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Amino Acid-Supplemented Diets and Amino Acid Requirements in Corn and Soybean Meal Diets for Broilers and Pigs

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AMINO ACID-SUPPLEMENTED DIETS AND AMINO ACID REQUIREMENTS IN CORN
AND SOYBEAN MEAL DIETS FOR BROILERS AND PIGS

A Dissertation

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
Doctor of Philosophy

in

The Interdepartmental Program in the School of Animal Sciences

by
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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	viii
ABSTRACT	x
CHAPTER	
1 INTRODUCTION	1
References	2
2 REVIEW OF LITERATURE	3
Introduction	3
Low Crude Protein, Amino Acid Supplemented Diets for Broilers.....	3
Roles of Glycine	4
Low Crude Protein, Amino Acid Supplemented Diets for Pigs.....	7
References	8
3 THE INTERACTIVE EFFECTS OF GLYCINE, CREATINE, AND FISHMEAL SUPPLEMENTATION IN CORN-SOYBEAN MEAL DIETS ON GROWTH PERFORMANCE OF BROILER CHICKS	14
Introduction	14
Materials and Methods	15
Results	20
Discussion	25
References	28
4 EFFECT OF GLYCINE, LYSINE, AND ANTIBIOTIC SUPPLEMENTATION IN CORN-SOYBEAN MEAL DIETS ON GROWTH PERFORMANCE, ORGAN RESPONSES, AND SERUM URIC ACID IN BROILER CHICKS.....	31
Introduction	31
Materials and Methods	33
Results	40
Discussion	45
References	50
5 EFFECT OF SUPPLEMENTAL LYSINE AND GLYCINE IN REDUCED CRUDE PROTEIN, CORN-SOYBEAN MEAL DIETS FOR BROILERS DURING THE STARTER PHASE AND FEEDING A COMMON DIET DURING THE SUBSEQUENT GROWER PHASE.....	53
Introduction	53
Materials and Methods	54
Results	58
Discussion	62
References	64

6	VALINE AND ISOLEUCINE REQUIREMENT OF TWENTY- TO FORTY- FIVE-KILOGRAM PIGS	66
	Introduction	66
	Materials and Methods	70
	Results	73
	Discussion	76
	References	83
7	SUMMARY AND CONCLUSIONS	87
	APPENDIX: LIST OF ABBREVIATIONS	89
	VITA	90

LIST OF TABLES

Table	
3.1	Percentage composition of corn-soybean meal diets fed to 0- to 12- or 18d broilers in experiments 1, 2, and 3, as-fed basis 17
3.2	The effects of supplemental Gly, creatine, or Menhaden fishmeal in corn-soybean meal (C-SBM) diets on growth performance of 0- to 18-d broilers in experiment 1 20
3.3	The additive effects of supplemental Gly, creatine, or Menhaden fishmeal in corn-soybean meal diets on growth performance of 0- to 18-d broilers in experiment 2 22
3.4	The effects of supplemental Gly, creatine, guanidino acetic acid (GAA), or Menhaden fishmeal in corn-soybean meal (C-SBM) diets on growth performance and serum metabolites of 0- to 12-d broilers in experiment 3..... 22
4.1	Percentage composition of corn-soybean meal diets fed to broilers d 0 to 14 posthatching in experiment 1, as-fed basis 35
4.2	Percentage composition of corn-soybean meal diets fed to broilers d 0 to 18 posthatching in experiment 2, as-fed basis 37
4.3	The effects on growth performance, serum uric acid, and organ weights of male and female broiler chicks fed diets without and with supplemental Lys and Gly in experiment 1 42
4.4	The effects on growth performance and organ weights of male broiler chicks fed diets with 2 levels of Lys (1.26 and 1.40%) without and with supplemental Gly and antibiotics in experiment 2..... 46
5.1	Percentage composition of corn-soybean meal diets for starter (0- to 10-d) and grower phase (10- to 24-d) broilers, as-fed basis 56
5.2	Calculated and analyzed percentage of Gly+Ser in starter (0- to 10-d) phase diets fed to Ross 708 broilers 58
5.3	Growth performance of d 0 to 10 (Phase 1) broiler chicks fed supplemental Lys without and with supplemental Gly in corn-soybean meal based diets..... 60
5.4	Subsequent growth performance of d 10 to 24 (Phase 2) and d 0 to 24 (overall) broiler chicks fed a common corn-soybean meal (C-SBM) diet that were previously fed diets with supplemental Lys without and with supplemental Gly..... 61
6.1	Isoleucine requirement estimates of growing pigs 67
6.2	Valine requirement estimates of growing pigs 69

6.3	Composition of negative control diet that was fed to 20- to 45-kg grower pigs in experiments 1, 2, and 3, as-fed basis	74
6.4	Valine and isoleucine composition of diets in experiments 1, 2, and 3	75
6.5	The effects of increasing standardized ileal digestible (SID) valine on growth performance and plasma urea N (PUN) of 20- to 45-kg pigs from experiment 1	77
6.6	The effects of increasing standardized ileal digestible (SID) isoleucine on growth performance and plasma urea N (PUN) of 22- to 44-kg pigs	78

LIST OF FIGURES

Figure

2.1	Guanidinoacetate (GAA) is formed in the kidney from Arg and Gly via the enzyme L-Arg:Gly amidinotransferase. Creatine is synthesized in the liver by methylation of GAA using S-adenosyl-methionine (AdoMet) as the methyl donor via the enzyme guanidinoacetate methyltransferase. Glycine is completely incorporated into the backbone of creatine. (Adapted and modified from Brosnan and Brosnan, 2007).....	5
2.2	Glycine is used in the formation of uric acid to facilitate N excretion in the excreta of broilers. The box represents the 2 carbons and 1 N that originate from Gly to synthesize uric acid. (Adapted and modified from Stevens, 1996).....	6
3.1	The interactive effects of supplemental Gly and creatine (Cre) in corn-soybean meal diets fed to 0- to 18-d posthatch broilers. (A) Creatine supplementation increased ADG ($P < 0.05$). (B) Glycine supplementation increased G:F ($P < 0.08$).....	23
3.2	The interactive effects of supplemental Gly and fishmeal (FM) in corn-soybean meal diets fed to 0- to 18-d posthatch broilers. (A) Fishmeal addition increased ADG ($P < 0.01$). (B) Fishmeal addition increased ADFI ($P < 0.02$). (C) Glycine supplementation increased ($P < 0.05$) G:F more in broilers fed the C diet than in those fed the diet with fishmeal.....	24
3.3	The interactive effects of supplemental creatine (Cre) and fishmeal (FM) in corn-soybean meal diets fed to 0- to 18-d posthatch broilers. Fishmeal addition to the diet increased ($P < 0.02$) ADG (A) and ADFI (B)	25
4.1	The effects on serum uric acid of feeding male and female broilers diets without and with supplemental Lys and Gly in experiment 1 (Treatment \times sex, $P = 0.051$).....	41
4.2	The effects on liver weight as a percentage of BW of feeding male and female broilers diets without and with supplemental Lys and Gly in experiment 1 (Treatment \times sex, $P < 0.073$).....	42
4.3	The effects on proventricular weight (as a percentage of BW) of feeding broilers diets with 2 levels of Lys (1.26 and 1.40%) without and with supplemental Gly and antibiotics in experiment 2 (Lys \times Gly \times antibiotic, $P < 0.05$).....	45
5.1	Subsequent effects of feeding increasing levels of supplemental Lys without and with supplemental Gly during Phase 1 (d 0 to 10) on average daily feed intake of Phase 2 (d 10 to 24) broilers (Gly \times Lys, $P < 0.01$)	62
6.1	Treatment means (●) are shown for Exp. 1. (A) Fitted broken-line of overall (d0 to 26) ADG as a function of standardized ileal digestible (SID) Val in the diet. Using broken-line analysis on treatment means and pen mean values (n = 6 observations per treatment mean), the SID Val	

	requirements (▲) were 0.58 ($P < 0.03$) and 0.58 ($P < 0.08$). (B) Fitted broken-line of overall (d0 to 26) G:F as a function of SID Val in the diet. Using broken-line analysis on treatment means and pen mean values (n = 6 observations per treatment mean), the SID Val requirements were 0.56 ($P = 0.03$) and 0.56 ($P = 0.63$).....	79
6.2	Standardized ileal digestible (SID) Val: SID Lys estimates by mean BW. (A) The linear regression equation including all 9 data points is $Y = 0.34140x + 60.083558$ ($R^2 = 0.414$; $P < 0.06$). (B) The linear regression equation with removal of the low and high outlier points is $Y = -0.23264x + 70.99814$ ($R^2 = 0.261$; $P < 0.25$)	81
6.3	Standardized ileal digestible (SID) Ile: SID Lys estimates by mean BW. The linear regression equation is $Y = -0.01795x + 56.54727$ ($R^2 = 0.01$; $P < 0.68$).....	82

ABSTRACT

The objectives of this research were to: 1) evaluate the role of Gly in low CP, AA supplemented diets for broilers, 2) determine subsequent effects of supplementing Gly in broiler diets, and 3) determine the Val and Ile requirement in an AA supplemented, C-SBM diet for 20- to 45-kg pigs. Three experiments were conducted to evaluate supplementation of Gly, creatine, GAA, and fishmeal in C-SBM diets on growth performance of broiler chicks and to determine if Gly, creatine, or GAA could be added to a C-SBM diet in place of fishmeal to produce similar growth performance. The results from these experiments indicate that supplemental Gly, creatine, or GAA can be supplemented in a C-SBM diet in replacement of fishmeal and produce similar growth performance to broilers fed a C-SBM, fishmeal diet. Two experiments were conducted to determine the effects of supplementing Lys and Gly and effects of feeding 2 dietary Lys levels without and with supplemental Gly and antibiotics on growth performance, serum uric acid, and intestinal and organ responses of broilers. Results from these experiments indicate that increasing dietary Lys in diets for broilers increases growth performance and supplementation of Gly in low CP, AA supplemented diets increases feed efficiency. Thus, this improvement in G:F coincides with an increase in serum uric acid and occurs in diets that are high (1.40%) or low (1.26%) in dietary Lys. The increase in G:F is not similar to the feed efficiency response seen when antibiotics are supplemented in broiler diets. An experiment was conducted to determine the effect of feeding increasing levels of supplemental Lys without and with supplemental Gly from 0- to 10-d and the subsequent effects of feeding these diets from 10- to 24-d. Results from these experiments indicate that the inclusion of up to 0.15% supplemental Lys can be added in a C-SBM diet without or with supplemental Gly without reducing growth performance of 0- to 10-d old broilers. These results also indicated that providing Gly in the starter phase (0- to 10-d) increased G:F during the subsequent grower phase depending on the supplemental Lys level that was provided in the starter phase. Three experiments were conducted to determine the Val and

Ile requirement in an AA supplemented, C-SBM diet for 20- to 45-kg pigs. The Val and Ile requirement for 20- to 45-kg pigs in an AA-supplemented, C-SBM diet is 0.56 to 0.58% SID Val (0.67 to 0.70 SID Val:Lys) and 0.43% SID Ile (0.52 SID Ile:Lys).

CHAPTER 1

INTRODUCTION

Continual fluctuation in ingredient costs for swine and poultry diets necessitates the development of different diet formulation options to have the most cost effective production. Maximizing supplemental AA use is one option to improve cost effective production for broilers and pigs. Reducing the intact protein sources in the diet and increasing crystalline or supplemental AA inclusion results in a reduction of dietary CP levels and an improvement in overall N utilization by the animals. This effect ultimately results in a reduction in the amount of N excreted in waste, which is a direct reduction in N loss into the environment. However, feeding these low CP, AA supplemented diets to broilers and pigs may result in decreased growth performance.

Previous attempts to correct the negative growth effects of broilers fed low CP diets have been relatively unsuccessful, especially beyond a 2% decrease in CP. However, researchers have reported that broilers fed low CP, AA supplemented diets had growth performance equal to those fed a conventional C-SBM diet and that the limiting factor in these low CP diets was Gly (Corzo et al., 2005; Dean et. al., 2006). Yet, the improvement in growth performance with supplemental Gly is not fully understood.

Research to determine the optimal dietary concentration of supplemental AA that can be added to diets for growing pigs with no negative effect on growth production is of great interest to the swine industry. A better understanding of the AA requirements beyond Lys, Thr, Trp, and Met and maximum inclusion rates of supplemental AA that can be used in diets for growing pigs will provide nutritionists more flexibility in least cost formulation. Also, the increased digestibility of AA supplemented diets will allow for more precise nutrition.

Therefore, the objectives of this dissertation were to: 1) evaluate the role of Gly in low CP, AA supplemented diets for broilers, 2) determine subsequent effects of supplementing Gly in

broiler diets, and 3) determine the Val and Ile requirement in an AA supplemented, C-SBM diet for 20- to 45-kg pigs.

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CHAPTER 2

REVIEW OF LITERATURE

INTRODUCTION

Increasing ingredient costs for swine and poultry diets results in the need to address different options for diet formulations to achieve the most cost effective production. Reducing intact protein sources and replacing them with increasing supplemental AA in swine and poultry diets decreases dietary CP levels as well as decreases N excretion (Moran et al., 1992; Deschepper and De Groote, 1995; Ferguson et al., 1998a,b; Bregendahl et al., 2002; Si et al., 2004; Corzo et al., 2005). Maximizing supplemental AA inclusion may make swine and poultry diets more cost effective. Methionine and Lys are two of the major AA that are supplemented in swine and poultry diets mostly due to the high production demand in the commercial markets; however, Thr and Trp are also used in the animal feed industry, but their volume of use is not as high as Met and Lys (Malveda et al., 2006). Commercial production of Val increased in 2009; however, economic feasibility of supplementing Val in swine and poultry diets is not to the level of Met, Lys, Thr, or Trp.

LOW CRUDE PROTEIN, AMINO ACID SUPPLEMENTED DIETS FOR BROILERS

While reducing intact protein sources and increasing AA supplementation in the diet may be economically feasible, there are some limitations in order to maintain growth performance. Some researchers have reported that 0- to 21-d broilers fed low CP, AA supplemented diets failed to support equal performance of broilers fed a standard or higher CP diet (Edmonds et al., 1985; Fancher and Jensen, 1989; Pinchasov et al., 1990; Ferguson et al., 1998a,b; Bregendahl et al., 2002). Many researchers have conducted research to attempt to correct the negative growth effects associated with feeding broilers low CP diets (see Aftab et al. [2006] for a comprehensive review).

Previous attempts to correct the negative growth effects of broilers fed low CP diets have been relatively unsuccessful, especially beyond a 2% decrease in CP; however, Gly supplementation in low CP diets for 0- to 21-d broilers has primarily yielded positive growth responses especially in terms of feed efficiency (Parr and Summers, 1991; Heger and Pack, 1996; Corzo et al., 2005; Dean et al., 2006; Waguespack et al., 2009). However, Sohail et al. (2003) reported that there was no effect on G:F of broilers fed 0.05 or 0.1% supplemental Gly in a 20.8% CP diet. Si et al. (2004) and Waldroup et al. (2005) reported that supplementing Gly in low CP diets improved broiler performance, but growth was not fully restored to the level of the broilers fed a conventional 22 or 24% CP diet. On the other hand, Corzo et al. (2005) reported that broilers fed an 18% CP diet with supplemental Gly or Leu had G:F equal to that of broilers fed a control diet. Lehman et al. (2009) supplemented 2% gelatin, which contains Gly, Ser, and Pro, in low CP diets for 0 to 3 wk old broilers and reported an increase in G:F by 8.7% compared with broilers fed a low CP diet without gelatin supplementation. Also, Dean et al. (2006) reported that broilers fed low CP, AA supplemented diets had growth performance equal to those fed a conventional C-SBM diet and that the limiting factor in these low CP diets was Gly. Within our lab, we also have shown that up to 0.2% L-Lys (resulting in greater than a 3% reduction in dietary CP) can be added to all vegetable broiler diets with no negative effects on growth performance as long as supplemental Met, Thr, and Gly are provided in the diet (Waguespack et al., 2009).

ROLES OF GLYCINE

Glycine is considered a conditionally essential AA for chicks because they are able to synthesize only 70% of Gly required for optimal growth (Graber and Baker, 1973) and the remaining 30% is supposed to be obtained from the diet. Recently, we have shown that Gly supplementation to seemingly nutritionally adequate, all vegetable C-SBM diets increases G:F of

broilers (Dean et al., 2006; Waguespack et al., 2008, 2009). The response to Gly also was observed in diets deficient in AA (Donsbough et al., 2010). These data indicate that the response to Gly is beyond its role as a component of protein.

Creatine Synthesis

Animal protein sources such as meat and bone meal or fishmeal contain Gly in addition to other AA and nutrients. Animal protein sources are routinely added to poultry diets because they result in an increase in growth performance and an improvement in carcass traits. Glycine is necessary in the metabolic pathway for creatine synthesis. Creatine is mainly synthesized in the liver from guanidino acetic acid, which is transported to the liver after it is synthesized in the kidney from Arg and Gly (Wyss and Kaddurah-Daouk, 2000; Figure 2.1). Thus, it is possible that the responses observed from Gly may be as a precursor for creatine synthesis, and that Gly may be able to replace the use of animal protein sources in situations where all vegetable diets are preferred.

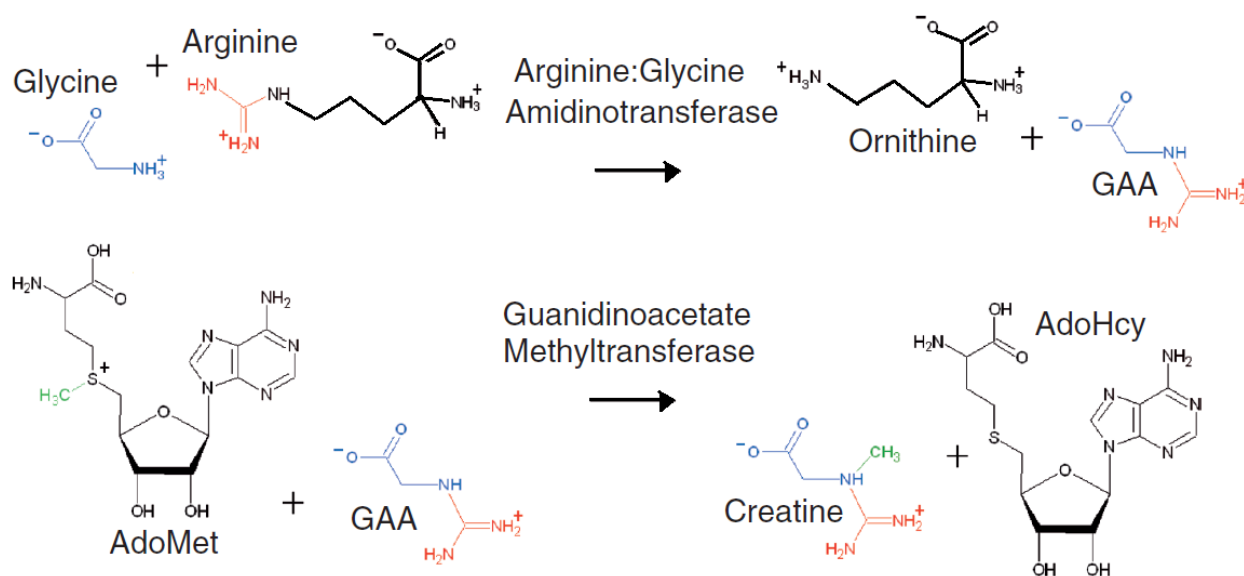


Figure 2.1. Guanidinoacetate (GAA) is formed in the kidney from Arg and Gly via the enzyme L-Arg:Gly amidinotransferase. Creatine is synthesized in the liver by methylation of GAA using S-adenosyl-methionine (AdoMet) as the methyl donor via the enzyme guanidinoacetate methyltransferase. Glycine is completely incorporated into the backbone of creatine. (Adapted and modified from Brosnan and Brosnan, 2007).

Uric Acid Synthesis

Glycine is also important for synthesis of purines such as formation of uric acid (Christman and Mosier, 1929), which is the end product of N metabolism in poultry. For every 1 molecule of uric acid produced, one molecule of Gly is needed (Stevens, 1996). Glycine contributes 2 C and 1 N to the synthesis of uric acid (Sonne et al., 1946; Matsuda et al., 1973; Figure 2.2). To remove 4 N atoms (in 1 molecule of uric acid), 15 ATPs are used and of these, 9 ATPs come from Gly synthesis (Stevens, 1996). Thus, it is possible that the increase in G:F when supplementing Gly in broiler diets is due to an improvement in feed utilization because the body is not using energy to produce Gly for uric acid production but rather using it for other metabolic needs.

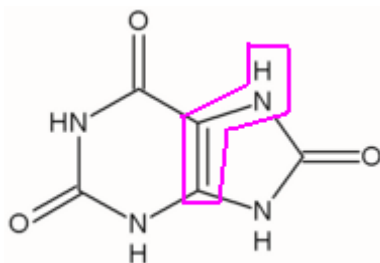


Figure 2.2. Glycine is used in the formation of uric acid to facilitate N excretion in the excreta of broilers. The box represents the 2 carbons and 1 N that originate from Gly to synthesize uric acid. (Adapted and modified from Stevens, 1996).

Antibacterial and Anticoccidial Properties

Glycine has been shown to have antibacterial properties in vitro. Glycine addition to in vitro bacterium solutions was shown to result in cell lysis (Maculla and Cowles, 1948). Minami et al. (2004) conducted in vitro research investigating Gly alone as an antibacterial as well as Gly in combination with amoxicillin in the presence of *Helicobacter pylori*. Their research indicates that Gly could be used to treat patients with *H. pylori*, which is a gram-negative bacterium that is associated with gastric ulcers and cancer in humans. The suggested mode of action that Gly utilizes to inhibit bacterial growth is similar to the mode of action of some antibiotics. For

example, a mode of action of the antibiotic penicillin is to target bacterial cell wall formation. Peptidoglycans are structural components in bacterial cell walls. Glycine has been shown to inhibit the synthesis of peptidoglycan (Hammes et al., 1973). Glycine also has been investigated in combination with anticoccidial vaccines in broiler chicks. Lehman et al. (2009) fed low CP diets supplemented with 2% gelatin, which contains Gly, Ser, and Pro, and indicated that gelatin supplementation helped to alleviate the negative growth response that can occur with coccidiosis live vaccines. Thus, the increase in G:F when supplementing Gly in low CP broiler diets that we have seen within our lab could be due to Gly functioning in an antibacterial or anticoccidial manner.

LOW CRUDE PROTEIN, AMINO ACID SUPPLEMENTED DIETS FOR PIGS

Reducing diet cost has always been a top priority in swine production, but with increasing ingredient cost it is even more important today. Also, environmental concerns with N runoff from excess protein intake of confined livestock have caused an increase in research to decrease N loss to the environment. Amino acid supplementation to diets fed to pigs is one strategy that has been shown to reduce dietary CP and N excretion, and it also can be a cost effective strategy. Research to determine the maximum dietary concentration of the available supplemental AA that can be added to diets for growing pigs that will have no negative effect on growth production is of great interest to the swine industry. Some research has shown a reduction of CP in diets for pigs results in decreased growth performance. To improve performance of pigs fed these diets, AA levels in the diet have to be closely evaluated.

Researchers have focused their efforts primarily on Lys, Thr, Met, and Trp, which are currently the most affordable AA available to swine producers. Roux et al. (2011) determined that a low CP, C-SBM diet with up to 0.23% supplemental Lys along with supplemental Thr, Trp, and Met could be fed to 20- to 45-kg pigs without reducing growth performance. The

opportunity to further reduce CP via increasing supplemental AA in the diet necessitates that the requirement for the limiting AA beyond Lys, Thr, Trp, and Met be known. Valine and Ile are not limiting in a C-SBM diet with no supplemental AA, but they may become limiting in low CP diets (Figuereroa et al., 2003; Lordelo et al., 2008). Valine and Ile may not be economically feasible to add in swine diets currently, but establishing the requirements can help set minimums in the feed matrix, as well as enable nutritionists to use these AA more quickly once they are economically available. There is limited research on the optimal inclusion levels of Val and Ile in low CP, AA supplemented C-SBM diets for 20- to 50-kg pigs. The majority of the research that has been conducted with 20- to 50-kg pigs to estimate the Ile requirement have been fed semi-purified or blood products in the diets (Brinegar et al., 1950; Bravo et al., 1970; Taylor et al., 1985; Lenis and van Diepen, 1997; Parr et al., 2003; and Dean et al., 2005). Research has shown that the Ile requirement for pigs fed a corn and blood product diet is higher than in a C-SBM diet (Fu et al., 2005a,b; Dean et al., 2005; Wiltafsky et al., 2009a; Barea et al., 2009b).

The Val requirement also has been estimated in weanling pigs (James et al., 2001; Mavromichalis et al., 2001; Theil et al., 2004; Kendall et al., 2004; Wiltafsky et al., 2009b; Barea et al., 2009a) and in finishing pigs (Lewis et al., 1995); however, there is limited research estimating the Val requirement in 20-to 50-kg pigs. Wang and Fuller (1989) estimated the SID Val:Lys for 27.5- to 47.5-kg pigs to be 0.75 in a semi-purified diet. Gaines et al. (2011) estimated the SID Val:Lys for 21.4- to 32.6-kg pigs to be 0.65 in a C-SBM diet. Because of the differing estimates (0.75 vs. 0.65), it would be beneficial to clarify conflicting research estimating the Val requirement in 20- to 50-kg pigs.

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CHAPTER 3

THE INTERACTIVE EFFECTS OF GLYCINE, CREATINE, AND FISHMEAL SUPPLEMENTATION IN CORN-SOYBEAN MEAL DIETS ON GROWTH PERFORMANCE OF BROILER CHICKS

INTRODUCTION

Previously within our lab, we have shown that the addition of Gly to C-SBM diets that are otherwise nutritionally adequate results in increased growth rate and increased feed efficiency (Dean et al., 2006; Waguespack et al., 2008). The increase in G:F was much more consistent than the response in ADG. In 26 experiments, G:F was increased with Gly supplementation in 22 experiments, but an increase in ADG was seen in only 20 experiments. Also, there is little to no change in ADFI with Gly supplementation in these 26 experiments.

Because previous research has shown an increased in ADG and G:F in broilers fed supplemental Gly, we are interested in determining why this response is occurring. Fishmeal or other animal protein sources are routinely added to broiler diets because they generally result in increased growth rate and feed efficiency compared with broilers fed a primarily vegetable-based diet (C-SBM). Diets with added fishmeal contain more Gly than a conventional vegetable-based diet. Fishmeal also contains creatine. Because Gly is an intermediate in the synthesis of creatine, it may have a similar response to Gly when supplemented into a vegetable-based diet fed to broilers.

Glycine has many roles in metabolic pathways in the chick. Glycine is used in protein and purine synthesis, formation of uric acid (Christman and Mosier, 1929), and porphyrin structure of heme groups (Shemin and Rittenberg, 1945). Glycine is also a precursor in the synthesis of creatine (Almquist et al., 1941; Bloch and Schoenheimer, 1941). Glycine and Arg are precursors for metabolic intermediates, GAA and ornithine synthesis. Guandino acidic acid is then methylated to form creatine.

The objective of this research was to evaluate growth performance of broilers fed supplemental Gly, creatine, GAA, and/or fishmeal in C-SBM diets, and to evaluate if supplemental Gly, creatine, or GAA can be added to a C-SBM diet in place of fishmeal to produce similar or increased growth performance compared with broilers fed a diet that contains fishmeal.

MATERIALS AND METHODS

General

Three experiments were conducted with male Ross × Ross 708 broilers. On d 0 posthatching, broilers were wing sexed, weighed, wing banded, and randomly allotted to treatments. Broilers were housed in temperature-controlled Petersime brooder batteries (Petersime Incubator Co., Gettysburg, OH) with continuous fluorescent lighting. Each experiment was conducted for 12- or 18-d posthatching. Feed in mash form and water were available on an ad libitum basis. Chicks and feeders were weighed on d 0 and 12 (experiment 3) or 18 (experiments 1 and 2) for the determination of ADG, ADFI, and G:F. All experimental animal use was in compliance with the Louisiana State University Agricultural Center Animal Care and Use Committee.

In all experiments, diets were C-SBM based (Table 3.1). Dietary treatments were formulated on a SID basis using coefficients for corn, soybean meal, and fishmeal from AminoDat[®] 3.0 (Evonik Degussa GmbH, Hanau, Germany). All diets were formulated to contain 1.27% SID Lys, which is based on the SID Lys recommendation for 0- to 10-d Ross 708 broilers (Aviagen, 2007). All C-SBM diets (without fishmeal) were formulated to contain 0.19 (experiment 3) or 0.20% (experiments 1 and 2) supplemental L-Lys, which our lab previously has reported to have no negative effects on growth performance of broilers (Waguespack et al., 2009). All diets that contained fishmeal were formulated to contain 0.15 (experiments 1 and 2) or

0.17% (experiment 3) supplemental L-Lys. All diets contained supplemental Met and Thr to maintain SID TSAA:Lys and SID Thr:Lys of 0.72 and 0.70, respectively, which met or exceeded suggested ratios (Baker, 1997; Dozier et al., 2008). All diets were formulated to contain 3,200 kcal ME per kg of diet, 1.0% Ca, 0.45% nonphytate P, and to meet the requirements of all other nutrients suggested for d 0 to 21 broilers (NRC, 1994).

Experiment 1

A total of 180 male broilers (42 and 672 g, initial and final BW, respectively) were allotted to 5 treatments with 6 replications of 6 broilers per replicate pen to evaluate the effects of supplemental Gly, creatine, and FM on growth performance of broilers. Dietary treatments included: 1) C-SBM control; 2) Diet 1 + 0.36% supplemental Gly; 3) Diet 1 + 0.05% creatine; 4) Diet 1 + 0.11% creatine; and 5) C-SBM + 3% Menhaden fishmeal. Diet 2 contained 0.36% supplemental Gly to achieve a total Gly+Ser of 2.32%, which has been shown to maximize growth performance of broilers fed a low CP, AA supplemented diet (Dean et al., 2006). Diet 3 and 4 contained 0.061 and 0.122% creatine monohydrate to provide 0.05 and 0.11% supplemental creatine, which were similar to levels that Lemme et al. (2007a) supplemented GAA in vegetable-based diets. Diet 5 was supplemented with 3% fishmeal based on an average inclusion level similar to commercial diets.

Experiment 2

A total of 252 male broilers (37 and 655 g, initial and final BW, respectively) were allotted to 7 treatments with 6 replications of 6 broilers per replicate pen to evaluate the additive effects of supplemental Gly, creatine, and fishmeal on growth performance of broilers. Dietary treatments included: 1) C-SBM control; 2) Diet 1 + 0.357% supplemental Gly; 3) Diet 1 + 0.05% creatine; 4) Diet 1 + 0.357% supplemental Gly + 0.05% creatine; 5) C-SBM + 3% Menhaden fishmeal; 6) Diet 5 + 0.252% supplemental Gly; and 7) Diet 5 + 0.05% creatine.

Table 3.1. Percentage composition of corn-soybean meal diets fed to 0- to 12- or 18d broilers in experiments 1, 2, and 3, as-fed basis^{1,2}

Item	Experiment 1		Experiment 2		Experiment 3	
	Control	3% fishmeal	Control	3% fishmeal	Control	3% fishmeal
Ingredient (%)						
Corn	52.949	54.413	52.890	54.042	53.027	55.344
Soybean meal (46.9%)	36.690	33.883	36.695	33.925	36.859	33.007
Soy oil	5.071	4.510	5.065	4.587	5.065	4.385
Fishmeal, Menhaden	----	3.000	----	3.000	----	3.000
Monocalcium phosphate	1.519	1.135	1.519	1.136	1.517	1.141
Limestone	1.446	1.229	1.446	1.228	1.446	1.233
Sodium chloride	0.500	0.500	0.500	0.500	0.500	0.500
Mineral mix ³	0.250	0.250	0.250	0.250	0.250	0.250
Vitamin mix ⁴	0.250	0.250	0.250	0.250	0.250	0.250
Choline chloride ⁵	0.050	0.050	0.050	0.050	0.050	0.050
Biolys ⁶	0.389	0.299	0.389	0.299	0.380	0.340
DL-Met ⁷	0.340	0.313	0.340	0.314	0.338	0.321
L-Thr ⁷	0.186	0.168	0.186	0.168	0.184	0.179
Cornstarch	0.360	----	0.420	0.252	0.135	----
Calculated composition						
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200
CP, %	22.07	22.49	21.98	22.48	22.06	22.20
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, %	1.39	1.40	1.39	1.40	1.39	1.40
Met, %	0.66	0.66	0.66	0.66	0.66	0.67
Met+Cys, %	1.00	1.01	1.00	1.01	1.00	1.01
Thr, %	1.01	1.02	1.01	1.02	1.01	1.02
Val, %	1.00	1.03	1.00	1.03	1.00	1.01
Arg, %	1.46	1.47	1.46	1.47	1.46	1.45
Ile, %	0.91	0.93	0.91	0.93	0.91	0.91
Total Gly+Ser, %	1.96	2.06	1.96	2.07	1.97	2.04
SID Lys ⁸ , %	1.27	1.27	1.27	1.27	1.27	1.27
SID Met, %	0.63	0.63	0.63	0.63	0.63	0.64
SID Met+Cys, %	0.91	0.91	0.91	0.91	0.91	0.91
SID Thr, %	0.89	0.89	0.89	0.89	0.89	0.89
SID Arg, %	1.35	1.36	1.35	1.36	1.36	1.34
SID Ile, %	0.82	0.83	0.82	0.83	0.82	0.82
SID Val, %	0.89	0.91	0.89	0.91	0.89	0.91
SID Leu, %	1.63	1.66	1.63	1.66	1.63	1.64
SID His, %	0.53	0.54	0.53	0.54	0.53	0.53
SID Trp, %	0.24	0.23	0.24	0.23	0.24	0.23
SID Met+Cys:SID Lys	0.72	0.72	0.72	0.72	0.72	0.72
SID Thr:SID Lys	0.70	0.70	0.70	0.70	0.70	0.70

¹ In experiments 1 and 3, a basal diet was mixed for diets 1 to 4 to contain the minimum of all ingredients except for supplemental Gly, creatine monohydrate, guanidino acetic acid (experiment 3) and cornstarch, which were added as needed to each diet. Diet 5, which contained 3% fishmeal, was mixed separately.

(Table 3.1 continued)

² In experiment 2, 2 separate basal diets were mixed for diets 1 to 4 and diets 5 to 7 to contain the minimum of all ingredients except for supplemental Gly, creatine monohydrate, and cornstarch, which were added as needed to each diet.

³ Provided per kilogram of diet: Cu (copper sulfate), 7 mg; I (calcium iodate), 1 mg; Fe (ferrous sulfate), 50 mg; Mn (manganese sulfate), 100 mg; Se (sodium selenite), 0.15 mg; and Zn (zinc sulfate), 75 mg.

⁴ Provided per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg.

⁵ Contains 600,000 mg/kg of choline chloride.

⁶ Biolys was obtained from Evonik Corporation (Kennesaw, GA) and contains 50.7% L-Lys.

⁷ DL-Met and L-Thr were provided by Evonik Corporation (Kennesaw, GA).

⁸ SID = standardized ileal digestible. Digestibility coefficients for corn, soybean meal, and fishmeal were from AminoDat[®] 3.0 (Evonik Degussa GmbH, Hanau, Germany).

Diets 2 and 6 contained 0.357 or 0.252% supplemental Gly, respectively, to achieve a total Gly+Ser of 2.32%, which has been shown to maximize growth performance of broilers fed low CP, AA supplemented diets (Dean et al., 2006). Diets 3 and 7 contained 0.057% creatine monohydrate to provide 0.05% supplemental creatine, which was the level chosen as a result of experiment 1 because of a numerical increase in ADG and ADFI compared with the 0.11% creatine level. Diet 5 was supplemented with 3% fishmeal based on an average inclusion level similar to commercial diets.

Experiment 3

A total of 270 male broilers (38 and 328 g, initial and final BW, respectively) were allotted to 5 treatments with 9 replications of 6 broilers per replicate pen to evaluate if supplemental Gly, GAA, or Cre can be added to a C-SBM diet in place of fishmeal. Dietary treatments included: 1) C-SBM control; 2) C + 0.068% supplemental Gly; 3) C + 0.119% creatine; 4) C + 0.106% GAA; and 5) C-SBM + 3% Menhaden fishmeal. Diet 2 contained 0.068% supplemental Gly to achieve 2.037% total Gly+Ser, which is equal to the total Gly+Ser in diet 5. Diet 3 contained 0.135% creatine monohydrate to provide 0.119% supplemental creatine, which is equimolar to 0.068% supplemental Gly in diet 2. Diet 4 contained 0.106% GAA, which is equimolar to 0.068% Gly in diet 2. Diet 5 was supplemented with 3% fishmeal based on an average inclusion level similar to commercial diets.

On d 12, blood was collected via cardiac puncture from 3 broilers per pen after a 20 minute fast/20 minute re-feeding to ensure a fed-state blood sample (Donsbough et al., 2010). Blood was collected and placed into 10-mL serum tubes (BD Vacutainer, Franklin Lakes, NJ) and samples were placed on ice before centrifugation at $3,000 \times g$ at 0°C for 20 min. After centrifugation, the serum was pooled from 3 broilers into 2 aliquots per pen and frozen until time of analysis. Serum creatinine was analyzed on each pooled sample using a commercial reagent kit (BioAssay

Systems, Hayward, CA). The other aliquot of pooled serum was analyzed for serum uric acid (Fossati et al., 1980) using a commercial reagent kit (Pointe Scientific, Canton, MI).

Statistical Analysis

Data were analyzed by ANOVA as a completely randomized design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Each pen of broilers served as the experimental unit. The PDIFF option of SAS was used to compare individual diets. For experiment 2, the treatments were analyzed as 3 2×2 factorial arrangements (Gly × creatine, Gly × fishmeal, and creatine × fishmeal). Treatment differences were considered significant with an $\alpha = 0.10$.

RESULTS

Experiment 1

Broilers fed the diet with 3% fishmeal had increased ($P < 0.10$) ADG compared with broilers fed the control diet (Table 3.2); however, broilers fed diets with supplemental Gly or either level of creatine had similar ADG to broilers fed the control or 3% fishmeal diet ($P > 0.10$). Daily feed intake was not affected by dietary treatment ($P > 0.10$). Broilers fed the diet supplemented with Gly, either level of creatine, or fishmeal had increased ($P < 0.10$) G:F compared with broilers fed the control diet.

Table 3.2. The effects of supplemental Gly, creatine, or Menhaden fishmeal in corn-soybean meal (C-SBM) diets on growth performance of 0- to 18-d broilers in experiment 1¹

Dietary treatments	ADG, g	ADFI, g	G:F, g/g
1) C-SBM (control)	33.83 ^b	42.49	0.797 ^b
2) Diet 1 + 0.36% supplemental Gly	35.49 ^{ab}	42.10	0.843 ^a
3) Diet 1 + 0.05% creatine	35.07 ^{ab}	42.62	0.823 ^a
4) Diet 1 + 0.11% creatine	34.96 ^{ab}	41.84	0.836 ^a
5) C-SBM with 3% fishmeal	35.75 ^a	43.26	0.827 ^a
SEM	0.749	0.977	0.008
Overall treatment <i>P</i> -value	0.439	0.871	0.008

^{a-b} Means with different superscripts within a column are significantly different ($P < 0.10$).

¹ Data are means of 6 replications with 6 chicks per replicate pen. Average initial and d 18 BW was 42 and 672 g, respectively.

Experiment 2

Table 3.3 contains all 7 treatments; however, the data were also analyzed as 3 2×2 factorials (Gly × creatine, Gly × fishmeal, and creatine × fishmeal), which are presented in Figures 3.1, 3.2, and 3.3, and to which we will focus our results and discussion. In the supplemental Gly × supplemental creatine analysis, broilers fed the diets supplemented with creatine had increased ($P < 0.05$) ADG compared with broilers fed the control or the control + Gly diet (Figure 3.1); however, broilers fed diets with supplemental Gly had increased G:F compared with broilers fed the control or creatine supplemented diet ($P < 0.08$). Daily feed intake was not affected ($P > 0.10$) by dietary treatment in the Gly × creatine analysis. In the supplemental Gly × fishmeal analysis, broilers fed the diets with 3% fishmeal had increased ADG ($P < 0.01$) and ADFI ($P < 0.02$) compared with broilers fed the control or the control + supplemental Gly diet (Figure 3.2); however, broilers fed diets with supplemental Gly had increased G:F ($P < 0.05$) compared with broilers fed the control or 3% fishmeal diet. In the supplemental creatine × fishmeal analysis, broilers fed the diets with 3% fishmeal had increased ADG and ADFI ($P < 0.02$) compared with broilers fed the control or the control + supplemental creatine diet (Figure 3.3). Feed efficiency was not affected ($P > 0.10$) by dietary treatment in the supplemental creatine × fishmeal analysis.

Experiment 3

Daily gain and ADFI were not affected ($P > 0.10$) by dietary treatment (Table 3.4). Broilers fed diets with supplemental creatine or GAA had increased G:F ($P < 0.10$) compared with broilers fed the control diet. However, broilers fed diets with supplemental Gly or 3% fishmeal had similar G:F to broilers fed the control, supplemental creatine, or GAA diet ($P > 0.10$).

Broilers fed the diet with 3% fishmeal had increased serum creatinine compared with all other dietary treatments ($P < 0.10$). Broilers fed the diet with supplemental Gly had increased serum uric acid ($P < 0.10$) compared with all other dietary treatments.

Table 3.3. The additive effects of supplemental Gly, creatine, or Menhaden fishmeal in corn-soybean meal diets on growth performance of 0- to 18-d broilers in experiment 2¹

Treatments	ADG, g	ADFI, g	G:F, g/g
1) Corn-soybean meal (C-SBM) control	32.31	39.91	0.810 ^b
2) Diet 1 + 0.357% supplemental Gly	33.09	38.97	0.852 ^a
3) Diet 1 + 0.05% creatine	33.99	40.60	0.837 ^c
4) Diet 1 + 0.357% supplemental Gly + 0.05% creatine	34.44 ^a	40.81	0.844 ^a
5) C-SBM with 3% fishmeal	35.36 ^{a,b}	42.25 ^{a,b}	0.837 ^c
6) Diet 5 + 0.252% supplemental Gly	35.61 ^{a,b}	41.85 ^b	0.851 ^a
7) Diet 5 + 0.05% creatine	35.56 ^{a,b}	43.08 ^{a,b}	0.836
SEM	0.734	0.952	0.013
Overall treatment P -value	0.016	0.100	0.204

¹ Data are means of 6 replications with 6 chicks per pen. Average initial and d 18 BW was 37 and 655 g, respectively.

^a Significantly different ($P < 0.10$) from the control.

^b Significantly different ($P < 0.04$) from diet 2 (C-SBM + 0.357% supplemental Gly).

^c Tended to be significantly ($P < 0.11$) from the control.

Table 3.4. The effects of supplemental Gly, creatine, guanidino acetic acid (GAA), or Menhaden fishmeal in corn-soybean meal (C-SBM) diets on growth performance and serum metabolites of 0- to 12-d broilers in experiment 3¹

Treatment	ADG, g	ADFI, g	G:F, g/g	Serum ²	
				Creatinine, mg/dL ²	Uric acid, mg/dL ²
1) C-SBM (control)	23.88	28.87	0.827 ^b	0.412 ^b	8.09 ^b
2) Diet 1 + 0.068% Gly	23.74	28.41	0.835 ^{ab}	0.403 ^b	9.60 ^a
3) Diet 1 + 0.119% creatine	24.35	28.71	0.848 ^a	0.415 ^b	7.27 ^b
4) Diet 1 + 0.106% GAA	24.69	28.96	0.853 ^a	0.415 ^b	7.65 ^b
5) C-SBM + 3% fishmeal	23.99	28.64	0.837 ^{ab}	0.482 ^a	7.71 ^b
SEM	0.514	0.514	0.008	0.014	0.542
Overall treatment P -value	0.688	0.952	0.221	0.002	0.037

^{a-b} Means with different superscripts within a column are significantly different ($P < 0.10$).

¹ Data are means of 9 replications with 6 chicks per replicate pen. Average initial and d 12 BW was 38 and 328 g, respectively.

² Serum creatinine and uric acid were determined from pooled serum samples of 3 chicks per replicate pen.

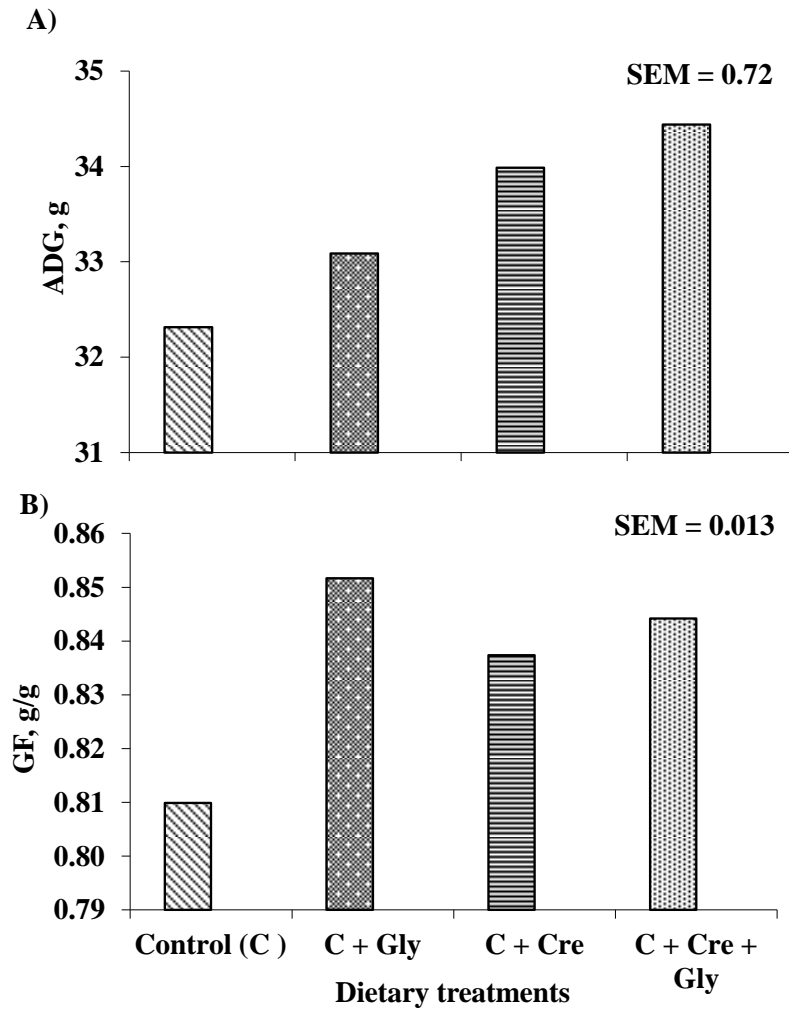


Figure 3.1. The interactive effects of supplemental Gly and creatine (Cre) in corn-soybean meal diets fed to 0- to 18-d posthatch broilers. (A) Creatine supplementation increased ADG ($P < 0.05$). (B) Glycine supplementation increased G:F ($P < 0.08$).

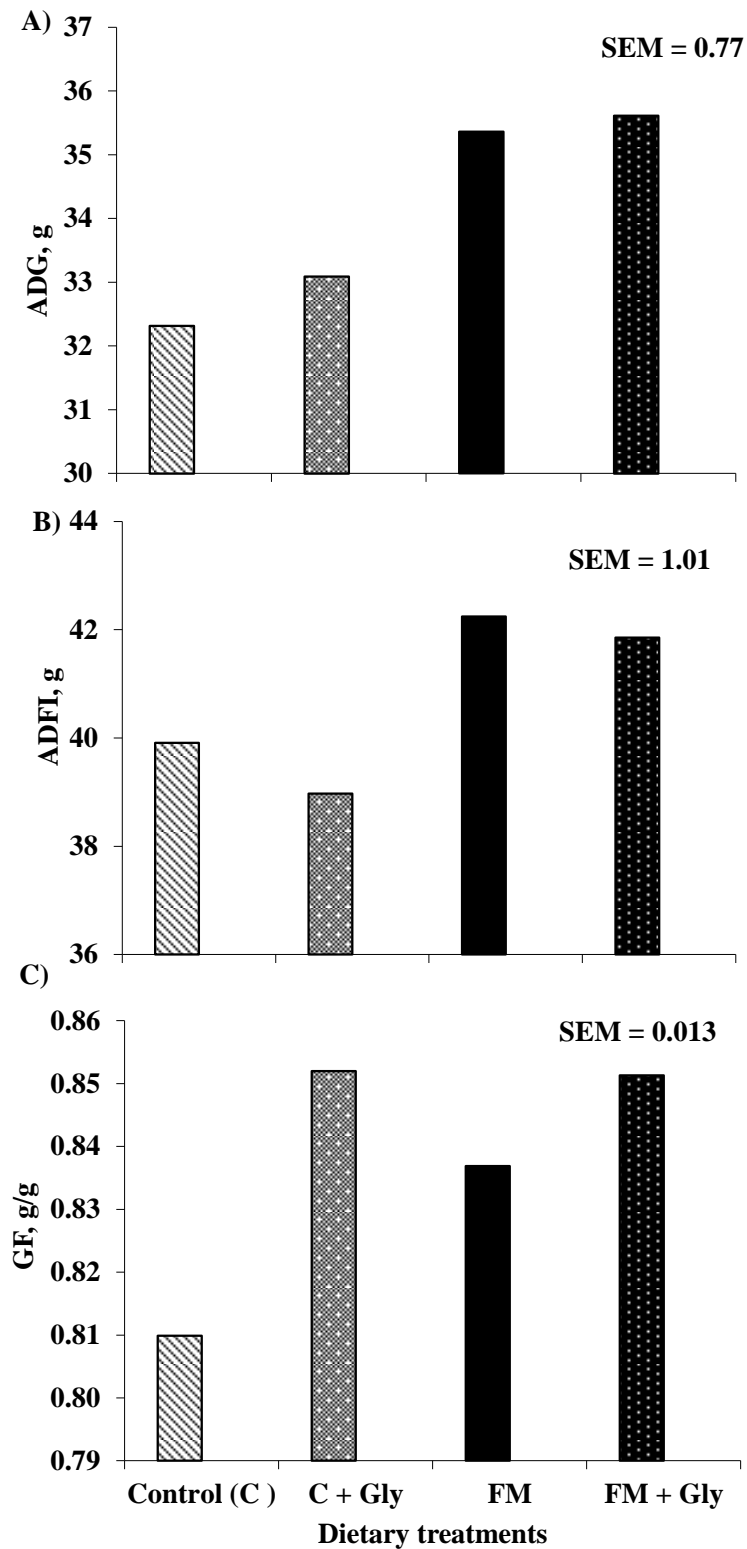


Figure 3.2. The interactive effects of supplemental Gly and fishmeal (FM) in corn-soybean meal diets fed to 0- to 18-d posthatch broilers. (A) Fishmeal addition increased ADG ($P < 0.01$). (B) Fishmeal addition increased ADFI ($P < 0.02$). (C) Glycine supplementation increased G:F ($P < 0.05$).

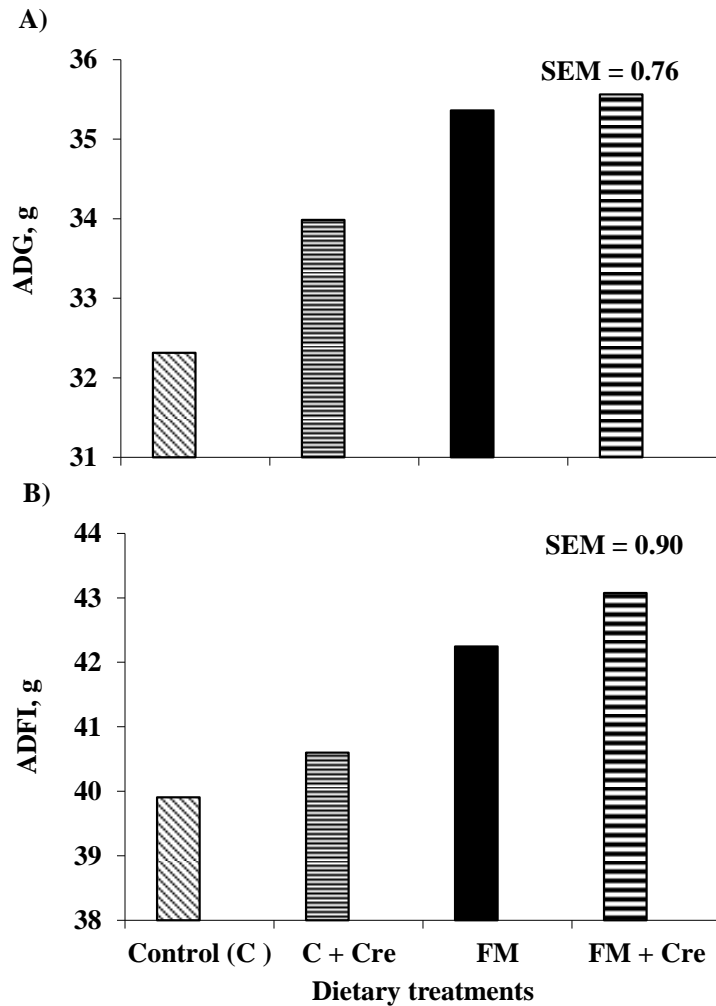


Figure 3.3. The interactive effects of supplemental creatine (Cre) and fishmeal (FM) in corn-soybean meal diets fed to 0- to 18-d posthatch broilers. Fishmeal addition to the diet increased ($P < 0.02$) ADG (A) and ADFI (B).

DISCUSSION

The objective of this research was to evaluate growth performance of broiler chicks fed supplemental Gly, creatine, GAA, and/or fishmeal in C-SBM diets. The results of experiments 1 and 2 indicate that broilers fed a diet with supplemental Gly or creatine individually or combined increased G:F compared with broilers fed the control diet. Previous research has determined a similar improvement in G:F of broiler chicks fed low CP, AA supplemented diets with Gly supplementation (Dean et al., 2006; Waguespack et al., 2008, 2009). Also, broilers fed diets

supplemented with Gly and/or creatine had G:F that was not different from broilers fed the 3% fishmeal diets.

Another purpose of this research was to evaluate the additive effects of supplementing Gly, creatine, and fishmeal. There was no further increase in ADG with the addition of supplemental Gly or creatine in the C-SBM diet with 3% fishmeal addition. This indicates that there is no additive effect of supplementing Gly or creatine in a 3% fishmeal diet.

In experiment 3, the objective of the research was to determine if the percentage of Gly that is present in a fishmeal, C-SBM diet could be supplemented in a C-SBM diet to produce similar or increased growth performance compared with broilers fed a diet that contains fishmeal, and to evaluate if Gly can be replaced with equimolar additions of creatine and GAA. Because the broilers fed the diet with 3% fishmeal had similar growth performance to those broilers fed the vegetable-based (C-SBM) diets, this objective could not be evaluated

The results of 2 of 3 experiments indicate that fishmeal addition to C-SBM diets results in improved growth performance. This somewhat inconsistent response is similar to what has been reported previously. Lemme et al. (2007a) reported that d 1 to 21 male broilers fed a vegetable-based (C-SBM, sorghum) diet had similar gain and feed intake compared with broilers fed a diet with 6% meat and bone meal. However, Ringel et al. (2007) reported that male broilers fed 5% fishmeal addition during the starter phase (d 0 to 21) and 3% fishmeal addition during the grower phase (d 22 to 42) had increased gain compared with broilers that were fed a vegetable-based (wheat, C-SBM) diet). The difference in gain might be due to the higher inclusion level of fishmeal that Ringel et al. (2007) fed in the starter phase.

The level of supplemental GAA (0.106%) in experiment 3 was similar to the GAA inclusion level that was determined by Lemme et al. (2007a), which suggested that optimal supplementation of GAA in a vegetable-based diet ranges from 0.06 to 0.12% to have similar

growth performance to broilers fed an animal-byproduct diet. Guanidino acetic acid was included in experiment 3 because GAA is a metabolic intermediate in creatine synthesis. Guanidino acetic acid is synthesized in the kidney from Gly and Arg and transported to liver, where it is used as a precursor for creatine synthesis (Wyss and Kaddurah-Daouk, 2000).

The increase in G:F in broilers fed diets supplemented with GAA or creatine compared with broilers fed the control vegetable-based diet might be explained by an improvement in the ability of broilers to utilize nutrients for growth. Lemme et al. (2007b) suggested that the improvement in feed efficiency as well as increased ATP and phosphorylated creatine in the breast meat of broilers fed diets with GAA supplementation might be due to improved energy availability. Brosnan and Brosnan (2007) agree that synthesis of creatine requires many precursors that are also used for AA metabolism; therefore, there are high demands for AA and other metabolites to produce creatine.

In experiment 3, broilers fed the diet with supplemental Gly had increased serum uric acid compared with broilers fed all other diets. Researchers have reported similar results when supplementing Gly and/or Glu in a low CP, C-SBM diet, plasma uric acid concentrations increased compared with broilers fed a low CP, C-SBM without Gly and/or Glu supplementation (Corzo et al., 2005; Namroud et al., 2008). Because each mole of uric acid produced by a broiler requires 1 mole of Gly to contribute 2 carbons and 1 N (Sonne et al., 1946), the increase in plasma or serum uric acid could be an indication of improved feed utilization. Corzo et al. (2005) reported an increase in G:F when Gly was supplemented in a low CP, C-SBM diet; however, Namroud et al. (2008) determined that Gly and Glu supplementation in the low CP, C-SBM diet had similar G:F. Similarly in experiment 3, G:F of broilers fed the diet with supplemental Gly was not different from broilers fed any of the other diets, but most of the time when Gly is supplemented in a C-SBM diet, there is an improvement in G:F (Waguespack et al., 2008; 2009).

Also, in experiment 3, broilers that were fed the diet with 3% fishmeal had increased serum creatinine compared with broilers fed all other dietary treatments. Although the percentage of creatine and GAA in 3% fishmeal is less than the percentage of Gly, all 3 of these metabolites are parts of creatine metabolism. Creatine and phosphorylated creatine are broken down into creatinine and removed via excretion (Brosnan and Brosnan, 2007). The increased serum creatinine in the broilers fed the fishmeal diet may be due to increased breakdown of creatine or there may be more creatinine being provided by the 3% fishmeal than what is synthesized by the broiler from supplementing Gly, creatine, or GAA in a C-SBM diet.

In conclusion, supplemental Gly, creatine, or GAA can be added to a C-SBM diet to produce similar growth performance to broilers fed a C-SBM, fishmeal diet, and there is no additive effect of supplementing Gly or creatine in a 3% fishmeal diet.

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CHAPTER 4

EFFECT OF GLYCINE, LYSINE, AND ANTIBIOTIC SUPPLEMENTATION IN CORN-SOYBEAN MEAL DIETS ON GROWTH PERFORMANCE, ORGAN RESPONSES, AND SERUM URIC ACID IN BROILER CHICKS

INTRODUCTION

The continuing rise in feed costs for broiler production results in the need to address different options for diet formulations to achieve the most cost effective production. Reducing intact protein sources and replacing them with increasing supplemental AA in broiler diets reduces dietary CP levels and N excretion (Moran et al., 1992; Deschepper and De Groote, 1995; Ferguson et al., 1998a,b; Bregendahl et al., 2002; Corzo et al., 2005). Many researchers have conducted research to attempt to correct the negative growth effects associated with feeding broilers low CP, AA supplemented diets (Aftab et al., 2006).

Dean et al. (2006) reported that broilers fed low CP, AA supplemented diets had growth performance equal to those fed a conventional C-SBM diet and that the limiting factor in these low CP diets was Gly. Within our lab, we have shown that Gly supplementation to seemingly nutritionally adequate, all vegetable C-SBM diets increases G:F of broilers (Dean et al., 2006; Waguespack et al., 2008, 2009a). Supplementing Gly in low CP, AA supplemented diets generally results in an increase in G:F. The reason for this is not completely known. Another point of interest is whether or not this improvement in G:F would still be observed if the dietary Lys was increased in the diet. Dean et al. (2006) and Waguespack et al. (2009a) determined this improvement in ADG and G:F in diets that contained 1.26% total dietary Lys, which might be at or slightly below the Lys requirement for Ross 708 broilers.

If G:F is increased, it could be due to increased nutrient absorption that could be measured by an increase in intestinal length (Jackson and Diamond, 1996). The small intestine plays an important role in the digestion and absorption of nutrients. Although an increase in intestinal

length would be a positive response, the need to measure weights of internal organs is necessary. If the improvement in ADG and G:F in response to supplementing Gly in broiler diets was due to an increase in organ weights, especially highly metabolically active organs such as the liver, this would be a negative response because it would indicate that energy that should be going towards protein deposition was being diverted.

Researchers have reported that when supplementing Gly and/or Glu in low CP, C-SBM diets, plasma uric acid concentrations increased compared with broilers fed a low CP, C-SBM diets without Gly and/or Glu supplementation (Corzo et al., 2005; Namroud et al., 2008). Because each mole of uric acid produced by broilers requires 1 mole of Gly to contribute 2 carbons and 1 N (Sonne et al., 1946), the increase in plasma or serum uric acid (SUA) could be an indication of improved feed utilization for protein deposition. Corzo et al. (2005) reported an increase in G:F when Gly was supplemented in a low CP, C-SBM diet as well as an increase in plasma uric acid concentrations.

Glycine has been shown to have antibacterial properties in vitro (Hammes et al., 1973; Minami et al., 2004). Glycine addition to in vitro bacterium solutions was shown to result in cell lysis (Maculla and Cowles, 1948). Glycine also has been investigated in combination with anticoccidial vaccines in broiler chicks. Lehman et al. (2009) fed low CP diets supplemented with 2% gelatin, which contains Gly, Ser, and Pro, and indicated that gelatin supplementation helped to alleviate the negative growth response that can occur with coccidiosis live vaccines. Thus, the increase in G:F when supplementing Gly in low CP broiler diets that we have seen within our lab could be due to Gly responding in an antibacterial or anticoccidial manner.

The objectives of this research were to determine: 1) the effects of supplementing Lys and Gly and 2) the effects of feeding 2 levels of total dietary Lys without and with supplemental

Gly and without and with antibiotics/anticoccidial on growth performance, serum uric acid, intestinal lengths, and organ weights of broiler chicks.

MATERIALS AND METHODS

General

Two experiments were conducted with Ross × Ross 708 broilers. In experiment 1, broiler chicks were obtained from House of Raeford in Gibsland, LA. In experiment 2, eggs were obtained from House of Raeford in Gibsland, LA and hatched at the Louisiana State University Agricultural Center Poultry Farm. Broilers used in experiment 2 were not vaccinated before allotment to treatment. On d 0 post-hatching, broilers were wing sexed, weighed, wing banded, and randomly allotted to treatments. Broilers were housed in temperature-controlled Petersime brooder batteries (Petersime Incubator Co., Gettysburg, OH) with continuous fluorescent lighting. Each experiment was conducted for 14- or 18-d posthatching. Feed in mash form and water were available on an ad libitum basis. Chicks and feeders were weighed on d 0 and 14 (experiment 1) or d 0 and d 18 (experiment 2) for the determination of ADG, ADFI, and G:F. All experimental animal use was in compliance with the Louisiana State University Agricultural Center Animal Care and Use Committee.

Dietary Treatments

In experiment 1, 144 male and female broilers (33.6 and 341.4 g, initial and final BW, respectively) were allotted to 4 treatments with 6 replications (3 male and 3 female) of 6 broilers per replicate pen to investigate the effects of Lys and Gly supplementation in C-SBM diets. The treatments were arranged as a 2 × 2 factorial without and with supplemental Lys and Gly. In experiment 1, the standardized ileal digestible (SID) Lys was kept constant in all diets, but only the source of Lys changed.

All diets were C-SBM based (Table 4.1), and contained 1.27% SID Lys, which is the SID Lys recommendation for 0- to 10-d Ross 708 broilers (Aviagen, 2007). Glycine supplementation was added to achieve 2.42% Gly+Ser, which was to achieve 1.909 Gly+Ser:SID Lys, which is based on a ratio of Gly+Ser obtained in previous research (Waguespack et al., 2009b). Supplemental Lys was added at 0.334% L-Lys·SO₄ (0.169% supplemental L-Lys) in diets 3 and 4. The supplemental Lys value was based on maintaining the minimum Val:Lys, Arg:Lys, and Ile:Lys at 0.73, 1.01, and 0.65, respectively, (Waguespack et al., 2009a) in all diets.

In experiment 2, 384 male broilers (46.2 and 673.3 g, initial and final BW, respectively) were allotted to 8 treatments with 8 replications of 6 broilers per replicate pen to investigate the effects of Gly supplementation in low and high Lys diets without and with antibiotics in C-SBM diets. The treatments were arranged as a 2 × 2 × 2 factorial with 2 levels of Lys (1.26 and 1.40%) without and with supplemental Gly and without and with antibiotic addition.

All diets were C-SBM based (Table 4.2), and contained 0.361% L-Lys·SO₄ (0.183% supplemental L-Lys). The supplemental Lys value was based on maintaining the minimum Val:Lys, Arg:Lys, and Ile:Lys at 0.73, 1.01, and 0.65, respectively, (Waguespack et al., 2009a) in both the low and high Lys diets. Diets 1 to 4 were formulated to contain 1.26% total Lys (Waguespack et al. (2009a), and diets 5 to 8 were formulated to contain 1.40% total Lys, which is based on the Lys recommendation for 0- to 10-d Ross 708 broilers (Aviagen, 2007). Glycine supplementation was added to achieve a 2.10% Gly+Ser (Waguespack et al., 2009b). Supplementation of antibiotics included 0.50% BMD+3Nitro and 0.05% Biocox as well as 0.05% ethoxyquin (even though it is an antioxidant and not an antibiotic). All 3 of these are typically included in diets fed to broilers raised in a floor pen environment.

Tissue Sampling and Measurements

On d 14 (experiment 1) and d 19 (experiment 2), 3 broilers per pen were weighed and

Table 4.1. Percentage composition of corn-soybean meal diets fed to broilers d 0 to 14 posthatching in experiment 1, as-fed basis¹

Item	0% supplemental L-Lys		0.17% supplemental L-Lys	
	- Gly	+ Gly	- Gly	+ Gly
Ingredients				
Corn	45.794	45.794	52.643	52.643
Soybean meal (47.5%)	42.703	42.703	36.021	36.021
Poultry fat	6.762	6.762	5.772	5.772
Monocalcium phosphate	1.718	1.718	1.766	1.766
Limestone	1.446	1.446	1.480	1.480
Sodium chloride	0.500	0.500	0.500	0.500
Mineral premix ²	0.250	0.250	0.250	0.250
Vitamin premix ³	0.250	0.250	0.250	0.250
Choline chloride ⁴	0.050	0.050	0.050	0.050
DL-Met ⁵	0.275	0.275	0.335	0.335
L-Thr ⁵	0.083	0.083	0.175	0.175
Biolys ⁶	0.000	0.000	0.334	0.334
Gly	0.000	0.169	0.000	0.424
Cornstarch	0.169	0.000	0.424	0.000
Calculated composition				
ME, kcal/kg	3,200	3,200	3,200	3,200
CP (%)	24.30	24.50	21.87	22.55
Ca (%)	1.05	1.05	1.05	1.05
Total P (%)	0.78	0.78	0.77	0.77
Nonphytate P (%)	0.50	0.50	0.50	0.50
K (%)	1.06	1.06	0.93	0.93
Cl (%)	0.36	0.36	0.36	0.36
SID Lys (%) ⁷	1.27	1.27	1.27	1.27
SID Met (%)	0.61	0.61	0.64	0.64
SID Met+Cys (%)	0.94	0.94	0.94	0.94
SID Thr (%)	0.89	0.89	0.89	0.89
SID Arg (%)	1.52	1.52	1.33	1.33
SID Ile (%)	0.97	0.97	0.86	0.86
SID Val (%)	1.04	1.04	0.93	0.93
SID Trp (%)	0.28	0.28	0.24	0.24
SID Leu (%)	1.86	1.86	1.70	1.70
SID His (%)	0.58	0.58	0.52	0.52
SID Phe+Tyr (%)	1.92	1.92	1.70	1.70
Gly+Ser (%)	2.26	2.42	2.00	2.42
Gly+Ser:SID Lys	1.78	1.91	1.58	1.91
SID Arg:SID Lys	1.20	1.20	1.05	1.05
SID Val:SID Val	0.82	0.82	0.73	0.73
SID Ile:SID Ile	0.76	0.76	0.67	0.67

¹ Two basal diets were mixed to contain the minimum of all ingredients except for supplemental Gly and cornstarch, which were added as needed to each diet. Supplemental Gly supplementation was based on 1.909 ratio of total Gly+Ser to SID Lys (Waguespack et al., 2009b; 2.10 total Gly+Ser to 1.26 total Lys, which is approximately 1.10 SID Lys).

(Table 4.1 continued)

² Provided per kilogram of diet: Cu (copper sulfate), 7 mg; I (calcium iodate), 1 mg; Fe (ferrous sulfate), 50 mg; Mn (manganese sulfate), 100 mg; Se (sodium selenite), 0.15 mg; and Zn (zinc sulfate), 75 mg.

³ Provided per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg.

⁴ Contains 600,000 mg/kg of choline chloride.

⁵ DL-Met and L-Thr were provided by Evonik Corporation (Kennesaw, GA).

⁶ Biolys was obtained from Evonik Corporation (Kennesaw, GA) and contains 50.7% L-Lys.

⁷ SID = standardized ileal digestible. Levels were calculated based on digestibility coefficients in NRC (1994).

Table 4.2. Percentage composition of corn-soybean meal diets fed to broilers d 0 to 18 posthatching in experiment 2, as-fed basis¹

Item	1.26% Lys diets				1.40% Lys diets				
	Antibiotic	-	-	+	+	-	-	+	+
	Gly	-	+	-	+	-	+	-	+
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	
Ingredients									
Corn	59.449	59.449	59.449	59.449	53.880	53.880	53.880	53.880	
Soybean meal (47.5% CP)	30.541	30.541	30.541	30.541	35.652	35.652	35.652	35.652	
Soy oil	4.433	4.433	4.433	4.433	5.078	5.078	5.078	5.078	
Monocalcium phosphate	1.564	1.564	1.564	1.564	1.527	1.527	1.527	1.527	
Limestone	1.293	1.293	1.293	1.293	1.267	1.267	1.267	1.267	
Sodium chloride	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	
Mineral premix ²	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	
Vitamin premix ³	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	
Choline chloride ⁴	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	
DL-Met ⁵	0.264	0.264	0.264	0.264	0.313	0.313	0.313	0.313	
L-Thr ⁵	0.145	0.145	0.145	0.145	0.164	0.164	0.164	0.164	
Biolys ⁶	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	
Gly	-----	0.300	-----	0.300	-----	0.108	-----	0.108	
BMD+3Nitro ⁷	-----	-----	0.500	0.500	-----	-----	0.500	0.500	
Ethoxyquin ⁸	-----	-----	0.050	0.050	-----	-----	0.050	0.050	
Biocox ⁹	-----	-----	0.050	0.050	-----	-----	0.050	0.050	
Sand	0.600	0.600	-----	-----	0.600	0.600	-----	-----	
Cornstarch	0.300	-----	0.300	-----	0.108	-----	0.108	-----	
Calculated composition									
ME (kcal/kg)	3,207	3,200	3,207	3,200	3,203	3,200	3,203	3,200	
Crude protein (%)	19.97	20.32	19.97	20.32	21.98	22.11	21.98	22.11	
Ca (%)	1.00	1.00	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	0.45	0.45	
Lys (%)	1.26	1.26	1.26	1.26	1.40	1.40	1.40	1.40	
Met+Cys (%)	0.91	0.91	0.91	0.91	1.01	1.01	1.01	1.01	
Thr (%)	0.88	0.88	0.88	0.88	0.98	0.98	0.98	0.98	
Val (%)	0.93	0.93	0.93	0.93	1.02	1.02	1.02	1.02	

(Table 4.2 continued)

Arg (%)	1.29	1.29	1.29	1.29	1.44	1.44	1.44	1.44
Ile (%)	0.83	0.83	0.83	0.83	0.92	0.92	0.92	0.92
Gly+Ser (%)	1.80	2.10	1.80	2.10	1.99	2.10	1.99	2.10
Met+Cys: Lys	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Thr: Lys	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Val: Lys	0.74	0.74	0.74	0.74	0.73	0.73	0.73	0.73
Arg: Lys	1.02	1.02	1.02	1.02	1.03	1.03	1.03	1.03
Ile: Lys	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Gly+Ser: Lys	1.43	1.67	1.43	1.67	1.42	1.50	1.42	1.50

¹ Two basal diets were mixed to contain the minimum of all ingredients except for crystalline Gly, BMD + 3Nitro, ethoxyquin, Biocox, sand, and cornstarch, which were added as needed to each diet.

² Provided per kilogram of diet: Cu (copper sulfate), 7 mg; I (calcium iodate), 1 mg; Fe (ferrous sulfate), 50 mg; Mn (manganese sulfate), 100 mg; Se (sodium selenite), 0.15 mg; and Zn (zinc sulfate), 75 mg.

³ Provided per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg.

⁴ Contains 600,000 mg/kg of choline chloride.

⁵ DL-Met and L-Thr were provided by Evonik Corporation (Kennesaw, GA).

⁶ Biolys was obtained from Evonik Corporation (Kennesaw, GA) and contains 50.7% L-Lys.

⁷ Bacitracin methylene disalicylate + 3-nitro-4-hydroxyphenylarsonic acid from Nutra Blend (Neosho, MO).

⁸ Ethoxyquin provides 66.6% 6-ethoxy-1,2-dihydro-2,2,4-trimethylquionine (Novus International, St. Louis, MO).

⁹ Biocox provides 132.3 g/kg salinomycin sodium (Roche, Parsippany, NJ).

killed by CO₂ asphyxiation after a minimum of a 2-h fasting period. After CO₂ asphyxiation, the length of the duodenum (gizzard to the entrance of bile duct/end of the duodenal loop), jejunum (entrance of bile duct to the Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileo-cecal junction) were taken before cleaning of the digesta. The digesta contents were gently squeezed to determine the empty weights. The heart, liver (with gallbladder), and pancreas were removed and weighed as well as empty weights of the proventriculus, gizzard, duodenum, jejunum, ileum, and ceca (left and right together). Weights are expressed as a percentage of live BW.

Blood Sampling and Serum Biochemistry

On d 14 (experiment 1) and d 18 (experiment 2), blood was collected via cardiac puncture from 3 broilers per pen after a 2-h fast. Blood was collected and placed into 10-mL serum tubes (BD Vacutainer, Franklin Lakes, NJ) and samples were placed on ice before centrifugation at $3,000 \times g$ at 0°C for 20 min. After centrifugation, serum from each broiler was collected and individually kept frozen until analysis to determine serum uric acid (**SUA**) concentrations (Fossati et al., 1980) using a commercial reagent kit (Pointe Scientific, Canton, MI). In order to assess AA utilization using SUA, a 2-h fast has been determined to be the best time to detect differences (Okumura and Tasaki, 1969; Donsbough et al., 2010).

Statistical Analysis

Data were analyzed by ANOVA as completely randomized designs using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Each pen of broilers served as the experimental unit. Treatment differences were considered significant with an $\alpha = 0.10$. The PDIFF option of SAS was used to compare individual diets. For experiment 1, the statistical model included treatment, sex, and treatment \times sex. Treatment \times sex interactions were initially included in the statistical model; however, if these contrasts were not significant for response variables, they

were removed. Contrast statements were used to determine significant effects of supplemental Lys, supplemental Gly, and the interaction. For experiment 2, the statistical model included the main effects of Lys, supplemental Gly, antibiotics, and all 2- and 3-way interactions. Duodenal, jejunal, ileal, and total small intestine lengths were analyzed with d18 BW as a covariable.

RESULTS

Experiment 1

Daily feed intake was increased and G:F was decreased in female broilers compared with male broilers ($P < 0.08$; data not shown). Daily gain and ADFI were not affected by any dietary treatment ($P > 0.10$; Table 4.3). Broilers fed – Lys diets had increased G:F compared with broilers fed + Lys diets ($P = 0.001$). Broilers fed + Gly diets had increased G:F compared with broilers fed – Gly diets ($P = 0.039$).

Serum uric acid for male and female broilers fed the diets without supplemental Lys were not different with Gly addition; however, in the diets with supplemental Lys addition, male broilers had decreased SUA and female broilers had increased SUA with Gly addition (Treatment \times sex, $P = 0.051$; Figure 4.1).

Heart, proventricular, gizzard, duodenal, jejunal, pancreas, and cecal weights were not affected by any dietary treatment ($P > 0.10$). Ileal weight as a percentage of BW was greater in male broilers than in female broilers ($P = 0.054$; data not shown). In the broilers fed the – Lys diets, male broilers had increased liver weight as a percentage of BW with Gly supplementation, but in the male broilers fed the + Lys diets, liver weight as a percentage of BW was decreased in male broilers fed - Gly diets (Treatment \times sex, $P < 0.073$; Figure 4.2). In the broilers fed the – Lys diets, Gly supplementation increased liver weight as a percentage of BW, but in the broilers fed the + Lys diets, Gly supplementation decreased liver weight as a percentage of BW (Gly \times Lys, $P = 0.084$).

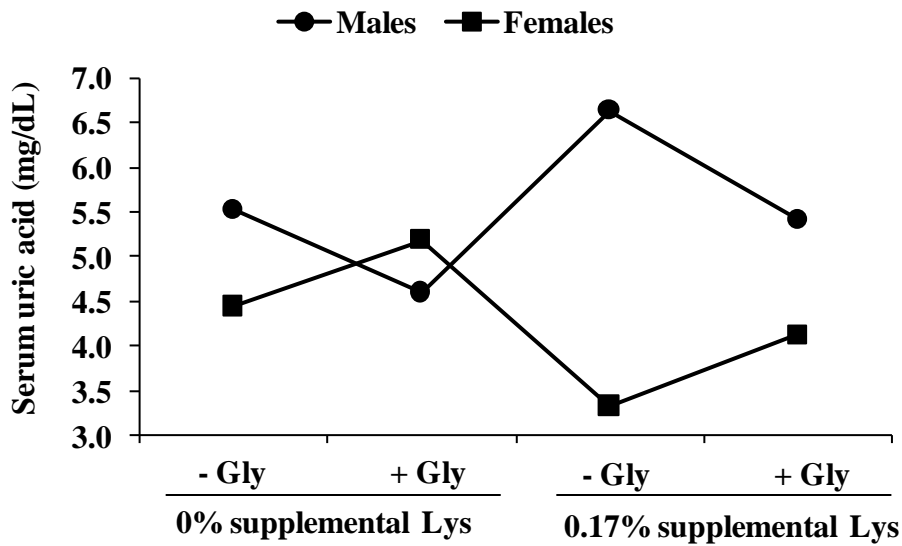


Figure 4.1. The effects on serum uric acid of feeding male and female broilers diets without and with supplemental Lys and Gly in experiment 1 (Treatment \times sex, $P = 0.051$).

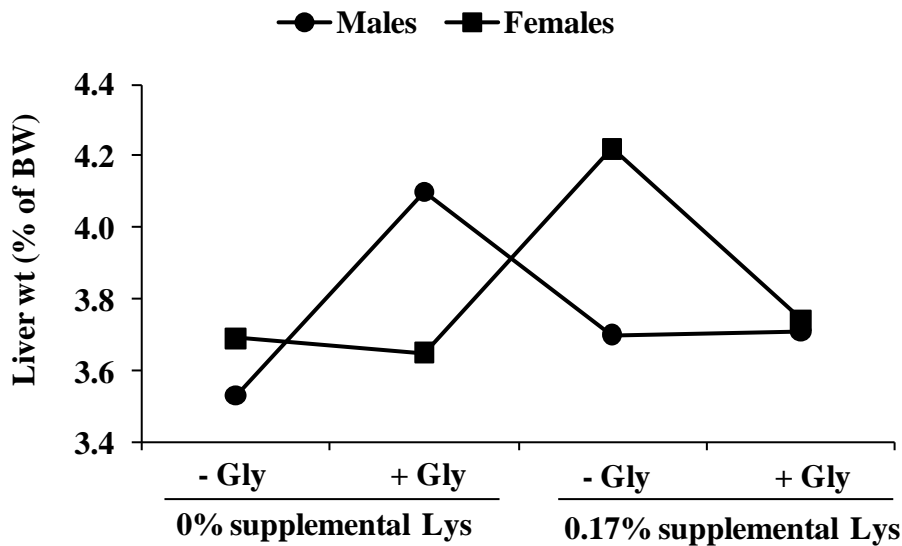


Figure 4.2. The effects on liver weight as a percentage of BW of feeding male and female broilers diets without and with supplemental Lys and Gly in experiment 1 (Treatment \times sex, $P < 0.073$).

Table 4.3. The effects on growth performance, serum uric acid, and organ weights of male and female broiler chicks fed diets without and with supplemental Lys and Gly in experiment 1^{1,2,3}

Response	0% supplemental Lys		0.17% supplemental Lys		SEM	<i>P</i> -value ⁴	Sex	Suppl. Lys	Gly
	- Gly	+ Gly	- Gly	+ Gly					
Growth performance									
d 0 to 14									
ADG (g)	22.48	21.75	22.05	21.53	0.850	0.870	0.217	0.707	0.469
ADFI (g)	26.92	25.32	27.37	26.27	0.952	0.472	0.072	0.469	0.173
G:F (g/g)	0.836	0.859	0.806	0.820	0.008	0.002	0.066	0.001	0.039
Serum uric acid (mg/dL) ⁵	4.99	4.90	4.99	4.78	0.446	0.984	0.012	0.893	0.736
Intestinal wet weights ⁶									
Heart (%)	0.716	0.665	0.693	0.680	0.017	0.949	0.222	0.846	0.655
Proventriculus (%)	0.712	0.756	0.694	0.710	0.024	0.251	0.619	0.303	0.299
Gizzard (%)	2.905	2.983	3.084	2.982	0.070	0.400	0.590	0.649	0.692
Duodenum (%)	1.071	1.149	1.140	1.102	0.047	0.969	0.525	0.876	0.829
Jejunum (%)	1.837	1.805	1.918	1.883	0.060	0.725	0.583	0.504	0.564
Ileum (%)	1.166	1.156	1.181	1.203	0.035	0.691	0.054	0.711	0.806
Pancreas (%)	0.386	0.388	0.374	0.381	0.012	0.745	0.387	0.361	0.677
Ceca (%)	0.355	0.378	0.349	0.371	0.014	0.514	0.418	0.519	0.215
Liver (%) ⁷	3.614	3.875	3.962	3.726	0.155	0.269	0.503	0.526	0.881

¹Data are means of 6 replications (3 male and 3 female replicates) with 6 chicks per replicate. Average initial and d 14 BW was 33.6 and 341.4 g, respectively.

²Treatment × sex was initially included in the model for all response variables, but was NS for any variable ($P = 0.19$ to 0.99); therefore, it was removed from the model, except for serum uric acid ($P = 0.051$) and liver weight as a % of BW ($P < 0.073$).

³Supplemental Gly × supplemental Lys was NS for any response variable ($P = 0.14$ to 0.92), except for liver weight as a % of BW ($P = 0.084$). In the broilers fed the diets without supplemental Lys, Gly increased liver weight as a % of BW, but in the broilers fed the diets with supplemental Lys diet, Gly decreased liver weight.

⁴Overall treatment P -value.

(Table 4.3 continued)

⁵Serum uric acid (SUA) for male broilers fed + Lys diets had increased SUA compared with female broilers fed + Lys diets, and male broilers fed + Lys + Gly diets had decreased SUA compared with female broilers fed + Lys + Gly diets (Treatment \times sex, $P = 0.051$).

⁶Weights are expressed as a % of total live BW.

⁷In the broilers fed the – Lys diets, male broilers had increased liver weight as a percentage of BW with Gly supplementation, but in the male broilers fed the + Lys diets, liver weight as a percentage of BW was decreased in male broilers fed - Gly diets (Treatment \times sex, $P < 0.073$).

Experiment 2

Daily gain ($P = 0.002$) and G:F ($P = 0.001$) were increased for broilers fed the 1.40% Lys diets compared with broilers fed the 1.26% Lys diets. Daily feed intake was decreased ($P = 0.077$) and G:F was increased ($P = 0.062$) for broilers fed diets with supplemental Gly compared with broilers fed diets without supplemental Gly. Antibiotic supplementation did not affect ADG, ADFI, or G:F ($P > 0.10$).

Serum uric acid for broilers fed the diets with antibiotic supplementation was decreased compared with broilers fed the diets without antibiotic supplementation in the 1.26% Lys diets; however, this response in SUA was reversed in broilers fed the 1.40% Lys diets (Lys \times antibiotic, $P < 0.02$). Serum uric acid was not affected by Lys level ($P > 0.10$), but SUA was increased when broilers were fed diets with Gly supplementation compared with broilers fed diets without Gly supplementation ($P = 0.066$).

Duodenal length was not affected by any dietary treatment ($P > 0.10$). Jejunal, ileal, and total small intestinal lengths were increased in broilers fed the 1.40% Lys diets compared with broilers fed the 1.26% Lys diets ($P < 0.09$). Antibiotic or Gly supplementation did not affect duodenal, jejunal, ileal, or total small intestine lengths ($P > 0.10$).

Heart, duodenal, jejunal, and liver weights were not affected by any dietary treatment ($P > 0.10$). Gizzard weight was increased in broilers fed the 1.40% Lys diet compared with broilers fed the 1.26% Lys diets ($P = 0.102$). Gizzard weight was decreased in broilers fed diets with Gly supplementation compared with broilers fed diets without Gly supplementation ($P = 0.071$). Ileal and pancreas weight were decreased in broilers fed diets supplemented with antibiotics compared with broilers fed diets without antibiotics ($P = 0.077$ and 0.041). Broilers fed the 1.26% Lys without antibiotics diets had increased cecal weight compared with broilers fed the 1.40% Lys without antibiotics diets; however, broilers fed the 1.26% Lys with antibiotics diets had

decreased cecal weight compared with broilers fed the 1.40% Lys with antibiotics diets (Lys \times antibiotic, $P = 0.092$). There was a Lys \times Gly \times antibiotic interaction for proventricular weight as a % of BW ($P < 0.05$; Figure 4.3).

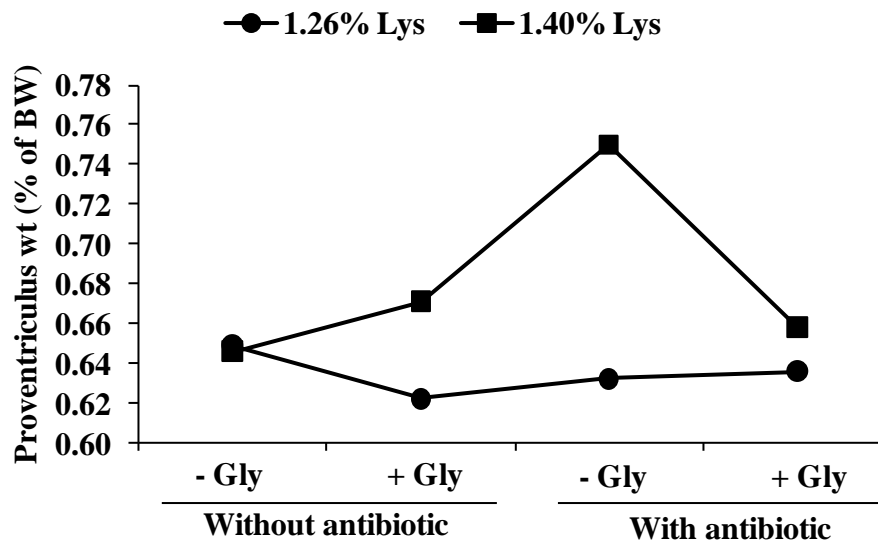


Figure 4.3. The effects on proventricular weight (as a percentage of BW) of feeding broilers diets with 2 levels of Lys (1.26 and 1.40%) without and with supplemental Gly and antibiotics in experiment 2 (Lys \times Gly \times antibiotic, $P < 0.05$).

DISCUSSION

The purpose of this research was to determine the effects of supplementing Lys and Gly on the growth performance, SUA, intestinal weights, and organ weights of broiler chicks, and to evaluate the effects of feeding 2 levels of dietary Lys without and with supplemental Gly and without and with antibiotics/anticoccidials.

Supplemental Lys did not affect ADG or ADFI of broilers in experiment 1. Similarly, researchers have shown that 0.20% supplemental Lys can be added in a C-SBM diet without decreasing growth performance (Waguespack et al., 2009a).

Table 4.4. Effects on growth performance and organ weights of male broiler chicks fed diets with 2 levels of Lys (1.26 and 1.40%) without and with supplemental Gly and antibiotics in experiment 2^{1,2}

Response	1.26% Lys diets				1.40% Lys diets				SEM	Lys	Gly	Antibiotic
	Antibiotic	-	-	+	+	-	-	+	+			
	Gly	-	+	-	+	-	+	-	+			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8				
Growth performance												
d 0 to 18												
ADG (g)	33.84	33.97	34.10	33.82	37.20	35.57	34.92	35.46	0.78	0.002	0.576	0.309
ADFI (g)	44.91	44.33	45.76	44.36	46.84	44.03	44.90	44.82	0.95	0.648	0.077	0.916
G:F (g/g)	0.755	0.767	0.746	0.762	0.794	0.807	0.778	0.791	0.01	0.001	0.062	0.116
Serum uric acid (mg/dL) ³	2.21	2.43	1.74	2.23	1.90	2.43	2.60	2.48	0.21	0.178	0.066	0.885
Intestinal length ⁴												
Duodenum (cm)	21.36	22.70	22.47	21.97	22.02	22.78	22.52	22.81	0.42	0.379	0.297	0.618
Jejunum (cm)	50.98	52.45	53.78	54.16	55.05	54.41	55.88	53.32	0.95	0.085	0.741	0.299
Ileum (cm)	49.07	51.52	52.37	52.29	55.38	54.53	55.39	53.87	1.11	0.004	1.000	0.456
Whole SI (cm)	121.52	126.02	126.99	126.71	132.35	131.64	133.72	129.95	1.99	0.007	0.978	0.528
Intestinal wet weights ⁵												
Heart (%)	0.68	0.65	0.66	0.63	0.64	0.65	0.67	0.66	0.01	0.972	0.308	0.891
Proventriculus (%) ⁶	0.65	0.62	0.63	0.64	0.65	0.67	0.75	0.66	0.03	0.015	0.229	0.232
Gizzard (%)	2.67	2.57	2.64	2.47	2.76	2.66	2.76	2.62	0.07	0.102	0.071	0.496
Duodenum (%)	0.71	0.66	0.65	0.61	0.64	0.68	0.65	0.65	0.02	0.941	0.686	0.490
Jejunum (%)	1.50	1.33	1.41	1.30	1.34	1.30	1.34	1.35	0.04	0.417	0.250	0.785
Ileum (%)	1.18	1.14	1.08	1.07	1.13	1.10	1.11	1.05	0.03	0.494	0.336	0.077
Pancreas (%)	0.33	0.35	0.32	0.31	0.33	0.32	0.32	0.32	0.01	0.712	0.963	0.041
Ceca (%) ⁷	0.40	0.42	0.35	0.35	0.39	0.39	0.38	0.36	0.01	0.882	0.903	0.001
Liver (%)	3.22	3.40	3.52	3.51	3.48	3.37	3.46	3.47	0.07	0.715	0.829	0.151

¹ Data are means of 8 replications with 6 chicks per replicate. Average initial, d 10, and d 18 BW was 46.2, 257.6, and 673.3 g, respectively.

² Glycine × Lys, Gly × antibiotic, Lys × antibiotic, and Lys × Gly × antibiotic were included in the model, but was NS for any response variable ($P = 0.16$ to 0.96), except for a Lys × antibiotic interaction for serum uric acid ($P < 0.02$), a Lys × antibiotic interaction for cecal weight as a % of BW ($P = 0.092$), and Lys × Gly × antibiotic interaction for proventricular weight ($P < 0.05$).

³ There was a Lys × antibiotic interaction for serum uric acid ($P < 0.02$).

(Table 4.4 continued)

⁴ Three birds per pen were killed by CO₂ asphyxiation after a minimum of a 2-h fast. Intestinal lengths were taken before cleaning out of digesta. Average duodenal, jejunal, ileal, and whole small intestinal (SI) length were 22.3, 53.8, 53.1, and 128.6 cm, respectively.

Data were analyzed with d 18 BW as a covariable.

⁵ Weights are expressed as a % of total live 18-d BW.

⁶ There was a Lys × Gly × antibiotic interaction for proventricular weight as a % of BW ($P < 0.05$).

⁷ Broilers fed the 1.26% Lys without antibiotics diets had increased cecal weight compared with broilers fed the 1.40% Lys without antibiotics diets; however, broilers fed the 1.26% Lys with antibiotics diets had decreased cecal weight compared with broilers fed the 1.40% Lys with antibiotics diets (Lys × antibiotic, $P = 0.092$).

As previous research has suggested (Corzo et al., 2005; Dean et al., 2006; Waguespack et al., 2008, 2009a), we observed that broilers fed diets with supplemental Gly had increased G:F compared with broilers fed diets without supplemental Gly. Also in experiment 1, there was an increase in G:F in broilers fed supplemental Gly in a seemingly nutritionally adequate, all vegetable C-SBM diet with no supplemental Lys (Dean et al., 2006; Waguespack et al., 2008, 2009a). Regardless of which level of dietary Lys level was fed in the diet in experiment 2, broilers had increased G:F with Gly supplementation.

Researchers have determined that increasing dietary Lys generally results in improvement in growth performance (Holsheimer and Veerkamp, 1992; Sterling et al., 2006; Berri et al., 2008). Similarly, we observed that ADG, ADFI and G:F were increased in broilers fed diets with 1.40% Lys compared with broilers fed the 1.26% diet.

In experiment 1, SUA for male broilers fed the supplemental Lys diets was increased compared with female broilers fed the same diet. Similarly, Donsbough et al. (2010) reported that SUA concentrations were higher in male broilers. We have no explanation for the treatment \times sex interaction in which male broilers fed + Lys + Gly diets had decreased SUA compared with females fed the same diet. We also have no explanation for the interaction of Lys and antibiotic with SUA response, especially because there was no effect on ADG, ADFI, or G:F of broilers fed diets without or with antibiotic supplementation. Serum uric acid was not affected by Lys level in experiment 2. Similarly, Corzo et al. (2003) reported that plasma uric acid of broilers did not change with increasing dietary Lys in the diets. The increase in SUA when feeding broilers diets with supplemental Gly is similar to results observed by others (Corzo et al., 2005; Namroud et al., 2008). This observed increase in SUA with Gly supplementation coincides with an increase in G:F with Gly supplementation.

In experiment 2, there was no effect of supplemental Gly or antibiotic on duodenal, jejunal, ileal, or whole intestinal length of broilers. Broilers fed the 1.40% Lys diet had increased length of jejunum, ileum, and whole SI compared with broilers fed the 1.26% Lys diet. The increase in intestinal length along with increased ADG and G:F for the broilers fed the 1.40% Lys diet might be due to increased nutrient absorption (Jackson and Diamond, 1996). There was no difference in individual SI section weights as a percentage of BW between broilers fed the 1.26 or 1.40% Lys diet.

Broilers fed diets with supplemental Gly had decreased gizzard weight compared with broilers fed diets without supplemental Gly. The gizzard is a metabolically active organ in the broiler. The decrease in gizzard weight as a percentage of BW coincided with an increase in G:F with Gly supplementation, which might be an indication that less energy from the feed is going toward maintaining organ growth. Some of the other metabolically active organs such as the heart and liver were not affected by Gly supplementation.

Because Gly has been shown to have antibacterial properties *in vitro* (Hammes et al., 1973; Minami et al., 2004) and has been investigated in combination with anticoccidial vaccines in broiler chicks (Lehman et al., 2009), we wanted to evaluate the effect of supplemental Gly on improving G:F and to determine if this response is similar to an antibacterial or anticoccidial in the diet. Bacitracin methylene disalicylate + 3-nitro-4-hydroxyphenylarsonic acid (BMD + 3 Nitro) was added to the diet as an antibiotic/anticoccidial as well as Biocox, which provides salinomycin sodium (anticoccidial). The combination of these 2 ingredients is one example of what is being commercially fed in broiler diets. The results of experiment 2 indicated that growth performance, SI length, duodenal weight, jejunal weight, and metabolically active organs weights (heart, liver, and gizzard) were not affected by antibiotic/anticoccidials supplementation;

however, the ileal and pancreas weights of broilers fed diets with the antibiotic/anticoccidials decreased compared with broilers fed diets without the antibiotic/anticoccidials.

In conclusion, increasing dietary Lys in broiler diets increases growth performance. Supplementation of Gly in low CP, AA supplemented diets increases G:F. This improvement in G:F coincides with an increase in SUA and occurs in diets that are high (1.40%) or low (1.26%) in dietary Lys. The increase in G:F is not similar to the response seen when antibiotic/anticoccidials are supplemented in broiler diets.

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CHAPTER 5

EFFECT OF SUPPLEMENTAL LYSINE AND GLYCINE IN REDUCED CRUDE PROTEIN, CORN-SOYBEAN MEAL DIETS FOR BROILERS DURING THE STARTER PHASE AND FEEDING A COMMON DIET DURING THE SUBSEQUENT GROWER PHASE

INTRODUCTION

The primary considerations for formulating broiler diets include maximizing growth performance and economic returns as well as decreasing environmental impact. With the cost of feed ingredients changing constantly, formulating broiler diets that are cost effective is important. Maximizing supplemental AA inclusion may make broiler diets more cost effective as well as reducing N loss in the excreta, which ultimately is lost into the environment (Moran et al., 1992; Namroud et al., 2008). Currently, DL-Met, L-Lys, and L-Thr are three of the commercially available supplemental AA that are being supplemented in most broiler diets. Determining the maximum use of these supplemental AA in C-SBM diets without compromising growth performance would be an advantage.

Some researchers have reported decreased feed efficiency and carcass yield when broilers were fed low CP, AA-supplemented diets (Pinchasov et al., 1990; Bregendahl et al., 2002). Many researchers have conducted research to attempt to correct the negative growth effects associated with feeding broilers low CP diets (Aftab et al., 2006). Amino acid supplementation, specifically Gly, in low CP diets has been shown to be successful in maintaining growth performance. Corzo et al. (2005) and Dean et al. (2006) reported that ADG and G:F were restored to the level of broilers fed a control diet when fed diets supplemented with Gly. In addition to the benefits of supplementing Gly in low CP diets, previous research has shown that the addition of Gly to C-SBM diets that are otherwise nutritionally adequate results in increased growth rate and feed efficiency (Dean et al., 2006; Waguespack et al., 2008).

Although supplemental Gly may not be economically feasible currently to add in broiler diets, supplementing Gly in the diet during the starter phase would be less costly than supplementing diets in the grower or finisher phases due to increased feed intake with increasing age of broilers. Also, research has been shown that the endogenous rate of Gly synthesis in the broiler chick cannot maintain optimal growth performance (Featherston, 1976). Ngo et al. (1977) suggested that the Gly requirement is the greatest during the first 10-d posthatching. Therefore, if supplementing Gly in the starter phase would have carryover growth performance effects in the grower phase, then it may be an advantage to supplement Gly in broiler chick diets.

The objective of this research was to determine the effect of feeding increasing levels of supplemental Lys without or with supplemental Gly in low CP, C-SBM diets from 0- to 10-d of age (P1), and the subsequent effects of feeding these diets from 10- to 24-d of age (P2).

MATERIALS AND METHODS

General

An experiment was conducted with male and female Ross \times Ross 708 broilers. Chicks were obtained from House of Raeford Hatchery in Gibsland, LA. Broilers were wing sexed and allotted on d of hatch to 10 treatments with 6 replications (3 male and 3 female pens) per treatment with 20 or 25 broilers per pen. The experiment was conducted for 24-d. Broilers were weighed on d 0, 10, and 24 for determination of ADG, ADFI, and G:F. The broilers were housed in 0.76×3.05 m pens at the Louisiana State University Agricultural Center Poultry Farm in one room of a tunnel-ventilated house equipped with cool cells. Hours of light for a 24 h period consisted of 4-d of 24 h of light, followed by 6-d of 20 h of light, and 18 h of light from d 10 to 24. The house temperature was maintained at 29 to 32°C for the first 7-d posthatching and was decreased by 2°C every 7-d until the house temperature was 23 to 26°C. Feed was fed in mash form. For the first 5-d, feed was provided by a feed tray and then by a hanging feeder with a

diameter of 43 cm. Water was provided via an automatic waterer with 6 nipple drinkers per pen. The broilers were fed a 2-period feeding program consisting of starter (0- to 10-d) and grower (10- to 24-d) periods. All experimental animal use was in compliance with the Louisiana State University Agricultural Center Animal Care and Use Committee.

Dietary Treatments

Diets (Table 5.1) were C-SBM based, and were formulated to provide 1.27% SID Lys for P1. Also, diets were formulated to maintain 3,025 kcal ME per kg of diet, 1.05% Ca, and 0.50% nonphytate P. During P1, the treatments consisted of 0, 0.05, 0.10, 0.15, and 0.20% supplemental Lys (provided by L-Lys·SO₄) without and with supplemental Gly to achieve a total Gly+Ser level of 2.42%. Supplemental Gly was added to achieve 1.909 Gly+Ser:SID Lys, which is based on a ratio of Gly+Ser obtained in previous research (Waguespack et al., 2009b). Supplemental Thr and Met were supplemented to maintain SID Thr:SID Lys and SID TSAA:SID Lys of 0.70 and 0.74, respectively, in all diets during P1. Supplemental Arg, Val, or Ile were not added to any dietary treatment, but diets were formulated to maintain a minimum of 1.01, 0.72, and 0.65 SID AA:SID Lys, respectively (Waguespack et al., 2009a).

During P2, a common C-SBM diet with 0.05% supplemental Lys (provided by L-Lys·SO₄) was fed to all broilers (Table 5.1). This diet was formulated to provide 1.10% SID Lys. Also, diets were formulated to maintain 3,150 kcal ME per kg of diet, 0.90% Ca, and 0.45% nonphytate P. Supplemental Thr and Met were supplemented to maintain SID Thr:SID Lys and SID TSAA:SID Lys of 0.70 and 0.76, respectively.

The AA composition in the diets was determined after acid hydrolysis (AOAC, 2002; Method 994.12). Total sulfur AA composition in the diets was determined after performic acid oxidation followed by acid hydrolysis (AOAC, 2002; Method 994.12). Nitrogen analysis to determine CP for diets was measured with a LECO nitrogen combustion analyzer (LECO Corp.,

Table 5.1. Percentage composition of corn-soybean meal diets for starter (0- to 10-d) and grower phase (10- to 24-d) broilers, as-fed basis¹

Item	Phase 1 diets					Phase 2 diet
	Supplemental Lys additions, %					Control
Ingredients	Control	0.05	0.10	0.15	0.20	Control
Corn	48.498	50.551	52.070	54.574	56.568	57.206
Soybean meal	42.496	40.493	38.569	36.567	34.570	33.797
Poultry fat	3.872	3.575	3.735	2.989	2.679	4.371
Monocalcium phosphate	1.710	1.725	1.740	1.753	1.767	1.538
Limestone	1.264	1.275	1.284	1.295	1.305	1.013
Sodium chloride	0.500	0.500	0.500	0.500	0.500	0.500
BMD + 3 nitro ²	0.500	0.500	0.500	0.500	0.500	0.500
Minerals mix ³	0.250	0.250	0.250	0.250	0.250	0.250
Vitamins mix ⁴	0.250	0.250	0.250	0.250	0.250	0.250
Biocox ⁵	0.050	0.050	0.050	0.050	0.050	0.050
Ethoxyquin ⁶	0.050	0.050	0.050	0.050	0.050	0.050
Choline chloride ⁷	0.050	0.050	0.050	0.050	0.050	0.050
DL-Met	0.269	0.287	0.306	0.322	0.340	0.244
Biolys ⁸	0.000	0.100	0.198	0.296	0.396	0.100
L-Thr	0.080	0.107	0.135	0.161	0.189	0.081
Cornstarch ⁹	0.161	0.237	0.313	0.393	0.536	-----
Calculated composition ¹⁰						
ME, kcal/kg	3,025	3,025	3,025	3,025	3,025	3,150
CP (%)	24.4 (23.4)	23.8 (22.8)	23.1 (23.1)	22.4 (22.2)	21.8 (21.2)	21.1 (21.2)
Ca (%)	1.05	1.05	1.05	1.05	1.05	0.90
Total P (%)	0.79	0.78	0.78	0.77	0.77	0.72
Nonphytate P (%)	0.50	0.50	0.50	0.50	0.50	0.45
K (%)	1.06	1.02	0.99	0.95	0.91	0.90
Cl (%)	0.36	0.36	0.36	0.36	0.36	0.36
Lys (%)	1.41 (1.27)	1.41 (1.26)	1.40 (1.32)	1.40 (1.33)	1.39 (1.28)	1.22 (1.13)
Met (%)	0.63 (0.68)	0.64 (0.65)	0.65 (0.65)	0.66 (0.62)	0.67 (0.64)	0.57 (0.55)
Met+Cys (%)	1.04 (1.05)	1.04 (1.01)	1.04 (1.01)	1.03 (0.98)	1.03 (0.99)	0.92 (0.88)
Thr (%)	1.01 (0.94)	1.00 (0.95)	1.00 (0.95)	1.00 (0.95)	0.99 (0.92)	0.87 (0.85)
Val (%)	1.15 (1.08)	1.12 (0.99)	1.08 (1.04)	1.05 (1.04)	1.01 (0.97)	0.99 (0.97)

(Table 5.1 continued)

Arg (%)	1.66 (1.50)	1.60 (1.42)	1.54 (1.44)	1.48 (1.42)	1.42 (1.32)	1.39 (1.32)
Ile (%)	1.05 (0.98)	1.02 (0.91)	0.98 (0.94)	0.94 (0.94)	0.91 (0.87)	0.89 (0.89)
SID Lys (%)	1.27	1.27	1.27	1.27	1.27	1.10
SID Met (%)	0.60	0.61	0.62	0.63	0.64	0.54
SID Met+Cys (%)	0.94	0.94	0.94	0.94	0.94	0.84
SID Thr (%)	0.89	0.89	0.89	0.89	0.89	0.77
SID Val (%)	1.04	1.01	0.98	0.94	0.91	0.90
SID Arg (%)	1.52	1.46	1.41	1.35	1.30	1.27
SID Ile (%)	0.97	0.94	0.90	0.87	0.84	0.82

¹Five basal diets for Phase 1 were mixed to contain the minimum of all ingredients except for supplemental Gly and cornstarch, which were added as needed to each diet.

²Bacitracin methylene disalicylate + 3-nitro-4-hydroxyphenylarsonic acid from Nutra Blend (Neosho, MO).

³Provided per kilogram of diet: Cu (copper sulfate), 7 mg; I (calcium iodate), 1 mg; Fe (ferrous sulfate), 50 mg; manganese (manganese sulfate), 100 mg; Se (sodium selenite), 0.15 mg; Zn (zinc sulfate), 75 mg.

⁴Provided per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B₁₂, 0.02 µg; biotin, 0.1 µg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg.

⁵Biocox provides 132.3 g/kg salinomycin sodium (Roche, Parsippany, NJ).

⁶Ethoxyquin provides 66.6% 6-ethoxy-1,2-dihydro-2,2,4-trimethylquionine (Novus International, St. Louis, MO).

⁷Contains 600,000 mg/kg of choline chloride.

⁸Contained 50.7% L-Lys (Evonik Corporation, Kennesaw, GA).

⁹Cornstarch was added to diets 1 to 5, and cornstarch was replaced with 0.161, 0.237, 0.313, 0.386, and 0.462% supplemental Gly in diets 6 to 10.

¹⁰SID = standardized ileal digestible. Levels were calculated based on digestibility coefficients in NRC (1994). Analyzed values for AA are in parentheses.

St. Joseph, MI) according to the Dumas procedure.

Statistical Analysis

Data were analyzed by ANOVA as completely randomized design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Each pen of broilers served as the experimental unit. Treatment differences were considered significant with an $\alpha = 0.10$. Contrast statements were used to determine significant effects of sex, Gly, supplemental Lys (linear and quadratic), Gly \times supplemental Lys (linear and quadratic). Treatment \times sex interactions were initially included in the statistical model; however, these contrasts were not significant for ADG, ADFI, and G:F and were removed from the model. The PDIFF option of SAS was used to compare individual diets to the control diet (C-SBM diet without supplemental Lys or Gly; diet 1).

RESULTS

The AA analysis of the diets closely agrees with the formulated values (Tables 5.1 and 5.2). Analyzed values for total Gly+Ser for diets without Gly supplementation (Diets 1 to 5) and with Gly supplementation (Diets 6 to 10) were all slightly less than calculated values.

Table 5.2. Calculated and analyzed percentage of Gly+Ser in starter (0- to 10-d) phase diets fed to Ross 708 broilers.

Item ¹	Supplemental Lys additions, %				
	0.00	0.05	0.10	0.15	0.20
Diets without supplemental Gly (1 to 5)					
Calculated Gly+Ser (%)	2.26	2.19	2.11	2.04	1.96
Analyzed Gly+Ser (%)	2.03	1.98	1.99	1.95	1.82
Diets with supplemental Gly (6 to 10)					
Calculated Gly+Ser (%)	2.42	2.42	2.42	2.42	2.42
Analyzed Gly+Ser (%)	2.26	2.22	2.19	2.25	2.22

¹ Cornstarch was added to diets 1 to 5, and cornstarch was replaced with 0.161, 0.237, 0.313, 0.386, and 0.462% supplemental Gly in diets 6 to 10, respectively.

Phase 1 (d 0 to 10)

Daily feed intake was greater in male broilers than in female broilers ($P = 0.001$; data not shown). Male broilers had decreased G:F compared with female broilers ($P = 0.018$; data not

shown). Daily gain was not affected by any dietary treatment ($P > 0.10$; Table 5.3). Broilers fed the 0.20% supplemental Lys diet had decreased ADFI (quadratic, $P = 0.022$) compared with broilers fed the control diet, but ADFI of broilers fed the lower inclusion levels of supplemental Lys was not affected ($P > 0.10$). Broilers fed diets with supplemental Gly had decreased ADFI compared with broilers fed diets without supplemental Gly except for broilers that were fed the 0.10% supplemental Lys diet (Gly \times Lys, $P = 0.062$). Broilers fed increasing levels of supplemental Lys had decreased G:F, except broilers fed the 0.15 or 0.20% supplemental Lys diets (quadratic, $P = 0.019$).

Phase 2 (d 11 to 24)

The purpose of P2 was to determine the subsequent effects of supplementing Lys and Gly in P1. Daily gain, ADFI, and G:F were greater in male broilers than in female broilers ($P < 0.07$; data not shown). Phase 1 treatments did not affect ADG during P2 ($P > 0.10$). Broilers that were fed diets with increasing supplemental Lys in P1 had decreased ADFI in P2 (linear, $P < 0.01$); however, this decrease in ADFI was not similar when Gly was supplemented to the diets (Gly \times Lys, $P < 0.01$; Figure 5.1). Broilers fed diets with supplemental Gly in P1 had decreased ADFI at the lower inclusion levels of Lys and increased ADFI at the 0.15 and 0.20% supplemental Lys levels. Broilers fed the increasing Lys supplemented diets in P1 had increased G:F in P2 (linear, $P = 0.082$), but the increase in G:F tended to be most evident with broilers fed 0% supplemental Lys with supplemental Gly or 0.20% supplemental Lys level with or without supplemental Gly (Gly, $P = 0.109$; Lys \times Gly, $P = 0.103$).

Overall (d 0 to 24)

Overall ADG and ADFI were greater in male broilers than in female broilers ($P = 0.001$; data not shown). Broilers that were fed diets with increasing supplemental Lys in P1 had decreased overall ADFI (linear, $P = 0.014$; Table 5.4); however, this decrease in overall ADFI

was not similar when Gly was supplemented to the P1 diets (Gly \times Lys, $P = 0.011$). Broilers fed diets with supplemental Gly in P1 had decreased overall ADFI at the lower inclusion levels of Lys and increased ADFI at the 0.10, 0.15 and 0.20% supplemental Lys levels (Gly, $P = 0.057$). Broilers fed the increasing Lys supplemented diets in P1 had increased overall G:F (linear, $P = 0.02$), but the increase in G:F was most evident with broilers fed 0% supplemental Lys with supplemental Gly or 0.20% supplemental Lys level with or without supplemental Gly (Gly, $P = 0.072$; Lys \times Gly, $P = 0.078$).

Table 5.3. Growth performance of d 0 to 10 (Phase 1) broiler chicks fed supplemental Lys without and with supplemental Gly in corn-soybean meal based diets^{1,2}

Treatments	ADG, g	ADFI, g	G:F, g/g
1) C-SBM (Control)	18.18	25.33	0.723
2) C-SBM with 0.05% supplemental Lys	17.41	24.63	0.710
3) C-SBM with 0.10% supplemental Lys	17.94	23.93 ^a	0.750
4) C-SBM with 0.15% supplemental Lys	17.48	24.25	0.725
5) C-SBM with 0.20% supplemental Lys	17.55	22.70 ^a	0.773 ^a
6) Diet 1 + supplemental Gly ³	17.34 ^a	22.64 ^a	0.769 ^a
7) Diet 2 + supplemental Gly	17.08 ^a	23.33 ^a	0.732
8) Diet 3 + supplemental Gly	17.72	25.03	0.712
9) Diet 4 + supplemental Gly	17.47	23.20 ^a	0.753
10) Diet 5 + supplemental Gly	17.38	22.46 ^a	0.774 ^a
SEM	0.340	0.568	0.018
Overall treatment P -value	0.546	0.003	0.076
Sex	0.108	0.001	0.018
Gly	0.153	0.024	0.310
Supplemental Lys (linear)	0.642	0.019	0.075
Gly \times supplemental Lys (linear)	0.275	0.047	0.302
Supplemental Lys (quadratic)	0.937	0.022	0.019
Gly \times supplemental Lys (quadratic)	0.490	0.062	0.223

^a Significantly different ($P < 0.10$) from the control.

¹ Data are means of 6 replications (3 male and 3 female pens) with 25 or 20 broilers per pen. All diets were formulated to contain 1.27% standardized ileal digestible Lys.

² Treatment \times sex interactions were NS for ADG, ADFI, and G:F; therefore, it was removed from the statistical model.

³ Supplemental Gly was added to achieve a total Gly+Ser level of 2.42%.

Table 5.4. Subsequent growth performance of d 10 to 24 (Phase 2) and d 0 to 24 (overall) broiler chicks fed a common corn-soybean meal (C-SBM) diet that were previously fed diets with supplemental Lys without and with supplemental Gly¹

Phase 1 treatments ²	Phase 2 (d 10 to 24)			Overall (d 0 to 24)		
	ADG, g	ADFI, g	G:F, g/g	ADG, g	ADFI, g	G:F, g/g
1) C-SBM (Control)	51.77	79.65	0.650	37.29	56.64	0.659
2) C-SBM with 0.05% supplemental Lys	50.97	77.85	0.654	36.64	55.53	0.660
3) C-SBM with 0.10% supplemental Lys	50.77	77.33 ^a	0.657	36.83	54.87 ^a	0.671
4) C-SBM with 0.15% supplemental Lys	51.32	76.24 ^a	0.673 ^a	37.17	54.54 ^a	0.682 ^a
5) C-SBM with 0.20% supplemental Lys	50.23	74.78 ^a	0.672 ^a	36.48	53.05 ^a	0.687 ^a
6) Diet 1 + supplemental Gly ³	50.94	75.72 ^a	0.673 ^a	36.71	43.42 ^a	0.688 ^a
7) Diet 2 + supplemental Gly	50.85	76.15 ^a	0.667	36.51	53.97 ^a	0.676
8) Diet 3 + supplemental Gly	51.13	76.76 ^a	0.666	37.06	55.09	0.673
9) Diet 4 + supplemental Gly	51.38	77.32 ^a	0.665	37.00	54.62 ^a	0.677
10) Diet 5 + supplemental Gly	50.80	75.15 ^a	0.675 ^a	36.86	53.19 ^a	0.693 ^a
SEM	0.720	0.924	0.008	0.529	0.697	0.008
Overall treatment <i>P</i> -value	0.958	0.024	0.317	0.981	0.015	0.081
Sex	0.001	0.001	0.069	0.001	0.001	0.586
Gly	0.988	0.111	0.109	0.875	0.057	0.072
Supplemental Lys (linear)	0.445	0.009	0.082	0.896	0.014	0.020
Gly × supplemental Lys (linear)	0.359	0.008	0.103	0.434	0.011	0.078
Supplemental Lys (quadratic)	0.825	0.297	0.394	0.879	0.114	0.120
Gly × supplemental Lys (quadratic)	0.756	0.277	0.508	0.848	0.172	0.255

^a Significantly different ($P < 0.10$) from the control.

¹ Data are means of 6 replications (3 male and 3 female pens) with 25 or 20 broilers per pen. Treatment × sex interactions were NS for ADG, ADFI, and G:F; therefore, it was removed from the statistical model.

² Phase 2 was conducted feeding a common C-SBM diet with 0.05% supplemental Lys to determine subsequent effects of Phase 1 treatments. All diets in Phase 2 were formulated to contain 1.10% standardized ileal digestible Lys.

³ Supplemental Gly was added to achieve a total Gly+Ser level of 2.42%.

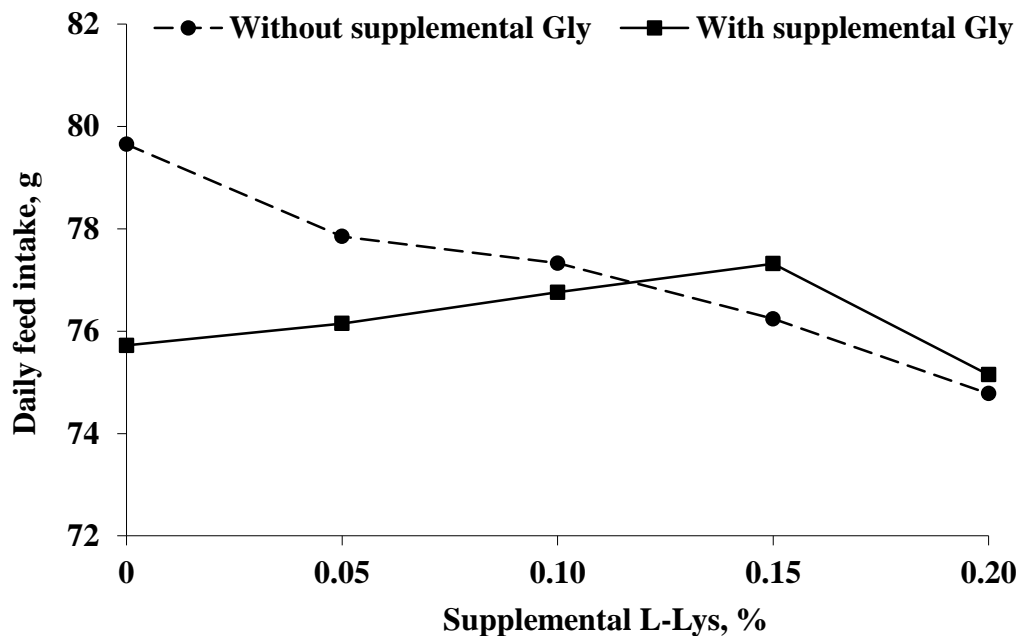


Figure 5.1. The subsequent effects of feeding increasing levels of supplemental Lys without and with supplemental Gly during Phase 1 (d 0 to 10) on average daily feed intake of Phase 2 (d 10 to 24) broilers (Gly \times Lys, $P < 0.01$).

DISCUSSION

One of the purposes of this research was to determine the maximum level of supplemental Lys that could be included in C-SBM diets for broiler chicks before an AA other than Met, Thr, and Lys becomes limiting and decreases growth performance. Previous research determined that feeding broilers a C-SBM diet supplemented with 0.20% L-Lys along with DL-Met, L-Thr, and Gly could support similar performance to broilers fed a C-SBM diet without supplemental Lys or Gly (Waguespack et al., 2009a). The results of this experiment indicate that up to 0.15% supplemental Lys can be added to a C-SBM diet without reducing growth performance of d 0 to 10 broilers. This decrease in maximum Lys supplementation compared with previous research may be due to the increased level of dietary Lys in this experiment.

The decrease in ADFI with broilers fed the 0.20% supplemental Lys diet may be due to a marginally deficient AA limiting growth performance. Most likely this AA limiting ADFI might

be Val. Previously within our lab, we determined that feeding broilers a C-SBM diet with 0.20% supplemental Lys with minimum ratios of 1.01 Arg:Lys, 0.73 Val:Lys, and 0.65 Ile:Lys was still able to support growth performance equal to broilers fed a control, C-SBM diet. In this experiment, the Arg:Lys and Ile:Lys in the diet with 0.20% supplemental Lys were at or above these values on a calculated or analyzed basis. However, the Val:Lys in the diet with 0.20% supplemental Lys was 0.727 (calculated) and 0.758 (analyzed). Berres et al. (2010) reported similar BW gain and feed intake when male broilers were fed a C-SBM diet with 0.20% supplemental Lys with Val or Val + Gly.

Broilers fed diets with supplemental Gly had decreased ADFI in P1 compared with broilers fed diets without supplemental Gly except for broilers that were fed the 0.10% supplemental Lys diet. We do not know the reason for the increased ADFI for the broilers fed the 0.10% supplemental Lys diet.

Supplementing Gly in the P1 diets resulted in the carryover effect of increased G:F of broilers in P2 and overall. The increased G:F may be due to Gly increasing the utilization of AA. Previous research has been shown that the endogenous rate of Gly synthesis in the broiler chick cannot maintain optimal growth performance (Featherson, 1976). Graber and Baker (1973) similarly report that chicks are able to synthesize approximately 70% of Gly needed for optimal growth performance. Providing Gly in the diet allows the broiler chick to not have to produce as much Gly de novo. Also, Gly is used in the formation of uric acid to facilitate N excretion in the excreta. To remove 4 N moles (in 1 mole of uric acid), 15 ATPs are used and of these 15, 9 ATPs come from Gly synthesis (Stevens, 1996). The increased G:F in broilers fed diets with supplemental Gly in the starter phase may allow broilers to divert more energy towards protein synthesis instead of using energy for general maintenance needs.

The results of this experiment indicate that up to 0.15% supplemental Lys can be added to a C-SBM diet without or with Gly supplementation without reducing growth performance of d 0 to 10 broilers; however, the Gly addition to the 0.15% supplemental Lys diet improved G:F of P1 broilers. Broilers fed diets with supplemental Gly during P1 had decreased ADFI, but increased G:F in P2. These responses were most evident in broilers previously fed diets supplemented with 0 to 0.10% Lys. These data suggest that providing Gly in the starter phase feed increases feed efficiency during the subsequent grower phase depending on the level of supplemental Lys fed.

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CHAPTER 6

VALINE AND ISOLEUCINE REQUIREMENT OF TWENTY- TO FORTY-FIVE-KILOGRAM PIGS

INTRODUCTION

To develop the most cost effective diets for swine, one option is to formulate diets to maximize supplemental AA inclusion that optimizes growth performance. Roux et al. (2011) determined that a low-CP, C-SBM diet with up to 0.23% supplemental Lys along with supplemental Thr, Trp, and Met could be fed to 20- to 45-kg pigs without reducing growth performance. The opportunity to further reduce CP via increasing supplemental AA in the diet necessitates that the requirement for the limiting AA beyond Lys, Thr, Trp, and Met be known. Figueroa et al. (2003) determined that Val was the fifth limiting AA followed by either His or Ile in a C-SBM diet with Lys, Met, Thr, and Trp supplementation. Similarly, Nørgaard and Fernández (2009) suggested that Val was limiting before Ile in a C-SBM, wheat, and barley diet. Based on these results, it is then necessary to estimate the requirements for Val and Ile as the next-limiting AA. Valine and Ile may not be economically feasible to add in swine diets currently, but establishing the requirements can help set limits or minimums in the feed matrix, as well as enable nutritionists to use these AA quicker once they are available.

Many researchers have recently determined the Ile requirement of pigs (7- to 109-kg) in diets containing SDBC, which contains high amounts of Lys, Leu, and Val and is deficient in Ile (see references in Table 6.1). Determining the Ile requirement in diets containing SDBC results in an overestimation of the Ile requirement because of the imbalance of branch chained AA (Dean et al., 2005). Unfortunately, there is limited research determining the Ile requirement in AA-supplemented, C-SBM diets without SDBC for 20- to 45-kg pigs.

Table 6.1. Isoleucine requirement estimates of growing pigs¹

BW, kg			Composition of the diet	SID	SID	Reference
Mean	Initial	Final		Ile, %	Ile:Lys ²	
7.0	5.6	8.4	C-SBM + whey + SDPP + fishmeal + SDBC	0.69	---	James et al. (2000) ³
7.0	5.6	8.4	C-SBM + whey + SDPP + fishmeal + SDBC	0.62	59	James et al. (2000) ⁴
7.0	5.5	8.4	C-SBM + fishmeal + SDPP + SDBM	---	60	Bergström et al. (1996b)
8.3	6.6	9.9	C-SBM + whey + SDBC + fishmeal	0.61	---	Kerr et al. (2004) Exp. 2
8.4	5.1	11.6	Semi-purified diet (blood flour)	0.70	---	Becker et al. (1963)
8.8	6.6	10.9	C-SBM + whey + fishmeal + SDBC	0.65	---	Kerr et al. (2004) Exp. 1
10.8	5.8	15.7	Semi-purified diet (blood flour + SBM)	0.43	---	Oestemer et al. (1973)
15.5	7.7	23.2	Wheat + barley + corn + SDBC	0.60	59	Wiltafsky et al. (2009a) Exp. 1
15.9	9.9	21.9	Purified diet (lactose + sucrose)	---	60	Chung and Baker (1992)
16.2	11.1	21.2	C-SBM + wheat + barley	0.44	46	Barea et al. (2009b) Exp. 2 ⁵
17.0	12.0	22.0	C-SBM + supplemental AA	0.75	68	Fu et al. (2006) (Abstr) Exp. 2
17.0	12.0	22.0	C-SBM + SDBC	0.51	46	Fu et al. (2006) (Abstr) Exp. 2
17.2	12.5	21.9	Corn + wheat + barley (+ CGM or SBM + SDBC)	0.51	51	Barea et al. (2009b) Exp. 3 ⁶
17.5	11.4	23.5	C-SBM + SDBM	0.37	---	Bergström et al. (1996a)
17.5	11.4	23.5	C-SBM + SDBM	0.55	---	Bergström et al. (1996a)
18.2	8.0	26.2	Wheat + corn + CGM + barley	0.51	54	Wiltafsky et al. (2009a) Exp. 3
25.8	18.2	33.3	Semi-purified diet (blood flour)	0.51	---	Brinegar et al. (1950)
29.0	18.0	40.0	NA	0.50	---	Lenis and van Diepen (1997) (Abstr) ⁷
31.0	20.5	41.5	Semi-purified diet (SBM + blood flour)	0.26	---	Bravo et al. (1970)
34.7	27.0	42.3	C-SBM + SDBC	0.50	?	Parr et al. (2003) Exp. 2
37.5	27.5	47.5	Semi-purified diet (corn + casein)	---	60	Wang and Fuller (1989)
40.0	25.0	55.0	Barley + corn + blood meal + yeast	0.38	---	Taylor et al. (1985) ⁸
52.9	44.6	61.1	Semi-purified diet (blood flour)	0.34	---	Becker et al. (1963) Exp. 4
93.0	87.0	99.0	Corn + SDBC	0.31	---	Parr et al. (2004) Exp. 2
97.7	80.7	114.6	C-SBM	0.24	46	Dean et al. (2005) Exp. 4 & 5
100.3	88.5	112.0	C-SBM + supplemental AA	0.28	54	Fu et al. (2005a) (Abstr) Exp. 1 & 2
100.3	88.5	112.0	C-SBM + SDBC	0.32	61	Fu et al. (2005a) (Abstr) Exp. 1 & 2
101.6	85.3	117.8	Corn + SDBC	0.34	---	Dean et al. (2005) Exp. 3
103.5	91.0	116.0	Corn + BC	0.36	---	Kendall et al. (2004) (Abstr)
105.0	89.9	120.0	C-SBM + SDBC	0.31	60	Fu et al. (2005b) (Abstr) Exp. 1 ⁹
108.5	96.9	120.0	Corn + supplemental AA	0.27	52	Fu et al. (2005b) (Abstr) Exp. 2 ¹⁰

(Table 6.1 continued)

¹SID = standardized ileal digestible. C-SBM = corn and soybean meal. SDPP = spray dried plasma protein. SDBM = spray dried blood meal. SDBC = spray dried blood cells. CGM = corn gluten meal. BC = blood cells.

²Valid SID Ile:SID Lys was reported or calculated based on the diets being formulated to be at or below the Lys requirement for the respective BW of pigs.

³Diet was formulated at 1.26% apparent digestible Lys (1.47% total Lys).

⁴Diet was formulated at 1.00% apparent digestible Lys (1.17% total Lys).

⁵Estimated based on SID values from feed ingredients. The reported values represent the basal SID Ile and Lys values because there was no response to Ile supplementation.

⁶The reported values represent the average basal SID Ile and Lys values from the CGM and SBM + SDBC diets because there was no response to Ile supplementation.

⁷Data taken from Dean et al. (2005).

⁸A digestibility value of 88% was used to calculate SID Ile.

⁹Calculated an average SID Ile:Lys based on ADG and ADFI estimates of 50.7 and 53, respectively.

¹⁰Calculated an average SID Ile:Lys based on ADG and G:F estimates of 61.7 and 57.9, respectively.

Table 6.2. Valine requirement estimates of growing pigs¹

BW, kg			Composition of the diet	SID Val, %	SID	Reference
Mean	Initial	Final			Val:Lys ²	
7.6	5.8	9.4	C-SBM + whey + lactose	0.86	---	Mavromichalis et al. (2001) Exp. 4
9.9	8.4	12.0	C-SBM + 0.63% L-Lys•HCl	0.92	---	Gaines et al. (2006) (Abstr) Exp. 1
13.0	8.9	17.0	C-SBM + whey	0.67	60	James et al. (2001) (Abstr)
14.1	8.1	20.0	Barley + wheat + soy protein concentrate	**	---	Theil et al. (2004) ³
14.4	8.1	20.6	C-SBM + barley + whey + tapioca	0.70	68	Warnants et al. (2001)
14.8	7.9	21.6	C-SBM + wheat + barley	0.65	66	Wiltafsky et al. (2009b) Exp. 2
15.1	10.9	19.2	Corn + whey + lactose + peanut meal	0.78	---	Mavromichalis et al. (2001) Exp. 5
15.9	9.9	21.9	Purified diet (lactose + sucrose)	---	68	Chung and Baker (1992)
16.0	11.9	20.0	C-SBM + 0.70% L-Lys•HCl	0.78	---	Gaines et al. (2006) (Abstr) Exp. 2
17.8	12.8	22.7	C-SBM + wheat + barley	0.64	70	Barea et al. (2009a) Exp. 4
18.8	14.1	23.4	C-SBM + wheat + barley	0.64	65	Wiltafsky et al. (2009b) Exp. 3
20.2	13.5	26.9	C-SBM	0.72	65	Gaines et al. (2011) Exp. 3
20.8	12.7	28.9	Corn + crystalline AA	0.37	---	Jackson et al. (1953)
27.0	21.4	32.6	C-SBM	0.72	65	Gaines et al. (2011) Exp. 2
37.5	27.5	47.5	Semi-purified diet (Corn + casein)	---	75	Wang and Fuller (1989)
73.5	67.0	80.0	Corn + cornstarch + gelatin	0.38	---	Lewis and Nishimura (1995) Exp. 2
---	59.8	---	C-SBM	0.37	53	Liu et al. (2000) (Abstr) Exp. 2

¹C-SBM = corn and soybean meal.

²Valid SID Val:SID Lys was reported or calculated based on the diets being formulated to be at or below the Lys requirement for the respective BW of pigs.

³Theil et al. (2004) reported an estimated requirement of 0.59 g AID Val/MJ of ME.

Many researchers have estimated the Val requirement in weanling (8- to 20-kg) pigs fed complex, highly digestible diets (see references in Table 6.2); however, there is limited and conflicting research estimating the Val requirement of grower (20- to 50-kg) pigs. Wang and Fuller (1989) estimated the SID Val:Lys for 27.5- to 47.5-kg pigs to be 0.75 in a semi-purified diet. Gaines et al. (2011) estimated the SID Val:Lys for 21.4- to 32.6-kg pigs to be 0.65 in a C-SBM diet. Because of the differing estimates, it would be beneficial to clarify conflicting research estimating the Val requirement.

In previous research within our lab, Roux et al. (2011) determined that 0.83% SID Lys was the Lys requirement of our 20- to 50-kg pigs. When developing diets to estimate AA requirements, it is important that diets are formulated at or below the Lys requirement (Southern et al., 2010). Through a series of experiments, Powell et al. (2011) validated a C-SBM diet, with 0.335% supplemental Lys that also contained supplemental Thr, Trp, Met, Gly, and Glu, that results in similar growth performance to a C-SBM diet that contains no supplemental AA. Supplementation of Val and Ile to the low CP, AA-supplemented diet also has been shown to result in similar growth performance to that of pigs fed a positive control diet. Therefore, this diet is an excellent diet to determine the Ile and Val requirement of pigs. The purpose of this research was to determine the Val and Ile requirement in an AA-supplemented, C-SBM diet for 20- to 45-kg pigs.

MATERIALS AND METHODS

General

Pigs (Yorkshire, Yorkshire \times Landrace, or Yorkshire \times Landrace \times Duroc) were obtained from the Louisiana State Agricultural Center Swine Unit. Pigs were housed in a completely enclosed building in 1.32×2.44 m pens with metal-slotted floors, one nipple waterer, and a 2-hole stainless-steel feeder with lids. Feed in meal form and water were provided ad libitum. All

experimental procedures were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

In all experiments, pigs were blocked by initial BW and allotted to treatments in a randomized complete block design. Littermates were balanced across treatments. To determine ADG, ADFI, and G:F, all pigs and feeders were weighed on d 0, 14, and 26 or 27. Before allotment to treatments (Exp. 1) and at end of each experiment, blood samples were collected from the anterior vena cava from each pig per pen. Blood was collected and placed into 10 mL tubes containing sodium heparin (BD Vacutainer, Franklin Lakes, NJ). Blood samples were placed on ice before centrifugation at $3,000 \times g$ at 0°C for 20 min. After centrifugation, plasma was removed and kept frozen at -80°C until analysis for PUN concentration (Mathies, 1964) using commercial reagent kits (Pointe Scientific, Canton, MI).

In all experiments, diets were C-SBM based. Dietary treatments were formulated on a SID basis using coefficients from AminoDat[®] 3.0 (Evonik Degussa GmbH, Hanau, Germany). All diets contained supplemental Thr, Trp, and Met to maintain SID Thr:Lys, SID Trp:Lys, and SID TSAA:Lys of 0.71, 0.20, and 0.62, respectively. All diets were formulated to contain 0.335% supplemental L-Lys, 0.53% Gly, and 1.027% Glu to achieve 0.83% SID Lys, 1.66% Gly+Ser, and 3.28% Glu, which is equal to the levels in the previously validated positive control diet (Roux et al., 2011; Powell et al., 2011).

For each experiment, total AA composition of diets was determined after acid hydrolysis (method 994.12; AOAC International, 2002a) to verify calculated composition as outlined above. Total sulfur AA content was determined after performic acid oxidation followed by acid hydrolysis (method 994.12; AOAC International, 2002a). Tryptophan content was determined after alkaline hydrolysis (method 988.15; AOAC International, 2002b).

Experiment 1

Experiment 1 was conducted to determine the Val requirement for 20- to 45-kg pigs fed an AA-supplemented, C-SBM diet. A total of 102 pigs with an average initial BW of 21.8 ± 2.9 kg were allotted to 6 dietary treatments. Each treatment was replicated with 5 pens of 3 or 4 pigs per replicate pen. Replicates 1 and 2 contained 2 barrows and 2 gilts, and replicates 3, 4, and 5 contained 2 barrows and 1 gilt. All diets contained 0.065% supplemental Ile to maintain 0.50% SID Ile (60% SID Ile:Lys) in the diet, which is 110% of NRC (1998) recommendation for 20- to 50-kg pigs. Dietary treatments were formulated to contain 0, 0.02, 0.04, 0.06, 0.08, or 0.10% supplemental Val to achieve 0.51, 0.53, 0.55, 0.57, 0.59, or 0.61% SID Val.

Experiments 2 and 3

Experiments 2 and 3 were conducted to determine the Ile requirement for 20- to 45-kg pigs fed an AA-supplemented, C-SBM diet. Pigs with an average initial BW of 24.4 ± 2.8 (Exp. 2; n=100) or 20.6 ± 2.7 kg (Exp. 3; n=120) were allotted to 5 dietary treatments. Each treatment was replicated with 5 (Exp. 2) or 6 (Exp. 3) pens of 4 pigs (2 barrows and 2 gilts) per replicate pen. In both experiments, all diets contained 0.106% supplemental Val to maintain 0.61% SID Val (0.74% SID Val:Lys) in the diet, which is 110% of NRC (1998) recommendation for 20- to 50-kg pigs. Dietary treatments were formulated to contain 0, 0.02, 0.04, 0.06, or 0.08% supplemental Ile to achieve 0.43, 0.45, 0.47, 0.49, or 0.51% SID Ile. The data from these two experiments were combined.

Statistical Analysis

Data were analyzed by ANOVA as randomized complete block designs with initial BW as the blocking factor using the MIXED procedure of SAS (Littell et al., 1996; SAS Inst., Inc., Cary, NC). Each pen of pigs served as the experimental unit. Treatment differences were considered significant with an $\alpha = 0.10$. In Exp. 1, treatment was considered a fixed effect, and

replicate was considered a random effect. Baseline PUN was determined before allotment to treatment and analyzed as a covariate on subsequent post-treatment PUN using MIXED procedures of SAS.

Experiments 2 and 3 were combined for statistical analysis. Treatment \times trial was initially included in the model but was not significant for any response variables ($P = 0.13$ to 0.95); therefore, it was removed from the model. For the combined data, treatment was considered a fixed effect, and trial and replicate within trial were considered random effects. Baseline PUN was not determined in Exp. 2 and 3.

Orthogonal contrasts were used to determine significant linear and quadratic effects, and the PDIFF option of SAS was used to compare individual diets to the negative control diet. Overall treatments and replicate pen means were analyzed by broken-line analysis using NLIN procedure of SAS to estimate the Val and Ile requirements (Robbins et al., 2006).

The SID AA:SID Lys data from Tables 1 and 2 were analyzed independently as simple linear regressions to determine the relationship between SID AA:Lys and increasing BW of pigs.

RESULTS

The AA analysis of the diets closely agreed with the formulated values (Tables 6.3 and 6.4); therefore, the formulated values are used for determination of requirements and for discussion purposes. For all 3 experiments, the results for growth performance will be presented for the 0- to 14-d and 14- to 26- or 27-d periods in the tables, but only the overall data (d 0 to 26 or 27) are discussed in the results.

Experiment 1

Valine addition increased ADG, ADFI, and G:F in pigs fed 0.51 to 0.59% SID Val (linear, $P < 0.08$; Table 6.5), but ADG and ADFI were decreased at 0.61% SID Val (quadratic, $P \leq 0.10$). Daily gain was increased among pigs fed diets with 0.55 to 0.61% SID Val ($P < 0.10$)

Table 6.3. Composition of negative control diet that was fed to 20- to 45-kg grower pigs in experiments 1, 2, and 3, as-fed basis¹

Item	Negative control (NC)	
	Exp. 1	Exp. 2 and 3
Ingredient, %		
Corn ²	79.913	79.890
Soybean meal ³	13.368	13.369
Monocalcium phosphate	1.347	1.348
Limestone	1.128	1.127
Vitamin premix ⁴	0.380	0.380
Sodium chloride	0.500	0.500
Sodium bentonite ⁵	0.500	0.500
Trace mineral premix ⁶	0.100	0.100
Biolys ⁷	0.660	0.660
L-Thr ⁸	0.192	0.192
DL-Met ⁸	0.138	0.138
L-Trp ⁸	0.052	0.052
Gly	0.530	0.530
L-Glu	1.027	1.027
L-Ile	0.065	----
L-Val	----	0.106
Cornstarch	0.100	0.080
Calculated composition ⁹		
ME, kcal/kg	3,210	3,210
CP, %	14.59	14.63
Ca, %	0.70	0.70
P, %	0.60	0.60
Lys, %	0.92 (0.92)	0.92 (0.93)
Met, %	0.35 (0.35)	0.35 (0.37)
TSAA, %	0.58 (0.60)	0.58 (0.61)
Thr, %	0.66 (0.63)	0.66 (0.65)
Trp, %	0.19 (0.18)	0.19 (0.18)
Ile, %	0.56 (0.56)	0.49 (0.50)
Val, %	0.59 (0.60)	0.69 (0.71)
Arg, %	0.76 (0.78)	0.76 (0.76)
Leu, %	1.23 (1.24)	1.23 (1.18)
Gly+Ser, %	1.66 (1.61)	1.66 (1.67)
Glu, %	3.28 (3.17)	3.28 (3.27)
SID Lys, ¹⁰ %	0.83	0.83
SID Met, %	0.33	0.33
SID TSAA, %	0.52	0.52
SID Thr, %	0.59	0.59
SID Trp, %	0.17	0.17
SID Ile, %	0.50	0.43
SID Val, %	0.51	0.61
SID Arg, %	0.69	0.69
SID Leu, %	1.08	1.08

(Table 6.3 continued)

SID Val:SID Lys	0.61	0.74
SID Ile:SID Lys	0.60	0.52

¹ A basal diet was mixed to contain the minimum of all ingredients except for supplemental L-Val (Exp. 1) or L-Ile (Exp. 2 and 3) and cornstarch, which were added as needed to each diet.

^{2,3} Nutrient values based on Evonik values (Kennesaw, GA).

⁴ Provided the following per kilogram of diet: vitamin A, 8,378 IU; vitamin D₃, 2,513 IU; vitamin E, 67 IU; menadione (menadione dimethylpyrimidinol bisulfite), 6.3 mg; riboflavin, 10.1 mg; pantothenic acid, 37.7 mg; niacin, 67 mg; vitamin B₁₂, 46 µg; biotin, 335 µg; folic acid, 2.5 mg; pyridoxine, 3.4 mg; thiamin, 3.4 mg; and vitamin C, 84 µg.

⁵ AB-20, provided by Prince Agri Products, Inc, Quincy, IL.

⁶ Provided the following per kilogram of diet: Zn, 127 mg as zinc sulfate; Fe, 127 mg as ferrous sulfate; Mn, 20 mg as manganese sulfate; Cu, 12.7 mg as copper sulfate; I, 0.80 mg as calcium iodate; and Se, 0.30 mg as sodium selenite with calcium carbonate as the carrier.

⁷ Biolys was obtained from Evonik Degussa Corporation (Kennesaw, GA) and contains 50.7% L-Lys·SO₄.

⁸ L-Thr, DL-Met, and L-Trp were provided by Evonik Degussa Corporation (Kennesaw, GA).

⁹ Analyzed values for AA are in parentheses.

¹⁰ SID = standardized ileal digestible.

Table 6.4. Valine and isoleucine content of diets in experiments 1, 2, and 3

Item ¹	Supplemental Val additions, %				
	0.02	0.04	0.06	0.08	0.10
Exp. 1					
Calculated values					
SID Val, %	0.53	0.55	0.57	0.59	0.61
Val, %	0.61	0.63	0.65	0.67	0.69
Analyzed values					
Val, %	0.64	0.63	0.66	0.68	0.70
	Supplemental Ile additions, %				
	0.02	0.04	0.06	0.08	
Exp. 2					
Calculated values					
SID Ile, %	0.45	0.47	0.49	0.51	
Ile, %	0.51	0.53	0.55	0.57	
Analyzed values					
Ile, %	0.52	0.54	0.58	0.61	
Exp. 3					
Calculated values					
SID Ile, %	0.45	0.47	0.49	0.51	
Ile, %	0.51	0.53	0.55	0.57	
Analyzed values					
Ile, %	0.51	0.54	0.57	0.58	

¹ SID = standardized ileal digestible.

compared with pigs fed the 0.51% SID Val diet. Daily feed intake was increased among pigs fed diets with 0.53, 0.57, or 0.59% SID Val ($P < 0.10$) compared with pigs fed the 0.51% SID Val diet. Plasma urea N was not affected by SID Val additions ($P > 0.10$). Using broken-line analysis on treatment and pen mean values for ADG and G:F, the SID Val requirements were 0.58 ($P < 0.03$ and 0.08; Figure 6.1) and 0.56% ($P = 0.03$ and 0.63), respectively.

Experiments 2 and 3

Daily gain, ADFI, and G:F were not affected by SID Ile addition ($P > 0.10$; Table 6.6); however, ADFI was decreased and G:F was increased in pigs fed the diet with 0.45% SID Ile ($P < 0.10$) compared with pigs fed the 0.43% SID Ile diet, but pigs fed levels of Ile greater than 0.45% were not affected by diet. Plasma urea N was not affected by SID Ile additions ($P > 0.10$). A requirement estimate could not be made with broken-line analysis using ADG, ADFI, G:F, or PUN due to lack of response to graded levels of Ile.

DISCUSSION

The purpose of this research was to determine the Val and Ile requirement in an AA-supplemented, C-SBM diet for 20- to 45-kg pigs. The initial step to determine the Val or Ile ratio to Lys was to formulate diets to be at or below the Lys requirement (Southern et al., 2010). Roux et al. (2011) determined that the Lys requirement of our 20- to 45-kg pigs was 0.83% SID, which is in agreement with NRC recommendations for 20- to 50-kg pigs. The next step is to develop a diet that is limiting in Val and Ile in order to achieve an improvement in growth performance with supplemental addition.

Roux et al. (2011) validated a C-SBM diet, with 0.335% supplemental Lys (+ supplemental Thr, Trp, Met, Val, and Ile) that results in similar growth performance to a positive control, C-SBM diet that contains no supplemental AA, but growth performance was reduced

Table 6.5. The effects of increasing standardized ileal digestible (SID) valine on growth performance and plasma urea N (PUN) of 20- to 45-kg pigs from experiment 1¹

Item	SID Val, %						SED ²	Linear	Quad
	0.51	0.53	0.55	0.57	0.59	0.61			
Initial BW, kg	21.82	21.76	21.76	21.70	21.84	21.92	0.22	0.578	0.380
14-d BW, kg	30.15	31.21	31.31 ⁴	31.70 ⁴	32.71 ⁴	32.02 ⁴	0.66	0.002	0.242
26-d BW, kg	40.50	42.02 ⁴	42.14 ⁴	42.88 ⁴	43.39 ⁴	42.43 ⁴	0.85	0.009	0.063
d 0 to 14									
ADG, g	595	677 ⁴	683 ⁴	714 ⁴	776 ⁴	721 ⁴	42	0.001	0.107
ADFI, g	1,512	1,685 ⁴	1,616	1,703 ⁴	1,760 ⁴	1,656 ⁴	70	0.022	0.067
G:F	0.392	0.407	0.423	0.419	0.445 ⁴	0.437 ⁴	0.02	0.007	0.552
d 14 to 26									
ADG, g	868	879	902	931	890	896	45	0.456	0.338
ADFI, g	2,115	2,289 ⁴	2,237	2,235	2,305 ⁴	2,213	94	0.348	0.183
G:F	0.410	0.389	0.406	0.418	0.388	0.405	0.02	0.880	0.986
Overall, d 0 to 26									
ADG, g	720	776	784 ⁴	814 ⁴	829 ⁴	803 ⁴	35	0.008	0.103
ADFI, g	1,788	1,956 ⁴	1,903	1,949 ⁴	2,011 ⁴	1,908	74	0.078	0.076
G:F	0.398	0.398	0.413	0.419	0.415	0.422	0.02	0.069	0.688
PUN, mg/dL ³	6.88	7.30	7.43	7.71	7.80	6.78	0.57	0.768	0.120

¹Data are means of 5 replicates with 3 or 4 pigs per pen (replicates 1 and 2 had 2 barrows and 2 gilts; replicates 3, 4, and 5 had 2 barrows and 1 gilt). Average initial and 26-d weights were 21.8 and 42.2 kg, respectively.

²Standard error difference.

³Baseline PUN was determined before allotment to treatment and was analyzed as a covariate.

⁴Significantly different ($P < 0.10$) from pigs fed the diet with 0.51% SID Val (negative control).

Table 6.6. The effects of increasing standardized ileal digestible (SID) isoleucine on growth performance and plasma urea N (PUN) of 22- to 44-kg pigs¹

Item	SID Ile, %					SED ²	Linear	Quad
	0.43	0.45	0.47	0.49	0.51			
d 0 to 14								
ADG, g	743	751	764	761	764	16	0.156	0.568
ADFI, g	1,791	1,765	1,760	1,781	1,757	35	0.511	0.753
G:F	0.416	0.429	0.436 ³	0.429	0.437 ³	0.01	0.028	0.330
d 14 to 26 (Exp. 2) or 27 (Exp. 3)								
ADG, g	873	848	811 ³	853	857	19	0.505	0.011
ADFI, g	2,305	2,167 ³	2,223	2,298	2,286	53	0.428	0.066
G:F	0.381	0.398 ³	0.369	0.373	0.378	0.01	0.174	0.786
d 0 to 26 (Exp. 2) or 27 (Exp. 3)								
ADG, g	805	804	786	805	808	14	0.778	0.233
ADFI, g	2,033	1,956 ³	1,979	2,025	2,007	40	0.860	0.194
G:F	0.397	0.415 ³	0.399	0.399	0.405	0.01	0.984	0.614
PUN, mg/dL	6.93	7.68	7.54	7.54	7.47	0.57	0.466	0.327

¹Data are means of 11 replicates with 4 pigs per pen (2 barrows and 2 gilts). Average initial and final weights were 22.4 and 43.6 kg, respectively.

²Standard error difference.

³Significantly different ($P < 0.10$) from pigs fed the diet with 0.43% SID Ile (negative control).

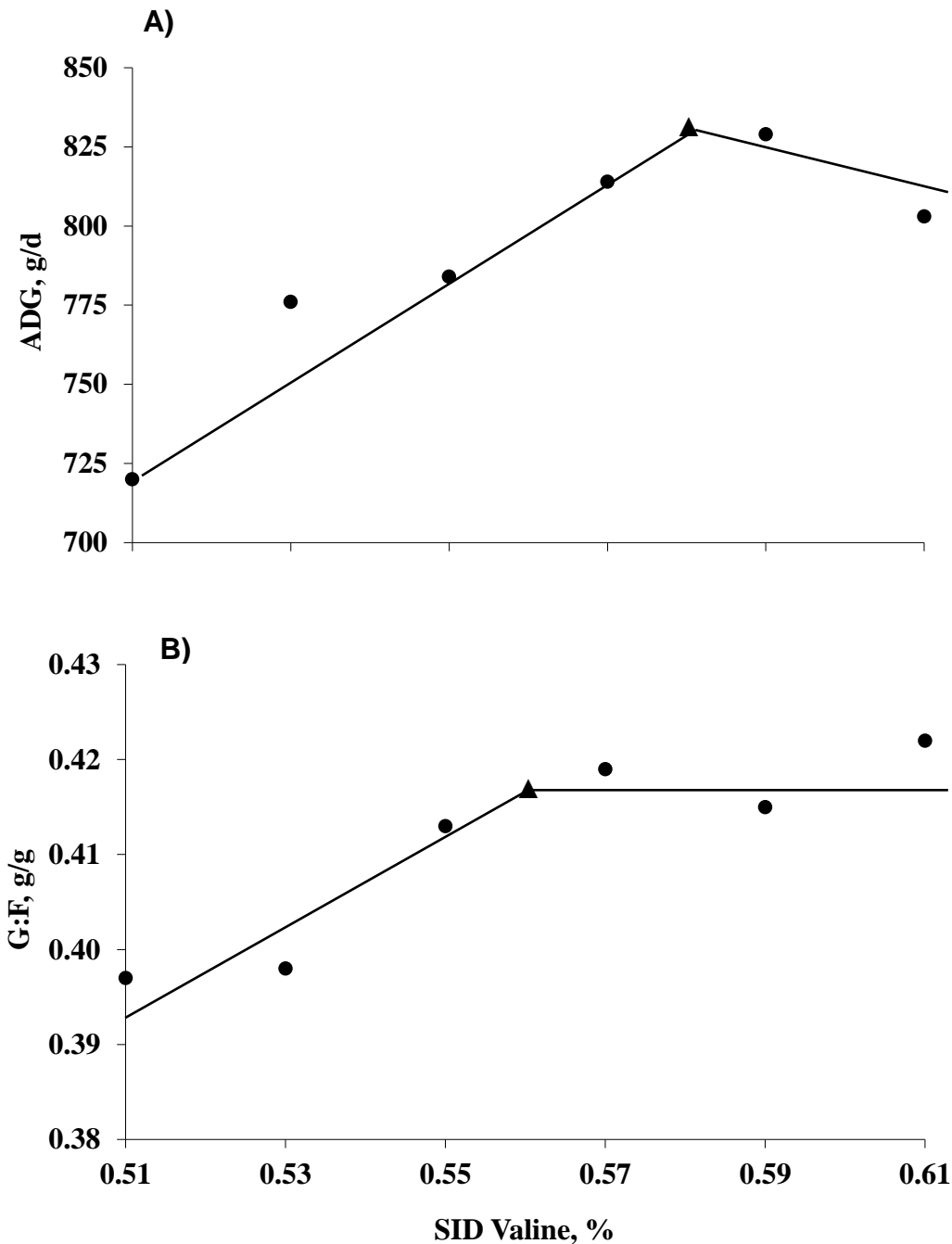


Figure 6.1. Treatment means (●) are shown for Exp. 1. (A) Fitted broken-line of overall (d0 to 26) ADG as a function of standardized ileal digestible (SID) Val in the diet. Using broken-line analysis on treatment means and pen mean values ($n = 6$ observations per treatment mean), the SID Val requirements (▲) were 0.58 ($P < 0.03$) and 0.58 ($P < 0.08$). (B) Fitted broken-line of overall (d0 to 26) G:F as a function of SID Val in the diet. Using broken-line analysis on treatment means and pen mean values ($n = 6$ observations per treatment mean), the SID Val requirements were 0.56 ($P = 0.03$) and 0.56 ($P = 0.63$).

when Ile and Val were independently removed. Therefore, this diet is ideal to determine the Val and Ile requirement of pigs.

In Exp. 1, based on ADG and G:F, the SID Val requirement is between 0.56 and 0.58% (0.67 to 0.70 SID Val:Lys). Table 6.2 is a list of published Val requirement estimates for growing pigs. A simple linear regression analysis of the published literature was conducted to determine the relationship between the SID Val:Lys and BW of pigs (Figure 2). The linear regression equation including the 9 data points was $Y = 0.34140x + 60.083558$ ($R^2 = 0.414$; $P < 0.06$). This regression line indicates that as BW increases the SID Val:Lys increases (Figure 6.2A). However, if the low (0.60 SID Val:Lys) and high (0.75 SID Val:Lys) estimates are removed from the dataset and reanalyzed as a regression, then the linear regression equation estimate was $Y = -0.23264x + 70.99814$ ($R^2 = 0.261$; $P < 0.25$). Because there is approximately a slope of zero, there is no evidence of a linear relationship between SID Val:Lys and BW of pigs; therefore, the SID Val:Lys does not change as BW increases. Our data were not included in this analysis; however, the ideal SID Val:Lys ratio based on this compiled data is estimated at 0.71, which is similar to our results.

Experiments 2 and 3 were conducted to determine the Ile requirement in an AA-supplemented, C-SBM diet. The combined data from Exp. 2 and 3 indicate that the supplemental Ile additions did not affect ADG, ADFI, or G:F. However, pigs fed the diet with 0.45% SID Ile had decreased ADFI compared with pigs fed the 0.43% SID Ile diet. This reduction in ADFI resulted in an increase in G:F. We do not have an explanation for these responses to the 0.45% SID Ile diet especially when the pigs fed levels of Ile greater than 0.45% were not affected by diet. Similarly, Barea et al. (2009b) reported that growth performance was not affected by Ile supplementation in a low-CP, cereal-SBM diet fed to 11- to 23-kg pigs. Barea et al. (2009b) and our results are similar because in both studies Val was provided at an adequate amount and SID

Ile was calculated to be deficient in the diets based on 0.52 SID Ile:Lys.

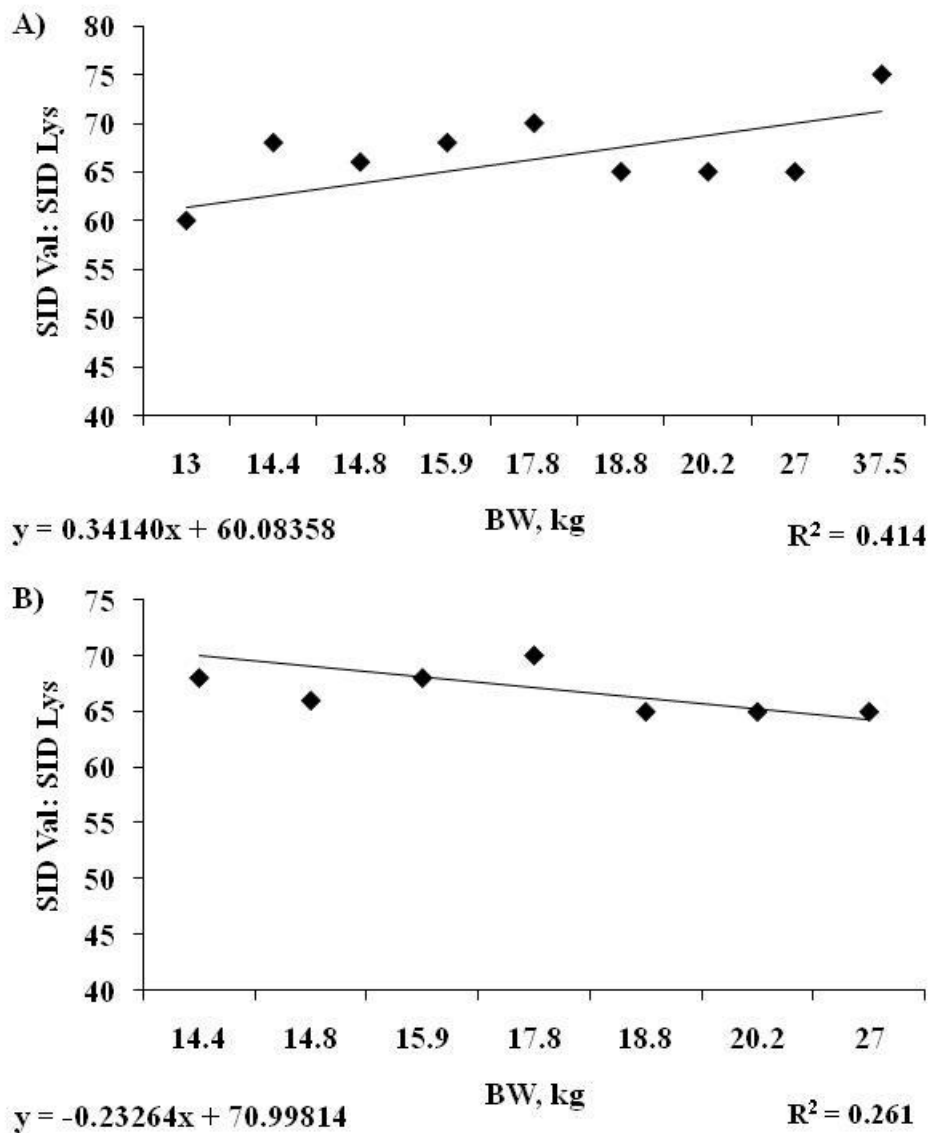


Figure 6.2. Standardized ileal digestible (SID) Val: SID Lys estimates by mean BW. (A) The linear regression equation including all 9 data points is $Y = 0.34140x + 60.083558$ ($R^2 = 0.414$; $P < 0.06$). (B) The linear regression equation with removal of the low and high outlier points is $Y = -0.23264x + 70.99814$ ($R^2 = 0.261$; $P < 0.25$).

The results of Exp. 2 and 3 indicate that 0.43% SID Ile (0.52 SID Ile:Lys) is adequate for 20- to 50-kg pigs. Table 6.1 is a list of published Ile requirement estimates for growing pigs. A simple linear regression analysis of this published data was conducted to determine the relationship between the SID Ile:Lys and BW of pigs (Figure 6.3). The linear regression equation

was $Y = -0.01795x + 56.54727$ ($R^2 = 0.01$; $P < 0.68$). Because there is a slope of zero, there is no evidence of a linear relationship between SID Ile:Lys and BW of pigs; therefore, the SID Ile:Lys does not change as BW increases.

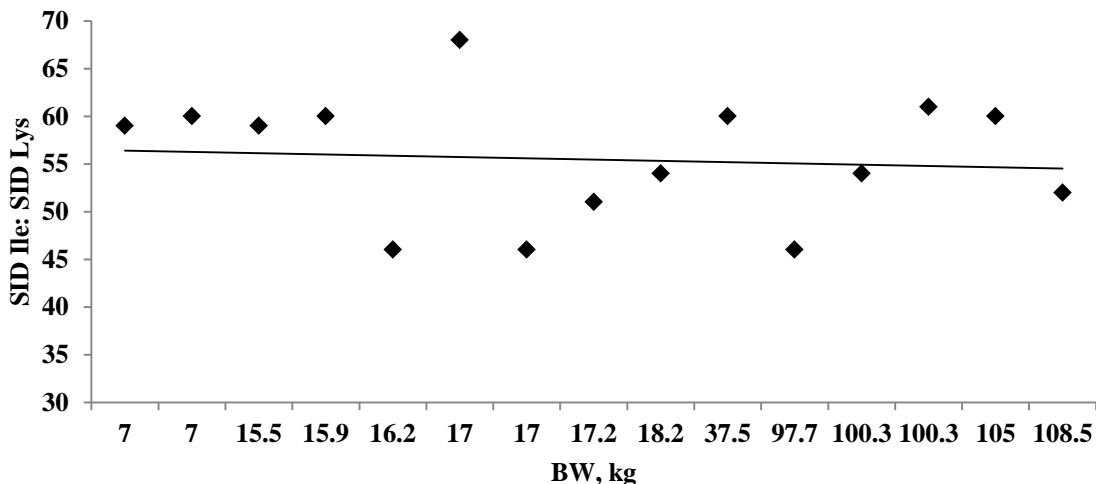


Figure 6.3. Standardized ileal digestible (SID) Ile: SID Lys estimates by mean BW. The linear regression equation is $Y = -0.01795x + 56.54727$ ($R^2 = 0.01$; $P < 0.68$).

The SID Ile requirement changes depending on the type of diet fed. Our estimate of 0.43% SID Ile is lower than Parr et al. (2003) estimate of 0.50% in 20- to 45-kg pigs; however, Parr et al. (2003) fed a corn SDBC based diet. The higher SID Ile estimate with the same BW pigs is due to an overestimation of the Ile requirement when feeding pigs diets containing blood products. Dean et al. (2005) suggested that this overestimation is due to an imbalance of branch chained AA. Fu et al. (2005a) agrees with the suggestion that pigs fed diets containing SDBC have a higher SID Ile requirement compared with pigs fed a C-SBM, AA-supplemented diet (0.32 vs. 0.28%).

Plasma urea N can be used as a rapid response variable to determine AA requirements in pigs (Coma et al., 1995; Knowles et al., 1997). Plasma urea N was determined in all experiments in an attempt to estimate the Val and Ile requirements. In Exp. 1, incremental Val additions resulted in a linear increase in ADFI which led to a linear increase in PUN concentrations;

therefore, a requirement could not be estimated. Similarly, Dean et al. (2005) and Parr et al. (2004) could not estimate an Ile requirement using PUN as a response variable because ADFI increased linearly with Ile addition. Parr et al. (2004) used ADFI as a covariate for PUN but was still unable to estimate an Ile requirement using PUN. Because ADFI increased with additions of Val and was widely variable with additions of Ile, plasma urea N is not an ideal response criterion for estimation of Ile or Val requirements.

In conclusion, the Val and Ile requirement for 20- to 45-kg pigs in an AA-supplemented, C-SBM diet is 0.56 to 0.58% SID Val (0.67 to 0.70 SID Val:Lys) and 0.43% SID Ile (0.52 SID Ile:Lys).

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CHAPTER 7

SUMMARY AND CONCLUSIONS

Replacing intact protein sources with supplemental AA is an option to decrease feed costs in swine and poultry production. Although feeding low CP, AA supplemented diets allows nutritionists to feed close to the animal's AA requirements and reduces excess N lost into the environment, many researchers have shown that feeding low CP, AA supplemented diets can cause a reduction in growth performance. Researchers have determined that Gly is limiting in low CP, AA supplemented diets for broilers. The improvement of growth performance due to Gly supplementation in these diets is not well understood.

Three experiments were conducted to evaluate supplementation of Gly, creatine, GAA, and fishmeal in C-SBM diets on growth performance of broiler chicks and to determine if Gly, creatine, or GAA could be added to a C-SBM diet in place of fishmeal to produce similar growth performance. The results from these experiments indicate that supplemental Gly, creatine, or GAA can be supplemented in a C-SBM diet in replacement of fishmeal and produce similar growth performance to broilers fed a C-SBM, fishmeal diet.

Two experiments were conducted to determine the effects of supplementing Lys and Gly and effects of feeding 2 dietary Lys levels without and with supplemental Gly and antibiotics on the growth performance, serum uric acid, and intestinal and organ responses of broilers. Results from these experiments indicate that increasing total dietary Lys in diets for broilers increases growth performance and small intestinal length. Also, Gly supplementation in low CP, AA supplemented diets as well as supplementation in an otherwise nutritionally adequate positive control diet increases feed efficiency of broiler chicks. Furthermore, this improvement in G:F coincides with an increase in serum uric acid and occurs in diets that are high (1.40%) or low (1.26%) in total dietary Lys. These results also are an indication that the increase in G:F is not

similar to the G:F response seen when antibiotic/anticoccidials are supplemented in broiler diets. Supplemental Gly decreased gizzard weights, which may be an indication of a shift in energy that is making the broiler more efficient in energy utilization.

An experiment was conducted to determine the effect of feeding increasing levels of supplemental Lys without and with supplemental Gly from 0- to 10-d and the subsequent effects of feeding these diets from 10- to 24-d. Results from these experiments indicate that the inclusion of up to 0.15% supplemental Lys can be added in a C-SBM diet without or with supplemental Gly without reducing growth performance of 0- to 10-d old broilers. These results also indicated that providing Gly in the starter phase (0- to 10-d) increased G:F during the subsequent grower phase depending on the supplemental Lys level that was provided in the starter phase.

A better understanding of the AA requirements beyond Lys, Thr, Trp, and Met and maximum inclusion rates of supplemental AA that can be used in diets for growing pigs will provide nutritionists more flexibility in least cost formulation. Three experiments were conducted to determine the Val and Ile requirement in an AA supplemented, C-SBM diet for 20- to 45-kg pigs. The Val and Ile requirement for 20- to 45-kg pigs in an AA-supplemented, C-SBM diet is 0.56 to 0.58% SID Val (0.67 to 0.70 SID Val:Lys) and 0.43% SID Ile (0.52 SID Ile:Lys).

APPENDIX:
LIST OF ABBREVIATIONS

Item	Abbreviation
Amino acid(s)	AA
Analysis of variance	ANOVA
Average daily feed intake	ADFI
Average daily gain	ADG
Adenosine triphosphate(s)	ATPs
Body weight	BW
Corn-soybean meal	C-SBM
Corn gluten meal	CGM
Creatine	Cre
Crude protein	CP
Fishmeal	FM
Gain:feed	G:F
General linear model	GLM
Guandino acidic acid	GAA
Plasma urea nitrogen	PUN
Serum uric acid	SUA
Spray dried blood cells	SDBC
Spray dried blood meal	SDBM
Spray dried plasma protein	SDPP
Standardized ileal digestible	SID
Total sulfur amino acid(s)	TSAA

VITA

April Marie Waguespack, daughter of Galyn and Michele Waguespack, was born in Baton Rouge, Louisiana, on April 25, 1983. April is the youngest of two children. She attended a college preparatory high school at St. Joseph's Academy in Baton Rouge, Louisiana. After graduating in 2001, she was accepted to Louisiana State University in Baton Rouge, Louisiana. In December of 2005, she completed a Bachelor of Science degree in animal, dairy, and poultry sciences. In December of 2007, she completed her Master of Science degree in animal, dairy, and poultry sciences evaluating low crude protein, amino acid supplemented diets and the Gly requirement in broilers at Louisiana State University. April continued her education at Louisiana State University evaluating Gly supplementation in broiler diets as well as amino acid requirements of growing pigs. On June 27, 2011, April got engaged to Noah Levy. April is currently a doctoral candidate in Interdepartmental Program in the School of Animal Sciences.