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LYSINE AND THREONINE RESPONSES IN ROSS X ROSS TP16 MALE BROILERS

By

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The purpose of this study was to evaluate the Lys and Thr responses of TP-16 male broilers. Experiment 1 (14-28 d of age) showed no significant live performance effects. In Experiment 2, Lys and Thr (P=0.021) interacted to affect 28 to 42 d body weight gain and (P=0.004) feed intake. Lys main effects improved body weight gain (P=0.002), and feed conversion (P=0.009). Thr fed at the 0.68% level improved body weight gain and feed conversion. Mortality did not differ among treatments and averaged 0.09% across all treatments. The study indicated the sensitivity of the Thr:Lys ratio when broilers are fed diets containing marginal Lys. Although Thr X Lys interactions occurred in this study, the Lys levels in the test diets may have been too high for determining a ratio to an amino acid in a factorial study. Interaction results indicate that the Thr:Lys ratio in broilers from 28 to 42 days of age is between 0.57 to 0.68.

DEDICATION

I would like to dedicate this research to my nieces, nephews, and the entire Gaines and Everett families.

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I wish to express thanks to my major professor, Dr. Michael Kidd for being my mentor and for believing in me when I did not believe in myself. I would like to thank my committee members, Dr. Alejandro Corzo, and Dr. William Dozier, III for their advice and support. Also I would like to express my utmost appreciation to the faculty, staff, poultry farm crew and fellow students in the Poultry Science Department for their willingness to assist me throughout my career as a student. I would like to express deep gratitude to Tim Ishee for leading me into poultry science. Finally, I would like to express thanks to Christopher Wallace and Charles Barnett for advice and encouragement throughout my years in college.

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CHAPTER I

INTRODUCTION

Threonine has been shown to be an essential amino acid for broiler chicks (Almquist and Grau, 1944). High crude protein prices have driven nutritionist to find alternative ways to grow bigger broilers for white meat production. Diets high in crude protein can have other drawbacks such as excess nitrogen excretion in broilers resulting in high ammonia readings throughout a house which could cause blindness in broilers (Kristensen and Wathes, 2000). The addition of supplemental Met and Lys (1st and 2nd limiting amino acids) to low crude protein broiler diets have shown increased meat production without sacrifice to the birds' live performance characteristics. Met and Lys metabolism also depend upon Thr in order for the broilers to achieve optimal physical characteristics. The fundamental explanation represents the ideal protein concept whereby all essential amino acids must be present in adequate amounts to satisfy tissues requiring enzymatic reactions and protein deposition. Practically, the concept has been explained and verified by commercial nutritionist striving to meet, but not exceed, Thr needs for broilers in their particular environments. Thr is typically the 3rd limiting amino acid in corn-soybean based broiler diets. Deficiencies of Thr can result in subpar white meat production and carcass growth, hence, reflecting the importance of diets to meet or be slightly deficient in the most limiting amino acids. The objective of this review is to inform readers about the basic biochemical aspects and functions of Thr, Thr needs in typical commercial broilers, Thr needs of broilers in different environments and Lys and Thr interactions that occur in broilers that affect production characteristics.

CHAPTER II

LITERATURE REVIEW

Biochemical Aspects of Threonine

Thr is a hydroxyl-containing amino acid. Both the α and β carbons of Thr are optically active. Poultry cannot synthesize Thr therefore making it an essential amino acid. Thr is used in the synthesis of protein which is very important in metabolism. Thr is very unique among amino acids because it does not undergo transamination or oxidative deamination (Blanchard *et al*., 1944). Thr (2-amino-3-hydroxybutyric acid, **($C_4H_9N0_3$)** has a molecular weight of 119.12 and contains 11.76% nitrogen. DL-Thr should provide no more than 25% of biological activity in broilers, which is based upon one-fourth of the molecule being L-Thr (Baker 1986). This means that poultry can only utilize L-Thr completely. During the catabolism of Thr, three products are formed and have been detected in tissues of various animals (Green and Elliott, 1964): Thr dehydratase (E.C. 4.2.1.16), Thr dehydrogenase (E.C. 1.1.1.103), and Thr aldolase (E.C. 4.1.2.5).

Thr dehydratase (also known as serine dehydratase) carries out the first step in Thr degradation and is one of several enzymes carrying out the first step in serine degradation. Thr dehydratase uses pyridoxal-5'-phosphate to degrade Thr to aketobutyrate and ammonia. This process is of importance when fasting occurs in the bird.

Ballevre *et al*. (1990) discovered that under normal biological conditions Thr dehydrogenase accounts for most of the hepatic catabolism of L-Thr. Thr aldolase (also known as serine hydroxymethyl) degrades Thr to Gly and acetaldehyde by a pyridoxal-5'-phosphate-catalyzed Schiff base cleavage. Davis and Austic (1982) established that Thr imbalance could be induced by dietary supplements of 3 % L-serine, 6 % branched chain amino acids or a 5.6 % mixture of amino acids lacking Thr, and that this imbalance could be corrected by the addition to the supplemented diet of ≤0.4 % Thr/100 g diet. The authors discovered that Thr aldolase and Thr dehydrogenase had the highest Thr degrading activity in the broiler chick. The authors used female single comb white leghorn chicks (Cornell K-strain) and found that Thr dehydrogenase was more active in the pancreas, and Thr dehydratase and aldolase were more active in the liver and muscle, respectively.

Davis and Austic (1994) experimented with rats and broilers to elevate the tissue distribution of Thr dehydrogenase and to determine whether increases in hepatic activity of this enzyme would occur in chicks irrespective of the amino acid supplement used to induce Thr imbalance they found that Thr dehydrogenase is increased rapidly and significantly in response to ingestion of a Thr imbalanced diet by both chicks and rats.

Threonine Need of Broilers

The Thr need for chicks has been studied extensively. The need for Thr, as with all amino acid needs, differs throughout the life cycle of the broiler. The NRC (1994) recommendation for Thr in chicks 0 to 3 wk of age is 0.80%, for 3 to 6 wk of age is 0.74%, and for 6 to 8 wk of age is 0.68%. Thr needs vary by the age and strain of the broiler. Thus, it is important to define the optimum levels of Thr for live performance and processing characteristics. As broilers age, Thr needs, as well as other amino acid needs, decrease as feed intake increases. Thr requirement studies assessing various time periods are presented in Table 1.

Starter

In earlier experiments, Krautmann *et al.* (1958) used Barred Plymouth Rock males reared in electrically heated battery brooders from 7 to 21 days and found that Thr at 0.55% and 0.60% of the diet was the optimal Thr concentration combined with a diet using 21% crude protein.

Thomas *et al*. (1986) used male broilers from 7 to 21 days of age in two experiments using graded levels of Thr that were added to a diet utilizing soybean meal, peanut meal corn, and supplemental amino acids. Eight levels of Thr and two levels of coccidiostats (salinomycin and stenoral) were used in both experiments. In the first experiment, a Thr level between 0.73% and 0.77% showed the optimum weight gain and feed efficiency for male broilers. In the second experiment, peanut meal was substituted for soybean meal thus, increasing the Thr level (0.55%) in the basal diet. Results indicate that the Thr requirement for feed efficiency was 0.73% as determined by a regression equation.

Rangel-Lugo *et al*. (1994) used Peterson X Hubbard broilers to determine the Thr requirement for weight gain and feed efficiency up to 14 days of age in birds fed wheat-peanut meal-based diets formulated to contain 20 and 25% crude protein. Results indicated that the Thr requirement for the 20% crude protein was 0.67% and the 25% crude protein diet required a level of 0.77%.

Robbins (1987) experimented with 14 d old female Peterson crossbred broiler chicks and found that the Thr requirement was as 3.7% of dietary crude protein. Birds received diets that were formulated to contain 11.0 to 18.3% crude protein which were supplemented with L-Thr. Thr requirements as a percentage of diet ranged from 0.58 to 0.78%. This study showed disagreement with the NRC (1994) value of 0.80% for 1 to 21 d old broilers. Chicks grow rapidly the first few days of life. Hence, much of the variation that exists in starter Thr needs may be attributable to the length of experimentation. Clearly, three experiments assaying Thr needs up to d 21 (e.g., 1 to 7, 7 to 14, and 14 to 21) would give a more precise estimate than that of 1 to 21 d of age. In addition, the research cited indicates that dietary crude protein may be a factor in variation of starter Thr needs. For example, Thr has been shown to spare Gly in chicks but the pathway of Thr to Gly is not reversible (Baker, 1972).

Smith and Waldroup (1988) experimented with 3wk old male Vantress X Arbor Acres broilers to ascertain the Thr requirement by feeding a Thr deficient (0.59%) milo-soybean meal diet. The diet was supplemented with crystalline amino acids and contained 15% crude protein to obtain a diet with a minimum of 110% of amino acid recommendations (NRC, 1984). Thr requirements for weight gain and feed efficiency for this strain of broilers were 0.68% and 0.79%, respectively.

Uzu (1986) fed broilers four European experimental diets that varied in Thr levels from 1 to 42 days of age. A variety of basal diets were tested (corn/soy vs.

corn/soy/peanut meal; wheat/soy vs. wheat/soy/peanut meal). The Thr requirement for growth and feed efficiency for birds 1 to 21 days of age averaged to be 0.73 to 0.75% across all experiments for the starter period. The author stated that the results indicate that Thr is not a limiting amino acid in typical or low protein corn-soybean meal diets.

Holsheimer *et al*. (1994) used European diets to test the Thr requirement of Hybrid male and female broiler chicks from 1 to 28 days of age. Two experiments were performed at which eight levels of Thr and two crude protein levels of either 16 and 20% or 16 and 22%. Experiment 1 contained female broilers that were fed Thr-supplemented diets from 10 to 28 days of age and the Thr requirement was 0.63%. In Experiment 2, male and female broilers were fed a Thr-supplemented diet from 7 to 21 days of age and the Thr requirement was estimated at 0.73%. Chicks that received a 16% crude protein diet supplemented with 0.25% Thr (total Thr 0.85%) grew equally as chicks receiving a 22% crude protein diet containing 0.85% Thr from corn and soybean meal. Also, chicks fed the low protein Thr-supplemented diet had excellent feed conversion compared to birds fed the 22% crude protein corn/soybean meal diet.

Grower

Webel *et al*. (1996) conducted an experiment to determine the digestible Thr need of Ross X Hubbard commercial broilers. Optimum feed efficiency was achieved when feeding birds a corn peanut meal basal diet containing 20% crude protein with 0.61% digestible Thr in 3 to 6 wk old birds. A true digestibility assay from cecectomized roosters indicated that Thr was 81% digestible for the diets used in their study (Webel et al. 1996). Thr requirements obtained were in close agreement with those from the ideal amino acid calculations whereby Thr should be 70% of Lys requirements for chicks 3 to 6 wk of age.

Penz *et al.* (1997) experimented with Peterson X Arbor Acres at age 21 to 42 days of age, mixed-sex broilers fed a wheat based basal diet (Experiment 1) or a grain sorghum based diet (Experiments 2 and 3) with 3,200 kcal ME/kg of diet. Analysis of the data indicated a requirement of 0.68% for maximum body weight gain and 0.70% for maximum feed efficiency. The author of the study indicates that the responses between the males may differ because of the time of year (Exp. 2-late summer and Exp.3-fall).

Kidd and Kerr (1997) experimented with 30 to 42 d Ross x Ross male broilers in which a Thr deficient diet was fed (sorghum, peanut meal, corn, and poultry meal). It was reported that the NRC (1994) Thr requirement of 0.74% of diet was too high for live performance parameters based on the weight gain and feed:gain response, but the Thr requirement for breast meat may be higher than that of 0.74% of diet.

Finisher

Kidd *et al.* (1999) experimented with 4096 male Ross 308 X Hubbard from 42 to 56 days of age to find the dietary Thr requirement for finishing broilers and the economic responses. The broilers received Thr deficient diets composed of corn, peanut meal, wheat middlings, poultry oil and supplemented amino acids. The experimental diets contained seven graded levels of Thr from 0.45% to 0.81% in increments of 0.06%. Results find that a total dietary Thr level of 0.66 to 0.67% is adequate for good growth and carcass response in finishing broilers. Results from the economic analysis specifies that the level of dietary Thr needed to gain maximum profit was near the level needed for optimum feed conversion and carcass composition,

Dozier *et al.* (2000a) documented the Thr requirement of seven hundred and fifty Ross X Ross 308 male broilers from 42 to 56 in a summer environment. Broilers were fed six experimental diet containing increments of Thr ranging from 0.50 to 0.80%. Results showed feed conversion was optimized using 0.67% total Thr in the diet; however, growth rate and feed consumption were not significantly affected by dietary Thr. The author suggested that the Thr need for male broilers is less for optimum breast meat recovery than feed conversion ratio during hot temperature condition.

Dozier *et al.* (2001) suggested that the total Thr need for male and female birds from 42 to 56 d, when fed a diet that contained 18% crude protein and 3,200 ME kcal/kg, was 0.74% and 0.63%, respectively.

In conjunction with a previous grower trial, Webel *et al*. (1996) used the Ross X Hubbard strain for a 6 to 8 wk old experiment and found that the digestible Thr need in a diet at 0.529% with 18.3% crude protein. These diets indicate that the dietary Thr level for females could be fed at a level 15% lower than that of males.

Threonine Need of Broilers of Differing Strains

In a more recent study, Kidd *et al*. (2004) tested the Thr need of three strains of broilers (A=multipurpose, B= high yield, C=high yield) that received a common vegetable diet based on corn, soybean, and peanut meal up to day 20 and fed supplemental Thr (0.52 to 0.87%) in increments of 0.07% for 21 to 42 d. Results indicated that the Thr needs from 21-42 days of age broilers across all three strains was estimated to be 0.74% total or 0.65% digestible. The authors also noted that since Lys was not in excess, the recommended Thr:Lys ratio was near 0.68. It appears that in the strains tested that the Thr need is very similar, presumably based on feed intake adjustments.

Threonine Need of Broilers in Different Environments

Thr requirements of broilers may be altered when the birds are exposed to conditions that are not normal such as extreme heat or different litter conditions. This problem can be particularly important because in the finishing period extreme heat can lead to decreased feed intake in which broilers will not ingest the needed amino acid requirement for optimal growth. Although all US broiler growers use nipple drinkers rather than open water systems, sub optimal litter conditions can be still prevalent. Litter with too much cake (i.e., moisture and excreta) can increase microbial pathogens and alter digestion functions thus affecting Thr needs.

Litter

Kidd *et al*. (2003) experimented with 42 to 56 days old Cobb X Cobb 500 fast feathering males in different environmental conditions (clean litter vs dirty litter). Live performance and carcass responses were evaluated. L-Thr was added at 0.21% to a common corn and peanut meal diet so that the feed had 0.66% total Thr. Results indicate that broilers reared in the clean environment and fed a Thr level of 0.67% had good live performance and carcass responses. Broilers reared in the dirty environment responded to increasing dietary Thr in a positive linear manner. The authors stated that the birds in the dirty environment responded to higher levels of Thr possibly due to an increased Thr need for gastrointestinal function.

Corzo *et al*. (2007) evaluated the Thr need of Ross X Ross 708 male broilers in different litter conditions (new litter) vs. (used built up soft wood shavings) by feeding a common corn soybean basal diet with six dietary Thr levels that ranged from 0.51 to 0.86%. Thr estimates for live performance indicate that results were maximized on new litter using 0.71 to 74% Thr and results for used litter were maximized at 0.73 to 0.78% depending on the variable for both types of litter. Immunity was also measured in this experiment and the results indicated that low Thr decreased relative thymus weight and increased monocyte nitric oxide production in built-up and new litter environments, respectively. Corzo *et al*. (2003) stated that the increase need for Thr in broilers under extreme environmental conditions can be due to the increased maintenance requirements associated with intestinal functions.

Cages

Kidd *et al.* (2001) performed an experiment with three hundred and eighty-four Ross X Ross 308 male broilers randomly housed in 48 pens of two starter battery cages from 5 to 15 days of age. The objective of this study was to evaluate Thr and crude protein responses of broilers in the starter period. The experimental feed consisted of two dietary crude protein (**CP**) levels (190 and 225 g/kg) and three dietary Thr levels (0.60, 0.70, and 0.80%) fed at 5 to 15 days of age. Results show that increasing Thr from 0.60 to 0.70% improved body weight gain and feed intake. Feed:gain was improved in chicks receiving both crude protein diets as Thr was increased from 0.60 to 0.70%. Contrastly, chicks fed the 0.80% Thr diet had lower feed:gain ratio when fed the 225g CP/kg diet than chicks fed the 190 Cp/kg diet. Chicks had improved growth performance measurements when fed 0.70 g Thr/kg diet. The author states that the effect of CP on Thr needs of chicks should be studied further.

Corzo *et al*. (2003) evaluated the Thr need for Ross X Ross 508 broiler females reared in cages (Petersime batteries) from 30 to 42 days. Diets consisted primarily of corn, peanut meal and soybean meal with L-Thr added at the expense of sand in increments generating 6 experimental treatments. Results indicated that weight gain and feed conversion from regression equations had similar optimization points (0.71% Thr). The author notes that a drastic increase of free plasma Thr concentration occured after 0.65% suggesting a requirement for Thr is within this range.

Kidd *et al*. (2003) experimented with Cobb 500 females from 42 to 56 days of age and reared in finishing battery units (three birds/pen). Three experiments were performed but only two experiments are relevant to this literary review (experiment 2 and 3). The study was conducted to determine the Thr requirements for growth and carcass responses in finishing broilers. In experiment 2, birds were fed total Thr in 0.07% increments from 0.45 to 0.80%, whereas experiment 3, birds were fed total Thr in 0.04% increments from 0.52 to 0.72%. Results indicate that Thr had minimal effects on carcass attributes in both experiments. In experiment 2, a dietary Thr level of 0.60% supported sufficient growth. In experiment 3, a dietary Thr level of 0.67% showed good growth. The results indicate that (1994) NRC Thr recommendation (0.68%) for female broilers from 42 to 56 of age is adequate.

Threonine:Lysine Ratios in Broilers

Meeting the Thr need in least cost formulation represents a significant cost but if this cost is not incurred and Thr is deficient then TSAA and Lys utilization may be impaired (Kidd *et al*., 2004). Diet formulation is more accurate when formulating broiler diets using ideal amino acid ratios because excesses of essential amino acids or an increase in nitrogen excretion from the bird may be minimized (Samadi and Liebert, 2006). Limited research has shown dietary Thr to Lys interactions in broilers (Kerr *et al*., 1999; Kidd *et al*., 1997). Lys and Thr are the second and third limiting amino acids in corn-soybean based broiler diets (Kidd and Kerr, 1996). Lys is primarily utilized for protein synthesis in broilers, therefore ideal amino acid ratios are based on all other essential amino acids as a percentage of Lys (Emmert and Baker, 1997). Precision feeding of amino acids to broilers depends on the sex, age, and strain of the bird being fed.

Kidd *et al*. (1997) performed an experiment using Ross X Ross 308 males, from 18 to 54 days of age, fed a sorghum-peanut meal diet with two levels of Lys and four levels of Thr. Results indicated that Lys and Thr interacted to increase weight gain and breast fillet yields with Lys and Thr levels at 100 and 83%, respectively, of NRC (1994). The main conclusion of this study indicates that feeding high dietary Lys without consideration of dietary Thr may limit breast fillet yield.

Kerr *et al*. (1999) performed an experiment that measured the effect of adequate and super adequate levels of dietary Lys as affected by Thr concentrations ranging from adequate to super adequate. Avian 34 X Avian male broilers from 21 to 52 days of age fed a corn and wheat diet was used for this study. Experimental diets were fed from 21 to

42, 42 to 46, and 46 to 52 days of age. Diets consisted of two levels of Lys (105 and 120% of NRC, 1994) and six levels of Thr (85, 92.5, 100,107.5, 115 and 122.5% of NRC 1994) Results indicate that dietary Lys and Thr interacted for 21 to 52 d processing body weight when broilers were fed 105% of the NRC (1994) Lys recommendation which is 0.98% Lys. Breast meat weight also showed a Lys:Thr interaction so that 100% of the NRC (1994) recommendation for Thr (0.71%) was required to maximize breast meat deposition in broilers fed the 105% Lys diets, but 107.5% Thr (0.76%) was necessary to maximize breast meat in broilers fed the 120% Lys (1.12%) diets. The author states that processing live weight, breast meat, and drumstick weights were optimized when the digestible Thr:Lys ratio was approximately 0.70.

In conclusion, numerous factors affect dietary Thr needs. However, expressing Thr needs relative to Lys across numerous ages may reduce Thr need variation.

CHAPTER III

MATERIALS AND METHODS

Experiment 1

Experimental Facility and Bird Husbandry

A total of 576 day old male Ross X Ross TP16 broilers was obtained from Aviagen (Albertville, AL) and placed in 48 pens. Eight treatments were randomized across the 48 pens. Pens were located inside of a house with negative air pressure, curtain sided walls with cool cells. Brooding temperature was adjusted from1 to14 days of age via gas brooders. There were 6 replications per treatment with 12 birds/pen (72 birds / treatment) and each bird was wing-banded with pen number. Each concrete pen measured 1.1 m^2 and contained a nipple watering system and a pan feeder. New pine shavings were placed over used "built-up" litter from previous trials. All birds were vaccinated for Marek's, Newcastle, and Bronchitis at the hatchery. All birds had *ad libitum* access to water and feed. Daily lighting schedule consisted of 23 hours of light and 1 hour of darkness.

Experimental Design and Diets

All birds received a common starter diet that was pelleted and then crumbled from 1 to 14 days of age. At 14 day of age, birds were wing-banded to correspond to assigned pen. The experimental diets were fed from 14 to 28 days of age and each diet was formulated to contain 22.49% crude protein and 3,125 ME kcal/kg. The test diet contained 1.12% digestible Lys and 0.69% digestible Thr. Treatment diets consisted of two levels of Lys (1.12 and 1.18%) and four levels of Thr (0.69, 0.73, 0.77, and 0.81%) of diet. Supplemental L-Lys HCL and L-Thr were added to experimental feed at the expense of 'washed builders' sand. Diets were analyzed for all amino acids except tryptophan. Supplemental Lys and Thr content of each sample were analyzed for each experimental diet.

Growth Measurements

On day 14, birds were collected, weighed, and began receiving treatment feed. The parameters calculated were body weight gain, feed consumption, and feed conversion for the experimental 14 to 28 days of age. Mortality was recorded throughout the experiment.

Experiment 2

Experimental Facility and Bird Husbandry

These broilers (576 male Ross X Ross TP16) were delivered with the broilers from Experiment 1, originated from the same breeder flock, and were reared in the same experimental facility which contained 96 floor pens (i.e., utilizing 48 floor pens). There were 6 replications per treatment with 12 birds/pen (72 birds / treatment) and each bird was wing-banded with pen number. Each concrete pen measured 1.1 m^2 and contained a nipple watering system and a pan feeder. New pine shavings were placed over caked litter from previous trial. All birds had *ad libitum* access to water and feed. Birds obtained 23 hours of light and 1 hour of darkness.

Experimental Design and Diets

All birds received a common diet from 1 to 28 days of age that was pelleted and then crumbled. The experimental diets were fed from 28 to 42 days of age and each diet contained 19.34% CP, 3,125 ME kcal/kg. Treatment diets consisted of two levels of digestible Lys (1.00 and 1.05%) and 4 levels of Thr (0.60, 0.64, 0.68, and 0.72%). Supplemental L-Lys HCL and L-Thr were added to experimental feed at the expense of ‘washed builders’ sand. Diets were analyzed for all amino acids except tryptophan by the method of cold acid wash. Supplemental Lys and Thr content of each sample was analyzed for each experimental diet. In both experiments, before feed samples are analyzed for amino acid content, samples were analyzed for nitrogen combustion on Kjeldahl and dry matter (AOAC International, 1984). Immediately following acid hydrolysis, amino acid contents were determined with a high performance cation exchange resin column. Each feed sample was analyzed in duplicate.

Growth Measurements

On day 28, each pen of birds was weighed collectively and began receiving treatment feed. On day 42, each pen of birds was weighed and feed consumption was determined. The parameters calculated were body weight gain, feed consumption, and feed conversion for the experimental 28 to 42 days of age. Mortality was recorded throughout the experiment. BW gain was calculated by total pen weight at day 42 divided by total number of birds on day 42 minus the total pen weight at day 28 divided by total number of birds on day 28. Feed conversion was calculated by dividing pen feed consumption (28 to 42 days of age) total by pen day 42 BW minus initial pen bird weight at day 28. Feed conversion was not adjusted for weight of mortality because mortality averaged only 0.9%.

Processing

At 42 days, six birds per pen were randomly selected for processing. Each pen of six broilers was weighed individually before being placed in coops to be transferred to the Mississippi State University poultry processing plant. Feed was removed from each pen 12 hours prior to processing. Broilers were manually hung on shackles, electrically stunned, bled for 1.5 min by cutting the jugular vein, scalded in hot water for 1.5 min, and De-feathered in an in-line picker for 1 min. Hocks, necks, and viscera were removed. Carcasses and abdominal fat pads were weighed. Carcasses were placed on ice 4 h before deboning was performed on each carcass whereby *Pectoralis major* and *Pectoralis minor* muscles were removed. The processing parameters measured were carcass yield, breast yield, and percentage fat. The yield was based upon the day 42 weight of 2.5 kg.

Statistical Analysis

In both experiments, a 2 X 4 factorial design was used with pen being the experimental unit. The factorial arrangement of treatments was analyzed using the MIXED procedure of SAS® Institute (2002) by the following model:

$$Y_{ijk} = \mu + L_i + T_j + LT_{ij} + e_{ijk}$$

Where:

μ	=	mean.
L_i	=	effect of the ith of Lys.
T_j	=	effect of the jth of Thr.
LT_{ij}	=	effect of the Lys X Thr interaction.
e_{ijk}	=	random error.

Analysis of covariance (ANCOVA) was used for 28 d BW. Means were separated using repeated *t* test.

CHAPTER IV

RESULTS AND DISCUSSION

Diets for Experiments 1 and 2 are represented in Table 2. Calculated Lys and Thr contents for both experiments and the respective analyzed values for the supplemental Lys and Thr are presented in Table 3. Analyzed values were in close agreement with the calculated values.

Dietary Lys in the diets fed from 14 to 28 and 28 to 42 days of age was 1.12 % and 1.00% calculated, whereas Thr was 0.69% and 0.60% calculated, respectively. Total Lys in the 14 to 28 and 28 to 42 day diets was 1.26% and 1.12%, and total Thr in the 14 to 28 and 28 to 42 day diets was 0.87% and 0.76%, respectively. The values are in agreement with analyzed values: 14 to 28 day Lys, 1.21%; 28 to 42 day Lys, 1.11%; 14 to 28 day Thr 0.84%; and 28 to 42 day Thr, 0.72%. Further, cold acid wash analysis of supplemental Lys and Thr indicated that dose additions to the test diet were achieved (Table 3).

Experiment 1

No significant interactions between Lys and Thr occurred (Table 4). Thr levels of 0.69 to 0.81% failed to improve any parameter measured. Lys also failed to show any improvement in live performance characteristics as a main effect. These results are in disagreement with Thomas *et al*., 1986 who used male broilers from 7 to 21 days of age and found the optimum Thr requirement for weight gain and feed efficiency to be between 0.73 and 0.77%. However, these results are in agreement with Dozier *et al*. 2008 who stated that Lys level may be higher than predicted for the TP16 broiler compared to other Ross X Ross strains. As differences in feed intake were not observed, these results indicate that 1.12% digestible Lys and 0.69 digestible Thr may be adequate for the 14 to 28 day period.

Experiment 2

Mortality averaged 0.9% during treatment period which indicates that the experimental environment minimized diseases and stressors. Increasing digestible lysine from 1.00 to 1.05% of the diet resulted in a significant ($P \leq 0.01$) increase in BW gain (1.21 kg v s 1.28 kg) (Table 5). Birds fed 0.64% digestible Thr at the Lys level of 1.05% weighed 0.16 kg more than birds fed digestible Thr of 0.64% at the 1.00% level. Numerical improvements ($P<0.07$) were observed for Lys in feed intake. However, feed conversion was improved ($P \leq 0.009$) as Lys was increased. These results are in agreement with Kidd *et al*., 1997 who saw a significant improvement in feed conversion when Lys was increased from 100 to 105% of the NRC (1994). Birds fed digestible Thr at 0.72% had improved BW gain (P<0.01) over birds fed 0.64% Thr, but 0.68% Thr yielded an intermediate response. Feed conversion (P<0.01) mimicked the BW gain response for the Thr main effect. Lys and Thr interacted (P<0.021) for 28 to 42 day BW Gain (Table 5). BW gain was significantly different in birds fed 0.64 vs 0.72% digestible Thr in the low Lys diet (1.00%) and Thr levels were not significant at any level in the high Lys diet (1.05%), pointing to the importance of meeting a Thr:Lys ratio in this period. Lys and Thr also interacted (P<0.004) for 28 to 42 day feed intake (Table 5). Feed intake at the low Lys level was significantly different at Thr levels of 0.60 and 0.64 vs 0.72% and the high Lys level shows a significant response at Thr levels of 0.64% vs. 0.72%.

There was no Thr X Lys interaction or a significant difference in main effects for processing parameters (Table 6). However, tender yield was improved numerically (P=0.06) by increasing dietary Lys. Analysis of the data indicated that the low level of digestible Lys optimized BW gain at an inclusion level of 1.00% and dietary Thr at 0.68% which results in an optimal Thr:Lys ratio of 0.68 which is higher than a previous recommendation by Mack *et al*., 1999 of 0.63. The high level of digestible Lys of 1.05 and digestible Thr at 0.64% results in a Thr:Lys ratio of 0.57 which is similar to previous research performed by Baker *et al*., 2002 who found the ratio to be 55.7 although the authors study focused on the time period of 8 to 21 days.

Feeding TP16 male broilers 1.05% digestible Lys and 0.68% digestible Thr from 28 to 42 days of age should support adequate live performance and carcass traits. Although feeding birds 1.05% digestible Lys increased some parameters, it may be too high for determining a ratio to an amino acid in a factorial study.

CHAPTER V

CONCLUSIONS

There has been little published research that evaluates Thr X Lys interactions in broilers. This research performed suggests that Lys and Thr can interact to affect certain parameters in broilers. Many parameters were improved but were not significant ($P \leq 0.05$) for this study.

The objective of Experiment 1 was to evaluate the Lys and Thr responses in TP16 male broilers from 14 to 28 days of age. In Experiment 1, there was no Lys and Thr interaction, nor was there a Lys or Thr main effect.

The objective of Experiment 2 was to evaluate the Lys and Thr responses in TP16 male broilers from 28 to 42 days of age. In Experiment 2, there was a Lys and Thr interaction for BW gain and feed intake. There was a Lys main effect ($P \leq 0.01$) for BW gain and ($P \leq 0.05$) feed conversion. There was a Thr main effect ($P \leq 0.01$) for BW gain and feed conversion which mimicked the BW gain response.

Future research should use lower Lys levels in interaction studies because this will be more efficient in determining a more accurate Thr:Lys ratio.

Table 1 Threonine requirement of broilers

Day of age	Body weight gain	Feed:gain	Breast meat	Abdominal fat	Reference
1-14	0.67[5] 0.77[6]	nd[2]	nd[2]	nd[2]	Rangel-Lugo *et al.* (1994)
5-15					
7-21	0.68	0.79	nd[2]	nd[2]	Smith *et al.* (1988)
7-21	0.73	0.73	nd[2]	nd[2]	Thomas *et al.* (1986)
1-21	0.58	0.78	nd[2]	nd[2]	Robbins (1987)
1-21	0.73	0.76	nd[2]	nd[2]	Uzu (1986)
7-21	0.73	nd[2]	nd[2]	nd[2]	Holsheimer *et al.*(1994)
1-21	0.55	0.60	nd[2]	nd[2]	Krautmann *et al* (1958)
20-40[1]	0.61[3]	0.61[3]	0.61[3]	≤0.55[3]	Leclercq (1998)
21-42	0.61[3]	0.61[3]	nd[2]	nd[2]	Webel *et al*. (1996)
21-42	0.70[4]	0.70[4]	nd[2]	nd[2]	Webel *et al*. (1996)
21-42	≤0.70[4]	≤0.70[4]	nd[2]	≤0.70[4]	Penz *et al*. (1997)
21-42	nd[2]	0.74[4]	nd[2]	nd[2]	Kidd *et al.* (2004)
21-42	0.71-0.74[7] 0.73-0.78[8]	nd[2]	nd[2]	nd[2]	Corzo *et al*. (2007)
30-42	0.70[4]	0.70[4]	0.78[4]	≤0.60[4]	Kidd and Kerr (1997)
30-42	0.71	0.71	nd[2]	nd[2]	Corzo *et al.* (2003)
42-56	0.52[3]	0.52[3]	nd[2]	nd[2]	Webel *et al*. (1996)
42-56	0.60[4]	0.60[4]	nd[2]	nd[2]	Webel *et al*. (1996)
42-56	0.67[4]	0.67[4]	0.66[4]	nd[2]	Kidd *et al*. (1999)
42-56	0.67	0.67	nd[2]	nd[2]	Dozier *et al*. (2000a)
42-56	0.74	0.74	0.70	nd[2]	Dozier *et al*. (2000b)
42-56	0.67[4]	0.66[4]	0.70[4]	nd[2]	Dozier *et al*. (2000c)
42-56	0.74	nd[2]	nd[2]	nd[2]	Dozier *et al.* (2001)
42-56	0.67[7]	nd[2]	nd[2]	nd[2]	Kidd *et al.* (2003)
42-56	0.63	nd[2]	nd[2]	nd[2]	Kidd *et al*. (2003)

[1]The mathematical test to determine the requirement was a monomolecular model.
[2] nd, not determined or requirement estimate was unable to be calculated based on data set
[3]Represents the dietary digestible threonine requirement.
[4]Represents the dietary total threonine requirement.
[5]Represents low protein diet
[6]Represents high protein diet
[7] Represents clean environment
[8] Represents dirty environment

Table 2. Composition of test diets differing in Lys and Thr for TP16 male broilers (Experiments 1 and 2)

Ingredients	14-28 d	28-42 d
Corn	50.77	60.60
Soybean meal (48% CP)	29.28	23.59
Peanut meal	8.00	5.00
Poultry fat	5.92	4.87
Poultry meal	2.00	2.00
Dicalcium Phosphate	1.68	1.67
Limestone	1.02	1.02
NaCl	0.48	0.49
DL-Met	0.26	0.25
Premix[1]	0.25	0.20
Filler[2]	0.22	0.20
L-Lys HCl	0.07	0.14
Sacox 60[3]	0.05	0.05
Calculated composition[4]		
ME, kcal/kg	3,125	3,125
CP, %	22.49	19.34
Ca, %	0.94	0.92
P, % available	0.47	0.46
Na, %	0.22	0.22
K, %	0.30	0.30
DEB, mEq/kg[6]	212	177

Amino acid composition[5]	Calculated digestible	Analyzed total	Calculated digestible	Analyzed total
Lys, %	1.12	1.21	1.00	1.11
Thr, %	0.69	0.84	0.60	0.72
Met, %	0.55	0.61	0.54	0.47
TSAA, %	0.85	0.93	0.73	0.76
Arg, %	1.52	1.70	1.10	1.44
Val, %	0.89	1.04	0.77	0.94
Ile, %	0.79	0.91	0.67	0.87
Trp, %	0.24	0.24	0.19	NA

[1]Premix provided the following per kg of diet: Vitamin A (Vitamin A acetate) 7,718 IU; cholecalciferol 2,200 IU; Vitamin E (source unspecified) 10 IU; menadione, 0.9 mg; B_{12}, 11g; choline, 379 mg; riboflavin, 5.0 mg; niacin, 33 mg; D-biotin, 0.06 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper,7 mg; iodine, 1 mg; selenium, 0.2 mg.

[2]Represents dietary space of the inert filler (sand) to which L-Lys and L-Thr were added.

[3]Sacox 60 provided 50g of salinomycin sodium per ton (907.2 kg) of feed.

[4]All amino acids are presented as calculated digestible.

[5] Analyzed total Amino Acids

[6]DEB=dietary electrolyte balance.

Table 3. Calculated and analyzed values of Lys and Thr dietary additions[1] (Experiments 1 and 2)

Calculated values, %[2]	Analyzed Lys, %	Analyzed Thr, %
14-28 d		
0.06% Lys	0.045	
0.12% Lys	0.116	
0.00		0.000
0.04		0.041
0.08		0.078
0.12		0.118
28-42 d		
0.11% Lys	0.107	
0.16% Lys	0.171	
0.00		0.000
0.04		0.070
0.08		0.100
0.12		0.110

[1] Cold acid wash – at a low pH amino acids are positively charged and bound to the resin by negative charged ion exchange sites, this rids all contaminants. Amino acids are then selectively eluted by increasing the pH and salt concentrations with different buffers.

[2]Values are presented as total.

Table 4. Live performance of TP16 male broilers fed diets differing in Lys and Thr from 14 to 28 days of age (Experiment 1)

Dietary treatments[1] Lys, %	Thr, %	BW gain	Feed intake	Feed conversion	Mortality
		(kg/period)	(g/bird/d)	(kg/kg)	(%)
1.12	0.69	0.913	120	1.69	3.20
1.12	0.73	0.918	118	1.61	0.13
1.12	0.77	0.929	119	1.59	1.49
1.12	0.81	0.908	118	1.65	2.73
1.18	0.69	0.910	119	1.64	2.54
1.18	0.73	0.914	124	1.66	1.73
1.18	0.77	0.908	118	1.62	1.47
1.18	0.81	0.894	118	1.64	0.54
SEM		0.044	3.4	0.042	1.326
Source of variation					
Lys		0.957	0.518	0.852	0.555
Thr		0.703	0.804	0.508	0.720
Lys X Thr		0.834	0.521	0.671	0.522
Thr linear		0.606	0.360	0.132	0.271
Thr quadratic		0.605	0.361	0.134	0.279

[1]Dietary treatments represent percentage digestible.

Table 5. Live performance of TP16 male broilers fed diets differing in Lys and Thr from 28 to 42 days of age (Experiment 2)

Dietary treatments[1]			Feed	Feed	
Lys, %	Thr, %	BW gain	intake	conversion	Mortality
		(kg/period)	(g/bird/d)	(kg/kg)	(%)
1.00	0.60	1.194[bc]	172[d]	2.02	0.00
1.00	0.64	1.146[c]	172[d]	2.10	1.50
1.00	0.68	1.241[ab]	176[bcd]	1.99	0.00
1.00	0.72	1.280[a]	179[ab]	1.92	0.00
1.05	0.60	1.242[ab]	175[bcd]	1.99	0.00
1.05	0.64	1.303[a]	180[a]	1.92	1.50
1.05	0.68	1.289[a]	178[abc]	1.94	0.00
1.05	0.72	1.293[a]	175[cd]	1.90	0.00
1.00		1.215[b]	175	2.00[a]	0.38
1.05		1.282[a]	177	1.93[b]	0.38
	0.60	1.218[b]	174	2.01[a]	0.00
	0.64	1.225[b]	176	2.01[a]	1.52
	0.68	1.265[ab]	177	1.96[ab]	0.00
	0.72	1.286[a]	177	1.91[b]	0.00
SEM		0.033	1.6	0.027	0.758
Source of variation					
Lys		0.0003	0.065	0.009	1.000
Thr		0.017	0.160	0.045	0.129
Lys X Thr		0.021	0.004	0.170	1.000
Thr linear		0.933	0.122	0.230	0.163
Thr quadratic		0.996	0.128	0.201	0.158

[1]Dietary treatments represent percentage calculated digestible.

Table 6. Processing yields of 42 day TP16 male broilers fed diets differing in Lys and Thr from 28 to 42 days of age (Experiment 2)

Dietary treatments[1] Lys, %	Thr, %	Carcass yield	Fillet yield	Tender yield	Total breast yield
		------------------------ (%) -----	------------------		
1.00	0.60	66.89	16.50	3.82	20.32
1.00	0.64	67.09	16.49	3.82	20.31
1.00	0.68	67.67	16.59	3.78	20.37
1.00	0.72	67.08	16.31	3.75	20.06
1.05	0.60	69.80	17.21	3.97	21.18
1.05	0.64	67.85	16.39	3.88	20.27
1.05	0.68	67.43	16.48	3.98	20.46
1.05	0.72	67.06	16.30	3.79	20.09
SEM		0.054	0.318	0.081	0.370
Source of variation					
Lys		0.359	0.749	0.175	0.817
Thr		0.952	0.861	0.836	0.870
Lys X Thr		0.218	0.997	0.753	0.995
Thr linear		0.410	0.227	0.929	0.291
Thr quadratic		0.432	0.244	0.958	0.311

[1]Dietary treatments represent percentage calculated digestible.

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