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The use of serum uric acid as an indicator of amino acid utilization in diets for broilers

Amanda L. Donsbough
Louisiana State University and Agricultural and Mechanical College

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THE USE OF SERUM URIC ACID AS AN INDICATOR OF AMINO ACID UTILIZATION
IN DIETS FOR BROILERS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program of Animal Sciences

by
Amanda L. Donsbough
B.S., Louisiana State University, 2006
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ABSTRACT

The purpose of this research was to determine if serum uric acid (SUA) could be used as an indicator of amino acid (AA) utilization in broilers and to determine the Lys requirement of broilers using SUA as a response variable. Treatments were replicated with at least 6 pens with at least 6 broilers per pen. Experiments (Exp.) were conducted from 0- to 14, 17, or 18- d posthatching in brooder batteries. Five experiments were conducted to determine if SUA and uric acid content of the excreta (UAE) could be used to determine AA adequacy of a diet. The results of these experiments indicate that after a 2 h fast, SUA can be used to determine the AA adequacy of a diet as well as UAE. Two experiments were conducted to determine the Lys requirement of female broilers from 0- to 17- d posthatching using growth performance and SUA as response variables. Using daily gain as the response, the Lys requirement of female broilers is 1.27% total Lys for diets formulated with the main source of Lys as SBM. Using gain:feed (GF) as a response variable, estimates of the Lys requirement are 1.30%, 1.42%, and 1.45% total Lys when supplemental Lys is the source of Lys. A final experiment was conducted to determine the effects of supplemental Lys and Met on SUA, serum urea N (SUN), and UAE concentrations. The results of this experiment indicate that supplemental Lys has no effect on SUA or SUN concentrations. The results of this research indicate that SUA concentrations can be used as an indicator of AA utilization in broilers fed AA adequate and deficient diets, but it is not a good response variable to estimate the Lys requirement of broilers.

CHAPTER 1

INTRODUCTION

Corn and soybean meal (SBM) prices are increasing for livestock production. It is therefore important to formulate diets to efficiently meet the needs of animals. Most importantly, diets must be formulated to contain the correct amount of AA for optimum performance. Previous research has used average daily gain (ADG), average daily feed intake (ADFI), GF to estimate AA requirements of broilers. In swine, the use of plasma urea N (PUN) has been shown to be an accurate variable for estimating AA requirements (Coma et al., 1995; Knowles et al., 1997; and Guzik et al., 2005). In broilers, uric acid (UA), and not urea, is produced as the main end product of N metabolism. Therefore plasma UA (PUA) and UAE should be viable response variables to determine AA requirements of broilers. Some research examining PUA and UAE has been conducted with broilers fed very low to excessive amounts of crude protein (CP) (Pudelkiewicz et al., 1968; Featherston, 1969; Okumura and Tasaki, 1969; and Hevia and Clifford, 1977). This research indicates that both PUA and UAE increase as dietary N increases. However, inconsistent results have been reported when using PUA as a response variable to assess AA utilization. Xie et al. (2004) reported both increases and decreases in PUA concentrations when Met was increased in the diet. Miles and Featherston (1974) reported decreases in both PUA and UAE when dietary Lys was increased in the diet. However, Corzo et al. (2003) and Corzo et al. (2005) reported no changes in PUA concentrations when dietary Lys or Trp were increased in the diet. Research also shows that fasting and refeeding schedules may need to be implemented to determine differences in PUA concentrations (Okumura and Tasaki, 1969; Wilson and Miles, 1988; and Kolmstetter and Ramsay, 2000).

The objective of this research was to determine if SUA could be used to determine the AA adequacy of a diet for broilers, much like that of PUN for swine, and to further determine if SUA could be used as an accurate variable to determine the Lys requirement of broilers.

CHAPTER 2
REVIEW OF LITERATURE
INTRODUCTION

Plasma urea N has been shown to be an accurate indicator of AA utilization in diets for swine (Coma et al., 1995; Knowles et al., 1997; and Guzik et al., 2005). Applying this concept to broilers is difficult because broilers are uricotelic and swine are ureotelic, meaning the main end product of nitrogen metabolism in broilers is UA, not urea. Broilers do not produce urea in large quantities because of low amounts of arginase activity in liver, which is responsible for the production of urea (Stevens, 1996). Uric acid in the plasma of birds is influenced by age, sex, reproductive state, and nutritional status (Featherston, 1969). It is thought that because birds lack uricase, which is essential to catabolize UA, that UA excreted and PUA would be accurate measures of the total amount of UA produced (Hevia and Clifford, 1977). Uric acid is efficiently removed from the blood and levels rarely exceed 10 mg/dL. This process is important due to the insolubility of UA, which if concentrated at high levels in the blood can precipitate into joints, under the skin, and into the kidney, which results in gout.

PLASMA URIC ACID AS AFFECTED BY PROTEIN LEVELS IN DIETS FOR POULTRY

Research has been conducted examining the effects of low to very high dietary N concentrations on PUA. Russell and Weber (1934) reported that blood urea N (BUN) and PUA concentrations were not affected in laying hens fed high or low protein diets. In more recent studies, Hevia and Clifford (1977) fed diets containing 11, 20, 43, or 80% CP and reported a linear increase in PUA concentrations as dietary N increased. Plasma UA and ammonia concentrations were evaluated in male chicks fed diets

containing 25 and 75% isolated soy protein (Featherston, 1969). Chicks from each treatment were placed into groups that would and would not be injected with ammonium acetate, and also groups that were allowed feed ad libitum or were fasted for 24 h before bleeding. Chicks fed the 25% isolated soy protein diet, not injected with ammonium acetate and fasted for 24 h had lower PUA concentrations than those with ad libitum access to feed.

Feeding low to high levels of dietary N was previously shown to affect PUA concentrations. Fasting and refeeding birds fed low and high N diets also affects PUA concentrations. Okumura and Tasaki (1969) fed a protein free diet along with 5, 10, 15, 20, 30, or 40% casein diets to cockerels. Plasma UA concentrations were examined in fasted and fed birds. After 5 d, cockerels were bled just before feeding and after a 1, 2, 3, or 6 h fast. The 20% casein diet was fed to all birds for 5 more days and birds were bled after 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h. Immediately before feeding, PUA concentrations were similar regardless of dietary casein level. After feeding, PUA increased sharply and peaked at 2 h after feeding. Also, as the level of casein increased, PUA increased. After the extended fast of birds fed the 20% casein diet, PUA increased gradually until 120 h and then increased at a greater rate until 240 h. When birds were refed, PUA concentrations returned to initial concentrations within 6 h. These results indicate that fasting and refeeding, and dietary protein level affect PUA concentrations. Also, the increase in PUA after an extended fast indicates that tissue protein is being catabolized, and after feed is reintroduced, tissue catabolism is reduced.

Research that has been conducted to examine the effect of supplemental AA on PUA has reported inconsistent results. Corzo et al. (2005) reported no changes in PUA

concentrations when dietary Trp was increased from 0.12 to 0.24% in male broilers. Although there were no significant differences in PUA concentrations, a numerical increase was seen in broilers fed higher levels of Trp, which was thought to be due to excess N from the excess Trp. It was also reported that PUA concentrations were higher in broilers with heavier body weights, which was thought to be due to a higher maintenance requirement. Corzo et al. (2003) also reported no differences in PUA concentrations when dietary Lys was increased from 0.85 to 1.25% in broilers at 50 d of age.

Miles and Featherston (1974) used PUA and UAE to determine the Lys requirement of broilers. Broilers were fed glucose-safflower meal diets supplemented with L-Lys•HCl to achieve the desired Lys levels. Plasma UA concentrations decreased as Lys increased and plateaued at dietary levels of Lys similar to where UAE and ADG plateaued. Although this response was reported, a Lys requirement was not determined using PUA as a variable.

Xie et al. (2004) examined the effects of Met and Cys on PUA in ducklings. Plasma UA decreased and then increased with increasing Met. The level of Met at which PUA was lowest was similar to the estimated Met requirement determined by ADG. Cysteine had no effect on PUA concentrations.

Effects of fasting and refeeding on PUA concentrations were examined by Kolmstetter and Ramsay (2000) in penguins. Pre-prandial and post-prandial PUA concentrations were determined in penguins that consumed no feed during the 2 h of access to fish, and penguins that repeatedly ate during the 2 h of access to fish. Penguins that did not consume any fish did not have different pre-prandial and post-prandial PUA concentrations. Penguins that consumed fish repeatedly had

significantly higher PUA concentrations 0- to 2- h post-prandial compared with pre-prandial and 4- to 6- h post-prandial concentrations. The PUA concentrations at 4- to 6- h post-prandial were also significantly higher than pre-prandial concentrations.

Wilson and Miles (1988) examined the effect of feeding time and lighting on PUA concentrations in male broiler breeders and single comb white leghorn cockerels. Plasma UA concentrations peaked 2 h after feeding and steadily decreased up to 24 h after feeding. When examining effects of light on PUA, a peak in PUA was observed 6 h after lights were turned on.

THE LYSINE REQUIREMENT OF BROILERS

Lysine is the second limiting AA in corn-soybean meal (C-SBM) diets for broilers. In order to apply the ideal protein concept to diet formulation, the Lys requirement must be determined (Baker and Han, 1994; Baker et al, 2002). When determining the Lys requirement for poultry, age, sex, strain, rearing environment, type of feed, and levels of other essential nutrients should be considered (Garcia et al., 2005).

Due to the demand of the industry for fast growing broilers, the Lys requirement of these broilers will be discussed. Aviagen (2007) recommends a total Lys requirement of Ross × Ross 308 and 708 broilers to be 1.43% for the starter phase of 0- to 10- d and 1.24% for the grower phase from 11- to 24- d.

The Lys requirement for Ross × Ross 508 male broilers 0- to 18- d was examined by Corzo et al. (2005) by feeding increasing levels of total Lys ranging from 0.95 to 1.40% at 0.05% increments. Regression analysis of ADG and feed conversion ratio (FCR) estimated total Lys requirements to be 1.24 and 1.27%, respectively.

URIC ACID CONTENT OF THE EXCRETA

Nitrogen excretion is directly related to the amount of N provided in the diet.

Pudelkiewicz et al (1968) reported that chicks fed diets with 4.02% N had lower levels of UAE compared with chicks fed 4.37 or 6.98% N. The UA levels reported ranged from 86 to 88 mg/g dry matter (DM) in chicks fed 4.02% N, 198 to 204 mg/g DM in chicks fed 4.37% N, and 330 to 355 mg/g DM in chicks fed 6.98% N. These ranges were due to the different methods used to determine the amount of UAE. Scholz and Featherston (1968) reported that the total amount of N and UAE were significantly higher in broilers fed high protein diets. Miles and Featherston (1974) reported a decrease in the amount of UAE as Lys increased in the diet. Marquardt (1983) and Marquardt et al. (1983), reported that UAE ranges from 27 to 55%. The amount of urinary N that comes from UA can be as high as 88% which accounts for most of the UAE in birds.

The amount of dietary N is not the only factor that affects UAE. Creek and Vasaitis (1961) reported that N excretion increased with age, which was due to the decrease in protein needs as birds grow. When broilers are fed poor quality protein, more UA is excreted and less N is incorporated into the body (Miles and Featherston, 1976). Miles and Featherston (1976) and Salter et al. (1974) reported that when a protein source was damaged, such as by applying heat, UAE was increased. When broilers were fed a diet without heat damaged protein and supplemented with limiting AA, UAE was decreased, which indicated better N utilization (Salter et al., 1974).

CHAPTER 3

THE USE OF SERUM URIC ACID AND SERUM UREA NITROGEN AS INDICATORS OF AMINO ACID ADEQUACY OF A DIET FOR BROILER CHICKS

INTRODUCTION

As corn and SBM prices continue to rise, formulating diets to meet AA requirements becomes more important. Previously, ADG, ADFI, and GF have been used as response variables to estimate AA requirements for broilers. In swine, PUN has become a widely accepted response variable to estimate AA requirements (Coma et al., 1995; Knowles et al., 1997; and Guzik et al., 2005). In broilers, UA is formed, not urea, as the end product of N metabolism. Therefore, PUA could possibly be used as a response variable to determine AA requirements of broilers.

Attempts to relate PUA concentrations to protein utilization have shown mixed results. Hevia and Clifford (1977) showed a linear increase in PUA as dietary protein increased from 11 to 80%. This effect was due to the excess N in the high protein diets. When examining more conventional diets with smaller changes in dietary protein, PUA concentrations do not change as drastically. Corzo et al. (2003) and Corzo et al. (2005) reported no changes in PUA as dietary Lys or Trp were increased. However, Miles and Featherston (1974) were able to show a decrease in PUA as dietary Lys increased to the Lys requirement.

Plasma UA concentrations are not only affected by dietary protein, but also by fasting and refeeding. Okumura and Tasaki (1969) reported that immediately after birds consumed feed, PUA concentrations were similar in birds fed diets ranging from a protein-free up to a 40% casein diet. Plasma UA increased and peaked after a 2 h fast

and then gradually decreased up to a 6 h fast. Plasma UA concentrations after a 6 h fast were similar to concentrations observed immediately after feeding.

As formulating diets to meet AA requirements becomes more important, it is important to find as many variables as possible that accurately estimate AA requirements. Therefore, the objective of this research was to determine if SUA could be used to determine the AA adequacy of a diet for broilers.

MATERIALS AND METHODS

General

All experimental animal use was in compliance with the LSU Agricultural Center Animal Care and Use Committee. Five experiments were conducted using Ross × Ross 308 or 708 male or female broiler chicks. Ross × Ross 308 broilers are fast growing broilers that are efficient and have high-quality meat yield. Ross × Ross 708 broilers are slower growing, but have good feed efficiency and are used for cut-up pieces and deboning (Aviagen, 2007). Broilers were allotted, wing-banded, and placed onto dietary treatments on d 0 posthatching. Each experiment lasted 14 or 18 d. Broilers were housed in environmentally-controlled brooder batteries with raised wire floors and continuous fluorescent lighting. All diets were corn and SBM (C-SBM) based and formulated to contain 1.0% Ca and 0.45% nonphytate P and to meet or exceed the requirements of all other nutrients except total Lys, Met, and Thr (Exp. 1) or Met (Exp. 2 to 5), as recommended by the NRC (1994). At the termination of each experiment, broilers and feeders were weighed for the determination of growth performance, and broilers were bled for determination of SUA, SUN, and serum ammonia (SA).

Excreta Collection and Analysis

Excreta samples were collected from each pen of broilers daily for the last 4 d of each experiment to determine the amount of UA excreted. Samples from all 4 d were combined and mixed thoroughly, dried and ground for analysis of UA content by methods described by Marquardt (1983).

Blood Sampling

At the termination of each experiment, broilers were bled via cardiac puncture for determination of SUA, SUN, and SA. Blood was placed into 10 mL serum tubes (BD Vacutainer, Franklin Lakes, NJ) and samples were held on ice until centrifugation at $1,734 \times g$ at 0°C for 20 min. Serum (0.5 mL) from each broiler was collected, but serum was pooled by replicate pen. Serum UA, SUN, and SA concentrations were determined using commercial reagent kits (Pointe Scientific, Canton, MI).

In Exp. 1, 84 male Ross × Ross 708 broilers were allotted to 2 dietary treatments (Table 3.1) to determine if SUA, SUN, or SA could be used to determine the AA adequacy of a diet. Diet 1 was formulated to be deficient in Lys, Thr, and Met and Diet 2 was formulated to be adequate in all AA. Cornstarch was added in the absence of AA in Diet 1. Treatments contained 7 replications with 6 broilers per replicate. At the termination of the experiment, individual broilers were bled. Before bleeding, all broilers had access to feed, and no fasting and refeeding schedule was implemented.

Experiments 2 to 5 were conducted to determine the SUA, SUN, or SA concentrations of broilers during various fasting and refeeding schedules. There were 6 replications of each treatment with 6 to 18 broilers per replicate. Dietary treatments (Table 3.2) were set up in a 2 × 2 factorial arrangement with or without supplemental Met or Gly to contain a TSAA:Lys ratio of 0.50 or 0.76 and a total Gly+Ser of 1.905% or

2.32% (Dean et al., 2006). All diets contained 1.35% total Lys with 0.25% L-Lys•HCl. Cornstarch was added in place of Met and Gly in diets without supplemental Met or Gly.

Experiment 2 was conducted to determine the blood N concentrations in fasted and fed birds. For this experiment, 144 Ross × Ross 308 female broilers were used. This experiment was conducted for 18 d. At the termination of the experiment, broilers were fasted for 2 h and 2 broilers from each pen were bled. Feeders were reintroduced to each pen and 2 broilers were bled again after 30 and 60 min.

Experiment 3 was similar to Exp. 2 except 360 male and female Ross × Ross 308 broilers were used and the experiment was conducted for 14 d. There were 3 replications of males and 3 replications of females. This experiment was conducted to determine the optimal time for blood collection after a fast to determine SUA, SUN, and SA concentrations. The fasting and refeeding schedule for this experiment consisted of an initial 2 h fast, replacement of feeders to each pen for 20 min to ensure each broiler had the opportunity to consume feed, and feeders were then removed. Three broilers per pen were bled at 0 (when feeders were removed), 1, 2, and 3 h post-feeding.

Experiment 4 was similar to Exp. 3 except 432 Ross × Ross 708 female broilers were used. This experiment was conducted to extend the fasting period that was used in Exp. 3. The fasting and refeeding schedule was similar to the previous experiment, except broilers were bled at 0, 2, 3, 4, and 5, h post-feeding.

Experiment 5 was similar to Exp. 4 except 144 Ross × Ross 708 female broilers were used. Three broilers were bled at 0 and 2 h post-feeding.

For Exp. 1, 2, 4, and 5, data were analyzed by ANOVA as completely randomized designs using the GLM procedure in SAS (SAS Inst. Inc., Cary, NC). The pen of broilers served as the experimental unit for all data. For Exp. 3, data were

analyzed by ANOVA as a randomized complete block with sex as the block using the GLM procedure in SAS. Treatment means for Exp. 2 to 5 were separated by orthogonal contrasts appropriate for a 2×2 factorial arrangement of treatments.

RESULTS

In Exp. 1 (Table 3.3), broilers fed the AA adequate diet had an increased ADG, ADFI, and GF compared with broilers fed the AA deficient diet ($P < 0.03$). Serum UA, SUN, SA and UAE (Table 3.4) were not affected by the addition of supplemental AA ($P > 0.10$).

In Exp. 2 (Table 3.5), broilers fed diets containing supplemental Met had increased ADG, ADFI, and GF compared with broilers fed diets without supplemental Met ($P < 0.03$). Feed efficiency was also increased in broilers fed diets containing supplemental Gly ($P < 0.07$). Serum UA and SUN (Table 3.6) were decreased after the 2 h fast in broilers fed diets containing supplemental Met and Gly or the combination ($P < 0.08$). Serum urea N was decreased after 60 min of access to feed in broilers fed diets containing supplemental Met ($P < 0.02$). Serum ammonia (Table 3.6) was decreased after a 2 h fast and after 30 min of access to feed in broilers fed diets containing supplemental Met ($P < 0.01$). Uric acid content of the excreta (Table 3.6) was decreased in broilers fed diets containing supplemental Met ($P < 0.01$) and increased when Gly was supplemented ($P < 0.02$).

In Exp. 3 (Table 3.7), broilers fed diets containing supplemental Met had increased ADG, ADFI, and GF ($P < 0.01$). Gain was greatest when Gly and Met were added together ($P < 0.03$). Feed efficiency was increased in broilers fed diets containing supplemental Gly ($P < 0.03$). Males had a greater ADG ($P < 0.06$) and GF ($P < 0.03$) than females (data not shown). Serum UA and SUN (Table 3.8) were decreased at all

times in broilers fed diets containing supplemental Met ($P < 0.02$) but the largest decrease in broilers fed Met was at 2 h post-feeding. Serum ammonia (Table 3.8) was decreased at 1 and 3 h post-feeding in broilers fed diets containing supplemental Met ($P < 0.05$) and was decreased most at 0 and 3 h post-feeding when Gly and Met were added together ($P < 0.09$). Serum UA concentrations were higher in males at 0 and 3 h post-feeding ($P < 0.05$). Serum urea N concentrations were higher in females at 1 h post-feeding ($P < 0.04$). At 0 h post-feeding, there was a treatment \times sex interaction for SUN ($P < 0.01$). Female broilers fed no supplemental Met had greater SUN concentrations than males. Serum ammonia concentrations were higher in females at all times ($P < 0.01$). Uric acid content of the excreta (Table 3.8) was decreased ($P < 0.01$) by the addition of supplemental Met and was increased by supplemental Gly ($P < 0.01$). Females had higher UAE ($P < 0.05$) compared with male broilers (303.47 mg/dL, 294.44 mg/dL).

In Exp. 4 (Table 3.9), broilers fed diets containing supplemental Met had increased ADG, ADFI, and GF ($P < 0.01$). Gain:feed was increased in broilers fed diets containing supplemental Gly ($P < 0.05$) and was highest in broilers fed Gly and Met added together ($P < 0.04$). Serum UA (Table 3.10) was decreased in broilers fed diets containing supplemental Met at 0 and 2 h post-feeding ($P < 0.04$), and was increased at 5 h post-feeding ($P < 0.05$). Serum urea N (Table 3.10) was decreased in broilers fed diets containing supplemental Met at all times ($P < 0.04$), and was also decreased in broilers fed diets containing supplemental Gly at 2 h post-feeding ($P < 0.01$). Serum ammonia (Table 3.10) was decreased in broilers fed diets containing supplemental Met

Table 3.1. Composition of corn-soybean meal diets with and without supplemental amino acids in Experiment 1, as-fed basis¹

Ingredient	AA deficient	AA adequate
Corn	58.61	58.61
Soybean meal (47.5%)	30.15	30.15
Soy oil	4.20	4.20
Monocalcium phosphate	1.58	1.58
Limestone	1.52	1.52
Salt	0.50	0.50
Mineral mix ²	0.25	0.25
Vitamin mix ³	0.25	0.25
Choline chloride ⁴	0.05	0.05
DL-Met	---	0.274
L-Lys•HCl	---	0.250
L-Thr	---	0.156
Gly	---	0.750
Cornstarch	2.90	1.47
Calculated composition		
ME, kcal/kg	3,164	3,200
CP, %	19.20	20.58
Ca, %	1.00	1.00
Nonphytate P, %	0.45	0.45
Lys, %	1.063	1.260
TSAA ⁵ , %	0.636	0.907
Thr, %	0.728	0.882
Trp, %	0.231	0.231
Leu, %	1.684	1.684
Gly+Ser, %	1.776	2.526
Arg, %	1.266	1.266
Ile, %	0.815	0.815
Val, %	0.913	0.913

¹ A basal diet was mixed to contain the minimum of all ingredients except supplemental Gly, DL-Met, L-Lys•HCl, L-Thr, and cornstarch, which were added as needed for each diet.

² Provided per kilogram of diet: copper (copper sulfate•5H₂O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate•7H₂O), 60 mg; manganese (manganese sulfate•H₂O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate•7H₂O), 44 mg; calcium (calcium carbonate), 723 mg.

³ Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,003 IU; vitamin D₃ (cholecalciferol), 3004 IU; vitamin E (DL- α -tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B₁₂, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine-HCl), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin-HCl), 3.00 mg.

⁴ Contains 750,000 mg/kg of choline.

⁵ TSAA = total sulfur amino acids.

Table 3.2. Composition of corn-soybean meal diets with and without supplemental DL-methionine and glycine in Experiments 2 to 5, as-fed basis¹

Ingredient	-Met -Gly	+Met -Gly	-Met +Gly	+Met +Gly
Corn	56.60	56.60	56.60	56.60
Soybean meal (47.5%)	33.31	33.31	33.31	33.31
Soy oil	4.66	4.66	4.66	4.66
Monocalcium phosphate	1.55	1.55	1.55	1.55
Limestone	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50
Mineral mix ²	0.25	0.25	0.25	0.25
Vitamin mix ³	0.25	0.25	0.25	0.25
Choline chloride ⁴	0.05	0.05	0.05	0.05
DL-Met	---	0.356	---	0.356
L-Lys•HCl	0.250	0.250	0.250	0.250
L-Thr	0.166	0.166	0.166	0.166
Gly	---	---	0.415	0.415
L-Val	0.064	0.064	0.064	0.064
L-Arg•HCl	0.059	0.059	0.059	0.059
L-Ile	0.027	0.027	0.027	0.027
Cornstarch	0.771	0.415	0.356	---
Calculated composition				
ME, kcal/kg	3,180	3,193	3,187	3,200
CP, %	21.23	21.25	21.52	21.73
Ca, %	1.00	1.00	1.00	1.00
Nonphytate P, %	0.45	0.45	0.45	0.45
Lys, %	1.350	1.350	1.350	1.350
TSAA ⁵ , %	0.670	1.026	0.670	1.026
Thr, %	0.945	0.945	0.945	0.945
Trp, %	0.250	0.250	0.250	0.250
Leu, %	1.779	1.779	1.779	1.779
Gly+Ser, %	1.905	1.905	2.320	2.320
Arg, %	1.418	1.418	1.418	1.418
Ile, %	0.905	0.905	0.905	0.905
Val, %	1.040	1.040	1.040	1.040

¹ A basal diet was mixed to contain the minimum of all ingredients except supplemental Gly, DL-Met and cornstarch were added as needed for each diet.

² Provided per kilogram of diet: copper (copper sulfate-5H₂O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate-7H₂O), 60 mg; manganese (manganese sulfate-H₂O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate-7H₂O), 44 mg; calcium (calcium carbonate), 723 mg.

³ Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,003 IU; vitamin D₃ (cholecalciferol), 3004 IU; vitamin E (DL- α -tocopheryl acetate), 25.00 IU; menadione

(continued on the next page)

(menadione sodium bisulfite), 1.50 mg; vitamin B₁₂, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine·HCl), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin·HCl), 3.00 mg.

⁴ Contains 750,000 mg/kg of choline.

⁵ TSAA = total sulfur amino acids.

at 0 and 4 h post-feeding ($P < 0.02$). Uric acid content of the excreta (Table 3.10) was decreased ($P < 0.01$) by the addition of supplemental Met.

In Exp. 5 (Table 3.11), broilers fed diets containing supplemental Met had higher ADG, ADFI, and GF ($P < 0.01$), and when Gly and Met were added together, the effect was greater ($P < 0.08$). Gain:feed was also increased in broilers fed diets containing supplemental Gly ($P < 0.07$). Serum UA and SUN (Table 3.12) concentrations were decreased in broilers fed diets containing supplemental Met and Gly at 2 h post-feeding ($P < 0.04$), and SUA was further decreased in broilers fed diets containing both Gly and Met ($P < 0.08$). Serum ammonia (Table 3.12) concentrations were not affected by treatment ($P > 0.10$). Uric acid content of the excreta (Table 3.12) was decreased ($P < 0.01$) by the addition of supplemental Met.

DISCUSSION

None of the diets that were fed in Exp. 1 to 5 had extreme excesses of AA and CP, which may be the reason there were no drastic changes in SUA as were observed by Okumura and Tasaki (1969) and Featherston (1969). No significant differences in PUA were observed by Russell and Weber (1934) in laying hens fed low or high protein diets of 13 or 19% protein, respectively.

The results from Exp. 1 to 5 indicate that SUA can be used as an indicator of AA utilization in diets for broilers. Decreases in ADG and GF were observed in broilers fed diets without supplemental AA in Exp. 1 and in broilers fed diets without Met or Gly in Exp. 2 to 5 which indicate that diets were deficient in AA. Differences in blood N concentrations were unable to be detected in Exp. 1. In Exp. 1 broilers were bled immediately after they had access to feed. Therefore, we do not know when each broiler last consumed feed relative to the time of bleeding. This inconsistency in time of

eating and access to feed may be the reason that SUA was not affected by AA adequacy or deficiency. Supplementing diets with Met decreased SUA in nearly all instances in fasted broilers in Exp. 2 through 5, indicating an increase in protein utilization by feeding supplemental Met. These results are similar to effects of Met on PUA observed by Xie et al. (2004) in ducks. Supplemental Gly did not exhibit the same effect as Met, but some decreases were observed in SUA concentrations when Gly was supplemented. Miles and Featherston (1974) reported a decrease in PUA as supplemental Lys increased in a Lys requirement study. The PUA concentrations plateaued at Lys levels that were in excess of the requirement.

The use of PUN in swine does not require a fasting or refeeding schedule to observe differences in PUN (Coma et al., 1995; Knowles et al., 1997; and Guzik et al., 2005). The fasting and refeeding schedules in our experiments were implemented due to no differences in blood N data in Exp. 1. In our experiments, there were very few significant differences in SUA concentrations in fed broilers. The effects of fasting and refeeding schedules on PUA and BUN have been examined and have shown that PUA increased and peaked after a 2 h fast and decreased as the fasting period increased (Okumura and Tasaki, 1969; Wilson and Miles, 1988; and Kolmstetter and Ramsay, 2000). Decreases in SUA concentrations were observed in Exp. 2 to 5 when fasting and refeeding schedules were implemented. Our results indicate that 2 h post-feeding is the optimal time for blood to be collected for determination of SUA. Although at 2 h post-feeding, broilers did not exhibit peak SUA concentrations, this result of differences being observed after a 2 h fast is similar to Okumura and Tasaki (1969) and Wilson and Miles (1988), who reported that PUA concentrations peaked at 2 h post-feeding. Kolmstetter and Ramsay (2000) reported that penguins given ad libitum access to their normal diets

Table 3.3. Growth performance of broiler chicks fed amino acid deficient and amino acid adequate diets in Experiment 1¹

Treatment	ADG, g	ADFI, g	GF, g/g
1) AA deficient	25.48	37.49	0.68
2) AA adequate	31.98	40.66	0.79
SEM	0.64	0.93	0.01
P-value	0.01	0.03	0.01

¹ Data are means of 7 replications of 6 broilers per replicate.

Table 3.4. Blood nitrogen data and uric acid content of the excreta of broiler chicks fed amino acid deficient and amino acid adequate diets in Experiment 1¹

Treatment	SUA ² , mg/dL	SUN ³ , mg/dL	SA ⁴ , mmol/L	UAE ⁵ , mg/g ⁶
1) AA deficient	6.58	1.78	2.78	407.09
2) AA adequate	6.39	1.86	3.55	397.50
SEM	0.33	0.10	0.56	9.65
P-value	0.70	0.56	0.34	0.50

¹ Data are means of 7 replications of 6 broilers per replicate.

² SUA= serum uric acid.

³ SUN= serum urea N.

⁴ SA= serum ammonia.

⁵ UAE= uric acid of the excreta.

⁶ Dry matter basis.

Table 3.5. Growth performance of broiler chicks fed supplemental DL-methionine and glycine in Experiment 2¹

Treatment	ADG, g	ADFI, g	GF, g/g
1) - Met - Gly	29.68	44.68	0.66
2) + Met	35.88	46.56	0.77
3) + Gly	30.26	43.45	0.70
4) + Met + Gly	36.93	46.96	0.79
SEM	1.22	1.17	0.01
P-value	0.01	0.17	0.01
Gly	0.52	0.73	0.07
Met	0.01	0.03	0.01
Gly × Met	0.85	0.50	0.53

¹ Data are means of 6 replications of 6 broilers per replicate.

Table 3.6. Blood nitrogen data and uric acid content of the excreta of broiler chicks fed supplemental DL-methionine and glycine in Experiment 2¹

Treatment	SUA ² , mg/dl			SUN ³ , mg/dL			SA ⁴ , mmol/L			UAE ⁵ , mg/g ⁶
	2 h	30 min	60 min	2 h	30 min	60 min	2 h	30 min	60 min	
1) - Met - Gly	3.83	4.31	3.67	2.36	2.16	2.13	3.33	5.05	4.76	327.96
2) + Met	2.85	5.09	3.43	1.83	1.92	1.92	2.75	3.58	4.67	302.38
3) + Gly	3.50	5.13	4.10	2.05	2.45	2.46	3.89	5.09	5.18	343.80
4) + Met + Gly	2.02	4.60	3.50	1.50	2.13	1.93	2.69	3.69	3.96	309.19
SEM	0.32	0.57	0.37	0.15	0.20	0.15	0.32	0.49	0.67	4.31
P-value	0.01	0.70	0.60	0.01	0.32	0.06	0.06	0.06	0.63	0.01
Gly	0.08	0.78	0.52	0.05	0.22	0.27	0.45	0.88	0.83	0.02
Met	0.01	0.83	0.27	0.01	0.17	0.02	0.01	0.01	0.34	0.01
Gly × Met	0.44	0.27	0.64	0.94	0.85	0.31	0.35	0.94	0.40	0.31

¹ Data are means of 6 replications with pooled serum of 2 broilers per replicate per time.

² SUA= serum uric acid.

³ SUN= serum urea N.

⁴ SA= serum ammonia.

⁵ UAE= uric acid of the excreta.

⁶ Dry matter basis.

Table 3.7. Growth performance of broiler chicks fed supplemental DL-methionine and glycine in Experiment 3¹

Treatment	ADG, g	ADFI, g	GF, g/g
1) - Met - Gly	24.29	34.26	0.71
2) + Met	30.38	37.15	0.82
3) + Gly	23.72	32.91	0.72
4) + Met + Gly	31.92	37.80	0.84
SEM	0.44	0.80	0.01
P-value	0.01	0.01	0.01
Sex ²	0.06	0.61	0.03
Gly	0.29	0.67	0.03
Met	0.01	0.01	0.01
Gly × Met	0.03	0.23	0.25

¹ Data are means of 6 replications of 15 broilers per replicate.

² Sex × Treatment interaction was not significant and was removed from the model.

Table 3.8. Blood nitrogen data and uric acid content of the excreta of broiler chicks fed supplemental DL-methionine and glycine in Experiment 3¹

Treatment	SUA ² , mg/dl				SUN ³ , mg/dL				SA ⁴ , mmol/L				UAE ⁵ , mg/g ⁶
	0 h	1 h	2 h	3 h	0 h	1 h	2 h	3 h	0 h	1 h	2 h	3 h	
1) - Met - Gly	5.17	4.47	4.25	4.43	2.45	2.42	1.98	1.70	4.51	3.64	3.09	3.55	302.90
2) + Met	4.86	3.34	2.69	2.22	1.95	1.89	1.60	1.26	4.63	3.08	2.95	2.92	280.17
3) + Gly	6.14	5.21	3.49	4.52	2.42	2.41	2.00	1.54	5.14	3.42	3.27	3.89	331.03
4) + Met + Gly	4.66	3.74	3.37	2.96	1.88	1.77	1.54	1.40	3.76	2.84	2.73	2.64	281.72
SEM	0.35	0.38	0.27	0.39	0.10	0.11	0.10	0.11	0.38	0.27	0.35	0.18	4.33
P-value	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.08	0.11	0.21	0.74	0.01	0.01
Sex	0.05	0.22	0.77	0.01	0.23	0.04	0.63	0.20	0.01	0.01	0.01	0.01	0.05
Sex × TRT ⁷	---	---	---	---	0.01	0.84	0.31	0.98	---	---	---	---	0.09
Gly	0.29	0.15	0.88	0.30	0.58	0.57	0.86	0.92	0.76	0.41	0.94	0.86	0.01
Met	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.11	0.05	0.34	0.01	0.01
Gly × Met	0.11	0.66	0.02	0.42	0.86	0.65	0.69	0.20	0.06	0.97	0.57	0.09	0.01

¹ Data are means of 6 replicates with pooled serum from 3 broilers per replicate per time.

² SUA= serum uric acid.

³ SUN= serum urea N.

⁴ SA= serum ammonia.

⁵ UAE= uric acid of the excreta.

⁶ Dry matter basis.

⁷ Sex × TRT was not significant for SUA and SA and was removed from the model.

Table 3.9. Growth performance of broiler chicks fed supplemental DL-methionine and glycine in Experiment 4¹

Treatment	ADG, g	ADFI, g	GF, g/g
1) - Met - Gly	22.95	28.99	0.79
2) + Met	27.64	31.90	0.87
3) + Gly	22.41	28.34	0.79
4) + Met + Gly	27.79	31.09	0.89
SEM	0.37	0.51	0.01
P-value	0.01	0.01	0.01
Gly	0.60	0.17	0.05
Met	0.01	0.01	0.01
Gly × Met	0.35	0.87	0.04

¹ Data are means of 6 replications with 18 broilers per replicate.

Table 3.10. Blood nitrogen data and uric acid content of the excreta of broiler chicks fed supplemental DL-methionine and glycine in Experiment 4¹

Treatment	SUA ² , mg/dl					SUN ³ , mg/dL					SA ⁴ , mmol/L					UAE ⁵ , mg/g ⁶
	0 h	2h	3 h	4 h	5 h	0 h	2h	3 h	4 h	5 h	0 h	2h	3 h	4 h	5 h	
1) - Met - Gly	5.33	4.20	4.24	5.00	5.01	2.06	1.87	1.57	1.43	1.07	6.35	3.94	2.88	3.73	3.96	487.08
2) + Met	4.17	3.41	3.82	5.16	6.25	1.69	1.46	1.15	0.99	0.99	5.03	3.37	2.49	3.07	3.49	412.40
3) + Gly	5.56	3.98	4.08	5.44	4.71	2.31	1.75	1.50	1.38	1.40	7.11	4.19	3.04	4.10	4.34	495.94
4) + Met + Gly	4.43	3.22	4.58	4.96	5.82	1.73	1.30	1.05	0.89	1.02	5.19	3.40	2.69	3.03	3.60	404.22
SEM	0.33	0.34	0.56	0.53	0.56	0.10	0.05	0.09	0.10	0.11	0.41	0.44	0.39	0.33	0.39	10.86
P-value	0.02	0.17	0.63	0.91	0.22	0.01	0.01	0.01	0.01	0.04	0.01	0.48	0.78	0.09	0.43	0.01
Gly	0.47	0.55	0.48	0.82	0.52	0.18	0.01	0.31	0.46	0.11	0.28	0.75	0.65	0.63	0.54	0.96
Met	0.01	0.04	0.92	0.77	0.05	0.01	0.01	0.01	0.01	0.04	0.01	0.13	0.36	0.02	0.14	0.01
Gly × Met	0.96	0.96	0.28	0.55	0.92	0.32	0.64	0.86	0.83	0.18	0.48	0.81	0.96	0.55	0.73	0.44

¹ Data are means of 6 replicates of pooled serum from 3 broilers per replicate per time.

² SUA= serum uric acid.

³ SUN= serum urea N.

⁴ SA= serum ammonia.

⁵ UAE= uric acid of the excreta.

⁶ Dry matter basis.

Table 3.11. Growth performance of broiler chicks fed supplemental DL-methionine and glycine in Experiment 5¹

Treatment	ADG, g	ADFI, g	GF, g/g
1) - Met - Gly	21.53	28.94	0.74
2) + Met	25.75	31.58	0.82
3) + Gly	19.96	26.82	0.74
4) + Met + Gly	27.17	32.13	0.85
SEM	0.49	0.63	0.01
P-value	0.01	0.01	0.01
Gly	0.88	0.22	0.07
Met	0.01	0.01	0.01
Gly × Met	0.01	0.04	0.08

¹ Data are means of 6 replications with 6 broilers per replicate.

Table 3.12. Blood nitrogen data and uric acid content of the excreta of broiler chicks fed supplemental DL-methionine and glycine in Experiment 5¹

Treatment	SUA ² , mg/dl		SUN ³ , mg/dL		SA ⁴ , mmol/L		UAE ⁵ , mg/g ⁶
	0 h	2h	0 h	2 h	0 h	2 h	
1) - Met - Gly	4.44	3.62	1.59	1.86	3.97	2.50	320.06
2) + Met	3.91	2.75	1.65	1.06	2.85	2.48	284.26
3) + Gly	4.31	3.49	1.87	1.38	3.99	2.68	322.44
4) + Met + Gly	4.43	1.83	1.59	0.88	3.18	2.38	287.40
SEM	0.32	0.21	0.12	0.15	0.64	0.47	9.31
P-value	0.60	0.01	0.35	0.01	0.51	0.98	0.01
Gly	0.55	0.02	0.37	0.04	0.79	0.93	0.77
Met	0.52	0.01	0.38	0.01	0.15	0.74	0.01
Gly × Met	0.31	0.08	0.19	0.35	0.82	0.77	0.97

¹ Data are means of 6 replications of pooled serum from 3 broilers per replicate per time.

² SUA= serum uric acid.

³ SUN=serum urea N.

⁴ SA= serum ammonia.

⁵ UAE= uric acid of the excreta.

⁶ Dry matter basis.

had significantly increased PUA 0- to 2- h post-prandial compared with pre-prandial and 4- to 6-h post-prandial PUA concentrations. It was noted that the penguin that ate constantly for the 2 h that feed was allowed had the highest post-prandial PUA concentration.

Little is known about the concentrations of SUN in broilers due to the lack of research in this area. In our experiments, decreases in SUN concentrations were observed at many different times post-feeding in broilers fed diets containing supplemental Met, but Gly had little effect on SUA. Kolmstetter and Ramsay (2000) observed differences in BUN concentrations in fed and fasted penguins. Two h post-prandial BUN concentrations were significantly lower than pre-prandial and 4- to 6- h post-prandial BUN concentrations. No significant differences in BUN were observed by Russell and Weber (1934) in laying hens fed low or high protein diets.

Serum ammonia concentrations provided little information about AA utilization in our experiments due to the inconsistency of significance among experiments.

Uric acid content of the excreta was decreased by the addition of Met in Exp. 2 to 5. In Exp. 2 and 3, Gly increased UAE which is thought to be due to a possible excess of Gly, a main precursor to UA formation. This effect is similar to previous research by Miles and Featherston (1974) and Pudelkiewicz et al. (1968) in that changing the amount of N intake by increasing supplemental AA in the diet or by increasing N from 4 to 7% changed the UAE. The values observed in these experiments agree with the percent of UAE reported by Pudelkiewicz et al. (1968), Marquardt et al. (1983), and Marquardt (1983). The UAE values observed in these experiments ranges from 28 to

49%. The decrease in UAE is related to the decrease in SUA that is observed in all experiments when broilers are fed diets containing supplemental Met. Therefore, supplemental Met decreases excess UA in the plasma and excreta.

The results of these 5 experiments indicate that SUA and to some extent SUN can be used as indicators of AA utilization in broilers. Differences in SUA and SUN were not detected in fed broilers and therefore a fasting and refeeding schedule must be implemented. The most optimal time for blood collection is 2 h post-feeding.

CHAPTER 4

DETERMINATION OF THE LYSINE REQUIREMENT OF BROILER CHICKS FROM 0- to 17- d POSTHATCHING USING SERUM URIC ACID AND GROWTH PERFORMANCE AS RESPONSE VARIABLES

INTRODUCTION

Determination of the Lys requirement is essential in order to apply the ideal protein concept to formulating diets (Baker and Han, 1994; Baker et al, 2002) for broilers. Gain, ADFI, and GF have been the most widely used variables in determining the AA requirements for broilers. In swine, however, PUN has been shown to be an accurate variable in determining AA requirements (Coma et al., 1995; Knowles et al., 1997; and Guzik et al., 2005). Uric acid is the end product of N metabolism in broilers and previously in Chapter 3, SUA was shown to be an accurate means of evaluating AA utilization.

Therefore, the objective of this research was to use growth performance and SUA and SUN concentrations in broilers to determine the Lys requirement of Ross × Ross 708 broilers.

MATERIALS AND METHODS

General

All methods used in these Experiments were approved by the LSU Agricultural Center Animal Care and Use Committee. Broilers were housed in environmentally-controlled brooder batteries with raised wire floors and continuous fluorescent lighting. Broilers were weighed, allotted, wing-banded, and placed onto dietary treatments on d 0 posthatching. Diets were formulated to contain 1.0% Ca and 0.45% nonphytate P, and to meet or exceed the requirements of all other nutrients as recommended by the NRC

(1994) except for Lys (Exp. 1 and 2) and Lys and Met (Exp. 3). Feed in mash form and water were provided ad libitum for all experiments. At the termination of each experiment, broilers and feeders were weighed for determination of ADG, ADFI, and GF.

Blood Sampling

At the termination of each experiment, 3 broilers were bled via cardiac puncture for determination of SUA and SUN. The fasting and refeeding schedule for these experiments consisted of an initial 2 h fast followed by 20 min of access to feed to ensure that each broiler consumed feed. After 20 min, feeders were removed and broilers were fasted for another 2 h and 3 broilers from each replication were bled. Blood was placed into 10 mL serum tubes (BD Vacutainer, Franklin Lakes, NJ). Samples were placed on ice until centrifugation at $1,734 \times g$ at 0°C for 20 min. Serum (0.5 mL) from each broiler was collected but serum was pooled by replicate pen. Serum UA and SUN concentrations were determined using commercial reagent kits (Pointe Scientific, Canton, MI).

Excreta Collection and Analysis

Excreta samples were collected from each pen of broilers daily for the last 4 d of Exp. 3 to determine the amount of UA excreted. Samples from all 4 d were combined and mixed thoroughly, dried and ground for analysis of UA content by methods described by Marquardt (1983).

Experiment 1 was conducted to determine the total Lys requirement of Ross \times Ross 708 female broilers. There were 12 dietary treatments with 8 replications of 6 broilers per replicate. Dietary treatments consisted of increasing levels of total Lys. The increase in total Lys was accomplished by 2 different methods. The first 6 treatments

(Table 4.1) increased the total Lys from 1.150 to 1.545% at 0.079% increments by increasing supplemental crystalline Lys. Corn and SBM levels in the diet were held constant. Total Gly+Ser was set at 2.08% (Waguespack et al., 2008). Diets 7 through 12 (Table 4.2) increased the total Lys from 1.154 to 1.529% at 0.075% increments by increasing SBM and decreasing corn. Supplemental crystalline Lys was held constant at 0.170% in diets 7 through 12. This amount of supplemental Lys was chosen because it was the highest level of supplemental Lys that could be added without Val becoming limiting in the diets. All treatments were formulated to contain a TSAA:Lys ratio of at least 0.76% and a Thr:Lys ratio of at least 0.67.

Experiment 2 was conducted similarly to Exp. 1, except diets were formulated on an sid Lys basis rather than on a total Lys basis. The first 6 treatments (Table 4.3) increased sid Lys from 1.00 to 1.40% at 0.08% increments by increasing supplemental Lys. Corn and SBM levels in the diet were held constant. Total Gly+Ser was held constant at 2.08%. Treatments 7 through 12 (Table 4.4) increased sid Lys from 1.00 to 1.40% at 0.08% increments by increasing SBM and decreasing corn. Supplemental Lys was held constant at 0.06%. This amount of supplemental Lys was chosen because it was the highest level of supplemental Lys that could be added to the diet without Val becoming limiting. All treatments were formulated to contain a TSAA:Lys ratio of at least 0.76 and a Thr:Lys ratio of at least 0.67.

Experiment 3 was conducted to determine the effects of supplemental Lys and Met on growth performance, SUA, SUN, and UAE. There were 6 replications of each treatment with 6 broilers per replicate. The experiment was conducted for 16 d. Dietary treatments (Table 4.5) were set up in a 2 × 2 factorial arrangement with or without supplemental Lys and Met. Diets with and without Lys contained 1.00 or 1.16% sid Lys,

and diets with or without Met contained sidTSAA:sidLys ratios of 0.57, 0.49, 0.88, and 0.76, respectively.

For all experiments, data were analyzed by ANOVA for completely randomized designs using the GLM procedure in SAS (SAS Inst. Inc., Cary, NC). The pen of broilers served as the experimental unit for all data. For Exp. 1 and 2, linear and quadratic contrasts were determined. The NLIN procedures of SAS were used to estimate the total (Exp. 1) or sid (Exp. 2) Lys requirement (Robbins et al., 2006). Treatment means for Exp. 3 were separated by orthogonal contrasts appropriate for a 2×2 factorial arrangement of treatments.

RESULTS

In Exp. 1, (Table 4.6) there were no significant linear or quadratic effects of increasing total Lys from supplemental Lys on ADG; however, ADG was increased ($P < 0.01$) as total Lys increased from SBM. There were significant increasing linear and quadratic effects ($P < 0.08$) of increasing total Lys from supplemental Lys and SBM on GF. A two-slope broken-line analysis (Table 4.7) of ADG estimated a total Lys requirement of 1.27% ($P < 0.07$) when the main source of Lys was SBM. When supplemental Lys was the main source of Lys, a one and two-slope broken-line analysis of GF estimated Lys requirements of 1.42 and 1.45% total Lys, respectively (Table 4.7). There was a linear increase ($P < 0.01$) in SUN by increasing total Lys from supplemental Lys and SBM, and a linear increase by increasing Lys from SBM on SUA (Table 4.6). Broilers fed diets increasing in total Lys by increasing supplemental Lys had decreased SUA concentrations ($P < 0.02$) compared with broilers fed diets increasing in total Lys by increasing SBM. Broilers fed diets increasing in total Lys by increasing SBM

Table 4.1. Composition of corn-soybean meal diets with increasing total lysine by increasing supplemental lysine in Experiment 1, as fed basis¹

Ingredient	Total Lys, %					
	1.150	1.229	1.308	1.387	1.466	1.545
Corn	54.78	54.78	54.78	54.78	54.78	54.78
Soybean meal (47.5%)	33.36	33.36	33.36	33.36	33.36	33.36
Soy oil	4.40	4.40	4.40	4.40	4.40	4.40
Monocalcium phosphate	1.55	1.55	1.55	1.55	1.55	1.55
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix ²	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mix ³	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride ⁴	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.208	0.266	0.323	0.380	0.437	0.494
Biolys ⁵	---	0.156	0.311	0.467	0.623	0.778
L-Thr	0.029	0.082	0.134	0.186	0.239	0.291
Gly	0.185	0.185	0.185	0.185	0.185	0.185
L-Val	---	---	0.028	0.086	0.144	0.202
L-Arg•HCl	---	---	---	0.093	0.188	0.282
L-Ile	---	---	---	0.046	0.097	0.148
Cornstarch	2.93	2.67	2.37	1.91	1.45	0.98

Calculated composition

ME, kcal/kg	3,204	3,203	3,204	3,203	3,202	3,202
CP, %	20.76	20.77	20.96	21.17	21.58	22.00
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00
Nonphytate P, %	0.45	0.45	0.45	0.45	0.45	0.45
Lys, %	1.150	1.229	1.308	1.387	1.466	1.545
Met, %	0.523	0.580	0.645	0.706	0.766	0.827
TSAA ⁶ , %	0.874	0.931	0.988	1.045	1.102	1.159
Thr, %	0.805	0.857	0.908	0.958	1.010	1.061
Trp, %	0.250	0.250	0.250	0.250	0.251	0.251
Leu, %	1.763	1.764	1.765	1.767	1.768	1.769
Gly+Ser, %	2.080	2.080	2.080	2.080	2.080	2.080
Arg, %	1.364	1.365	1.366	1.444	1.522	1.601
Ile, %	0.874	0.875	0.875	0.921	0.971	1.022
Val, %	0.971	0.972	1.001	1.059	1.116	1.174
sid Arg, %	1.249	1.249	1.250	1.328	0.407	1.483
sid Lys, %	1.032	1.111	1.190	1.269	1.348	1.427
sid Ile, %	0.805	0.806	0.806	0.852	0.903	0.953
sid Val, %	0.877	0.878	0.907	0.965	1.023	1.080
sid Met, %	0.497	0.554	0.610	0.667	0.724	0.781
sid TSAA, %	0.788	0.845	0.902	0.959	1.016	1.073
sid Leu, %	1.628	1.628	1.629	1.630	1.631	1.632
sid Trp, %	0.225	0.225	0.225	0.225	0.226	0.226

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sid Thr, %	0.706	0.758	0.810	0.863	0.915	0.968
Arg:Lys	1.186	1.111	1.045	1.041	1.038	1.037
Ile:Lys	0.760	0.712	0.669	0.664	0.663	0.662
TSAA:Lys	0.760	0.758	0.755	0.754	0.752	0.750
Thr:Lys	0.700	0.697	0.694	0.691	0.689	0.687
Trp:Lys	0.217	0.203	0.191	0.180	0.171	0.163
Val:Lys	0.844	0.791	0.765	0.764	0.762	0.760

¹ A basal diet was mixed to contain the minimum of all ingredients.

² Provided per kilogram of diet: copper (copper sulfate·5H₂O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate·7H₂O), 60 mg; manganese (manganese sulfate·H₂O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate·7H₂O), 44 mg; calcium (calcium carbonate), 723 mg.

³ Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,003 IU; vitamin D₃ (cholecalciferol), 3004 IU; vitamin E (DL- α -tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B₁₂, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine·HCl), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin·HCl), 3.00 mg.

⁴ Contains 750,000 mg/kg of choline.

⁵ Contains 50.7% L-Lys.

⁶ TSAA = total sulfur amino acids.

Table 4.2. Composition of corn-soybean meal diets with increasing total lysine by increasing soybean meal in Experiment 1, as fed basis¹

Ingredient	Total Lys, %					
	1.154	1.229	1.304	1.379	1.454	1.529
Corn	60.76	57.77	54.78	51.76	48.63	45.49
Soybean meal (47.5%)	30.14	32.88	35.62	38.36	41.12	43.87
Soy oil	4.21	4.55	4.90	5.25	5.62	6.00
Monocalcium phosphate	1.57	1.55	1.53	1.51	1.49	1.47
Limestone	1.25	1.51	1.49	1.48	1.46	1.45
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix ²	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mix ³	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride ⁴	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.232	0.262	0.291	0.320	0.350	0.380
Biolys ⁵	0.170	0.170	0.170	0.170	0.170	0.170
L-Thr	0.071	0.082	0.092	0.103	0.114	0.125
Gly	0.289	0.186	0.083	---	---	---
Calculated composition						
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200
CP, %	20.02	20.97	21.93	22.91	23.99	25.06
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00
Nonphytate P, %	0.45	0.45	0.45	0.45	0.45	0.45
Lys, %	1.154	1.229	1.304	1.379	1.454	1.529
Met, %	0.535	0.578	0.620	0.663	0.705	0.748
TSAA ⁶ , %	0.874	0.931	0.988	1.045	1.102	1.159
Thr, %	0.805	0.858	0.910	0.963	1.015	1.068
Trp, %	0.233	0.249	0.265	0.281	0.297	0.313
Leu, %	1.706	1.776	1.847	1.918	1.987	2.057
Gly+Ser, %	2.080	2.080	2.080	2.100	2.203	2.306
Arg, %	1.275	1.359	1.443	1.527	1.612	1.696
Ile, %	0.822	0.873	0.923	0.974	1.025	1.076
Val, %	0.922	0.973	1.023	1.074	1.124	1.174
sid Arg, %	1.166	1.244	1.322	1.400	1.477	1.555
sid Lys, %	1.042	1.111	1.180	1.249	1.318	1.387
sid Ile, %	0.756	0.803	0.851	0.899	0.946	0.994
sid Val, %	0.832	0.879	0.925	0.971	1.017	1.063
sid Met, %	0.510	0.551	0.593	0.634	0.676	0.717
sid TSAA, %	0.791	0.844	0.897	0.951	1.004	1.057
sid Leu, %	1.575	1.640	1.705	1.769	1.833	1.897
sid Trp, %	0.209	0.224	0.238	0.253	0.267	0.281
sid Thr, %	0.710	0.758	0.806	0.853	0.901	0.949
Arg:Lys	1.105	1.106	1.106	1.107	1.108	1.109
Ile:Lys	0.712	0.710	0.708	0.706	0.705	0.704
TSAA:Lys	0.757	0.757	0.758	0.758	0.758	0.758

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Thr:Lys	0.697	0.698	0.698	0.698	0.698	0.698
Trp:Lys	0.202	0.203	0.203	0.203	0.204	0.205
Val:Lys	0.799	0.792	0.784	0.779	0.773	0.768

¹ A basal diet was mixed to contain the minimum of all ingredients.

² Provided per kilogram of diet: copper (copper sulfate·5H₂O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate·7H₂O), 60 mg; manganese (manganese sulfate·H₂O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate·7H₂O), 44 mg; calcium (calcium carbonate), 723 mg.

³ Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,003 IU; vitamin D₃ (cholecalciferol), 3004 IU; vitamin E (DL- α -tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B₁₂, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine-HCl), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin-HCl), 3.00 mg.

⁴ Contains 750,000 mg/kg of choline.

⁵ Contains 50.7% L-Lys.

⁶ TSAA = total sulfur amino acids.

Table 4.3. Composition of corn-soybean meal diets with increasing standard ileal digestible (sid) lysine by increasing supplemental lysine in Experiment 2, as fed basis¹

Ingredient, %	sid Lys, %					
	1.000	1.080	1.160	1.240	1.320	1.400
Corn	56.19	56.19	56.19	56.19	56.19	56.19
Soybean meal (47.5%)	32.08	32.08	32.08	32.08	32.08	32.08
Soy oil	4.17	4.17	4.17	4.17	4.17	4.17
Monocalcium	1.56	1.56	1.56	1.56	1.56	1.56
Limestone	1.51	1.51	1.51	1.51	1.51	1.51
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix ²	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mix ³	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride ⁴	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.192	0.253	0.314	0.375	0.436	0.497
Biolys ⁵	---	0.158	0.316	0.473	0.631	0.789
L-Thr	0.011	0.064	0.118	0.171	0.225	0.278
Gly	0.233	0.233	0.233	0.233	0.233	0.233
L-Val	---	---	0.060	0.123	0.187	0.250
L-Arg•HCl	---	---	---	0.075	0.174	0.272
L-Ile	---	---	0.005	0.060	0.114	0.169
L-His•HCl	---	---	---	---	---	0.007
L-Trp	---	---	---	---	---	0.005
Cornstarch	3.000	2.727	2.391	1.926	1.438	0.938

Calculated composition

ME, kcal/kg	3,200	3,199	3,200	3,199	3,199	3,198
CP, %	20.30	20.49	20.73	21.13	21.57	22.03
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00
Nonphytate P,	0.45	0.45	0.45	0.45	0.45	0.45
Lys, %	1.115	1.195	1.275	1.355	1.435	1.515
Met	0.500	0.561	0.622	0.682	0.743	0.804
TSAA ⁶ , %	0.845	0.905	0.966	1.027	1.088	1.149
Thr, %	0.767	0.821	0.875	0.928	0.982	1.035
Trp, %	0.242	0.242	0.243	0.243	0.243	0.248
Leu, %	1.730	1.732	1.733	1.734	1.735	1.736
Gly+Ser, %	2.080	2.080	2.080	2.080	2.080	2.080
Arg, %	1.324	1.325	1.326	1.389	1.472	1.554
Ile, %	0.850	0.851	0.856	0.911	0.965	1.019
Val, %	0.947	0.949	1.008	1.071	1.135	1.198
sid Arg, %	1.212	1.213	1.214	1.277	1.360	1.442
sid Lys, %	1.000	1.080	1.160	1.240	1.320	1.400
sid Ile, %	0.783	0.784	0.789	0.843	0.898	0.952
sid Val, %	0.856	0.857	0.916	0.980	1.043	1.106
sid Met, %	0.475	0.535	0.596	0.657	0.717	0.778

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sid TSAA, %	0.760	0.821	0.882	0.942	1.003	1.064
sid Leu, %	1.598	1.599	1.600	1.601	1.602	1.603
sid Trp, %	0.218	0.218	0.218	0.219	0.219	0.224
sid Thr, %	0.670	0.724	0.777	0.831	0.884	0.938
Arg:Lys	1.212	1.123	1.047	1.030	1.030	1.030
Ile:Lys	0.783	0.726	0.680	0.680	0.680	0.680
TSAA:Lys	0.760	0.760	0.760	0.760	0.760	0.760
Thr:Lys	0.670	0.670	0.670	0.670	0.670	0.670
Trp:Lys	0.218	0.202	0.188	0.176	0.166	0.160
Val:Lys	0.856	0.793	0.790	0.790	0.790	0.790

¹ A basal diet was mixed to contain the minimum of all ingredients.

² Provided per kilogram of diet: copper (copper sulfate·5H₂O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate·7H₂O), 60 mg; manganese (manganese sulfate·H₂O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate·7H₂O), 44 mg; calcium (calcium carbonate), 723 mg.

³ Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,003 IU; vitamin D₃ (cholecalciferol), 3004 IU; vitamin E (DL- α -tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B₁₂, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine·HCl), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin·HCl), 3.00 mg.

⁴ Contains 750,000 mg/kg of choline.

⁵ Contains 50.7% L-Lys.

⁶ TSAA = total sulfur amino acids.

Table 4.4. Composition of corn-soybean meal diets increasing standard ileal digestible (sid) lysine by increasing soybean meal in Experiment 2, as fed basis¹

	sid Lys, %					
Ingredient, %	1.000	1.080	1.160	1.240	1.320	1.400
Corn	59.93	56.47	53.01	49.55	46.09	42.63
Soybean meal (47.5%)	30.69	33.86	37.04	40.21	43.39	46.57
Soy oil	4.37	4.77	5.17	5.57	5.97	6.37
Monocalcium	1.56	1.54	1.52	1.49	1.47	1.45
Limestone	1.52	1.50	1.48	1.47	1.45	1.43
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix ²	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mix ³	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride ⁴	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.197	0.230	0.263	0.297	0.330	0.363
BioLys ⁵	0.060	0.060	0.060	0.060	0.060	0.060
L-Thr	0.024	0.035	0.045	0.056	0.066	0.077
Gly	0.598	0.479	0.359	0.239	0.120	---

Calculated composition

	3,200	3,200	3,200	3,200	3,200	3,200
ME, kcal/kg	20.43	21.54	22.64	23.75	24.86	25.97
CP, %	1.00	1.00	1.00	1.00	1.00	1.00
Ca, %	0.45	0.45	0.45	0.45	0.45	0.45
Nonphytate P,	1.113	1.200	1.287	1.374	1.461	1.548
Lys, %	0.503	0.551	0.599	0.647	0.696	0.744
Met	0.844	0.909	0.974	1.039	1.104	1.170
TSAA ⁶ , %	0.766	0.825	0.884	0.943	1.002	1.061
Thr, %	0.236	0.254	0.273	0.291	0.310	0.328
Leu, %	1.717	1.799	1.881	1.963	2.045	2.127
Gly+Ser, %	2.408	2.408	2.408	2.408	2.408	2.408
Arg, %	1.290	1.388	1.485	1.583	1.681	1.779
Ile, %	0.831	0.890	0.949	1.008	1.067	1.125
Val, %	0.931	0.989	1.048	1.107	1.165	1.224
sid Arg, %	1.180	1.270	1.361	1.451	1.541	1.632
sid Lys, %	1.000	1.080	1.160	1.240	1.320	1.400
sid Ile, %	0.764	0.820	0.875	0.930	0.985	1.041
sid Val, %	0.840	0.894	0.947	1.001	1.055	1.109
sid Met, %	0.477	0.524	0.571	0.618	0.665	0.712
sid TSAA, %	0.760	0.821	0.882	0.942	1.003	1.064
sid Leu, %	1.585	1.661	1.736	1.811	1.886	1.961
sid Trp, %	0.212	0.229	0.245	0.262	0.279	0.296
sid Thr, %	0.670	0.724	0.777	0.831	0.884	0.938
Arg:Lys	1.180	1.176	1.173	1.170	1.168	1.165
Ile:Lys	0.764	0.759	0.754	0.750	0.747	0.743
TSAA:Lys	0.760	0.760	0.760	0.760	0.760	0.760

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Thr:Lys	0.670	0.670	0.670	0.670	0.670	0.670
Trp:Lys	0.212	0.212	0.212	0.211	0.211	0.211
Val:Lys	0.840	0.828	0.817	0.807	0.799	0.792

¹ A basal diet was mixed to contain the minimum of all ingredients.

² Provided per kilogram of diet: copper (copper sulfate·5H₂O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate·7H₂O), 60 mg; manganese (manganese sulfate·H₂O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate·7H₂O), 44 mg; calcium (calcium carbonate), 723 mg.

³ Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,003 IU; vitamin D₃ (cholecalciferol), 3004 IU; vitamin E (DL- α -tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B₁₂, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine·HCl), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin·HCl), 3.00 mg.

⁴ Contains 750,000 mg/kg of choline.

⁵ Contains 50.7% L-Lys.

⁶ TSAA = total sulfur amino acids.

Table 4.5. Composition of corn-soybean meal diets with and without supplemental DL-methionine and lysine in Experiment 3, as-fed basis¹

Ingredient	-Lys -Met	+Lys -Met	-Lys +Met	+Lys +Met
Corn	56.19	56.19	56.19	56.19
Soybean meal (47.5%)	32.08	32.08	32.08	32.08
Soy oil	4.17	4.17	4.17	4.17
Monocalcium phosphate	1.56	1.56	1.56	1.56
Limestone	1.51	1.51	1.51	1.51
Salt	0.50	0.50	0.50	0.50
Mineral mix ²	0.25	0.25	0.25	0.25
Vitamin mix ³	0.25	0.25	0.25	0.25
Choline chloride ⁴	0.05	0.05	0.05	0.05
DL-Met	---	---	0.314	0.314
Biolys ⁵	---	0.316	---	0.316
L-Thr	0.118	0.118	0.118	0.118
Gly	0.233	0.233	0.233	0.233
L-Val	0.060	0.060	0.060	0.060
L-Ile	0.005	0.005	0.005	0.005
Cornstarch	3.021	2.705	2.707	2.391
Calculated composition				
ME, kcal/kg	3,176	3,193	3,187	3,200
CP, %	20.31	21.25	21.52	21.73
Ca, %	1.00	1.00	1.00	1.00
Nonphytate P, %	0.45	0.45	0.45	0.45
Lys, %	1.115	1.275	1.115	1.275
Met, %	0.311	0.311	0.621	0.622
TSAA ⁶ , %	0.654	0.654	0.965	0.965
Thr, %	0.874	0.875	0.874	0.875
Trp, %	0.243	0.243	0.243	0.243
Leu, %	1.731	1.733	1.731	1.733
Gly+Ser, %	2.080	2.080	2.080	2.080
Arg, %	1.324	1.326	1.324	1.326
Ile, %	0.855	0.856	0.855	0.856
Val, %	1.006	1.008	1.006	1.008
sid Arg, %	1.212	1.214	1.212	1.214
sid Lys, %	1.000	1.160	1.000	1.160
sid Ile, %	0.789	0.789	0.788	0.789
sid Val, %	0.914	0.916	0.914	0.916
sid Met, %	0.285	0.285	0.595	0.596
sid TSAA, %	0.570	0.571	0.881	0.882
sid Leu, %	1.600	1.600	1.598	1.600
sid Trp, %	0.218	0.218	0.218	0.218
sid Thr, %	0.776	0.777	0.776	0.777

(continued on the next page)

¹ A basal diet was mixed to contain the minimum of all ingredients except supplemental Lys and DL-Met were added as needed for each diet.

² Provided per kilogram of diet: copper (copper sulfate·5H₂O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate·7H₂O), 60 mg; manganese (manganese sulfate·H₂O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate·7H₂O), 44 mg; calcium (calcium carbonate), 723 mg.

³ Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,003 IU; vitamin D₃ (cholecalciferol), 3004 IU; vitamin E (DL-α-tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B₁₂, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine·HCl), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin·HCl), 3.00 mg.

⁴ Contains 750,000 mg/kg of choline.

⁵ Contains 50.7% L-Lys.

⁶ TSAA = total sulfur amino acids.

had decreased SUN concentrations ($P < 0.06$) compared with broilers fed increasing total Lys from increasing supplemental Lys. A Lys requirement estimate could not be made by SUA or SUN for either source of Lys.

In Exp. 2, (Table 4.8) ADG and GF increased (linear and quadratic, $P < 0.01$) by increasing sid Lys from supplemental Lys and SBM. Average daily feed intake increased (linear and quadratic, $P < 0.03$) by increasing sid Lys from SBM. A one-slope broken-line analysis (Table 4.7) of GF estimated a sid Lys requirement of 1.17% ($P < 0.01$) when supplemental Lys was the main source of Lys. However, GF continued to increase as the Lys level increased regardless of the source of Lys. An estimate of the sid Lys requirement could not be made by ADG or ADFI for either source of Lys. There was a quadratic increase ($P < 0.06$) in SUA by increasing sid Lys from SBM (Table 4.8). There was a linear increase ($P < 0.08$) in SUN by increasing sid Lys from increasing supplemental Lys. Broilers fed diets increasing in sid Lys by increasing supplemental Lys had decreased SUN concentrations ($P < 0.03$) compared with broilers fed diets increasing in sid Lys by increasing SBM. A Lys requirement estimate could not be made by SUA or SUN for either source of Lys.

In Exp. 3, (Table 4.9) broilers fed supplemental Met had increased ADG, ADFI, and GF ($P < 0.01$) compared with broilers fed diets without supplemental Met. Broilers fed supplemental Lys had increased GF ($P < 0.03$). Serum UA (Table 4.10) was not affected by the addition of supplemental Lys or Met. However, SUN was decreased in broilers fed diets containing supplemental Met ($P < 0.07$), and was lowest when Lys and Met were added together ($P < 0.07$). Uric acid content of the excreta (Table 4.10) was decreased by the addition of supplemental Met ($P < 0.01$).

Table 4.6. Growth performance and blood nitrogen data of broiler chicks fed increasing levels of total lysine in Experiment 1^{1,2}

Treatment	ADG, g	ADFI, g	GF, g/g	SUA ³ , mg/dL	SUN ⁴ , mg/dL
Supplemental Lys					
1) 1.150% Lys	29.64	39.68	0.75	3.50	1.39
2) 1.229% Lys	29.87	39.24	0.76	3.77	1.49
3) 1.308% Lys	31.68	40.09	0.79	3.83	1.48
4) 1.387% Lys	30.24	38.04	0.80	4.78	1.52
5) 1.466% Lys	31.45	38.53	0.82	4.15	1.81
6) 1.545% Lys	30.60	38.11	0.80	4.12	1.88
Increasing SBM					
7) 1.154% Lys	29.22	38.20	0.77	3.51	1.14
8) 1.229% Lys	30.05	38.32	0.78	4.77	1.31
9) 1.304% Lys	29.68	36.59	0.81	4.22	1.40
10) 1.379% Lys	31.48	38.50	0.82	4.67	1.51
11) 1.454% Lys	31.68	38.22	0.83	5.83	1.68
12) 1.529% Lys	32.42	38.85	0.83	5.49	1.62
SEM	0.71	0.90	0.01	0.54	0.14
P-value	0.03	0.52	0.01	0.05	0.02
Source	0.67	0.11	0.01	0.02	0.06
Supp. Lys linear	0.17	0.11	0.01	0.25	0.01
Supp. Lys quadratic	0.23	0.87	0.02	0.40	0.43
SBM linear	0.01	0.52	0.01	0.01	0.01
SBM quadratic	0.79	0.32	0.08	0.82	0.54
Level (both sources)	0.02	0.97	0.01	0.07	0.01
Source × level	0.15	0.24	0.90	0.47	0.95

¹ Data are means of 8 replications with 6 broilers per replicate.

² Blood nitrogen data are means of 8 replications of pooled serum from 3 broilers per replicate.

² SUA= serum uric acid.

³ SUN= serum urea N.

Table 4.7. Broken-line estimates of the total lysine requirements in Experiments 1 and 2

Response	One-slope estimate	Two-slope estimate
Supplemental Lys		
Experiment 1		
GF	1.42 ^a	1.45 ^a
Experiment 2		
GF	---	1.30 ^a
SBM		
Experiment 1		
ADG	---	1.27 ^a

^a Model was significant $P < 0.07$.

Table 4.8. Growth performance and blood nitrogen data of broiler chicks fed increasing levels of standard ileal digestible (sid) lysine in Experiment 2^{1,2}

Treatment	ADG, g	ADFI, g	GF, g/g	SUA ³ , mg/dL	SUN ⁴ , mg/dL
Supplemental Lys					
1) 1.000% sid Lys	27.59	38.61	0.71	3.55	1.33
2) 1.080% sid Lys	28.23	38.04	0.74	4.12	1.32
3) 1.160% sid Lys	30.64	39.62	0.77	3.93	1.21
4) 1.240% sid Lys	29.67	38.09	0.78	3.47	1.48
5) 1.320% sid Lys	30.57	38.91	0.79	4.34	1.38
6) 1.400% sid Lys	29.86	37.43	0.80	4.03	1.66
Increasing SBM					
7) 1.000% sid Lys	28.18	38.61	0.73	3.25	1.52
8) 1.080% sid Lys	29.75	38.42	0.77	5.17	1.39
9) 1.160% sid Lys	31.12	39.46	0.79	4.70	1.56
10) 1.240% sid Lys	31.89	38.89	0.82	4.64	1.66
11) 1.320% sid Lys	30.13	36.70	0.82	4.48	1.59
12) 1.400% sid Lys	30.60	36.43	0.84	4.18	1.73
SEM	0.51	0.65	0.01	0.55	0.14
P-value	0.01	0.01	0.01	0.41	0.18
Source	0.01	0.33	0.01	0.12	0.03
Supp. Lys linear	0.01	0.37	0.01	0.57	0.08
Supp. Lys quadratic	0.01	0.21	0.01	0.97	0.24
SBM linear	0.01	0.01	0.01	0.59	0.12
SBM quadratic	0.01	0.03	0.01	0.06	0.77
Level (both sources)	0.01	0.44	0.01	0.30	0.13
Source × level	0.92	0.70	0.10	0.72	0.92

¹ Data are means of 8 replications with 6 broilers per replicate.

² Blood nitrogen data are means of 6 replications of pooled serum from 3 broilers per replicate.

³ SUA=serum uric acid.

⁴ SUN= serum urea N.

Table 4.9. Growth performance of broiler chicks fed supplemental DL-methionine and lysine in Experiment 3¹

Treatment	ADG, g	ADFI, g	GF, g/g
1) - Lys - DL-Met	23.55	33.18	0.71
2) + Lys	23.99	33.49	0.72
3) + DL-Met	28.22	36.96	0.76
4) + Lys + DL-Met	29.18	37.01	0.79
SEM	0.69	0.98	0.01
P-value	0.01	0.01	0.01
Lys	0.32	0.86	0.03
Met	0.01	0.01	0.01
Lys × Met	0.71	0.90	0.26

¹ Data are means of 6 replications with 6 broilers per replicate.

Table 4.10. Blood nitrogen data and uric acid content of the excreta of broiler chicks fed supplemental DL-methionine and lysine in Experiment 3¹

Treatment	SUA ² , mg/dL	SUN ³ , mg/dL	UAE ⁴ , mg/g ⁵
1) - Lys - DL-Met	3.61	2.91	390.51
2) + Lys	3.87	3.13	390.59
3) + DL-Met	3.56	2.91	332.77
4) + Lys + DL-Met	3.24	2.57	321.34
SEM	0.38	0.15	13.90
P-value	0.70	0.08	0.01
Lys	0.95	0.69	0.69
Met	0.39	0.07	0.01
Lys × Met	0.46	0.07	0.68

¹ Data are means of 6 replications of pooled serum of 3 broilers bled per replicate

² SUA= serum uric acid.

³ SUN= serum urea N.

⁴ UAE= uric acid of the excreta.

⁵ Dry matter basis.

DISCUSSION

The results of the Lys requirement experiments indicate requirements of 1.27% total Lys for diets formulated with the main source of Lys as SBM. When supplemental Lys is the main source of Lys, the estimated Lys requirements are 1.30%, 1.42%, and 1.45% total Lys. These results are close to or lower than the recommended Lys requirement of 1.43% total Lys as advised by Aviagen (2007) for Ross × Ross 708 broilers during the starter period. This could be due to the length of the starter period. Aviagen suggests a starter period of 0- to 10- d and the broilers used in these experiments were raised to 17 d. Comparing the starter and grower requirements recommended by Aviagen which is 1.24% total Lys, the results for these experiments indicate the Lys requirement to be between the two phases. The Lys requirement in SBM and supplemental Lys diets is similar indicating that there is no difference in the Lys requirement of broilers fed SBM or supplemental Lys as the main source of Lys.

Serum UA and SUN concentrations increased linearly when SBM was increased as the main source of Lys in Exp 1. This is thought to be due to the excess of AA from the high levels of SBM. Serum urea N increased linearly in Exp. 1 and 2 when supplemental Lys was the main source of Lys. This effect is thought to be due to the increasing availability of Lys from the supplemental source.

An estimate of the Lys requirement was not able to be determined using SUA as a response variable. Experiment 3 showed no response of SUA to supplemental Lys. Although Miles and Featherston (1969) were able to show a decrease in PUA and UAE as Lys increased, we were unable to show these same responses to Lys.

The results of these experiments indicate the Lys requirement for female Ross × Ross 708 broilers to be 1.27% total Lys for diets formulated with the main source of Lys

as SBM and between 1.30 and 1.45% total Lys when the main source of Lys is supplemental Lys. Also, SUA could not be used as a response variable to determine the Lys requirement. This was confirmed by the results of Exp. 3, where supplemental Lys had no effect on SUA concentrations.

CHAPTER 5

SUMMARY AND CONCLUSIONS

This research was conducted to determine if SUA and UAE could be used as indicators of AA utilization, and to use SUA as a response variable to determine the Lys requirement of broilers.

Five experiments were conducted to determine if SUA and UAE could be used as indicators of AA utilization in diets for broilers. Various fasting and refeeding schedules were examined and it was determined that after a 2 h fast, SUA could be used as an indicator of AA utilization. It was also determined that UAE can also be used to determine AA utilization.

Using what was observed in the first set of experiments, the Lys requirement of female broilers using SUA as a response variable was examined. It was determined that SUA was not a suitable response variable to estimate the Lys requirement of broilers. This was confirmed in a final experiment where Lys and Met were supplemented in the diet and no effect of supplemental Lys was observed on SUA concentrations.

In conclusion, SUA and UAE can be used to indicate AA adequacy or deficiency of a diet; however, SUA cannot be used to estimate the Lys requirement. Also, the optimal time for blood collection for analyzing SUA as a means to evaluate AA utilization is after a 2 h fast.

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APPENDIX:
LIST OF ABBREVIATIONS

Item	Abbreviation
Amino acid (s)	AA
Average daily feed intake	ADFI
Average daily gain	ADG
Corn	C
Crude protein	CP
Blood urea nitrogen	BUN
Blood uric acid	BUA
Experiment (s)	Exp
Feed conversion ratio	FCR
Gain:feed	GF
Metabolizable energy	ME
Nitrogen	N
Plasma urea nitrogen	PUN
Plasma uric acid	PUA
Serum ammonia	SA
Serum urea nitrogen	SUN
Serum uric acid	SUA
Soybean meal	SBM
Standard ileal digestibility	sid
Total sulfur amino acids	TSAA
Uric acid	UA
Uric acid of the excreta	UAE

VITA

Amanda L. Donsbough was born in August, 1984, in Meriden, Connecticut. Amanda lived in Meriden throughout high school. She graduated from Lyman Hall High School in June 2002 and moved to Louisiana to obtain her undergraduate degree. Amanda received her Bachelor of Science degree in May 2006 from Louisiana State University in animal sciences. Currently, Amanda is a candidate for her Master of Science degree in animal sciences.