EFFECT OF DIETARY ENERGY, PROTEIN, LYSINE, VERSATILE ENZYME

AND PEPTIDES ON COMMERCIAL LEGHORNS

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EFFECT OF DIETARY ENERGY, PROTEIN, LYSINE, VERSATILE ENZYME AND PEPTIDES ON COMMERCIAL LEGHORNS

Priyantha Kumara Gunawardana

A Dissertation

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Doctor of Philosophy

Auburn, Alabama

May 09, 2009

EFFECT OF DIETARY ENERGY, PROTEIN, LYSINE, VERSATILE ENZYME

AND PEPTIDES ON COMMERCIAL LEGHORNS

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VITA

Priyantha Kumara Gunawardana, son of M. Buddhadasa and S.Jayasinghe was born June 21, 1970. He received his bachelor degree in Veterinary medicine and animal sciences from University of Peradeniya, Peradeniya, Sri Lanka. After completion of his degree, he worked as veterinary internee at veterinary research institute, Peradeniya for one year and as a technical service officer at Ceylon Grain Elevators Ltd. for five years. He entered the Ph. D. Program in Poultry Science under the direction of Dr. David A. Roland, Sr. He will complete his Ph. D. by May of 2009 and plans a career as an animal nutritionist. He is happily married to Kanchana Ranasinghe, daughter of Douglas Ranasinghe and Sujatha Perera, and is blessed with sons Viran and Manoj.

DISSERTATION ABSTRACT

EFFECT OF DIETARY ENERGY, PROTEIN, LYSINE, VERSATIL ENZYME AND PEPTIDES ON COMMERCIAL LEGHORNS

Priyantha Gunawardana

Doctor of Philosophy, May 9, 2009
(B. V. Sc, University of Peradeniya, Sri Lanka. August 1999)

168 Typed Pages

Directed by David A. Roland, Sr.

Two experiments were conducted to determine the effect of increasing dietary lysine on performance, egg composition, egg solids, egg quality, in seven commercial brown egg layer strains, and to determine the nutrient (lysine) requirements that allow for the best performance in phase one and two. Lysine had significant effects on egg weight, egg mass, feed conversion, percent albumen solids, yolk color, shell color, albumen weight, egg shell and albumen components. There were significant strain effects on egg production, feed consumption, egg weight, egg mass, feed conversion, specific gravity,

yolk weight, shell color, shell, albumen and yolk components, yolk, albumen and whole egg solids. All strains peaked in production over 94% and were laying 94 to 96% at 36 weeks of age. Average egg weight (21wk to 36wk) was 60.3g, varying from 59.0 to 62.8 g between strains. Average feed intake was 112.3g/hen/day varying from 109.6 to 116.7g/hen/day between strains. Increasing dietary lysine from 0.747 to 0.917% significantly improved feed conversion from 2.20 to 2.06 g feed/g egg and increased egg mass from 51.8 to 54.32 g/hen/day. Average lysine intake of hens fed 0.917% level was 1023mg/hen/day varying from 1005 to 1070mg/hen/day between strains.

During phase two, increasing dietary lysine increased egg production, egg weight, egg mass and improved feed conversion as observed in phase one. However, increasing dietary lysine increased feed consumption during phase two. Increasing dietary lysine from 0.680 to 0.828% significantly improved feed conversion from 2.03 to 1.91 g feed/g egg and increased egg mass from 54.0 to 59.30 g/hen/day. Average lysine intake of hens fed 0.828% level was 939 mg/hen/day varying from 907 to 964 mg/hen/day between strains. Because egg and ingredient prices often change, there can be no fixed dietary lysine level for optimal profits.

Protein had a significant effect on egg production, egg mass, feed intake, feed conversion, egg weight, percentage of egg shell components, yolk color , and yolk and albumen weight. Increasing dietary energy to 238 kcal ME/kg by addition of poultry oil, feed intake linearly decreased. Increasing dietary energy also significantly increased body weight and egg yolk color.

ACKNOWLEDGEMENTS

The author would like to thank his major professor, Dr. Roland, for his four years of patient and helpful direction and support in completing this program. The author would like to thank Dr. Davis, Dr. Chiba, Dr. Giambrone and Dr. Tung-Shi Huang for their help and suggestions in completing this program. Thanks are also due to Matilda Bryant, Dwain Holt, Sedat Bilgili, Steven Martin, Joseph Mitchell and Tommy Stinson for their assistance to the experiments.

The author wishes to express his love and gratitude to his wife Kanchana for her patience and support for the graduate study period. The author also wishes to express his appreciation to his loving sons, father and mother, father in law and mother in law and brothers and sisters for their moral support during the course of this program.

Style manual or journal used : Journal of Applied Poultry Research and Journal of Poultry Science

Computer software used : Microsoft word 2003 and SAS, 9.1

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1. INTRODUCTION

The knowledge and understanding of poultry nutrition is indispensable for efficient operation of large scale poultry enterprises. Commercial Poultry Nutrition provides extensive information on the nutrient requirements, ingredient evaluation and diet formulation for poultry of all types. This includes layer pullets, laying hens, broilers and broiler breeders, turkeys, ducks, geese, game birds, ratites and pet birds. Advances in genetic selection make today's commercial layers quite different from those of a decade ago. Body weight is less, age at housing and age at 5 percent production are earlier, total egg numbers have increased, egg mass is greater, and feed conversion has improved considerably. At present with competitive market in which producers are forced to aim at profitability rather than maximizing performance, optimization of nutrient utilization has become a necessity. Therefore the primary objective of poultry nutrition is to obtain highest level of performance, reduce nutrient burden on the environment and maximize profits.

Dietary energy is one of the two expensive and essential nutrients. There is a broad range of energy levels (2680 – 2990 kcal ME/kg) currently being used by egg producers. A large number of trials have been conducted to investigate dietary energy effect on performances of commercial leghorns. It is well established that hens generally adjust their feed intake according to their energy requirements. However, the results of studies of the effects of dietary energy on the egg production are conflicting.

For example, Ciftci et al. (2003) found that decreasing the energy content of feed from 2,751 to 2,641 kcal of ME/kg increased egg production from 86.44 to 88.27%. But Mathlouthi et al. (2002) reported increased egg production at an energy content of 2,753 kcal of ME/kg of feed compared with 2,653 kcal of ME/kg of feed. Responses of egg weight to changes in feed energy content are typically insignificant (Vogt, 1986; Summers and Leeson, 1993; Keshavarz and Nakajima, 1995; Grobas et al., 1999b; Mathlouthi et al., 2002; Ciftci et al., 2003). However, some authors have reported significant, although small, increases in egg weight caused by increased dietary energy (Marsden et al., 1987; Peguri and Coon, 1991). Feed intake significantly decreased with increasing dietary energy or supplemental fat (Grobas et al., 1999; Harms et al., 2000 Wu et al., 2005a, b, c). However, Summers and Leeson (1993), Jalal et al. (2006) and Wu et al. (2006) reported that there was no significant effect of dietary energy on feed intake.

Decreased feed intake could have a big impact on cost of production. Feed intake and egg weight plays a significant roll in cost of production and profits. If feed intake can not be decreased by increased dietary energy, increasing dietary energy in the diets may not be economical. With the markedly increase in feed ingredient prices during last few years, it is more important for producers to get information that would allow them to optimize dietary energy use. Even though egg further processing such as dried and liquid egg industry has tremendously developed during last few years, few studies have been conducted to investigate the effect of dietary energy on egg solids and composition of commercially available leghorn strains. Therefore some of our experiments were conducted to determine the effect of dietary energy on egg composition, performance and profits in commercially available leghorns (Section III and V).

Protein (lysine) is one of the two major nutrients representing a high percentage of total cost of the diets. Liu et al. (2005) and Wu et al (2007a) reported that increasing Protein (lysine) level significantly improved egg production, egg weight; egg mass, feed consumption, feed conversion, egg specific gravity and body weight of hens. Dietary protein and individual amino acid consumption rates directly influence the protein constituents of egg yolk and albumen. Calvery and Titus (1934) observed differences in egg weight, albumen and yolk yield, solids, and protein composition of eggs produced by hens fed diets differing in wheat, corn, or soybean meal. They also recorded differences in amino acid composition and total nitrogen content of the eggs. Butt and Cunningham (1972) reported that total nitrogen in whole egg and albumen increased as dietary protein increased from 12 to 18%. Gardner and Young (1972) observed that increasing dietary protein from 12 to 18% resulted in significant increases in the weights, solids, and protein content of yolk and albumen. Higher levels of dietary protein elicit higher protein concentrations in yolk and albumen (Andersson, 1979).

Increasing dietary protein from 15 to 19%, increased yield of albumen and decreased yield of yolk thereby increasing the albumen: yolk ratio (Akbar et al., 1983). As hens age, the nutrient (protein and amino acids) requirement decreases (Sell et al., 1987 and Wu et al., 2005). If the nutrient contents of diets fed to old hens are the same as that of diets fed to young hens, some of nutrients may be wasted and cost of production may increase. Therefore, it is important for commercial leghorn industry to know the lysine requirement, variation in strain performance, and if there are any interaction between strain and lysine on performance.

Hens are normally fed lysine ranging from 0.956 to 0.828% during phase one. Current high cost of protein (lysine), emphasize the need to know the lysine requirement for optimal performance and profits. The amino acid composition of poultry diets influences the efficiency of protein utilization. Lysine is the second limiting amino acid in corn-soy diets, next to Methionine. The ideal protein theory is often used in the poultry diet formulation. This concept assumes that all amino acids are balance and are equally limiting (Harms et al., 1993; Viera et al., 2004). Even though the absolute requirement of amino acids might change in practical situation, the ratios between amino acids must remain constant. Therefore, lysine is often used as the reference amino acid and the other essential amino acids are calculated by using the respective ratio to lysine (Viera et al., 2004).

Lysine requirement of hens varied from 650 to 900mg/hen per day (Jensen et al.,1974; Latshaw, 1976; Nathanael and Sell, 1980; NRC, 1994; and Shutte and Swink, 1998). Some studies (Bateman et al., 2000; Yadalam et al., 2000; Yadalam, 2001) reported that the optimal Methionine+Cystine/Lysine (Met + Cys/Lys) ratio for laying hens was 0.75. If the protein level of diets changes, the natural lysine content also varies. When the protein content of a typical corn-soy diet decrease from 19 to 14%, lysine content decreases from 1.02 to 0.69% while methionine level decreases from 0.29 to 0.24%.

Technological advances in genetics, managements, animal health and hen behavior have allowed laying hens to have better feed efficiency, larger egg size, and longer persistence of peak production. Thus, it is necessary to conduct research in nutrient requirement determination and in optimization of the use of nutrients so that

laying hens can have maximum genetic potential expression. However, very few studies have been conducted to determine the lysine requirement across brown egg layer strains.

Brown-egg-laying hens predominate in many parts of the world, and their use is rapidly growing in the USA and other South American countries as well. Different brown strains have different production characteristics, egg components, egg solids, and egg quality (Wu et al., 2008). Some strains may be beneficial for further processing such as dried and liquid egg production where as some strains may be more beneficial for table egg production. Several commercial Brown-egg-layer strains are currently used by egg producers. Egg quality has become an important aspect of egg marketing as retail outlets are now demanding high standards for conventional internal and external quality characteristics.

Throughout the world, preference for shell color in table eggs differs and is based mainly on the visual appearance of the egg. Although shell color has little to do with the nutritional value of a table egg, uniformity of color of brown eggs, together with a certain minimum depth of color are important considerations for consumers. Pale–shelled eggs are often deemed unacceptable causing some highly productive strains to be rejected. Egg shell color between strains has received very limited or no research attention (Odabasi et al., 2007). Therefore, two experiments (section I and II) were conducted to determine the effect of increasing dietary lysine on performance, egg composition, egg solids, egg quality, in seven commercial brown egg layer strains, and to determine the nutrient (lysine) requirements that allow for the best performance in phase one and two.

Enzymes have changed the way nutritionists select ingredients for a nutritionally balanced, least-cost diet. Using feed enzymes can also alleviate the problem of

environmental pollution and control certain diseases. Enzymes will play an indispensable role in 21st-century animal production. In poultry operations, feed cost has always been a major issue. Enzyme supplementation as a feed additive has become common during the last five decades (Jensen et al., 1957). Enzymes are proteins, having unique abilities to catalyze biochemical reactions. Their usage is popular because of positive effects on hen performance and lack of harmful effects on consumers. Utilization of most grains is influenced by the presence of indigestible complex carbohydrates, such as non-starch polysaccharides (NSP). Enzymes are supplemented to the feed to improve nutritive value in those grains. Legume seeds also contain NSP-like hemicelluloses, mannan and raffinose (Irish et al., 1995 and Veldman et al., 1994). Chickens do not produce some enzymes like galactosidases, thus corn-soybean based diets without supplemented enzymes like xylanases and pectinases might result in gas accumulation in the gut and diarrhea (Wu et al., 2005 and Jaroni et al., 1999). It is reported a mixture of enzymes is more effective than a single enzyme (Wu et al., 2005).

Although the economic and social benefits of enzymes have been well established, more research and development are needed if enzymes are to reach their full potential in the industry. Specifically, more research is needed to explore the mechanisms by which enzymes produce their beneficial effects to identify the sites in the gastrointestinal tract where enzymes are most effective; and to determine the types and amounts of enzymes required for different ages of laying hens and for a wide spectrum of feedstuffs. In addition, we need to develop reliable ways to assess enzyme potency before and after addition to diets, model systems to accurately predict how laying hens will respond to the use of enzymes and analytical programs to assess the economic and social

benefits of enzyme treatments. Many exciting developments can be expected in the future, particularly with the use of recombinant enzymes for laying hens and animal feedstuffs. Enzymes not only will enable poultry producers to economically use new feedstuffs, but will also prove to be environmentally friendly, as they reduce the pollution associated with laying hen industry. Dry-matter digestibility (DMD) in laying hens ranges from 50 to 80%; the remainder of the dry matter (DM) is lost via the excreta. In the poultry industry, this represents huge amount of N manure.

In densely populated parts of the world, such as Asia and Europe, excretion of large amounts of organic matter, especially organic matter containing high levels of nitrogen and phosphorus, presents serious environmental problems. In recent years, enzymes have been widely used in laying hen diets to increase nutrient digestibilities and to decrease nutrient waste in the excreta. The effect of enzyme supplementation on DMD in laying hens depends on the type of diet. Wet excreta is a big problem in the poultry industry, especially in the case of laying hens, where increased percentages of dirty eggs are associated with wet droppings. In many countries, dirty eggs are unsuitable for sale, as a result they are sold as second-grade eggs and therefore represent a substantial loss for the industry. Wet droppings may increase the production of gases (that is, ammonia and hydrogen sulfide) and fly and rodent populations in poultry houses. These can affect the well-being of the animals by increasing stress and lowering air quality, and they can affect the health of the staff who work in the poultry houses (Donham 1995).

A reduction in the moisture content of poultry excreta is often noted when glycanases are included in the diet. The significance of gut microflora to the nutrition of chickens is not well documented. Excessive fermentation in the small intestine may

interfere with the normal physiological process of nutrient digestion. As often noted, adding antibiotics to poultry diets that have highly soluble NSPs markedly improved bird performance (Misir and Marquardt 1978). Elevated levels of intact soluble NSPs detrimentally increased the activity of fermentative microorganisms in the small intestine (Choct et al. 1996). Xylanase supplementation largely eliminated fermentation in the small intestine and improved the performance of the birds. Morgan and Bedford (1995) reported that coccidiosis problems could be prevented by using enzymes. Birds fed a wheat-based diet with and without glycanase supplementation showed vastly different responses to coccidiosis challenge. Growth was depressed by 52.5% in the control group but by only 30.5% in the enzyme group, which also had a much better lesion score. An increase in digesta passage rate and a reduction in excreta moisture are often noted when glycanases are added to laying hen diets, which may be detrimental to the life cycle of the organism.

In a recent trial, enzyme supplementation increased the AME of wheat from 13.7 MJ/kg DM to 14.5 MJ/kg DM and reduced between-bird variation by 74% (M. Choct et al., unpublished). Significant reductions in the coefficient of variation in performance of birds fed barley diets have also been reported (Classen et al. 1988). The practical significance of this is that it allows increased precision in least-cost feed formulation, resulting in more uniform poultry performance. Enzymes also allow a wide range of ingredients to be used in a diet for a desired outcome. This gives the producer a great deal of flexibility in formulating a nutritionally balanced, least-cost diet. An experiment (section IV) was conducted to evaluate the effect of dietary energy, protein and a versatile enzyme on hen performance, egg solids, egg composition and egg quality of Hy-

Line W- 36 hens during second cycle phase two. It was thought that all dietary proteins needed to be hydrolyzed to free amino acids in order to be absorbed. This theory changed when the first intestinal oligo-peptide transporter, PepT1, was identified by two separate groups in rats and rabbits (Boll et al., 1994; Fei et al., 1994). The peptide transporter was then identified and characterized in domestic animals like pigs (Klang et al., 2005), chickens (Chen et al., 1999), turkey (Van et al., 2005), and ruminant animals (Chen et al., 1999). The PepT1 protein is located at the brush border membrane of intestinal epithelial cells (Leibach and Ganapathy, 1996), and has been shown to have rather broad substrate specificity, compared to the relatively narrow substrate specificity of most free amino acids transporters.

Peptide absorption from the lumen is faster and more efficient than amino acids transportation (Johnson, 1997a). Hypothetically, it is therefore possible that incorporation of small peptides or hydrolyzed protein into the diet would be beneficial for layers. However, very limited information is available about the peptides as protein sources in laying hen diets because of the lack of manufacturing and the difficulty of peptide detection methods. There are few commercial peptide products available for use in the laying hen diets. Peptiva® is produced by Vitech BioChem Corporation (San Fernando, CA), by blending appropriate amount of porcine mucosa peptides, fish peptides, and microbial peptides. It is claimed to contain feed stimulating peptides, small intestine activity peptides, exorphine peptides, immune modulating peptides and anti-microbial peptides (www.vitechusa.com). Even though results of previous studies and company field trials showed some benefit of the addition of peptide products in laying hen diets, more research is needed before it is used commercially. Therefore, an experiment

(section VI) was conducted to evaluate the effect of peptide in corn-soy diets on hen performance, egg solids, egg composition and egg quality of Hy-line W- 36 hens during second cycle phase three.

I I. PERFORMANCE COMPARISON AND LYSINE REQUIREMENTS OF SEVEN COMMERCIAL BROWN EGG LAYER STRAINS DURING PHASE ONE

ABSTRACT

This study was a 3 × 7 factorial arrangement of 3 lysine levels (0.917, 0.828, and 0.747) and seven commercial brown egg layer strains. The objective of this experiment was to determine the effect of increasing dietary lysine on performance, egg composition, egg solids, egg quality and profits in seven commercial brown egg layer strains and to determine the lysine requirement during phase one (from 21 to 36 wk of age). This experiment lasted 16 weeks. Seven strains of hens (n = 240 of each strain) at 21 week of age were randomly divided into 21 treatments (8 replicates of 10 birds/ treatment). The results showed that there were no interactions between lysine and strain on any parameter. Lysine had significant effects on egg weight, egg mass, feed conversion, percent albumen solids, yolk color, shell color, albumen weight, egg shell and albumen components. There were significant strain effects on egg production, feed consumption, egg weight, egg mass, feed conversion, specific gravity, yolk weight, shell color, shell, albumen and yolk components, yolk, albumen and whole egg solids. Strain 1 had the best overall performance. All strains peaked in production over 94% and were laying 94 to 96% at 36 weeks of age. Average egg weight (21wk to 36wk) was 60.3g, varying from 59.0 to 62.8 g between strains. Average feed intake was 112.3g/hen/day varying from

109.6 to 116.7g/hen/day between strains. Average egg weight of hens fed diets containing the highest lysine level was 2.04 g heavier than the hens fed the diets containing the lowest lysine level. Increasing dietary lysine from 0.747 to 0.917% significantly improved feed conversion from 2.20 to 2.06 g feed/g egg and increased egg mass from 51.8 to 54.32 g/hen/day. Average lysine intake of hens fed 0.917% level was 1023mg/hen/day varying from 1005 to 1070mg/hen/day between strains. Because egg prices and ingredient prices often change, there can be no fixed dietary lysine level for optimal profits.

Key words: Brown layer Strain, Lysine requirement, Shell color

INTRODUCTION

Brown-egg-laying hens predominate in many parts of the world, and their use is rapidly growing in the USA and other South American countries as well. Different brown strains have different production characteristics, egg components, egg solids, and egg quality (Wu et al., 2008). Some strains may be beneficial for further procession such as dried and liquid egg production where as some strains may be beneficial for table egg production. Several commercial Brown-egg-layer strains are currently used by egg producers.

Protein (lysine) is the major nutrient representing a high percentage of total cost of the diets. Liu et al. (2005) and Wu et al (2007a) reported that increasing Protein (lysine) level significantly improved egg production, egg weight; egg mass, feed consumption, feed conversion, egg specific gravity and body weight of hens. As hens age, the nutrient requirement decreases (Sell et al., 1987 and Wu et al., 2005). If the nutrient contents of diets fed to old hens are the same as that of diets fed to young hens, some of nutrients may be wasted and cost of production may increase. It is important for commercial leghorn industry to know the lysine requirement, variation in strain performance, and if there are any interaction between strain and lysine on performance. Hens are normally fed lysine ranging from 0.828 to 0.956% during phase one. Current high cost of protein (lysine), emphasize the need to know the lysine requirement for optimal performance and profits. However very few studies have been conducted to determine the lysine requirement across brown egg layer strains during phase one.

Egg quality has become an important aspect of egg marketing as retail outlets are now demanding high standards for conventional internal and external quality characteristics. Throughout the world, preference for shell color in table eggs differs and is based mainly on the visual appearance of the egg. Although shell color has little to do with the nutritional value of a table egg, uniformity of color of brown eggs, together with a certain minimum depth of color are important considerations for consumers. Pale–shelled eggs are often deemed unacceptable causing some highly productive strains to be rejected. Egg shell color between strains has received very limited or no research attention (Odabasi et al., 2007).

Even though brown egg laying hens are not used for liquid and breaker egg industry, there may be a potential of using them in the future. Increasing amino acids such as methionine and lysine significantly increase percent albumen in Hy-line W-36 hens during phase 1 (Shafer et al., 1998 and Novak et al., 2004). It might be beneficial for the egg processing industry to know how to manipulate dietary lysine to improve liquid egg and dried egg production. There are very few if any studies available on the effect of dietary lysine on egg composition and egg solids of brown egg layers.

Technological advances in genetics, management, animal health and behavior have allowed laying hens to have better feed efficiency, larger egg size and longer persistence of production. Thus it is necessary to conduct research in nutritional requirement determination and in optimization of the use of nutrients, so that laying hens can have the maximum genetic potential expression (William et al., 2005). Therefore the objective of this experiment was to determine the effect of increasing dietary lysine on

performance, egg composition, egg solids, egg quality, in seven commercial brown egg layer strains, and to determine the nutrient (lysine) requirements that allow for the best performance in phase one (from 21 to 36 wk of age).

MATERIAL AND METHODS

This study was a 3 × 7 factorial arrangement with three dietary lysine levels (0.917, 0.828, and 0.747) and seven commercial brown egg layer strains. The seven brown commercial or experimental egg laying strains (obtained from Centurion poultry Inc.[1]) were identified as strain 1 to 7. Strain 5 was the Bovans Brown classic. Ingredients and nutrient composition of experimental diets are shown in table 1. Feed and feed ingredient samples were analyzed for amino acids[2]. Dietary energy (2840 ME kcal/kg) was maintained the same in all diets. Energy and lysine levels of experimental diets were determined to meet the minimum nutrients requirements specified by (NRC, 1994). In this experiment, seven brown egg laying strains (total n = 1680) at 21 week of age were randomly assigned into 21 treatments (8 replicates of 10 hens per treatment). The trial lasted 16 weeks. Hens were housed two per cage in a 40.6 X 45.7cm cage. Each replicate consisted of five adjoining cages. Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. All hens were housed in an environmentally controlled house with temperature maintained as close to 26^{o}C as possible. Pullets were housed at 18 wk of age. Light was increased by 15 minutes per week from 12 hours per day to 16 hours per day. The house had controlled ventilation and lighting (16 h/d). All hens were supplied with feed and water *ad libitum*. Animal housing and handling procedures during experimentation were in accordance with guidelines of Auburn University's Institutional Animal Care and Use Committee

(IACUC). Feed consumption was recorded weekly and calculated average daily feed consumption, egg production was recorded daily, and egg weight and specific gravity were recorded once every two weeks. Egg weight and egg specific gravity were measured using all eggs produced during 2 consecutive days. Feed consumption was determined by subtracting the ending feed weight of each trough (each replicate) from beginning feed weight weekly. Egg specific gravity was determined using 9 gradient saline solutions varying in specific gravity from 1.060 to 1.100 in 0.005 unit increments (Holder and Bradford, 1979). Mortality was determined daily, and feed consumption was adjusted accordingly. Body weight was obtained by weighing 3 hens per replicate at the end of the experiment. Egg mass (g of egg/hen per day) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption.

Egg components were measured using 3 randomly selected eggs from each treatment replicate at the middle and end of the experiment. Eggs were weighed and broken. The yolks were separated from the albumen. Before yolk weight was determined, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned of any adhering albumen and dried for 5 days. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

Three eggs from each treatment replicate were randomly collected at the middle and at the end of the experiment for measuring solids. The yolk and albumen were mixed and 5 to 6 g of homogenate was pipetted into an aluminum dish with weight recorded to 0.001g.The sample was dried in an oven for 24h at 40.5°C (AOAC, 1990) and then weighed. Three eggs which randomly selected from each treatment replicate were used to

analyze yolk and albumen solid. After yolk was separated from albumen, three yolks and albumen per replicate were mixed separately. The procedure for analyzing albumen and yolk solid was the same as the procedure for whole egg solid content. Yolk color and haugh units were measured (3 eggs from each treatment replicate) at the middle and at the end of the experiment using an egg multitester EMT-5200 (Robotmation,co, Ltd. Tokyo, Japan). Haugh units were calculated from the records of albumen height and egg weight using the formula: $HU=100 \log_{10} (H-1.7 W^{0.37}+7.56)$, where HU=Haugh unit, H=height of the albumen (mm) and W=egg weight (g). Shell color was measured (3 eggs of each treatment replicate) at the middle and at the end of the experiment using CHROMA METER CR-300 (Minolta co, Ltd. Osaka, Japan). The egg shell color reported as L^*, a^*, and b^*. The L^* value represents lightness and ranges from 0 to 100, with 0 corresponding to black and 100 to white. Redness-greenness and yellowness-blueness, were measured by a^* and b^*, respectively.

Data were analyzed by ANOVA using proc mixed of Statistical Analysis System (SAS Institute, 2000) for a randomized complete block with a factorial arrangement of treatments. Dietary lysine and strains were fixed, whereas blocks were random. The factorial treatment arrangement consisted of three dietary lysine levels and seven strains. The following model was used to analyze the data:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + P_k + \varepsilon_{ijk}$$

Where Y_{ijk} = individual observation, μ = overall mean, α_i = dietary lysine effect, β_j = strain effect, $(\alpha\beta)_{ij}$ = interaction between dietary lysine and strain, P_k = effect of block, ε_{ijk} = error component. If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. A significance level of $P\leq$

0.05 was used for analysis.

[1]Centurion poultry, Inc. Lexington, GA 30648.

[2] Degussa Corporation, Allendale, NJ 07401

RESULTS AND DISCUSSION

Feed consumption:

There was no significant interaction between strain and dietary lysine on feed consumption (Table 2). There was a significant strain effect on feed consumption. Feed consumption of Strain 5 was significantly higher than those of strain 1, 4, and 7 but was similar to strain 2, 3, and 6. There was no significant effect of dietary lysine on feed consumption. This result was in agreement with Wu et al., 2007b, who reported that there was no significant effect of dietary protein (lysine) on feed consumption with Hy-line W-36 hens.

Egg production:

There was a significant strain effect on egg production (Table 2). Strain 1 had the highest egg production whereas strain 2 had the lowest. Dietary lysine did not significantly affect egg production and there was no significant interaction between strain and dietary lysine on egg production. This result was in agreement with Bateman et al., 2002a, 2002b, and 2003 who reported that there was no significant interaction between strain and lysine on egg production with white leghorns. Wu et al 2007b also reported that there was no effect of dietary lysine on egg production.

Egg mass:

Significant strain and dietary lysine effects were observed on egg mass (Table 2). Strain 1 had the highest egg mass among seven strains. Increasing dietary lysine linearly increased egg mass from 51.79 to 54.32 g resulting in a 4.88% increase in egg mass. There was no significant interaction between strain and dietary lysine on egg mass.

Egg weight:

Both dietary lysine and strain had a significant effect on egg weight (Table 2). Strain 4 had the lowest egg weight. Dietary lysine linearly increased egg weight by 3.44% from 59.37 to 61.41g. There was no significant interaction between strain and dietary lysine on egg weight. This result was consistent with the previous reports that there was no significant interaction between strain and lysine on egg weight with white leghorns (Bateman et al., 2002a, 2002b, 2003).

Feed conversion:

Dietary lysine and strain significantly effected feed conversion. However there was no interaction between strain and lysine on feed conversion (Table 2). Strain 1 had the best feed conversion whereas strain 2 had the worst. Hens fed high lysine diets had better feed conversion than hens fed low lysine levels. This result was in agreement with that of Liu et al (2005) and Wu et al. (2007b) who reported increasing dietary lysine improved feed conversion with Hy-line W-36 hens.

Egg specific gravity, final body weight and mortality:

There was a significant effect of strain on egg specific gravity. However dietary lysine or interaction between lysine and strain had no significant effect on egg specific gravity (Table 4). Egg specific gravity of strain 1 was significantly greater than that of the other six strains. There was no significant effect of dietary lysine, strain or interaction between strain and lysine on body weight and mortality.

Increasing dietary lysine significantly increased percent albumen, percent albumen solids and albumen weights (Table 3). However, increasing dietary lysine decreased percent shell. There was no significant interaction between lysine and strain on

percent components, percent solids or albumen, yolk and shell weight (Table 3). Strain had a significant effect on percent egg components, percent egg solids and yolk weight (Table 3). Strain 5 and strain 1, and 7 had the highest and lowest percent yolk respectively. Strain 1, 2, 6, and 7 had highest percent albumen and strain 1, had the highest percent shell. Strain 4 had the highest yolk and whole egg solids whereas strain 1, 2, 4, and 5 had the highest albumen solids.

Both dietary lysine and strain had a significant effect on shell color (Table 4). Strain 4 and 5 had the darkest and lightest shell color respectively (Table 4). Increasing dietary lysine significantly decreased yolk color due to a reduction in corn use, as the level of protein increased. This result was in agreement with that of Karunajeewa. (1972), who reported that increasing dietary lysine from 0.747 to 0.825%, significantly decreased yolk color from 6.58 to 6.45 with white leghorn hens.

Strain 1 had the best overall performance (Table 2). Compared to brown egg laying hens of other six strains, Strain 1 hens had the best efficiency in utilizing nutrients to produce one gram egg (Table 5). The best performance of all seven strains was obtained with hens fed diets containing 0.0917% lysine. Strain 5 (Bovans brown classic) hens consumed 20.0 g protein, 1063 mg lysine, 798 mg TSAA, and 329 kcal ME per hen daily or 0.36 g protein, 19.32 mg lysine, 14.51 mg TSAA, and 5.98 kcal ME per g egg for the best performance (Table 5). Strain 5 required 15% more protein, 17% more TSAA, and 33% more lysine than the values recommended by NRC (1994).

Hy-line brown and Bovans brown strains are the most popular brown egg laying strains in the world. Comparing the nutrient requirements of Hy-line brown with the nutrient requirements of strain 5 (Bovans brown classic), the Bovans brown classic

(strain 5) required 13.9% more protein, 8.7% more TSAA, and 17.6% more lysine than the values recommended by Hy-line brown management guide (2006-2008) and 2.6% more protein, 10.1% more TSAA, and 18.4% more lysine than the values recommended by Bovans brown management guide (2008) respectively.

The Econometric Feeding and Management Program developed by Roland et al., (1998, 2000) was used to calculate profits at different dietary lysine levels and egg prices (Table 8). Current feed prices were used. Price spreads, between medium and large eggs were 10, 15, 30, and 37 cents/dozen. Maximum profits per dozen of eggs were obtained in Bovans brown classic hens (Strain 5) fed the lowest lysine diet (0.747%) at small (10 and 15 cents/dozen) price spreads (0.200 and 0.140 $/dozen) whereas, highest profits were obtained by feeding the high lysine diet (0.917%) at larger (30 and 37 cents/dozen) price spreads (0.212 and 0.449 $/dozen). Since feed prices and egg price vary, there can be no fixed dietary lysine level for optimal profits during phase 1 (21 wk to 36 wk of age).

In this study, nutrient requirement of Bovans brown classic hens' (strain 5) for optimal performance was obtained with the highest dietary lysine level (0.917). However, nutrient requirement for optimal profits vary according to the current ingredient and egg prices. According to the profit analysis (Table 8) highest profits with larger price spreads were obtained with the highest dietary lysine diet (0.917%) whereas with smaller price spread, highest profits were obtained with the lowest lysine diet (0.747%). In addition to the lysine requirement for optimal profits varying with ingredients and egg prices, the lysine requirement for optimal performance and profits also varies with energy intake (Table 5, 6 and 7); this complicates efforts to determine requirements for optimal

performance and profits. In conclusion, the results showed that there were no interactions between lysine and strain on any parameter. Lysine had significant effects on egg weight, egg mass, feed conversion, percent albumen solids, yolk color, shell color, albumen weight, egg shell and albumen components. There were significant strain effects on egg production, feed consumption, egg weight, egg mass, feed conversion, specific gravity, yolk weight, shell color, shell, albumen and yolk components, yolk albumen and whole egg solids. All strains peaked in production over 94% and were laying 94 to 96% at 36 weeks of age. Average egg weight (21wk to 36wk) was 60.3g, varying from 59.0 to 62.8g between strains. Average feed intake was 112.3g/hen/day varying from 109.6 to 116.7g/hen/day between strains. Average egg weight of hens fed diets containing the highest lysine level was 2.04g heavier than the hens fed the diets containing the lowest lysine level. Increasing dietary lysine from 0.747 to 0.917% significantly improved feed conversion from 2.20 to 2.06 g feed/g egg and increased egg mass from 51.8 to 54.32 g/hen/day. Average lysine intake of hens fed 0.917% level was 1023mg/hen/day varying from 1005 to 1070mg/hen/day between strains. Because egg and ingredient prices and energy intake often change, there can be no fixed dietary lysine level for optimal profits.

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Table 1 Ingredients and nutrient content of the experimental diets

Ingredient (%)	Diet 1	Diet 2	Diet3
Corn	68.39	64.80	60.82
Soy bean meal	19.42	22.40	25.69
Hard shell[1]	4.0	4.0	4.0
Limestone	5.0	5.0	5.0
Dicalcium phosphate	1.73	1.73	1.74
Poultry oil	0.51	1.12	1.79
NaCl	0.39	0.39	0.39
Vitamin Premix[2]	0.25	0.25	0.25
Mineral Premix[3]	0.25	0.25	0.25
DL-Methionine	0.034	0.07	0.10
Total	100	100	100
Calculated Analysis			
ME (Kcal/kg)	2840	2840	2840
Crude protein	14.81	15.97	17.26
Ca	4.0	4.0	4.0
Available phosphorus	0.42	0.42	0.42
Na	0.18	0.18	0.18
Methionine + Cystine	0.560	0.621	0.688
Lysine	0.747	0.828	0.917

1 Hard shell = large particle limestone (passing US mesh #4 and retained by US mesh #6) $CaCo_3$ supplied by Franklin Industrial Minerals, Lowell ,FL.

[2] Provided per kilogram of diet: Vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200ICU, vitamin E (as DL – α - tocopheryl acetate), 8 IU; vitamin B12,
0.02mg; riboflavin, 5.5mg; D-calcium pentothenic acid, 13mg; niacin, 36mg; choline, 500mg; folic acid, 0.5mg; vitamin B1(thiamin mononitrate), 1mg; pyridoxine, 2.2mg; biotin, 0.05mg; vitamin K (menadione sodium bisulfate complex), 2mg.

[3] Provided per kilogram of diet: manganese, 65mg; iodine,1mg; iron, 55mg; copper, 6mg; zinc, 55mg; selenium, 0.3mg.

Table 2. Effect of lysine on feed intake, egg production, egg mass, feed conversion and egg weight of seven brown egg layer strains during first cycle phase 1.

Lysine (%)	Strain	Feed intake (g/hen day)	Egg Production (%)	Egg mass (g of egg/h per day)	Feed conversion (g feed /g egg)	Egg Weight (g)
0.747		113.61	87.21	51.79 [b]	2.20 [a]	59.37 [c]
0.828		111.64	87.45	52.47 [b]	2.13 [b]	60.09 [b]
0.917		111.58	88.45	54.32 [c]	2.06 [c]	61.41 [a]
	Strain 1	109.63 [b]	90.59 [a]	54.81 [a]	2.00 [c]	60.49 [a]
	Strain 2	111.93 [ab]	82.50 [c]	49.46 [c]	2.27 [a]	60.20 [a]
	Strain 3	112.52 [ab]	88.12 [ab]	53.68 [ab]	2.10 [b]	60.87 [a]
	Strain 4	111.09 [b]	88.43 [ab]	52.14 [b]	2.13 [b]	58.97 [b]
	Strain 5	116.65 [a]	89.16 [ab]	53.84 [ab]	2.17 [b]	60.38 [a]
	Strain 6	114.01 [ab]	89.26 [ab]	53.76 [ab]	2.12 [b]	60.22 [a]
	Strain 7	110.18 [b]	86.05 [ab]	52.02 [b]	2.12 [b]	60.44 [a]
0.747 × Strain	Strain 1	111.83	89.54	53.22	2.10	59.43
	Strain 2	112.70	81.66	48.10	2.35	58.87
	Strain 3	111.90	84.85	50.72	2.22	59.73
	Strain 4	113.44	88.30	51.92	2.19	58.78
	Strain 5	117.56	89.32	53.17	2.21	59.52
	Strain 6	116.05	90.50	54.33	2.14	60.04
	Strain 7	111.81	86.26	51.08	2.19	59.22
0.828 × Strain	Strain 1	109.22	91.71	55.95	1.95	61.01
	Strain 2	111.95	82.37	49.13	2.29	60.44
	Strain 3	112.13	89.62	54.67	2.05	60.97
	Strain 4	111.61	89.97	52.52	2.13	58.37
	Strain 5	116.46	88.68	53.34	2.19	60.12
	Strain 6	111.92	87.04	51.74	2.16	59.45
	Strain 7	108.20	82.80	49.92	2.18	60.28
0.917 × Strain	Strain 1	107.85	90.53	55.25	1.95	61.03
	Strain 2	111.14	83.46	51.16	2.17	61.29
	Strain 3	113.52	89.89	55.64	2.04	61.91
	Strain 4	108.20	87.01	51.98	2.08	59.75
	Strain 5	115.92	89.46	55.01	2.11	61.49
	Strain 6	114.06	90.23	55.21	2.06	61.16
	Strain 7	110.52	89.08	55.06	2.01	61.82
Pooled SEM		1.85	1.73	1.2	0.032	0.48
				Probability		
Lysine		NS	NS	0.0008	< 0.0001	< 0.0001
Strain		0.045	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Lysine×Strain		NS	NS	NS	NA	NA

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($P \leq 0.05$).

Table 3. Effect of lysine on egg components, egg solids, and albumen, yolk and shell weights of seven brown egg layer strains during first cycle phase 1 (21wk to 36 wk of age)

Lysine (%)	Strain	% Egg components			% of solids			Albumen, Yolk, Shell wt. (g)		
		Yolk	Albumen	Shell	Whole egg	Albumen	Yolk	Albumen	Yolk	Shell
0.747		24.87	65.55 [b]	9.59 [a]	24.16	12.94 [b]	54.37	39.15 [c]	14.84	5.71
0.828		24.40	65.97 [ab]	9.40 [ab]	24.17	13.24 [a]	54.29	40.57 [b]	14.97	5.77
0.917		24.32	66.46 [a]	9.32 [b]	23.96	13.21 [a]	54.11	41.57 [a]	15.22	5.83
	Strain 1	23.93 [c]	66.18 [a]	9.79 [a]	24.08 [bcd]	13.23 [a]	54.37 [ab]	40.22	14.52 [b]	5.94
	Strain 2	24.24 [bc]	66.49 [a]	9.29 [b]	24.22 [abc]	13.31 [a]	54.49 [ab]	40.06	14.57 [b]	5.59
	Strain 3	24.30 [bc]	66.17 [ab]	9.46 [ab]	23.91 [cdc]	13.02 [ab]	54.05 [b]	40.82	14.99 [ab]	5.83
	Strain 4	25.18 [ab]	65.45 [ab]	9.24 [b]	24.55 [a]	13.31 [a]	54.86 [a]	40.66	15.62 [a]	5.73
	Strain 5	25.46 [a]	65.00 [b]	9.53 [ab]	24.37 [ab]	13.20 [a]	54.00 [b]	40.12	15.71 [a]	5.87
	Strain 6	24.72 [abc]	66.44 [a]	9.30 [b]	24.03 [bcd]	12.98 [ab]	53.89 [b]	40.69	15.14 [ab]	5.69
	Strain 7	23.81 [c]	66.38 [a]	9.40 [ab]	23.58 [e]	13.02 [ab]	54.28 [ab]	40.46	14.47 [b]	5.72
Pooled SEM		0.44	0.55	0.18	0.23	0.16	0.34	0.83	0.32	0.13
	Probability									
Lysine		0.08	0.02	0.04	NS	0.001	NS	< 0.0001	NS	NS
Strain		0.0006	0.05	0.05	< 0.0001	0.02	0.04	NS	< .0001	NS
Lys×Stra		NS	NS	NS	NS	NS	NS	NS	NS	NS

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($P \leq 0.05$).

Table 4 Effect of lysine on specific gravity, body weight, mortality, shell color [lightness (L*), redness (a*), yellowness (b*)] [1] and egg quality of seven brown egg layer strains during first cycle phase 1 (21wk to 36 wk of age).

					Egg shell color			Egg quality	
Lysine (%)	Strain	Egg Specific Gravity (Unit)	Body Weight (Kg)	Mortality (%)	L*	a*	b*	Haugh Unit	Yolk color
0.747		1.0906	2.05	0.11	60.55[a]	17.91[b]	30.34[b]	78.37	5.45[a]
0.828		1.0907	2.00	0.10	59.96[b]	18.35[a]	30.63[a]	78.15	5.26[b]
0.917		1.0906	2.03	0.07	60.28[a b]	18.15[a b]	30.59[a]	79.45	5.17[b]
	Strain 1	1.0919[a]	1.92	0.08	59.61[cd]	18.57[bc]	30.40[b]	77.69	5.19
	Strain 2	1.0900[b]	2.06	0.16	58.86[d]	19.03[b]	31.13[a]	81.21	5.41
	Strain 3	1.0905[b]	2.03	0.03	61.02[b]	17.74[d]	30.49[b]	80.69	5.30
	Strain 4	1.0900[b]	1.96	0.18	58.04[e]	19.58[a]	31.26[a]	80.41	5.33
	Strain 5	1.0906[b]	2.09	0.08	62.10[a]	16.98[e]	30.17[bc]	77.22	5.31
	Strain 6	1.0905[b]	2.09	0.05	60.78[b]	17.65[d]	30.30[b]	76.37	5.25
	Strain 7	1.0909[b]	2.02	0.08	61.53[ab]	17.28[de]	29.89[c]	77.75	5.23
Pooled SEM		0.00051	0.07	0.084	0.85	0.53	0.37	2.47	0.13
		Probability							
Lysine		NS	NS	NS	0.04	0.007	0.005	NS	0.0002
Strain		0.0003	NS	NS	< 0.001	< 0.001	< 0.001	NS	NS
Lysine×Strain		NA	NS	NS	NS	NS	NS	NS	NS

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($p \leq 0.05$).

[1] A higher L* value means lighter color; a higher a* value means a redder color; a higher b* value means a more yellow color.

Table 5: Nutrient requirement of seven brown egg layer strains fed diets containing 0.917% lysine during first cycle phase 1 (21wk to 36 wk of age).

	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7
Nutrients required per hen daily							
Protein (g)	18.6	19.2	19.6	18.7	20.0	19.7	19.1
Lysine (mg)	989	1019	1041	992	1063	1046	1013
TSAA (mg)	742	765	781	744	798	785	760
Dietary energy (kcal)	306	316	322	307	329	324	314
Nutrients required to produce one gram egg							
Protein (g)	0.34	0.38	0.35	0.36	0.36	0.36	0.35
Lysine (mg)	17.90	19.92	18.71	19.08	19.32	18.95	18.40
TSAA (mg)	13.43	14.95	14.04	14.31	14.51	14.22	13.80
Dietary energy (kcal)	5.54	6.18	5.79	5.91	5.98	5.87	5.70

Table 6: Nutrient requirement of seven brown egg layer strains fed diets containing 0.828% lysine during first cycle phase 1 (21wk to 36 wk of age).

	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7
Nutrients required per hen daily							
Protein (g)	17.4	17.9	17.9	17.8	18.6	17.9	17.3
Lysine (mg)	904	927	928	924	964	927	896
TSAA (mg)	678	695	696	693	723	695	672
Dietary energy (kcal)	310	318	318	317	331	318	307
Nutrients required to produce one gram egg							
Protein (g)	0.31	0.36	0.33	0.34	0.35	0.35	0.35
Lysine (mg)	16.16	18.87	16.98	17.60	18.08	17.91	17.95
TSAA (mg)	12.12	14.15	12.74	13.20	13.56	13.43	13.46
Dietary energy (kcal)	5.54	6.47	5.82	6.04	6.20	6.14	6.16

Table 7: Nutrient requirement of seven brown egg layer strains fed diets containing 0.747% lysine during first cycle phase 1 (21wk to 36 wk of age).

	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7
Nutrients required per hen daily							
Protein (g)	16.6	16.7	16.6	16.8	17.4	17.2	16.6
Lysine (mg)	836	842	836	847	878	867	835
TSAA (mg)	627	631	627	635	658	650	626
Dietary energy (kcal)	318	320	318	322	334	330	318
Nutrients required to produce one gram egg							
Protein (g)	0.31	0.35	0.33	0.32	0.33	0.32	0.32
Lysine (mg)	15.71	17.50	16.48	16.32	16.52	15.96	16.35
TSAA (mg)	11.78	13.12	12.35	12.24	12.38	11.96	12.26
Dietary energy (kcal)	5.97	6.65	6.27	6.21	6.28	6.07	6.22

Table 8. Influence of dietary lysine on profits [1] of Bovans brown classic (Strain 5) hens during first cycle phase 1 (21wk to 36 wk of age)

Diet (Lysine %)	Price spread			
	10 cents[2]	15 cents[3]	30 cents[4]	37 cents[5]
	----------Returns [5] ($/dozen)----------			
0.917	0.168	0.139	0.212	0.449
0.828	0.164	0.137	0.200	0.437
0.747	0.200	0.140	0.199	0.435

[1] Based on a feed price of $106.44 per to for the 0.917 lysine diet, $ 102.65 per ton for the 0.828 lysine diet, and $99.28 per ton for the 0.747 lysine diet.

[2] Based on an Urner Barry egg price of 31 cents/doz. for check and peewee, 40 cents/doz. for small, 57 cents/doz. for medium, 67 cents/doz. for large, 68 cents/doz. for extra large and 69 cents/doz. for jumbo eggs.

[3] Based on an Urner Barry egg price of 31 cents/doz. for check and peewee, 40 cents/doz. for small, 65 cents/doz. for medium, 80 cents/doz. for large, 81 cents/doz. for extra large and 84 cents/doz. for jumbo eggs.

[4] Based on an Urner Barry egg price of 31 cents/doz. for check and peewee, 40 cents/doz. for small, 57 cents/doz. for medium, 87 cents/doz. for large, 91 cents/doz. for extra large and 110 cents/doz. for jumbo eggs.

[2] Based on an Urner Barry egg price of 31 cents/doz. for check and peewee, 40 cents/doz. for small, 75 cents/doz. for medium, 112 cents/doz. for large, 117 cents/doz. for extra large and 120 cents/doz. for jumbo eggs.

[5] Returns (R) were calculated using the equation R = UBEP – NR – PC – FdC, where UBEP = Urner Berry Egg Price, NR = nest run into package product delivered, PC = production cost and FdC = feed cost as described by Roland et al.(1998, 2000).

III . PERFORMANCE COMPARISON AND LYSINE REQUIREMENTS OF SEVEN COMMERCIAL BROWN EGG LAYER STRAINS DURING PHASE TWO

ABSTRACT

This study was a 3 × 7 factorial arrangement with 3 lysine levels (0.828, 0.747, and 0.680) and seven commercial brown egg layer strains. The objective of this experiment was to determine the effect of increasing dietary lysine on performance, egg composition, egg solids, egg quality and profits in seven commercial brown egg layer strains and to determine the lysine requirement during phase two (from 39 to 52 wk of age). This experiment lasted 14 weeks. Seven strains of hens (n = 240 of each strain) at 39 week of age were randomly divided into 21 treatments (8 replicates of 10 birds/ treatment). The results showed that there were no interactions between lysine and strain on any parameter. Lysine had significant effects on feed consumption, egg production, egg mass, feed conversion, egg weight, egg shell components, percent yolk and whole egg solids, albumen and yolk weight, egg specific gravity, yolk color, and haugh units. There were significant strain effects on feed consumption, egg mass, feed conversion, egg weight, albumen and yolk components, whole egg solids, albumen and shell weight, egg specific gravity, body weight, shell color, and haugh unit. Strain 1 had the best overall performance. All strains were laying 89.5 to 92.5% at 52 weeks of age. Average egg weight (39wk to 52wk) was 63g, varying from 61.5 to 63.6 g between strains.

Average feed intake was 112.1g/hen/day varying from 108 to 114 g/hen/day between strains. Average egg weight of hens fed diets containing the highest lysine level was 3.38g heavier than hens fed the diets containing the lowest lysine level. Increasing dietary lysine from 0.680 to 0.828% significantly improved feed conversion from 2.03 to 1.91 g feed/g egg and increased egg mass from 54.0 to 59.30 g/hen/day. Average lysine intake of hens fed 0.828% level was 939 mg/hen/day varying from 907 to 964 mg/hen/day between strains. Because egg and ingredient prices often change, there can be no fixed dietary lysine level for optimal profits.

Key words: Brown layer Strain, Lysine requirement, Shell color

INTRODUCTION

Brown-egg-laying hens predominate in many parts of the world, and their use is growing in the USA and South American countries as well. Different brown strains have different production characteristics, egg components, egg solids, and egg quality (Wu et al., 2005a). Some strains may be beneficial for further processing such as dried and liquid egg production where as some strains may be beneficial for table egg production. Several commercial Brown-egg-layer strains are currently used by egg producers. There is a surprising lack of information on comparative nutrition and their response to various feeding strategies.

Protein (lysine) is a nutrient for laying hens representing a significant percentage of total cost of the diets. Liu et al (2005), and Wu et al (2005b) reported that increasing protein (lysine) level significantly affected egg production, egg weight, egg mass, feed consumption, feed conversion, egg specific gravity and body weight of hens. The requirement for amino acids, especially lysine of brown egg laying hens varies as functions of the rate of growth determined by the genotype and age (Hurwitz et al., 1978). The various strains of hens, each with its own lysine requirement, are kept for extended periods during which the requirements may continuously change. Brown-egg strains produce considerably more egg mass and generally convert feed to egg mass more efficiently than white leghorns (Hurwitz et al., 1978). So it might be expected that their nutrient requirements are more exacting than white leghorns.

The amino acid content of diets affects the efficiency of protein utilization. Methionine is the first limiting amino acid in corn-soybean diets followed by lysine (Harms et al., 2000). The ideal protein concept is often used in the formulation of diets.

This concept assumes that all amino acids are in balance and are equally limiting (Barker, 2003; Vieira et al., 2004). Although the absolute requirements of amino acids may change in different practical situations, the ratio between amino acids should remain stable. Therefore, lysine is often used as the reference amino acid and the other essential amino acids such as TSAA are calculated by using the respective ratio to lysine. However no estimates of the lysine requirements of different brown-egg layer strains have been made. It is important for commercial layer industry to know the nutritional requirements of laying hens at different ages. Hens are normally fed high lysine diets ranging from 0.828 to 0.956% during phase two. Although feeding high lysine diets can optimize performance, the cost of high protein diets and egg prices determine the level needed for optimal profits (Wu et al., 2007). Therefore it is important to know the response of hens (egg weight, egg production and feed consumption) above and below the lysine requirement to be able to determine the requirement for optimal profits as feed and egg prices change. Few if any studies have been conducted to compare responses to lysine across current brown-egg layer strains to determine lysine requirement during phase two.

Egg quality has become an important aspect of egg marketing as retail outlets are now demanding very high standards for conventional internal and external quality characteristics. Throughout the world, preference for shell color in table eggs differs and is based mainly on the visual appearance of the egg. Although shell color has nothing to do with the internal quality of egg, many consumers throughout the world prefer brown eggs over white eggs (Odabasi et al., 2007). Uniformity of color of brown eggs, together with a certain minimum depth of color are important considerations for consumers. Pale–

shelled eggs are often deemed unacceptable causing some highly productive strains to be rejected. However, egg shell color between strains has received very limited or no research attention. Therefore the objective of this experiment was to determine the effect of increasing dietary lysine on performance, egg composition, egg solids, egg quality, in seven commercial brown egg layer strains and to determine the nutrients (lysine) requirements that allow for best performance in phase two (from 39 to 52 wk).

MATERIAL AND METHODS

This study was a 3 × 7 factorial arrangement with three dietary lysine levels (0.828, 0.747, and 0.680) and seven commercial brown egg layer strains. The seven brown commercial or experimental egg laying strains (obtained from Centurion poultry Inc.[1]) were identified as strains 1–7. Strain 5 was the Bovans Brown classic. Ingredients and nutrient composition of experimental diets are shown in Table 1. Feed and feed ingredient samples were analyzed for amino acids[2]. Dietary energy (2866 ME kcal/kg) was maintained the same in all diets. Diets were formulated according to the minimum nutrient requirements specified by (NRC, 1994). In this experiment, seven brown egg laying strain of hens (total n = 1680) at 39 week of age were randomly assigned into 21 treatments (8 replicates of 10 hens per treatment). The trial lasted 14 weeks. Hens were housed two per cage in a 40.6 X 45.7 cm cage. Each replicate consisted of five adjoining cages. Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. All hens were housed in an environmentally controlled house with temperature maintained as close to 26^{o}C as possible. The house had controlled ventilation and lighting (16L:8D). All hens were supplied with feed and water *ad libitum*. Animal housing and handling procedures during experimentation were in accordance with guidelines of Auburn University's Institutional Animal Care and Use Committee (IACUC). Feed consumption was recorded weekly and calculated average daily feed consumption, egg production was recorded daily, and egg weight and specific gravity were recorded once every two weeks. Egg weight and egg specific gravity were measured using all eggs produced during two consecutive days. Feed consumption was determined by subtracting the ending feed weight of each trough (each replicate) from beginning feed

weight weekly. Egg specific gravity was determined using 9 gradient saline solutions varying in specific gravity from 1.060 to 1.100 in 0.005 unit increments (Holder and Bradford, 1979). Mortality was determined daily, and feed consumption was adjusted accordingly. Body weight was obtained by weighing 3 hens per replicate at the end of the experiment. Egg mass (g of egg/hen per day) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption.

Egg components were measured using 3 randomly selected eggs from each treatment replicate at the middle and end of the experiment. Eggs were weighed and broken. The yolks were separated from the albumen. Before yolk weight was determined, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned of any adhering albumen and dried for 5 days. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

Three eggs from each treatment replicate were randomly collected at the middle and at the end of the experiment for measuring solids. The yolk and albumen were mixed and 5 to 6 g of homogenate was pipetted into an aluminum dish with weight recorded to 0.001g. The sample was dried in an oven for 24h at 40.5^{0}C (AOAC, 1990) and then weighed. Three eggs which randomly selected from each treatment replicate were used to analyze yolk and albumen solid. After yolk was separated from albumen, three yolks and albumen per replicate were mixed separately. The procedure for analyzing albumen and yolk solid was the same as the procedure for whole egg solid content. Yolk color and haugh units were measured (3 eggs from each treatment replicate) at the middle and at the end of the experiment using an egg multitester EMT-5200 (Robotmation,co, Ltd.

Tokyo, Japan). Haugh units were calculated from the records of albumen height and egg weight using the formula: $HU=100 \log_{10} (H-1.7 W^{0.37}+7.56)$, where HU=Haugh unit, H=height of the albumen and W=egg weight. Shell color was measured (3 eggs of each treatment replicate) at the middle and at the end of the experiment using CHROMA METER CR-300 (Minolta co, Ltd. Osaka, Japan). The egg shell color reported as L^*, a^*, and b^*. The L^* value represents lightness and ranges from 0 to 100, with 0 corresponding to black and 100 to white. Redness-greenness and yellowness-blueness, were measured by a^* and b^*, respectively.

Data were analyzed by ANOVA using proc mixed of Statistical Analysis System (SAS Institute, 2000) for a randomized complete block with a factorial arrangement of treatments. Dietary lysine and strains were fixed, whereas blocks were random. The factorial treatment arrangement consisted of three dietary lysine levels and seven strains. The following model was used to analyze the data:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + P_k + \varepsilon_{ijk}$$

Where Y_{ijk} = individual observation, μ = overall mean, α_i = dietary lysine effect, β_j = strain effect, $(\alpha\beta)_{ij}$ = interaction between dietary lysine and strain, P_k = effect of block, ε_{ijk} = error component. If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. A significance level of $P \leq 0.05$ was used for analysis.

[1]Centurion poultry Inc. Lexington, GA 30648

[2] Degussa Corporation, Allendale, NJ 07401

RESULTS AND DISCUSSION

Feed consumption:

Increasing dietary lysine significantly increased feed consumption (Table 2). As dietary lysine increased from 0.680 to 0.828%, feed consumption increased by 3.02%. Strain also had a significant effect on feed consumption. Feed consumption of strain 1 was significantly lower than the other six strains (Table 2). Highest feed consumption occurred in strain 2 and 6. There were no significant differences in feed consumption among strains 3, 4 and 7. Although lysine level and strain influenced feed consumption, there was no significant interaction between strain and dietary lysine on feed consumption.

Egg production

Increasing dietary lysine significantly increased egg production (Table 2). As dietary lysine increased from 0.680 to 0.828%, egg production linearly increased from 88.5 to 92.1, resulting in a 4.1% increase of egg weight (Table 2). However, neither strain nor interaction between dietary lysine and strain had a significant effect on egg production.

Egg mass:

Both strain and dietary lysine had a significant effect on egg mass (Table 2). However there was no significant interaction between strain and dietary lysine on egg mass. As dietary lysine increased, egg mass linearly increased by 9.85% from 53.9 to 59.3g. Strain 1 had the highest egg mass and strain 4 had the lowest. There were no significant differences in egg mass among strain 1, 2, 3, 5 and 7.

Egg weight:

Both dietary lysine and strain had a significant effect on egg weight (Table 2). However there was no significant interaction between strain and dietary lysine on egg weight. Increasing dietary lysine increased egg weight by 5.54% from 61.02 to 64.40g. Strain 4 had the lowest egg weight. There were no significant differences among other strains.

Feed conversion:

Dietary lysine and strain significantly affected feed conversion. However there was no interaction between strain and lysine on feed conversion (Table 2). Strain 1 had the best feed conversion whereas strain 5 had the worst. Feed conversion of strains 3 and 7 is lower than strains 4 and 6. Hens fed high lysine diets had better feed conversation than the other two levels.

Egg specific gravity, final body weight and mortality:

Strain and dietary lysine had a significant effect on specific gravity (Table 4). Increasing dietary lysine linearly decreased egg specific gravity. This reduction in specific gravity can be attributed to the increased egg size caused by increased dietary lysine consumption. Sohail et al (2003) also reported that increasing dietary lysine from 0.75% to 0.92%, significantly decreased specific gravity from 1.0848 to 1.0842 with Hy-line W-36 hens in phase 2. Strain 1 had the highest specific gravity whereas strain 2 had the lowest. There were no significant differences of specific gravity among strain 5, 6, and 7. A significant effect of strain on body weight was observed (Table 4). Strain 5 and 6 gained the highest body weight whereas strain 1 had the lowest. There was no significant effect of dietary lysine, strain or interaction between strain and lysine on mortality.

Strain had a significant effect on percent albumen and yolk (Table 3). Strain 4 had the highest percent yolk and strain 7 had the lowest. Strain 2 and 7 had the highest percent albumen and strain 4 and 6 had the lowest. Increasing dietary lysine significantly decreased percent shell and increased albumen and whole egg solids (Table 4). A significant strain effect was observed on whole egg solids; strain 4 had the highest whole egg solids whereas strain 3 and 7 had the lowest. As dietary lysine increased, albumen and yolk weights increased significantly.

Strain had a significant effect on shell color (Table 4). Strain 2 and 4 had the darkest egg shells whereas strain 5 had the lightest egg shells. As dietary lysine increased, both haugh unit and yolk color significantly decreased (Table 4). Increasing dietary lysine significantly decreased yolk color due to a reduction in corn use, as the level of protein increased. This result was in agreement with that of Karunajeewa.1972, who reported that increasing dietary lysine from 0.747 to 0.825%, significantly decreased yolk color from 6.58 to 6.45 with white leghorn hens.

Strain 1 had the best overall performance (Table 2). Compared to brown egg laying hens of other six strains, strain 1 hens had the best efficiency in utilizing nutrients to produce one gram egg (Table 5). The best performance of all seven strains was obtained with hens fed diets containing 0.828% lysine. Strain 5 (Bovans Brown classic) hens consumed 18.4 g protein, 954 mg lysine, 714 mg TSAA, and 330 kcal ME per hen daily or 0.31 g protein, 16.18 mg lysine, 12.11 mg TSAA, and 5.6 kcal ME per egg for the best performance (Table 5). Strain 5 required 6.5% more protein, 5.7% more TSAA, and 19.9% more lysine than the values recommended by NRC (1994).

The Econometric Feeding and Management Program developed by Roland et al., (1998, 2000) was used to calculate profits at different dietary lysine levels and egg prices (Table 8). Current feed prices and low, medium and high price spreads were used. Maximum profits per dozen of eggs were obtained in Bovans brown classic hens (Strain 5) fed the diets containing 0.828% lysine at high price spread, 0.747% lysine at medium price spread and 0.680% lysine at low price spread. Because feed and egg prices vary, there can be no fixed dietary lysine level for optimal profits during phase 1 (39 wk to 52 wk of age).

Lysine requirements to produce one gram of egg increased as dietary lysine increased (Table 6). However, energy requirement and feed conversion decreased (Table 2). This complicates effort to determine lysine requirements for optimal profits. That explains the need of an econometric program (Roland et al., 1998, 2000) to determine requirements for optimal profits as feed and egg prices change.

In conclusion, the results showed that there were no interactions between lysine and strain on any parameter. Lysine had significant effects on feed consumption, egg production, egg mass, feed conversion, egg weight, egg shell components, percent yolk and whole egg solids, albumen and yolk weight, egg specific gravity, yolk color, and haugh unit. There were significant strain effects on feed consumption, egg mass, feed conversion, egg weight, albumen and yolk components, whole egg solids, albumen and shell weight, egg specific gravity, body weight, shell color, and haugh unit. Strain 1 had the best overall performance. All strains were laying 89.5 to 92.5% at 52 weeks of age. Average egg weight (39wk to 52wk) was 63g, varying from 61.5 to 63.6 g between strains. Average feed intake was 112.1g/hen/day varying from 108 to 114 g/hen/day

between strains. Average Egg weight of hens fed diets containing the highest lysine level was 3.38g heavier than the hens fed the diets containing the lowest lysine level. Increasing dietary lysine from 0.680 to 0.828% significantly improved feed conversion from 2.03 to 1.91 g feed/g egg and increased egg mass from 54.0 to 59.30 g/hen/day. Average lysine intake of hens fed 0.828% level was 939 mg/hen/day varying from 907 to 964 mg/hen/day between strains. Because egg prices and ingredient prices often change, there can be no fixed dietary lysine level for optimal profits.

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Table 1 Ingredients and nutrient content of the experimental diets

Ingredient (%)	Diet 1	Diet 2	Diet3
Corn	70.91	67.9	64.35
Soy bean meal	16.9	19.5	22.4
Hard shell[1]	4.0	4.0	4.0
Limestone	5.1	5.1	5.1
Dicalcium phosphate	1.6	1.6	1.6
Poultry oil	0.5	1.0	1.6
NaCl	0.36	0.36	0.36
Vitamin Premix[2]	0.25	0.25	0.25
Mineral Premix[3]	0.25	0.25	0.25
DL-Methionine	0.008	0.03	0.07
Total	100	100	100
Calculated Analysis			
ME (Kcal/kg)	2866	2866	2866
Crude protein	13.8	14.8	15.96
Ca	4.0	4.0	4.0
Available phosphorus	0.4	0.4	0.4
Na	0.17	0.17	0.17
Methionine + Cystine	0.51	0.56	0.62
Lysine	0.680	0.747	0.828

1 Hard shell = large particle limestone (passing US mesh #4 and retained by US mesh #6) $CaCo_3$ supplied by Franklin Industrial Minerals, Lowell ,FL.

[2] Provided per kilogram of diet: Vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200ICU, vitamin E (as DL – α - tocopheryl acetate), 8 IU; vitamin B12, 0.02mg; riboflavin, 5.5mg; D-calcium pentothenic acid, 13mg; niacin, 36mg; choline, 500mg; folic acid, 0.5mg; vitamin B1(thiamin mononitrate), 1mg; pyridoxine, 2.2mg; biotin, 0.05mg; vitamin K (menadione sodium bisulfate complex), 2mg.

[3] Provided per kilogram of diet: manganese, 65mg; iodine,1mg; iron, 55mg; copper, 6mg; zinc, 55mg; selenium, 0.3mg.

Table 2. Effect of lysine on feed intake, egg production, egg mass, feed conversion and egg weight of seven brown egg layer strains during first cycle phase 2 (39wk to 52 wk of age)

Lysine (%)	Strain	Feed intake (g/hen per day)	Egg Production (%)	Egg mass (g of egg/h per day)	Feed conversion (g of feed /g of egg)	Egg Weight (g)
0.680		109.75 [b]	88.49 [b]	53.98 [c]	2.03 [a]	61.02 [c]
0.747		113.06 [a]	90.84 [a]	57.45 [b]	1.97 [b]	63.40 [b]
0.828		113.37 [a]	92.10 [a]	59.30 [a]	1.91 [c]	64.40 [a]
	Strain 1	108.04 [c]	90.89	57.07 [ab]	1.90 [c]	62.77 [a]
	Strain 2	114.24 [a]	90.44	57.04 [ab]	2.01 [ab]	63.04 [a]
	Strain 3	111.73 [bc]	89.84	57.20 [ab]	1.96 [b]	63.64 [a]
	Strain 4	110.97 [bc]	90.59	55.82 [b]	1.99 [ab]	61.63 [b]
	Strain 5	113.45 [b]	89.80	56.02 [ab]	2.03 [a]	62.72 [a]
	Strain 6	114.48 [a]	92.49	58.03 [a]	1.97 [ab]	62.74 [a]
	Strain 7	110.97 [bc]	89.29	56.58 [ab]	1.96 [b]	63.38 [a]
0.680 × Strain	Strain 1	104.79	87.27	53.39	1.97	61.17
	Strain 2	112.33	89.66	54.85	2.05	61.17
	Strain 3	111.32	88.44	55.02	2.02	62.19
	Strain 4	109.83	89.52	53.27	2.06	59.52
	Strain 5	113.28	88.41	54.12	2.10	61.19
	Strain 6	111.36	90.89	54.92	2.03	60.44
	Strain 7	105.35	85.26	52.26	2.02	61.44
0.747 × Strain	Strain 1	109.78	91.52	57.91	1.90	63.30
	Strain 2	113.92	90.01	57.45	1.99	63.76
	Strain 3	111.75	89.49	57.04	1.96	63.73
	Strain 4	112.12	93.00	58.10	1.93	62.50
	Strain 5	111.90	88.80	54.97	2.04	62.97
	Strain 6	116.83	92.21	58.40	2.00	63.37
	Strain 7	115.15	90.88	58.27	1.98	64.15
0.828 × Strain	Strain 1	109.54	93.89	59.93	1.83	63.84
	Strain 2	116.47	91.66	58.81	1.98	64.17
	Strain 3	112.12	91.57	59.53	1.89	65.00
	Strain 4	110.95	89.26	56.07	1.98	62.86
	Strain 5	115.17	92.18	58.95	1.95	63.99
	Strain 6	115.25	94.36	60.77	1.90	64.41
	Strain 7	112.42	91.73	59.20	1.90	64.55
Pooled SEM		1.36	1.58	1.07	0.02	0.53
		----------	----------	**Probability**	----------	----------
Lysine		0.0017	<0.0001	<0.0001	<0.0001	<0.0001
Strain		0.0011	NS	0.0017	0.0002	<0.0001
Lysine×Strain		NS	NS	NS	NS	NS

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($P \leq 0.05$).

Table 3. Effect of lysine on egg components, egg solids, and albumen, yolk and shell weights of seven brown egg layer strains during first cycle phase 2 (39wk to 52 wk of age)

Lysine (%)	Strain	% Egg components			% of solids			Albumen, Yolk, Shell weight (g)		
		Yolk	Albumen	Shell	Whole egg	Albumen	Yolk	Albumen	Yolk	Shell
0.680		25.89	64.58	9.53 [a]	23.90 [b]	12.06 [b]	54.60	40.04 [b]	16.02 [b]	5.90
0.747		26.17	64.62	9.20 [b]	24.23 [a]	12.39 [a]	54.95	40.81 [b]	16.48 [ab]	5.80
0.828		25.84	64.96	9.20 [b]	24.21 [a]	12.64 [a]	54.64	42.35 [a]	16.77 [a]	5.99
	Strain 1	25.95 [cd]	64.47 [ab]	9.58	24.26 [ab]	12.30	54.52	40.13 [ab]	16.10	5.95 [ab]
	Strain 2	25.69 [cd]	65.25 [a]	9.06	24.22 [ab]	12.40	54.84	41.25 [ab]	16.21	5.72 [b]
	Strain 3	25.55 [cd]	65.00 [ab]	9.45	23.84 [b]	12.42	54.85	42.99 [a]	16.80	6.23 [a]
	Strain 4	27.05 [a]	63.68 [c]	9.27	24.50 [a]	12.53	54.95	39.66 [b]	16.84	5.77 [b]
	Strain 5	26.30 [abc]	64.58 [ab]	9.12	24.22 [ab]	12.36	54.57	40.90 [ab]	16.61	5.76 [b]
	Strain 6	26.90 [ab]	63.75 [c]	9.35	24.10 [ab]	12.25	55.10	39.67 [b]	16.75	5.81 [b]
	Strain 7	24.73 [d]	65.94 [a]	9.33	23.88 [b]	12.20	54.36	42.15 [ab]	15.72	5.94 [ab]
Pooled SEM		0.63	0.72	0.23	0.24	0.23	0.55	1.37	0.46	0.17
		Probability								
Lysine		NS	NS	0.012	0.0056	0.0007	NS	0.03	0.01	NS
Strain		0.002	0.003	NS	0.0041	NS	NS	0.012	NS	0.01
Ly×Strain		NS	NS	NS	NS	NS	NS	NS	NS	NS

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($P \leq 0.05$).

Table 4 Effect of lysine on specific gravity, body weight, mortality, shell color [lightness (L*), redness (a*), yellowness (b*)] [1] and egg quality of eight brown egg layer strains during first cycle phase 2 (39wk to 52 wk of age).

					Egg shell color			Egg quality	
Lysine (%)	Strain	Egg Specific Gravity (Unit)	Body Weight (Kg)	Mortality (%)	L*	a*	b*	Haugh Unit	Yolk color
0.680		1.0891 [a]	2.05	0.13	63.43	17.49	29.77	76.00 [a]	6.26 [a]
0.747		1.0878 [b]	2.00	0.15	63.27	17.53	29.89	73.54 [a]	6.15 [ab]
0.828		1.0870 [c]	2.03	0.12	62.94	17.78	29.75	70.39 [b]	6.02 [b]
	Strain 1	1.0901 [a]	1.93 [b]	0.18	62.82 [c]	17.80 [b]	29.33 [cb]	74.91 [ab]	5.98
	Strain 2	1.0861 [d]	2.05 [ab]	0.09	61.45 [d]	18.69 [a]	30.70 [a]	76.13 [a]	6.08
	Strain 3	1.0884 [b]	2.03 [ab]	0.03	63.99 [b]	17.20 [cb]	29.60 [cb]	71.68 [ab]	6.13
	Strain 4	1.0869 [cd]	1.96 [ab]	0.15	61.08 [d]	18.93 [a]	30.63 [a]	75.98 [a]	6.27
	Strain 5	1.0882 [cb]	2.09 [a]	0.18	65.09 [a]	16.49 [d]	29.45 [cb]	71.91 [ab]	6.22
	Strain 6	1.0876 [cb]	2.10 [a]	0.24	64.06 [ab]	17.22 [cb]	29.73 [cb]	70.69 [b]	6.15
	Strain 7	1.0878 [cb]	2.02 [ab]	0.06	64.05 [ab]	16.95 [cd]	29.12 [c]	71.02 [ab]	6.17
Pooled SEM		0.01	0.07	0.11	0.89	0.60	0.50	2.50	0.16
		----------**Probability**----------							
Lysine		<0.0001	NS	NS	NS	NS	NS	0.0001	0.009
Strain		<0.0001	<0.0001	NS	<0.0001	<0.0001	<0.0001	0.025	NS
Lys×Strain		NS	NS	NS	NS	NS	NS	NS	NS

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($p \leq 0.05$).

[1] A higher L* value means lighter color; a higher a* value means a redder color; a higher b* value means a more yellow color.

Table 5: Nutrient requirement of seven brown egg layer strains fed diets containing 0.828% lysine during first cycle phase 2 (39wk to 52 wk of age).

	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7
Nutrients required per hen daily							
Protein (g)	17.5	18.6	17.9	17.7	18.4	18.4	17.9
Lysine (mg)	907	964	928	919	954	954	931
TSAA (mg)	679	722	695	688	714	715	697
Dietary energy (kcal)	314	334	321	318	330	330	322
Nutrients required to produce one gram egg							
Protein (g)	0.29	0.32	0.30	0.32	0.31	0.30	0.30
Lysine (mg)	15.13	16.40	15.59	16.38	16.18	15.70	15.72
TSAA (mg)	11.33	12.28	11.68	12.27	12.11	11.76	11.77
Dietary energy (kcal)	5.24	5.68	5.40	5.67	5.6	5.44	5.44

Table 6: Nutrient requirement and nutrients required to produce one gram egg of Bovans brown classic (Strain 5) hens during first cycle phase 2

Lysine (%)	Protein (g)	Lysine (mg)	TSAA (mg)	Energy (kcal)
Nutrients required to produce one gram egg				
0.680	0.29	14.23	10.67	6.00
0.747	0.30	15.21	11.40	5.83
0.828	0.31	16.18	12.11	5.60
Nutrients required per hen daily				
0.680	15.6	770	578	325
0.747	16.6	836	627	321
0.828	18.4	954	714	330

Table 7. Influence of dietary lysine on profits [1] of Bovans brown classic (Strain 5) hens during first cycle phase 2 (39wk to 52 wk of age)

	Price spread		
Diet (Lysine %)	Low[2]	Medium[3]	High[4]
	Returns[5] ($/dozen)		
0.828	0.302	0.429	0.751
0.747	0.306	0.431	0.750
0.680	0.306	0.429	0.738

[1] Based on a feed price of $106.44 per ton for the 0.828 lysine diet, $ 102.65 per ton for the 0.747 lysine diet, and $99.28 per ton for the 0.680 lysine diet.

[2] Based on an Urner Barry egg price of 31 cents/doz. for check and peewee, 40 cents/doz. for small, 57 cents/doz. for medium, 67 cents/doz. for large, 68 cents/doz. for extra large and 69 cents/doz. for jumbo eggs.

[3] Based on an Urner Barry egg price of 31 cents/doz. for check and peewee, 40 cents/doz. for small, 65 cents/doz. for medium, 80 cents/doz. for large, 81 cents/doz. for extra large and 84 cents/doz. for jumbo eggs.

[4] Based on an Urner Barry egg price of 31 cents/doz. for check and peewee, 40 cents/doz. for small, 75 cents/doz. for medium, 112 cents/doz. for large, 117 cents/doz. for extra large and 120 cents/doz. for jumbo eggs.

[5] Returns (R) were calculated using the equation R = UBEP – NR – PC – FdC, where UBEP = Urner Berry Egg Price, NR = nest run into package product delivered, PC = production cost and FdC = feed cost as described by Roland et al.(1998, 2000).

IV. EFFECT OF ENERGY AND PROTEIN ON PERFORMANCE, EGG COMPONENTS, EGG SOLIDS, EGG QUALITY, AND PROFITS IN MOLTED HYLINE W-36 HENS

SUMMARY

An experiment was conducted to determine the influence of dietary energy on performance, egg composition, egg solids, egg quality and profits of Hy-line W-36 hens fed different protein levels. The experiment was designed as a 4 × 3 factorial arrangement with four added dietary energy levels (0, 79, 158, and 238 kcal ME/kg) and three protein levels (14.89, 16.06 and17.38%). The basal diets of the 17.38, 16.06 and 14.89% protein contained 2751, 2784 and 2815 kcal ME/kg, respectively. This study lasted 12 weeks. Molted Hy-line W-36 hens (n=1440) phase 1 (70weeks of age) were randomly divided into 12 treatments (8 replicates of 15 hens per treatment). Protein had a significant effect on egg production, egg mass, feed intake, feed conversion, egg weight, percentage of egg shell components, yolk color, and yolk and albumen weight. As dietary energy increased from 0 to 238 kcal ME/kg by addition of poultry oil, feed intake linearly decreased. Increasing dietary energy also significantly increased body weight and egg yolk color. As dietary energy increased, percent yolk solids increased at the two higher dietary protein levels, while egg specific gravity linearly decreased at the 17.38% protein level.

Increasing dietary energy and protein significantly improved feed conversion. Increasing protein intake significantly increased albumen and yolk weight but had no influence on yolk, albumen or whole egg solids. Because feed ingredient and egg prices vary, there can be no fixed ideal dietary energy level for optimal profits during molt phase 1 (70 to 81wk)

Key words: Protein, Dietary energy, Hens

DESCRIPTION OF PROBLEM

Dietary energy and protein represent approximately 85% of total feed cost. At present, there are wide ranges of dietary energy (2685 – 3100 kcal ME/kg) and protein levels (14.5 – 19%) being used by the egg industry during molt phase 1[1]. Numerous investigations have focused on methods of influencing egg weight through diet manipulation during various production phases. Increasing levels of protein [2, 3 and 4], fat [5, 1, 6, 7], methionine [5], lysine [8] and linoleic acid [9, 10] have resulted in improvements in egg weight. However there are inconsistent results on the effect of supplemental fat or dietary energy on egg weight. Some research [11, 12] indicated that addition of supplemental fat increased egg weight. In contrast other research [13] reported that added fat had no effect on egg weight.

Regulating dietary energy may be the most effective dietary method to manipulate feed intake of laying hens. Earlier studies [1, 6, 14] reported that increasing dietary energy decreased feed intake and improved feed efficiency. Dietary energy level can be easily manipulated with supplemental fat, but some poultry producers do not use fat because of inadequate storing and mixing facilities [11].

Yolk color has a considerable influence on egg marketing. The study of factors affecting the intensity of yolk color is therefore of economic significance for egg producers. The color of yolk depends on the fat soluble carotenoids in dietary fats. Experimental evidence concerning the effect of added fat on yolk color is contradictory. Sullivan and Holleman. [15] and Madiedo and sunde [16] reported that added dietary fat had no affect on egg yolk color; however Mackay et al.[17] and Stevans et al.[18] reported supplemental fat had a significant effect on egg yolk color.

The liquid egg and breaker egg industry have grown during the last 10 years. However, there are very few studies where the effects of the dietary energy and protein on egg composition and egg solids of Hy-line W-36 hens have been studied. With sharp increases in energy cost, it is important to have a better understanding of how to maximize the use of dietary energy at different protein levels to optimize performance and yield especially for the egg breaker industry. Therefore, The objective of this study was to determine the effect of dietary energy on performance, egg components, egg solids and profits, at different protein levels in Hy-line W-36 hens during molt phase 1 (from 70 weeks to 81 weeks).

MATERIAL AND METHODS

This study was a 4 × 3 factorial arrangement with four added dietary energy levels (0, 79, 158 and 238 kcal ME/kg) and three dietary protein levels (14.89, 16.06 and17.38%). Ingredients and nutrient composition of experimental diets are shown in table 1. In this experiment, 1440 Hy-Line W-36 hens [19] just out of molt (70wk old) were randomly divided into 12 combinations of energy and protein (8 replicates of 15 hens per treatment). Hens were housed three per cage in a 40.6 X 45.7 cm cage. Each treatment replicate consisted of five adjoining cages. Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. All hens were housed in an environmentally controlled house with temperature maintained as close to 26^{o}C as possible. The house had controlled ventilation and lighting (16 h/d). All hens were supplied with feed and water *ad libitum*. Animal housing and handling procedures during experimentation were in accordance with guidelines of Auburn University's Institutional Animal Care and Use Committee (IACUC). Feed consumption was recorded weekly for calculation of average daily feed consumption. Egg production was recorded daily, and egg weight and specific gravity were recorded once every two weeks. Egg weight and egg specific gravity were measured using all eggs produced during 2 consecutive days. Egg specific gravity was determined using 9 gradient saline solutions varying in specific gravity from 1.060 to 1.100 in 0.005 unit increments [20]. Mortality was determined daily, and feed consumption was adjusted accordingly. Body weight was obtained by weighing 3 hens per treatment replicate at the end of the experiment. Egg mass (gram of egg/hen per day) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption.

Egg components were measured using 3 randomly selected eggs from each replicate at the middle and end of the experiment. Eggs were weighed and broken. The yolks were separated from the albumen. Before yolk weight was determined, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned of any adhering albumen and dried for 5 days. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

Three eggs from each treatment replicate were randomly collected at the middle and at the end of the experiment for measuring solids. The yolk and albumen were mixed and 5 to 6 g of homogenate was pipetted into an aluminum dish with weight recorded to 0.001g.The sample was dried in an oven for 24h at 40.5^0C [21] and then weighed. Three eggs which randomly selected from each treatment replicate were used to analyze the yolk and albumen solid. After yolk was separated from albumen, three yolks and albumen per treatment replicate were mixed separately. The procedure for analyzing albumen and yolk solid was the same as the procedure for whole egg solid content. Yolk color and haugh units were measured (3 eggs from each treatment replicate) at the middle and at the end of the experiment using an egg multitester EMT-5200 [22]. Haugh units were calculated from the records of albumen height and egg weight using the formula: $HU=100 \log_{10} (H-1.7 W^{0.37}+7.56)$, where HU=Haugh unit, H=height of the albumen (mm) and W=egg weight (g).

Data were analyzed by ANOVA using proc mixed of statistical analysis system [23] for a randomized complete block with factorial arrangement of treatments. The factorial arrangement consisted of four dietary energy levels and three protein levels.

Dietary energy and protein were fixed; whereas blocks (Location of cages) were random. The following model was used to analyze the data:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + P_k + \varepsilon_{ijk}$$

Where Y_{ijk} = individual observation, μ = overall mean, α_i = dietary energy effect, β_j = protein effect, $(\alpha\beta)_{ij}$ = interaction between dietary energy and protein, P_k = effect of block, ε_{ijk} = error component. If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. Contrast statements were utilized to test for linear or quadratic dietary energy effects. A significance level of $P \leq 0.05$ was used.

RESULTS AND DISCUSSION

There was a significant effect of dietary protein and energy on feed intake. (Table 2). As added dietary energy increased, feed intake decreased from 96.9 to 94.9 g/hen per day, which resulted in a 2.1% decrease in feed intake. Results of feed intake vs. dietary energy in this study are in agreement with that of Wu et al. [7], Sohail et al.[11], Grobas et al.[1] and Parsons et al. [2]. Increasing dietary protein increased feed intake from 93.2 to 98.8g/hen/day, resulting in a 6.01% increase of feed intake. (Table 2). This result was consistent with that of Parsons et al. [2] who reported increasing crude protein from 18 to 20%, increased feed intake from 104 to 107g/bird/day and Wu et al. [14] who indicated that, with the increase of dietary protein from 13.99 to 16%, feed intake increased from 102.9 to 105.6 g/hen/day.

Increasing dietary protein intake from 13.8 to 17.1 g/hen/day, increased egg production from 65.2 to 71.7% (Table 2). Similarly, Liu et al. [24] and Wu et al. [7] reported that increasing dietary protein improved egg production. Zou et al. [25] reported that increasing dietary protein intake from 15.3 to 16.3 g/hen/day, increased egg production by 3.2%. Keshavarz [5] indicated that with an increase of dietary protein intake from 17.4 to 21.4 g/hen/day, egg production increased by 1.9%. However there was no significant effect of dietary energy on egg production; this result was consistent with that of Harms et al.[6], Wu et al. [7, 14], Parsons et al. [2], Sell et al. [26] and Sohail et al. [11] who reported that egg production was not influenced by supplemental fat.

There was a significant interaction between dietary energy and protein on egg specific gravity (Table 2). When added dietary energy increased from 0 to 238 kcal/kg, egg specific gravity linearly decreased at the 17.38% protein level; but not at the other two protein levels. This may be related to reduce feed (Ca) intake with increased supplemented fat. There was a significant linear effect of added dietary energy but not dietary protein on body weight (Table 2). The effect of protein on body weight was in agreement with Sohail et al. [11], Grobas et al. [1] and Keshavarz & Nakajima. [3] who reported no significant effect of increasing dietary protein levels on body weight.

As dietary protein and energy levels increased, feed conversion improved significantly, because egg weight and production increased significantly with increased dietary protein and feed intake decreased significantly with increased dietary energy. This result was in agreement with that of Liu et al. [24] and Wu et al. [7, 14], who reported increasing dietary protein and energy improved feed conversion.

Increasing dietary protein intake from 13.8 to 17.1 g/hen/day, increased egg mass 5.75g/hen day and egg weight by 2.38g, respectively (Table 2). The mechanism by which protein improves egg size is well understood [2, 3, 27, 28]. In this study dietary energy had no influence on egg weight. However conflicting results arise concerning the influence of supplemental energy on egg weight. De Groote. [29], Harms et al. [6] and Wu et al. [14] reported that egg weight increased as dietary energy increased, Leeson. [4], also indicated that energy intake had no effect on egg size. Further more Keshavarz. [5] reported that young hens respond better to added fat than old hens. The effect of dietary protein on egg weight in this study was consistent with Parsons et al. [2], Keshavarz. [5], Leeson. [4], Wu et al. [14] and Sohail et al. [11]; who reported egg weight of hens fed

higher protein was greater than of the hens fed diets containing lower protein. The increased egg weight was due to increased albumen and yolk weight (Table 3). Shafter et al. [30] also indicated that increasing amino acid (Lysine and TSAA) intake had a significant effect on albumin weight.

Increasing dietary protein significantly decreased percent egg shell (Table 3). This was due to a significant increase of egg weight as protein level increased. As dietary protein increased albumen and yolk weight significantly increased (Table 3). As dietary energy increased, percent yolk solids significantly increased at the higher dietary protein levels (16.06 and 17.38%) but not at the lowest (Table 3). These results were in agreement with that of Wu et al. [14] and Liu et al. [37]. Prochaska et al. [38]. Furthermore Novak et al. [39] also reported that increasing TSSA and lysine intake per hen daily had a significant influence on albumen weight. These results could be important tools for influencing profits of the breaker, liquid and powdered egg industries.

Dietary protein and energy had a significant effect on egg yolk color. Increasing dietary energy significantly increased egg yolk color where as increasing dietary protein significantly decreased egg yolk color (Table 4). These results suggest a strong relationship between added dietary fat, protein and egg yolk color. Xanthophil is the major colorant responsible for the egg yolk color and it is highly fat soluble; [31, 32, 33]. Because xanthophylls are fat soluble, with the increase of dietary fat, more xanthophylls may be deposited in the egg yolk as pigments. These results agree with those reported by Morihiro et al. [34], Masahiro et al. [35] and Abu Serewa [33]. Increasing dietary protein, decreased egg yolk color due to less corn in the diet. This result was in agreement with the results reported by Karunajeewa [36].

The economic feeding and management program developed by Roland et al. [40, 41] was used to calculate profits at different dietary energy and protein levels at different poultry oil prices. As protein levels increased, profits increased at all dietary energy levels, regardless of poultry oil prices (Table 5).Maximum profits were obtained at the highest dietary protein level (17.38) and highest added energy level (258 kcal ME/kg) with low oil price. With high oil prices, highest profits were obtained at the lowest dietary energy and highest protein level. Because feed ingredient and egg prices vary, there can be no fixed ideal dietary energy level for optimal profits during phase 1 (70 to 81wk).

CONCLUSION AND APPLICATIONS

1. Increasing dietary energy by addition of poultry oil had no significant effect on egg production, egg weight, and percent egg components. However increasing dietary energy had a significant effect on feed intake, egg specific gravity, feed conversion, body weight and yolk color. Increasing dietary energy significantly increased yolk solids at the two higher levels of protein but not at the lower level. This could prove useful to the breaker egg industry.
2. Increasing dietary protein significantly increased feed consumption, egg production, egg weight; egg mass, and albumen and yolk weight. However, increasing dietary protein significantly decreased egg specific gravity, yolk color and percent shell. Feed conversion improved with increased dietary protein.
3. An ideal dietary energy level for optimal performance could not be determined for laying hens during molt phase 1 (from wk70 to 81wk of age).
4. There can be no fixed ideal dietary energy level for optimal profits during molt phase 1, due to varying feed ingredient and egg prices.

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Table 1. Ingredients and nutrient content of the experimental diets.

Ingredients (%)	**P[1]: 17.38 E: 0**	**P: 17.38 E: 79**	**P: 17.38 E: 158**	**P: 17.38 E: 238**	**P: 16.06 E: 0**	**P: 16.06 E: 79**	**P: 16.06 E: 158**	**P: 16.06 E: 238**	**P: 14.89 E: 0**	**P: 14.89 E: 79**	**P: 14.89 E: 158**	**P: 14.89 E: 238**
Corn (8.6%)	62.68	60.86	59.02	57.17	65.95	64.12	62.29	60.44	68.98	67.15	65.31	63.47
Soy bean meal 48%	25.65	25.79	25.95	26.09	22.39	22.54	22.68	22.83	19.48	19.63	19.78	19.92
Limestone	6.99	6.98	6.98	6.98	7.00	6.99	6.99	6.99	7.12	7.13	7.12	7.12
Hard shell[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium Phosphate	1.70	1.71	1.72	1.72	1.72	1.73	1.73	1.74	1.52	1.53	1.53	1.54
Poultry oil	0.00	1.67	3.35	5.04	0.00	1.67	3.35	5.04	0.00	1.67	3.35	5.04
NaCl	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.36	0.36	0.37	0.37
Vitamin Premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.01	0.10	0.12	0.11	0.06	0.07	0.07	0.08	0.03	0.04	0.04	0.05
Analysis (%)												
Crude protein	17.38	17.31	17.24	17.16	16.06	15.99	15.91	15.84	14.89	14.83	14.74	14.67
ME (Kcal/kg)	2751	2830	2910	2989	2784	2861	2941	3020	2815	2894	2974	3053
Ca	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Avilable phosphorus	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.38	0.38	0.38	0.38
Sodium	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17
Methionine	0.38	0.38	0.39	0.39	0.33	0.34	0.34	0.34	0.29	0.29	0.29	0.30
Methionine + Cystine	0.69	0.69	0.69	0.69	0.69	0.62	0.62	0.62	0.56	0.56	0.56	0.56
Lysine	0.92	0.92	0.92	0.92	0.83	0.83	0.83	0.83	0.75	0.75	0.75	0.75

P1: - Dietary Protein, **E:** - Added dietary energy. Assumed Energy level for poultry oil – 1690 kcal/ kg

[2] Hard shell = large particle limestone (passing US mesh #4 and retained by US mesh #6) $CaCo_3$ supplied by Franklin Industrial Minerals, Lowell ,FL.
[3] Provided per kilogram of diet: Vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200ICU, vitamin E (as DL – α - tocopheryl acetate), 8 IU; vitamin B12, 0.02mg; riboflavin, 5.5mg; D-calcium pentothenic acid, 13mg; niacin, 36mg; choline, 500mg; folic acid, 0.5mg; vitamin B1 (thiamin mononitrate), 1mg; pyridoxine, 2.2mg; biotin, 0.05mg; vitamin K (menadione sodium bisulfate complex), 2mg.
[4] Provided per kilogram of diet: manganese, 65mg; iodine,1mg; iron, 55mg;copper, 6mg;zinc, 55mg; selenium, 0.3m

Table 2.Effect of protein, and dietary energy on performance of Hy-Line w-36 hens during molt phase 1 (70wk to 81 wk of age)

Protein (%)	Added Dietary Energy (kcal/kg)	Feed intake (g/hen day)	Egg Production (%)	Egg Specific Gravity (Units)	Body Weight (Kg)	Mortality (%)	Egg mass (g of egg/h day)	Feed conversion (g feed /g egg)	Average Egg Weight (g)
14.89		93.2[c]	65.2[b]	1.083[a]	1.67	0.16	40.47[c]	2.31[a]	62.08[c]
16.06		96.6[b]	68.7[a b]	1.082[b]	1.71	0.07	43.78[b]	2.21[b]	63.70[b]
17.38		98.8[a]	71.7[a]	1.082[b]	1.76	0.10	46.22[a]	2.14[c]	64.46[a]
	0	96.9	67.8	1.083	1.61	0.09	42.74	2.28	63.05
	79	97.6	67.8	1.082	1.70	0.05	43.22	2.26	63.74
	158	95.2	68.6	1.082	1.77	0.12	43.40	2.20	63.24
	238	94.9	70.1	1.082	1.77	0.19	44.61	2.13	63.62
Pooled SEM		0.85	1.37	0.0003	0.05	0.08	0.86	0.03	0.48
		----------	----------	----------	**Probability**	----------	----------	----------	----------
Protein		<0.001	<0.001	<0.001	0.638	NS	<0.001	<0.001	<0.001
Energy		0.0218	NS	0.0032	0.0017	NS	0.0617	0.0009	NS
Protein×Energy		NS	NS	0.0175	NS	NS	NS	NS	NS
Contrasts									
Energy Linear		0.0093	0.0317	0.0004	0.0003	NS	0.0112	<0.001	NS
Energy quadratic		NS	NS	NS	NS	NS	NS	NS	NS

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($p \leq 0.05$).

Table 3. Effect of protein, and dietary energy on egg components, egg solids, and albumen, yolk, shell weights of Hy-Line w-36 hens during molt phase 1 (70wk to 81 wk of age)

Protein (%)	Added dietary Energy (kcal/kg)	Egg components (%)			Egg solids (%)			Albumen, Yolk, Shell weight(g)		
		Yolk	Albumen	Shell	Whole egg	Albumen	Yolk	Albu	Yolk	Shell
14.89		28.56	62.56	8.88 [a]	24.80	12.57	51.45	37.10 [b]	16.94 [b]	5.26
16.06		28.36	62.97	8.67 [a b]	24.70	12.50	51.72	38.79 [a]	17.48 [a b]	5.34
17.38		28.22	63.27	8.50 [b]	24.72	12.41	51.25	39.65 [a]	17.68 [a]	5.33
	0	28.01	63.31	8.68	24.74	12.56	51.74	38.53	17.05	5.28
	79	28.42	62.85	8.73	24.70	12.43	51.30	39.07	17.67	5.42
	158	28.66	62.74	8.60	24.71	12.39	51.38	37.99	17.34	5.20
	238	28.44	62.84	8.72	24.80	12.60	51.47	38.45	17.39	5.34
Pooled SEM		0.51	0.52	0.18	0.25	0.21	0.42	0.77	0.43	0.13
	----------	----------	----------	**Probability**	----------	----------	----------	----------	----------	----------
Protein		NS	NS	0.0155	NS	NS	NS	<0.0001	0.0497	NS
Energy		NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein×Energy		NS	NS	NS	NS	NS	0.033	NS	NS	NS
Contrasts										
Energy Linear		NS	NS	NS	NS	NS	NS	NS	NS	NS
Energy quadratic		NS	NS	NS	NS	NS	NS	NS	NS	NS

[a, b] Means within a column and under each main effect with no common superscripts differ significantly ($p \leq 0.05$).

Table 4. Effect of protein, and dietary energy on nutrient Intake, and egg quality of Hy-Line w-36 hens during molt phase 1 (70wk to 81 wk of age).

Protein (%)	Added Dietary Energy (kcal/kg)	Nutrient intake per hen daily				Egg quality	
		Energy (kcal)	Protein (g)	TSAA (mg)	Lysine (mg)	Haugh Unit	Yolk color
14.89		273 [b]	13.77 [c]	522 [c]	699 [c]	76.12	6.05 [a]
16.06		280 [a]	15.40 [b]	599 [b]	801 [b]	75.28	5.69 [b]
17.38		283 [a]	17.06 [a]	681 [a]	909 [a]	75.02	5.50 [c]
	0	269	15.65	606	810	75.31	5.57
	79	279	15.68	610	815	75.74	5.78
	158	280	15.21	594	794	75.68	5.86
	238	287	15.10	593	792	75.16	5.78
Pooled SEM		2.46	0.14	5.24	7.01	1.62	0.11
		----------Probability----------					
Protein		0.0008	<0.0001	<0.0001	<0.0001	NS	<.0001
Energy		<0.0001	0.0006	0.0162	0.0163	NS	0.0139
Protein×Energy		NS	NS	NS	NS	NS	0.0697
Contrasts							
Energy Linear		<0.0001	0.0001	0.0071	0.0071	NS	0.0147
Energy quadratic		NS	NS	NS	NS	NS	0.0272

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($p \leq 0.05$).

Table 5.Influence of dietary energy and protein on profits[1,2] at different poultry oil prices from 70 to 81 wk of age

		Added dietary energy (kcal ME/kg)			
		0	79	158	258
	Protein, %	----------Returns[3] ($/dozen)----------			
High poultry oil	14.89	0.152	0.152	0.152	0.154
Price ($0.40/kg)	16.06	0.176	0.162	0.162	0.167
	17.38	0.182	0.174	0.177	0.176
Low poultry oil	14.89	0.152	0.157	0.162	0.169
Price ($0.22/kg)	16.06	0.176	0.167	0.173	0.181
	17.38	0.182	0.179	0.186	0.191

[1] Corn price = $0.12/kg, soy price = $0.39/kg, $CaCo_3$ = $0.03/kg, hard shell = $0.03/kg, dicalcium phosphate = $0.027/kg, salt = $0.06/kg, vitamin premix = $2.67/kg, mineral premix = $0.59/kg, DL-methionine = $2.59/kg.

[2] Urner Berry egg price: jumbo size = 105 cents, extra large size = 101cents, large size = 97 cents, medium size = 75 cents and small size = 54 cents.

[3] Returns (R) were calculated using the equation R = UBEP – NR – PC – FdC, where UBEP = Urner Berry Egg Price, NR = nest run into package product delivered, PC = production cost and FdC = feed cost as described by Roland et al.(40, 41).

V. EFFECT OF DIETARY ENERGY, PROTEIN AND A VERSATILE ENZYME ON HEN PERFORMANCE, EGG SOLIDS, EGG COMPOSITION AND EGG QUALITY OF HY-LINE W- 36 HENS DURING SECOND CYCLE PHASE TWO

SUMMARY

This study was conducted to evaluate the effect of Rovabio™, dietary energy and protein on performance, egg composition, egg solids, and egg quality of commercial leghorns in phase two second cycle. A 4 × 2 × 2 factorial arrangement of treatments comprising four dietary energy levels (2791, 2857, 2923 and 2989 kcal of ME/kg) and two protein levels (15.5 and 16.1%) with and without Rovabio™ were used. Hy-line W-36 hens (n=1920, 87 wk old) were randomly divided into 16 dietary treatments (8 replicates of 15 hens per treatment). The trial lasted 12 weeks. Dietary protein significantly increased feed consumption but decreased yolk color. As dietary energy increased from 2791 to 2989 kcal ME/kg, feed consumption decreased from 98.0 to 94.9 g per hen daily and yolk color increased from 5.27 to 5.56. There was a significant interaction among dietary protein, energy and Rovabio™ on egg production, body weight, egg mass, feed conversion and yolk solids. Egg weight of hens fed the diets supplemented with Rovabio™ was significantly higher than that of hens fed the diets without Rovabio™ during wk 3 and 4. However Rovabio™ did not significantly influence average egg weight (87-98 wk of age).

Rovabio™ supplementation significantly increased body weight of hens. These results suggest Rovabio™ had a small but significant influence on nutrient utilization of commercial Leghorns during phase two of second cycle.

Key words: Rovabio™, Energy utilization, Laying hen

DESCRIPTION OF PROBLEM

In poultry operations, feed cost has always been a major issue. Enzyme supplementation as a feed additive has become common during the last five decades [1]. Enzymes are proteins, having unique abilities to catalyze biochemical reactions. Their usage is popular because of positive effects on hen performance and lack of harmful effects on consumers. Utilization of most grains is influenced by the presence of indigestible complex carbohydrates, such as non-starch polysaccharides (NSP). Enzymes are supplemented to the feed to improve nutritive value in those grains. Legume seeds also contain NSP-like hemicelluloses, mannan and raffinose [2, 3]. Chickens do not produce some enzymes like galactosidases, thus corn-soybean based diets without supplemented enzymes like xylanases and pectinases might result in gas accumulation in the gut and diarrhea [4, 5].

Rovabio™ is a natural mixture of enzymes produced by the organism *Penicillium funiculosum* [6]. Sims et al. [7] and Jakob et al. [8] reported that Rovabio™ significantly increased final body weight and average daily body weight gain of broilers and swine respectively. However no research has been conducted to evaluate the effect of Rovabio™ on laying hens.

Rovabio™ contains xylanases (endo-1,4-β-xylanase, α-arabinofuranosidase, β-xylosidase, feruloyl esterase, endo-1,5-α-arabinanase) β- glucanases (endo-1,3(4) β-glucanases, β-1,3-glucanase, endo-1,4-β-glucanase, cellobiohydrolase, β-glucosidase), mannanases (endo-1,4-β-mannanase, β-mannosidase), pectinases (pectinase, polygalacturonase, pectin esterase) and proteases (aspartic protease, metallo protease) [6].

The enzymes in this mixture work together to improve the utilization of feed ingredients. It is reported a mixture of enzymes is more effective than a single enzyme [4]. Feed intake significantly decreased with increasing dietary energy or supplemental fat [9, 10, and 11]. However, Summers and Leeson [12] and Jalal et al. [13] reported that there was no significant effect of dietary energy on feed intake. Decreased feed intake may have a big impact on cost of production. If feed intake cannot be linearly decreased by increased energy, increasing dietary energy by the addition of fat may not be economical. In addition, egg weight is also an important factor that can affect profits. Egg weight increased with increasing dietary energy [10, 11, 14, and 15]. However, Jalal et al. [13] reported that there was no response of egg weight to increasing dietary energy by the addition of fat. There is very limited literature on the effect of dietary energy on hen performance, egg composition, egg solids, egg quality, in second cycle phase two. Therefore it is necessary to have a better understanding on how to optimize the use of dietary energy to get optimal performance and profits of laying hens in second cycle.

Numerous studies have been conducted to determine the effect of enzymes on availability of nutrients in broilers fed diets containing cereal grains that are rich in NSP. However very few studies are available concerning the effect of enzymes in laying hens. Therefore the goal of this study was to evaluate the effect of Rovabio™, dietary energy and protein on performance, egg composition, egg solids, and egg quality of commercial leghorns in phase two second cycle (87 to 98 wk of age).

MATERIAL AND METHODS

A 4 × 2 × 2 factorial arrangement with four dietary energy levels (2791, 2857, 2923 and 2989 kcal/kg ME) and two protein levels (15.5 and 16.1%) with and without Rovabio™ was used in this experiment. Those energy and protein levels of the experimental diets were calculated to meet the nutrient requirement specified by the Hy-Line W-36 management guide [16]. Ingredients and nutrient composition of the experimental diets are shown in Table 1. Hy-Line [17] W-36 hens (n=1920, molted at 66 wk) in their second cycle (87 wk old) were randomly divided among 16 treatments (8 replicates of 15 hens per treatment). Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. Three hens were housed in a 40.6 × 45.7 cm cage. All hens were housed in an environmentally controlled house with temperature maintained at approximately 26^{o}C. The house had controlled ventilation and lighting (16L:8D). All hens were supplied with feed and water *ad libitum*. Animal housing and handling procedures during experimentation were in accordance with guidelines of Auburn University's Institutional Animal Care and Use Committee (IACUC). Feed consumption was recorded weekly for calculation of average daily feed consumption. Egg production was recorded daily and egg weight and specific gravity were recorded once every two weeks. Egg weight and egg specific gravity were measured using all eggs produced during 2 consecutive days. Egg specific gravity was determined using 9 gradient saline solutions varying in a specific gravity from 1.060 to 1.100 in 0.005 unit increments [18]. Mortality was determined daily, and feed consumption was adjusted accordingly. Body weight was obtained by randomly weighing 3 hens (1 of 5 cages) per replicate. Egg mass (gram of egg/hen per day) and feed conversion (gram of feed/g of

egg) were calculated from egg production, egg weight, and feed consumption. Feed samples were analyzed for enzyme activity [19].

Egg components were measured using 3 eggs from each treatment replicate at the middle (92 wk of age) and end (98 wk of age) of the experiment. Eggs were weighed and then broken. The yolks were separated from the albumen. Before yolk weight was determined, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned of any adhering albumen and dried for 5 days. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

Three eggs from each treatment replicate were randomly collected at the middle (92 wk of age) and at the end (98 wk of age) of the experiment for measuring whole solids. The yolk and albumen were mixed and 5 to 6 g of homogenate was pipetted into an aluminum dish with weight recorded to 0.001 g. The sample was dried in an oven for 24 h at 40.5°C [20] and then weighed. Three eggs per treatment replicate were used to analyze the yolk and albumen solids. After yolk was separated from albumen, three yolks and albumen per treatment replicate were mixed separately. The procedure for analyzing albumen and yolk solids was the same as the procedure for whole egg solids content. Yolk color and Haugh unit were measured (3 eggs from each replicate) at the middle (92 wk of age) and at the end (wk 98 of age) of the experiment using a egg multitester EMT-5200 [21]. Haugh units were calculated from the records of albumen height and egg weight using the formula: $HU=100 \log_{10} (H-1.7 W^{0.37}+7.56)$, where HU=Haugh unit, H=height of the albumen (mm) and W=egg weight (g).

Data were analyzed by ANOVA using proc mixed of Statistical Analysis System [22] for a randomized complete block with a factorial arrangement of treatments. Dietary energy, dietary protein and Rovabio were fixed, whereas blocks were random. The factorial treatment arrangement consisted of four dietary energy levels and two protein levels with and without Rovabio™ with eight replicates per treatment. The following model was used to analyze the data:

$$Y_{ijk} = \mu + E_i + P_j + R_k + EP_{ij} + ER_{ik} + PR_{ik} + EPR_{ijk} + B_l + e_{ijk}$$

Where Y_{ijk} = individual observation, μ = overall mean, E_i = dietary energy effect, P_j = protein effect, R_k = Rovabio™ effect, EP_{ij} = interaction between datary energy and protein, ER_{ik} = interaction between dietary energy and Rovabio™, PR_{ik} = interaction between protein and Rovabio™, EPR_{ijk} = interaction among dietary energy, dietary protein and Rovabio™, B_l = effect of block, e_{ijkl} = error component.

If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. Contrast statements were used to test for linear or quadratic dietary energy effects. A significance level of $P \leq 0.05$ was used.

RESULT AND DISCUSSION

There were no significant effects of dietary energy, protein or Rovabio™ on average egg weight (Table 2). However, egg weight of hens fed the diets supplemented with Rovabio™ was significantly higher than that of hens fed the diets without Rovabio during wk 3 and 4 (hens at 89 and 90 wk of age). Egg weight of hens fed the high protein diet was significantly higher than that of hens fed the low protein diet during wk 97 of age and 98 of age (Table 2). The influence of dietary protein on egg weight in this study was consistent with that of Parsons et al. [23], Keshavarz. [24], Leeson [25], Wu et al. [11] and Sohail et al. [15] who reported that egg weight of hens fed higher protein had a higher egg weight than the hens fed lower protein diets.

The mechanism by which dietary protein and energy improves egg weight is well understood; Wu et al. [26] reported that increasing only dietary energy without the increase of other nutrient (protein and amino acids) level did not improve egg weight and both dietary energy and protein (amino acids) are important to optimize egg weight. However conflicting results arise concerning the influence of supplemental energy on egg weight. Wu et al. [26] reported that egg weight linearly increased with increasing dietary energy in Bovans and Dekalb hens during second cycle. Whereas De Groote. [27], Harms et al. [10], Jalal et al. [13] and Leeson [25] reported that egg weight was not influenced by dietary energy. In this study, dietary energy had no influence on average egg weight. This result was in agreement with of Jalal et al. [13], who reported that there was no response of egg weight to dietary energy in Hy-line W-36 hens during second cycle. Linoleic acid content of all experimental diets in this study was more than 1.3%. Grobas et al. [9] reported that linoleic acid content (more than 1.15%) in the diet had no

effect on egg weight, and NRC [28] recommended that the linoleic acid requirement for laying hen is 1.0%. Therefore, it is believed variations in linoleic acid content between diets had no influence on egg weight in this study.

Rovabio™ significantly increased egg weight during wk 89-90 of age and there was a significant interaction among three factors on egg weight during wk 91-92 of age. The effect of Rovabio™ on egg weight was in agreement with Yoruk et al. [29] who reported that egg weight of hens fed diets supplemented with a multi-enzyme similar to Rovabio™ had a increased egg weight in some weeks. Similarly Wu et al. [4] reported that diets supplemented with β-mannanase, which is a constituent in Rovabio™, significantly increased egg weight in some weeks but not all. Effect of multi-enzymes on performance of laying hens may not be explained by simply making NSPs available as an energy source. Multi-enzymes help to improve the utilization of the nutrients found in the feed ingredients by decreasing the viscosity of digesta which impair diffusions of nutrients and reduce availability of nutrients for digestion and absorption [29].

There were no significant effect of Rovabio™ on feed intake (Table 3). However dietary energy and protein had significant effects on feed intake. As dietary energy increased, feed intake linearly decreased by 3.1% from 98.0 to 94.9 g/hen per day. These results agree with those of Wu et al. [26], Sohail et al. [15], Grobas et al. [9] and Parsons et al. [23]. Increasing dietary protein increased feed intake from 95.9 to 97.5 g/hen per day, corresponding to 1.65% increase in feed intake (Table 3). Parsons et al [23] reported that increasing dietary protein from 18 to 20%, increased feed intake from 104 to 107 g/bird per day and Wu et al. [11] also reported that, increasing dietary protein from 14 to 16%, increased feed intake from 102.9 to 105.6 g/hen per day.

Increasing dietary energy linearly decreased protein, TSAA, and lysine intake and increased dietary energy intake (Table 4). Although nutrient intake such as protein and TSAA decreased as dietary energy increased, egg production, egg weight, and egg mass did not decrease. Increasing fat content has an effect of slowing passage rate which lead to increase digestibility of other nutrients such as protein and amino acids [30]. This effect is called "extra caloric effect" of fat. Reginatto et al. [31] reported that increasing dietary energy improved protein utilization in broilers. In this study, also, increasing dietary energy by addition of poultry oil appeared to improve nutrient (protein, lysine or TSAA) utilization.

Hens fed diets supplemented with Rovabio™ with low dietary energy levels (2791 and 2857 kcal of ME/kg) had a significantly higher body weight than the hens fed diets without Rovabio™ at high protein level (Table 5). These results suggest that Rovabio™ helps increase ileal digestibility of feed ingredients, increasing amino acids and/or energy availability. Yoruk et al. [29] also reported a significant effect of a multi-enzyme, which was similar to Rovabio™, on body weight. Marsman et al. [32] found that improvement in the nutritional value of soybeans could be achieved with protease and glucanase enzyme supplementation which are also constituents of Rovabio™. Results of this study suggest that Rovabio™ has the ability to increase energy and/or amino acid availability from feed ingredients. Similarly, Sims et al. [7] and Jacob et al. [8] also reported that broilers and swine fed diets supplemented with Rovabio™ had significantly increased body weights.

There was a significant interaction among dietary protein, energy, and Rovabio™ on egg production (Table 5). Hens fed Rovabio™ supplemented diets with high dietary

protein had a higher egg production than hens fed diets without Rovabio™ at high energy levels (2923 and 2989 kcal of ME/kg). However, hens fed Rovabio™ supplemented diets with low dietary protein had a lower egg production than hens fed diets without Rovabio™ at high energy levels. Results of dietary protein and energy on egg production in this study were consistent with that of Harms et al. [33] and Parsons et al. [23] who reported that egg production was not affected by dietary energy. Similarly, Parsons et al. [23], Sell et al. [34], Wu et al [26] and Sohail et al. [15] reported that there was no consistent effect of dietary energy or dietary protein on egg production.

There was a significant interaction between dietary energy and Rovabio™ on egg albumen solids (Table 6). Rovabio™ significantly increased albumen solids at the lower energy level. There was a significant interaction among dietary protein, energy, and Rovabio™ on egg yolk solids (Table 6). However, significant interaction observed only with low dietary protein level (15.5%). Hens fed Rovabio™ with low dietary protein, had higher egg yolk solids than hens fed diets without Rovabio™ at high energy levels (2923 and 2989 kcal of ME/kg). These results are important for the egg breaker industry as Rovabio™ could be used in some situations to increase solids with diets containing lower dietary protein. However, more research is needed to further explore the influence of Rovabio™ on hen performance, particularly in regard to the potential influence of Rovabio™ on egg weight in young hens and percent yolk solids which would useful to the breaker egg industry.

Dietary protein had a significant effect and dietary energy had a significant linear effect on egg yolk color (Table 4); as dietary energy increased by increasing poultry oil content in the diets from 0 to 4.2%, yolk color index increased from 5.27 to 5.56,

resulting in a net increase of 0.29 units. These results suggest a relationship between dietary fat and egg yolk color. Xanthophyll is the major colorant responsible for the egg yolk color and it is highly fat soluble [35, 36 and 37]. Because xanthophylls are fat soluble, with an increase of dietary fat, more xanthophylls may be absorbed and deposited in the egg yolk. These results agree with those reported by Morihiro et al. [38], Masahiro et al. [39] and Abu Serewa et al. [37]. Increasing dietary protein decreased egg yolk color due to a reduction in corn use, as the level of protein increased. This result was in agreement with that reported by Karunajeewa [40].

There was a significant interaction between dietary protein and energy on egg specific gravity (Table 5). As dietary energy increased, egg specific gravity decreased at higher protein level. This may be due to reduced feed (Ca) intake with the increased supplemented fat. Harms et al. [10] also reported that as dietary energy increased egg specific gravity decreased. There was a significant interaction among dietary protein, energy, and Rovabio™ on egg mass and feed conversion (Table 5). Hens fed diets supplemented with Rovabio™ at the high protein level had a higher egg mass and improved feed conversion at high energy level.

The influence of Rovabio™ on hen performance is complex as indicated by interactions with protein and energy. Because increasing dietary energy significantly reduced feed intake, one would expect to see a decrease in feed intake with the addition of Rovabio™ if it increased dietary energy 66 kcal/lb as suggested by the manufacturer [6]. However, because the difference (66 kcal/lb) in dietary energy levels between diets with and without Rovabio™, is much smaller than that which can be created with fat makes it difficult to statistically show an influence of Rovabio™ on feed intake even if it

were there. Hens fed Rovabio™ had increased body weight apparently because they did not adjust dietary energy intake.

CONCLUSION AND APPLICATIONS

1. Increasing dietary energy by addition of poultry oil significantly decreased feed intake and increased yolk color.
2. Increasing dietary protein significantly increased feed intake, egg weight during last two weeks (wk 97-98 of age), and decreased yolk color.
3. The significant influence of Rovabio™ on hen performance was complex as indicated by the significant Rovabio™, dietary energy and protein interactions on egg weight (91-92 wk of age), egg production, body weight, egg mass, feed conversion and albumen and yolk solids. These interactions suggest that Rovabio™ has at least some influence on utilization of energy, amino acids, or both.

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ACKNOWLEDGEMENT

The authors thank Adisseo Inc., for funding support of this research.

Table 1. Ingredients and nutrient content of the experimental diets

Ingredient	P[1]:16.1 R: -	P:16.1 R: +	P:16.1 R: -	P:16.1 R: +	P:16.1 R: -	P:16.1 R: +	P:16.1 R: -	P:16.1 R: +	P:15.5 R: -	P:15.5 R: +	P:15.5 R: -	P:15.5 R: +	P:15.5 R: -	P:15.5 R: +	P:15.5 R: -	P:15.5 R: +
Corn (8.5%)	66.07	66.07	64.51	64.48	62.98	62.94	61.44	61.42	67.60	67.60	66.08	66.05	64.54	64.49	63.01	63.00
Soybean meal (48.5%)	22.39	22.34	22.50	22.48	22.62	22.61	22.75	22.72	20.86	20.81	20.98	20.96	21.10	21.10	21.24	21.20
Limestone	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12
Hard shell[2]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Dicalcium phosphate	1.50	1.50	1.51	1.51	1.52	1.52	1.52	1.52	1.51	1.51	1.52	1.52	1.52	1.52	1.52	1.52
Poultry oil	0.00	0.00	1.43	1.43	2.83	2.83	4.23	4.23	0.00	0.00	1.39	1.39	2.80	2.80	4.20	4.20
NaCl	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.05	0.05	0.05	0.05	0.06	0.06	0.05	0.05
Rovabio[TM5]	-	0.05	-	0.05	-	0.05	-	0.05	-	0.05	-	0.05	-	0.05	-	0.05
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Calculated analysis																
Crude protein	16.07	16.07	16.00	16.00	15.94	15.94	15.88	15.88	15.45	15.45	15.39	15.39	15.33	15.33	15.26	15.26
Crude fat	2.78	2.78	4.12	4.12	5.44	5.44	6.75	6.75	2.78	2.78	4.12	4.12	5.44	5.44	6.75	6.75
ME (Kcal/kg)	2791	2791	2857	2857	2923	2923	2989	2989	2791	2791	2857	2857	2923	2923	2989	2989
Ca	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Available phosphorus	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Linoleic acid	1.31	1.31	1.61	1.61	1.90	1.90	2.20	2.20	1.32	1.32	1.62	1.62	1.92	1.92	2.21	2.21
Methionine	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.34	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Methionine + Cystine	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59
Tryptophan	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Isoleucine	0.79	0.79	0.78	0.78	0.78	0.78	0.78	0.78	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Lysine	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79

P**1**: Dietary protein, R : Rovabio TM

1 Hard shell = large particle limestone (passing US mesh #4 and retained by US mesh #6) $CaCo_3$ supplied by Franklin Industrial Minerals, Lowell ,FL.

[2] Provided per kilogram of diet: Vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200ICU, vitamin E (as DL – α - tocopheryl acetate), 8 IU; vitamin B12, 0.02mg; riboflavin, 5.5mg; D-calcium pentothenic acid, 13mg; niacin, 36mg; choline, 500mg; folic acid, 0.5mg; vitamin B1(thiamin mononitrate), 1mg; pyridoxine, 2.2mg; biotin, 0.05mg; vitamin K (menadione sodium bisulfate complex), 2mg.

[3] Provided per kilogram of diet: manganese, 65mg; iodine,1mg; iron, 55mg; copper, 6mg; zinc, 55mg; selenium, 0.3mg.

Table 2. Influence of Rovabio^{TM}, protein and energy on egg weight (g) of Hy-Line W-36 hens of 87 to 98 wk of age.

Protein (%)	Dietary energy (kcal/kg)	87-88 wk	89-90 wk	91-92 wk	93-94 wk	95-96 wk	97-98 wk	Mean egg weight (87-98 wk)
15.5		64.42	64.08	63.85	64.26	64.10	64.30	64.14
16.1		64.64	64.34	64.19	64.40	64.47	65.12	64.53
	2791	64.36	63.74	63.97	64.15	64.00	64.37	64.10
	2857	64.12	63.91	63.84	64.11	64.00	64.50	64.08
	2923	64.74	64.68	64.63	64.76	64.61	64.98	64.73
	2989	64.89	64.50	63.65	64.29	64.53	65.00	64.48
Rovabio™	-	64.33	63.86	63.89	64.13	64.05	64.65	64.15
	+	64.72	64.56	64.16	64.53	64.52	64.76	64.54
Pooled SEM		0.55	0.69	0.66	0.65	0.76	0.69	0.5
	----------**Probability**----------							
Protein		NS	NS	NS	NS	NS	0.02	NS
Energy		NS	NS	NS	NS	NS	NS	NS
Rovabio™		NS	0.04	NS	NS	NS	NS	NS
Protein×Energy		NS	NS	NS	NS	NS	NS	NS
Protein×Rovabio		NS	NS	NS	NS	NS	NS	NS
Rovabio×Energy		NS	NS	NS	NS	NS	NS	NS
Protein×Energy×Rovabio™		NS	NS	0.03	NS	NS	NS	NS
Contrasts								
Energy Linear		NS	0.05	NS	NS	NS	NS	NS
Energy quadratic		NS	NS	NS	NS	NS	NS	NS

Table 3. Influence of Rovabio™, protein and energy on feed intake, mortality, and egg composition of Hy-Line W-36 hens of 87 to 98 wk of age.

Protein (%)	Dietary energy (kcal/kg)	Feed Intake (g/henday)	Mortality (%)	Egg composition (%)			Egg composition (g)		
				Yolk	Albumen	Shell	Yolk	Albumen	Shell
15.5		95.92	0.17	28.45	63.12	8.42	18.35	40.86	5.42
16.1		97.50	0.21	28.24	63.38	8.38	18.61	41.84	5.52
	2791	97.96	0.09	28.12	63.38	8.50	18.29	41.40	5.53
	2857	97.34	0.28	28.46	63.26	8.29	18.60	41.44	5.42
	2923	96.63	0.18	28.31	63.38	8.31	18.42	41.34	5.40
	2989	94.90	0.23	28.50	62.99	8.51	18.60	41.23	5.55
Rovabio™	-	96.35	0.19	28.52	63.14	8.35	18.61	41.32	5.44
	+	97.07	0.19	28.17	63.37	8.46	18.35	41.38	5.50
Pooled SEM		0.98	0.13	0.54	0.62	0.19	0.42	1.08	0.12
	----------Probability----------								
Protein		0.03	NS	NS	NS	NS	NS	NS	NS
Energy		0.02	NS	NS	NS	NS	NS	NS	NS
Rovabio™		NS	NS	NS	NS	NS	NS	NS	NS
Protein×Energy		NS	NS	NS	NS	NS	NS	NS	NS
Protein×Rovabio		NS	NS	NS	NS	NS	NS	NS	NS
Rovabio×Energy		NS	NS	NS	NS	NS	NS	NS	NS
Protein×Energy×Rovabio™		NS	NS	NS	NS	NS	NS	NS	NS
Contrasts									
Energy Linear		0.01	NS	NS	NS	NS	NS	NS	NS
Energy quadratic		NS	NS	NS	NS	0.03	NS	NS	0.03

Table 4. Influence of Rovabio™, protein, and energy on energy, protein, TSAA, and lysine intake and egg quality of Hy-Line W-36 hens of 87 to 98 wk of age.

Protein (%)	Dietary energy (kcal/kg)	Nutrient intake per hen daily				Egg quality	
		Energy (kcal)	Protein (g)	TSAA (mg)	Lysine (mg)	Haugh unit	Yolk color
15.5		278	14.9	565.9	757.7	73.57	5.50
16.1		281	15.7	604.5	809.3	72.96	5.34
	2791	274	15.5	592.7	793.6	74.74	5.27
	2857	278	15.4	588.9	788.5	73.73	5.33
	2923	283	15.3	584.8	782.9	70.96	5.52
	2989	284	15.0	574.4	768.9	73.64	5.56
Rovabio™	-	279	15.2	583.0	780.5	74.07	5.41
	+	281	15.3	587.4	786.5	72.46	5.43
Pooled SEM		2.83	0.15	5.95	7.97	2.13	0.14
	----------	----------	----------	**Probability**	----------	----------	----------
Protein		NS	0.01	0.01	0.01	NS	0.02
Energy		0.03	0.02	0.02	0.02	NS	0.01
Rovabio™		NS	NS	NS	NS	NS	NS
Protein×Energy		NS	NS	NS	NS	NS	NS
Protein×Rovabio		NS	NS	NS	NS	NS	NS
Rovabio×Energy		NS	NS	NS	NS	NS	NS
Protein×Energy×Rovabio™		NS	NS	NS	NS	NS	NS
Contrasts							
Energy Linear		0.03	0.02	0.02	0.02	NS	0.01
Energy quadratic		NS	NS	NS	NS	NS	NS

Table 5. Influence of Rovabio™, protein and energy on egg production, egg specific gravity, body weight, egg mass, and feed conversion of Hy-Line W-36 hens of 87 to 98 wk of age.

Protein (%)	Dietary energy (kcal/kg)	Egg production (%)	Specific gravity (Unit)	Body weight (kg)	Egg mass (g/hen/d)	Feed conversion (g feed/g egg)
15.5		77.84	1.0784	1.74	49.96	1.92
16.1		78.65	1.0781	1.80	50.75	1.92
	2791	79.11	1.0786	1.71	50.70	1.93
	2857	78.20	1.0781	1.79	50.12	1.94
	2923	78.34	1.0782	1.73	50.70	1.91
	2989	77.34	1.0781	1.89	49.89	1.91
Rovabio						
	-	78.22	1.0783	1.71	50.19	1.92
	+	78.27	1.0782	1.82	50.52	1.92
Interactions						
Pro: × Ene: × Rov						
15.5 × 2791 × -		79.16	1.0783	1.75	50.58	1.90
15.5 × 2791 × +		79.31	1.0789	1.74	50.72	1.95
15.5 × 2857 × -		76.05	1.0777	1.62	48.29	1.99
15.5 × 2857 × +		79.19	1.0777	1.86	51.06	1.93
15.5 × 2923 × -		79.40	1.0791	1.52	51.28	1.90
15.5 × 2923 × +		76.67	1.0785	1.76	49.83	1.89
15.5 × 2989 × -		77.74	1.0779	1.85	50.03	1.87
15.5 × 2989 × +		75.21	1.0788	1.81	47.86	1.95
16.1 × 2791 × -		80.60	1.0783	1.62	51.68	1.91
16.1 × 2791 × +		77.35	1.0788	1.72	49.81	1.97
16.1 × 2857 × -		79.10	1.0790	1.67	50.42	1.92
16.1 × 2857 × +		78.45	1.0780	2.00	50.72	1.94
16.1 × 2923 × -		77.97	1.0779	1.84	50.32	1.93
16.1 × 2923 × +		79.31	1.0773	1.81	51.37	1.91
16.1 × 2989 × -		75.77	1.0781	1.85	48.91	1.96
16.1 × 2989 × +		80.63	1.0771	1.90	52.77	1.85
Pooled SEM		1.19	0.0004	0.03	0.89	0.02
		----------**Probability**----------				
Protein		NS	NS	0.03	NS	NS
Energy		NS	NS	0.01	NS	NS
Rovabio		NS	NS	0.01	NS	NS
Protein × Energy		NS	0.01	0.02	NS	NS
Protein × Rovabio		NS	NS	NS	NS	NS
Rovabio × Energy		NS	NS	0.01	NS	NS
Pro × En × Rov		0.02	NS	0.02	0.02	0.03
Contrasts						
Energy Linear		NS	NS	0.03	NS	NS
Energy Quadratic		NS	NS	NS	NS	NS

Table 6. Influence of Rovabio™, protein and energy on egg solids of Hy-Line W-36 hens of 87 to 98 wk of age.

Protein (%)	Dietary energy (kcal/kg)	% Solids Whole egg	albumen	yolk
15.5		24.93	12.12	53.26
16.1		25.61	12.30	52.69
	2791	25.10	12.66	52.51
	2857	24.95	12.03	52.97
	2923	24.99	12.14	53.80
	2989	26.05	12.01	52.63
Rovabio				
	-	24.88	12.02	53.07
	+	25.66	12.40	52.89
Interactions				
Pro: × Ene: × Rov				
15.5 × 2791 × -		25.31	11.85	52.20
15.5 × 2791 × +		25.29	12.63	52.76
15.5 × 2857 × -		24.91	11.95	54.35
15.5 × 2857 × +		24.85	11.99	52.36
15.5 × 2923 × -		24.15	12.33	53.69
15.5 × 2923 × +		24.69	12.18	54.93
15.5 × 2989 × -		24.99	12.29	52.74
15.5 × 2989 × +		25.29	11.76	53.08
16.1 × 2791 × -		25.26	11.84	52.83
16.1 × 2791 × +		24.53	14.32	52.25
16.1 × 2857 × -		24.89	12.13	52.81
16.1 × 2857 × +		25.16	12.07	52.37
16.1 × 2923 × -		24.92	11.87	53.22
16.1 × 2923 × +		26.19	12.17	53.36
16.1 × 2989 × -		24.62	11.92	52.71
16.1 × 2989 × +		29.29	12.08	51.98
Pooled SEM		1.15	0.42	0.36
		----------**Probability**----------		
Protein		NS	NS	0.01
Energy		NS	NS	0.02
Rovabio		NS	NS	NS
Protein × Energy		NS	NS	NS
Protein × Rovabio		NS	NS	NS
Rovabio × Energy		NS	0.01	0.03
Prot × En × Rov		NS	NS	0.01
Contrasts				
Energy Linear		NS	NS	NS
Energy Quadratic		NS	NS	0.01

VI. EFFECT OF DIETARY ENERGY ON PERFORMANCE, EGG COMPONENTS, EGG SOLIDS, EGG QUALITY AND PROFITS IN SEVEN COMMERCIAL LEGHORN STRAINS DURING SECOND CYCLE PHASE TWO

ABSTRACT

This study was a 3 X 7 factorial arrangement with three dietary energy levels (low, medium and high) and seven commercial Leghorn strains. The objective of this experiment was to determine the effect of increasing dietary energy on performance, egg composition, egg solids, egg quality, and profits in seven commercial Leghorn strains during second cycle phase 2 (from 88 to 97 week of age). This experiment lasted 10 weeks. Seven strains of hens (n=245 of each strain) at 88 week of age were randomly divided into 21 treatments (6 replicates of 15 birds per treatment). Strain had a significant effect on feed intake, egg production, egg specific gravity, egg weight, percent whole egg solids, and haugh unit. There were no interactions between strain and dietary energy on any parameters during second cycle phase 2 (88 to 97weeks of age). Dietary energy had no significant effect on any parameter. However as dietary energy increased, egg production, final body weight of hens, egg mass, egg yolk color and egg yolk weight numerically increased; moreover feed conversion numerically improved from 2.06 to 2.02, resulting in a 1.94% improvement of feed conversion.

It is difficult to determine an ideal dietary energy level for the hens in second cycle phase 2 because increasing dietary energy had no significant effect on feed intake, egg mass and feed conversion. Because feed ingredient and egg price vary, there can be no fixed ideal dietary energy requirement for optimal profits.

Key words: Protein, Dietary energy, Hens

INTRODUCTION

Protein and energy are the major nutrients of laying hens diets. As much as 85% of total costs of the diet come from protein and energy ingredients. Some studies showed that increasing dietary energy significantly decreased feed intake (Grobas et al., 1999; Harms et al., 2000; Bohnsack et al., 2002; Wu et al., 2005a, b) and improved feed conversion (Wu et al., 2005b), but others have shown that there is no significant effect of dietary energy on feed intake (Summer and Leeson 1993, and Jalal et al., 2006).

Some earlier research indicated that increasing dietary energy significantly increased egg mass (Wu et al., 2005b and Harms et al., 2000) whereas other researchers indicated that there was no significant effect on egg mass (Wu et al., 2005a; Summers and Leeson 1993).

Many studies have shown that increasing dietary energy had no significant effect on egg weight (Jalal et al., 2006; Summer et al., 1993) or on egg production (Grobas et al., 1999; Harms et al., 2000). These results might be due to decreased nutrient (protein and amino acids) intake. As energy content increased in the diet, feed intake normally decreased, resulting in decreased nutrient (protein and amino acids) intake (Guangbing et al., 2007). A better understanding of the effect of increasing dietary energy might help to maximize profits by optimizing egg weight and egg production.

Several commercial Leghorn strains are currently used by egg producers. However, each strain has different production characteristics, percent egg components, egg solids and quality (Wu et al., 2005a, b), some strains may be beneficial for table egg production whereas others may be beneficial for liquid and dried egg processing. Few studies have been conducted to compare responses to dietary energy across strains.

There is little research on the effect of dietary energy on performance, egg components, egg solids, and egg quality in commercial leghorn strains. It is necessary to have a better understanding on how to optimize the use of dietary energy to get optimal performance and profits. Therefore the objective of this experiment was to determine the effect of dietary energy on performance, egg composition, egg solids, egg quality and profits in seven Leghorn strains during second cycle phase 2 (from 88 to 97 week of age).

MATERIALS AND METHODS

This study was a 3 X 7 factorial arrangement with three dietary energy levels (Low, medium and High) and seven commercial Leghorn strains. Ingredients and nutrient composition of experimental diets are shown in table 1.The dietary energy/lysine ratio (334 kcal/g) was maintained the same in three diets.

In this experiment, seven strains of hens (total n=1890) at 88 weeks of age were randomly assigned into 21 treatments (6 replicates of 15 birds per treatment). Hy-line W-36, Dekalb, and several experimental Bovans strains were used in this trial. The trial lasted 10 weeks. Three hens were housed in a 40.6 X 45.7 cm cage, and 5 adjoining cages consisted of a replicate. Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. All hens were housed in an environmentally controlled house with temperature maintained at approximately 26^{o}C as possible.

The house had controlled ventilation and lighting (16L:8D). Hens were supplied with feed and water *ad libitum*. Animal housing and handling procedures during experimentation were in accordance with guidelines of Auburn University's Institutional Animal Care and Use Committee (IACUC). Feed consumption was recorded weekly for calculation of average daily feed consumption. Egg production was recorded daily, and egg weight and specific gravity were recorded once every two weeks. Egg weight and egg specific gravity were measured using all eggs produced during 2 consecutive days. Egg specific gravity was determined using 9 gradient saline solutions varying in specific gravity from 1.060 to 1.100 in 0.005 unit increments (Holder and Bradford, 1979). Mortality was determined daily, and feed consumption was adjusted accordingly. Body weight was obtained by randomly weighing three hens (1 of 5 cages) per replicate at the

end of the experiment. Egg mass (g of egg/hen per day) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption.

Egg components were measured using 3 randomly selected eggs from each treatment replicate at the middle and end of the experiment. Eggs were weighed and broken. The yolks were separated from the albumen. Before the yolk weight was determined, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned of any adhering albumen and dried for 5 days. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

Three eggs from each treatment replicate were randomly collected at the middle and at the end of the experiment for measuring solid. The yolk and albumen were mixed and 5 to 6g of homogenate was pipetted into aluminum dish with weight recorded to 0.001g. The sample was dried in an oven for 24h at 40.5^{0}C (AOAC, 1990) and then weighed. Three eggs which randomly selected from each treatment replicate were used to analyze yolk and albumen solid. After yolk was separated from albumen, three yolks and albumen per treatment replicate were mixed separately. The procedure for analyzing albumen and yolk solid was the same as the procedure for whole egg solid content. Yolk color and haugh unit were measured (3 eggs from each treatment replicate) at the middle and at the end of the experiment using an egg multitester EMT-5200 (Robotmation,co,Ltd. Tokyo, Japan). Haugh unit was calculated from the records of albumen height and egg weight using formula: $HU=100 \log_{10} (H-1.7 W^{0.37}+7.56)$, where HU=Haugh unit, H=height of the albumen (mm) and W=egg weight (g).

Data were analyzed by ANOVA using proc mixed of statistical analysis system (SAS institute, 2000) for a randomized complete block with factorial arrangement of treatments. The factorial treatment arrangement consisted of three dietary energy levels and seven leghorn strains. Dietary energy and strains were fixed; whereas blocks were random, the following model was used to analyze the data:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + P_k + \varepsilon_{ijk}$$

Where Y_{ijk} = individual observation, μ = overall mean, α_i = dietary energy effect, β_j = strain effect, $(\alpha\beta)_{ij}$ = interaction between dietary energy and strain, P_k = effect of block, e_{ijk} = error component. If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. Contrast statements were utilized to test for linear or quadratic dietary energy effects. A significance level of $P \leq 0.05$ was used for analysis.

RESULTS AND DISCUSSION

There were no interactions between strain and dietary energy on all parameters during second cycle phase 2 (88 to 97 weeks of age). Strain had a significant effect on feed intake. There was no significant effect of dietary energy on feed intake, (Table 2). This result is inconsistent with that of Grobas et al (1999), Keshavarz K.&Nakajima et al (1995), Zou geng et al (2005) and Wu et al.(2005a); this might be due to the smaller gap between dietary energy levels (approximately 44 kcal ME/kg) in this experiment, compared to that (approximately over 80 kcal ME/kg) of other experiments. Strain A had the highest feed consumption (106.6g/hen per day) whereas strain C was the lowest feed consuming strain (100.7g/hen per day).

Strain had a significant effect on egg production whereas dietary energy had no significant effect on egg production (Table 2). However, increasing dietary energy increased egg production numerically from 74.6 to 75.8% resulting in a 1.6% increase. This result was consistent with that of Wu et al (2007), who reported that there was no significant effect of dietary energy on egg production. Strain D had the highest egg production (79.0%) and Strain F had the lowest egg production (70.4%).

Dietary energy had no significant effect on egg weight. However, dietary energy numerically increased egg weight from 66.71 to 67.55g, resulting in a 1.3% increase. Strain had a significant affect on egg weight (Table 2). Strain A had the highest egg weight (69.18g) whereas strain D had the lowest egg weight (66.36g). Result of dietary energy on egg weight of this study was inconsistent with that of Wu et al (2007), who reported that there was a significant effect of dietary energy on egg weight. This may be due to the comparatively larger gap between dietary energy levels (2747, 2874, and 3002

kcal/kg ME). However, percent egg yolk did not increase with the increasing dietary energy (Table 3). This suggested that older hens as in this experiment may have the ability to synthesize sufficient lipoprotein. Thus, increasing dietary fat may not help to increase egg yolk weight in older hens.

Neither strain nor dietary energy had a significant effect on egg mass (Table 2). However, egg mass was numerically increased from 49.8 to 51.16 g of egg/hen per day. Similarly both strain and dietary energy had no significant effect on feed conversion (Table 2). However, as dietary energy increased, feed conversion numerically improved from 2.06 to 2.02, resulting in a 1.94% improvement of feed conversion. It is difficult to determine an ideal dietary energy for the hens in second cycle phase 2, because increasing nutrient density had no significant effect on feed intake, egg mass and feed conversion.

Strain significantly affected egg specific gravity (Table 2). Egg specific gravity ranged from 1.075 to 1.077. Dietary energy had no significant effect on egg specific gravity probably because egg weight did not significantly increase with increasing dietary energy.

Dietary energy had no significant effect on haugh unit (Table 3). However, As dietary energy increased, haugh unit numerically decreased from 73.72 to 73.16 units, this may be due to increased egg weight with increased dietary energy. Strain had a significant effect on haugh unit. Strain B had the highest quality eggs whereas strain E and F had the lowest. Increasing dietary energy had no effect on egg components, egg solids, egg quality and albumen, yolk, and shell weights in second cycle phase 2 (88 to 97 week of age). Wu et al., 2007 also reported that there was no significant effect of dietary

energy on egg composition, shell weight, yolk and whole egg solids and yolk color during second cycle phase 1. Strain significantly affected whole egg solids. Strain C had the highest whole egg solids and strain A had the lowest whole egg solids.

The Econometric Feeding and Management Program developed by Roland et al., (1998, 2000) was used to calculate profits of different dietary energy levels at different poultry oil prices and egg prices (Table 4). When egg price was high, at both high and low poultry oil prices, maximum profit per dozen eggs was obtained in the hens fed low energy diets. Similarly, both at high and low poultry oil prices, equal profits (0.220 $/dozen) obtained in the hens fed low energy diets at low egg prices. Since feed ingredient prices and egg price vary, there can be no fixed ideal energy or a constant energy/lysine ratio for optimal profits during phase 2 (wk 88 to 97).

In conclusion, Strain had a significant effect on feed intake, egg production, egg specific gravity, egg weight, percent whole egg solids, and haugh unit. There were no interactions between strain and dietary energy on any parameters during second cycle phase 2 (88 to 97weeks of age). Dietary energy had no significant effect on any parameter. However as dietary energy increased, egg production, final body weight of hens, egg mass, egg yolk color and egg yolk weight numerically increased; moreover feed conversion numerically improved from 2.06 to 2.02, resulting in a 1.94% improvement of feed conversion. It is difficult to determine an ideal dietary energy level for the hens in second cycle phase 2 because increasing dietary energy had no significant effect on feed intake, egg mass and feed conversion. Because feed ingredient and egg price vary, there can be no fixed ideal dietary energy requirement for optimal profits.

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Table 1**:** Ingredients and nutrient content of Experimental diets.

Ingredient (%)	Diet 1	Diet 2	Diet3
Corn (8.6%)	65.65	63.66	61.65
Soy bean meal (48%)	22.42	23.02	23.62
Hard shell[1]	4.00	4.00	4.00
Limestone	5.51	5.66	5.82
Dicalcium phosphate	1.50	1.54	1.58
Poultry oil	0.00	1.17	2.36
NaCl	0.36	0.37	0.38
Vitamin Premix[2]	0.25	0.25	0.25
Mineral Premix[3]	0.25	0.25	0.25
DL-Methionine	0.06	0.07	0.08
Total	100	100	100
Calculated analysis			
ME (Kcal/kg)	2,776	2820	2864
Crude protein	16.05	16.18	16.32
Ca	4.15	4.22	4.29
Available Phosphorus	0.39	0.39	0.39
Sodium	0.17	0.17	0.18
Methionine	0.33	0.34	0.35
Methionine + Cystine	0.62	0.63	0.64
Lysine	0.83	0.84	0.86
Dietary energy/Lysine ratio (ME/g)	334	334	334

[1] Hard shell = large particle limestone (passing US mesh #4 and retained by US mesh #6) $CaCo_3$ supplied by Franklin Industrial Minerals, Lowell ,FL.
[2] Provided per kilogram of diet: Vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200ICU, vitamin E (as DL – α - tocopheryl acetate), 8 IU; vitamin B12, 0.02mg; riboflavin, 5.5mg; D-calcium pentothenic acid, 13mg; niacin, 36mg; choline, 500mg; folic acid, 0.5mg; vitamin B1(thiamin mononitrate), 1mg; pyridoxine, 2.2mg; biotin, 0.05mg; vitamin K (menadione sodium bisulfate complex), 2mg.
[3] Provided per kilogram of diet: manganese, 65mg; iodine,1mg; iron, 55mg;copper, 6mg;zinc, 55mg; selenium, 0.3mg.

Table 2.Effect of strain and dietary energy on performance of seven commercial white leghorns during second cycle phase 2 (88wk to 97wk of age)

Dietary energy	Strain	Feed intake (g/hen day)	Egg production (%)	Egg specific gravity (Unit)	Body Weight (Kg)	Mortality (%)	Egg mass (g egg/h day)	Feed conversion (g feed /g egg)	Egg weight (g)
Low		103.1	74.6	1.076	1.80	0.18	49.81	2.06	66.71
Medium		103.5	75.0	1.076	1.87	0.33	50.72	2.04	67.63
High		103.8	75.8	1.076	1.89	0.17	51.16	2.02	67.55
	Strain A	106.6 [a]	73.3 [bc]	1.077[ab]	1.88	0.30	50.67	2.11	69.18 [a]
	Strain B	105.2 [ab]	77.6 [ab]	1.077 [a]	1.91	0.09	52.11	2.05	67.02 [b]
	Strain C	100.7 [c]	73.4 [bc]	1.076 [abc]	1.84	0.22	48.33	2.06	66.49 [b]
	Strain D	104.3 [ab]	79.0 [a]	1.075 [c]	1.89	0.13	52.44	1.99	66.36 [b]
	Strain E	102.4 [bc]	76.9 [ab]	1.077 [ab]	1.87	0.35	51.28	2.00	66.68 [b]
	Strain F	102.6 [bc]	70.4 [c]	1.075 [bc]	1.87	0.30	48.55	2.12	68.99 [a]
	Strain G	102.4 [bc]	75.4 [ab]	1.075 [c]	1.73	0.19	50.06	2.05	66.37 [b]
Pooled SEM		1.38	2.94	0.0091	0.048	0.17	2.13	0.07	0.86
		----------**Probability**----------							
Strain		0.0123	0.0083	0.0064	NS	NS	NS	NS	<0.0001
Dietary Energy		NS	NS	NS	NS	NS	NS	NS	0.0935
Strain×Energy		0.0838	NS	NS	NS	NS	NS	NS	NS
Contrasts									
Energy Linear		NS	NS	NS	NS	NS	NS	NS	NS
Energy quadratic		NS	NS	NS	NS	NS	NS	NS	NS

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($p \leq 0.05$).

Table 3. Effect of strain and dietary energy on egg components, egg solids, egg quality and albumen, yolk, shell weight of seven commercial white leghorns during second cycle phase 2 (88wk to 97wk of age)

Dietary energy	Strain	Egg components (%)			Egg solids (%)			Egg quality		Albumen, Yolk, Shell weight(g)		
		Yolk	Albumen	Shell	Whole egg	Albumen	Yolk	Haugh Unit	Yolk color	Albumen	Yolk	Shell
Low		26.51	65.22	8.26	24.48	11.95	52.72	73.72	5.31	42.99	17.42	5.44
Medium		26.72	65.03	8.25	24.04	11.49	52.28	73.40	5.32	42.89	17.59	5.44
High		26.65	65.30	8.05	24.34	11.47	51.56	73.16	5.42	43.42	17.69	5.34
	Strain A	26.90	64.76	8.33	23.49 [c]	12.04	52.46	73.06 [abc]	5.33	43.70	18.15	5.62
	Strain B	27.03	64.57	8.39	24.27 [abc]	11.46	52.20	77.22 [a]	5.19	42.00	17.59	5.46
	Strain C	27.32	64.60	8.08	24.89 [a]	11.57	53.02	72.64 [bc]	5.31	42.61	17.99	5.33
	Strain D	26.88	65.04	8.08	24.69 [ab]	11.58	51.36	73.06 [abc]	5.28	42.60	17.53	5.26
	Strain E	26.41	65.53	8.06	24.58 [ab]	11.47	52.19	69.57 [c]	5.42	42.55	17.06	5.21
	Strain F	25.44	66.46	8.10	24.06 [bc]	11.81	51.78	71.77 [c]	5.44	44.74	17.08	5.45
	Strain G	26.40	65.32	8.27	24.04 [bc]	11.55	52.30	76.69 [ab]	5.47	43.51	17.56	5.50
Pooled SEM		0.63	0.71	0.24	0.49	0.30	0.75	2.73	0.20	0.37	0.48	0.18
		Probability										
Strain		NS	NS	NS	0.0153	NS	NS	0.0112	NS	NS	NS	NS
Dietary Energy		NS	NS	NS	NS	0.06	NS	NS	NS	NS	0.06	NS
Strain×Energy		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Contrasts												
Energy Linear		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ene:quadratic		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a – c] Means within a column and under each main effect with no common superscripts differ significantly ($p \leq 0.05$)

Table 4. Influence of dietary energy and poultry oil price on profits[1] from 88 to 97wk of age

		Nutrient density		
		Low	Medium	High
		----------Returns[4] ($/dozen) ----------		
High poultry oil Price ($ 0.40/kg)	High egg price[2]	0.368	0.362	0.355
	Low egg price[3]	0.220	0.214	0.208
Low poultry oil Price ($ 0.22/kg)	High egg price	0.368	0.365	0.362
	Low egg price	0.220	0.218	0.215

[1] Corn price = $0.12/kg, soy price = $0.39/kg, $Caco_3$ = $0.03/kg, hard shell = $0.03/kg,Dicalcium phosphate = $0.027/kg, salt = $0.06/kg, vitamin premix = $2.67/kg, mineral premix = $0.59/kg, DL-methionine = $2.59/kg.

[2] High Urner Berry egg price: jumbo size = 120 cents, extra large size = 117cents, large size = 112 cents, medium size = 75 cents and small size = 54 cents.

[3] Low Urner Berry egg price: jumbo size = 105 cents, extra large size = 101cents, large size = 97 cents, medium size = 75 cents and small size = 54 cents.

[4] Returns (R) were calculated using the equation R = UBEP – NR – PC – FdC, where UBEP = Urner Berry Egg Price, NR = nest run into package product delivered, PC = production cost and FdC = feed cost as described by Roland et al.(1998, 2000).

VII. EFFECT OF DIETARY PROTEIN AND PEPTIDE IN CORN-SOY DIETS ON HEN PERFORMANCE, EGG SOLIDS, EGG COMPOSITION AND EGG QUALITY OF HY – LINE W-36 HENS DURING SECOND CYCLE PHASE THREE

ABSTRACT

A 5 × 2 factorial arrangement of five protein levels with and without Peptiva was conducted to evaluate the effect of Peptiva on performance, egg composition, egg solids, and egg quality of commercial Leghorns. Hy-white W-36 hens (n=1200, 98 weeks old) were randomly divided into 10 dietary treatments (8 replicates of 15 hens per treatment). The experiment lasted 12 weeks.

Protein had a significant effect on feed consumption, egg weight, egg production, egg mass, egg specific gravity, egg albumen solids, and percent egg components. As dietary protein increased from 13.53 to 15.62%, egg production, feed consumption and egg weight increased by 6.14%, 8.2% and 5.18% respectively. Feed consumption of hens fed the diets supplemented with Peptiva was significantly lower than that of hens fed the diets without Peptiva. Peptiva supplementation also significantly increased egg production of hens during week 98 and numerically higher in week 99, 103, 105, 106, 107, 109 and overall egg production. There was also a significant effect of peptiva on egg mass and feed conversion during first week but the significant effects were lost after second week.

Peptiva significantly decreased feed intake without causing any adverse effects on egg weight and egg production. Peptiva might be more beneficial for young hens. More research is needed with young hens to evaluate performance and profits of commercial layers at different egg and ingredient prices.

Key words: Protein, Dietary energy, Hens, Peptiva

INTRODUCTION

Protein is a critical nutrient for laying hen growth, production and health. The quality of protein depends on AA balance as well as digestion and absorption in the small intestine. Soy protein is the most commonly used protein source in the laying hen industry. However, layers are not well adapted to soy protein because of the presence of anti-nutritional factors, such as glycinin and β- conglycinin (Sissons and Smit, 1976). Laying hen nutritionists are interesting in finding appropriate substitutes for soy protein, due to the high cost of animal protein, and public concerns about feeding animal products back to animals. Since the identification of peptide transporters and increased efforts directed at peptide research, utilization of small peptides as a protein source in the animal industry becomes a reality; however, limited information regarding their effects on laying hen performance is available.

It was thought that all dietary proteins need to be hydrolyzed to free amino acids in order to be absorbed. This theory changed when the first intestinal oligo-peptide transporter, PepT1, was identified by two separate groups in rats and rabbits (Boll et al., 1994; Fei et al., 1994). The peptide transporter was then identified and characterized in domestic animals like pigs (Klang et al., 2005), chickens (Chen et al., 1999), turkey (Van et al., 2005), and ruminant animals (Chen et al., 1999). The PepT1 protein is located at the brush border membrane of intestinal epithelial cells (Leibach and Ganapathy, 1996), and has been shown to have rather broad substrate specificity, compared to the relatively narrow substrate specificity of most free amino acids transporters.

Peptide absorption from the lumen is faster and more efficient than amino acids transportation (Johnson, 1997a). Hypothetically, it is therefore possible that incorporation

of small peptides or hydrolyzed protein into the diet would be beneficial for layers. However, very limited information is available using peptides as protein sources in laying hen diets because of the lack of manufacturing and the difficulty of peptide detection methods. There are few commercial peptide products available for use in the laying hen diets. Peptiva® is produced by Vitech BioChem Corporation (San Fernando, CA), by blending appropriate amount of porcine mucosa peptides, fish peptides, and microbial peptides. It is claimed to contain feed stimulating peptides, small intestine activity peptides, exorphine peptides, immune modulating peptides and anti-microbial peptides (www.vitechusa.com).

Protein is a major nutrient representing a high percentage of total cost of the laying hen diets. Liu et al.(2004; 2005) and Wu et al. (2005) reported that increasing protein level significantly improved egg production, egg weight, feed consumption, feed conversion, egg specific gravity, and body weight of hens. Even though feeding high protein diets can optimize performance, the cost of high protein diets and egg prices determine the level needed for optimal profits. As protein requirement decrease as hens age, normally, hens during second cycle are fed lower protein diets ranging from 14 to 16%.

The liquid and breaker egg industry have grown during last 10 years. Egg components and egg solids might be altered by manipulation of protein. Increasing protein significantly increased percent albumen (Shafter et al.,1998 and Novak et al.,2004). More research is needed to investigate the effect of protein on egg solids to improve the profits of egg further processing industry. Therefore the objective of this study was to determine the effect of dietary protein and Peptide on performance, egg

solids, egg composition and egg quality of Hy-line W- 36 hens during second cycle phase three (98 to 109wk).

MATERIAL AND METHODS

A 5 × 2 factorial arrangement with five protein levels (13.56, 14.01, 14.52 15.06 15.62%) with and without Peptiva was used in this experiment. Ingredients and nutrient composition of the experimental diets are shown in Table 1. Hy-Line W-36 hens (n=1200, molted at 66wk) in their second cycle (98wk old) were randomly divided among 10 treatments (8 replicates of 15 hens per treatment). Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. Three hens were housed in a 40.6 X 45.7cm cage, and 5 adjoining cages consisted of a replicate. Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. All hens were housed in an environmentally controlled house with temperature maintained at approximately 26^{o}C. The house had controlled ventilation and lighting (16L:8D). All hens were supplied with feed and water *ad libitum*. Animal housing and handling procedures during experimentation were in accordance with guidelines of Auburn University's Institutional Animal Care and Use Committee (IACUC).

Feed consumption was recorded weekly for calculation of average daily feed consumption. Egg production was recorded daily and egg weight and specific gravity were recorded once every two weeks. Egg weight and egg specific gravity were measured using all eggs produced during two consecutive days. Egg specific gravity was determined using 9 gradient saline solutions varying in a specific gravity from 1.060 to 1.100 in 0.005 unit increments (Holder and Bradford, 1979). Mortality was determined daily, and feed consumption was adjusted accordingly. Body weight was obtained by randomly weighing three hens (1 of 5 cages) per replicate at the end of the experiment.

Egg mass (g of egg/hen per day) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption. Feed samples were sent for Peptide activity analysis[1].

Egg components were measured using 3 eggs from each treatment replicate at the middle (103 wk of age) and end (109 wk of age) of the experiment. Eggs were weighed and the broken. The yolks were separated from the albumen. Before the yolk weight was determined, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned of any adhering albumen and dried for 5 days. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

Three eggs from each treatment replicate were collected at the middle (103 wk of age) and at the end (109 wk of age) of the experiment for measuring whole solid. The yolk and albumen were mixed and 5 to 6g of homogenate was pipetted into aluminum dish with weight recorded to 0.001g. The sample was dried in an oven for 24h at 40.5°C (AOAC,1990) and then weighed. Three eggs per treatment replicate were used to analyze yolk and albumen solid. After yolk was separated from albumen, three yolks and albumen per treatment replicate were mixed separately. The procedure for analyzing albumen and yolk solid was the same as the procedure for whole egg solid content. Yolk color and haugh unit were measured (3 eggs of each treatment replicate) at the middle (103 wk of age) and at the end (109 wk of age) of the experiment by a egg multitester EMT-5200 (Robotmation,co,Ltd.Tokyo,Japan). Haugh units were calculated from the records of albumen height and egg weight using the formula: $HU=100 \log_{10} (H-1.7 W^{0..37}+7.56)$, where HU=Haugh unit, H=height of the albumen (mm) and W=egg weight

Data were analyzed by proc ANOVA using proc mixed of Statistical Analysis System (SAS institute, 2000) for a randomized complete block with factorial arrangement of treatments. Dietary protein and Petiva were fixed, whereas blocks were random. The factorial treatment arrangement consisted of five protein levels with and without Peptiva. The following model was used to analyze the data:

$$Y_{ijk} = \mu + P_j + R_k + PR_{ik} + B_1 + e_{jk}$$

Where Y_{ijk} = individual observation, μ = experimental mean, P_j = protein effect, R_k = Peptiva effect, PR_{ik} = interaction between protein and Peptiva, B_1 = effect of block, e_{ijk} = error component.

If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. A significance level of $p \leq 0.05$ was used during analysis.

[1] Vitech Bio-Chem Corporation California, USA

RESULTS AND DISCUSSION

There were no interactions between dietary protein and Peptiva on any factor. Protein had a significant linear effect on feed consumption (Table 2). Hens fed 15.62% protein level had the highest feed consumption. Similarly, Wu et al. (2005) and Liu et al. (2004 and 2005) reported that feed consumption significantly increased with increased dietary protein levels. Increased feed consumption of laying hens fed the high dietary protein might be due to low dietary energy content in the high protein diets. Increasing dietary protein significantly increased egg weight (Table 2). The influence of dietary protein on egg weight in this study was consistent with that of Parsons et al. (1993), Keshavarz. (1995), Leeson (1989), Wu et al. (2005) and Sohail et al. (2003) who reported that egg weight of hens fed higher dietary protein had a higher egg weight than the hens fed lower protein diets.

Increasing dietary protein intake from 11.8 to 14.7 g/hen/day, increased egg production from 63.5 to 68.6% (Table 3). Similarly, Liu et al. (2004) and Wu et al. (2005) reported that increasing dietary protein improved egg production. Zou et al. (2005) reported that increasing dietary protein intake from 15.3 to 16.3 g/hen/day, increased egg production by 3.2%. Keshavarz (1995) indicated that with an increase of dietary protein intake from 17.4 to 21.4 g/hen/day, egg production increased by 1.9%. Increasing dietary protein intake from 11.8 to 14.7 g/hen/day, increased egg mass 4.97g/hen day. (Table 4).

As dietary protein increased from 13.56 to 15.62%, percent albumen, percent albumen solids linearly increased and percent yolk linearly decreased (Table 6). Similarly, Shafer et al. (1998) and Novak et al. (2004) reported that increasing amino

acids such as methionine and lysine significantly increased percent albumen. These results are important for the egg breaker industry.

Peptiva significantly decreased feed consumption from 91.8 to 90.4 g/hen per day or by 1.49% (Table 2). The mechanism on how peptiva influence feed consumption without adversely affect on performance is not yet known well. Peptiva supplementation also significantly increased egg production of hens during week 98 and numerically higher in week 99, 103, 105, 106, 107, 109 and overall egg production (Table 3). There was also a significant effect of peptiva on egg mass (Table 4) and feed conversion (Table 5) during first week.

In conclusion, Protein had a significant effect on feed consumption, egg weight, egg production, egg mass, egg specific gravity, egg albumen solids, and percent egg components. As dietary protein increased from 13.53 to 15.62%, egg production, feed consumption and egg weight increased by 6.14%, 8.2% and 5.18% respectively. Feed consumption of hens fed the diets supplemented with Peptiva was significantly lower than that of hens fed the diets without Peptiva. Peptiva supplementation also significantly increased egg production of hens during week 98 and numerically higher in week 99, 103, 105, 106, 107, 109 and overall egg production. There was also a significant effect of peptiva on egg mass and feed conversion during first week but the significant effects were lost after second week.

Peptiva significantly decreased feed intake without causing any adverse effects on egg weight and egg production. Peptiva might be more beneficial for young hens. More research is needed with young hens to evaluate performance and profits of commercial layers at different egg and ingredient prices.

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Table 1. Ingredients and nutrient content of the experimental diets.

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
Corn (8.6%)	66.30	66.30	68.05	68.05	69.76	69.76	71.40	71.40	72.90	72.90
Soy bean meal 48%	20.59	20.59	19.14	19.14	17.72	17.72	16.38	16.38	15.12	15.12
Limestone	7.24	7.24	7.25	7.25	7.25	7.25	7.26	7.26	7.30	7.30
Hard shell[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium Phosphate	1.30	1.30	1.30	1.30	1.32	1.32	1.32	1.32	1.33	1.33
Poultry oil	1.62	1.62	1.33	1.33	1.04	1.04	0.77	0.77	0.51	0.51
NaCl	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Vitamin Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.06	0.06	0.04	0.04	0.03	0.03	0.01	0.01	0.00	0.00
Peptiva[4]	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis (%)										
Crude protein	15.62	15.62	15.06	15.06	14.52	14.52	14.01	14.01	13.53	13.53
ME (Kcal/kg)	1310	1310	1310	1310	1310	1310	1310	1310	1310	1310
Ca	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Avilable phosphorus	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Methionine	0.32	0.32	0.30	0.30	0.27	0.27	0.25	0.25	0.24	0.24
Methionine + Cystine	0.59	0.59	0.56	0.56	0.53	0.53	0.51	0.51	0.48	0.48
Lysine	0.79	0.79	0.75	0.75	0.71	0.71	0.68	0.68	0.64	0.64

1 Hard shell = large particle limestone (passing US mesh #4 and retained by US mesh #6) $CaCo_3$ supplied by Franklin Industrial Minerals, Lowell ,FL.

[2] Provided per kilogram of diet: Vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200ICU, vitamin E (as DL – α - tocopheryl acetate), 8 IU; vitamin B12, 0.02mg; riboflavin, 5.5mg; D-calcium pentothenic acid, 13mg; niacin, 36mg; choline, 500mg; folic acid, 0.5mg;vitamin B1(thiamin mononitrate), 1mg; pyridoxine, 2.2mg; biotin, 0.05mg; vitamin K (menadione sodium bisulfate complex), 2mg.

[3] Provided per kilogram of diet: manganese, 65mg; iodine,1mg; iron, 55mg; copper, 6mg; zinc, 55mg; selenium, 0.3mg.

[4]Vitech Bio-Chem Corporation California, USA recommended addition rate =0.2%

Table 2. Influence of Peptiva and protein, on feed consumption, egg specific gravity, body weight, egg weight, and mortality of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

Protein (%)		Feed consumption (g/hen day)	Egg Specific Gravity (Unit)	Body Weight (Kg)	Egg weight (g)	Mortality (%)
13.56		86.98	1.0754	1.76	61.33	0.31
14.01		88.58	1.0751	1.82	62.45	0.21
14.52		92.07	1.0777	1.77	63.31	0.16
15.06		93.73	1.0751	1.78	64.74	0.11
15.62		94.12	1.0755	1.75	64.51	0.21
Peptiva	-	91.78	1.0755	1.79	63.33	0.15
	+	90.41	1.0760	1.76	63.21	0.25
Pooled SEM		0.77	0.00076	0.057	0.78	0.12
		----------Probability----------				
Protein		<0.0001	0.0045	NS	<0.0001	NS
Peptiva		0.05	NS	NS	NS	NS
Protein×Peptiva		NS	NS	NS	NS	NS

Table 3. Influence of Peptiva and protein, on egg production (%) of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

Protein (%)		98 wk	99 wk	100 wk	101 wk	102 wk	103 wk	104 wk	105 wk	106 wk	107 wk	108 wk	109 wk	Average egg production (%)
13.56		66.96	67.92	65.83	64.17	62.41	61.91	57.62	60.95	61.74	63.58	64.82	64.20	63.51
14.01		69.64	68.99	65.95	64.46	64.58	63.51	64.20	63.72	61.46	63.26	65.73	64.58	65.01
14.52		64.82	65.51	66.43	68.93	67.08	64.49	67.08	65.66	62.57	63.66	68.35	68.48	66.09
15.06		71.73	69.94	71.07	70.74	69.17	65.98	68.12	68.19	63.47	64.47	69.75	69.61	68.52
15.62		70.54	68.99	69.76	69.61	69.76	68.48	69.82	69.94	62.71	64.01	68.24	70.74	68.55
Peptiva	-	66.95	66.84	67.90	67.75	66.62	64.30	65.46	65.43	62.28	63.19	67.41	66.26	65.87
	+	70.52	69.69	67.71	67.42	66.58	65.45	65.27	65.95	62.50	64.40	67.35	68.79	66.80
Pooled SEM		2.62	2.45	2.32	2.76	2.29	2.41	2.45	2.04	2.80	2.64	2.24	2.21	1.82
		Probability												
Protein		0.06	NS	0.07	0.05	0.04	0.05	0.001	0.001	NS	NS	0.04	0.001	0.004
Peptiva		0.03	0.07	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein×Peptiva		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 4. Influence of Peptiva and protein, on egg mass (g egg/h/d) of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

Protein (%)		98 wk	99 wk	100 wk	101 wk	102 wk	103 wk	104 wk	105 wk	106 wk	107 wk	108 wk	109 wk	Average egg mass
13.56		42.16	42.41	41.16	39.18	37.72	37.55	34.83	37.26	38.87	39.39	39.56	39.39	39.12
14.01		44.09	43.47	41.73	39.69	40.21	39.39	40.24	39.40	38.88	39.53	41.18	41.91	40.81
14.52		41.15	41.37	42.85	42.82	42.86	40.79	42.32	41.49	40.19	40.96	43.39	44.35	42.05
15.06		46.56	46.06	46.48	45.09	44.69	42.89	43.73	43.83	41.24	42.14	44.80	42.84	44.20
15.62		44.92	45.25	45.15	44.23	45.24	44.26	44.98	45.28	40.63	41.65	43.88	43.59	44.09
Peptiva	-	42.59	42.96	43.63	42.75	42.02	40.53	41.31	41.22	39.78	40.22	42.62	42.08	41.81
	+	44.97	44.47	43.32	41.66	42.28	41.43	41.13	41.68	40.15	41.25	42.51	42.75	42.30
Pooled SEM		1.86	1.71	1.67	1.92	1.72	1.71	1.81	1.46	1.86	1.72	1.70	1.52	1.38
		Probability												
Protein		0.03	0.04	0.009	0.007	0.001	0.002	0.001	0.001	0.05	NS	0.01	0.01	0.0001
Peptiva		0.04	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pro×Peptiva		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 5. Influence of Peptiva and protein, on feed conversion (g feed/g egg) of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

Protein (%)		98 wk	99 wk	100 wk	101 wk	102 wk	103 wk	104 wk	105 wk	106 wk	107 wk	108 wk	109 wk	Average feed conversion
13.56		2.18	2.12	2.12	2.19	2.30	2.29	2.43	2.40	2.40	2.24	2.24	2.28	2.27
14.01		2.04	2.02	2.11	2.17	2.19	2.21	2.26	2.36	2.35	2.18	2.16	2.21	2.19
14.52		2.26	2.25	2.16	2.11	2.16	2.30	2.18	2.26	2.30	2.14	2.14	2.12	2.20
15.06		1.99	2.05	2.03	2.03	2.14	2.24	2.17	2.14	2.32	2.19	2.11	2.22	2.14
15.62		2.07	2.09	2.07	2.09	2.12	2.14	2.14	2.13	2.38	2.20	2.16	2.19	2.15
Peptiva	-	2.18	2.15	2.11	2.10	2.21	2.27	2.24	2.30	2.34	2.24	2.17	2.24	2.21
	+	2.03	2.06	2.08	2.13	2.15	2.21	2.23	2.21	2.36	2.14	2.15	2.16	2.16
Pooled SEM		0.07	0.06	0.05	0.06	0.06	0.07	0.07	0.07	0.09	0.07	0.07	0.07	0.05
		Probability												
Protein		0.08	NS	NS	NS	NS	NS	0.04	0.03	NS	NS	NS	NS	NS
Peptiva		0.03	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Prot×Peptiva		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 6. Influence of Peptiva and protein, on egg components, egg solids, and egg quality of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

	Egg components (%)			Egg solids (%)			Egg Quality	
Protein (%)	Yolk	Albumen	Shell	Whole egg	Albumen	Yolk	Haugh Unit	Yolk color
13.56	29.71	61.71	8.58	25.35	11.20	54.36	71.29	5.69
14.01	28.95	63.06	7.99	25.75	11.49	53.72	72.93	5.50
14.52	28.33	63.58	8.09	25.56	11.57	54.34	70.36	5.47
15.06	27.99	63.95	8.06	26.05	11.72	54.72	68.79	5.53
15.62	27.80	64.35	7.85	25.45	11.95	54.06	68.23	5.25
Peptiva -	28.58	63.41	8.02	25.64	11.63	54.51	71.61	5.55
+	28.53	63.26	8.21	25.62	11.54	53.97	69.03	5.43
Pooled SEM	0.71	0.54	0.23	0.42	0.24	0.53	2.37	0.17
	----------Probability----------							
Protein	0.05	0.006	0.03	NS	0.04	NS	NS	NS
Peptiva	NS	NS	NS	NS	NS	NS	NS	NS
Protein × Peptiva	NS	NS	NS	NS	NS	NS	NS	NS

VIII. CONCLUSIONS

Brown Egg Layer Strains Experiment 1 (Section II)

The results showed that there were no interactions between lysine and strain on any parameter. Lysine had significant effects on egg weight, egg mass, feed conversion, percent albumen solids, yolk color, shell color, albumen weight, egg shell and albumen components. There were significant strain effects on egg production, feed consumption, egg weight, egg mass, feed conversion, specific gravity, yolk weight, shell color, shell, albumen and yolk components, yolk, albumen and whole egg solids. Strain 1 had the best overall performance. All strains peaked in production over 94% and were laying 94 to 96% at 36 weeks of age. Average egg weight (21wk to 36wk) was 60.3g, varying from 59.0 to 62.8 g between strains. Average feed intake was 112.3g/hen/day varying from 109.6 to 116.7g/hen/day between strains. Average egg weight of hens fed diets containing the highest lysine level was 2.04 g heavier than the hens fed the diets containing the lowest lysine level. Increasing dietary lysine from 0.747 to 0.917% significantly improved feed conversion from 2.20 to 2.06 g feed/g egg and increased egg mass from 51.8 to 54.32 g/hen/day. Average lysine intake of hens fed 0.917% level was 1023mg/hen/day varying from 1005 to 1070mg/hen/day between strains. Because egg prices and ingredient prices often change, there can be no fixed dietary lysine level for optimal profits.

Brown Egg Layer Strains Experiment 2 (Section III)

The results showed that there were no interactions between lysine and strain on any parameter. Lysine had significant effects on feed consumption, egg production, egg mass, feed conversion, egg weight, egg shell components, percent yolk and whole egg solids, albumen and yolk weight, egg specific gravity, yolk color, and haugh units. There were significant strain effects on feed consumption, egg mass, feed conversion, egg weight, albumen and yolk components, whole egg solids, albumen and shell weight, egg specific gravity, body weight, shell color, and haugh unit. Strain 1 had the best overall performance. All strains were laying 89.5 to 92.5% at 52 weeks of age. Average egg weight (39wk to 52wk) was 63g, varying from 61.5 to 63.6 g between strains. Average feed intake was 112.1g/hen/day varying from 108 to 114 g/hen/day between strains. Average egg weight of hens fed diets containing the highest lysine level was 3.38g heavier than hens fed the diets containing the lowest lysine level. Increasing dietary lysine from 0.680 to 0.828% significantly improved feed conversion from 2.03 to 1.91 g feed/g egg and increased egg mass from 54.0 to 59.30 g/hen/day. Average lysine intake of hens fed 0.828% level was 939 mg/hen/day varying from 907 to 964 mg/hen/day between strains. Because egg and ingredient prices often change, there can be no fixed dietary lysine level for optimal profits.

Dietary Energy and Protein Experiment 3 (Section IV)

Protein had a significant effect on egg production, egg mass, feed intake, feed conversion, egg weight, percentage of egg shell components, yolk color, and yolk and albumen weight. As dietary energy increased from 0 to 238 kcal ME/kg by addition of

poultry oil, feed intake linearly decreased. Increasing dietary energy also significantly increased body weight and egg yolk color. As dietary energy increased, percent yolk solids increased at the two higher dietary protein levels, while egg specific gravity linearly decreased at the 17.38% protein level.

Increasing dietary energy and protein significantly improved feed conversion. Increasing protein intake significantly increased albumen and yolk weight but had no influence on yolk, albumen or whole egg solids. Because feed ingredient and egg prices vary, there can be no fixed ideal dietary energy level for optimal profits during molt phase 1 (70 to 81wk).

Versatile Enzyme, Dietary Protein and Energy Experiment 4 (Section V)

Dietary protein significantly increased feed consumption but decreased yolk color. As dietary energy increased from 2791 to 2989 kcal ME/kg, feed consumption decreased from 98.0 to 94.9 g per hen daily and yolk color increased from 5.27 to 5.56. There was a significant interaction among dietary protein, energy and Rovabio™ on egg production, body weight, egg mass, feed conversion and yolk solids. Egg weight of hens fed the diets supplemented with Rovabio™ was significantly higher than that of hens fed the diets without Rovabio™ during wk 3 and 4. However Rovabio™ did not significantly influence average egg weight (87-98 wk of age). Rovabio™ supplementation significantly increased body weight of hens. These results suggest Rovabio™ had a small but significant influence on nutrient utilization of commercial Leghorns during phase two of second cycle.

Dietary Energy and Seven Strains Experiment 5 (Section VI)

Strain had a significant effect on feed intake, egg production, egg specific gravity, egg weight, percent whole egg solids, and haugh unit. There were no interactions between strain and dietary energy on any parameters during second cycle phase 2 (88 to 97weeks of age). Dietary energy had no significant effect on any parameter. However as dietary energy increased, egg production, final body weight of hens, egg mass, egg yolk color and egg yolk weight numerically increased; moreover feed conversion numerically improved from 2.06 to 2.02, resulting in a 1.94% improvement of feed conversion. It is difficult to determine an ideal dietary energy level for the hens in second cycle phase 2 because increasing dietary energy had no significant effect on feed intake, egg mass and feed conversion. Because feed ingredient and egg price vary, there can be no fixed ideal dietary energy requirement for optimal profits.

Dietary Protein and Peptiva Experiment 6 (Section VII)

Protein had a significant effect on feed consumption, egg weight, egg production, egg mass, egg specific gravity, egg albumen solids, and percent egg components. As dietary protein increased from 13.53 to 15.62%, egg production, feed consumption and egg weight increased by 6.14%, 8.2% and 5.18% respectively. Feed consumption of hens fed the diets supplemented with Peptiva was significantly lower than that of hens fed the diets without Peptiva. Peptiva supplementation also significantly increased egg production of hens during week 98 and numerically higher in week 99, 103, 105, 106, 107, 109 and overall egg production.

There was also a significant effect of peptiva on egg mass and feed conversion during first week but the significant effects were lost after second week.

Peptiva significantly decreased feed intake without causing any adverse effects on egg weight and egg production. Peptiva might be more beneficial for young hens. More research is needed with young hens to evaluate performance and profits of commercial layers at different egg and ingredient prices.

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