THE IMPACT OF SUPPLEMENTAL L-THREONINE IN LAYING HEN DIETS ON EGG COMPONENT YIELD, COMPOSITION, AND FUNCTIONALITY

A Dissertation

by

PAIGE REYNOLDS NIEMEYER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2005

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	John Carey
Committee Members,	David Caldwell
	Christine Stanley
	Rhonda Miller
Head of Department,	Alan Sams

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ABSTRACT

The Impact of Supplemental L-Threonine in Laying Hen Diets on Egg Component Yield, Composition, and Functionality. (August 2005)

Paige Reynolds Niemeyer, B.S., Texas A&M University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. John Carey

The impacts of supplemental L-threonine in laying hen diets were evaluated. Over three experiments, control hens were fed a corn-soybean commercial layer diet containing 0.56% threonine (Thr). Experimental diets containing 0.66, 0.76, 0.86, and 0.96% Thr were fed for experiment 1. Experimental diets containing 0.76, 0.96 and 1.16% Thr were fed for experiment 2. Experiment 1 and 2 hens were 42 weeks of age. In experiment 3, experimental diets containing 0.76 and 0.96% Thr were fed to aged hens (61 weeks at beginning of experiment). Data collection methods were the same for all three experiments. Beginning and ending hen weight, egg production, and feed consumption data were collected. Egg samples were analyzed for egg weight, yolk and albumen yield, protein, and functionality. In experiments 1 and 2, egg production increased with increasing dietary threonine levels up to 0.76% Thr in the diet and subsequently decreased suggesting a production threshold for the amino acid. Shell cracking strength increased with increasing threonine levels in all three experiments. In experiment 3, shell thickness increased with increasing threonine levels. Albumen protein was significantly increased when hens were fed increased levels of dietary threonine. Angel food cake volume was significantly increased in experiments 1 and 3

with increasing dietary threonine, as were other texture profile parameters. Sponge cake volume was significantly increased in experiments 2 and 3 as a result of increased threonine levels. In experiment 3, yolk gel hardness was significantly increased by increasing the level of dietary threonine. These data clearly indicate a potential important impact on egg composition and functionality by increasing dietary threonine nutrition of a laying hen.

DEDICATION

To those who make the most of themselves

ACKNOWLEDGMENTS

I would like to extend thanks to Dr. John Carey for his counseling and patience during the past three years of graduate school. I would also like to extend gratitude to Dr. David Caldwell, Dr. Christine Stanley, and Dr. Rhonda Miller for serving on my committee. Thank you to Craig Coufal for constant maintenance on the Leco and other general lab help. Thank you to Liz Hirschler for working with my schedule for Instron set-up and for helping with some of the concepts of this research. Thank you to Dr. Jimmy Keeton for aiding with the concepts of texture profile analysis and torsion, and the use of lab space and equipment for torsion. I would like to extend gratitude to Betsy Booren in the Animal Science Sensory Lab for oven and lab use and for coming in early to work on baking days. I would like to extend much love and gratitude to my darling and patient husband, Truitt, who spent many Saturdays keeping me company in the lab, helping me weigh feed, and staying away while I tended to my studies. I would like to thank my father and mother for providing monetary and emotional support during the last three years. It is always the little things in life that matter.

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CHAPTER I

INTRODUCTION

In 2002, per capita egg consumption rose to 245.6 eggs, which is 20 more eggs per person compared to the mid 1990's. This rapid increase in egg consumption is primarily due to increased demand for breaking eggs by commercial confection, baking, hotels, and fast food industries. Liquid egg (LE) product consumption has increased 25% since 1996 to a soaring 73 LE per capita (USDA 2003). Commercial liquid egg standards rely on the functional and compositional parameters of the yolk and albumen. The dramatic increase in LE consumption leads to a need for research on the rate of laying hen nutrition on the quality of liquid egg yield, composition, and functionality.

The Nutritional Requirement Compendium (NRC) requirement for threonine is 0.56% of the diet, which is an estimate due to the lack of experimental data (NRC 1994). Thus a level of 0.56% Thr will serve as control in this research. The primary objective of this research is to evaluate the impact of increased levels of dietary threonine on the quantity, composition and functionality of albumen and yolk. Other production parameters evaluated will include body weight, feed and consumption, egg production, egg weight, and shell quality.

The style and format of this dissertation follow Poultry Science.

CHAPTER II

REVIEW OF LITERATURE

Overview

In general, dietary requirements for metabolizable energy and amino acids are based on environment, maintenance and production parameters (Coon and Zang, 1999). Increasing the overall protein content of laying hens diets has been shown to increase component yields and functionality parameters of eggs (Butts and Cunningham, 1972). However, protein is an expensive source of nutrition in poultry diets (Butts and Cunningham, 1972) and methods to decrease protein in layer diets are being investigated. When protein levels are decreased, amino acid concentration decreases as well. By supplementing amino acids in the diet, poultry may maintain production, growth, and homeostasis. Extensive research has been conducted in improvement of egg quality parameters. The effects of supplemental amino acids, alone or in combination have been evaluated for impacts on egg production and quality.

Amino Acids

Past research has primarily focused on dietary essential amino acids methionine and lysine. Shafer et al. (1998) reported that methionine fed at a level of 507 mg/HD positively affected egg weight and albumen and yolk solids when compared to Nutritional Requirement Compendium (NRC) requirements for the amino acid in normal layer diets. Similar results were reported by Prochaska et al. (1996) who added lysine

to a normal sorghum - soybean diet at a level of 1,613 mg/HD. Recent research has focused on the use of a combination of amino acids added to a typical layer diet: lysine, isoleucine, threonine, and tryptophan (LITT). This supplemental combination had no effect on egg mass, egg production, or egg weight (Sohail et al., 2002). These researchers did not evaluate the yield, composition or functionality of the egg components.

The essential amino acid, threonine (Thr), is used in important metabolic processes such as protein synthesis and uric acid formation. Threonine is the third most limiting amino acid, especially in a low crude protein diet (Kidd and Kerr, 1996; Schutte 1998). Poultry cannot synthesize threonine making it a nutritionally essential amino acid. Poultry can utilize only L-Threonine (Kidd and Kerr, 1996), making it metabolically expensive. Threonine has also been shown to hinder methionine influx and stimulate lysine influx into the epithelial cells of the intestinal lumen (Lerner 1971).

Until recently, research including threonine as a supplemental amino acid has been vague or included with the effects of other amino acids. In 1991, Huyghebaert and Butler reported that 0.51% Thr or 637mg/HD was adequate for egg mass and feed efficiency for a producing flock of medium weight laying hens of 28 – 38 weeks of age. Ishibashi et al. (1998) studied a flock of 2000 Single Comb White Leghorns (Dekalb XL Link) and found improved feed intake, egg mass, and feed efficiency with increasing threonine levels (0.31% - 0.61%). Body weight decreased with increasing threonine levels and egg size was not affected by the dietary treatments. Graduating the level of dietary threonine from 0.35% to 0.58% in a typical layer diet increased egg production without affecting egg weight and bird weight (Faria et al., 2002). Koelkebeck et al. (1991) fed laying hens a commercial corn – soy diet with the addition of 1%

supplemental threonine and reported significantly improved hen day egg production, egg weight, and feed intake.

Post peak production hens fed five graded levels of Thr (0.47% - 0.63%) in a sunflower- sorghum- soybean diet were reported to have improved egg production and feed efficiency with increasing levels of threonine in aged hens (Martinez-Amezcua et al., 1999). Past threonine research has focused on egg production, hen weight, and feed consumption. There is a lack of evidence concerning the effect of supplemental threonine in laying hen diets on the internal contents of the egg.

Egg Parameters

Whole, Yolk, and Albumen

Peak production large breed hen eggs have been reported to weigh 60 grams. The albumen and yolk of these eggs averaged 38 and 16 grams, respectively, when hens were fed recommended commercial diets (Cotterill and Geiger, 1977; Fletcher et al., 1983). Fletcher et al., 1983 reported average egg weight of a 63 week old hen to be 65.5 grams, with albumen and yolk weighing 40.6 and 19.1 grams, respectively. Increasing protein content of the diet from 12% to 18% significantly increased egg production and decreased feed consumption (Butts and Cunningham, 1972). Solids content of albumen has been reported to be 12.1% (Cotterill and Geiger, 1977). Albumen solids and protein content is reported to increase with graduated protein levels (Butts and Cunningham, 1972; Gardner and Young, 1972; Andersson 1979). Solids content of the yolk has been reported to be 50% (Mine 2002) and 51.8% (Cotterill and

Geiger, 1977). Solids content of the yolk is also reported to increase with increasing levels of crude protein in a laying hen diet (Gardner and Young, 1972; Andersson 1979). Protein content of the yolk on a wet basis has been reported to be between 15.7% and 16.6% (Mine 2002).

Shell Quality

Shell quality and shell strength are interchangeable terms and are defined as the ability of the shell to withstand externally applied forces (Hamilton 1982). Factors affecting shell quality include but are not limited to; hen age, environmental factors, caging systems, flock health, and nutrition. Increased dietary protein has been reported to have a negative effect on shell protein content and consequently shell strength (Tyler 1961).

Egg shell strength can be measured by crushing, puncturing or impacting methods. Typically, egg shells have been crushed or cracked around the equatorial regions, as these are typically the area of high impact and thin shell quality (Hamilton 1982). Because shell strength and thickness varies extensively at the equator (Hamilton 1982), new methods which crack the blunt or small end of the egg are recommended due to increased uniformity of shell thickness in this region. Quasi-static compression fracture force is a direct method of measurement of shell strength and simulated the type of major damage an egg shell encountered in a mechanized processing facility (Hamilton 1982). Such a method could be studied using an Instron Universal Testing[1] (Instron) machine. Keshavarz (1986) reported average shell

[1] Model 1011, Instron Corp., Canton, MA 02021

strength to be 3.38 kg for peak production hens using this machine. Using a flat steel compression anvil on the Instron, a force of 4.0kg was required to crack shells of eggs of 44 week old hens while a force of 2.8kg was required to crack shells of eggs of 78 weeks old hens (Hamilton et al., 1979).

Texture Profile Analysis

Food is eaten and selected for enjoyment and nutritive value (Bourne 1978). Therefore food technologists seek to develop food items that appeal to the masses of consumers. To do this, the food technologist uses a variety of methods that define texture, appearance and flavor of a food product. This review focuses on the textural parameters of food items baked from eggs. Cake baking yields homogenous samples of a standard shape and size (Breene 1975). Cake functionality, assessed by texture profile analysis (TPA) and rapeseed displacement, relates to protein characteristics as affected by the baking process.

History

The originators of texture profile analysis worked with General Foods and prototyped a General Foods Texturometer (GFT), which used a flat faced cylinder to compress a bite-size piece of food, usually a cube to 75% of its original height (Bourne 1968). The GFT imitated the mastication process and results were provided by a strip-chart recorder expressing force-time curves (Breene 1975). This test gave

measurements of hardness, springiness, and brittleness. Bourne (1966), adapted the Instron Universal testing machine to perform a modified TPA test. Instead of both ends of the compression area moving yielding curved scales, the Instron allowed for compression by only the anvil while the compression plate remained in place. Other superior parameters in using the Instron include a constant compression speed and immediate reversal at the end of the down strokes, resulting in sharp peaks (Bourne 1966). While this method does not imitate the action of the jaw like the GFT (Bourne 1978), extensive research across many food types has yielded similar results to sensory evaluation. Common texture parameters include hardness, cohesiveness, springiness, gumminess, and chewiness (Bourne 1978). Chart force-distance curves for each cake core were measured for hardness, cohesiveness, springiness, gumminess, and chewiness as defined by Bourne (1978). Hardness correlates to the 'first bite' or force that is required to deform a food particle (Bourne 1978). Springiness is the distance in height (mm) the sample will recover between the first and second compression or 'bite' (Bourne 1978). Cohesiveness is defined as the ratio of the positive force areas under the curves of both compressions and is dimensionless (Bourne 1978). Cohesiveness was necessary to define in order to determine gumminess and chewiness. Gumminess is defined as hardness multiplied by cohesiveness (Breene 1975). Chewiness is defined as the product of gumminess multiplied by springiness (N mm) (Breene 1975). These attributes have been studied among a variety of food items.

Application to Cake

Dry egg whites are commonly used in manufacturing of angel food cakes (Forsythe 1970). Foaming ability of albumen for angel food cakes, whipping speed and duration are critical. Whipping is commonly defined as the capacity to form stable foams with air. Additional whips past a medium peak in batter will have a marked decrease in stability (MacDonnell et al., 1954). Optimum volume for a given whipping time is not the same for each batch of eggs. Foaming power measures the increase in the volume upon the introduction of a gas into a protein solution. Foam stability refers to the ability of the formed foam to retain its maximum volume over time and cooking (Woodward and Cotterill, 1987). Ovalbumin functions as a volumizer while ovomucin functions as a stabilizer as long as whipping time is kept to a minimum (MacDonnell et al., 1954). Albumen foams consist of air droplets encased in a liquid protein containing a soluble surfactant (Mine 2002). There is a critical balance between the proteins ability to engage in intermolecular cohesion to form a stable membrane around the air and its ability to associate exclusively with proteins of its kind (Kinsella 1976). This is especially important to the liquid egg industry because, an increase in albumen protein content could lead to an increase in volume and TPA parameters. Essentially, this could mean a baker can use less batter to make the same amount of product.

Application to Gels

All amino acids and therefore proteins have different solubility, pH, and susceptibility to denaturation that will contribute to their functionality in a food product. Therefore, it is noted that the functionality associated with protein preparations like cakes and gels may not be the properties of the total proteins, but only a fraction of their components. Protein gel formation may require some prior heating of a protein to cause unfolding of polypeptide chains (Mine 2002). A denatured gel can only be formed when a balance of attractive and repulsive forces is attained at a given thermodynamically suitable condition. Because denaturation of proteins differs, it may be possible that gelation may occur in higher than normal heat forming a network of formations making firmer gels, followed immediately by cooling allowing uncoiled polypeptides to form the cross-linking network of hydrogen bonds, ionic attractions, disulfide bonds, and hydrophobic interactions that trap water in a matrix of sorts to form the gel (Mine 2002). Woodward and Cotterill (1987) reported that at 80C, the albumen forms the “normal” appearance of commercially available cooked egg whites. At higher protein concentrations, a protein network or denatured polypeptides that are cross-linking during a cooling stage may increase the stability.

Two-cycle compression of protein gels made from native egg albumen, albumen mixed with oleic acid, and succinylated albumen were evaluated at 50% of their original height using a 50kg load cell with a 10cm/min crosshead speed (Montejano et al., 1985). Hardness, springiness, cohesiveness, gumminess and chewiness were evaluated. Albumen gels that were mixed with oleic acid or succinylated were found to be significantly superior in all TPA categories compared to the native egg white

(Montejano et al., 1985). This suggests that the addition of chemicals may aid the proteins in retaining a tight bond, and decrease unfolding during heat stress and cooking. Hatta et al. (1986) researched the hardness of purified egg ovalbumin gels. These researchers used a stainless steel tube, which was boiled with the contents of the ovalbumin for one hour at 80C. The sample remained in the tube during compression. These researchers found a harder gel at pH 3.5 and 7.0 compared to others. Albumen gel plugs made in glass beakers from eggs of hens fed increased levels of supplemental lysine, yielded increased hardness, and no difference in springiness (Prochaska et al., 1996).

The yolk of hard cooked eggs is crumbly in texture. When the yolk is disturbed, for example stirred, the gels formed from cooking are springier and harder in texture compared to boiled whole yolks (Woodward and Cotterill, 1987). Woodward and Cotterill (1987) reported that cooked yolk hardness increased with time cooking time. Gels formed at 10 minutes and were able to hold their form. This group also reported that increasing protein content of the yolk resulted in an increase in hardness. Springiness of yolk were not affected by protein level or cooking times and temperatures studied. Woodward and Cotterill (1987) recommended cooking at 80 C for 30 m. Prochaska et al. (1996) boiled yolk gels from eggs of hens fed increasing levels of dietary lysine and reported that hardness of the yolk gel was significantly improved with increasing lysine levels in the diets.

While two-cycle compression using an Instron Universal testing machine has been extensively studied, measurement of torsion strain and stress of egg gel plugs is a neoteric TPA method. Stress correlates to hardness (Hamann et al., 1990). Strain is the ratio of deformation to sample length (Hamann et al., 1990). Deformation is a

length in centimeters of the distance the spindles travel in a counter-twist (Gel Consultants 2004). Thus, the greater the length, the greater the strain required to fracture a sample. Strain is reported as an indicator of gelling quality for proteins and machining characteristics of food gels (Gel Consultants 2004). Torsion stress and strain at fracture of native egg albumen was reported to be 12.61 kPa and 1.21, respectively (Montejano et al., 1984). The instrument used for these measurements was adapted to the Instron Universal testing machine. More recent methods of torsion testing have utilized an adaptation of a Brookfield Viscometer. The gels are cut to 1.5 cm, and then milled to yield a 1cm bar-bell shaped gel (Hamann et al., 1990). The ends are glued to notched styrene discs and placed in the Brookfield Viscometer, also known as the Hamann Torsion/Vane Gelometer. Beginning with negative speed, the instrument counter-twists the sample until fracture (deformation), at which time peak stress and strain are recorded. Torsion testing has been shown to strongly correlate with sensory methods (Hamann et al., 1990). When egg albumen was added to Surimi, the stress and strain values of the gels nearly double, compared to native Surimi gels or Surimi gels with Plasma Hydrolsayte (Hamann et al., 1990). As albumen protein level increases, the hardness of the subsequent gel increased (Woodward and Cotterill, 1986).

Rapeseed Displacement

Rapeseed displacement is a method of determining volume displacement in a cooked product. Butts and Cunningham (1972) fed hens increased levels of dietary crude protein and reported no effect on sponge cake functionality as measured by

rapeseed displacement. Shafer et al. (1998) reported increased angel cake and sponge cake volumes based on rapeseed displacement when hens were fed increased levels of supplemental methionine. Foaming ability of albumen is in direct relation to the proteins ability to maintain form and shape during cooking of angel food cakes. Foaming ability is typically measured while the cakes are in the uncooked form by measuring the separation of liquid from foam over varying periods of time.

CHAPTER III

THE IMPACT OF SUPPLEMENTAL L-THREONINE IN LAYING HEN DIETS ON EGG COMPONENT YIELD, COMPOSITION, AND FUNCTIONALITY

Overview

Two eighteen week experiments were conducted each utilizing one hundred 42 week old Single Comb White Leghorn laying hens housed individually in an open sided cage laying facility with groups of 5 hens sharing access to a common feed trough. A typical layer diet, containing 0.56% threonine (Thr) was fed to control hens. Four experimental diets containing 0.66, 0.76, 0.86, and 0.96% Thr were fed in experiment one. Three experimental diets containing 0.76, 0.96, and 1.16% Thr were fed in experiment two. Beginning and ending hen weights, egg production, and feed consumption data were collected. Egg samples were analyzed for egg weight, shell strength, yolk and albumen yield, protein, and functionality. Yolk and albumen were separated and pooled by experimental unit, homogenized, and analyzed for protein content and solids at 2 week intervals. Egg samples collected at 3 week intervals were used to prepare angel food and sponge cakes. Cake volume was determined by rapeseed displacement. Cakes were cored, up to 5 cores per cake and subjected to two-cycle compression on an Instron Universal Testing machine for Texture Profile Analysis (TPA) parameters of hardness, springiness, gumminess, chewiness, and cohesiveness. Egg production increased with increasing dietary threonine levels up to 0.76% Thr in the diet. Whole egg weight, yolk, and albumen weight were increased by increasing dietary threonine levels. More force was required to crack egg shells from

hens that consumed increased levels of dietary threonine. Sponge and angel food cakes baked from eggs of hens consuming increased levels of dietary threonine were harder then those baked from the control diet. These data clearly indicate a potentially important impact of threonine nutrition of laying hens on egg parameters, functionality, and shell strength.

Introduction

In 2002, per capita egg consumption rose to 245.6, which is 20 more eggs per person compared to the mid 1990's. This rapid increase in egg consumption is primarily due to increased demand by commercial confection, baking, hotels, and fast food industries. The majority of these markets utilize liquid egg (LE) products. Liquid egg consumption has increased 25% since 1996 to 73 per capita (USDA 2003). Commercial LE standards rely on the functional and compositional parameters of the yolk and albumen. The dramatic increase in LE consumption leads to a need for research on the impact of laying hen nutrition on liquid egg yield, composition, and functionality.

The essential amino acid, threonine (Thr), is used in important metabolic processes such as protein synthesis and uric acid formation. Until recently, research including threonine as a supplemental amino acid has included the effects of other amino acids. Huyghebaert and Butler (1991) reported that 0.51% Thr or 637mg/HD was adequate for egg mass and feed efficiency for a flock of medium weight laying hens 28 to 38 weeks of age. Ishibashi et al. (1998) reported improved feed intake, egg mass, and feed efficiency with increasing threonine levels (0.31% - 0.61%). However, body weight decreased with increasing threonine levels and no change in egg size was

noted among dietary treatments. Incrementally increasing the level of dietary threonine from 0.35% to 0.58% in a typical layer diet yielded increased egg production with varying egg weight and bird weight impacts (Faria et al., 2002). Koelkebeck et al. (1991) fed laying hens a commercial corn – soy diet with the addition of 1% supplemental threonine and reported significantly lower feed consumption and higher egg production compared to the control diet. The NRC requirement for threonine is 0.56% of the diet, which is an estimate because experimental data are lacking (NRC 1994).

Food technologists are concerned with production of food products that will remain intact to their destination while remaining desirable to consumers (Bourne 1966). Imitative tests involving the machined extrapolation of sensory data have been developed and are known as texture profile analysis (TPA). Hardness correlates to the 'first bite' or force that is required to deform a food particle (Bourne 1978). Springiness is the distance in height (mm) the sample will recover between the first and second compression or 'bite' (Bourne 1978). Cohesiveness is defined as the ratio of the positive force areas under the curves of both compressions and is dimensionless (Bourne 1978). Cohesiveness was necessary to define in order to determine gumminess and chewiness. Gumminess is defined as hardness multiplied by cohesiveness (Breene 1975). Chewiness is defined as the product of gumminess multiplied by springiness (N mm) (Breene 1975).These test best evaluate and imitate mastication and are highly correlated with sensory evaluation (Bourne 1978). Cake mixing has been shown as a good measure of whipping and protein stability (MacDonnell et al., 1955). Cake volume measurements are a means of determining protein stability (Butts and Cunningham, 1972).

Approximately 8% of eggs collected in the Southern US are shell-less, soft-shelled, or thin-shelled (Grizzle et al., 1992) causing problems for farmers and commercial egg processing facilities. Shell strength is affected by hen age, temperature, and nutrition. Shell strength is correlated to the impact or insult the shell encounters (Hamilton 1982). A significant correlation exists in the breaking strength and membrane strength of an egg (Essary et al., 1977). The objective of this research is to evaluate the impact of supplemental dietary threonine above NRC recommendations on hen body weight, feed consumption, egg production, egg weight, shell quality, liquid yield, protein content, and functionality of albumen and yolk.

Materials and Methods

Experimental Design

Two eighteen week experiments were conducted utilizing one hundred 42 week old commercial strain laying hens[2]. Hens were housed in individual wire cages in an open sided laying facility with experimental units consisting of 5 hens sharing access to a common feed trough. The lighting schedule was maintained at 17 hours of daylight and 7 hours of darkness throughout the studies. Hens utilized in experiment 1 were randomly assigned into four blocks with 5 dietary treatments per block (twenty hens per diet). Experiment 2 hens were randomly assigned into five blocks with four dietary treatments per block (twenty-five hens per diet).

[2] Dekalb XL, Centurion Poultry, Lexington, GA

A layer diet containing 0.56% threonine was fed to control hens throughout both experiments (Table 3.1). Experiment 1 diets were formulated at 0.66, 0.76, 0.86, and 0.96% threonine. Experiment 2 diets were formulated at 0.76, 0.96 and 1.16% threonine. Supplemental threonine was supplied by synthetic 98% feed quality L-Threonine[3]. Feed was formulated to contain approximately 16.5% crude protein and 2800kcal/kg, as fed in a typical layer diet (NRC 1994) and was mixed every 3 weeks. Threonine was added to the diets at the expense of soybean meal. Samples of the control diet were pooled by experiment and subjected to amino acid analysis at the Texas A&M Protein Chemistry Laboratory. Threonine in the control diet was 0.50 and 0.63 for experiments 1 and 2, respectively. Feed samples were collected at each mixing date, pooled, and 5 sub samples were subjected to nitrogen analysis using a Leco FP-428 Nitrogen Determinator System.[4] Percent nitrogen was multiplied by 6.25 to determine protein content of the feed samples. Crude protein in the control diets was 15.1 and 17.4% for experiments 1 and 2, respectively. Prior to the initiation of each experiment the hens were fed the control diet for a two-week acclimation period. Feed and water were provided *ad libitum.* Hens were individually weighed at the initiation and termination of each experiment. Hen-day egg production and feed consumption were calculated on a weekly basis.

[3] AJINOMOTO Heartland, Inc., Eddyville, IA
[4] Leco Corp., St. Joseph, MI 49085-2396

TABLE 3.1. Composition of layer diets[1] containing increased levels of threonine, Experiments 1 and 2

	Dietary Threonine Level					
Ingredient	0.56[5,6]	0.66[5]	0.76[5,6]	0.86[5]	0.96[5,6]	1.16[6]
	----------(% of diet)----------					
Corn	69.5	69.5	69.5	69.5	69.5	69.5
Soybean meal 48	18.8	18.7	18.6	18.5	18.4	18.2
Limestone	8.5	8.5	8.5	8.5	8.5	8.5
Lysine HCL	1.5	1.5	1.5	1.5	1.5	1.5
Phosphorus	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met98	0.12	0.12	0.12	0.12	0.12	0.12
Thr[3]	0.0	0.1	0.2	0.3	0.4	0.6
Mineral Premix[4]	0.05	0.05	0.05	0.05	0.05	0.05

[1]Diet calculated to contain 2800kcal of ME/kg, and 16.5% crude protein.
[2]Vitamin premix added at this rate yielded 11,023 IU of vitamin A, 3,858 IU of vitamin D, 46 IU of vitamin E, 0.0165 mg of vitamin B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg of D-pantothenic acid, 477.67 mg choline, 1.47mf of menadione, 1.75mf of folic acid, 7.17 mg of pyroxidine, 2.94 mg of thiamin, 0.55 mg of biotin per kilogram of diet. The carrier was ground rice hulls.
[3]98% feed grade L - threonine.
[4]Mineral premix added at this rate yielded 149.6 mg of manganese, 125.4 mg of zinc, 16.5 mg of iron, 1.7 mg of copper, 1.05 mg of iodine, 0.25 mg of selenium, a minimum of 6.27 mg of calcium, and a maximum of 8.69 mg of calcium per kilogram of diet. The carrier was calcium carbonate, and the premix contained les than 1% mineral oil.
[5]Experiment one diets.
[6]Experiment two diets.

Experimental Methods

Egg Composition. In each experiment at 2 week intervals, eggs were evaluated for weight, yolk and albumen yield, protein content, and solids. Egg contents (yolk and albumen) were manually separated using a plastic egg separator. The yolk was rolled on a damp paper towel to remove any chalazae or remaining albumen and then weighed. Yolks were punctured with a metal spatula, pooled by experimental unit and stirred using a glass rod prior to further analysis. Albumen yield was determined by subtraction of the yolk and shell with shell membranes intact from the whole egg weight (Shafer et al., 1996). Albumen was pooled by experimental unit and homogenized using an upright hand-held household blender[5] on low speed for 2 five second pulses to reduce froth. Solids content for albumen and yolk were determined from dual 10 gram sub samples of the each pooled samples. The sub samples were dried in aluminum drying pans at 105C for 24 hours. Protein content of dried albumen and yolk was determined from 5 sub samples of dried albumen and yolk using a Leco FP-428 Nitrogen Determinator System[6]. The percent nitrogen was multiplied by 6.25 to yield protein content of albumen and yolk samples.

Texture Profile Analysis. In experiment 1, eggs were sampled at weeks 3, 10, and 16 for cake baking. In experiment 2, eggs were sampled at weeks 3, 6, 9, 12, 15, and 18 for cake baking. Yolk and albumen were separated with a plastic egg separator and pooled by nutritional treatment to make three replicates each of sponge and angel

[5] Cuisinart, E. Windsor, NJ 08520
[6] Leco Corp., St. Joseph, MI 49085-2396

food cakes, respectively, by methods reported by Shafer et al. (1998) (Appendix A; Appendix B). Due to insufficient yolk material at week 10, in experiment 1, only 2 sponge cakes were prepared. The yolks were strained through cheesecloth to remove the yolk membranes for sponge cake preparation. Sponge cakes were prepared using a stand mixer[7] on medium (7) speed. Angel food cakes were prepared using the same mixer on speed 9 until medium peaks were formed. Sponge cakes were baked for 25 minutes at 176C. Angel food cakes were baked for 22 minutes at 176C. All cakes were baked in the same oven. Prior to each baking session, ovens were preheated and checked for constant heat with a calibrated oven thermometer. Cakes were allowed to cool for approximately 4 hours and volume results were recorded by rapeseed displacement (Shafer et al., 1998). Thereafter, cakes were cored (1.75mm corer), up to 5 cores per cake and subjected to two-cycle compression on an Instron Universal Testing Machine[8] equipped with a 500 Newton (N) load cell and 200N load range with a crosshead speed of 100 millimeters per minute (mm/min). Cores for week 10, experiment 1 were not compressed. Chart force-distance curves for each cake core were measured for hardness, cohesiveness, springiness, gumminess, and chewiness as defined by Bourne (1978).

For each dietary treatment, eggs were tested for shell strength using an Instron Universal Testing Machine equipped with a 50 kilogram (kg) load cell at a 10kg load range with a crosshead speed of 50 mm/min. Shell strength was conducted during weeks 3, 10, and 16 for experiment 1, and weeks 3, 8, 12, 15, and 18 for experiment two.

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[7] Model 168949, Wal-Mart Stores, Inc., Bentonville, AK 72716
[8] Model 1011, Instron Corp., Canton, MA 02021

Rupture strength of the vitelline membrane was measured on an Instron Universal Testing Machine equipped with a 5kg load cell and 500gram load range, for sensitivity, with a crosshead speed of 50mm/min during weeks 9, 11, 13, 15, and 17 of experiment two. Each egg yolk was separated from the albumen using a plastic egg separator, rolled on a damp paper towel to remove any adhering albumen and weighed in grams. The yolk was then placed in the center of filter paper lying in the bottom of a 100ml beaker. Prior to compression, the beaker containing the yolk was placed beneath the anvil. The vitelline membrane was allowed to rupture, exposing yolk contents, yielding a rupture strength number recorded in kilograms for each egg yolk sampled.

Statistical Analysis

Data were analyzed using ANOVA with General Linear Model (GLM) procedure of SAS[9], with main effects of diet, week, and replication. Mean differences were separated using the PDIFF option, which uses pair-wise *t* tests of the GLM procedure. All statistical comparisons were considered significant at $P < 0.05$.

[9] SAS version 8.01, SAS Institute, Cary, N.C.

Results

Experiment 1

No significant differences among dietary treatments were detected for beginning or ending hen weights or weight loss (Table 3.2). Hen day feed consumption was not significantly different among dietary treatments. Actual threonine levels consumed daily by hens were 549 (control), 657, 758, 857, and 940 mg Thr/HD for the 0.56, 0.66, 0.76, 0.86, and 0.96 diets, respectively. Subsequently all references to threonine treatments are identified by these mg/HD intake levels. Egg production of hens that consumed 758 mg Thr / HD was significantly higher than egg production of hens that consumed 549, 657, and 940 mg Thr / HD (Table 3.3).

Significantly higher whole egg weights were attained from hens that consumed 549 and 758 mg Thr / HD compared to all other diets (Table 3.3). Albumen weight was significantly higher in eggs from hens fed the control diet compared to those from hens that consumed 940 mg Thr /HD. Yolk weight of eggs from hens that consumed 758 and 549 mg Thr /HD was significantly higher than that of eggs from hens that consumed 657, 857, and 940 mg Thr /HD (Table 3.3). Percent albumen was significantly higher in eggs of hens that consumed 857 mg Thr/HD compared to that of eggs from hens that consumed 758 mg Thr /HD. Yolk liquid percentage of eggs from hens that consumed 758 and 940mg Thr /HD was significantly higher compared to liquid yolks of eggs from hens that consumed 857 mg Thr /HD (Table 3.3).

TABLE 3.2. The impact of dietary threonine on feed consumption and hen weight, Experiment 1

Thr Level (%)	Feed Consumption (g/H/D[2])	Thr Intake (mg/HD)	Beginning Hen Weight[1]	Ending Hen Weight[1]	Weight Gain/Loss
			----------(g)----------		
0.56%	98.0[a]	549[e]	1602[a]	1547[a]	-55[a]
0.66%	99.6[a]	657[d]	1605[a]	1576[a]	-29[a]
0.76%	99.7[a]	758[c]	1601[a]	1569[a]	-32[a]
0.86%	99.6[a]	857[b]	1608[a]	1533[a]	-75[a]
0.96%	97.9[a]	940[a]	1611[a]	1552[a]	-59[a]
Pooled SEM	0.91	7.1	18.6	26.2	22.3

[a-e] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]n = 197: 100 beginning hens + 97 ending hens.
[2]HD = per live hen per day.

TABLE 3.3. The impact of dietary threonine on egg production, egg weight, component yield by weight and percentage of liquid egg, Experiment 1

Thr Intake (mg/HD[5])	Egg Production[1] (%)	Whole Egg Weight[2] (g/egg)	Albumen Weight[3] (g/egg)	Yolk Weight[4] (g/egg)	Albumen Liquid[3] (%)	Yolk Liquid[4] (%)
549	78.0[b]	60.7[a]	34.9[a]	16.8[a]	57.2[ab]	27.6[ab]
657	77.3[b]	59.4[b]	34.1[ab]	16.2[b]	57.3[ab]	27.4[ab]
758	81.0[a]	60.7[a]	34.4[ab]	16.9[a]	56.4[b]	27.8[a]
857	79.1[ab]	59.4[b]	34.2[ab]	16.1[b]	57.5[a]	27.2[b]
940	78.1[b]	58.9[b]	33.6[b]	16.4[b]	56.8[ab]	27.8[a]
Pooled SEM	0.81	0.41	0.35	0.12	0.33	0.18

[a,b] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]n = 20 hens per treatment.
[2]n = 745
[3]n = 724
[4]n = 691
[5]HD = per live hen per day.

Albumen solids from hens that consumed 940 mg Thr /HD was significantly higher than all other dietary treatments (Table 3.4). Albumen protein of eggs from hens consuming 758 and 940 mg Thr/ HD was significantly higher than all other dietary treatments. Yolk solids content was not significantly different among dietary treatments. Yolk protein was significantly higher in egg yolks of hens that consumed 758, 857, and 940 mg Thr/HD compared to 549 and 657 mg Thr / HD. Stronger shells were detected among eggs from hens that consumed 857mg Thr/HD compared to egg shells of hens that consumed 549 and 657 mg Thr / HD (Table 3.4).

Angel food cakes baked from egg albumen of hens that consumed 940mg Thr/HD displaced significantly more volume than all other treatments (Table 3.5). Angel food cakes baked from egg albumen of hens that consumed 549mg Thr/HD displaced significantly less volume than all other dietary treatments (Table 3.5). Angel food cakes baked from egg albumen of hens that consumed 940mg Thr/HD were significantly harder compared to those from hens that consumed 549 and 657mg Thr/HD (Table 3.5). Angel food cakes baked from egg albumen of hens that consumed 657mg Thr/HD were significantly springier compared to all other dietary treatments. Angel food cakes baked from egg albumen of hens which consumed 857mg Thr/HD were significantly more cohesive compared to that of hens that consumed 940 mg Thr/HD. Angel food cakes baked from egg albumen of hens that consumed 758 and 857 mg Thr/HD yielded significantly more gummy cakes compared to that of hens that consumed 549 and 657 mg Thr/HD. Angel food cakes baked from egg albumen of hens that consumed 657 and 857 mg Thr/HD yielded significantly chewier cakes compared to the control diet.

TABLE 3.4. The impact of dietary threonine on egg component total solids, protein content, and shell strength, Experiment 1

Thr Intake (mg/HD[1])	Albumen Solids[2]	Albumen Protein[3]	Yolk Solids[2]	Yolk Protein[3]	Shell Strength[4] Kg Force
	----------(%)----------				
549	11.3[b]	87.8[b]	51.8[a]	33.6[b]	3.4[b]
657	11.3[b]	87.7[b]	51.4[a]	33.5[b]	3.4[b]
758	11.3[b]	88.2[a]	51.8[a]	33.8[a]	3.6[ab]
857	11.3[b]	88.0[ab]	51.6[a]	33.7[a]	3.9[a]
940	11.5[a]	88.1[a]	51.7[a]	33.9[a]	3.6[ab]
Pooled SEM	0.04	0.08	0.18	0.06	0.11

[a,b] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per live hen per day.
[2]n = 360
[3]n = 900
[4]n = 240

TABLE 3.5. The impact of dietary threonine on texture profile analysis parameters of angel food cake, Experiment 1

Thr Intake (mg/HD[1])	Rapeseed Displacement[2] (ml)	Hard[3] (N)	Springy[3] (mm)	Gummy[3] (N)	Chewy[3] (N mm)	Cohesive[3,4]
549	297[c]	16.7[c]	25.4[b]	6.8[b]	178.5[b]	0.406[ab]
657	390[b]	21.1[c]	47.7[a]	8.1[b]	324.2[a]	0.399[ab]
758	414[b]	34.2[ab]	19.6[b]	13.6[a]	260.4[ab]	0.414[ab]
857	372[b]	30.7[b]	24.3[b]	13.9[a]	322.4[a]	0.457[a]
940	527[a]	37.3[a]	21.7[b]	11.3[ab]	261.6[ab]	0.316[b]
Pooled SEM	36.82	2.02	2.56	1.43	32.88	0.035

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per hen per live day.
[2]n = 45
[3]n = 133
[4] Defined as Area 2:Area 1.

Sponge cake rapeseed displacement was not significantly among dietary treatments (Table 3.6). Sponge cakes baked from egg yolks of hens that consumed 758 mg Thr/ HD required more force to compress resulting in a significantly harder cake compared to all other dietary treatments. A significantly softer cake compared to all other dietary treatments was baked from egg yolk of hens that consumed 549 mg Thr/HD. Sponge cakes baked from egg yolks of hens that consumed 758 and 657 mg Thr/HD were significantly springier compared to those of hens that consumed 940mg Thr/HD. Cohesiveness was significantly higher in sponge cakes baked from egg yolks of hens that consumed 857 and 657 mg Thr/HD compared to those of hens that consumed 549 mg Thr/HD. Egg yolks of hens that consumed the 657, 758, 857, and 940 mg Thr/HD produced significantly gummier sponge cakes compared to those of hens that consumed the control diet. Significantly chewier sponge cakes were baked from egg yolks of hens that consumed 758mg Thr/HD compared to the control diet which were significantly less chewy compared to all dietary treatments.

TABLE 3.6. The impact of dietary threonine on texture profile analysis parameters of sponge cake, Experiment 1

Thr Intake (mg/HD[1])	Rapeseed Displacement[2] (ml)	Hard[3] (N)	Springy[3] (mm)	Gummy[3] (N)	Chewy[3] (N mm)	Cohesive[3,4]
549	325[a]	10.4[c]	30.6[ab]	4.0[b]	122.1[c]	0.405[b]
657	349[a]	17.2[b]	33.2[a]	7.9[a]	261.3[ab]	0.477[a]
758	320[a]	23.7[a]	33.9[a]	8.6[a]	327.5[a]	0.429[ab]
857	325[a]	17.6[b]	30.3[ab]	7.5[a]	246.0[b]	0.483[a]
940	311[a]	19.3[ab]	27.0[b]	8.8[a]	249.6[b]	0.465[ab]
Pooled SEM	18.6	1.67	1.47	0.55	25.86	0.022

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per live hen per day
[2]n = 40
[3]n = 124
[4] Defined as Area 2:Area 1.

Experiment 2

Feed consumption was not affected among dietary treatments (Table 3.7). Actual threonine consumed by the hens was 561, 766, 964, and 1167 mg Thr/HD for 0.56, 0.76, 0.96, and 1.16 diets, respectively. Subsequently all references to threonine treatments are identified by these mg/HD intake levels.

Egg production was significantly higher for hens that consumed 766mg Thr/HD compared to hens that consumed 1167 and 561mg Thr/HD (Table 3.8). Egg production for the control diet was significantly lower compared to all other dietary treatments. Albumen weight (g/egg) and albumen liquid yield (%) were not significantly affected by dietary treatment. Yolk weight was significantly higher for hens that consumed 561 and 964 mg Thr/HD compared to those of hens that consumed 766 mg Thr/HD. Yolk liquid yield of hens that consumed 964 mg Thr/HD was significantly higher than those of hens that consumed 766 mg Thr/HD (Table 3.8).

Albumen and yolk solids (%) were not significantly affected by dietary treatments (Table 3.9). Albumen protein was significantly higher for eggs of hens that consumed 1167 mg Thr/HD compared to all other dietary treatments. Yolk protein was significantly higher among eggs of hens that consumed the control diet (561 mg Thr/HD) compared all other treatments. Stronger shells were detected among eggs of hens that consumed 1167 mg Thr/HD compared to eggs of hens that consumed 766 and 561 mg Thr/HD. Vitelline membrane rupture strength was unaffected by levels of dietary threonine.

Angel food cake rapeseed displacement was not affected by the dietary treatments (Table 3.10). Angel food cakes baked from egg albumen of hens that consumed 1167 mg Thr/HD were significantly harder requiring more force to deform

TABLE 3.7. The impact of dietary threonine on feed consumption and hen weight, Experiment 2

Thr Level (%)	Feed Consumption (g/H/D)	Thr Intake (mg/HD)	Beginning Hen Weight	Ending Hen Weight	Weight Gain/Loss
			----------(g)----------		
0.56	100.3[a]	561[d]	1564[b]	1519[b]	-45[b]
0.76	100.8[a]	766[c]	1667[a]	1629[a]	-38[ab]
0.96	100.4[a]	964[b]	1612[ab]	1624[a]	12[ab]
1.16	100.6[a]	1167[a]	1649[ab]	1682[a]	33[a]
Pooled SEM	0.39	3.3	34.1	34.4	27.8

[a-d] Values within columns with no common superscript differ significantly ($p<0.05$)
[1]HD = per live hen per day

TABLE 3.8. The impact of dietary threonine on egg production, egg weight, component yield by weight and percentage of liquid egg, Experiment 2

Thr Intake (mg/HD[1])	Egg Production	Whole Egg Weight[2]	Albumen Weight[3]	Yolk Weight[4]	Albumen Liquid[3]	Yolk Liquid[4]
		----------(g/egg)----------			----------(%)----------	
561	82.5[c]	63.2[a]	36.8[a]	17.4[a]	58.1[a]	27.6[ab]
766	87.2[a]	62.4[ab]	36.5[a]	17.0[b]	58.4[a]	27.3[b]
964	86.3[ab]	62.1[b]	36.1[a]	17.4[a]	58.0[a]	28.0[a]
1167	85.1[b]	62.3[ab]	36.2[a]	17.1[ab]	58.0[a]	27.6[ab]
Pooled SEM	0.62	0.35	0.27	0.10	0.19	0.14

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$)
[1]HD = per live hen per day
[2]n = 900
[3]n = 797
[4]n = 764

TABLE 3.9. The impact of dietary threonine on egg component total solids, protein content, shell strength, and vitelline membrane strength, Experiment 2

Thr Intake (mg/HD[1])	Albumen Solids[2]	Albumen Protein[3]	Yolk Solids[2]	Yolk Protein[4]	Shell Strength[5]	Vitelline Membrane Strength[6]
	----------(%)----------				----------Kg Force----------	
561	11.4[a]	87.3[b]	52.1[a]	33.9[a]	3.0[b]	8.2[a]
766	11.5[a]	87.3[b]	52.2[a]	33.5[b]	3.0[b]	8.3[a]
964	11.4[a]	87.4[b]	52.1[a]	33.5[b]	3.1[ab]	8.8[a]
1167	11.5[a]	87.6[a]	52.0[a]	33.7[ab]	3.3[a]	7.9[a]
Pooled SEM	0.06	0.90	0.30	0.08	0.07	0.46

[a,b] Values within columns with no common superscript differ significantly ($p<0.05$)
[1]HD = per live hen per day
[2]n = 320
[3]n = 740
[4]n = 730
[5]n = 600
[6]n = 405

TABLE 3.10. The impact of dietary threonine on texture profile analysis parameters of angel food cake, Experiment 2

Thr Intake (mg/HD[1])	Rapeseed Displacement[2] (mL)	Hard[3] (N)	Springy[3] (mm)	Gummy[3] (N)	Chewy[3] (N mm)	Cohesive[3,4]
561	277[a]	15.7[c]	22.0[a]	10.1[a]	218[a]	0.63[a]
766	276[a]	18.9[b]	21.0[a]	11.6[a]	236[a]	0.64[a]
964	285[a]	19.1[b]	21.5[a]	8.9[a]	193[a]	0.46[a]
1167	291[a]	21.9[a]	22.4[a]	10.1[a]	197[a]	0.49[a]
Pooled SEM	13.6	0.89	0.84	1.17	23.6	0.07

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per live hen per day.
[2]n = 60
[3]n = 299
[4] Defined as Area 2:Area 1.

compared to all other dietary treatments. A significantly softer angel food cake was baked from egg albumen of hens that consumed 561 mg Thr/HD compared to other dietary treatments. Springiness, cohesiveness, gumminess, and chewiness were unaffected by the treatments. (Table 3.10)

Sponge cakes baked from egg yolks of hens that consumed 964mg Thr/HD and 1167 mg Thr/HD had significantly more volume compared to the other diets. (Table 3.11) Egg yolks of hens that consumed 561 mg Thr/HD baked sponge cakes that displaced significantly less volume compared to other dietary treatments. Sponge cakes baked from egg yolks of hens that consumed 1167 mg Thr/HD required more force yielding a harder cake compared to those of hens that consumed 964, 766, and 561 mg Thr/HD. More springy sponge cakes were baked from egg yolks of hens that consumed 766 and 561 mg Thr/HD compared to those of hens that consumed 964 and 1167 mg Thr/HD. Significantly less springy sponge cakes were baked from egg yolks of hens that consumed 964mg Thr/HD. No significant differences were found among dietary treatments for sponge cake cohesiveness, gumminess, or chewiness.

TABLE 3.11. The impact of dietary threonine on texture profile analysis parameters of sponge cake, Experiment 2

Thr Intake (mg/HD[1])	Rapeseed Displacement[2] (ml)	Hard[3] (N)	Springy[3] (mm)	Gummy[3] (N)	Chewy[3] (N mm)	Cohesive[3,4]
561	169[c]	18.0[b]	21.2[a]	9.8[a]	185[a]	0.59[a]
766	216[b]	18.3[b]	21.8[a]	11.5[a]	191[a]	0.56[a]
964	268[a]	18.5[b]	16.4[c]	9.6[a]	148[a]	0.50[a]
1167	262[a]	24.8[a]	18.5[b]	12.2[a]	215[a]	0.58[a]
Pooled SEM	14.8	1.69	0.97	25.10	1.29	0.086

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$)
[1]HD = per live hen per day
[2]n = 60
[3]n = 246
[4] Defined as Area 2:Area 1.

Discussion

Egg production increased with increasing dietary threonine levels up to 758 and 766 mg/HD for experiments 1 and 2, respectively. Threonine levels higher than these resulted in decreased egg production. Schutte (1998) reported that increasing the supply of one amino acid improves performance only if no other amino acid is limiting. Performance of hens receiving higher levels of threonine may have been hindered when other amino acid levels were not increased as well. Threonine has been shown to hinder methionine influx and stimulate lysine influx into the epithelial cells of the intestinal lumen (Lerner 1970) thus the higher levels of threonine may have altered the metabolic balance of methionine and lysine.

Egg, albumen, and yolk weights were not affected in a significant manner within nor a consistent manner between experiments. Albumen and yolk yield as a percentage of egg weight were likewise not impacted in a consistent manner. Experiment 1 albumen solids increased with increasing threonine levels, reaching 11.5% for hens consuming 940 mg Thr/HD although the differences among the treatments were small. Experiment 2 albumen and yolk solids were not different among dietary treatments. It should be noted that analysis of the diets revealed that the levels of threonine and crude protein were lower in the control diet of experiment 1 compared to experiment 2. The threonine levels studied had minimal impact on solids content of albumen and yolk. Shell strength increased with increasing dietary threonine levels. Shell strength and shell membrane strength are positively correlated (Essary et al., 1977). Klingensmith et al. (1988) reported no significant differences in the Thr content of dried shell membranes of hard shelled, soft-shelled, and shell-less eggs. These findings along

with the increase in shell strength found in these experiments suggest that increasing the amount of dietary threonine enhances shell strength.

Cake height is related to the foam stability of a batter. Foam stability is the ability of foam to retain its maximum volume over cooking or heating (Woodward and Cotterill, 1987). Angel cake batter relies on the ability of the albumen proteins to form stable foams in order to withstand heating. Albumen foam consists of air droplets formed by whipping the egg albumen. These droplets are encased in a liquid protein containing a soluble surfactant that aids in the foam stability (Kinsella 1976). As shown by rapeseed displacement in experiment 1, increasing the level of dietary threonine increased the angel cake batter stability. Therefore, with a stronger, more stable protein complex occurring during cooking with increased dietary threonine levels, cake cores subjected to two-cycle compression of increased threonine levels required more force to compress, yielding a harder or more sturdy cake for confection. This protein matrix can also be related to the ability of the cake samples to be springier, gummier, and chewier with increasing dietary threonine levels. Based on this research finding, it is evident that increasing the level of dietary threonine can have positive impacts on the functional properties of albumen and yolk.

CHAPTER IV

THE IMPACT OF DIETARY SUPPLEMENTAL L-THREONINE ON EGG COMPONENT YIELD, COMPOSITION, AND FUNCTIONALITY IN 61-77 WEEK OLD LAYING HENS

Overview

Ninety 61 week old commercial laying hens were housed in individual cages in an open sided laying facility with experimental units of 5 hens sharing access to a common feed trough. A typical layer diet, containing 0.56% threonine (Thr) served as control. Two experimental diets containing 0.76 and 0.96% Thr were fed to the hens for sixteen weeks. Egg samples were analyzed for egg weight, shell strength, shell thickness, vitelline membrane strength, and yolk and albumen yield and functionality. Yolk and albumen were separated, pooled by experimental unit, homogenized, and analyzed for solids at 2 week intervals. Yolk and albumen were separated and pooled by experimental unit at weeks 2, 6, 10, 15 to make three replicates each of sponge and angel food cakes, respectively. Cake samples were cored, 5 cores per cake and subjected to two-cycle compression on an Instron Universal Testing machine for texture profile analysis. Cakes were measured for hardness, springiness gumminess, chewiness, and cohesiveness. Yolk and albumen gels were made for each egg and boiled in 30ml beakers at 2 week intervals and subjected to the same measures as the cake cores. Albumen gels were made at 2 week intervals and subjected to torsion methods to determine strain and stress values. Whole egg weight was found to be significantly ($P<0.05$) higher in the diet containing 0.76% Thr compared to all other

diets. Significantly stronger shells were found among hens fed the diet containing 0.96% Thr compared to the control diet. Significantly thicker egg shells were found in the diet fed to hens containing 0.96% Thr. These data clearly indicate a potentially important impact of threonine nutrition of laying hens on egg component functionality and shell characteristics.

Introduction

A recent trend of high protein diets has increased consumer demand for nutritionally sound food items in the commercial confection, baking, hotels, and fast food industries. This demand has resulted in an increase of about 25% in the consumption of liquid egg (USDA 2003). Liquid egg (LE) standards rely on the functionality and compositional parameters of the yolk and albumen. As hens age, egg quality parameters decrease, while egg size and weight increases (Akbar et al., 1983). These larger eggs are often sold for utilization in the LE industries in lieu of the table egg market.

The essential amino acid, threonine (Thr), is used in important metabolic processes such as protein synthesis and uric acid formation. Threonine is the third most limiting amino acid (Kidd and Kerr, 1996). Ishibashi et al. (1998) found improved feed intake, egg mass, and feed efficiency with increasing threonine levels (0.31% - 0.61%). However, body weight decreased with increasing threonine levels and no change in egg size was noted among dietary treatments. Graduating the level of dietary threonine from 0.35% to 0.58% in a typical layer diet yielded increased egg production (Faria et al., 2002). Until recently, research including threonine as a

supplemental amino acid has not included LE parameters. While extensive research has been conducted on eggs of aging hens, very little research has considered the impact of supplemental threonine as means of increasing component yield and functionality. The NRC requirement for threonine is 0.56% of the diet, which is an estimate because experimental data are lacking (NRC 1994). Research reported in the previous chapter indicate that increasing the level of dietary threonine can have positive impacts on egg production and the functional properties of albumen and yolk in 42-60 wk old hens. Because most post peak production eggs are utilized in the LE market, the role of increasing the level threonine in the diet of older hens on LE functional properties warrants investigation.

Shell strength decreases with increasing hen age (Tyler 1961). While the force required to crack a peak production egg is 4.0 kg, only 2.8 kg of force is required to crack the egg shell of a 78 week old hen (Hamilton et al., 1979). Because shell strength is strongly correlated with membrane strength (Essary et al., 1977) and amino acids are found in the membranes, it stands to reason that increasing the level of dietary threonine may increase the force required to crack an egg shell. The LE industry could benefit from research of supplemental threonine above NRC requirements in the diet of older hens. The objective of this research is to evaluate the impact of supplemental threonine on body weight, feed consumption, egg production, egg weight, shell quality, liquid yield, protein content, and functionality of albumen and yolk in eggs of 61 – 77 wk old hens.

Materials and Methods

Experimental Design

A sixteen week experiment was conducted utilizing ninety 61 week old commercial strain laying hens[10]. Hens were housed in individual wire cages in an open sided laying facility with experimental units of 5 hens sharing access to a common feed trough. The lighting schedule was maintained at 17 hours of daylight and 7 hours of darkness throughout the study. Hens were randomly assigned into 6 blocks with 3 dietary treatments per block (thirty hens per diet).

A layer diet containing 0.56% threonine was fed to control hens throughout the experiment (Table 4.1). Experimental diets contained 0.76 and 0.96% threonine supplied by synthetic 98% feed quality L-Threonine[11]. Feed was formulated to contain approximately 16.5% crude protein and 2800kcal/kg, as fed in a typical layer diet (NRC 1994) and was mixed every three weeks. Threonine was added to the diets at the expense of soybean meal. Samples of the control diet were pooled and subjected to amino acid analysis at the Texas A&M Protein Chemistry Laboratory. Threonine in the control diet was 0.62%. Feed samples were collected at each mixing date, pooled and 5 sub samples were subjected to nitrogen analysis using a Leco FP-428 Nitrogen Determinator System[12]. Percent nitrogen was multiplied by 6.25 to determine protein content of the feed samples. Crude protein in the control diets was 16.6%. Feed and water were provided *ad libitum.* Hens were individually weighed at the initiation and

[10] Dekalb XL, Centurion Poultry, Lexington, GA
[11] Ajinomoto Heartland, Inc., Eddyville, IA 52553
[12] Leco Corp., St. Joseph, MI 49085-2396

TABLE 4.1. Composition of layer diets[1] containing increased levels of threonine

	Dietary Threonine Level		
Ingredient	0.56	0.76	0.96
	----------(% of diet)----------		
Corn	69.5	69.5	69.5
Soybean meal 48	18.8	18.6	18.4
Limestone	8.5	8.5	8.5
Lysine HCL	1.5	1.5	1.5
Phosphorus	1.0	1.0	1.0
Salt	0.25	0.25	0.25
Vitamin Premix[2]	0.25	0.25	0.25
DL-Met98	0.12	0.12	0.12
Thr[3]	0.0	0.20	0.40
Mineral Premix[4]	0.05	0.05	0.05

[1]Diet maintained 2800kcal of ME/kg, and 16.5% crude protein.

[2]Vitamin premix added at this rate yielded 11,023 IU of vitamin A, 3,858 IU of vitamin D, 46 IU of vitamin E, 0.0165 mg of vitamin B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg of D-pantothenic acid, 477.67 mg choline, 1.47mf of menadione, 1.75mf of folic acid, 7.17 mg of pyroxidine, 2.94 mg of thiamin, 0.55 mg of biotin per kilogram of diet. The carrier was ground rice hulls.

[3]98% feed grade L - threonine.

[4]Mineral premix added at this rate yielded 149.6 mg of manganese, 125.4 mg of zinc, 16.5 mg of iron, 1.7 mg of copper, 1.05 mg of iodine, 0.25 mg of selenium, a minimum of 6.27 mg of calcium, and a maximum of 8.69 mg of calcium per kilogram of diet. The carrier was calcium carbonate, and the premix contained les than 1% mineral oil.

termination of the experiment. Hen-day egg production was calculated on a weekly basis. Feed consumption was calculated on a tri-weekly basis.

Experimental Methods

Egg Composition. Eggs were analyzed for egg weight, yolk and albumen yield, protein content, and solids at 2 week intervals. Egg contents (yolk and albumen) were manually separated using a plastic egg separator. The yolk was rolled on a damp paper towel to remove any chalazae or remaining albumen and then weighed. Yolks were punctured with a metal spatula, pooled by experimental unit, and stirred using a glass rod prior to further analysis. Albumen yield was determined by subtraction of the yolk and shell with shell membranes intact from the whole egg weight (Shafer et al., 1996). Albumen was pooled by experimental unit and homogenized using an upright hand-held household blender[13] on low speed for 2, five second pulses to reduce froth. Solids content for albumen and yolk were determined from dual 10 gram sub samples of the each pooled samples. The sub samples were dried in aluminum drying pans at 105C for 24 hours. Protein content of dried albumen and yolk was determined from five sub samples of dried albumen and yolk using a Leco FP-428 Nitrogen Determinator System[14]. Fives sub samples per pooled sample of dried albumen and yolk were analyzed for nitrogen. The percent nitrogen reported was multiplied by 6.25 to yield protein content of albumen and yolk samples.

[13] Cuisinart, E. Windsor, NJ 08520
[14] Leco Corp., St. Joseph, MI 49085-2396

For each dietary treatment, eggs were tested for shell strength using an Instron Universal Testing Machine equipped with a 50 kilogram (kg) load cell at a 10kg load range with a crosshead speed of 50 mm/min. Shell strength and thickness were conducted at 2 week intervals. Shell thickness was measured by averaging three measurements of thickness recorded from the equator of the each egg shell using an AMES[15] thickness measurer.

Rupture strength of vitelline membranes were measured on an Instron Universal Testing Machine equipped with a 5 kg load cell and 500 g load range, for sensitivity, with a crosshead speed of 50 mm/min during weeks 3, 6, 9, 12, and 15. Egg yolks were separated from the albumen using a plastic egg separator, rolled on a damp paper towel to remove any adhering albumen or chalazae and weighed in grams. Individual yolks were placed in the center of filter paper lying in the bottom of a 100 ml beaker. Prior to compression, beakers containing the yolk were placed beneath the anvil. Vitelline membranes were allowed to rupture, exposing yolk contents, yielding a rupture strength number recorded in kilograms for each egg yolk sampled.

Texture Profile Analysis. Eggs were sampled at wks 2, 6, 10 and 15 for cake baking. Albumen and yolk were separated with a plastic egg separator and pooled by nutritional treatment to make three replicates each of sponge and angel food cakes, respectively, by methods reported by Shafer et al. (1998) (Appendix A; Appendix B). The yolks were strained through cheesecloth to remove the yolk membranes for sponge cake preparation. Sponge cakes were prepared using stand mixer[16] on medium (7)

[15] B.C. AMES Co, Waltham, Mass
[16] Model 168949, Wal-Mart Stores, Inc., Bentonville, AK 72716

speed. Angel food cakes were prepared using the same mixer on speed 9 until medium peaks were formed. Sponge cakes were bake for 25 min at 176C. Angel food cakes were baked for 22 minutes at 176C. All cakes were baked in the same oven. Prior to each baking session, ovens were preheated and checked for constant heat with a calibrated oven thermometer. Cakes were allowed to cool for approximately 4 hours and volume results were recorded by rapeseed displacement (Shafer et al., 1998). Thereafter, cakes were cored (1.75mm corer), up to five cores per cake and subjected to two-cycle compression on an Instron Universal Testing Machine[17] equipped with a 500 Newton (N) load cell and 200N load range with a crosshead speed of 100 millimeters per minute (mm/min). Chart force-distance curves for each cake core were measured for hardness, cohesiveness, springiness, gumminess, and chewiness as defined by Bourne (1978). Hardness correlates to the 'first bite' or force that is required to deform a food particle. Springiness is the distance in height (mm) the sample will recover between the first and second compression or 'bite'. Cohesiveness is defined as the ratio of the positive force areas under the curves of both compressions and is considered dimensionless (Bourne 1978). Cohesiveness was necessary to define in order to determine gumminess and chewiness. Gumminess is defined hardness multiplied by cohesiveness Chewiness is defined as the product of gumminess multiplied by springiness (N mm) (Breene 1975).

Eggs were sampled at 2 week intervals for gel TPA. Albumen and yolk were separated using a plastic egg separator. During separation, the albumen of each egg was allowed to drop into individual weigh boats and stirred with a glass rod prior to being placed in 30 ml beakers. Yolks were rolled on damp paper towels to removed

[17]Model 1011, Instron Corp., Canton, MA

adhering albumen and chalazae. Yolks were punctured with a metal spatula and the contents were allowed to spill into a beaker. Prior to receiving egg components, each beaker was lightly coated with non-stick cooking spray[18]. Beakers were covered with heavy duty aluminum foil to reduce moisture loss (Shafer et al., 1998). Beakers were placed in 80C water bath for 30 min (Kinsella 1976; Hickson et al., 1980; Woodward and Cotterill, 1987;). Gels were cooled at room temperature for 1 h. Gels were removed from beakers using a thin metal spatula and placed on a labeled tray covered with heavy duty aluminum foil and refrigerated for a maximum of 4 h prior to sampling. Gels were removed from the refrigerator and trimmed to 2.5 cm in length using a modified egg slicer directly prior to testing (Shafer et al., 1998). Cooled gels have been reported to be more elastic (van Kleef 1986). Gels were compressed to 50% of their original height on an Instron Universal Testing Machine equipped with a 500N load cell with a cross head speed of 125 mm/min. Albumen gels were compressed with a 50 N load range and yolk gels were compressed with a 100N load range (Shafer et al., 1998). Chart force-distance curves for each gel were measured for hardness, cohesiveness, springiness, gumminess, and chewiness as defined by Bourne (1978).

Albumen gels for torsion were made by methods previous described during weeks 4, 10, 12, 14, and 16. After refrigeration, gels were trimmed using a modified egg slicer to 3.0 cm in height. Gels were then trimmed to 1 cm in length and width (maintaining height). Ends of gels were super-glued to polystyrene notched ½ inch discs. Gels were milled into a barbell–like shape maintaining a 0.5 cm diameter then

[18] PAM, ConAgra Foods, Irvine, CA

immediately placed on a modified Brookfield Viscometer[19] and measured for torsional forces of strain and stress at fracture (Hamann et al., 1990).

Statistical Analysis

Data were analyzed using ANOVA with General Linear Model (GLM) procedure of SAS[20], with main effects of diet, week, and replication. Mean differences were separated using the PDIFF option, which uses pair-wise *t* tests of the GLM procedure. All statistical comparisons were considered significant at $P < 0.05$.

Results

Feed consumption was not affected by dietary treatments. (Table 4.2) Calculated threonine intake of the hens was 540, 724, and 926 mg/HD for 0.56, 0.76, and 0.96% diets, respectively. Subsequently, all references to threonine treatments are identified by these mg/HD intake levels.

Egg production was not affected by dietary treatment. (Table 4.3) Whole egg, albumen, and yolk weights were significantly higher for eggs of hens that consumed 724 mg Thr/HD compared to all other treatments. Yolk weight of eggs of hens that consumed 540 mg Thr/HD was significantly lower than those of other dietary treatments. Yolk liquid was significantly higher in eggs of hens that consumed 926 mg Thr/HD compared to eggs of hens that consumed 540 mg Thr/HD. Albumen solids

[19] Model DV-I +, Brookfield Eng. Labs Inc., Stoughton, MA, 02072
[20] SAS version 8.01, SAS Institute, Cary, N.C

TABLE 4.2. The impact of dietary threonine on feed consumption and hen weight

Thr Level (%)	Feed Consumption (g/H/D)	Thr Intake (mg/HD[2])	Beginning Hen Weight[1]	Ending Hen Weight[1]	Weight Gain/Loss
			----------(g)----------		
0.56	96.5[a]	540[c]	1606[a]	1540[a]	-66[a]
0.76	95.2[a]	724[b]	1636[a]	1567[a]	-68[a]
0.96	96.4[a]	926[a]	1645[a]	1580[a]	-65[a]
Pooled SEM	0.64	4.7	27.3	28.8	38.7

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]n = 176: 90 beginning hens + 86 ending hens.
[2]HD = per live hen per day.

TABLE 4.3. The impact of dietary threonine on egg production, egg weight, component yield by weight and percentage of liquid egg

Thr Intake (mg/HD[5])	Egg Production[1] (%)	Whole Egg Weight[2]	Albumen Weight[3]	Yolk Weight[4]	Albumen Liquid[3]	Yolk Liquid[4]
		----------(g/egg)----------			----------(%)----------	
540	78.3[a]	63.5[b]	37.1[b]	17.1[c]	58.3[a]	27.1[b]
724	79.6[a]	65.3[a]	38.4[a]	17.8[a]	58.6[a]	27.4[ab]
926	77.8[a]	63.4[b]	36.9[b]	17.5[b]	58.2[a]	27.6[a]
Pooled SEM	0.09	0.37	0.29	0.11	0.17	0.14

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]n = 30 hens per treatment.
[2]n = 581
[3]n = 566
[4]n = 530
[5]HD = per live hen per day.

were significantly higher in eggs of hens that consumed the control diet compared to all other treatments. (Table 4.4) Significantly higher yolk solids were detected in eggs of hens that consumed 724 mg Thr/ HD compared to those of hens that consumed 926 mg Thr/HD. Albumen protein was significantly higher among eggs of hens that consumed 926 mg Thr/HD compared to other dietary treatments. Significantly higher yolk protein was detected among eggs of hens that consumed 926 and 540 mg Thr/HD compared to those of hens that consumed 724 mg Thr/HD. Significantly stronger shells were detected among eggs of hens that consumed 926 mg Thr/HD compared to those of hens that consumed the control diet. Significantly thicker shells were also detected among the eggs of hens that consumed 926 mg Thr/HD compared to those of all other dietary treatments (Table 4.4).

Angel food cake baked from albumen of eggs of hens that consumed 926 and 724 mg Thr/HD had significantly more volume compared to the control. (Table 4.5) Angel food cakes baked from albumen of eggs of hens that consumed 926 mg Thr/HD were significantly harder compared those baked from other dietary treatments. However, these cakes were also detected to be significantly less springy compared to those of the control and 724 mg Thr/HD. These findings suggest that a harder cake may not spring back to its original form as well as a less hard cake given a deformation force. Significantly chewier angel food cakes were baked from albumen of eggs of hens that consumed 540 and 724 mg Thr/HD compared to those of hens that consumed 926 mg Thr/HD.

TABLE 4.4. The impact of dietary threonine on egg component total solids, protein content, shell strength, and shell thickness

Thr Intake	Albumen Solids[2]	Albumen Protein[3]	Yolk Solids[2]	Yolk Protein[3]	Shell Strength[4]	Shell Thickness[5]
	----------(%)----------				Kg	mm
540	11.3[a]	87.7[b]	52.3[ab]	34.2[a]	2.73[b]	0.0147[b]
724	11.1[b]	87.6[b]	52.4[a]	34.0[b]	2.86[ab]	0.0146[b]
926	11.1[b]	88.0[a]	52.0[b]	34.2[a]	3.01[a]	0.0153[a]
Pooled SEM	0.06	0.05	0.12	0.05	0.07	0.0002

[a,b] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per live hen per day.
[2]n = 288
[3]n = 720
[4]n = 388
[5]n = 540

TABLE 4.5. The impact of dietary threonine on texture profile analysis parameters of angel food cake

Thr Intake (mg/HD[1])	Rapeseed Displacement[2] (ml)	Hard[3] (N)	Springy[3] (mm)	Gummy[3] (N)	Chewy[3] (N mm)	Cohesive[3,4]
540	164[b]	9.7[b]	22.9[a]	5.1[a]	113[a]	0.52[a]
724	274[a]	10.6[b]	21.5[a]	4.8[a]	98[a]	0.45[ab]
926	275[a]	12.8[a]	15.0[b]	5.1[a]	72[b]	0.39[b]
Pooled SEM	12.0	0.42	0.74	0.39	6.9	0.038

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per hen per live day.
[2]n = 36
[3]n = 180
[4] Defined as Area 2:Area 1

TABLE 4.6. The impact of dietary threonine on texture profile analysis parameters of sponge cake

Thr Intake (mg/HD[1])	Rapeseed Displacement[2] (ml)	Hard[3] (N)	Springy[3] (mm)	Gummy[3] (N)	Chewy[3] (N mm)	Cohesive[3,4]
540	122[b]	20.7[a]	22.3[a]	11.1[a]	280[a]	0.53[a]
724	186[a]	16.3[b]	21.4[ab]	8.0[a]	199[a]	0.51[a]
926	217[a]	14.6[b]	17.7[b]	11.9[a]	334[a]	0.68[a]
Pooled SEM	11.8	1.30	1.65	2.11	72.1	0.084

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per live hen per day
[2]n = 36
[3]n = 117
[4] Defined as Area 2:Area 1.

Sponge cakes baked from yolks of eggs of hens that consumed 926 and 724 mg Thr/HD had significantly more volume compared to those of the control diet. (Table 4.6) A significantly harder sponge cake was baked from yolks of eggs of hens that consumed the control diet compare to those of other dietary treatments. Significantly more springy sponge cakes were baked from yolks of eggs of hens that consumed the control diet compared to those of hens that consumed 926 mg Thr/HD. Gumminess and chewiness of sponge cakes was not significantly affected by dietary treatment.

No significant differences on texture profile parameters were detected for albumen gels among dietary treatment. (Table 4.7) However, gels prepared from yolks of eggs of hens that consumed 724 mg Thr/HD were significantly harder compared to those of hens that consumed the control diet. (Table 4.8) Springiness, gumminess and chewiness of yolk gels were not significantly different among dietary treatment. Significantly higher strain at fracture of albumen gels prepared from eggs of hens that consumed 724 mg Thr/HD were detected compared to those of the control diet. (Table 4.9) No significantly difference was detected among dietary treatment for stress at fracture for albumen gels.

TABLE 4.7. The impact of dietary threonine on texture profile analysis parameters of albumen gels

Thr Intake (mg/HD[1])	Hard[2] (N)	Springy[2] (mm)	Gummy[2] (N)	Chewy[2] (N mm)	Cohesive[2,3]
540	20.8[a]	14.2[a]	11.1[a]	158.4[a]	0.54[a]
724	20.0[a]	14.1[a]	11.6[a]	158.8[a]	0.58[a]
926	20.5[a]	14.6[a]	12.4[a]	189.5[a]	0.59[a]
Pooled SEM	0.66	0.49	0.97	18.30	0.035

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per live hen per day
[2]n = 369
[3] Defined as Area 2:Area 1

TABLE 4.8. The impact of dietary threonine on texture profile analysis parameters of yolk gels

Thr Intake (mg/HD[1])	Hard[2] (N)	Springy[2] (mm)	Gummy[2] (N)	Chewy[2] (N mm)	Cohesive[2,3]
540	34.7[b]	9.3[a]	22.0[a]	220[a]	0.62[a]
724	37.5[a]	10.6[a]	22.0[a]	239[a]	0.59[a]
926	36.7[ab]	9.5[a]	23.4[a]	236[a]	0.64[a]
Pooled SEM	0.88	0.54	0.91	18.4	0.02

[a-c] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per live hen per day
[2]n = 358
[3] Defined as Area 2:Area 1

TABLE 4.9. The impact of dietary threonine on albumen gels for torsion

Thr Intake (mg/HD[1])	Strain[2,3]	Stress[2] (kPa)
540	1.36[b]	5.90[a]
724	1.59[a]	6.50[a]
926	1.48[ab]	6.47[a]
Pooled SEM	0.08	0.61

[a,b] Values within columns with no common superscript differ significantly ($p<0.05$).
[1]HD = per live hen per day
[2]n = 360
[3]Strain is defined as deformation: sample height

Discussion

Typically, feed consumption, egg production and egg interior quality parameters decline as the hens age, while whole egg and component weight increase. Egg production for hens in this cycle of lay is typically 72.75% (Chowdhury and Smith, 2001) having an average egg weight of 64 grams, yolk weight of 18.35 grams, and albumen weight of 40.63 grams (Silversides and Budgell, 2004). Feed consumption and egg production were not affected by increasing dietary threonine levels. However, egg production of the hens in this experiment is higher compared to Chowdhury and Smith, (2001). This could be attributed to differences in strain of hens or environmental factors between this and other studies. Whole egg weights are comparable to past research findings however, yolk and albumen weights were lower. Hens consuming 724 mg Thr/HD produced significantly larger egg components (38.37 g of albumen and 17.82 g of yolk) compared to other dietary treatments. Whole egg weight may be a contributing factor, which was also significantly higher in this diet compared to the other treatments. The significantly higher whole egg weight of hens that consumed 724 mg Thr/HD could also explain the increase in yolk solids observed for the same dietary treatment. Yolk solids of eggs from 63 week old hens have been reported to be 52.27% (Akbar et al., 1983), which is comparable to these research findings. Whole egg weight can be influenced by hen body weight. However, hen body weights were not significantly different among dietary treatment at the beginning or ending of the experiment.

The significantly larger egg and component weight of the Thr intake level 724 mg/HD was not comparable to the impact of an intake of 926 mg Thr/HD on other studied parameters. While this intake level produced significantly lighter whole egg,

albumen and yolk weight compared to the 724 mg Thr/HD eggs, these eggs differed in composition and functionality. Albumen and yolk protein were significantly higher in this diet compared to the other dietary treatments. An albumen protein of 84.3% on a dry matter basis from Leghorn hens (Woodward and Cotterill, 1986) is lower than hens fed 926mg Thr/HD. This indicates increasing the amount of threonine in the diet may positively impact protein content of albumen.

Egg shell cracking strength increased significantly with increasing dietary threonine levels. Egg shell thickness also exhibited these attributes with hens consuming 926 mg Thr/HD. This is supported by Tyler (1961), Essary et al. (1977) and Strong (1989), who found a correlation in hen age, cracking strength, and shell thickness.

Angel food cake functionality was positively affected by increased threonine levels. The increase in albumen protein content in the eggs of hens that consumed 926mg Thr/HD may explain the significant increase in volume and hardness of angel food cakes baked from these diets. Because food technologist and confectioners are concerned with transportation of mass quantities of consumer appealing food items (Bourne 1966) a harder cake may be advantageous because it can withstand more compression weight. Sponge cakes baked from eggs of hens fed increased levels of threonine were significantly less hard and less springy compared to the control diet. The control diet provided a hard cake for transportation purposes that would spring back following deformation. However, the control diet was had significantly less volume compared to the other treatments.

On heating, egg proteins serve as binding agents for items such as custards and pie filings (Mine 2002). As protein level in egg white increases, hardness of albumen

gels should increase (Woodward and Cotterill, 1987). Despite the increase in albumen protein with increasing threonine levels, no significant differences in TPA were found among dietary treatments. An explanation for this is other methods adjust pH or blend and freeze the albumen source prior to sampling (Woodward and Cotterill, 1987).

The yolk of hard cooked eggs is crumbly, thus if the yolk is disrupted by stirring prior to heating, a firm rubbery gel may be formed (Woodward and Cotterill, 1987). Yolk gels of eggs of hens that consumed 724 mg Thr/HD were significantly harder compared to those of other dietary treatments. These yolks contained significantly less protein content compared to the other treatments suggesting that upon heating, the protein complexes were better able to denature and aggregate forming a harder gel than those of yolks containing the higher protein content. It is evident that increasing the level of dietary threonine in older hens can have positive impacts on composition and functional properties of albumen and yolk.

Albumen gels yielded no significant differences in torsion stress. Stress is related to the TPA parameter of hardness (Hamann et al., 1990). This supports the findings that albumen gels were not significantly different among the diets hardness or torsion stress. Strain is the counter twist length at deformation to sample length (Hamann et al., 1990). Length of all samples was the same, thus the greater the length of the counter-twist to acquire deformation (fracture), the greater the strain. Strain is also an indicator of gelling quality of proteins (Gel Consultants 2004). Significantly more strain was detected to fracture albumen gels of eggs of hens that consumed 724 mg Thr / HD compared to those of the control diet suggesting a stronger gel compared to gels of the control diet.

CHAPTER V

SUMMARY

Whole egg, albumen and yolk weight were not consistently or significantly impacted by increasing dietary threonine levels. Hens consuming 940, 1167, and 926 mg Thr/HD for experiments 1, 2, and 3, respectively, laid eggs that had significantly higher albumen protein content compared to other dietary treatments. Threonine has been shown to stimulate lysine influx into the epithelial cells of the intestinal lumen (Lerner 1971), which may lead to the incorporation of more protein in the albumen through the incorporation of more amino acids into the epithelial cells of the oviduct lumen.

Experiment 1 yolk protein increased with increasing dietary threonine levels and was found significant in all dietary levels above 758 mg. Experiment 2 yolk protein was not affected by dietary treatments. Experiment 3 hens consuming 926 and 561 mg Thr/HD yielded significantly higher yolk protein, suggesting no benefit to yolk protein content for aging hens that consume increased levels of threonine.

Shell strength increased with increasing dietary threonine levels in all three experiments. Strong (1989) reported 2.6 kg Force required to crack eggshells of 56 week old hens and 2.8 kg Force for shells of 30 week old hens. Keshavarz (1986) reported average shell strength of 3.38 kg Force for peak production hens. The forces required to crack egg shells of experiments 1 and 2 were close to the forces reported by Keshavarz (1986). This data can be extremely important to the table egg or hatching egg industries, as increased losses due to cracked or broken eggs can be costly. This is also important for producers who maintain late aging pre-molt flocks. In experiment

3, egg shells of hens that consumed 926 mg Thr / HD required 3.01 kg Force to crack compared to the control diet. These data indicate a significant impact on shell strength with increasing threonine levels. In all experiments, the control diet was significantly lower in shell breaking strength. Experiment 3 shell thickness increased with increasing threonine levels. Klingensmith (1988), reported an unchanged threonine content of shell membranes among shelled, soft-shelled, and shell-less eggs. Threonine increases the incorporation of other amino acids into the epithelial cells of the intestinal lumen (Lerner 1971) and may incorporate them into the cells of the reproductive tract allowing for a stronger shell membrane. Because shell strength, membrane strength, and shell thickness are correlated (Strong 1989), future research in this area of threonine incorporation may prove valuable to the commercial egg industry.

Determining functionality parameters was one major goal of this research. Texture profile analysis yielded hardness, springiness, cohesiveness, gumminess, and chewiness for angel food and sponge cakes (all experiments), and albumen and yolk gels (experiment three). For all experiments, the highest level of threonine consumed by hens consistently yielded significantly harder angel food cakes compared to other dietary treatments. The opposite effect was reported for springiness which was always significantly greater in the significantly softer angel food cakes. This suggests that a harder cake, once compressed with force, may not spring back as well as a softer cake. The same applies to sponge cake TPA.

Egg proteins on heating serve as binding agents for items such as custards and pie filings (Mine 2002). As protein level in egg white increases, hardness of albumen gels should increase (Woodward and Cotterill, 1987). Despite the increase in albumen protein with increasing threonine levels, no significant differences in albumen gel TPA

were found among dietary treatments. An explanation for this is that other methods adjust pH or blend and freeze the albumen source prior to sampling (Woodward and Cotterill, 1987).

The yolk of hard cooked eggs is crumbly, thus if the yolk is disrupted by stirring prior to heating, a firm rubbery gel may be formed (Woodward and Cotterill, 1987). Yolk gels of eggs of hens that consumed 724 mg Thr/HD were significantly harder compared to those of other dietary treatments. These yolks contained significantly less protein content compared to the other treatments suggesting that upon heating, the protein complexes were better able to denature and aggregate forming a harder gel than those of yolks containing the higher protein content.

Torsion of foods yields stress and strain parameters. Experiment 3 albumen gels yielded no significant differences in stress. Stress is related to the TPA parameter of hardness (Hamann et al., 1990). This supports the findings of these albumen gels experiments which yielded no significant differences in two-cycle compression hardness or torsion stress. Strain is the counter twist length at deformation to sample length (Hamann et al., 1990). Length of all samples was the same, thus the greater the length of the counter-twist to acquire deformation (fracture), the greater the strain. Strain is also an indicator of gelling quality of proteins (Gel Consultants 2004). Significantly more strain was detected to fracture albumen gels of eggs of hens that consumed 724 mg Thr / HD compared to those of the control diet suggesting that a stronger gel may have been made because more distance was covered prior to fracture compared to gels of the control diet. Supplemental threonine at 0.76% of the diet significantly increases albumen protein, shell strength, and functionality parameters.

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APPENDIX A

ANGEL FOOD CAKE

Ingredients

- 90 ml egg white
- 0.45g salt + 1.35g cream of tartar; sifted twice
- 69g granulated sugar
- 23g granulated sugar + 33g all-purpose flour; sifted twice

Procedure

- Preheat oven to 177C
- Add 90ml egg white to mixing bowl
- Beat at a medium speed
- Immediately sift salt + cream of tartar mixture over egg white
- Continue to beat at a medium speed until medium peaks form
- Sift sugar over foam in three increasing portions while mixer remains on medium speed
- Turn off mixer
- Sift flour + sugar mixture onto foam in three increasing portions, folding with a clean, rubber spatula 20 strokes after each addition
- Weigh 125g into tared loaf pan
- Bake for 20 minutes
- Remove from oven and invert onto a wire rack to cool

APPENDIX B

SPONGE CAKE

Ingredients

- 120g egg yolk: strain yolks through 2 layers of cheesecloth to remove membrane
- 82 ml boiling water
- 0.75g salt
- 75g sugar
- 48g flour + 60g sugar; sifted
- 36g flour + 2.7g baking powder; sifted twice

Procedure

- Preheat oven to 177C
- Place yolk, water, and salt in mixing bowl
- Beat on medium speed for 8 minutes
- Beat for 1.5 minutes while adding 75g sugar, 1 tablespoon at a time
- Beat at a slow speed for 1 minute while sifting flour + sugar mix into bowl
- Beat at a slow speed for 1 minute while sifting flour + baking powder mix into bowl
- Beat at slow speed for two more minutes
- Weigh 84g into tared loaf pan
- Bake for 25 minutes
- Remove from oven, place on wire rack

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Born Paige Lea Reynolds in Dallas, Texas in June of 1978, she became a Texas Aggie in 1996. Enrolling as a business major, she soon changed to poultry science in 1997. After becoming an active member of Alpha Phi Omega, Xi Delta chapter, the national coed service fraternity at TAMU, she soon realized a passion for research in the poultry industry. Under the direction of Dr. John Carey, she began microbiology research as an undergraduate and presented the research at the annual US Poultry and Egg conference held in Atlanta, GA. After graduating in May 2000 with a Bachelor of Science in poultry science, Paige interned with Longmont Foods- ConAgra in Colorado. Upon return to Texas A&M University, Paige began her master's degree research and coursework and worked at Sanderson Farms as the microbiologist. Paige graduated with her Master of Science in poultry science in May 2002. In November 2002, while beginning the first semester of her doctorate program, Paige wed Truitt Preston Niemeyer of Brenham, Texas, who is also a former Aggie. During her graduate career, Paige served as an Aggie Buddy at a local elementary school. Paige has also attained a certificate of college teaching, published six abstracts and four papers, and participated in six industry presentations.