DIETARY GUANIDINO ACETIC ACID SPARES ARGinine AND DIETARY L-HOMOSERINE SPARES THREONINE IN THE CHICK

BY

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THESIS

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Abstract

Guanidino acetic acid (GAA) is synthesized in the liver and kidney from Arg and Gly. It is subsequently methylated by S-adenosylmethione to creatine. L-Homoserine (HS) has been identified as an intermediate compound in the metabolism of Thr, Met, and Asp. Bacteria, plants and yeast can synthesize Thr and Met from HS, but this has not been shown in higher organisms. Several bioassays were carried out to determine the capacity of GAA to spare dietary Arg or HS to spare dietary Thr. Crossbred chicks were fed Arg-deficient dextrose-casein (0.86% Arg), Arg-deficient corn-corn coproduct-soybean meal (1.0% Arg), Thr-deficient dextrose-casein (0.22% Thr), or Thr-deficient corn-peanut meal (0.46%) basal diets during 9-d battery feeding trials involving 5 pens of 4 chicks per treatment. Growth and gain:feed responses were obtained when GAA was added to a purified Arg-deficient diet and when HS was added to both Thr-deficient diets. In the corn-corn coproduct-soybean meal diet, GAA yielded a gain:feed response, but not an increase in gain. These results demonstrate that 0.12% supplemental GAA, like creatine, produces consistent growth and feed efficiency responses in young chicks fed Arg-deficient diets and indicate that low levels of HS (0.08-0.1%) elicit a growth and feed efficiency response in young chicks fed Thr-deficient diets.
DEDICATION

This thesis is dedicated to my grandmother, Irene Bryant, who passed away shortly before I was able to defend it. I did it, Grandma. I love and miss you.

I would also like to dedicate this thesis to the rest of my family. You have inspired me to be a better person and have always believed in me, even when I may have doubted myself. I love you all.

And in the loving memory of

Dr. David H. Baker

who was not able to see this thesis completed.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Introduction and Literature Review</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1</td>
<td>Guanidino Acetic Acid</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>Homoserine</td>
<td>5</td>
</tr>
<tr>
<td>1.3</td>
<td>Literature Cited</td>
<td>7</td>
</tr>
<tr>
<td>1.4</td>
<td>Figure</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td><strong>Dietary Guanidino Acetic Acid Spares Arginine in Chicks</strong></td>
<td>12</td>
</tr>
<tr>
<td>2.1</td>
<td>Abstract</td>
<td>12</td>
</tr>
<tr>
<td>2.2</td>
<td>Introduction</td>
<td>13</td>
</tr>
<tr>
<td>2.3</td>
<td>Materials and Methods</td>
<td>14</td>
</tr>
<tr>
<td>2.4</td>
<td>Results</td>
<td>16</td>
</tr>
<tr>
<td>2.5</td>
<td>Discussion</td>
<td>18</td>
</tr>
<tr>
<td>2.6</td>
<td>Literature Cited</td>
<td>20</td>
</tr>
<tr>
<td>2.7</td>
<td>Tables</td>
<td>22</td>
</tr>
<tr>
<td>2.8</td>
<td>Figures</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td><strong>Dietary L-Homoserine Spares Threonine in Chicks</strong></td>
<td>30</td>
</tr>
<tr>
<td>3.1</td>
<td>Abstract</td>
<td>30</td>
</tr>
<tr>
<td>3.2</td>
<td>Introduction</td>
<td>30</td>
</tr>
<tr>
<td>3.3</td>
<td>Materials and Methods</td>
<td>31</td>
</tr>
<tr>
<td>3.4</td>
<td>Results</td>
<td>34</td>
</tr>
<tr>
<td>3.5</td>
<td>Discussion</td>
<td>36</td>
</tr>
<tr>
<td>3.6</td>
<td>Literature Cited</td>
<td>39</td>
</tr>
<tr>
<td>3.7</td>
<td>Tables</td>
<td>43</td>
</tr>
<tr>
<td>3.8</td>
<td>Figure</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td><strong>General Summary</strong></td>
<td>49</td>
</tr>
<tr>
<td>5</td>
<td><strong>Appendix</strong></td>
<td>51</td>
</tr>
<tr>
<td>6</td>
<td><strong>Biography</strong></td>
<td>52</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction and Literature Review

Amino acids (AA) are an important part of diet formulation in animal nutrition. Approximately 45-50% of the total feed costs are composed of AA and protein, which can become quite expensive. If other nutrients could be established as AA precursors, this could be economically efficient and could also free up more of the AA to be used specifically for protein deposition in the body (e.g. muscle). For example, dietary S-Methylmethionine spares choline in chickens, excess methionine can spare choline in mammals (Augspurger et al., 2005), Cys spares methionine (Finkelstein et al., 1988) and Tyr can spare Phe. It is theoretically possible that many or all AA in the diet could be partially spared by a precursor or related metabolite. Since AA are expensive to include in the diet, feeding precursors could become a means of lowering the cost of the diet. Two compounds that might spare AA are proposed to be evaluated in this thesis.

Guanidino Acetic Acid

Guanidino acetic acid (GAA) is a precursor of creatine and has potential for being a feed additive for chicks. Guanidino acetic acid has also been referred to as glycocamine, guanidoacetic acid, or guanidinoacetate. Ringel et al. (2008) proposed that GAA may have growth and feed efficiency promoting properties when added to corn-soybean meal diets in broilers. There is also interest in GAA and creatine because it has the potential to spare dietary Arg. In the 1960s, casein was shown to be deficient in Arg and rich in Lys for chick growth. This prompted a plethora of research with casein-based diets showing the Lys-Arg antagonism, and showing that supplemental Arg and creatine could elicit a growth response in these high-Lys low-Arg casein-based diets (Allen et al., 1972; Austic and Nesheim, 1972; Austic and Scott,
Creatine was discovered in 1835 by Michel-Eugène Chevreul (Rose, 1933). It is likely that the requirement for creatine is greater in growing animals versus adults because there may be a need to supply creatine to growing muscles (Brosnan et al., 2009). Creatine is an important energy buffer in vertebrates, serving as an energy shuttle where high-energy phosphates are brought to sites of rapid ATP utilization in cells with high energy demands (Brosnan et al., 2009). It is a constant component of voluntary muscle, and it has been found in skeletal muscle, cardiac and smooth muscle, and it has been isolated in the brain and testes of vertebrates (Rose, 1933). Some researchers have also been able to isolate creatine in the blood and plasma (Austic and Nesheim, 1972; Rose, 1935). The latter has been confirmed by more recent research showing that creatine is still only found in animal products and creatine excretion depends on two major factors 1) muscle mass and 2) diet (Stead et al., 2006). Guanidino acetic acid had been found in urine of humans, dogs and rats (Thomas, 1938). Unlike creatine, GAA had not been seen in muscle, heart or liver of the rat, but traces may have been present in kidney and the intestinal tract. Still at this point in time, GAA could not be regarded as the immediate precursor of creatine (Thomas, 1938).

Creatine is formed in the avian liver and the kidney (Borsook and Dubnoff, 1940b) (Figure 1.1) by Arg combining with Gly being acted upon by the enzyme transamidinase, forming GAA. Guanidino acetic acid is then methylated to creatine by S-adenosyl-methionine (SAM). An ATP then donates a phosphorus moiety to creatine to form phosphocreatine. Any excess phosphocreatine is catabolized into creatinine and then excreted in the urine (Meister, 1965). For years, the precursors of creatine
and its biosynthesis were not completely known. Not until 1940 was it finalized that Arg and Gly were the specific AA precursors of creatine and that GAA was formed in an intermediate step (Bloch and Schoenheimer, 1940a,b; Bloch and Schoenheimer, 1941; Borsook and Dubnoff, 1940a,b,c). Borsook and Dubnoff (1941) were the first to publish that the formation of GAA occurred from Arg and Gly by a new chemical reaction called “transamidination.” The latter authors claimed that this new reaction was direct proof that Arg and Gly are precursors of creatine.

Much of the early work with creatine was done in humans or rats. Creatine was found in human hearts obtained at autopsy; the left ventricle, in general, contained more than the right ventricle (Thomas, 1938). It was found that liver slices of rat, cat and rabbit were able to convert GAA to creatine (Borsook and Duboff, 1940a). Borsook and Dubnoff (1940b) also found that slices of liver and kidneys from cat, dog, guinea pig, pigeon, rabbit, and rat formed creatine in vitro. These authors interpreted these findings as creatine being formed by the methylation of GAA in the liver.

Researchers knew that high creatine values in muscle and increased growth resulted from feeding creatine. Thus, it was then postulated that creatine should be able to replace or spare the portion of Arg and Gly that was required for creatine formation, allowing the Arg and Gly to be utilized for growth (Hegsted et al., 1941). The first mention of GAA possibly being able to spare Arg and Gly, in the same manner that creatine could, came in 1941 when Almquist and coworkers showed that GAA yielded muscle creatine values that were similar to those obtained from feeding creatine (Almquist et al., 1941).

Wietlak et al. (1954) proposed that from a nutritional standpoint, creatine and Arg are interchangeable. Through their research, they concluded that creatine could
replace most of the arginine that the chick required; the response to Gly was only relatively slight. They also concluded that if the diet contains the optimum amount of Arg, there is no further improvement when creatine is added (Wietlake et al., 1954). In contrast, Fisher et al. (1956a,b) found that creatine only spares the amount of Arg that is normally utilized for creatine biosynthesis and some Arg is always being diverted to creatine synthesis. They also established that creatine alone only slightly increased growth of chicks fed Arg-deficient unsupplemented casein diets and that for optimum creatine formation, both Arg and Gly were required (Fisher et al. 1956a,b).

Edwards et al. (1958) suggested that any sparing action that GAA had could be contributed to creatine formation, thereby sparing Arg for protein anabolism. In their research, they found that at deficient levels of Arg for growth, supplemental GAA provided a response. And, when creatine alone was supplemented, a significant growth response was observed, but there was no further improvement when creatine was added to a diet containing a low level of supplemental L-Arg.

Savage and O'Dell (1960) found that when the basal diet contained 1.2% Arg, creatine spared about 0.4% of Arg. They also found that creatinine spared slightly less Arg than creatine, and GAA spared about half as much as creatine and, when sufficient Arg but insufficient Gly were fed, creatine improved growth rate and thus may spare Gly. However, Waterhouse and Scott (1961) reported that the growth response to supplemental creatine occurred both in the presence and absence of supplemental Gly, suggesting that creatine was not “sparing” glycine but was functioning entirely independent of glycine.

Austic and Nesheim (1972) claimed that creatine is not growth limiting per se in Arg-deficient diets, and the growth promoting effect of creatine is due to the sparing of the Arg being used for creatine synthesis. In these studies, they used two strains of
chicks that had either a high or low arginine requirement. They also fed α-aminoisobutyric acid (AIB) as an additive in attempt to prevent any increase of arginase activity which can occur when high Arg requirement (HA) chicks are fed an Arg-deficient diet. It was found that AIB improved the growth of the HA strain. Creatine supplementation, however, improved growth for both strains of chicks, and the combination of creatine and AIB fed in an arginine-supplemented diet appeared to be growth depressing (Austic and Nesheim, 1972).

Creatine does not make a good feed additive because of its instability and high cost (Wietlake et al., 1954). Guanidino acetic acid is more stable and less expensive than creatine. Because GAA is an immediate precursor of creatine, requiring only a methyl group transfer from S-adenosylmethionine (SAM), it is hypothesized that GAA should spare dietary Arg in the same manner as creatine (Baker, 2009). If so, this could be economically important because commercial reduced-protein corn-soybean meal diets for broiler chicks are usually third or fourth limiting in Arg. In addition, Ringel et al. (2008) reported that GAA improved growth performance of broiler chicks even when fed Arg-adequate corn-soybean meal diets.

**Homoserine**

Homoserine (HS) has been identified as an intermediate compound in the metabolism of Thr, Met, and Asp (Meister, 1965). The first evidence of HS being a precursor of Thr and Met was accomplished using a mutant strain of *Neurospora crassa* mold which required both Thr and Met for growth; HS was able to spare both amino acids for growth (Teas et al., 1948).

Homoserine occurs free (unbound to protein) in many plants and particularly in pea seedlings, it is found in large quantities (Meister, 1965). Homoserine is also
formed during the catabolism of the carbon skeleton of Met in higher organisms (Meister, 1965) (Figure 1.1). Through previous research, it has been well established that bacteria, plants, and yeast can synthesize Thr and Met from HS (Flavin and Kono, 1960; Flavin and Slaughter, 1960a,b; Kim et al., 2004; Rinder et al., 2008; Watanabe and Shimura, 1965, 1960).

Homoserine is formed when L-β-aspartic acid semialdehyde is reduced by the enzyme, homoserine dehydrogenase, to L-homoserine (Meister, 1965). Watanabe and Shimura (1956, 1960) showed that two enzymes were required in yeast and Neurospora for HS to be synthesized into Thr. The first is HS kinase, which catalyzes the phosphorylation of HS via ATP. The second enzyme is Thr synthetase, which requires pyridoxal phosphate. However, evidence of these reactions in higher organisms does not exist. A large amount of research has been completed on the biosynthesis of HS (Meister, 1965; Teas, 1948; Watanabe and Shimura, 1965, 1960), but no researchers have specifically evaluated the ability of HS to spare Thr in animals. It is hypothesized that HS may have Thr replacement bioactivity in young chicks. This is important economically because Thr is one of the most limiting AA in reduced protein corn-soybean meal diets for chicks (Fernandez et al., 1994).
**Literature Cited**


Figure 1.1. Guanidino acetic acid and creatine sparing arginine, and homoserine sparing threonine.
Chapter 2

Dietary Guanidino Acetic Acid Spares Arginine in Chicks

Abstract

Guanidino acetic acid (GAA) is synthesized in the liver and kidney from Arg and Gly. It is subsequently methylated by S-adenosylmethionine to creatine. Four bioassays were carried out to determine the capacity of GAA to spare dietary Arg. Crossbred chicks were fed Arg-deficient dextrose-casein (0.86% Arg) or corn-corn coproduct-soybean meal (1.0% Arg) basal diets during 9-d battery feeding trials involving 5 pens of 4 chicks per treatment. The first assay showed that the dextrose-casein diet was markedly deficient in Arg and would elicit marked (P < 0.01) responses in both weight gain and gain/feed to added Arg, GAA or creatine. The optimal level of added GAA was 0.12% of the diet, but this level of GAA or 1.0% supplemental creatine-H$_2$O did not improve growth performance when added to the diet made adequate in Arg. A second assay was then designed using the purified diet in attempt to repeat the previous results as a 2 x 2 factorial, feeding 0.12% L-Arg, 0.12% GAA, or the combination. The third assay, using the practical-type diet based on corn, corn gluten meal, distillers dried grains with solubles, and soybean meal produced gain:feed responses (P < 0.05) to 0.25% added Arg, 0.12% GAA or 0.15% creatine-H$_2$O, and the responses to these additions were similar. These results demonstrate that 0.12% supplemental GAA, like creatine, produces consistent growth responses in young chicks fed Arg-deficient diets and may also yield improvements in growth performance when added to Arg-adequate diets. A fourth assay was then completed involving 7 graded doses of supplemental Arg in the dextrose-casein diet containing either 0 or 0.12% added GAA (14 total diets). Weight gain and gain:feed responses to Arg were quadratic (P < 0.01). The GAA increased both weight gain and gain:feed and
the Arg quadratic x GAA interaction was not significant (P > 0.10). Thus, in this assay, GAA yielded responses in chicks fed either deficient or adequate levels of Arg.

**Introduction**

Guanidino acetic acid (GAA) is a compound that is formed from Gly and Arg by the enzyme transamidinase in the avian kidney and liver. The GAA is then methylated to creatine by S-adenosyl methionine (SAM) giving up a methyl group to form S-adenosyl homocysteine (SAH); this occurs in the liver. Adenosine triphosphate (ATP) donates a phosphorus moiety to creatine, and phosphocreatine is formed. This is a reversible reaction. Excess phosphocreatine is then catabolized into the ring structure formation of creatinine, which is excreted in the urine (Meister, 1965).

Creatine and its sparing dietary Arg effects have been studied (Austic and Nesheim, 1972; Fisher et al., 1956a,b; Waterhouse and Scott, 1961). Guanidino acetic acid was only mentioned in those publications when discussing the role of Arg in the biosynthesis of creatine. But recently, Ringel et al. (2008) proposed that GAA may also have growth and feed efficiency promoting properties when added to corn-soybean meal diets in Brazil.

As discussed above, GAA is a precursor of creatine and creatine biosynthesis from GAA may explain at least part of the GAA responses. Guanidino acetic acid is a more suitable feed additive than creatine and arginine because it is less expensive than both and is more stable than creatine. In addition, GAA may be beneficial in poultry diets because it may be able to spare Arg. The latter is important because Arg is the fifth limiting AA in corn-soybean diets for poultry (Fernandez et al., 1994; Han et al., 1992; Waguespack et al., 2009). The objective of these assays was to evaluate the ability of GAA to spare Arg in young chicks.
Materials and Methods

All animal procedures were reviewed and approved by the University of Illinois Institutional Animal Care and Use Committee. Three bioassays were conducted using male chicks (New Hampshire × Columbian) obtained from the University of Illinois Poultry Research Farm. Chicks were housed in thermostatically controlled starter batteries with raised-wire flooring in an environmentally controlled room with continuous lighting. Water and experimental diets were provided ad libitum throughout the trial period.

From hatch to d 7 posthatch, chicks were fed a diet adequate in all dietary nutrients (NRC, 1994). Following an overnight fast, chicks were weighed, wing-banded, and randomized to dietary treatments on d 8 such that average initial pen weights and weight distributions were similar across treatments. At the conclusion of the experiment, chicks and feeders were weighed. Body weight gain, feed intake, and feed efficiency (gain:feed ratio) were calculated for each replicate pen of chicks.

Basal Diets. Two separate basal diets (Table 2.1) were formulated to evaluate the Arg replacement value of GAA. The purified and the practical corn-soybean meal basal diets were formulated to be singly deficient in Arg, but were otherwise nutritionally complete for chicks of this age (NRC, 1994).

Assay 1. The objective of this assay was to determine whether or not GAA could spare the need for Arg in young chicks and to compare the GAA response to that obtained from creatine. Dietary treatments included a common purified basal diet, singly deficient in Arg, and the basal diet plus 0.06 or 0.12% GAA, or 1.0% L-Arg. The singly deficient basal diet and the basal diet plus 1.0% L-Arg were further supplemented with 0.39 or 0.78% GAA, or 1.0% creatine•H₂O. The 1.0% creatine•H₂O provided 0.88%
creatine which is isomolar to 0.78% GAA. Five replicate pens of 4 chicks received each of the 10 experimental diets during a 9-d feeding period (d 8 to 17 post-hatch).

**Assay 2.** This assay was conducted to confirm the results obtained with 0.12% GAA in Assay 1 and to compare the responses from 0.12% GAA to an equal amount of L-Arg. Dietary treatments were arranged in a 2 x 2 factorial design. Dietary treatments included a common purified basal diet, singly deficient in Arg, and the basal diet plus 0.12% Arg, the same level of GAA and the combination. Five replicate pens of 4 chicks received each of the 4 experimental diets during a 9-d feeding period (d 8 to 17 post-hatch).

**Assay 3.** This assay sought to determine whether GAA can spare Arg in a practical-type diet (Table 2.1). Dietary treatments included a practical-type basal diet, deficient only in Arg, and the basal diet supplemented with 0.25% Arg, 0.12% GAA, 0.25% Arg plus 0.12% GAA, or 0.153% creatine (isomolar to 0.12% GAA). Additionally, a methionine-fortified corn-soybean meal broiler starter diet (23% CP, 1.25% Arg) was included as a positive control diet. Five replicate pens of 4 chicks received each of the 6 experimental diets during a 9-d feeding period (d 8 to 17 post-hatch).

**Assay 4.** This assay was an Arg requirement study, which was done in the absence and presence of 0.12% supplemental GAA. The first 7 dietary treatments were the unsupplemented purified dextrose-casein diet with 7 graded doses of supplemental Arg from 0 to 0.72%, and this resulted in a dietary range of 0.86% to 1.58% Arg. The second seven treatments were the same as the first seven except that all diets contained 0.12% GAA. Five replicate pens of 4 chicks received each of the 14 experimental diets during a 9-d feeding period (d 8 to 17 post-hatch).

**Statistical Analysis.** All data were subjected to ANOVA using the General Linear Model procedure of SAS (SAS Institute, 2004). Data were analyzed using pen means
with procedures appropriate for a completely randomized design. Data are presented as mean values with pooled SEM estimates, and significance was set at $\alpha = 0.05$. Differences among treatment means were evaluated using the least significance procedure (Carmer and Walker, 1985). Data from Assay 4 were subjected to one-slope broken-line regression analysis (weight gain vs. supplemental Arg concentration and gain:feed vs. supplemental Arg concentration) to establish the breakpoint (requirement) for Arg (Robbins, 1986; Robbins et al., 1979).

**Results**

**Assay 1.** Weight gain and feed efficiency, or gain:feed ratio, responded markedly to addition of Arg to the Arg-deficient basal diet (Table 2.2). There was also a significant growth and feed efficiency response when 0.12% GAA was added to the Arg-deficient basal diet. At higher levels of GAA, no further response in weight gain or feed efficiency was observed; thus, the responses were maximized at 0.12% GAA in this assay. Higher levels of GAA were also added to the Arg-adequate diet, with no significant responses. For the 1.0% creatine added to the Arg-deficient diet, there was an increase in gain and feed efficiency. The latter responses were significantly greater than those obtained with an isomolar level of 0.78% GAA. When 1.0% creatine was added to the Arg-adequate diet, no significant responses were observed. These results established that, under our circumstances with the casein purified diet, 0.12% GAA yielded the optimal response.

**Assay 2.** The 0.12% L-Arg and 0.12% GAA yielded similar increases in weight gain and an identical increase in feed efficiency (Table 2.3). When the combination of GAA and Arg were added, an additional increase in gain and feed efficiency were obtained. Thus, the main effect of both Arg and GAA were significant for both gain ($P < 0.05$) and gain:feed ($P < 0.01$), and there was an additive response.
**Assay 3.** Weight gain was not significantly affected by any of the dietary treatments in this assay (Table 2.4). However, similar gain:feed responses (P < 0.05) were produced when levels of 0.25% Arg, 0.12% GAA or 0.153% creatine were added to the Arg-deficient practical-type diet. Weight gain of chicks fed the corn-soybean meal positive control diet was similar to that of the latter supplemental diets, whereas the corn-soybean meal positive control diet yielded feed efficiencies that were higher than that obtained from all other diets.

**Assay 4.** For diets with and without 0.12% GAA, weight gain responded to Arg addition up to approximately the fourth dose of 0.36% supplemental Arg, after which an apparent plateau occurred. Feed efficiency responded to Arg supplementation up to approximately the fifth dose of 0.48%. Overall, gain and gain:feed responses to Arg were quadratic, and the main effect of GAA was highly significant (P < 0.01). The overall Arg x GAA interaction was not significant, indicating that GAA responses occurred at both deficient and adequate levels of Arg.

When GAA was added to Arg-deficient diets (less than 0.40% supplemental L-Arg), it improved gain:feed by 8.2% over those with no GAA (Figure 2.1). When GAA was added to Arg-adequate diets (more than 0.40% supplemental L-Arg), it improved gain:feed by only 4.3% over those with no GAA. Although the overall Arg x GAA interaction was not significant, there was a (P < 0.01) interaction when comparing GAA responses in Arg-deficient versus adequate diets (Figure 2.1).

The one-slope broken-line regression analyses to estimate Arg requirements in diets without and with 0.12% added GAA are illustrated in Figures 2.2 and 2.3. The break point, or requirement estimate, for weight gain (Figure 2.2) was essentially the same with (0.30%) or without (0.29%) GAA. The total Arg requirement for weight gain was 1.16% Arg; this was calculated by adding the 0.30% to the 0.86% Arg in the basal
diet. For gain:feed ratio, the estimated break point requirement was the same (0.40%) between the diets with or without GAA (Figure 2.3). The required amount of total Arg for gain:feed was 1.26%, calculated by adding 0.40% and the 0.86% Arg in the basal diet.

**Discussion**

When GAA was added to the Arg-deficient casein diets, there was a large response in both weight gain and efficiency of feed utilization. These results clearly show that GAA can spare Arg in Arg-deficient diets, which is in agreement with those of Savage and O'Dell (1960).

In the Arg-deficient practical diets, there was no gain response to the supplementation of GAA. However, there were significant gain:feed responses when L-Arg, GAA, and the combination were supplemented. These gain:feed responses were similar to the responses to creatine (Table 2.4). These results are in agreement with the work of Snyder and coworkers (1956), who observed that adding Arg and Gly to a practical corn-soybean meal diet did not produce a growth response, but increased gain:feed ratio. They further suggested that the presence of creatine in practical feed ingredients could explain the lower requirement for Arg in practical rations compared to an apparent higher Arg requirement when fed a purified diet.

When GAA and creatine were fed in isomolar doses, the gain and gain:feed responses to GAA were similar to those of creatine for both the purified diet (Table 2.2) and the practical diet (Table 2.4). Thus, the reason for a GAA response is likely from GAA being the precursor to creatine. Edwards et al. (1958) also suggested that any sparing action of GAA is contributed to creatine formation, which spared Arg for other functions, such as protein anabolism.
In agreement with the work in the 1950s (Fisher et al., 1956a,b), inclusion of GAA in a purified casein diet did not reduce the dietary requirement for Arg. In general, the greater the deficiency of Arg, the greater were the weight gain and gain:feed responses to GAA in the current study. However, GAA produced responses even in Arg-adequate diets in Assay 4 (higher plateau), particularly for gain:feed ratio; this resulted in no reduction in the Arg requirement. The latter responses to GAA are in agreement with Ringel et al. (2008) who reported that GAA increased growth performance of broiler chicks fed Arg-adequate corn-soybean meal diets.

The digestible Arg requirement for maximal gain:feed (1.26%) estimated by broken line analysis was higher than the requirement for maximal weight gain, which was estimated at 1.16%. These results are in agreement with earlier work with Lys (Han and Baker, 1991). The reason for the higher requirement of amino acids for feed efficiency versus weight gain is unknown but may be associated with changes in feed intake and body composition (lean versus fat) as dietary concentrations of the limiting amino acid are increased (Baker et al., 1996).
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Robbins, K. R. 1986. A method, SAS program, and example for fitting the broken line to growth data. Univ. of Tenn. Res. Rep. 86-09. University of Tennessee of Agricultural Experiment Station, Knoxville, TN.


<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Purified diet(^1)</th>
<th>Practical diet(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>-----</td>
<td>0.37</td>
</tr>
<tr>
<td>Dextrose</td>
<td>59.43</td>
<td>-----</td>
</tr>
<tr>
<td>Casein (84.8% CP)</td>
<td>25.00</td>
<td>-----</td>
</tr>
<tr>
<td>Corn (8% CP)</td>
<td>-----</td>
<td>55.40</td>
</tr>
<tr>
<td>Soybean meal (47% CP)</td>
<td>-----</td>
<td>15.20</td>
</tr>
<tr>
<td>Distiller's dried grains with soluble (25.5% CP)</td>
<td>-----</td>
<td>16.00</td>
</tr>
<tr>
<td>Corn gluten meal (61% CP)</td>
<td>-----</td>
<td>4.00</td>
</tr>
<tr>
<td>Solka floc</td>
<td>3.00</td>
<td>-----</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>4.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>-----</td>
<td>1.70</td>
</tr>
<tr>
<td>Limestone</td>
<td>-----</td>
<td>1.40</td>
</tr>
<tr>
<td>NaCl</td>
<td>-----</td>
<td>0.40</td>
</tr>
<tr>
<td>NaHCO(_3)</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td>Amino acid mixture</td>
<td>2.30(^3)</td>
<td>1.78(^4)</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>5.37(^5)</td>
<td>0.15(^5)</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>0.20(^5)</td>
<td>0.20(^5)</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>DL-(\alpha)-tocopherol acetate, 20 mg/kg</td>
<td>+</td>
<td>-----</td>
</tr>
<tr>
<td>Ethoxyquin, 125 mg/kg</td>
<td>+</td>
<td>-----</td>
</tr>
<tr>
<td>Bacitracin premix</td>
<td>-----</td>
<td>0.05(^6)</td>
</tr>
</tbody>
</table>

\(^1\)Contained 0.86% Arg and 21.2% CP.

\(^2\)Contained 1.0% Arg and 20.0% CP.

\(^3\)Contained 0.30% DL-Met and 2.00% Gly.

\(^4\)Contained 0.62% L-Lys•HCl, 0.26% DL-Met, 0.12% L-Thr, 0.03% L-Trp, 0.06% L-Val, 0.09% L-Ile, and 0.60% Gly.

\(^5\)See Bryant et al. (2009) for composition of the mineral and vitamin mixes used for the purified and practical diets.

\(^6\)Provided 25 mg/kg bacitracin methylene disalicylate.
Table 2.2. Performance of chicks fed graded doses of guanidino acetic acid (GAA) when added to an arginine-deficient dextrose-casein diet (Assay 1)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain, g</th>
<th>Feed intake, g</th>
<th>Gain:feed, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Basal diet(^2)</td>
<td>111(^e)</td>
<td>195(^g)</td>
<td>569(^d)</td>
</tr>
<tr>
<td>2. As 1 + 1.0% L-Arg</td>
<td>212(^a)</td>
<td>275(^a)</td>
<td>773(^a)</td>
</tr>
<tr>
<td>3. As 1 + 0.06% GAA</td>
<td>108(^e)</td>
<td>195(^g)</td>
<td>553(^d)</td>
</tr>
<tr>
<td>4. As 1 + 0.12% GAA</td>
<td>145(^d)</td>
<td>233(^de)</td>
<td>622(^c)</td>
</tr>
<tr>
<td>5. As 1 + 0.39% GAA</td>
<td>139(^d)</td>
<td>220(^de)</td>
<td>631(^c)</td>
</tr>
<tr>
<td>6. As 1 + 0.78% GAA</td>
<td>134(^d)</td>
<td>209(^fg)</td>
<td>641(^c)</td>
</tr>
<tr>
<td>7. As 2 + 0.39% GAA</td>
<td>209(^ab)</td>
<td>265(^abc)</td>
<td>789(^a)</td>
</tr>
<tr>
<td>8. As 2 + 0.78% GAA</td>
<td>197(^b)</td>
<td>249(^cd)</td>
<td>792(^a)</td>
</tr>
<tr>
<td>9. As 1 + 1.00% Creatine•H(_2)O(^3)</td>
<td>173(^c)</td>
<td>254(^bc)</td>
<td>680(^b)</td>
</tr>
<tr>
<td>10. As 2 + 1.00% Creatine•H(_2)O(^3)</td>
<td>212(^a)</td>
<td>268(^ab)</td>
<td>794(^a)</td>
</tr>
</tbody>
</table>

SEM  5.0   6.2   12.5

\(^1\)Data are means of 5 pens of 4 chicks fed the experimental diets from 8 to 17-d posthatch; average initial weight was 94 g. Means in columns with no common superscript letters are significantly different (\(P < 0.05\)).

\(^2\)The basal Arg-deficient diet (Table 2.1) contained 0.86% Arg.

\(^3\)Provided 0.88% creatine, an amount isomolar to 0.78% GAA.
Table 2.3. Response to guanidino acetic acid (GAA) in chicks fed a dextrose-casein diet containing two deficient levels of arginine (Assay 2)\(^1\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain, g</th>
<th>Feed intake, g</th>
<th>Gain:feed, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Basal diet(^2)</td>
<td>97</td>
<td>184</td>
<td>524</td>
</tr>
<tr>
<td>2. As 1 + 0.12% L-Arg</td>
<td>124</td>
<td>211</td>
<td>587</td>
</tr>
<tr>
<td>3. As 1 + 0.12% GAA</td>
<td>112</td>
<td>191</td>
<td>587</td>
</tr>
<tr>
<td>4. As 2 + 3</td>
<td>139</td>
<td>211</td>
<td>657</td>
</tr>
</tbody>
</table>

SEM\(^3\) 7.3 6.5 18.7

\(^1\)Data are means of 5 replicate pens of 4 male chicks during the period of 8 to 17-d posthatch; average initial weight was 77g.

\(^2\)The basal Arg-deficient diet contained 0.86% Arg.

\(^3\)Main effect of both Arg and GAA for weight gain (\(P < 0.05\)) and gain:feed (\(P < 0.01\)). There was no significant interaction between L-Arg and GAA for either weight gain or gain:feed.
Table 2.4. Performance of chicks fed guanidino acetic acid (GAA) in an arginine-deficient practical-type diet (Assay 3)\textsuperscript{1}

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain, g</th>
<th>Feed intake, g</th>
<th>Gain:feed, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Basal diet\textsuperscript{2}</td>
<td>219</td>
<td>316\textsuperscript{a}</td>
<td>692\textsuperscript{c}</td>
</tr>
<tr>
<td>2. As 1 + 0.25% L-Arg</td>
<td>224</td>
<td>307\textsuperscript{ab}</td>
<td>728\textsuperscript{b}</td>
</tr>
<tr>
<td>3. As 1 + 0.12% GAA</td>
<td>220</td>
<td>300\textsuperscript{bc}</td>
<td>733\textsuperscript{b}</td>
</tr>
<tr>
<td>4. As 2 + 3</td>
<td>217</td>
<td>294\textsuperscript{bc}</td>
<td>739\textsuperscript{b}</td>
</tr>
<tr>
<td>5. As 1 + 0.153% Creatine•H\textsubscript{2}O\textsuperscript{3}</td>
<td>221</td>
<td>302\textsuperscript{abc}</td>
<td>730\textsuperscript{b}</td>
</tr>
<tr>
<td>6. Corn-soybean meal diet\textsuperscript{4}</td>
<td>222</td>
<td>291\textsuperscript{c}</td>
<td>760\textsuperscript{a}</td>
</tr>
</tbody>
</table>

| SEM | 4.23 | 5.0 | 4.99 |

\textsuperscript{1}Data are means of 5 pens of 4 chicks fed the experimental diets from 8 to 17-d posthatch; average initial weight was 112 g. Means in columns with no common superscript letters are significantly different (\(P < 0.05\)).

\textsuperscript{2}The basal Arg-deficient practical diet (Table 2.1) contained 1.0% Arg.

\textsuperscript{3}Provided 0.134% creatine, an amount isomolar to 0.12% GAA.

\textsuperscript{4}Methionine fortified corn-soybean meal positive-control diet (23% CP, 1.25% Arg).
Table 2.5. Performance of chicks fed increasing levels of arginine with or without guanidino acetic acid (GAA) in a dextrose-casein diet (Assay 4)\(^1\)

<table>
<thead>
<tr>
<th>Arg, %</th>
<th></th>
<th>Suplemental</th>
<th>Dietary(^2)</th>
<th>Weight gain, g(^3)</th>
<th>Feed intake, g(^3)</th>
<th>Gain:feed, g/kg(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.12</td>
<td>0</td>
<td>0.12</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SEM 4.2 6.0 13.0

\(^1\)Data are means of 5 pens of 4 chicks fed the experimental diets from 8 to 17-d posthatch; average initial weight was 77 g.

\(^2\)The basal Arg-deficient diet (Table 2.1) contained 0.86% Arg.

\(^3\)Main effect of GAA (\(P < 0.01\)); quadratic (\(P < 0.01\)) response to supplemental Arg.
Figure 2.1. Plot of the interaction ($P < 0.01$) of dietary Arg concentration x 0.12% GAA supplementation for gain:feed of chicks in Assay 4 (Table 2.5). Arg deficient diets are those containing less than 0.40% supplemental L-Arg and Arg-adequate diets are those containing more than 0.40% supplemental L-Arg. Values are means of 5 pens of 4 chicks.
Figure 2.2. Best-fit broken line plot of weight gain as a function of supplemental L-Arg concentration in diets with (▲) or without (●) 0.12% added GAA. Data points are means ± SEM values of 5 pens of 4 chicks (Assay 4).
Figure 2.3. Best-fit broken-line plot of gain:feed as a function of supplemental L-Arg concentration in diets with (▲) or without (●) 0.12% added GAA. Data points are means ± SEM values of 5 pens of 4 chicks (Assay 4).
Chapter 3

Dietary L-Homoserine Spares Threonine in Chicks

Abstract

Four chick bioassays were conducted to evaluate the Thr replacement value of L-homoserine (HS). Growth rate was increased (P < 0.05) by dietary addition of 800 mg L-HS/kg diet to a purified diet severely deficient in Thr or by the addition of 800 or 1000 mg of L-HS/kg diet to a corn-peanut meal diet distinctly deficient in Thr. The addition of an isomolar level of α-ketobutyrate, a catabolic product of both Thr and HS, did not elicit a response. Standard-curve methodology predicted a Thr replacement value of 38 ± 9% for HS. Interactions (P < 0.01) were observed in assays 2 and 4 between dietary Thr adequacy and 800 or 1000 mg L-HS/kg supplementation. Thus, HS improved growth performance when added to a Thr-deficient diet (0.46 g Thr/100 g diet), but it decreased growth performance when added to the same diet containing surfeit Thr (0.80 g Thr/100 g diet). The results indicate that low levels of HS elicit a growth response in young chicks fed Thr-deficient diets.

Introduction

Homoserine occurs free (unbound to protein) in a number of plants and it is particularly rich in pea seedlings (Meister, 1965). It is also formed during catabolism of the carbon skeleton of Met in higher organisms (Meister, 1965). It is well established (Flavin and Kono, 1960; Flavin and Slaughter, 1960a,b; Kim et al., 2004; Rinder et al., 2008; Watanabe and Shimura, 1956, 1960) that plants, bacteria, and yeast can synthesize not only Thr but also Met from HS. Watanabe and Shimura (1956, 1960) showed that at least 2 separate enzyme reactions are needed in yeast and Neurospora for synthesis of Thr from HS. The first involves HS kinase, which catalyzes the phosphorylation of HS via ATP. The second key enzyme in Thr

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biosynthesis from HS is Thr synthetase and this enzyme requires pyridoxal phosphate. Evidence for these conversions in higher organisms, however, does not exist. Thus, evidence is presented here that HS has Thr replacement bioactivity in young chicks.

**Materials and Methods**

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Four bioassays were conducted using male chicks (New Hampshire × Columbian) obtained from the University of Illinois Poultry Farm. Chicks were housed in thermostatically controlled starter batteries with raised-wire flooring in an environmentally controlled room with continuous lighting. Water and experimental diets were provided on and *ad libitum* basis throughout the trial period.

From hatch to d 7 posthatch, chicks were fed a diet adequate in all dietary nutrients (NRC, 1994). Following an overnight fast, chicks were weighed, wing-banded, and randomized to dietary treatments on d 8 such that average initial pen weights and weight distributions were similar across treatments. At the conclusion of the experiment, chicks and feeders were weighed. Body weight gain, feed intake, and feed efficiency (gain:feed ratio) were calculated for each replicate pen of chicks.

**Basal Diets.** Two separate basal diets *(Table 3.1)* were formulated to evaluate the Thr replacement value of L-HS. The purified and the corn-peanut meal-basal diets were formulated to be singly deficient in Thr but were otherwise nutritionally complete for chicks of this age (NRC, 1994). Individual proteinaceous ingredients as well as basal diets were analyzed for crude protein (CP), Thr, Met, Cys, and HS as previously described (Chung and Baker, 1992). The L-HS used herein was synthesized by Evonik Degussa (Hanau-Wolfgang, Germany). Amino acid analysis indicated the product was >99% pure HS and contained no detectable Thr.
**Assay 1.** The objective of this assay was to determine whether or not L-HS can spare the need for Thr in young chicks. The purified basal diet, singly deficient in Thr, was supplemented with either 800 mg Thr/kg diet or isomolar (800 mg/kg) or twice isomolar (1,600 mg/kg) concentrations of L-HS at the expense of cornstarch (Thr and HS have identical molecular weights). Five replicate pens of 4 chicks received each of the 4 experimental diets during a 12-d feeding period (d 8 to 20 post-hatch).

**Assay 2.** This assay sought to determine whether or not L-HS or α-ketobutyrate (α-KB) can spare the need for Thr in young chicks fed a Thr-deficient corn-peanut meal diet. Analysis of both corn and peanut meal revealed that there was no detectable free or bound HS in either ingredient. Graded doses of L-Thr (0, 400, or 800 mg/kg) were added to the basal diet to produce a standard curve. As a positive control, the basal diet was also supplemented with 3,400 mg L-Thr/kg diet to exceed the dietary Thr requirement for this age chick. L-homoserine or α-KB was added to the basal diet or positive control diet at concentrations isomolar to 800 mg L-Thr/kg diet. Each of the dietary treatments (8 total) were assigned to 4 replicate pens of 4 chicks during a 9-d feeding period (d 8 to 17 post-hatch).

**Assay 3.** In this assay, the Thr-sparing effect of L-HS was evaluated both directly and indirectly, i.e., via contribution from L-Met (i.e., Met conversion to HS). The Thr-deficient corn-peanut meal basal diet was supplemented with 0 or 800 mg L-Thr/kg diet or 800 mg L-HS/kg diet (i.e., isomolar to 800 mg L-Thr/kg diet). Additionally, 1,000 or 2,000 mg L-Met/kg diet (isomolar and twice isomolar that of 800 mg L-HS/kg diet, respectively) was added to the Thr-deficient basal diet. Five replicate pens of 4 chicks were fed each experimental diet during an 8-d feeding period (d 8 to 16 post-hatch).
**Assay 4.** To confirm the positive growth response to L-HS in chicks fed Thr-deficient diets as well as the negative growth response in chicks fed diets with surfeit Thr, 3 replicate pens of 4 chicks were fed 5 different treatment diets in a 9-d growth bioassay. The Thr-deficient corn-peanut meal diet (0.46 g Thr/kg diet) was fortified with either 1,000 or 3,400 mg L-Thr/kg diet. As in assay 2, the Thr-deficient basal diet and the diet with surfeit Thr (3,400 mg L-Thr/kg diet) were also supplemented with 1,000 mg L-HS/kg diet. At the end of the 9-d feeding period, heparinized blood was obtained via heart puncture from individual chicks. Plasma was prepared, after which an equal volume of plasma from each of the 4 chicks in a pen-replicate was pooled, and then deproteinized with an equal volume of sulfosalicylic acid. The 15 deproteinized plasma samples (5 treatments x 3 replicates) were then frozen until analyzed chromatographically for AA (Fekkes, 1996).

**Statistical Analysis.** All data were subjected to ANOVA using the General Linear Model procedure of SAS (SAS Institute, 2004). Data were analyzed using pen means with procedures appropriate for a completely randomized design. Data are presented as mean values with pooled SEM estimates, and significance was set at α = 0.05. Differences among treatment means were evaluated using the least significance procedure (Carmer and Walker, 1985).

In Assay 2, the ability of L-HS to spare Thr was quantified using standard-curve methodology (Augspurger et al., 2005; Pahm et al., 2009). Briefly, gain:feed (g/kg, dependent variable) was regressed on supplemental L-Thr intake (mg, independent variable) to produce a linear regression equation. Supplemental intake of L-Thr equivalents was then predicted for L-HS (i.e., diet 5) using the regression equation, after which this calculated value was divided by the mean total supplemental intake of L-HS (i.e., 800 mg/kg x feed intake) and multiplied by 100. Four of the diets in assays
2 and 4 formed a 2 x 2 factorial arrangement of treatments. Hence, these 4 treatment diets were evaluated by single degree-of-freedom contrasts to assess the main effects of HS supplementation and Thr concentration, and also the interaction.

**Results**

**Assay 1.** Weight gain and gain:feed increased ($P < 0.05$) when the Thr-deficient purified diet was supplemented with 800 mg Thr/kg diet or an isomolar concentration (800 mg/kg) of L-HS (**Table 3.2**). Addition of 1,600 mg HS/kg diet also increased both gain and gain:feed, but the response was no greater than that observed with 800 mg HS/kg.

**Assay 2.** Supplementation of the Thr-deficient corn-peanut meal diet with the first three doses of L-Thr resulted in linear ($P < 0.01$) responses in weight gain, feed intake, and gain:feed ratio (**Table 3.3**). Addition of 800 mg L-HS/kg to the basal diet improved ($P < 0.05$) gain:feed. Diets 1, 4, 5, and 6 represented a 2 x 2 factorial arrangement of treatments, and assessment of the interaction for these treatments revealed significance ($P < 0.01$) for weight gain, feed intake, and gain:feed. Thus, when dietary Thr was deficient, 800 mg L-HS/kg produced a positive response, but when dietary Thr was superadequate, 800 mg L-HS/kg depressed growth performance (**Figure 3.1**). Addition of 686 mg α-KB/kg, isomolar to 800 mg HS/kg, had no effect on growth performance of chicks fed the Thr deficient basal diet or the diet containing surfeit Thr.

Gain:feed ratio of chicks fed diets 1, 2 and 3 was used to establish a linear regression equation ($Y = 482.1 + 0.506X$, $r^2 = 0.93$, with $Y =$ gain:feed in g/kg and $X =$ supplemental L-Thr intake in mg). This equation was then used as a standard curve to estimate the L-Thr replacement activity of L-HS for chicks receiving diet 5, which was $38.2 \pm 9.0\%$. 
Assay 3. Addition of 800 mg L-Thr/kg to the Thr-deficient corn-peanut meal diet resulted in increased ($P < 0.05$) gain, feed intake, and gain:feed (Table 3.4). Likewise, chicks fed diets fortified with 800 mg L-HS/kg also responded with increased ($P < 0.05$) weight gain, feed intake, and gain:feed, although the responses to L-Thr were greater ($P < 0.05$) than those to L-HS. An approximate calculation of Thr sparing by HS (gain response to 800 mg HS/kg ÷ gain response to 800 mg Thr/kg) resulted in a HS replacement value of 40%. Supplementation of isomolar or twice isomolar levels of (excess) DL-Met had no effect on chick performance.

Assay 4. Supplemental L-HS at 1,000 mg/kg increased ($P < 0.05$) both gain and gain:feed, but only when added to the Thr-deficient diet (Table 3.5). A HS replacement value of 39% was calculated (as for assay 3) based upon gain:feed responses to 1,000 mg HS/kg vs 1,000 mg Thr/kg, respectively. Addition of 1,000 mg HS/kg to the diet containing surfeit Thr decreased weight gain, feed intake, and gain:feed ratio. Thus, in agreement with the results of assay 2, the interaction of Thr concentration x HS was significant ($P < 0.01$) for all growth criteria.

Homoserine was analyzed to be present in plasma of chicks fed the 2 diets containing supplemental HS, and the combination of 1,000 mg L-HS/kg plus 3,400 mg L-Thr elevated plasma HS to a greater extent ($P < 0.05$) than addition of 1,000 mg L-HS/kg alone. No HS was found in plasma of chicks fed diets without added L-HS. Plasma Thr was similar in chicks receiving diets with no added Thr or those with 1,000 mg Thr/kg, but addition of supplemental L-Thr (3,400 mg Thr/kg) beyond the chick’s requirement resulted in elevated ($P < 0.05$) plasma Thr. Also, dietary addition of 1,000 mg L-HS/kg together with 3,400 mg L-Thr/kg increased ($P < 0.05$) plasma Thr more than addition of 3,400 mg L-Thr/kg alone. There were no significant changes in other plasma AA (e.g., Gly, Ser, Met, Cys) due to dietary treatment.
Discussion

The results clearly indicate that dietary L-HS has Thr replacement bioactivity. Threonine biosynthesis is a possible explanation, but whether this might be occurring in the liver or other body tissues, or perhaps in the gut via gut microbes (Raj et al., 2008; Torrallardona et al., 2003) is open to question. Plants and microbes synthesize Thr from HS (Flavin and Kono, 1960; Flavin and Slaughter, 1960a,b; Kim et al., 2004; Rinder et al., 2008; Watanabe and Shimura, 1956, 1960). The results of assay 2 clearly point to the conclusion that the HS response results from HS per se and not from α-KB, the immediate deamination product of HS. Since the carbon skeleton of Met is metabolized to HS, and then α-KB (Carroll et al., 1949; Matsuo and Greenberg, 1959; Meister, 1965), the objective of assay 3 (Table 3.4) was to show a growth response to excess dietary Met, i.e., as a source of HS. It was realized, however, that HS produced via metabolism is not the same as providing L-HS in the diet. Also, relative to the Thr deficiency in the basal diet, this diet already contained a considerable excess of Met. Thus, the lack of a growth response to additional excess Met is probably not surprising. The Thr-deficient corn-peanut meal diet used herein was supplemented with 5,000 mg L-Met/kg but tests were unable to detect free HS in blood plasma (Appendix Table A.1). Moreover, no HS was found in plasma of chicks receiving either Thr deficient or Thr surfeit diets not containing supplemental L-HS in assay 4 herein (Table 3.5). Thus, Met catabolism may not yield HS per se.

Cystathionine gamma-lyase (EC 4.4.1.1), also known as homoserine deaminase-cystathionase, is the enzyme that degrades cystathionine to Cys, α-KB and ammonia (Carroll et al., 1949; Matsuo and Greenberg, 1959; Meister, 1965). The HS formed in this reaction is bound to the enzyme and, in fact, may never be released as free HS, only as α-KB.
What remains to be done is to orally administer 1-\(^{14}\)C labeled HS (and Met) to see if the radiolabel can be identified in body Thr – or perhaps also in gut mucin, a protein known to be rich in Thr and to decrease under conditions of Thr deficiency (Faure et al., 2005; Horn and Adeola, 2007; Nichols and Bertolo, 2008). Up to 30% of the AA content of mucin is comprised of Thr, and mucin biosynthesis accounts for a significant portion of the total dietary Thr requirement (Bertolo et al., 1998; Faure et al., 2005; Horn and Adeola, 2007; Nichols and Bertolo, 2008; Schaart et al., 2005). If either gut mucosal tissue or gut bacteria are capable of metabolizing HS to Thr to facilitate synthesis of gut mucins, this could (indirectly) allow more of the Thr in portal circulation (or that made available from protein degradation) to be available for muscle protein synthesis and growth.

Klasing (2009) in commenting on the lysine knockout work of Cleveland et al. (2008), concluded that a reduction in the catabolism of a given AA would likely result in a sparing (and reduced requirement) for that AA. Hence, the Thr sparing effect of HS observed herein may have resulted from HS causing a reduction in Thr catabolism. Nishimura and Greenberg (1961) found that HS decreases the activity of sheep liver Thr dehydratase (EC 4.2.1.19), and House et al. (2001) showed that Thr 1-\(^{14}\)C-Thr oxidation in rat hepatocytes could be suppressed by either cysteamine or \(\alpha\)-cyanocinnamate. It is possible that these compounds, like HS, would elicit growth responses when given to Thr-deficient chicks. Clearly, whether in hepatocytes or in the intact animal, a reduction in 1-\(^{14}\)C-Thr oxidation due to HS administration would offer support for the reduced catabolism mechanism of Thr sparing by HS.

An explanation is not obvious for why 800 or 1,000 mg/kg supplemental L-HS was growth depressing when added to a diet superadequate in Thr. Most of the HS flux involves its deamination to \(\alpha\)-KB (Carroll et al., 1949; Matsuo and Greenberg,
1959; Meister, 1965), but as shown in assay 2 (Table 3.3), equimolar addition of 686 mg/kg $\alpha$-KB to the diet containing surfeit Thr did not depress growth performance. Moreover, Thr conversion to $\alpha$-KB via Thr dehydratase (EC 4.2.1.19) is only one of 3 pathways of Thr degradation, with the Thr dehydrogenase (EC 1.1.1.103) and Thr aldolase (EC 2.1.2.1) pathways also involved in Thr degradation (Ballèvre et al., 1990; Bird et al., 1984; Davis and Austic, 1994). Hence, a build up of $\alpha$-KB from Thr (and HS) catabolism does not explain the HS-induced growth depression in chicks receiving diets containing surfeit Thr. Does HS, representing the carbon skeleton of Met during Met catabolism, somehow disrupt transsulfuration? Is HS per se toxic when added to diets adequate to superadequate in Thr and all other AA? The decreased weight gain resulting from as little as 800 or 1,000 mg/kg HS added to the diet with surfeit Thr was due largely to a 20% decrease in voluntary feed intake (Tables 3.3 and 3.5).

Previous research done in the laboratory of the University of Illinois 32 years ago involved graded dietary additions of L-HS or $\alpha$-KB to a complete crystalline AA chick diet. Supplemental L-HS (0, 4,000, 8,000 mg/kg) caused a precipitous and linear ($P < 0.01$) decrease in daily weight gain, i.e., 12.1, 6.6, 4.7 g/d, respectively; equimolar additions of $\alpha$-KB, however, had no effect on growth performance.

In conclusion, oral HS spares dietary Thr, but very modest doses (800 mg/kg) of supplemental L-HS are noxious when provided in diets adequate to superadequate in Thr. The sparing effect of HS may result from Thr biosynthesis from HS or from a HS-induced decrease in Thr catabolism. Why such a small dietary addition of L-HS reduces feed intake and growth of chicks fed diets with adequate to surfeit Thr remains to be determined.
**Literature Cited**


Table 3.1 Composition of threonine-deficient basal diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Purified diet (Assay 1)</th>
<th>Corn-peanut meal diet (Assays 2, 3 and 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>54.37</td>
<td>0.36</td>
</tr>
<tr>
<td>Corn, 8.4 g/100 g CP</td>
<td>-</td>
<td>61.08</td>
</tr>
<tr>
<td>Peanut meal, 45.6 g/100 g CP</td>
<td>-</td>
<td>27.50</td>
</tr>
<tr>
<td>Casein, 84.8 g/100 g CP</td>
<td>2.50</td>
<td>-</td>
</tr>
<tr>
<td>Soy protein isolate, 82.4 g/100 g CP</td>
<td>4.00</td>
<td>-</td>
</tr>
<tr>
<td>Amino acid mixture</td>
<td>19.56^3</td>
<td>2.26^4</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Solka floc</td>
<td>3.00</td>
<td>-</td>
</tr>
<tr>
<td>Purified mineral mix^5</td>
<td>5.37</td>
<td>-</td>
</tr>
<tr>
<td>Trace-mineral mix^6</td>
<td>-</td>
<td>0.15</td>
</tr>
<tr>
<td>Purified vitamin mix^6</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin mix^6</td>
<td>-</td>
<td>0.20</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>1.00</td>
<td>0.40</td>
</tr>
<tr>
<td>Limestone</td>
<td>-</td>
<td>1.40</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>-</td>
<td>2.10</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>-</td>
<td>0.40</td>
</tr>
<tr>
<td>Bacitracin premix^7</td>
<td>-</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1 Analyzed to contain (g/100 g diet); CP, 20.3; Thr, 0.22; Met, 0.32; Cys, 0.20.
2 Analyzed to contain (g/100 g diet): CP, 19.0; Thr, 0.46; Met, 0.42; Cys, 0.38.
3 Provided (g/100 g diet): L-Arg, 0.95; L-His, 0.33; L-Lys•HCl, 1.14; DL-Met, 0.20; L-Cys, 0.15; L-Phe, 0.50; L-Tyr, 0.45; L-Trp, 0.15; L-Leu, 1.00; L-Ile, 0.60; L-Val, 0.69; Gly, 1.00; L-Pro, 0.40; and L-Glu, 12.00.
4 Provided (g/100 g diet): L-Lys•HCl, 1.00; DL-Met, 0.20; L-Cys, 0.17; L-Val, 0.20; L-Ile, 0.23; L-Arg, 0.06; L-Trp, 0.07; and Gly, 0.33.
5 FS & D Corp., Urbana, OH.
6 See Dilger and Baker (2008) and Dilger et al. (2007) for composition.
7 Provided 55 mg of bacitracin methylene disalicylate per kilogram of complete diet.
Table 3.2  Response of young chicks to L-homoserine (HS) when added to a purified diet severely deficient in threonine (Assay 1)\(^1\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain</th>
<th>Feed intake</th>
<th>Gain:feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Thr-deficient basal diet(^2)</td>
<td>6(^b)</td>
<td>96(^b)</td>
<td>62(^c)</td>
</tr>
<tr>
<td>2. As 1 + 800 mg L-Thr/kg diet</td>
<td>23(^a)</td>
<td>110(^a)</td>
<td>210(^a)</td>
</tr>
<tr>
<td>3. As 1 + 800 mg L-HS/kg diet(^3)</td>
<td>17(^a)</td>
<td>103(^{a,b})</td>
<td>165(^b)</td>
</tr>
<tr>
<td>4. As 1 + 1,600 mg L-HS/kg diet</td>
<td>16(^a)</td>
<td>97(^b)</td>
<td>165(^b)</td>
</tr>
<tr>
<td>SEM(^4)</td>
<td>2.1</td>
<td>4.4</td>
<td>15</td>
</tr>
</tbody>
</table>

\(^1\)Data are means of 5 replicate pens of 4 male chicks during a 12-d feeding period from 8 to 20-d posthatch; mean initial weight was 89 g.

\(^2\)Contained 0.22 g Thr/100 g diet.

\(^3\)Isomolar to 800 mg Thr/kg diet.

\(^4\)Means within a column with unlike superscript letters are different \((P < 0.05)\).
Table 3.3  Response of young chicks to L-homoserine (HS) and α-ketobutyrate (α-KB) when added to a threonine-deficient or threonine surfeit corn-peanut meal diet (Assay 2)\(^1\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain(^2)</th>
<th>Feed intake</th>
<th>Gain:feed(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g/kg</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Thr-deficient basal diet(^3)</td>
<td>98(^{e,f})</td>
<td>205(^{b,c})</td>
</tr>
<tr>
<td>2.</td>
<td>As 1 + 400 mg L-Thr/kg diet</td>
<td>122(^d)</td>
<td>228(^b)</td>
</tr>
<tr>
<td>3.</td>
<td>As 1 + 800 mg L-Thr/kg diet</td>
<td>159(^b)</td>
<td>270(^a)</td>
</tr>
<tr>
<td>4.</td>
<td>As 1 + 3,400 mg L-Thr/kg diet</td>
<td>180(^a)</td>
<td>270(^a)</td>
</tr>
<tr>
<td>5.</td>
<td>As 1 + 800 mg L-HS/kg diet(^4)</td>
<td>111(^{d,e})</td>
<td>215(^{b,c})</td>
</tr>
<tr>
<td>6.</td>
<td>As 4 + 800 mg L-HS/kg diet(^4)</td>
<td>137(^c)</td>
<td>217(^{b,c})</td>
</tr>
<tr>
<td>7.</td>
<td>As 1 + 686 mg α-KB/kg diet(^4)</td>
<td>95(^f)</td>
<td>203(^c)</td>
</tr>
<tr>
<td>8.</td>
<td>As 4 + 686 mg α-KB/kg diet(^4)</td>
<td>176(^a)</td>
<td>265(^a)</td>
</tr>
<tr>
<td></td>
<td>SEM(^5)</td>
<td>5.0</td>
<td>7.7</td>
</tr>
</tbody>
</table>

\(^1\)Data are means of 4 replicate pens of 4 male chicks during a 9-day feeding period from 8 to 17-d posthatch; mean initial weight was 88 g.

\(^2\)Diets 1, 4, 5, and 6 formed a 2 x 2 factorial arrangement of treatments. Statistical analysis indicated an interaction (\(P < 0.01\)) between Thr adequacy and HS supplementation. Response to L-Thr (diets 1, 2, and 3) was linear (\(P < 0.01\)).

\(^3\)Contained 0.46 g Thr/100 g diet.

\(^4\)Isomolar to 800 mg Thr/kg diet.

\(^5\)Means within a column with unlike superscript letters are different (\(P < 0.05\)).
Table 3.4. Efficacy of L-homoserine (HS) or excess DL-methionine to replace threonine in young chicks fed a threonine-deficient corn-peanut meal diet (Assay 3)\(^1\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain</th>
<th>Feed intake</th>
<th>Gain:feed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g</td>
<td>g/kg</td>
</tr>
<tr>
<td>1. Thr-deficient basal diet(^2)</td>
<td>100(^c)</td>
<td>202(^c)</td>
<td>495(^c)</td>
</tr>
<tr>
<td>2. As 1 + 800 mg L-Thr/kg diet</td>
<td>153(^a)</td>
<td>259(^a)</td>
<td>590(^a)</td>
</tr>
<tr>
<td>3. As 1 + 800 mg L-HS/kg diet</td>
<td>121(^b)</td>
<td>220(^b)</td>
<td>550(^b)</td>
</tr>
<tr>
<td>4. As 1 + 1,000 mg DL-Met/kg diet(^3)</td>
<td>96(^c)</td>
<td>193(^c)</td>
<td>498(^c)</td>
</tr>
<tr>
<td>5. As 1 + 2,000 mg DL-Met/kg diet(^3)</td>
<td>95(^c)</td>
<td>192(^c)</td>
<td>494(^c)</td>
</tr>
</tbody>
</table>

SEM\(^4\) 3.6 5.1 7.7

\(^1\)Data are means of 5 replicate pens of 4 male chicks during an 8-day feeding period from 8 to 16-d posthatch; mean initial weight was 100 g.

\(^2\)Contained (g/100 g diet): Thr, 0.46; Met, 0.42; Cys, 0.38.

\(^3\)1,000 and 2,000 mg DL-Met/kg are isomolar and twice isomolar, respectively, to 800 mg L-HS/kg diet.

\(^4\)Means within a column with unlike superscript letters are different (\(P < 0.05\)).
**Table 3.5.** Growth performance and plasma free amino acid concentrations in chicks fed supplemental L-homoserine (HS) in corn-peanut meal diets either deficient or surfeit in threonine (Assay 4)\(^1\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain(^2)</th>
<th>Feed intake(^2)</th>
<th>Gain:feed(^2)</th>
<th>Plasma AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g</td>
<td>g/kg</td>
<td>HS  Thr</td>
</tr>
<tr>
<td>1.</td>
<td>Thr-deficient basal diet(^3)</td>
<td>71(^d)</td>
<td>169(^{d,e})</td>
<td>418(^d)</td>
</tr>
<tr>
<td>2.</td>
<td>As 1 + 1,000 mg L-Thr/kg diet</td>
<td>136(^b)</td>
<td>243(^b)</td>
<td>559(^b)</td>
</tr>
<tr>
<td>3.</td>
<td>As 1 + 3,400 mg L-Thr/kg diet</td>
<td>175(^a)</td>
<td>270(^a)</td>
<td>649(^a)</td>
</tr>
<tr>
<td>4.</td>
<td>As 1 + 1,000 mg L-HS/kg diet(^4)</td>
<td>85(^c)</td>
<td>180(^d)</td>
<td>473(^c)</td>
</tr>
<tr>
<td>5.</td>
<td>As 3 + 1,000 mg L-HS/kg diet(^4)</td>
<td>131(^b)</td>
<td>221(^c)</td>
<td>592(^b)</td>
</tr>
<tr>
<td>SEM(^5)</td>
<td>4.5</td>
<td>5.2</td>
<td>14</td>
<td>1.7</td>
</tr>
</tbody>
</table>

\(^1\)Data are means of 3 replicate pens of 4 male chicks during a 9-d feeding period from 8 to 17-d posthatch; mean initial weight was 75 g.

\(^2\)Diets 1, 3, 4, and 5 formed a 2 x 2 factorial arrangement of treatments. Statistical analysis indicated an interaction (\(P < 0.01\)) between Thr adequacy and HS supplementation.

\(^3\)Contained 0.46 g Thr/100 g diet.

\(^4\)Isomolar to 1,000 mg Thr/kg diet.

\(^5\)Means within a column with unlike superscript letters are different (\(P < 0.05\)).
Figure 3.1. Plot of the interaction ($P < 0.01$) of dietary Thr concentration x 800 mg/kg HS supplementation for weight gain of chicks in assay 2 (Table 3.3). Values are means of 4 pens of 4 chicks (pooled SEM = 5.0 g).
Amino Acids (AA) are a very important part of an animal’s diet, both nutritionally and economically. In some cases, AA precursors may be less expensive than the pure AA. Thus, if AA precursors are as effective in promoting growth and feed efficiency, they may replace a portion of the AA required to be fed, reduce the amount of intact protein or pure AA being added to the diet, and thus make the diet less expensive to produce and feed. For example, Cys has a sparing effect on Met (Finkelstein et al., 1988) and choline can be spared by S-Methylmethionine in chickens. In mammals, including humans, excess Met is able to spare choline (Augspurger et al., 2005).

In regards to guanidino acetic acid (GAA), it was shown to spare Arg in chick diets. In the first assay, the optimum level of inclusion in the diet was 0.12%. Subsequently, that level of GAA was added to Arg-deficient purified and practical basal diets in order to evaluate the ability of GAA to spare Arg. In the purified diets, GAA exhibited a large response in weight gain and gain:feed. In contrast, when GAA was added to the Arg-deficient practical-type diets, there was no growth response observed; however, there was a gain:feed response. Surprisingly, GAA produced responses even when fed in an Arg-adequate purified diet. These results confirm that GAA can spare Arg and may yield performance responses even when included in Arg-adequate diets. The reason for the GAA response in Arg-adequate diets is unknown.

The results from feeding L-homoserine (HS) show that it has Thr replacement bioactivity. When HS was added to a Thr-deficient purified diet, it increased both gain and gain:feed. In Thr-deficient practical diets, HS was able to increase gain and gain:feed; however, the responses to added Thr were better than those to HS. In
contrast to the results seen when GAA was fed to an adequate diet, when even a small level of HS was fed in a Thr-adequate diet, growth performance decreased. The reason why HS reduces feed intake and growth of chicks fed diets with adequate to surfeit Thr remains to be determined.
### Chapter 5

#### Appendix

**Table A.1.** Plasma free amino acids (μg/ml) of chicks fed supplemental L-homoserine (HS) in corn-peanut meal diets that were either deficient or adequate in threonine\(^1,2\)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Dietary supplement, mg/kg</th>
<th>1,000</th>
<th>3,400</th>
<th>1,000</th>
<th>3,400 Thr + 1,000 HS</th>
<th>5,000</th>
<th>Met</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0</td>
<td>0</td>
<td>2.1</td>
<td>3.8</td>
<td>0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td></td>
<td>5.3</td>
<td>8.4</td>
<td>63.3</td>
<td>5.6</td>
<td>104.0</td>
<td>7.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
<td>6.3</td>
<td>7.2</td>
<td>7.0</td>
<td>6.9</td>
<td>7.8</td>
<td>21.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td></td>
<td>7.8</td>
<td>8.5</td>
<td>8.9</td>
<td>8.4</td>
<td>10.8</td>
<td>8.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
<td>0</td>
<td>0.02</td>
<td>0.09</td>
<td>0</td>
<td>0.06</td>
<td>0.10</td>
<td>4.8</td>
</tr>
<tr>
<td>Taurine</td>
<td></td>
<td>52.3</td>
<td>33.2</td>
<td>42.9</td>
<td>40.9</td>
<td>60.1</td>
<td>72.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td>42.6</td>
<td>33.8</td>
<td>28.0</td>
<td>44.3</td>
<td>35.0</td>
<td>43.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td>65.9</td>
<td>56.8</td>
<td>48.9</td>
<td>68.2</td>
<td>61.0</td>
<td>70.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td>80.9</td>
<td>62.0</td>
<td>34.0</td>
<td>100.3</td>
<td>40.3</td>
<td>93.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Arginine</td>
<td></td>
<td>35.0</td>
<td>53.3</td>
<td>66.0</td>
<td>38.5</td>
<td>69.7</td>
<td>36.2</td>
<td>8.2</td>
</tr>
<tr>
<td>Urea</td>
<td></td>
<td>29.2</td>
<td>38.3</td>
<td>55.4</td>
<td>47.5</td>
<td>41.7</td>
<td>45.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(^1\)Data are mean values of 3 replicate pens of 4 chicks (n = 3, i.e., pooled blood from the 4 chicks in each of 3 pens) after 9 d of feeding.

\(^2\)See Table 3.5 for details of the growth data for this bioassay.
Chapter 6

Biography

Kasey Irene Bryant was born December 29, 1984 in Springfield, Illinois. She is the daughter of Bridget Bryant and granddaughter of Merritt and Irene Bryant Jr. At the age of 2, she moved with her mother to Salem, Illinois. She graduated in May 2003 from Salem Community High School and started her undergraduate career at the University of Illinois in August 2003. In September 2005, she was hired as an undergraduate laboratory assistant in Dr. Parsons’ poultry nutrition lab. During her undergraduate years, she was able to travel internationally and noticed that she was becoming more aware of the amount of poultry where she traveled and starting to wonder how that poultry population could be better utilized to feed people. She graduated with a Bachelor of Science degree in May 2007 in Animal Science from the University of Illinois.

Bryant entered graduate school in August of 2007 to pursue a Master of Science degree in Animal Science with focus on Poultry Nutrition under the guidance of Dr. Carl M. Parsons. She was co-advised by Dr. David H. Baker. In October 2009, she married her fiancé, Tony Angeloni III, in Champaign, Illinois. Upon completion of her master's program, Mrs. Bryant-Angeloni will be moving with her husband to the Lewisburg, Ohio area, where she has accepted a Nutrition Assistant position with Akey, part of Provimi North America.

Publications and Presentations

Peer-reviewed journal articles


Abstracts/Presentations