until recently, several studies have reported an enhanced immune response as dietary linoleic acid is replaced with dietary linolenic acid in humans (Harbige, 1998), rats (Jeffery *et al.,* 1996; Peterson *et al.,* 1998), and chickens (Fritsche and Cassity, 1992).

Deficiency or excess of a number of individual nutrients have been linked to altered immune responses. A deficiency of both linolenic and linoleic acids lowered several indices of immune response in rats, including spleen and thymus weights, delayed hypersensitivity skin response, T- and B-cell blastogenesis, macrophage cytotoxicity and neutrophil chemotaxis (Gyllenhammar *et al.,* 1988). Studies of the effects of dietary polyunsaturated fatty acids (PUFA) on the immune responses in poultry, however, are limited. Linoleic acid and its derivatives have been reported to have desirable effects on immune responses (Crevel and Saul, 1992); however, excess linoleic acid in diets causes immunosuppression (Yaqoob *et al.,* 1994) in animal models.

An important function of linolenic and linoleic acids is to serve as precursor substrates to produce active metabolites, as depicted in Figures 1 and 2. Linoleic acid is a precursor of arachidonic acid ($C_{20:4n-6}$). In the body, arachidonic acid interacts with cyclooxygenase or lipoxygenase to yield active eicosanoids including prostaglandin E_2 (PGE_2), thromboxanes, prostacyclins and leukotriene B_4 (LTB_4). These arachidonic acid-derived mediators, especially prostaglandin E_2 and leukotriene B_4, are involved in immune responses. Prostaglandin E_2 regulates the production of interleukin 1 and tumor necrosis factor. Leukotriene B_4 enhances T and B cell proliferation, natural killer cell activity, and cytokine release. Significant amounts of eicosanoids metabolized from overproduction of arachidonic acid suppress immune

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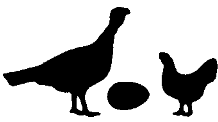


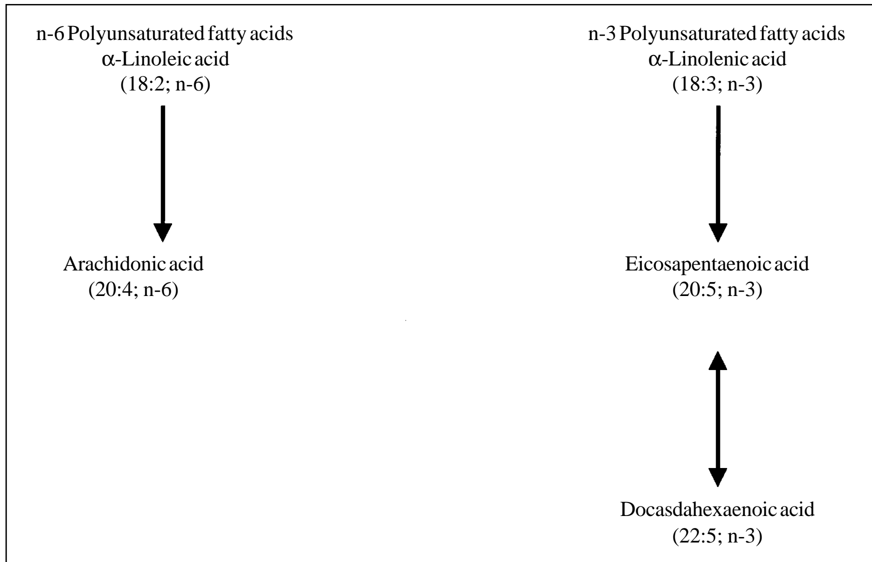


Figure 1. Metabolism of a-linoleic and linolenic acids.

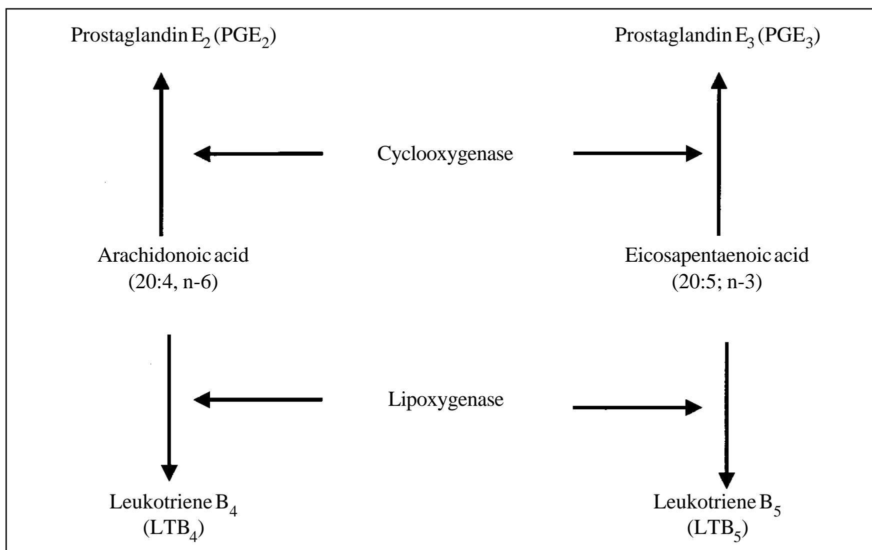


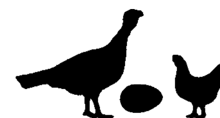
Figure 2. The arachidonic and eicosapentaenoic acid cascade.

responses. This undesirable effect has been shown to be alleviated by dietary linolenic acid.

Linolenic acid is also an essential fatty acid for poultry. Linolenic acid has been demonstrated to inhibit a number of lymphocyte functions and proliferation, interleukin-2 production, natural killer cell activity, and antigen presentation (Jeffery *et al.,* 1996). Eicosapentanoic acid (EPA; $C_{20:5n-3}$) and docosahexanoic acid (DHA; $C_{22:6n-3}$), metabolized forms of linolenic acid react with cyclooxygenase or lipoxygenase to yield prostaglandin E_3 (PGE_3) and leukotriene B_5 (LTB_5) whose immune effects are less harmful than those of PGE_2 and LTB_4. By sharing the same enzyme pathways, EPA and DHA can compete with arachidonic acid as substrates for the cyclooxygenase or lipoxygenase systems, resulting in reduction of eicosanoid production and an increase of PGE_3 and LTB_5.

The combination of dietary linoleic and linolenic acids in optimum proportions, therefore, may produce positive immune responses. Supplementing dietary lipids varying in polyunsaturated fatty acid (PUFA) content can modify the composition of tissue fatty acids. Replacement of proportions of linoleic acid with either EPA or DHA in rat diets has been reported to yield a decreased production of PGE_2 (Peterson *et al.,* 1998). Feeding chicks 50 g/kg of linseed oil, which is rich in α-linolenic acid, decreased the amounts of arachidonic acid and eicosanoid, but concomitantly raised eicosapentanoic acid in organ lipids by enhancing formation of n-3 derivatives (Phetteplace and Watkins, 1989). The investigators also reported that feeding animals with diets rich in n-3 PUFA suppressed lymphocyte proliferation. Similarly, Fritsche and Cassity (1992) reported that chicks fed 7% flaxseed oil had decreased activity of antibody-dependent cell cytotoxicity in splenocytes and lower eicosanoid production than chicks fed 7% corn oil. Jeffery *et al* (1996) fed rats with 20% of a mixed sunflower-linseed oil calculated to contain n-6/n-3 PUFA ratios of 112.5:1. 14.8:1, 6.5:1, 0.8:1, or 0.33:1. The investigators found there was a progressive decrease in the amount of linoleic and arachidonic acids, a progressive increase in the amount of α-linolenic acid and a progressive decrease of lymphocyte functions as the n-6/n-3 PUFA ratio of the diet decreased. These studies indicate that replacement of dietary linoleic acid with linolenic acid can decrease overreaction of immune responses in rats. The optimum ratios of dietary linoleic to linolenic acids for the immune system, however, have not been determined for pullets.

A preliminary study at the University of Nebraska (Scheideler and Puthpongsiriporn, 1999) investigated the optimum ratio of dietary linoleic to linolenic acids in pullet diets. In the study, pullets were fed the following dietary linoleic to linolenic acid ratios: 17:1 (control), 8:1, 4:1, or 2:1, beginning at 1 day old and continuing to 16 weeks. Pullets were vaccinated against specific diseases following a standard industry vaccination program. The study indicated that antibody titers in



response to the ratios of linoleic to linolenic acids at 8:1, 4:1 and 2:1 were greater than that of 17:1. The decreased n-6/n-3 ratios tended to properly stimulate T-lymphocyte proliferation, whereas B-lymphocyte proliferation was not influenced by the fatty acid ratios. In conclusion, the optimum ratio of dietary linoleic to linolenic acids should result in a balance of immunomodulatory response. This balance will induce high levels of immunity against disease in pullets. A study of varying ratios of dietary linoleic to linolenic acids to maintain high levels of immune responses throughout the production cycle of older hens will be conducted at the University of Nebraska in the near future.

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GMOs in Today's Animal Agricultural Industry

Jodi Ash[1]

Genetically modified organisms (GMOs) are a center topic of debate in todays agri-political forum. Concerns based on environmental impact, food safety and corporate control have stimulated a great deal of interest in current agricultural policy and reform in this proliferating industry. A wide range of GMOs has been developed over a few short years. More than 600 patents are held on genetically modified organisms around the world. Most of the patents are based on transgenic plant and crop improvements using recombinant DNA technology.

In traditional crossbreeding programs, the genetic resources available for improvement have been limited. Only the same or closely related species can be used. Moreover, when plants are crossbred, all 100,000 plus of each plant's genes are mixed, producing a completely randomized combination. Traditional plant breeders ultimately desire only a few genes or traits to be transferred, hence this methodology is extremely inefficient and imprecise. Through recent improvements in biotechnology and the science of genetic modification, however, both precision and efficiency have been achieved.

Modifications are being introduced into conventional genetics in ways not possible by traditional means of reproduction and/or natural recombination. Instead of spending years on a crossbreeding program to elicit a desired trait, scientists can identify and insert a single gene associated with a select trait with relative speed. In addition, genetic material can be incorporated from other plant species, animal, bacterial, or viral sources.

Using recombinant DNA technology, a vast array of products are being made. GMOs range from a strain of

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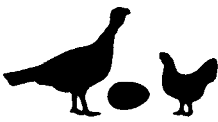

strawberries that contain shrimp DNA for color to a corn variety that has integrated viral DNA to produce pesticidal effects. This technology may aid in increasing global food production by reducing crop losses and increasing yields while conserving cropland. Already, improvements have been developed allowing crops to maintain herbicidal tolerance, be drought resistant and exhibit enhanced nutritional value.

GMO variety crops have been planted in the U.S. only since 1996, but they have been well received by the farmer. In the 1999 growing season, 50% of the nation's soybeans and cotton and 30% of the corn planted was GMO. The elevators, on the other hand, don't seem so enthusiastic. Only 8% of Midwest elevators last year segregated the GMO from conventional soybeans and only 11% for corn. Of the elevators segregating, only 3% were giving premiums for non-GMO beans, and 1% for non-GMO corn. Premiums ranging from 5-35 cents/bushel for non-GMO beans and 8-15 cents/bushel for non-GMO corn. There were no reported discounts for GMO.

This interest in segregation and premiums for non-GMO grains has been precipitated by recent developments in foreign market regulations. These regulations have been fueled by foreign consumer concerns based on environmental, human health and market share issues. Environmental concerns are derived from uneasiness over the potential hazards related to gene transfer from GMOs to indigenous organisms and prospects of secondary ecological effects. Consumers fear the development of enhanced resistance in weeds and insects to the chemical and herbicides now found in GMO corn and soybeans.

Human health concerns are based on the possibility of GMOs affecting the immune system, and a development of resistance to antibiotics. The possibilities of unforeseen allergic reactions in response to foreign genetics, as well as ethical and religious concerns, also are an issue.

Politically speaking, the prevention of a monopolistic market is possibly the root of foreign-based concern. Currently only a handful of American organizations hold most of the patents on transgenic plant and crop technology improvements. As a result these American organizations control most of the supply, certainty of supply, pricing and quality of GMO materials. Without access to this technology, foreign governments are very concerned about maintaining or improving their market share. The European Union, Mexico, Australia, New Zealand and many Asian countries have begun to integrate labeling requirements on genetically modified organisms or food products containing GMOs. In October of 1999 the European Union adopted a 1% threshold, meaning that it was only those foods that contain more than 1% GMO in any ingredient that have to be labeled as containing GMO. So far no governmental regulation has established the threshold below which foods could be labeled "non-GMO"; discussions indicate that such a threshold will most likely be "less than 0.1%."

These foreign regulations have stimulated political and economic tensions between governments. The U.S has strongly backed the development and use of genetic modification for the improvement of nutrient value and more efficient use of ecological and chemical resources. Currently in the U.S., the FDA requires labeling of food made from GM grains only if the crops differ in composition, nutritional profile, safety or allergenicity.

As the animal agricultural industry here in the U.S. uses corn and/or soybeans as major constituents in animal production diets, large percentages of which are GMO, the next concern is that foreign markets will begin to scrutinize poultry meat and egg product exports. In 1998, about 60% of the soybean meal and corn went to domestic livestock production. The biotechnology controversy could expand to U.S. meat produced from poultry fed GM grain. However, the research community is confident that the animal products will be void of any identifiable transgenic material. Although limited work on the subject has been published by academia, the biotech companies support the theory that poultry meat or eggs will not be affected by consumed modified genetics, though they are retaining the information.

Currently two tests are predominantly being used to test for genetically altered materials. The first test is an immunoassay test that uses antibody sandwich to indicate a particular identifying segment of a protein strand. However, when an animal ingests a protein source, the protein usually is broken down during normal digestion. It is degraded into di- and tripeptides as well as single amino acids which are absorbed in the lower hindgut. Due to this degradation researchers believe that protein from the transgenic portion of DNA is destroyed along with other proteins. Hence, the antibody sandwich no longer is able to find the identifying sequence. The second test is a polymerase chain reaction (PCR) test that looks for a particular sequence of nucleotides in a DNA strand unique to that particular GMO. Similarly, when DNA is broken down into nucleotides, the sequence is no longer intact and the PCR test cannot detect it.

At the University of Nebraska, we are using soybean meal, poultry feed and eggs to test the aforementioned theory of complete digestion of GMO protein by the laying hen. We hypothesize that no intact DNA or protein fragments from the modified portions of Roundup Ready soybean meal consumed by the hen will be deposited in the egg. This study and others like it aim to alleviate consumer concerns of GM protein being incorporated into meat and egg products. In addition, with the responsible use of this new technology, environmental, food safety and corporate control concerns also will be dispelled in time.

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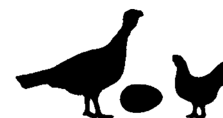

Behavior and Immune Response to n-3 Fatty Acid Supplementation in Preschooler Diet

Elizabeth Marsh
Sheila Scheideler[1]

Research has documented many associations between diet, early childhood development, behavior and overall health. The primary focus of the research presented herein is n-3 fatty acids, or, more specifically, alpha linolenic acid (ALA) and docosahexanoic acid (DHA), both essential nutrients. The n-3 fatty acids, otherwise referred to as omega-3 fatty acids, are found in their most concentrated forms in fish oils; however, flaxseed and "omega eggs" are two other valuable sources.

By adding flax seed, fish oil, or bioengineered algae into the hen's diet, fatty acid profiles of the hen's yolk can be modified to incorporate ALA and DHA in relatively large quantities to produce the designed Omega Egg. On average, a standard egg would have only 40mg and 20mg of ALA and DHA; however, Omega eggs contain 250mg and 100mg respectively. In a test of eggs from flax or fish oil-fed hens, taste was not greatly different from that of regular hens (Scheideler and Cuppett, 1997).

Americans generally do not consume adequate fish or fish oils to meet their n-3 fatty acid needs; therefore, ways of incorporating flax seed and omega eggs in the diet may provide a natural and healthy n-3 fatty acid dietary alternative. Current research suggests that an n-3 fatty acid deficiency is common among all age groups, and deficiency can lead to behavior problems.

Although infants, adolescents, and adults are commonly studied for such deficiencies, little research has been done on preschool children. Among the behavioral disorders exhibited by children, attention deficit/hyperactivity disorder (ADHD) is one of the most prevalent, affecting 3-5% of the school-age population. Incidence of learning and health problems appears greater in boys (ages 6-12 years-old) with lower total n-3 concentrations (Enslen et.al. 1991). The proposed research will add to current knowledge by examining the level of essential n-3 fatty acids in the diet and the effects of the supplementation with respect to hyperactivity and immune response in preschool-age children.

Initial assessment of dietary n-3 fatty acid intake was assessed in children ages 2-5 at Ruth Staples Childcare Center at UNL in the fall of 1998. Children were found to be moderately deficient in n-3 fatty acids due to lack of essential fatty as recommended by Canadian recommendations. For n-3 fatty acids, Health and Welfare Canada suggests 0.5g n-3 fatty acids per day for infants and 1.8g per day for adolescent boys. Choosing a moderate midpoint for preschoolers, 1g per day was not met in the average preschoolers' diet. With this comparison, concern was raised of the possibility of developmental problems among the preschool children due to n-3 deficiencies.

In the fall of 1999 a thorough study of dietary enrichment of n-3 fatty acids in preschool diets began at the Ruth Staples Laboratory. Children were randomly divided into "warm" or "cool" groups. During the first five weeks the "warm" group served as the experimental and the "cool" as the control. During snack time at the childcare center, children were given a normal baked good such as banana bread or brownies. Food given to the experimental group contained about 1000mg ALA and 100mg DHA, supplemented with Omega eggs and ground flaxseed. Food given to the control group contained virtually none of the n-3 fatty acids. On weekends and holidays, children were provided baked goods and Omega Eggs to eat at home. After the five-week trial period and a washout period of almost two months, the "warm" group became the control and the "cool" the experimental for a second five-week trial.

During this four-month period, parents were asked to complete three total health surveys — one before the first five-week trial, one after the first five-week trial, and one after the second five-week trial. The health surveys asked about frequency of colds, flu, doctor's visits and missed class due to illness to assess the dietary effects of the immune system of the child. Parents also were asked to complete a Conners' Rating Scale the same three times during the experiment. Conners' Rating Scale is a self-administered survey style test completed by both parents and teachers and is considered the gold standard in the clinical setting to quantify hyperactivity in 2-12-year-olds. Additional insight will be gained from the three-day food records of parents and teachers on randomly chosen days during both five-week trials. Knowing the intake of n-3 fatty acids will provide a direct correlation between the intake and perceived hyperactivity and immune response.

Although the average American of virtually every age group does not consume adequate n-3 fatty acids with fish or fish oil, omega egg supplementation could be an easy cost effective way to lower hyperactivity of preschoolers and improve the efficiency of their immune system. It is believed that consumers will be willing to pay slightly more for the omega eggs once they realize the importance of adequate n-3 fatty acids in the diet for all age groups.

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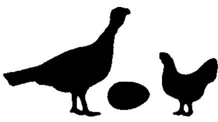
[1]Elizabeth Marsh, graduate student, and S. E. Scheideler, Professor and Extension Poultry Specialist, Animal Science.



Effects of a Mn & Zn Proteinate (Eggshell-49) on Eggshell Quality in Laying Hens

Sheila Scheideler
N. Ceylan[1]

Introduction

Eggshell quality has always been a problem in the layer industry. Economic losses because of poor shell quality are estimated to be greater than $250 million per year (Gomez-Basauri, 1997; and Bell, 1998). Numerous studies have been conducted to solve poor shell quality. Many of these studies have focused on macro minerals, especially calcium (Ca) and phosphorus (P) (Keshavarz 1988; and Roush et al. 1986). Trace minerals such as manganese (Mn) and zinc (Zn) play an important role in egg shell and shell membrane formation because these are co-factor and(or) structural products of enzyme systems responsible for carbonate formation and mucopolysaccharide synthesis, respectively.

Gill (1997) reported that chelated trace mineral (organic) sources are more biologically available in an animal's digestive system than inorganic minerals, perhaps resulting in less mineral excretion and pollution of the environment. In a field study conducted by Miles (1998) with 52-61 week old layers, there was an improvement in egg grading when Eggshell 49 (a Mn-Zn proteinate) was added at a rate of 1 kg/ton. Others have emphasized that the shell to organic membrane relationship is critical to good shell quality and must be considered. Eggshell 49 has been recommended as a supplement particularly after week 49 of egg production.

The mixed results of the above concepts are that macro and trace minerals (organic) can have significant effects on eggshell quality. It was the objective of this research to test the effects of a Mn and Zn proteinate supplementation from Eggshell 49 on eggshell quality throughout a laying cycle (20-60 wks.).

Experimental Design

Each diet was fed to Hy-Line W-36 hens from 20 weeks to 60 weeks of age. Two levels of Ca (3.5 and 4.0%) and two levels of Eggshell 49 (0 or 2 lb/ton) were fed during 20-40 or 40-60 weeks of age in a factorial arrangement of 2x2x2. Each treatment was replicated 6 times with 6 hens per replicate pen for a total of 48 pens. After 44 weeks of age, diets were reformulated to decrease the density (Hy-Line Management Guide, 1995). Egg production, feed intake and cracked eggs were recorded daily. Egg weights, egg specific gravity, Haugh units, dry shell percent and eggshell breaking strength (Instron) were measured biweekly. Hens were weighed at the start of the experiment and at three-week intervals thereafter. To ascertain potential effects of zinc supplementation on carbonic anhydrase activity, an *in vitro* assay was conducted with shell gland tissue at 40 and 60 weeks of age.

Research Results

Dietary Eggshell 49 (ES49) and/or Ca had no significant (P>0.05) effect on hen weight, egg mass, feed conversion, egg weight, or Haugh unit. A significant Ca×ES49 interaction effect on egg production and egg mass occurred during the 20-40 week time period, such that, within the 4.0% Ca treatments, ES49 supplementation decreased egg production by nearly 2%. However, within the 3.5% Ca level, ES49 had a more positive effect on egg production and egg mass. The hens receiving 4% Ca consumed more feed (P=.06). There was an improvement in feed conversion with ES49 supplementation, and the interaction with Ca was significant (P<0.05).

Average specific gravity, dry shell percent, egg shell breaking strength, cracked eggs, serum Ca, and shell gland carbonic anhydrase activity results from 20-40 weeks are presented in Table 1. There were no significant differences in specific gravity or serum Ca level by treatments. ES49 supplementation had a positive impact on dry shell percent (P=0.08), egg shell breaking strength (P=0.01), and percent cracked eggs (P<0.05). Carbonic anhydrase activity increased nearly 10% with ES49 supplementation. Higher dietary Ca also increased carbonic anhydrase activity significantly (P=0.01). Wang et al. (1996) reported carbonic anhydrase activity in hens with high quality eggs to be greater (P<0.01) than in hens producing low quality eggs. In our results, it can be seen that there is a good relationship between carbonic anhydrase activity and shell quality.

Effects of ES49 supplementation and dietary Ca on production parameters for the entire production period (20-60 wks) were minimal. Significant interaction effects of ES49 and Ca on egg production for the entire period

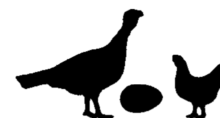


Table 1. Effects of Dietary Ca Level and ES49 Supplementation on Egg Shell Quality Measurements from 20-40 Weeks of Age

Diet Ca	ES-49	Specific gravity	Dry shell percent	Eggshell breaking strength	Cracked eggs	Carbonic anhydrase activity (40 week)
(%)	20-40 wks		(%)	(Newton)	(%)	(Unit/g tissue)
4	N	1.085	8.89	30.13	0.815	217.7
4	Y	1.086	9.07	31.46	0.701	221.4
4	N	1.085	8.94	30.25	1.190	219.4
4	Y	1.085	8.83	31.26	0.623	239.2
3.5	N	1.085	8.74	29.26	0.973	194.5
3.5	Y	1.085	8.91	30.26	0.651	227.1
3.5	N	1.085	8.82	29.49	0.849	187.3
3.5	Y	1.085	8.99	32.33	0.856	204.2
SEM		0.0007	0.09	0.81	0.125	20.2
P-value		NS	NS	NS	NS	0.05
ES49						
Without ES49		1.085	8.85	29.79	0.957	204.7
With ES49		1.085	8.95	31.33	0.708	222.9
P-value		NS	0.07	0.01	0.05	0.03

Table 2. Effects of Dietary Ca Levels and ES49 Supplementation on Egg Shell Quality Measurements from 20-60 Weeks of Age

Diet Ca	ES-49		Dry shell percent	Eggshell breaking strength	Cracked eggs	Carbonic anhydrase activity (60 weeks)
(%)	20-40 wks	40-60 wks	(%)	(Newton)	(%)	(Unit/g tissue)
4	N	N	8.77	28.48	0.695	145.5
4	Y	N	8.97	29.41	0.899	155.4
4	N	Y	8.76	28.41	1.229	178.1
4	Y	Y	8.72	29.47	0.607	200.1
3.5	N	N	8.50	27.22	1.268	158.1
3.5	Y	N	8.77	28.43	1.048	188.9
3.5	N	Y	8.64	28.15	1.356	214.9
3.5	Y	Y	8.86	29.52	0.715	195.3
SEM			0.08	0.58	0.162	13.9
P-value			0.001	0.07	0.05	0.01
ES-49						
Control		8.64	27.85	0.982	151.8	
ES49	Y	N	8.87	28.92	0.974	172.2
ES49	N	Y	8.70	28.27	1.293	196.5
ES49	Y	Y	8.79	29.50	0.661	197.7
P-value			0.002	0.028	0.02	0.01

were similar to those discussed from 20-40 weeks of age. Egg weight, Haugh unit and specific gravity were not affected ($P>0.05$) by either ES49 or Ca level. Although the difference was not significant, the heaviest egg weight and highest albumen quality (Haugh unit) was found in the groups receiving ES49 at both 20-40 and 40-60 weeks. Calcium level had no significant effect on these criteria.

Shell quality parameters (20-60 weeks) are presented in Table 2. Dry shell percent, egg shell breaking strength and percent cracked eggs were influenced significantly ($P<0.05$) by dietary ES49 supplementation. Dry shell percent was found to be the highest in the diet which contained ES49 from 20-40 weeks and Ca level at 4.0%, followed by the diet which contained ES49 from 20-60 weeks. The lowest percent shell was found in the diet with 3.5% Ca with no ES49 during the entire experiment. Higher dietary Ca (4.0%) also improved percent dry shell. Shell breaking strength showed a positive effect of ES49 from 20-60 weeks of age compared to no ES49 supplementation at all. Both 3.5 and 4.0% Ca without ES49 for the entire experiment had the poorest egg shell breaking strength. Percent cracked eggs was significantly reduced when ES49 was included the entire time from 20-60 weeks of age. Higher dietary Ca (4.0%) also decreased ($P=0.08$) percent cracked eggs.

Shell gland carbonic anhydrase activity was affected by all treatments. Hens receiving ES49 in both 20-40 and 40-60 weeks (20-60) had the greatest enzyme activity ($P<0.01$) at 60 weeks while the hens receiving no ES49 had the lowest activity. Levels of dietary Ca also affected activity such that hens receiving 4% Ca had higher activity than those receiving 3.5% Ca. There was a decrease in carbonic anhdrase activity (Tables 1 and 2) as hens become older. When we look at the 40-week and 60-week results we can see that eggshell quality parameters are declining. Hence, we can postulate that the decline in enzyme activity may be related to decreased shell quality.

Summary

From these results we can conclude that supplementation of a Mn and Zn proteinate from ES-49 starting at 20 weeks until 60 weeks of age had a positive impact on all shell quality criteria. Further, although 3.5% Ca was found to be adequate for 20-40 weeks, 4.0% Ca was recommended after 40 weeks of age.

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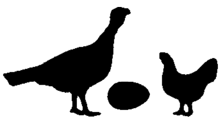
[1]Sheila E. Scheideler, Professor and Extension Poultry Specialist, Animal Science, Lincoln, and N. Ceylan, Associate Professor, Poultry Nutrition, University of Ankara, Turkey.



Effects of Feed Enzymes in Corn-Soy Layer Diets on Digestion and Nutrient Availability

Sheila E. Scheideler[1]

Supplementary feed enzymes in corn-soy diets for poultry have captured the interest of poultry nutritionists across the country as availability of such enzymes has increased. Traditionally, supplemental enzymes such as glucanase have been used primarily in wheat-based diets in North America. However, new research findings have indicated some value to adding amylase/proteinase enzymes to corn-soybean meal-based poultry diets. It is surprising to discover how much corn or soybean meal may escape the chicken's digestive effort intact. Figure 1 shows undigested particles of corn and soybean meal taken from the lower part of a hen's small intestine. Large cell walls are readily apparent that have not been completely broken down by the endogenous enzymes in this part of the small intestine 1 1/2 hours after feed consumption. Figure 2 shows digesta from the same strain of hen 1 1/2 hours after consumption of a diet supplemented with Avizyme 1500 (amylase + proteinase). There are still cell walls apparent in Figure 2, but there are not as many densely packed areas of undigested cell walls as were apparent in Figure 1 in digesta from hens not supplemented with Avizyme 1500. So even with a mature chicken such as the laying hen, there is still potential for improved digestion of corn and soybean meal-based diets.

We have been conducting research trials at UNL with Avizyme 1500 in laying hen diets for the past couple of years and have found some positive results of such supplementation on nutrient use. A trial in which Avizyme

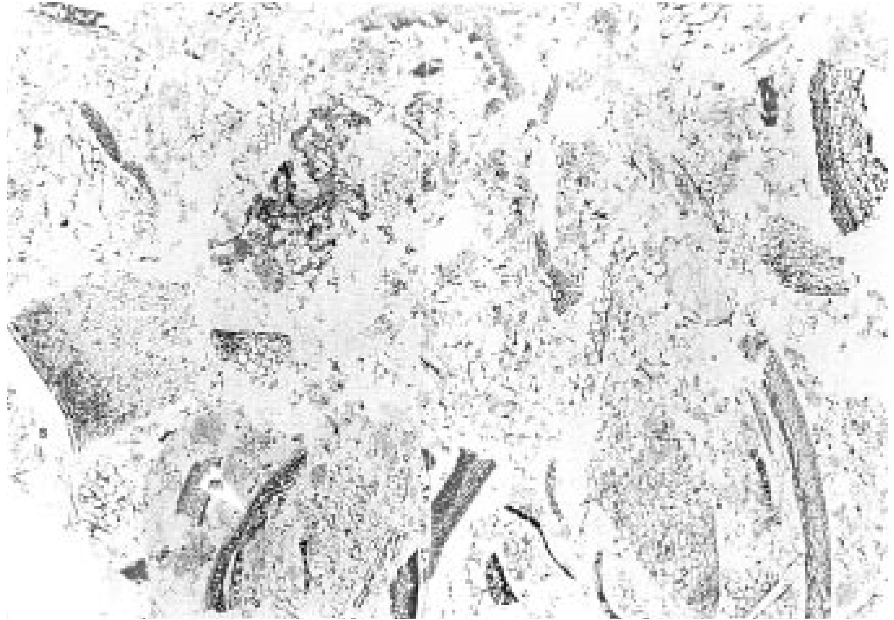


Figure 1. Small Intestine Digesta from Babcock B-300 Control Diet. M = Corn S = Soybeans O = Outer hull of corn

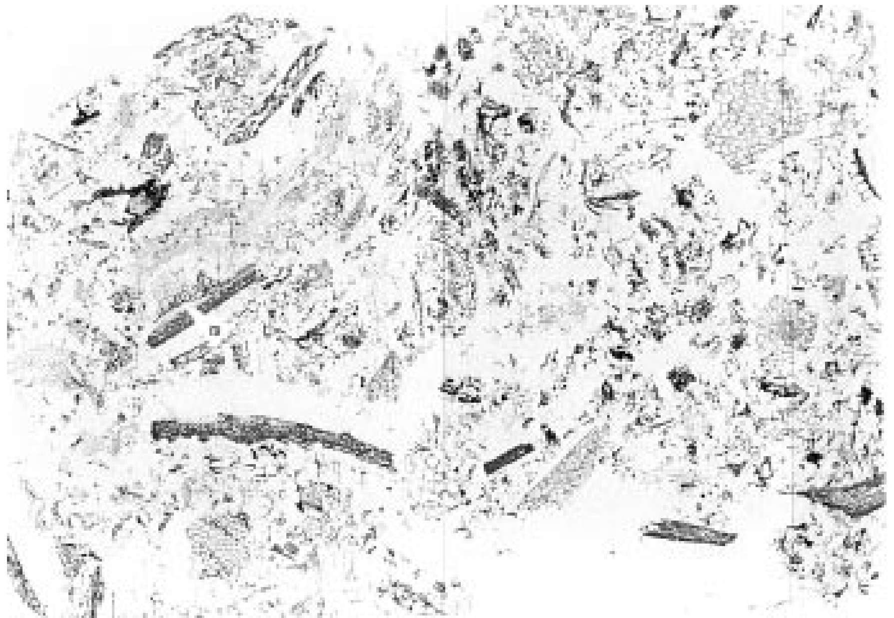


Figure 2. Small Intestine Digesta from Babcock B-300 Diet + Avizyme 1500. M = Corn S = Soybeans O = Outer hull of corn

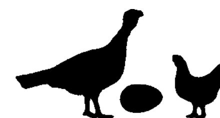


Table 1. Nutrient digestibilities of laying hens fed varying ME with and without Avizyme 1500 supplementation

Strain	Avizyme 1500	M.E.	Protein Dig.	Calcium Dig.	Apparent M.E.
	(%)	(Kcal/lb)	(%)	(%)	(Kcal/lb)
Hy-Line W-36	0	1314	53.4	36.4	1420
	.75	1314	55.1	44.8	1421
	0	1275	44.3	16.4	1325
	.75	1275	51.6	48.3	1344
Babcock B-300	0	1314	51.8	31.8	1408
	.75	1314	51.8	40.2	1380
	0	1275	45.6	34.0	1358
	.75	1275	48.2	45.5	1335
P-Value*			.04	.0005	NS

*P-value for enzyme main effect, NS means non-significant.

1500 was supplemented to Babcock B300 and Hy-Line W-36 laying hens beginning at 25 weeks old at two different diet densities (1314 kcal/lb or 1275 kcal/lb) in a factorial design, showed improvements in several nutrient digestibilities (Table 1). A significant improvement was observed for protein and calcium use (digestibility) in both strains, especially while on the low ME diet (1275 kcal/kg). No consistent improvement was observed for apparent metabolizable energy (AME) with or without Avizyme supplementation. The results of these data would indicate that the exogenous enzymes amylase and proteinase may be aiding digestion of the corn cell wall and soybean meal, releasing amino acids and lessening the negative interaction of calcium with fibrous cell walls. The lack of positive effect on the apparent metabolizable energy is a bit questionable. In the Hy-Line W-36 strain, the Avizyme 1500 was effective in raising AME on the low ME diets, whereas in the Babcock B300, Avizyme 1500 actually slightly lowered AME values. It should be noted though, that the differences in ME among diets, with or without Avizyme 1500 supplementation, and strains were not significant. Only the expected difference in AME among differing formulated ME levels was significant.

Improving nutrient digestibility should have some subsequent effect on production parameters. In this particular study, egg production and egg mass were significantly increased in the Hy-Line W-36 on the low-energy diet with Avizyme 1500 and in the Babcock B300 strain on the normal energy diet with Avizyme 1500 supplementation. Other studies in older (40 weeks +) Hy-Line W-36 hens at UNL have shown significant improvements in feed efficiency (g feed:g egg mass) when Avizyme 1500 was supplemented to diets with ME values of 1260 kcal/lb.

A number of questions still need to be addressed for the poultry producer and nutritionist to feel confident about the use of corn-soy enzymes in poultry diets. One is the approach to formulation: Should the enzyme be in addition to the base ingredients ("over the top") or should we be giving the enzyme some nutritive credits when formulating? If we give the enzyme some nutritive values, what should the nutritive values be and are they the same for all strains and at all ages? It will take time to answer these questions through objective research, but there is no doubt a future for enzyme technology in the poultry industry.

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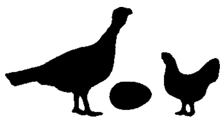

The Effect of Phytase Supplementation: A: on Egg Production Parameters in Laying Hens

Sheila A. Scheideler
Mohammad A. Jalal[1]

Introduction

Dietary requirements of phosphorus (P) and its availability in feedstuffs of plant origin are key issues in poultry nutrition. It is well known that P from plant sources is only 30-40% available. This is because so much of the P is in the form of phytate (*myo*-inositol hexaphosphate) which is poorly used by poultry. Poultry lack enough endogenous phytase in their gastrointestinal tracts to hydrolyze phytate and release bound phosphorus. To meet P requirements of the chicken, diets usually are supplemented with an inorganic source of phosphorus. This is not only expensive but also fails to address the problem of over-supplementation, leading to potential environmental P pollution in soil and ground water. As a result of economic and environmental concerns, there is renewed interest in using endogenous phytase to reduce the need for inorganic P supplements and to improve use of P present in feedstuffs.

The objective of this study was to assess the effect of phytase supplementation in older laying hens' diets on hen performance and production parameters.

Table 1. Composition of the basal diets

Ingredient	Control (Diet 1)	0.25% NPP (Diet 2, 3 & 4)	0.15% NPP (Diet 5, 6 & 7)	0.10% NPP Diet (8, 9 & 10)
	(%)			
Corn	63.01	63.59	64.16	64.45
Soybean meal	22.86	22.79	22.68	22.63
Calcium carbonate	9.15	9.45	9.75	9.90
Dicalcium phosphate	1.49	0.93	0.38	0.10
Salt	0.35	0.35	0.35	0.35
Vitamin premix	0.05	0.05	0.05	0.05
Mineral premix	0.10	0.10	0.10	0.10
Tallow	2.82	2.60	2.39	2.28
Methionine	0.13	0.13	0.13	0.13
Nutrient Analysis:				
ME, kcal/kg	2,865	2,865	2,865	2,865
Protein, analyzed	16.24	16.25	16.04	16.00
Calcium, analyzed	3.89	3.82	3.82	3.82
Nonphytate P	0.35	0.25	0.15	0.10
Total P, calculated	0.56	0.46	0.36	0.31
Total P, analyzed	0.60	0.51	0.39	0.34
Methionine, calculated	0.41	0.42	0.45	0.39
Methionine, analyzed	0.41	0.40	0.41	0.41
TSAA, analyzed	0.71	0.69	0.71	0.71
Lysine, analyzed	0.85	0.79	0.79	0.85

Materials and Methods

Hy-Line W-36 hens were fed corn-soybean diets from 40 to 60 weeks of age (Table 1). The treatments consisted of a control with 0.35% nonphytate phosphorus (NPP), plus 3 x 3 factorial arrangement with three levels of NPP (0.10, 0.15, and 0.25%) and three sources of phytase (0, phytase A, phytase B [250-300 FTU/kg]). Each treatment was randomly assigned to 8 replicates pens for a total of 80 pens. Each pen represented an experimental unit and contained 5 hens. The experimental design was a randomized complete block design.

Measurements

Egg production and feed intake were measured daily. Hens were fed *ad libitum* up to 100 grams per hen per day. One-day egg production was saved

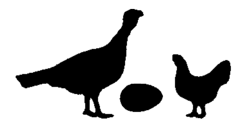

Table 2. Effect of phytase supplementation on feed intake, feed conversion, nonphytate phosphorus (NPP) intake, and hen weights

Dietary Treatments		Parameters			
Enzyme	NPP	Feed intake	Feed conversion	NPP intake	Hen weights
	(%)	(g/hen/day)	(g feed:g egg)	(mg/hen/day)	(kg)
None	0.35	91.60	1.88	320.67	1.51
None	0.25	94.11	1.86	233.87	1.50
FFI[1] (A)	0.25	93.45	1.91	229.96	1.49
BASF[2] (B)	0.25	93.42	1.93	233.15	1.49
None	0.15	93.98	1.87	140.63	1.49
FFI (A)	0.15	92.48	1.94	137.70	1.48
BASF (B)	0.15	93.43	1.88	139.71	1.51
None	0.10	89.55	2.04	89.52	1.48
FFI (A)	0.10	91.62	1.89	90.72	1.47
BASF (B)	0.10	93.42	1.86	92.30	1.48
NPP averages					
0.35%		91.60	1.88	320.67	1.51
0.25%		93.66	1.90	232.33	1.49
0.15%		93.30	1.86	139.35	1.49
0.10%		91.53	1.93	90.85	1.48
Phytase averages					
FFI (A)		92.52	1.88	152.70	1.49
BASF (B)		93.42	1.89	155.05	1.49
SEM		1.36	0.035	2.35	0.027
Statistical Probabilities					
Main Effects					
Diet		0.06	0.01	0.0001	NS
Contrasts					
Control vs 2 - 10		NS	NS	0.0001	NS
FFI (A) vs BASF (B)		NS	NS	NS	NS
Phytase vs No Phytase		NS	NS	NS	0.01
Control vs FFI		NS	NS	0.0001	NS
Control vs BASF		NS	NS	0.0001	NS
FFI vs 2,5,8		NS	NS	NS	0.01
BASF vs 2,5,8		NS	NS	NS	0.06
P Linear		NS	NS	0.0001	0.01
P Quadratic		NS	NS	0.001	NS

[1]Finnfeeds International Ltd., Marlbough, Wiltshire SN8 1AA, England, United Kingdom.
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weekly for egg weight and egg specific gravity measurements. Two eggs were saved biweekly per pen to measure eggshell breaking strength and percent egg components (albumen, yolk, shell). Hens were weighed monthly on an individual basis.

Results and Discussion

Phytase supplementation slightly increased feed intake of hens (Table 2). Hens fed 0.10% NPP diets with phytase consumed more than those fed 0.10% NPP without phytase. Feed conversion improved with phytase supplementation for hens fed 0.10% NPP diets (Table 2). Hens became more efficient when phytase was added to their diets. As expected, mg of nonphytate phosphorus intake decreased as dietary NPP decreased from 0.35 to 0.10%.

Egg production was not influenced by phytase supplementation at any NPP level even though hens fed 0.10% NPP without enzyme had lower egg production than those that received the other treatments (Table 2). Egg weight was not affected by phytase supplementation, but there was an improvement in 0.10% NPP diets with phytase (Table 3). Egg mass was significantly improved by phytase supplementation at all NPP levels. Eggs laid by hens receiving 0.10% NPP had the lowest egg mass value (45.16 g). Egg specific gravity and eggshell strength were not affected by phytase supplementation at any NPP level (Table 3). Dry shell percent was higher at all NPP levels when phytase was supplemented (Table 4), while dry yolk percentages for hens fed 0.10% NPP were significantly improved by phytase supplementation (Table 5).

In summary, phytase supplementation to regular corn-soybean meal diets improved feed intake and feed conversion of laying hens. Egg mass was significantly enhanced by the inclusion of phytase at all NPP levels. Dry yolk weights were increased in diets supplemented with phytase, and similar results were observed in wet and dry shell.

From our results it is concluded that supplemental phytase has beneficial effects on the performance of laying hens. It is recommended that laying hen diets be formulated to provide 0.15-0.20% NPP, with supplemental phytase, to hens late in the production cycle. There is a high risk in reducing the available P levels to 0.10% and adding phytase. Egg production and egg quality were severely affected at low levels of NPP even with added phytase, which would be catastrophic to commercial producers.

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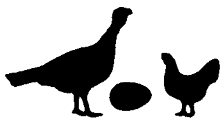

Table 3. Effect of phytase supplementation on egg production, egg weight, and egg mass

Dietary Treatments		Parameters		
Enzyme	NPP	Egg production	Egg weight	Egg mass
(%)	(%)	(g)	(g)	
None	0.35	91.52	59.58	48.96
None	0.25	94.07	58.76	50.73
FFI[1] (A)	0.25	91.44	58.60	48.62
BASF[2] (B)	0.25	93.00	58.52	48.98
None	0.15	93.63	58.55	50.68
FFI (A)	0.15	92.65	58.09	47.94
BASF (B)	0.15	93.23	58.96	50.25
None	0.10	84.76	57.76	45.16
FFI (A)	0.10	91.15	58.43	48.93
BASF (B)	0.10	92.94	58.64	49.80
NPP Averages				
0.35%		91.52	59.58	48.96
0.25%		92.84	58.63	49.44
0.15%		93.17	58.53	49.63
0.10%		89.62	58.28	47.86
Phytase Averages				
FFI (A)		91.74	58.37	48.50
BASF (B)		93.06	58.70	49.68
SEM		1.36	0.56	1.04
Statistical Probabilities				
Main Effects				
Diet		0.09	NS	0.004
Contrasts				
Control vs 2 - 10		NS	0.03	NS
FFI (A) vs BASF (B)		NS	NS	NS
Phytase vs No Phytase		NS	NS	NS
Control vs FFI		NS	0.03	NS
Control vs BASF		NS	NS	NS
FFI vs 2,5,8		NS	NS	NS
BASF vs 2,5,8		NS	NS	NS
P Linear		0.06	NS	0.06
P Quadratic		NS	NS	NS

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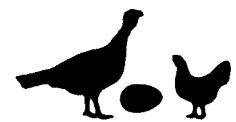

Table 4. Effect of phytase supplementation on shell breaking strength, specific gravity, and Haugh units

Dietary Treatments		Parameters			
Enzyme	NPP Strength	Shell breaking Shell	Wet Shell	Dry gravity	Specific
	(%)	(kg/cm^2)	(%)	(%)	
None	0.35	7.00	12.44	8.71	1.0764
None	0.25	6.68	12.73	9.08	1.0785
FFI[1] (A)	0.25	6.79	12.79	9.17	1.0774
BASF[2] (B)	0.25	6.65	12.97	9.21	1.0776
None	0.15	6.88	12.83	8.97	1.0775
FFI (A)	0.15	6.98	12.68	9.12	1.0771
BASF (B)	0.15	6.59	12.56	9.08	1.0773
None	0.10	6.48	13.09	9.05	1.0776
FFI (A)	0.10	6.78	12.52	8.89	1.0769
BASF (B)	0.10	6.59	12.33	8.89	1.0770
NPP Averages					
0.35%		7.00	12.44	8.79	1.0764
0.25%		6.71	12.83	9.15	1.0778
0.15%		6.82	12.69	9.06	1.0773
0.10%		6.62	12.65	8.94	1.0772
Phytase Averages					
FFI (A)		6.85	12.66	9.06	1.0771
BASF (B)		6.61	12.62	9.06	1.0773
SEM		0.2400.26	0.10	0.0005	
Statistical Probabilities					
Main Effects					
Diet		NS	0.05	0.05	NS
Contrasts					
Control vs 2 - 10		NS	NS	0.01	0.05
FFI (A) vs BASF (B)		NS	NS	NS	NS
Phytase vs No Phytase		NS	NS	0.02	NS
Control vs FFI		NS	NS	0.02	NS
Control vs BASF		NS	NS	0.02	0.07
FFI vs 2,5,8		NS	NS	NS	NS
BASF vs 2,5,8		NS	NS	NS	NS
P Linear		NS	NS	NS	NS
P Quadratic		NS	NS	NS	NS

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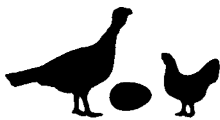
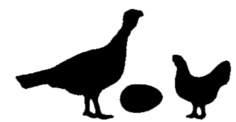


Table 5. Effect of phytase supplementation on egg composition

Dietary Treatments		Parameters		
Enzyme	NPP	Albumen	Wet yolk	Dry yolk
	(%)	(%)	(%)	(%)
None	0.35	58.17	27.87	13.90
None	0.25	57.38	29.79	14.50
FFI[1] (A)	0.25	57.17	28.02	14.47
BASF[2] (B)	0.25	57.61	28.80	14.51
None	0.15	57.42	28.55	14.59
FFI (A)	0.15	57.19	28.03	14.24
BASF (B)	0.15	57.02	28.42	14.44
None	0.10	55.88	28.53	15.36
FFI (A)	0.10	56.92	28.56	14.43
BASF (B)	0.10	58.01	30.13	14.49
NPP Averages				
0.35%		58.17	27.87	13.90
0.25%		57.39	28.87	14.49
0.15%		57.21	28.33	14.42
0.10%		56.94	29.07	14.76
Phytase Averages				
FFI (A)		57.09	28.20	14.38
BASF (B)		57.55	29.12	14.48
SEM		0.48	0.92	0.22
Statistical Probabilities				
Main Effect				
Diet		0.07	NS	0.04
Contrasts				
Control vs 2 - 10		0.05	NS	0.02
FFI (A) vs BASF (B)		NS	NS	NS
Phytase vs No Phytase		NS	NS	0.02
Control vs FFI (A)		NS	NS	NS
Control vs BASF (B)		NS	NS	0.06
FFI (A) vs 2,5,8		NS	NS	0.01
BASF (B) vs 2,5,8		NS	NS	NS
P Linear		NS	NS	NS
P Quadratic		NS	NS	NS

[1]Finnfeeds International Ltd., Marlbough, Wiltshire SN8 1AA, England, United Kingdom.
[2]BASF Corporation, 1609 Biddle Avenue, Wynadotte, MI 48192, USA.



The Effect of Phytase Supplementation: B: on Nutrient Digestibility in Laying Hens

Sheila A. Scheideler
Mohammad A. Jalal[1]

Introduction

Phytate, a naturally occurring organic compound found in plants, has tremendous chelating properties. Phytate can complex with several cations such as calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), potassium (K), and copper (Cu), as well as amino acids, and render them less available. Phytate forms these insoluble complexes in the small intestines and prevents absorption of the minerals and amino acids. The exact mechanism is not known but is pH dependent. The reduced availability of nutrients depends on several factors: nutritional status of animals, concentration of minerals and phytate in feedstuffs, age of animals, ability of animal to hydrolyze phytate and level of intestinal phytase activity.

The objective of this study was to assess the effect of phytase supplementation in older laying hens' diets on nutrient digestibility.

Materials and Methods

Hy-Line W-36 hens were fed corn-soybean diets from 40 to 60 weeks of age (Table in review article A). The treatments consisted of a control with 0.35% nonphytate phosphorus (NPP), plus 3 x 3 factorial arrangement with three levels of NPP (0.10, 0.15, and 0.25%) and three sources of phytase (0, phytase A, phytase B [250-300 FTU/kg]). Each treatment was randomly assigned to 8 replicates pens for a total of 80 pens. Each pen represented an experimental unit and contained 5 hens. The experimental design was a randomized complete block design.

Table 1. Fecal calcium and phosphorus digestibilities at 45 and 60 weeks.

Dietary Treatments		Parameters			
Enzyme Source	NPP	Digestible Ca (45 wk)	Digestible Ca (60 wk)	Digestible P (45 wk)	Digestible P (60 wk)
	(%)	(%)	(%)	(%)	(%)
None	0.35	49.27	43.25	19.15	27.89
None	0.25	39.65	65.12	32.90	40.65
FFI[1] (A)	0.25	45.72	62.19	33.79	47.31
BASF[2] (B)	0.25	40.62	51.40	24.91	31.73
None	0.15	29.30	46.27	30.90	32.15
FFI (A)	0.15	42.69	58.59	31.08	26.95
BASF (B)	0.15	36.98	60.87	33.63	37.31
None	0.10	47.89	54.81	32.86	31.48
FFI (A)	0.10	60.48	60.39	48.83	23.54
BASF (B)	0.10	36.22	51.04	28.30	21.02
NPP Averages					
0.35%		49.27	43.25	19.15	27.89
0.25%		41.99	51.57	30.53	39.90
0.15%		36.32	55.24	31.87	32.14
0.10%		48.20	55.41	36.66	25.35
Phytase Averages					
FFI		49.46	60.39	37.90	32.60
BASF		37.94	54.44	28.95	30.02
SEM		4.52	3.20	3.72	4.32
Statistical Probabilities					
Main Effects					
Diet		0.001	0.001	0.001	0.001
Contrasts					
Control vs 2 – 10		NS	0.001	0.001	NS
FFI (A) vs BASF (B)		0.01	NS	0.01	NS
Phytase vs No Phytase		NS	0.001	NS	NS
Control vs FFI (A)		NS	0.001	0.0001	NS
Control vs BASF (B)		0.05	0.001	0.05	NS
FFI (A) vs 2,5,8		0.01	0.001	NS	NS
BASF (B) vs 2,5,8		NS	0.001	NS	NS
P Linear		0.09	NS	0.05	0.001
P Quadratic		0.01	NS	NS	NS

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[2]BASF Corporation, 1609 Biddle Avenue, Wynadotte, MI 48192, USA.

Measurements

Digestibility of Ca, P and amino acids (AA) was determined using chromic oxide, an inert digestible marker. The marker was added to the diets at a level of 0.30% and fed for 5 days. Representative fecal samples were collected from each pen on the last day of feeding to determine digestibility of Ca, P, protein and amino acids. The excreta samples were freeze-dried and sieved through a screen to remove feathers, and then ground and stored for nutrient analysis. Calcium and P digestibility was determined at 45 and 60 weeks while protein and AA were examined at 60 weeks only.

Results and Discussion

Phytase supplementation with phytase A or B significantly improved Ca digestibility at 45 and 60 weeks at all NPP levels (Table 1). This was evident in hens fed 0.10% NPP diets with phytase A at 60 weeks where Ca digestibility was as high as 60.39%. Phytase A had significantly higher Ca digestibility values compared to phytase B at 45

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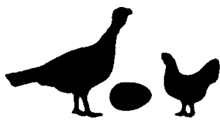

Table 2. Crude protein and essential amino acids digestibility percentages

Dietary Treatments		Amino Acids											
Enzyme Source	NPP	CP	Met	Cys	TSAA Met + Cys	Lys	Thr	Phe	Leu	Arg	His	Ile	Val
	(%)	(%)											
None	0.35	49.40	85.65	79.36	82.77	82.96	77.35	86.45	85.14	91.42	86.99	78.64	77.83
None	0.25	59.45	88.30	86.80	86.03	85.49	80.41	88.50	87.52	93.86	89.43	81.66	80.98
FFI[1] (A)	0.25	59.09	85.52	83.42	84.08	83.22	79.25	86.76	86.68	89.49	88.89	81.34	78.97
BASF[2] (B)	0.25	51.89	87.40	81.05	84.52	84.91	80.68	89.12	88.57	92.77	88.81	84.50	81.86
None	0.15	53.70	87.25	80.80	84.19	83.74	77.52	88.39	87.24	89.63	87.67	82.99	76.85
FFI (A)	0.15	57.45	90.03	79.81	82.99	82.21	77.59	87.26	86.37	92.37	87.27	81.76	78.05
BASF (B)	0.15	53.70	86.61	84.74	87.86	87.67	82.85	90.52	91.22	93.99	63.70	88.42	83.03
None	0.10	47.19	81.69	77.02	79.49	80.35	74.57	86.21	84.98	89.86	85.22	80.18	71.41
FFI (A)	0.10	60.41	89.25	83.62	86.84	87.47	82.06	91.59	90.14	93.92	90.10	87.58	81.87
BASF (B)	0.10	53.49	84.85	80.32	82.97	83.89	77.22	87.69	87.34	89.60	87.12	83.68	77.66
NPP Averages													
0.35		49.40	85.65	79.36	82.77	82.96	77.35	86.45	85.14	91.42	86.99	78.64	77.83
0.25		56.81	87.07	83.76	84.88	84.54	80.11	88.79	87.57	92.04	89.04	82.50	80.60
0.15		54.95	87.96	81.78	85.01	84.54	79.29	88.72	88.30	91.99	86.21	84.39	79.31
0.10		53.70	85.26	80.32	83.10	83.90	77.96	88.50	87.50	91.13	87.48	83.81	76.99
Phytase Averages													
FFI		58.98	88.30	82.28	84.64	84.30	79.63	88.54	87.73	91.93	88.75	83.56	79.64
BASF		53.03	86.30	82.04	85.12	85.49	80.27	89.11	89.04	92.12	86.54	85.53	80.85
SEM		3.88	1.66	1.75	1.70	1.79	2.07	1.78	1.40	1.65	1.65	1.94	2.16
Main Effects													
Diet		NS	0.05	0.01	0.07	NS	NS	NS	NS	NS	NS	0.05	0.03

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Table 3. Nonessential amino acids digestibility percentages

Dietary Treatments		Nonessential Amino Acids					
Enzyme Source	NPP	Ala	Glu	Gly	Asp	Ser	Pro
	(%)	(%)					
None	0.35	81.58	88.05	79.85	82.29	82.55	87.19
None	0.25	82.86	90.04	80.71	84.97	85.68	89.43
FFI[1] (A)	0.25	84.13	89.18	79.93	82.97	83.07	89.92
BASF[2] (B)	0.25	84.07	89.90	83.73	84.48	85.20	89.02
None	0.15	78.33	88.99	77.16	83.49	82.50	88.95
FFI (A)	0.15	79.53	88.42	76.04	82.19	83.39	89.45
BASF (B)	0.15	84.56	91.49	79.52	87.06	85.77	90.74
None	0.10	74.74	85.28	71.32	80.53	80.77	87.64
FFI (A)	0.10	84.26	91.34	81.35	87.30	86.18	90.89
BASF (B)	0.10	79.82	89.13	77.09	83.59	82.07	88.62
NPP Averages							
0.35%		81.58	88.05	79.85	84.14	82.55	87.19
0.25%		83.70	89.71	81.50	84.25	84.65	89.50
0.15%		80.81	89.63	77.57	76.59	83.89	89.71
0.10%		79.61	88.58	76.60	83.01	83.01	89.05
Phytase Averages							
FFI		84.64	89.76	79.11	84.15	84.21	90.09
BASF		82.82	90.17	80.11	85.04	84.33	89.46
SEM		2.09	1.24	2.10	1.60	1.73	1.20
Main Effects							
Diet		0.02	0.06	0.01	NS	NS	NS

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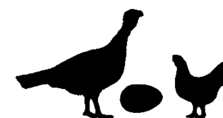

weeks, but no difference was noticed at 60 weeks.

Phosphorus digestibility was significantly improved by phytase A in contrast to B at 45 weeks especially at 0.10% NPP level (Table 1). At 60 weeks P digestibilities were significantly higher at lower NPP levels in contrast to the control. Diets supplemented with phytase A at 0.25% NPP level had the highest P digestibility of 47.31% at 60 weeks.

Crude protein digestibility was not significantly affected by the dietary NPP and phytase treatments (Table 2). Phytase supplementation significantly increased digestibility of essential amino acids methionine, cysteine, total sulfur amino acids, and valine at 0.10 and 0.15% NPP levels (Table 2), while isoleucine digestibility was improved at all NPP levels. Digestibility of nonessential amino acids alanine, glutamic acid and glycine were significantly higher at 0.10 and 0.25% NPP with supplemental phytase in contrast to no phytase at the same levels (Table 3).

In summary, phytase supplementation to regular corn-soybean meal diets improved a number of nutrient digestibilities. This is because of catabolism of the phytate molecules freeing both the minerals and proteins to more complete digestion. The effect of phytase on improving P retention was evident at the low (0.10%) NPP level, with about 23.8% of phytate P released by supplemental phytase.

Our results indicate a decrease in P excretion when low P diets were supplemented with phytase. This is very important in terms of decreasing the phytate P excretion in the manure and improving nutrient retention. There is an exciting potential for widespread application of phytase technology to improve the overall nutrient utilization in monogastric animals. This will be important in reducing soil and water contamination with potential environment pollutants.

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The Effect of Dietary Lysine Level and TSAA:Lysine Ratio on Egg Production Parameters and Egg Yield

Curtis Novak
Haitham Yakout
Sheila Scheideler[1]

Introduction

Ideal protein levels in diets for production animals is not a new concept. Considerable work in turkeys, broilers and pigs has been done to determine the optimal level of each limiting amino acid on a digestibility basis with the proper ratio to diminish interactions between amino acids. There are many ways to define ideal protein, but theoretically it is the exact balance of amino acids that meets the animal's needs. There should be no excess, and no deficiency, and a minimum of the amino acids should be used for energy. Overall nitrogen excretion would be reduced as a result of a reduction of total crude protein in the diet, reducing feed cost and pollution.

At the University of Nebraska, it has been determined that the current lysine recommendation of the NRC '94 is substantially lower than that required for optimal production performance. From our research, the recommended level of dietary lysine should be 850-900 mg intake per day for optimal egg mass, feed efficiency and egg production. In one research trial, a linear response with increased dietary lysine from 500 to 1000 mg/hen/day (100 mg increments) was reported for all egg production parameters. It also was determined that maximum egg production and egg mass can be maintained with a lysine intake of 900 mg/hen/day in late- producing hens. Prochaska et al. (1996) also reported a significant increase in egg and albumen weights with increased lysine intake, when fed from 42-64 weeks of age. Preliminary studies dealing with interactions between lysine and total sulfur amino acids (methionine and cystine-TSAA) have indicated some detrimental effects of high lysine levels combined with minimal TSAA intake. Novak and Scheideler (1998) determined that a TSAA:lysine ratio of .55 is detrimental during early egg production in hens and that .91 is optimal. Changes in dietary protein intake also may change the components of the egg, resulting in either an increase or a decrease in egg yield. The importance of determining amino acid ratios for optimal production lead us to the objective of determining the optimal ratio of TSAA:lysine for feed efficiency and egg yield.

Experimental Design and Measurements

A total of 320 DeKalb Delta hens were randomly assigned to one of 8 dietary treatment groups varying in lysine and TSAA levels. Hens were housed in an environmentally controlled room with *ad libitum* feed and water. A phase feeding program was used during the experiment from 20 to 60 weeks of age (Phase I - 20 to 40 wks and Phase II - 41 to 60 wks). Diets (Table 1) were formulated based on expected feed consumption and age of hens according to the DeKalb Delta[2] breeder guide. The experiment consisted of a 2 x 4 factorial arrangement of treatments: two levels of dietary lysine (800 or 900 mg/hen/day) and four SAA:lysine amino acid ratios (.71, .81, .91 or 1.01 mg/hen/

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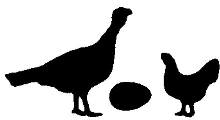

day). During phase II, the level of lysine was lowered to 700 or 800 mg/hen/day while maintaining TSAA:lysine amino acid ratios. TSAA:lysine ratios were altered by increasing TSAA level with supplemental methionine.

Feed consumption and egg production were recorded daily, while hens were weighed individually on a monthly basis. One day's egg production each week was used for measuring egg weight. Specific gravities were determined bi-weekly using one day's egg production, and two eggs per pen were used for either Haugh unit or egg component determination (wet and dry) bi-weekly.

Table 1. Phase I Diets (100 g/hen/day).

Ingredient	1	2	3	4	5	6	7	8
	----- % of Diet -----							
Corn	***62.4***	***62.0***	***61.7***	***61.3***	***61.1***	***61.2***	***61.3***	***61.3***
Soybean Meal	***20.7***	***21.1***	***21.4***	***21.7***	***22.0***	***21.8***	***21.6***	***21.4***
Meat & Bone M.	***5.3***	***4.9***	***4.4***	***4.0***	***3.9***	***3.9***	***3.9***	***3.9***
Limestone	6.7	6.8	6.9	6.9	6.9	6.9	6.9	6.9
Tallow	2.4	2.6	2.8	2.9	3.0	3.0	3.0	3.1
Oyster Shell	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Dical. Phos.	.5	.6	.7	.8	.8	.8	.8	.8
Salt	.30	.30	.30	.30	.30	.30	.30	.30
Methionine	***.06***	***.14***	***.23***	***.31***	***.14***	***.23***	***.31***	***.40***
Lysine	—	—	—	—	***.12***	***.13***	***.13***	***.14***
Vitamin Premix	. 05	.05	.05	.05	.05	.05	.05	.05
Mineral Premix	.1	.1	.1	.1	.1	.1	.1	.1
Nutrients (Formulated)								
M. E. (kcal/kg)	2889	2889	2889	2889	2889	2889	2889	2889
Protein, %	17.7	17.7	17.7	17.7	17.7	17.7	17.7	17.7
Lysine, %	***.80***	***.80***	***.80***	***.80***	***.90***	***.90***	***.90***	***.90***
TSAA, %	***.57***	***.65***	***.73***	***.81***	***.65***	***.73***	***.81***	***.89***
Ratio	***.71***	***.81***	***.91***	***1.01***	***.71***	***.81***	***.91***	***1.01***
Calcium, %	3.90	3.90	3.90	3.90	3.90	3.90	3.90	3.90
Avail. Phos, %	.43	.43	.43	.43	.43	.43	.43	.43

Results and Discussion

During the experiment, overall egg production and feed consumption were not significantly affected by either lysine or TSAA:lysine ratio (Table 2). The average egg production and feed consumption for the study were 83.5% and 97.2 g/day, respectively. Although not significant, as a result of poor pen uniformity, egg production was numerically greater for the high TSAA:lysine ratios (.81-1.01) within the 900 mg lysine/day treatments. Overall, hen weights (Table 2) were significantly affected by dietary lysine intake ($P<.02$); hens consuming the high lysine diet (1.569 kg) were significantly heavier compared to hens consuming the low lysine diet (1.534 kg). Due to lysine's role in tissue protein synthesis, the increased lysine may have contributed to increased growth while maintaining egg production.

Egg weights (Table 2) were significantly affected by dietary lysine intake ($P<.007$). Increasing the level of lysine increased average egg weights from 59.02 g to 60.34 g for low and high lysine intakes, respectively. Egg weights

Table 2. Effect of lysine and TSAA:Lysine ratios on egg production parameters (20-60 weeks of age).

	Lysine (mg)	Ratio	Egg Prod (%)	Feed Cons. (g)	Hen Wt. (Kg)	Egg Wt. (g)	Dry Shell (%)	Wet Shell (%)	Specific Gravity (Phase II)
1	800	.71	82.86	97.4	1.526	58.06	9.21	12.30	1.0830
2	800	.81	82.86	96.7	1.527	58.69	9.15	12.38	1.0813
3	800	.91	84.60	96.9	1.560	59.64	9.36	12.39	1.0822
4	800	1.01	79.71	95.6	1.524	59.70	9.02	12.29	1.0802
5	900	.71	81.27	97.5	1.589	60.70	9.21	12.29	1.0835
6	900	.81	86.64	97.2	1.578	60.01	9.21	12.35	1.0813
7	900	.91	84.12	97.0	1.542	60.39	9.14	12.40	1.0813
8	900	1.01	86.63	99.3	1.565	60.27	9.19	12.42	1.0815
Main Effects									
Lysine			NS	NS	.02	.007	NS	NS	NS
Ratio			NS	NS	NS	NS	.09[2]	NS	.02[2]
Lysine x Ratio			NS	NS	NS	NS	.05[1]	NS	NS
Estimates - Ratio									
Linear			NS	NS	NS	.09[2]	.08[2]	NS	.09; .008[2]
Quadratic			NS	NS	NS	NS	NS	NS	NS
Cubic			NS	NS	NS	NS	NS	NS	NS
SEM			2.36	1.38	.028	.71	.086	.139	.001

[1]Phase I (20 to 40 wks of age)
[2]Phase 2 (40 to 60 wks of age)

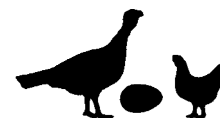

Table 3. Effect of lysine and TSAA:Lysine ratios on egg mass and components.

	Lysine (mg)	Ratio	Egg Mass (g)	Albumen (%)	Albumen Solids (%)	Yolk (%)	Yolk Solids (%)
1	800	.71	48.2	60.2	11.19	27.4	50.94
2	800	.81	48.6	60.4	11.09	27.1	51.39
3	800	.91	50.3	59.9	11.04	27.5	52.12
4	800	1.01	47.5	60.3	11.12	27.2	53.47
5	900	.71	49.2	61.5	11.19	26.1	51.17
6	900	.81	51.9	60.5	11.28	26.9	51.94
7	900	.91	50.9	60.2	11.23	27.1	52.22
8	900	1.01	52.1	60.1	11.38	27.2	52.03
Main Effects							
Lysine			.03	.02[1]	.04	.05	.07[1]
Ratio			NS	NS	NS	.09	NS
Lysine x Ratio			NS	NS	NS	NS	NS
Estimates - Ratio							
Linear			NS	.04	NS	.03	.01
Quadratic			NS	NS	NS	NS	NS
Cubic			NS	NS	NS	NS	NS
SEM			1.59	.356	.113	.288	.711

[1]Phase I (20 to 40 wks of age).

during phase II (data not shown) showed a significant lysine effect (*P*<.0005), again with heavier eggs from the hens consuming the high lysine diet. Also during phase II, a linear ratio effect approached significance (*P*<.09). As the TSAA:lysine ratio was increased, there was a linear increase in egg weights, with 60.6, 60.7, 61.6 and 61.6 g for .71, .81, .91 and 1.01, respectively. Wet and dry shell percentages and specific gravity were not affected overall (Table 2), but were affected during individual phases by dietary treatments (data not shown). During phase I, dry shell percentage showed a lysine by ratio interaction (*P*<.05); the highest dry shell percent was obtained at the low level of lysine and the .91 TSAA:lysine ratio. It also was significantly higher than the high lysine group within the same ratio. During phase II, the effect of TSAA:lysine ratio on dry shell percent approached significance (*P*<.09), where .71 and .91 were higher compared to 1.01 ratio treatments. During phase II, specific gravity (Table 2) values showed a significant ratio (*P*<.02) and linear ratio effect (*P*<.008) of increasing TSAA:lysine ratio. TSAA:lysine ratios of .81, .91 and 1.01 were significantly lower compared to .71. Also, as the ratio was increased from .71 to 1.01, there was a linear decrease in specific gravity from 1.083 to 1.080.

Overall egg mass (Table 3) also was significantly affected by dietary lysine intake (*P*<.03). As dietary lysine intake increased, there was a corresponding 2.5 g increase in egg mass. Egg components were affected by both dietary lysine intake and TSAA:lysine ratio. Overall, albumen percentage was significantly affected linearly by ratio (*P*<.04). As dietary TSAA:lysine ratio increased, there was a linear decrease in albumen percentage. Also during phase I there was a significant effect of lysine intake (*P*<.02) in addition to the significant linear effect of ratio (*P*<.02) (data not shown). As dietary lysine intake increased, albumen percentage increased from 60.8 % to 61.3 % for the low and high dietary lysine intake, respectively. Percent albumen solids (Table 3) were significantly increased by increasing dietary lysine intake (*P*<.04), but not by ratio. This may be an indication of increased protein synthesis in the magnum resulting in increased albumen percent as lysine increased in the diet. The opposite effect was noted for percent yolk (Table 3). Overall, percent yolk was affected by dietary lysine intake (*P*<.05), ratio (*P*<.09) and linearly by ratio (*P*<.03). As the level of dietary lysine was increased, there was a significant decrease in yolk percentage from 27.2 % to 26.8 % for the low and high dietary lysine intake, respectively. There was a significant linear increase in yolk percentage as the TSAA:lysine ratio increased from .71 to 1.01. Yolk percentages for the respective ratios of .71, .81, .91 and 1.01 were 26.5, 27.0, 27.3 and 27.2 %. Lysine and linear ratio effects were evident in both phases I and II. Percent yolk solids (Table 3) overall were not affected by lysine intake, but were significantly affected linearly by ratio. As the ratio increased in the diet, there was a linear increase in yolk solids. During phase I (data not shown), the effect of lysine intake approached significance (*P*<.07) with an increase in yolk solids with increased dietary lysine intake.

In conclusion, significant improvements in hen weights, egg weight, and egg components were observed when lysine intake were increased above NRC requirements. These data support previous recommendations from UNL of 900 mg and 800 mg lysine intake per day for hens 20-40 and 40-60 weeks of age, respectively. TSAA:lysine ratio is an important element when optimizing yolk and albumen synthesis and maintaining egg size. TSAA:lysine ratios of .91 and .81 are recommended for optimum egg yields for Dekalb Delta hens from 20-40 and 40-60 wks of age, respectively.

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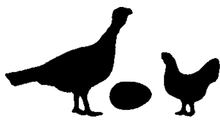
[1]Curtis Novak, graduate student; Haitham Yakout, graduate student; and Sheila E. Scheideler, Professor and Poultry Extension Specialist, Animal Science, Lincoln.

[2]DeKalb Delta Pullet & Layer Management Guide, Third Edition (1994), DeKalb, IL.



Total Sulfur Amino Acids: Lysine Ratios and Low Protein Diets' Effects on Laying Hens Performance During Early Stage of Production

Haitham M. Yakout
Curtis L. Novak
Sheila E. Scheideler[1]

Introduction

In practical poultry diets, methionine is the first limiting amino acid, followed by lysine. Supplementation of methionine and lysine to poultry diets provides a means of improving the efficiency of protein utilization. Recently, more attention has been given to the ideal amino acid profile in poultry diets. In the ideal amino acid concept for poultry, lysine is used as a standard, while the requirements for other amino acids are expressed as a percentage of the need for lysine.

Feeding strategies for poultry production have been given some new priorities with the advent of environmental problems related to nitrogen (N) excretion from animal waste. Previously, dietary adjustments to poultry requirements aimed to maximize production performance without special concern for nutrient oversupply, especially protein and amino acids. Recent environmental constraints have forced nutritionists to base protein/amino acid levels not only on terms of N retained in animal products, but also in terms of non-utilized fraction of N excreted.

With this in mind, the objective of

Table 1. Basal diets.

	TSAA:Lysine Ratio								
	0.71			0.81			0.91		
Crude Protein (%)	18	16	14	18	16	14	18	16	14
Ingredients	(%)								
Corn, yellow, %	59.20	64.34	70.54	59.29	64.41	70.62	59.37	64.49	70.70
Soybean meal, %	22.70	19.42	13.67	22.46	19.21	13.44	22.21	18.98	13.21
Corn gluten meal, %	5.00	3.00	3.00	5.00	3.00	3.00	5.00	3.00	3.00
Tallow, %	2.370	2.150	1.290	2.140	2.190	1.330	2.450	2.230	1.360
Cal. Carbonate, %	7.98	7.98	7.99	7.98	7.98	7.99	7.98	7.98	7.99
Dical. Phosphate, %	2.120	2.160	2.210	2.120	2.150	2.210	2.130	2.170	2.210
Salt, %	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
DL-Methionine, %	0.075	0.143	0.199	0.170	0.237	0.294	0.265	0.322	0.388
Lysine, %	---	0.286	0.464	0.164	0.294	0.472	0.172	0.035	0.479
Threonine, %	---	0.085	0.175	---	0.085	0.180	0.012	0.090	0.185
Tryptophan, %	---	0.035	0.065	0.010	0.035	0.065	0.010	0.035	0.065
Mineral premix[1], %	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Vitamin premix[2], %	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045
Nutrient Composition									
M.E., kcal/kg	2885	2885	2885	2885	2885	2885	2885	2885	2885
Protein, %	18.00	16.00	14.00	18.00	16.00	14.00	18.00	16.00	14.00
Ca, %	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55
Total P, %	0.69	0.68	0.67	0.69	0.68	0.66	0.69	0.68	0.66
Lysine (calculated), %	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Lysine (analyzed)[3], %	0.90	0.93	0.90	1.03	0.99	0.98	0.99	0.96	1.03
Methionine (calculated), %	0.36	0.39	0.42	0.44	0.48	0.51	0.55	0.57	0.60
Methionine (analyzed)[3], %	0.38	0.37	0.42	0.50	0.52	0.54	0.65	0.65	0.68
TSAA (calculated), %	0.64	0.64	0.70	0.73	0.73	0.73	0.82	0.82	0.82
TSAA (analyzed)[3], %	0.72	0.71	0.70	0.87	0.85	0.84	0.90	1.02	0.97
TSAA:Lysine Ratio (actual)	0.80	0.76	0.77	0.84	0.86	0.86	0.91	1.06	0.94

[1]Mineral premix provided Mn, 88 mg; Cu, 6.6 mg; Fe, 8.5 mg; Zn, 88 mg; Se, 0.30 mg.
[2]Vitamin premix provided vitamin A, 6,600 IU; cholecalciferol 2,805 IU; vitamin E, 10 IU; vitamin K, 2.0 mg; riboflavin, 4.4 mg; pantothenic acid, 6.6 mg; niacin, 24.2 mg; choline, 110 mg; vitamin B_{12}, 8.8 mg; ethoxyquin, 1.1 mg/kg.
[3]Amino acids were analyzed at Degussa Laboratories, Allendale, NJ 07401.

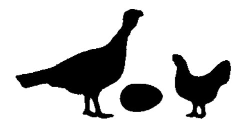


Table 2. Effects of dietary protein and TSAA:lysine ratio on egg production parameters.

Crude Protein	Ratio	Feed Consumption	Egg Production	Feed Efficiency	Body Weight	Egg Weight	Egg Mass
(%)		(g/day)	(%)	(g feed:g egg)	(kg)	(g)	(g/hen/day)
18	0.71	95.57	87.70	1.74	1.542^{b}	56.14	49.22
18	0.81	96.67	87.02	1.76	1.534	55.92	48.64
18	0.91	96.89	88.92	1.75	1.551	56.56	50.30
16	0.71	94.26	90.80	1.70	1.585^{a}	55.55	50.27
16	0.81	96.39	88.29	1.77	1.503	55.67	49.17
16	0.91	95.40	91.55	1.74	1.532	56.13	51.37
14	0.71	94.63	88.65	1.73	1.521^{b}	55.79	49.41
14	0.81	94.77	89.62	1.74	1.529	55.62	49.84
14	0.91	92.34	90.13	1.70	1.514	55.14	49.12
SEM	1.01	1.01	0.02	0.01	0.45	0.68	
Crude Protein, %							
18		96.38^{a}	87.88^{b}	1.75	1.542	56.21	49.38
16		95.35^{ab}	90.22^{a}	1.74	1.540	56.12	50.27
14		93.91^{b}	89.13^{ab}	1.76	1.521	55.51	49.45
Ratio							
0.71		94.82	89.05	1.72^{b}	1.549	56.16	49.63
0.81		95.94	88.31	1.76^{a}	1.522	55.74	49.22
0.91		94.88	89.87	1.73^{ab}	1.532	55.94	50.26
Statistical Probabilities							
Crude protein		0.01	0.02	NS	NS	NS	NS
Ratio		NS	NS	0.09	NS	NS	NS
Crude protein x ratio		NS	NS	NS	0.07	NS	NS

^{abc}Means within a column with no common superscripts differ significantly P=0.05 or P=0.01 based on least significance difference (LSD) test.

the current study was to test the effects of lowering dietary protein, combined with variable total sulfur amino acids (TSAA) to lysine ratios on hen performance during early egg production, from 20-40 weeks of age.

Experimental Design and Measurements

Four hundred and thirty two Single Comb White Leghorn hens (Hy-LineW98®) were used in this experiment. Hens were randomly assigned to nine dietary treatments within a factorial arrangement of three levels of crude protein (18, 16 and 14% CP), and three TSAA:lysine ratios of (0.71, 0.81 and 0.91), respectively, throughout the experiment.

Experimental diets (Table 1) were formulated to be isocaloric (2,885 kcal ME/kg) and only differ in crude protein content. TSAA:lysine ratio calculations were based on lysine intake of 900 and three levels of TSAA intake (640, 730 and 820 mg/kg). Diets were formulated on a digestible amino acid basis, using the Degussa prediction model[2].

Feed consumption and hen-day egg production were recorded daily. Hens were individually weighed once each month. One day's egg production weekly was used to measure egg weight. Two eggs per pen were used either for specific gravity or egg component measurements biweekly.

Results

Lowering dietary protein from 18 to 14% resulted in a significant decrease in feed consumption (P< 0.01) (Table 2). TSAA:lysine ratios did not affect consumption. However, hens fed 0.71 ratio had the best feed efficiency (1.72 g. feed/ g. egg) compared to those fed the other two ratios. Lowering dietary crude protein significantly improved egg production (P< 0.02) with the highest egg production of 90.22% at 16% CP compared to 87.88 and 89.13% at 18% and 14% respectively. TSAA:lysine ratio did not significantly affect rate of egg production in this trial.

A significant crude protein X ratio interaction (P<0.07) was observed for hen weight, such that the lower TSAA:lysine ratio 0.71 combined with 16% protein improved the body weight by (4.03%) compared to the same ratio with 14 or 16% protein. This increase in body weight might be a difference between hens in lipid stores. Egg weight (Table 2) was not significantly affected by any of the dietary treatments. Egg mass increased with increasing TSAA:lysine ratios, but it did not reach significance at the P=0.05 level, as the 0.91 ratio increased egg mass by 2.11% compared to the other ratios.

Increasing TSAA:lysine ratio significantly increased specific gravity (P<0.03) and percent wet shell (P<0.01), with the highest value at the higher ratio (0.91). These findings could be a direct response to the effect of TSAA on the shell matrix. Calcium binding ability of the shell matrix is enhanced by the presence of sulfate groups, which may positively increase calcium binding in the shell, leading to an increase in both percent shell and specific gravity. Percent albumen and yolk were not affected by dietary treatments.

Percent yolk solids were significantly improved (P<0.08) by 0.61% with lowering dietary crude protein levels from 18-14% (Table 3). Ratio did not

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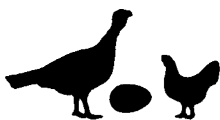

Table 3. Effects of dietary protein and TSAA:Lysine ratio on egg size and quality.

Crude Protein	Ratio	Specific gravity	Albumen	Albumen solids	Yolk	Yolk solids	Wet shell
			(%)	(%)	(%)	(%)	(%)
18	0.71	1.085	61.62	13.11	25.45	55.94	12.69
18	0.81	1.085	61.07	13.31	24.97	55.16[b]	12.58
18	0.91	1.085	61.76	13.02	25.09	55.65	12.94
16	0.71	1.084	61.95	13.42	25.24	55.90	12.58
16	0.81	1.083	61.88	13.12	25.42	55.68[ab]	12.48
16	0.91	1.085	61.39	12.93	25.55	56.08	12.84
14	0.71	1.084	61.81	12.86	25.17	55.61	12.70
14	0.81	1.084	61.17	13.08	25.42	56.15[a]	12.76
14	0.91	1.084	61.11	12.71	25.34	56.00	12.90
SEM		0.0004	0.45	0.28	0.21	0.20	0.12
Crude protein, %							
18		1.085	61.48	13.15	25.17	55.58[b]	12.73
16		1.085	61.74	13.16	25.40	55.89[ab]	12.63
14		1.084	61.36	12.88	25.31	55.92[a]	12.80
Ratio							
0.71		1.084[ab]	61.79	13.13	25.29	55.82	12.66[ab]
0.81		1.084[b]	61.37	13.17	25.27	55.66	12.61[b]
0.91		1.085[a]	61.42	12.89	25.32	55.91	12.89[a]
Statistical Probabilities							
Crude protein		NS	NS	NS	NS	0.08	NS
Ratio		0.03	NS	NS	NS	NS	0.01
Crude protein x ratio		NS	NS	NS	NS	0.03	NS

[abc]Means within a column with no common superscripts differ significantly P=0.05 or P=0.01 based onleast significant difference (LSD) test.

affect percent yolk solids but a significant (P<0.3) interaction affect occurred such that within the .81 ratio diet, percent solids increased largely as dietary protein decreased.

In conclusion, results reported herein suggest that lowering dietary protein levels in the range of 18 to 14% seemed to have no adverse effects on production performance parameters including egg production, egg mass and egg components' yield. A high TSAA:lysine ratio is recommended for high protein diets. Using these representative diets, this study has demonstrated that a reduction of crude protein using supplemental lysine and methionine, threonine and trypophan can sustain egg production and subsequently reduce N excretion into the environment.

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[1]Haitham M. Yakout and Curtis L. Novak, graduate students, and Sheila E. Scheideler, Professor and Extension Poultry Specialist, Animal Science, Lincoln.

[2]Degussa prediction model, *Degussa Laboratories,* Allendale, NJ 07401.

Physiology Notes

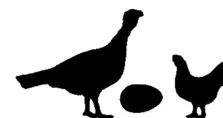
A Comparative Study of the Metabolic Responses to Heat Stress of Hy-Line Brown and White Varieties

Jennifer Bridger
Mary Beck[1]

Hy-Line white and brown variety birds have been selected for different production characteristics. Both hens lay approximately the same number of eggs/hen housed to 80 weeks. However, body size, feed intake, and age at 50% production varies greatly. The white hen is typically smaller, more efficient and reaches 50% production around 155 days. In contrast the brown hen is larger framed, heavily feathered, less efficient and reaches 50% production at 153 days. Hens were placed two at a time in an indirect calorimeter for two hour trials at 22°C, 33°C, and 38°C; each trial was replicated five times. Oxygen consumption and carbon dioxide production were measured, (respiratory quotients) RQ's determined, and heat production calculated as indicators of metabolic strain. At 22°C white hens (mean body weight 1.4 kg) had average RQ of 0.69 and produced 14.2 W. At 30°C (mean body weight 1.3 kg), RQ had increased to 0.73, and 17.6 W produced. At 38°C (mean body weight 1.5 kg), RQ had increased to 0.79 and W to 12.9. Brown hens had an RQ of 0.84 at 22°C, with 15.2 W produced. At 30°C, brown hens had an RQ of 0.81 and W of 18.5. At 38°C, browns had an RQ of 0.74 and W equal to 19.55. The heavier brown birds, with larger eggs and greater intake, had greater heat production (HP) as ambient temperature rose, and HP at all temperatures was greater in brown than in white hens.

Introduction

Over the years different strains of layers have been selected for various production characteristics. White layers have been selected for small size, high efficiency and increased production. Brown layers also have been



selected for better performance but are still a heavier breed that is often much less efficient. The selection for these different characteristics has led to very different responses to heat stress among these strains of layers.

The loss of production and decreased layer performance because of stress are responsible for millions of dollars in loss every year. These losses, though hard to calculate, are a burden to industry. In this experiment a comparative study was conducted between white and brown Hy-Line layers. The two strains were placed under identical heat stress conditions and metabolic responses were recorded. Determining the amount of carbon dioxide produced and oxygen consumed allowed for heat production (HP) and respiratory quotients (RQ) to be calculated. A comparison of the HP and RQ's was done as an indicator of metabolic stress for each strain.

Materials and Methods

During this experiment 30 brown hens and 30 white hens were compared at three temperatures; 22ºC, 30ºC, and 38ºC. At each temperature, two hens from each strain were placed in an indirect calorimeter for two hours and measurements were recorded. During the two hours measurements were taken of carbon dioxide, oxygen, body temperature, gas flow rates of the chamber and respiration rates. This procedure was replicated five times at each temperature.

Results

At 22ºC the white layers (mean body weight 1.4 kg) had an average RQ of 0.69 and produced 14.2 W, compared to the brown layer with an average RQ of 0.84 and 15.2 W. At 30ºC the white birds (mean body weight 1.3 kg) averaged an RQ of 0.73 and produced 17.6 W, while the brown birds averaged an RQ of 0.81 and produced 18.5 W. At 38ºC the white strain (mean body weight 1.5 kg) had an increased average RQ of 0.79 and produced 12.9 W, whereas the brown strain had an RQ of 0.74 and produced 19.55 W.

Conclusions

The heavier strain of brown layers had a greater HP as the ambient temperature rose and always had a greater HP than the white layers. The results indicate that the brown birds are at a physiological disadvantage in times of heat stress and may help us understand why greater mortality occurs in these birds during heat stress.

Endocrine Reproductive Properties in the Laying Hen Change with Age

K. K. Franzen
M. M. Beck[1]

Numerous studies have been conducted to examine reproductive hormone profiles and estrogen (E_2)-calcium relationships in the laying hen. Older hens in production (70+ weeks) produce fewer eggs and lay more soft-shelled or broken shell eggs, likely because of decreased reproductive hormones and decreased calcium uptake (CaT) by duodenal cells. Under heat stress (HS) conditions, which also negatively affect egg production, E_2 implants are able to improve both plasma estrogen and calcium uptake by duodenal cells (Forman et al., 1996; Hansen, 1998). In older hens with decreased egg production, E_2 significantly improved CaT and egg production parameters (Hansen and Beck, 1997). In this study, Hy-Line W-36 hens at three ages, pre-puberty (20 weeks, 0% production), peak production (29 weeks, ~93% production), and older (76 weeks, ~80% production) were maintained in 16h light and 8h dark. Blood samples were collected 4-6 hours prior to oviposition via brachial vein cannulation for plasma E_2 and progesterone (P_4) determination; and duodena were collected for *in vitro* CaT determination. Both absolute and the rate of CaT in duodenal cells from pre-pubertal birds were significantly greater than that of peak and older laying hens (absolute: P=.0545 and .0029, respectively; rate: P=.0069 and .0009, respectively). E_2 concentrations were significantly lower in the older hens compared to both peak and pre-pubertal hens (P=.0361 and .0715, respectively). P_4 concentrations in the older hens were significantly lower than in either peak or pre-pubertal hens (P=.0053 and .0035, respectively). In pre-pubertal hens compared to peak, equal plasma concentrations of E_2 appear to be associated with differential CaT, with efficiency in peak hens being lower. In contrast, the old hens appear to sustain CaT at a rate equal to that in peak hens with much less E_2. These results may suggest a change in efficacy of E_2 as hens age; one possibility might be the number and/or sensitivity of E_2 receptors.

IL-1 Suppresses Progesterone Production *in vitro* by Granulosa Cells of Laying Hens

M. A. Alodan
M. M. Beck[1]

It is known that high environmental temperature, heat stress (HS), significantly reduces egg production, in part at least through disruption of reproductive hormones, progesterone P_4,

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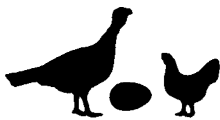

luteinizing hormone (LH) and estrogen. There are many well-documented systemic effects of HS that may affect these hormones, but very little is known about local mechanisms through which HS acts. IL-1 is increased by stress, including HS, and recently, in mammals, it has been shown that cytokines interleukin-1_α and β (IL-1_α, IL-1β) play a role in ovarian function. The role(s) of cytokines in the bird remains unclear. The aim of this study was to determine whether IL-1β has a role in ovarian function of the laying hen and whether it might be a viable candidate for HS-induced reductions in the granulosa cell production of P_4. The largest preovulatory follicles (F1) were collected from laying hens, and the granulosa cells dispersed enzymatically. The granulosa cell preparation was divided into two equal portions and each was incubated in RPMI medium with (IL treatment) or without (Control) 100*ng* IL-1β per ml for 5h at 39°C, washed, and viability determined. Approximately 100,000-viable cell aliquots from both IL and Control incubations were further incubated in duplicate in 1.8 ml RPMI; cells from the IL treatment were incubated with 70I*ng*/ml IL-1β and with (IL + LH) or without (IL-LH) 100*ng* LH for an additional 4h at 39°C. Control cell aliquots were also incubated for the additional 4h with (Control + LH) or without (Control) LH. P_4 was measured in the supernatant by RIA. Compared to basal production of P_4 (from Control cells), P_4 in Control + IL samples was significantly (P=0.0001) lower. LH stimulation increased (P=0.0001) P_4 in both Control + LH (by 600%) and IL + LH (by 500%) samples, but P_4 was lower (P=0.057) in samples incubated with IL-1β. We hypothesize that IL-1β does play a role in ovarian function of hens and that HS may induce macrophages to produce it.

VIP-immunoreactive Cells Increase in Hypothalamic Nucleus of Heat-Stressed and Hypothyroid Hens

M. M. Beck
S. A. Elnagar[1]

Studies were conducted to determine the effects of heat stress (HS) on the VIP-PRL regulatory pathway and, by comparison with chemical hypothyroidism, determine whether the HS effects on P_4 and E_2 could be caused by HS-inducted hypothyroidism. Hy-Line W-36 hens were used in two studies, one using mild (35°C, 50% RH) HS and the other a slightly higher temperature (36.5°C, 50% RH). In both, blood samples were obtained daily and analyzed for T_3, T_4, E_2, P_4, PRL, and VIP; brains were removed on day 5 and analyzed for VIP by immunocytochemistry. In the initial study, hens were subjected to HS or thermonentural (TN) conditions for five consecutive days. In the second study, hens were subjected to HS or TN conditions for 5 days. Two additional treatment groups were hypothyroid (Hy), 0.1% thiouracil for one week before and during the experimental period; and Hy + T_4 replacement (thiouracil then 0.5 ppm T_4 during the experimental period). HS at 35°C was not sufficient to cause the sustained and dramatic reproductive hormone response typically seen in HS hens; although there were transient effects on some days, there was full recovery of all hormones by day 5. Plasma E_2 and VIP were altered on day 2 and day 1, respectively, but this did not last. Brain VIP, in terms of numbers and apparent staining intensity, was not affected by HS in this experiment (P = .974). When hens were subjected to 36.5°C/50% RH, however, the effects on E_2 and P_4 were clear (P = .0001, .0001). T_3 was depressed (P = .0001), and PRL was elevated (P = .0019). Reproductive hormones responded similarly to Hy, with decreased E_2, and increased PRL and VIP (P = .0019, .0003). In all cases, T_4 replacement was able to overcome effects of Hy. In brain, VIP-ir cells increased in numbers in both HS and Hy (P = .0036) over controls (C); T_4 replacement resulted in VIP-cell numbers similar to C. These results indicate that HS could act by creating a functional hypothyroidism with subsequent activation of the VIP-PRL axis to suppress reproduction.

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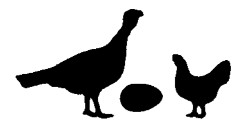

E. coli Infections of Laying Hens: A Disease Overview and Results of An Investigation

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E. coli bacteria are a rather diverse group of bacteria in the family Enterobacteriaceae This article discusses some of the factors involved in *E. coli* diseases in poultry, then reports on an investigation of *E. coli* septicemia in laying hens.

Introduction

E. coli infections are probably the most common and economically costly diseases of broilers and turkeys (meat-type poultry) in much of the United States. They also occur to a lesser degree in egg-laying type chickens. Several syndromes are recognized in all birds including: yolk-sac infections of chicks, septicemia, salpingitis (reproductive tract infections), airsacculitis and peritonitis, coligranuloma, cellulitis (infection of the skin and underlying tissues) and localized inflammation of the joints, eyes, or other tissues.

They are common inhabitants of the intestinal tract of all poultry and are excreted in the droppings. Hence, all poultry buildings contain these organisms on the floor or cages, in the pits, in the building dust, in the water and feeders, on the birds themselves, and virtually all indoor surfaces. The number of *E. coli* in the environment probably correlates with the level of fecal build-up which is related to bird density, manure removal practices, clean-up and disinfection practices and ventilation. However, the presence of high numbers of these bacteria in the environment does not automatically indicate that disease will occur.

Several factors are believed to predispose birds to infections with *E. coli* including:

1. Damage to the birds' natural defense (immune) system

Examples would include exposure to many intestinal and respiratory viruses, and other bacteria such as Bordatella, Pasteurella and Mycoplasma. In these instances, the primary infections can damage the protective lining of the respiratory tract and gut and allow secondary bacteria to invade. Some viruses go further by attacking immune cells involved with defense against infections, such as Hemorrhagic Enteritis Virus or Infectious Bursal Disease.

Poor air quality in a building such as high ammonia, or heavy dust levels can damage the respiratory tract by both injuring the cells lining the airways and burdening the existing clearing mechanisms of these tissues.

Increased stress factors such as temperature extremes, social stress from overcrowding also can weaken the bird's immune system.

External injuries such as breaks in the skin from cage trauma, jumping or fighting among birds can allow bacteria to gain entrance into the deeper tissues.

2. Heavy environmental contamination with *E. coli*

While exposure to high organism numbers alone does not always cause disease, it may be an important contributing factor. As fecal *E. coli* contamination goes up, the likelihood of exposure to other infectious agents and reduction of environmental quality go up as well.

Heavy environmental contamination can cause disease in birds with poorly functioning immune systems such as baby chicks and developing embryos. Hence much emphasis is placed on breeder farm, hatchery and brooding sanitation.

3. Virulence Factors of *E. coli* Strains

E. coli infections in poultry traditionally have been thought to be secondary. That is, these infections would otherwise not occur if host factors and environmental conditions were optimal. This theory is supported by observations that *E. coli* disease is very difficult to reproduce in healthy birds under controlled laboratory conditions. Often severe challenge routes such as intravenous inoculation are needed to produce disease.

However, even under laboratory conditions, differences in organism virulence appear to be present. Occasionally, there are *E. coli* outbreaks where no other primary diseases are identified. Researchers have wondered in these cases if unusually virulent strains of *E. coli* are involved. Highly virulent strains are known to exist in humans and in other animals.

In the latter cases several potential virulence factors have been identified. These virulence factors allow the organisms to invade, survive and multiply in the host. A few examples are discussed below.

a. Embryo mortality

Many researchers have used embryonated chicken eggs to separate virulent and non-virulent *E. coli* strains using embryo mortality as a virulence marker.

b. Complement resistance

Ability to survive the killing effects of serum in the animal is an important virulence factor for many microorganisms. Several genes have been identified in some *E. coli* strains that encode

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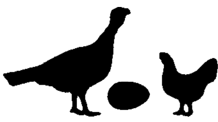


for outer membrane proteins that are associated with complement resistance. These include *tra*T, *iss*, and *omp*A genes. Presence of a capsule may also be associated with complement resistance.

c. Presence of toxins

Several strains of *E. coli* are known to produce toxins that are responsible for many of the clinical signs seen with disease such as diarrhea, enteritis and kidney damage to name a few. These toxins have been better studied in human and mammalian *E. coli* infections. Two examples are the shiga-like toxins, SLTI and SLTII, encoded by the genes *slt*I and *slt*II.

d. Presence of colicins

Colicins are antibiotic-like substances produced by some bacteria to inhibit other competing bacteria in the environment. ColV is a particular type of colicin produced by some *E. coli* strains encoded by the gene *cva*C.

e. Serotyping

Certain *E. coli* serotypes have been associated more often with disease than others. Serotyping has also been an important epidemiological tool to track outbreaks.

f. Antimicrobial sensitivity

Resistance to commonly used antimicrobials may be an important virulence factor and might be helpful to trace similarities between organisms.

Layer *E. coli* Field Investigation

This investigation was undertaken by the request of a producer because of observations that mortality due to *E. coli* appeared to be increasing. Numerous outbreaks were followed over a several month period. The goals of the investigation were to:

1. determine if possible, any primary environmental or infectious disease problems predisposing birds to *E. coli* infections.
2. determine if the outbreaks and organisms involved were related epidemiologically.
3. identify virulence factors in these organisms
4. identify any prevention and/or treatment strategies for future flocks.

History and Clinical Findings

Eleven flocks were monitored over three seasons. 7/11 outbreaks (63%) occurred between 19 and 24 weeks of age, coinciding with onset of egg production. 2/11 flocks were older hens, between 60-63 weeks of age. The remaining two flocks had been molted and were 92-98 weeks of age.

No specific adverse environmental conditions were identified in the houses that were inspected. Excess dust was considered in some of the outbreaks, generated during floor sweeping. However, outbreaks had continued even after this practice was discouraged by management. An attempt was made to identify location of mortality in the houses. However, no definitive mortality pattern was detected.

Mortality on 2/11 houses was 4-6 times higher than expected for the age group and persisted for up to 12 weeks. In 8/11 houses, the mortality was roughly double the expected level for 4-5 weeks. In two houses with *E. coli*, the mortality did not exceed the expected limit.

Egg production was considered low-normal for most of the flocks. However, increases in shelless eggs were observed. Wrinkling of shells was not evident.

Necropsy Findings

Affected birds sometimes were found sitting in their cages, gasping and showing dark discoloration of combs. In most instances, birds were found dead with no apparent weight loss and in full egg production. Necropsy findings included yellow thickened airsacs, yellow caseous peritonitis, enlarged congested livers and spleens and sometimes diffusely congested lungs. Rarely, a yellow caseous plug could be found within the lumen of a primary bronchus. The intestinal walls were reddened, and orange-red mucus was frequently present in the small intestines.

Microscopically, changes consistent with bacterial peritonitis, airsacculitis, pericarditis and septicemia were seen. The portal of entry for the bacteria was never determined. Lungs occasionally contained foreign debris with associated peribronchiolar inflammation, suggesting aspiration of dust may have been a contributing factor in development of illness. These findings were not consistent in every outbreak.

Results of Laboratory Testing

Bacteriology:

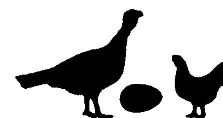
E. coli was always isolated, frequently in pure culture from peritoneal swabs and livers.

Serology:

Serological testing for Newcastle Disease, Infectious Bronchitis, Avian Influenza, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* were performed. No antibodies were detected for Avian Influenza or *Mycoplasma synoviae*. Antibodies were present in sampled birds for the remaining three infectious agents consistent with the company's vaccination program. No unusual titers or rising titers were detected.

Virus Isolation:

Pooled tissues submitted from chickens that died during an outbreak were submitted to the National Veterinary Services Laboratory for virus isolation. Feces were cultured separately from pooled viscera. Virus isolation from both groups was negative. No further virus isolation was attempted from any other affected flocks.



Identification of Virulence Factors from *E. coli* Organisms Isolated from Diseased Flocks.

Twenty-eight isolates were further tested for multiple virulence factors listed below.

Antibiotic Sensitivities:	*sensitive*	*resistant*
-floroquinolones	96%	4%
-ceftiophur	100%	0%
-gentamycin	100%	0%
-tylosin	0%	100%
-neomycin	93%	7%
-tetracyclines	14%	86%
-sulfa's	36%	64%
-spectinomycin	98%	2%

Lactose fermentation:

Subjective observations by company managers suggested that lactose negative strains were possibly more virulent than lactose positive strains. Of the 28 isolates we examined 10 (36%) were lactose negative and 18 (64%) were lactose positive. No correlation was seen between total flock mortality and lactose fermentation status of the bacteria isolated.

E. coli *serotyping:*

Organisms were serotyped for O somatic antigen. 14 isolates were O78, 12 isolates were untypable, one isolate was O2, another isolate was O123.

Other virulence factors:

The *iss* gene was present in all 28 isolates. Colicin was also identified in all examined organisms. No other significant virulence characteristics were identified.

Discussion

An investigation of several *E. coli* outbreaks within a company revealed that organisms isolated were all positive for the *iss* gene. This gene is important because it encodes for serum resistance. It allows the bacteria to spread throughout the bird's body, once it enters the blood stream. A vaccine developed against this gene may prove to be effective against systemic *E. coli* infections which are quite common in all poultry. Tetracycline resistance to *E. coli* was unfortunately quite prevalent. Almost all isolates were sensitive to spectinomycin, neomycin, gentomycine, cephtiofur and the floroquinolones. Unfortunately, laying hen use of these products is mostly prohibited.

More research is needed to develop an effective preventative strategy against this disease as treatment options are extremely limited for this costly problem.

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Microbial Characteristics of Immersion-Chilled Broilers and Air-Chilled Broilers

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Introduction

During processing of raw chicken, one of the most critical steps for inhibiting the growth of microorganisms is carcass chilling. The main purpose of chilling is to lower the broiler temperature. USDA requires that carcass temperature must be reduced to 4°C within 4 hours (USDA, 1973).

The two most common methods of chilling used in industry are immersion chilling (IC), in which birds are submerged in cold counter current water tanks, and air chilling (AC), in which broilers are individually chilled using drafts of cold air. IC is primarily used in the United States, while AC is commonly used in Europe.

In the IC process, as the chicken exits the tank, it comes in contact with the coldest, cleanest water. In addition to reducing carcass temperature, the IC process helps rinse away some of the bacterial load. However, two controversial issues of the IC process are risk of bacteria cross-contamination and water retention. When the broiler enters the communal water tank the risk of cross-contamination is increased due to the contact between birds and the contaminated water. The use of cold water also may increase selection for the survival of psychrotrophic bacteria, which are the principal spoilage organisms, having a net negative effect on shelf life. Additionally, water is added to the bird in the IC process when the bird gets in contact with cold water in the tank. The pores, which are opened in the warm broiler, trap moisture when closing by contacting cold water. Thus some water weight is added to the birds, which are then sold on a weight basis increasing costs to the consumer.

An alternative to IC is air chilling. In an air chill system the lack of a communal water bath may reduce the risk of cross-contamination and selection of psychrotrophic bacteria may be decreased. Broilers are chilled individually on an assembly line and contact between birds is minimized. Lack of a cold communal submersion step may reduce levels of psychrotrophic organisms. Moreover, the absence of the IC

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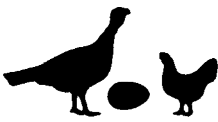

step minimizes water retention, resulting in a "no water-added" product, which may have different physical properties than that of the IC birds.

In October 1998 the first federally inspected AC facility began operations. Because there is only one AC plant in the US, no current data is available comparing both processes for quality and microbiological characteristics. Early studies comparing both processes suggested that spoilage occurred sooner in IC birds (Knoop 1971). The same study concludes that psychrotrophic counts were lower in AC broilers, resulting in a longer shelf life.

Of greatest concern among food borne pathogens associated with poultry products are *Salmonella* spp. and *Campylobacter* spp. (Deming *et al*., 1987; CDC, 1990.). *Salmonella enteritidis* is considered to be the deadliest enteric pathogen followed by *Campylobacter* spp. and *E. coli* O:157 H:7. (Food Protection Report, 1998). *Campylobacter* spp. is considered to be the leading cause of diarrhea, affecting 2 to 8 million individuals each year. This results in approximately 800 deaths annually (Maharaj, 1997; CDC 1990). These organisms originate in the digestive tract and can be spread by cross contamination during poultry production and processing.

Cross contamination can occur at many points in the process of bringing the product to the consumer, beginning at the farm and continuing during processing. Steps such as picking, scalding and chilling could be a source of cross contamination (Lillard 1989). Reports indicate that over 80% of the raw commercial broilers tested positive for *Campylobacter jejuni/ coli* (USDA 1995). In addition about 20% of ready to market broilers tested positive for *Salmonella spp*, whereas only 2 to 5% of samples were positive before entering the plant.

In the current study an equal number of samples was taken from each plant. These chicken broilers were tested for incidence of *Salmonella* spp. and *Campylobacter* spp. In addition, microbial populations for total aerobic microorganisms, coliforms, psychrotrophs and generic *E. coli* were determined. The study covered a six-month period to account for seasonal variations.

Results indicated that the incidence of pathogens tended to be lower in AC broilers compared to their IC counterparts. A 10% decrease was found in the incidence of *Salmonella* spp. and *Campylobacter* spp. levels. This could be explained by the possibility of cross-contamination in the immersion bath for the IC process, while this step is absent in AC birds.

No significant difference was detected for total aerobic populations. This may be explained by the fact that both processes are fairly similar until the chilling step. Slightly higher counts of coliforms and *E. coli* were found in the AC broilers. We attribute this result to the lack of a rinsing step that is present in the IC process that may remove some of the coliforms. Finally, psychrotroph counts were significantly higher in the IC chickens possibly because the cold water in the IC selects for psychrotrophic organisms.

The purpose of this study was to compare microbiological loads of ready-to-market immersion chilled (IC) broilers and air chilled (AC) broilers and to determine the pathogen incidence of both processes, accounting for the presence of *Salmonella* spp. and *Campylobacter* spp.

Materials & Methods

A total of 300 birds were collected. In each of 5 replications, 30 birds from an AC facility and 30 birds from an IC plant were sampled. IC birds are chilled in a three-stage immersion tank for a total time of 85 minutes. The first stage used water at 17.2 ºC (63ºF), the second at 5.6 to 6.7ºC (42 to 44ºF) and the third stage at –1.1 to 0ºC (30 to 32ºF). On the other hand AC broilers were chilled for 120 minutes using an air-chilling room divided in two stages, the first one with temperatures of -7.7 to –5.5ºC (18 to 22ºF) and the second one at -4.4 to –1.1ºC (24 to 30ºF).

Samples were collected after the chilling step using sterile 3500 stomacher bags. Broilers were packed on ice and transported to the lab where they were held overnight at 4º C. *Salmonella* spp. *Campylobacter* spp., generic *E.coli*, psychrotrophs, total coliforms and total APC were evaluated for each carcass.

Chicken carcasses were sampled using the whole rinse method by shaking the sample in a rocking motion using a sterile stomacher bag and 400ml of Butterfield buffered solution. The rinsate was then divided into three parts for each analysis. All microbial analysis was based on methods described in the USDA/FSIS Microbiology Laboratory Guidebook and in the FDA Bacteriological Analytical Manual.

1) Determination of Microbial Populations

Ten ml of rinse was used to prepare serial dilutions for each sample. Dilutions were prepared with Buffered Peptone Water (BPW) at 0.1%. Plate Count Agar (PCA) was used to determine total and psychrotroph counts.

For total counts, plates were incubated at 37ºC for 48 hours, while psychrotroph plates were incubated for 10 days at 7ºC. To determine coliform levels Violet Red Bile Agar (VRBA) was used supplemented with 0.4 ìg of 4-methyl umnellyferyl-beta-D-glucuronide (MUG). This allowed us to determine total coliforms and also the level of generic *E.coli* when counted under to UV light. These plates were incubated for 24 hours at 37ºC.

2) *Campylobacter* spp. detection

Campylobacter spp. presence was determined using 200 ml of rinse. Rinsate was sterile-filtered through cheesecloth into a sterile 250-ml centrifuge bottle. This was then centrifuged at 16,000 G for 15 minutes, supernatant was discarded and the pellet was

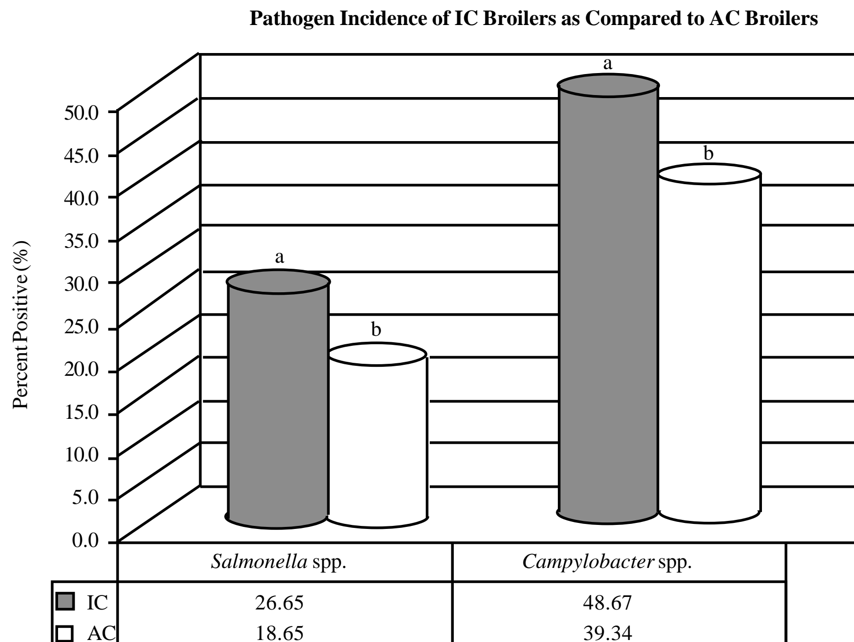

Figure 1. Pathogen incidence for IC and AC broilers. Different subscripts within each pathogen indicate significant (P<0.05) differences.

re-suspended in 10 ml of BPW. One ml of the inoculated-peptone water was pre-enriched adding in 100ml of Hunt Enrichment Broth (HEB) in a Quik Seal Bag, air was removed from the bag and a mixture of 5%O2, 10%CO2 and 75% N2 gas was added to inflate the bag and produce a microaerobic environment. The bag then was sealed and incubated at 37°C for 4 hours in a shaker incubator. After four hours sterile cefoperazone solution was added to yield a final concentration of 30mg/L. The microaerobic atmosphere was re-established and the bag was incubated for 20 hours at 42°C in the same incubator.

Selective plating was performed in Modified Campylobacter Charcoal Differential Agar (MCCDA) by swab-streaking solution to the plates to obtain isolated colonies. MCCDA plates were incubated at 42°C for 24 to 48 hours in a microaerobic environment using anaerobic jars with pressure gauge valves. Colonies produced were tested for oxidase reaction and also identification was performed with visual and biochemical tests. Three colonies were inoculated in Brucella Broth supplemented with Ferrous Sulphate (F), Potassium Biphosphate (B) and Sodium Piruvate (P). B-FBP tubes were incubated at 42°C for 24 to 48 hours in microaerobic atmosphere. A wet mount was examined under phase-contrast microscope, looking for a spiral shaped rod with screw-motility under 100X objective. Several drops of B-FBP solution were added to Semisolid Glucose Medium (SGM) and incubated in microaerobic conditions at 42°C for 1 to 3 days to observe glucose fermentation. These tubes also were used to determine catalase reaction using hydrogen peroxide solution, which would produce the formation of bubbles. Several drops of the same solution were spread over B-FBP agar plates using sterile cotton swabs. An antibiotic susceptibility test was performed in these plates using nalidixic acid and cephalothin disks and further incubation for 24 to 48 hours at 42°C. A sample was considered positive when positive for oxidase and catalase tests and negative for glucose fermentation. *Campylobacter* spp. should be nalidixic acid susceptible but resistant to cephalothin.

3) *Salmonella* spp. detection

Fifty ml of original rinsate was used to detect *Salmonella* spp. Sample was pre-enriched adding 50 ml of BPW and incubating for 24 hours at 37(C. Pre-enriched samples were divided in two enrichment broths; 0.5ml into Tetrathionate TT broth tubes and 0.1ml in Rappaport Vassiliadis Broth RV tubes. Incubation lasts 24 hours at 41(C. Selective plating is performed in Xylose Lysine Tetrathionate supplemented with Tergitol 4 (XLT4) and Brilliant Green Sulfa Agar (BGS) by streaking enrichment solutions and incubating for 24-48 hours at 37(C. Characteristic colonies were inoculated in slant tubes of Lysine Iron Agar (LIA) and Triple Sugar Iron Agar (TSI) and incubated 24 hours at 37°C. Characteristic colonies, which are black centered in XLT4 and translucent red in BGS; are used to perform urease and indol tests. Finally positive colonies were serologically confirmed with polyvalent serum for *Salmonella* spp. (Appendix 4)

Statistical Analysis

Sample number was determined based on the method described by Dormedy et al, 1999, and the number of birds per replication constitutes a representative sample of the 40,000 birds processed on a daily basis in both plants. Bacterial counts were converted to $\log_{10}$ counts per ml of rinse and analyzed using the General Linear Model (GLM) of SAS (SAS 1995). Differences in *Salmonella* spp. and *Campylobacter* spp. incidence between chilling regimens was determined using Chi Square Analysis of SAS (SAS 1995).

Results

Figure 1 illustrates that the incidence of pathogens were significantly lower in AC samples. The presence of

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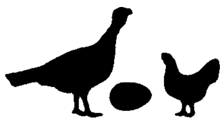
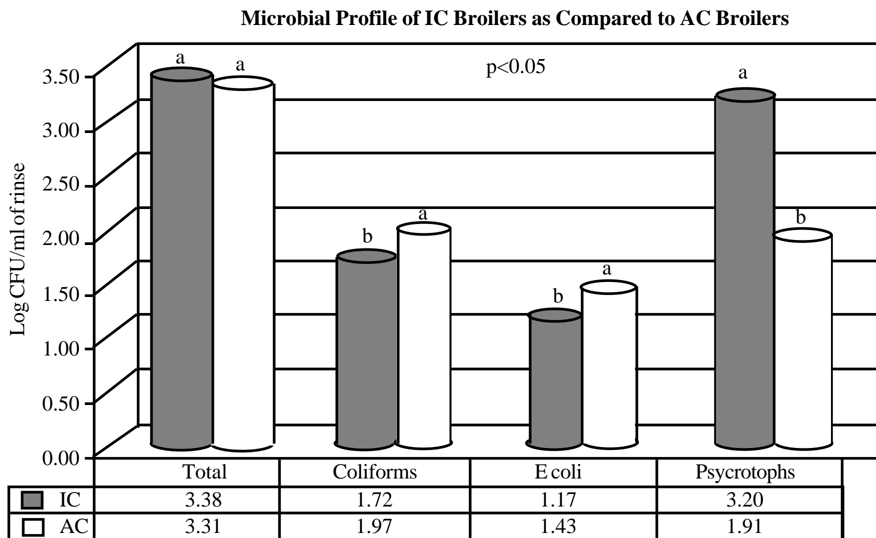




	Total	Coliforms	E coli	Psycrotophs
IC	3.38	1.72	1.17	3.20
AC	3.31	1.97	1.43	1.91

Figure 2. Results for counts of aerobic total, coliforms, generic E.coli and psychrotrophs for AC and IC broilers. Different subscripts within each bar indicate significant (P<0.05) differences.

Salmonella spp. in AC broilers was 18.7%, compared with 26.7% in IC-birds; also *Campylobacter* spp. positive samples constitute 39.3% in AC process, while 48.7% in IC-broilers.

Results indicated no significant differences in total aerobic counts comparing both processes. However, coliforms and generic *E. coli* counts were higher on AC broilers compared with the IC broilers. There is a significant difference of 0.2 log numbers in the number of coliforms for the AC over the IC birds. Psychrotrophs counts differ in more than 1 Log CFU/ml of rinse, being significantly higher in IC broilers (3.20) compared to 1.91 Log CFU/ml of rinse in AC broilers.

An additional finding was a higher incidence of *Campylobacter* spp. isolates that were resistant to fluoroquinolones in the IC birds. These isolates were preserved for future research.

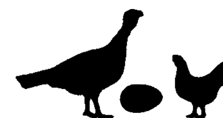
Discussion

The incidence of pathogens in IC broilers appears higher compared with AC birds. A 10% difference in the incidence of *Salmonella* spp. and *Campylobacter* spp. was found. These results suggest that IC birds have a greater chance of bacterial cross-contamination in the immersion tank. Contaminated birds spread bacteria to the water and to the other birds in the tank. On the contrary in AC birds, chilling is performed individually reducing contact between birds, and there is no communal media to spread bacteria. One contaminated broiler could only contaminate a few of the birds that are in direct contact to it, but not the remaining of the batch. Although chilling is only one step in poultry processing, it could be highly important in reducing overall pathogen levels. However, it should be noted that there is a difference in the physical properties of both birds. IC birds contain additional water picked up during chilling, increasing the water activity and hence the conditions for microbial growth. In contrast, in AC birds there is reduction of water added in the skin of the bird. The recovery of microorganisms during carcass rinse might also be influenced by attachment of organisms to the chicken skin, making removal of bacteria to the media for enumeration difficult. Watery skin in IC birds appeared to help in the recovery of organisms from the bird during analysis; however, a comparative study was performed using the whole carcass rinse and stomaching a sample of skin aseptically removed. The study resulted in a better recovery using the whole rinse method.

The higher levels of coliforms and generic *E. coli* in AC birds could be explained by the immersion-rinsing step that is absent in the AC process. Water flowing in counter current direction may wash away some of the bacteria originally present in the bird, including coliforms. There is no chance of adding an additional washing step in AC birds, because it is supposed to be sold as "no-water added product," and additional rinses increase water retention. Low temperature may select for psycrotrophic bacteria. This could explain the similar number in total counts in both groups.

Finally, the fact that the level of psycrotrophic organisms is higher in IC broilers at more than one log may be due to the fact that the water in the tank selects for these organisms, reducing levels of mesophilic bacteria. Some bacteria are removed, while psychrotrophs are homogeneously distributed not only in the exterior of the bird, but also inside the carcass and deeper in the pores of the skin. On the other hand AC birds retain the original bacterial flora including coliforms and psychrotrophs. This could result in a longer shelf life of AC birds compared with the IC counterparts. Since psychrotrophs account for most of the spoilage in poultry products, the higher the presence in the broiler, the shorter the shelf life.

Future studies will compare shelf life for both processes and will evaluate farm management practices. Also the level of cross contamination will be determined at each step during processing in order to establish the profile of each step. A farm-to-table HACCP plan will be developed for AC broilers. In addition a microbial baseline for air-chilled chickens will be provided for



future reference for the USDA and the poultry industry.

An unexpected finding we encountered during the study was a higher incidence of nalidixic acid-resistant *Campylobacter* spp. isolates in IC broilers. Reasons for this may be the fact that the IC birds came from a wider source of farms while the AC birds were less diversely distributed and/or the heterogeneity of the strains present in the water tank creating a larger gene pool from which to draw upon. However, this will need to be confirmed by future research.

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