ABSTRACT

CORDOVA NOBOA, HERNAN ALEJANDRO. Effects of Guanidinoacetic Acid in Broilers. (Under the direction of Dr. Edgar O. Oviedo Rondón.)

Guanidinoacetic acid (GAA) is a precursor of creatine and it is considered to be a more stable molecule as feed additive compared to creatine. Creatine is an important component of the energy delivery process in several tissues, particularly those characterized by a high and/or fluctuating energy demand. Two experiments were conducted to assess the effects of GAA on broiler live performance, carcass and cut up yields, meat quality (drip and cook loss, shear force, color, post-mortem pH), pectoral myopathies (white striping, wooden breast, and *spaghetti* muscle), blood hematology, clinical chemistry and GAA serum metabolites concentration. For both trials, chickens were raised on used litter. For the first experiment, a total of 800 one-d-old male Ross 708 chicks were randomly placed in 40 floor pens. Type of grain (corn or sorghum) and two levels of GAA supplementation (0 *vs.* 0.06%) were considered as main factors in a completely randomized block design with a 2 x 2 factorial arrangement of treatments. The second experiment consisted in two levels of inclusion of poultry by-products (0 *vs*. 5%) and two levels of supplementation of GAA (0 *vs*. 0.06%) considered as main factors in a completely randomized block design with a 2 x 2 factorial arrangement of treatments. For this trial, a total of 1,280 one-d-old male Ross 708 chicks were randomly placed in 64 floor pens. Data were analyzed in JMP 12 (SAS Inst. Inc., Cary, NC, 2016) using ANOVA in a mixed model. Differences between means were obtained using Tukey's or t-student test at a level of significance of alpha = 0.05. Generally,

the supplementation with GAA improved BW gain and FCR ($P < 0.05$) on broilers fed GAA supplemented diets, regardless of grain type and poultry by-products inclusion in the diet. Overall, breast meat yield improved ($P < 0.05$) in corn diets and diets with poultry by products. Meat quality was not affected ($P > 0.05$) by GAA supplementation except for the ultimate pH in the first experiment, in which the addition of the feed additive lowered the ultimate breast meat pH at 51 and 55 d of age. For both experiments, the probability distribution of wooden breast scores was affected ($P < 0.05$) by GAA supplementation. Blood collection was done only for the second experiment. Serum GAA and creatine concentrations were higher ($P < 0.05$) in broilers that ate supplemented diets. In conclusion, GAA addition could be used to improve live performance in corn or sorghum-based diets and in diets with or without poultry by-products inclusion. In addition, the use of feed additives that are comprised by metabolites or compounds involved in muscle development, growth and metabolism could be of interest on reducing current pectoral myopathies in the poultry industry due to the alleviation response observed in the severity of wooden breast myopathy in the current trials.

Effects of Guanidinoacetic Acid in Broilers

by
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DEDICATION

I dedicate this dissertation to my beloved parents Bolivar Cordova and Cecilia Noboa who supported and helped me in each step of the way. Also to my brothers Juan Carlos and Erik, for being my constant motivation. Lastly, I would like to dedicate this thesis to my lab colleagues and friends.

BIOGRAPHY

Hernan Alejandro Cordova Noboa is the first of the three siblings of Bolivar Cordova and Cecilia Noboa. He was born in Quito-Ecuador, and lived there for 29 years before he moved to Raleigh-North Carolina to continue his education. He obtained his Bachelor of Science degree with a Major in Farming Science from the Army University of Ecuador in 2011. At the same year he started working with the National Food Processor Company – PRONACA in the department of animal nutrition as an assistant. He received a Specialist in Animal Nutrition graduate degree in 2015 from Central University of Ecuador. In August 2015, he was accepted to start his Master's degree in Poultry Science at North Carolina State University where he joined Dr. Oviedo's lab as a graduate student and research assistant. He hopes to continue his doctoral studies at the same university in order to improve his knowledge about poultry industry related with nutrition.

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CHAPTER I

LITERATURE REVIEW

I.1 Introduction

Poultry selection has progressed continuously since the early 1970s and resulted in a very rapid increase in chicken growth rate and meat production. Over the past 45 years body weight (BW) gain, feed conversion ratio (FCR), and meat yield has been improved. Comparing 2016 with 2015 broiler's performance results in the U.S., live weight has increased by 27.3 g and at the same time FCR was reduced yearly by around 2 points (National Chicken Council, 2017).

At the same time, the growth, development, structure and overall metabolism of muscles has been also modified by such selection which probably resulted in modifications affecting biochemical and sensory characteristics of meat (Santoso, 2002; Rémignon and Bihan-Duval, 2003, Petracci *et al.*, 2014b). In the past years, several studies evidenced that fast growing strains exhibited a high incidence of idiopathic myopathies, an increased susceptibility to stress-induced myopathies which may have great implications for meat quality and incidence of abnormal conditions, such as deep pectoral myopathy, pale, soft and exudative-like meat, and an increase in pectoral myopathies like wooden breast, white striping and spaghetti muscle, has been reported (Owens *et al.*, 2009; Petracci and Cavani, 2012; Bailey *et al.*, 2015; Russo *et al.*, 2015; Trocino *et al.*, 2015). Several management and

nutritional solutions have been proposed to minimize and solve these myopathies and meat quality issues. Among these techniques, the reduction of growth rate by using feed restriction programs or lowering the energy value of the diets (Kuttappan *et al.*, 2012a; Trocino *et al.*, 2015; Radaelli *et al.*, 2017), higher dietary vitamin E and selenium (Guetchom *et al.*, 2012; Kuttappan *et al.*, 2012b), have been tested and show some benefits to improve meat quality and reduce myopathy occurrence. However, reduction of growth rate can be detrimental for productivity.

Additionally, it has been proposed that nutrients and molecules with specific activity in skeletal muscles could have a bigger effect on reducing these myopathies while improving live performance. Among these molecules, a precursor of creatine, the guanidinoacetic acid (GAA), was evaluated in the studies described in this project. The main reason for choosing this molecule is due to the changes observed in the use of feed ingredients in the poultry industry going from the more common use of all vegetable diets to the replacement of traditional feedstuffs like corn by some less common like sorghum (Kwari *et al.*, 2012, Torres *et al.*, 2013, Beski *et al.*, 2015). Under these two conditions the dietary supply of creatine or the digestibility of some key amino acids in muscle and nitrogen metabolism can be reduced (Rostagno *et al.*, 1973; Talmadge *et al.*, 1975; Mitaru *et al.*, 1985; Ebadi *et al.*, 2005) affecting growth, live performance, muscle metabolism and its development (Butler *et al.*, 1992; Hancock, 2000; Selle *et al.*, 2010).

This literature review will include topics like metabolism of creatine and GAA, its relationship with use of all poultry by-products or vegetable diets and replacement of corn for sorghum. Additionally, aspects of meat quality, muscle development and metabolism pre-

and post-mortem, and myopathies will be revised since it is expected to be one of the main effects in the subsequent studies presented herein.

Creatine and guanidinoacetic acid

Creatine is a naturally occurring component in animals and plays a major role in energy metabolism. In mammals, creatine is naturally formed mainly in the liver from GAA, which in turn is mainly in the kidney synthesized from the amino acids: arginine and glycine, that is mainly used in muscle tissues. Arginine is formed within the kidney and it is then either released into the blood and consumed by other tissues or used within the kidney itself for guanidinoacetate synthesis (Wyss and Kaddurah-Daouk, 2000). Avian species are able to synthesize GAA either from the kidney or from the liver (Van Pilsum *et al.*, 1972). GAA is a compound synthesized by the enzyme L-arginine:glycine amidinotransferase (AGAT) in the avian kidney and liver. The formation of GAA, also referred as glycoamine or guanidinoacetate (Michiels *et al.*, 2012), is normally the rate-limiting step of creatine biosynthesis. Consequently, the AGAT reaction is the most likely control step in the pathway. Based on different studies, it is hypothesized the existence of a feedback repression of AGAT by creatine, the end-product of the pathway (Figure I.1). Therefore, most probably creatine serves to conserve the dietary essential amino acids arginine and methionine (Wyss and Kaddurah-Daouk, 2000). Once GAA is obtained, it is methylated by S-adenosylmethionine (SAM) to form creatine, which is not regulated by a feedback mechanism (McBreairty *et al.*, 2015) and finally, adenosine triphosphate (ATP) donates a

phosphorus moiety to form the high-energy compound phosphocreatine as it is shown in Figure I.1 (Meister, 1965). Phosphocreatine is a major source of ATP replenishment in tissues with rapidly fluctuating energy demand. This supply is mediated by the creatine kinase reaction (McGuire *et al.*, 1984), in which creatine and ADP are reversibly phosphorylated to phosphocreatine and ATP, respectively (Wallimann *et al.*, 1998; Nabuurs *et al.*, 2013).

Supplemental creatine has the potential to spare methyl groups via negative feedback on AGAT activity, lowering GAA production and the demand for methyl groups used in creatine synthesis, as well as lowering homocysteine production, which has been demonstrated in creatine supplemented rats (Deminice *et al.*, 2009). S-Methylmethionine (SMM) is an analog of S-adenosyl-methionine (SAM), with a methyl group substituted for the adenosyl group. This compound is unique to plants and is found in considerable quantity in several vegetable-based foods, including soybeans and soybean meal (Augspurger *et al.* 2005; Grunau and Swiader 1991; Kovatscheva and Popova 1977). Radiolabeling experiments have suggested that the methyl group(s) of SMM are available for choline or creatine biosynthesis, but not for methionine biosynthesis (Stekol, 1955).

In an experiment conducted by Baker (2009), they developed a soy-protein isolate semi-purified diet devoid of choline (also SMM) and deficient in methionine. When the diet was made singly deficient in choline or in both choline and methionine, dietary SMM produced a marked growth response in young chicks. However, when the diet was adequate in choline and singly deficient in methionine, SMM supplementation did not elicit a

response. Thus, it appears that the re-methylation of homocysteine to methionine via betaine-homocysteine methyl transferase prefers betaine as a methyl donor and that the methyl from SMM may be used only if choline or betaine are inadequate in the diet. Thus, soybean meal contains not only choline and betaine, but also 0.165% SMM (Augspurger *et al.*, 2005). Because choline and creatine biosynthesis are thought to account for most the methyl demand from S-adenosylmethionine, it seems likely that SMM will also be found capable of methylating GAA to form creatine.

Previous studies had reported the efficacy of creatine and GAA to spare arginine (Fisher *et al.*, 1956 a,b; Waterhouse and Scott, 1961; Austic and Nesheim, 1972; Dilger *et al.*, 2013). However, creatine as a feed additive has several drawbacks, such as instability and high cost, as compared to GAA, which is more stable and less expensive (Baker, 2009). Considering this, GAA should be more suitable for use in animal nutrition (Michiels *et al.*, 2012). Consequently, GAA as precursor of creatine may be important for poultry nutrition not only as a replacement for dietary arginine, but also to support overall energy homeostasis of the bird, especially in skeletal muscles (Nabuurs *et al.*, 2013). The economics of energy utilization in the body could be more efficient by supplementing creatine or GAA as a precursor of creatine because the lack of sufficient creatine can limit methionine availability for protein and phosphatidylcholine synthesis, thereby limiting muscle growth (Wallimann *et al.*, 2011). Methionine is an indispensable amino acid that when not incorporated into protein will be converted into the methyl donor S-adenosylmethionine (SAM). When methionine is limited, the nutrients folate (folic acid) and betaine (derived from choline) donate a methyl

group during re-methylation, which provides an endogenous supply of methionine to meet the methionine demand (McBreairty and Bertolo, 2016). Cofactors involved in creatine metabolism include Zn^{2+}, Mn^{2+}, vitamin $B_{12,}$ pyridoxine (B_6) (Lee *et al*., 2010) and in SMM metabolism, copper and nicotinamide or niacinamide play an important role (Li *et al.*, 2007).

It has been reported based on mice trials that creatine may have a protective role in certain neuromuscular (Chung *et al.*, 2007; Tarnopolsky, 2007) and neuro-degenerative (Bender *et al.*, 2006; Kolling and Wise, 2010; Beal, 2011) diseases, as well as potentially being able to reverse muscular dystrophy (Nabuurs *et al.*, 2013). Creatine supplementation reduces plasma homocysteine levels and lipid peroxidation biomarkers, suggesting a protective role against oxidative damage (Deminice *et al.*, 2009) and this may be its main effect on muscle degenerative diseases.

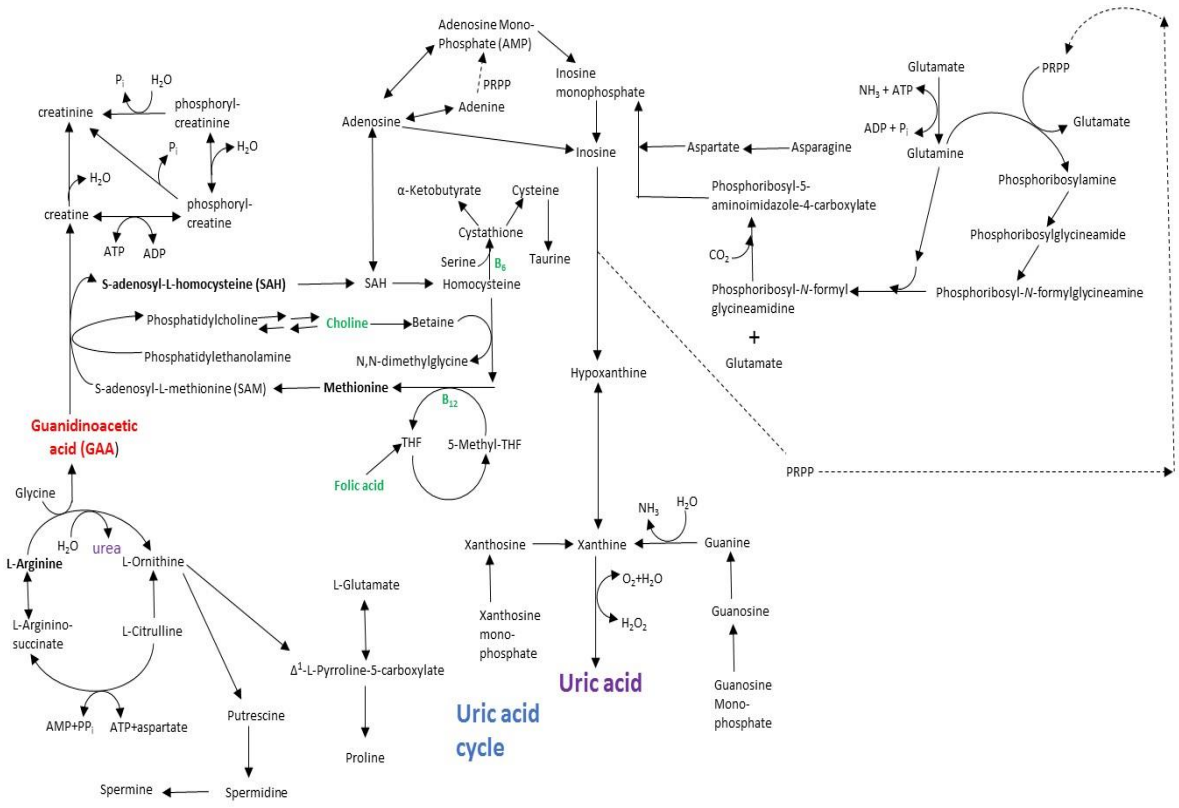

Figure I.1. Major pathways of the GAA, creatine and uric acid cycle (Adapted from Stevens, 1996; Bories *et al.*, 2009).

All vegetable diets and creatine

It is recognized that the quality and chemical composition of poultry meat may be affected by some nutrients of chicken feed. Dietary carbohydrates are important sources of energy for poultry. Cereal grains (corn, sorghum, wheat, and barley) contribute most of the carbohydrates in poultry diets (Jubbarah and Elzubeir, 2006). Recently, an increase in feeding broilers diets containing only plant-based ingredients has been observed (Vieira and

Lima, 2005; Hossain *et al.*, 2015). It has been reported that if animal by-products, which are rich in creatine, were included in poultry diets then no signs of a creatine deficiency should be detected. As a result of the ban of meat and bone meal in 2001, European poultry producers observed a certain drop in performance (European Community, 2000). This could have been caused by the lack of creatine supply because vegetable based feed ingredients do not contain this semi-essential nutrient. Therefore, with the absence of animal proteins in pure vegetable diets also called "all vegetable diets", the risk for a creatine deficiency has increased (Lemme *et al.*, 2007). According to Hossain *et al.* (2015), the live performance of chickens fed diets containing animal by-products can be achieved also by feeding vegetable diets. However, additional nutritional and economic impacts need to be considered.

The majority of an animal's dietary protein requirement is supplied by plant protein sources. Worldwide, traditionally, the most used energy and protein sources are respectively, maize and soybeans. Cereals, such as wheat and sorghum, and some plant protein meals are used all over the world as well. Soybean meal (SBM) is the preferred protein source used in poultry feed manufacturing (Beski *et al.*, 2015). The crude protein of SBM is about 40-48%, which depends on the quantity of hulls removed and the oil extraction process. Compared to the protein meal of other oilseed grains, soybean protein is favored due to its well-balanced amino acid profile, especially the essential ones that are needed to balance most cereal-based diets (Ravindran, 2013). Plant proteins are usually cheaper than animal proteins; however, there is a limitation to their use because of their content of anti-nutritional factors (Beski *et al.*, 2015). The most commonly found anti-nutrients in plant protein sources are phytic acid, undigestible proteins like kafirins, glycinin and conglycinin, toxic amino acids, saponins,

cyanogenic glycosides, tannins, gossypol, oxalates, goitrogens, lectins, protease inhibitors, chlorogenic acids, and amylase inhibitors (Akande *et al.*, 2010).

In general, vegetable protein sources are nutritionally unbalanced and poor in certain essential amino acids, and this decreases their biological value as they may not furnish the required limiting amino acids needed by birds for egg and meat production. Part of the solution to this amino acid imbalance was solved by the use of animal protein sources to create balanced diets (Akhter *et al.*, 2008). However, the current prohibition of the use of animal protein sources in poultry nutrition in many countries, has led to a possible inefficient supply of creatine (Lemme *et al.*, 2007; Ringel *et al.*, 2007; Michiels *et al.*, 2012; Heger *et al.*, 2014), and amino acids (Hossain, *et al.*, 2012; Beski *et al.*, 2015) being available for animal's metabolism.

Due to the inadequacy or lack of endogenous synthesis, arginine, glycine, and proline are essential for chickens (Baker, 2009). Previous studies (Fernandez *et al.*, 1994; Baker, 2009) determined the order of amino acid limitation in both corn and dehulled soybean meal for chicks during the period 8–22 days post-hatching. For corn, the limiting order was: lysine, threonine, tryptophan, arginine, valine and isoleucine (equally 4th limiting), sulfur amino acids, phenylalanine + tyrosine, and histidine. The order of limiting amino acids in soybean meal (SBM) was: sulfur amino acids, threonine, lysine and valine (equally 3rd limiting). In addition, the mixture of corn-SBM diets results suggested that the limiting order was: methionine, lysine, and threonine (Fernandez *et al.*, 1994). In grain sorghum, it has been reported (Shelton *et al.*, 1951) that lysine and threonine are the corresponding first and

second limiting amino acids. However, this can vary according to tannin content in the grain (Ebadi *et al.*, 2005).

Sorghum-based diets

Corn commonly is the main source of dietary energy in poultry nutrition. Although it is produced throughout the world, there is a stiff competition for maize among human's livestock, and the industry. This is because maize is high in energy as compared to other cereal grains (Mohamed *et al.*, 2015). Therefore, the need to evaluate partial or total replacements of corn and its impact on poultry production become of interest. Sorghum based-diets could be used as an alternative to the conventional corn based-diets.

Worldwide, sorghum grain is an important ingredient in poultry diets (Rooney, 1990). More than 90 percent of all sorghum consumed in the United States (Figure I.1, I.2) is used for livestock feed (Hamman *et al.*, 2002) and its inclusion in poultry diets is expected to increase in the near future. Sorghum (*Sorghum bicolor* L. Monch) is considered the fifth most important crop in the world after wheat, rice, corn and barley (Bryden *et al.*, 2009). According to Beyer (2010), production of grain sorghum in the U.S. is significantly lower than corn. However, sorghum is still the second most used cereal grain for commercial growers of broilers, turkeys and egg layers in regions where both are grown in the U.S.

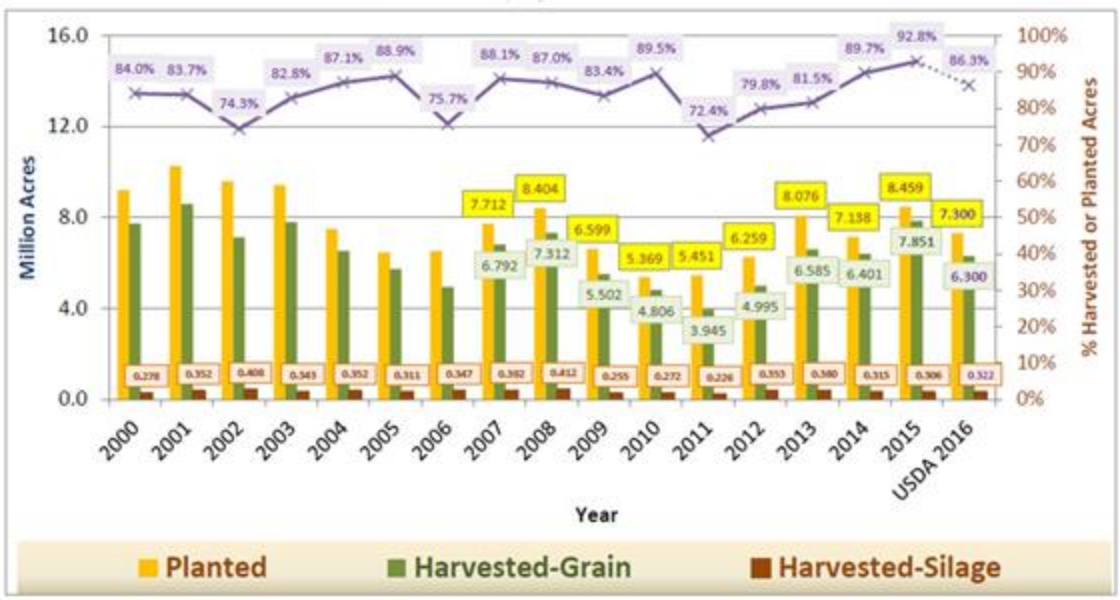

Figure I.2. U.S. grain sorghum planted & harvested acreage (1973-2016) as of March 9, 2016 USDA WASDE and December 14, 2015 USDA projections (O'Brien and Taylor, 2016).

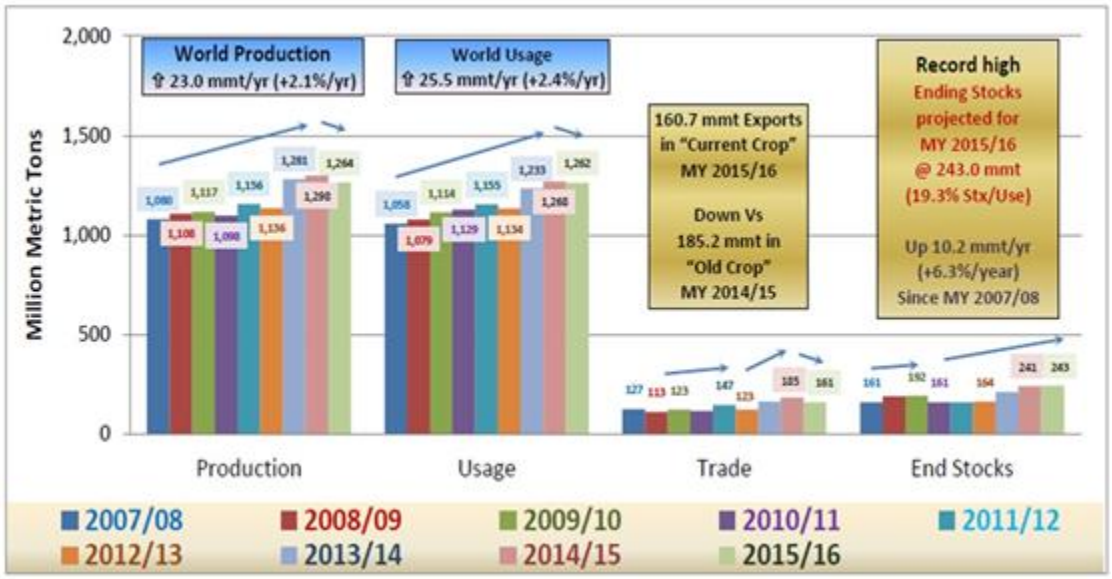

Figure 1.3 World coarse supply-demand: MY 2007/08 "current crop" 2015/16 as of March 9, 2016. USDA WASDE projections (O'Brien and Taylor, 2016).

Sorghum is included in animal diets primarily as an energy source type of ingredient, derived from starch. However, the efficiency of utilization of energy from sorghum can be variable and may affect animal production (Liu *et al.*, 2013). Although, the chemical composition of sorghum is similar to corn, sorghum has been associated with sub-optimal, or inconsistent poultry performance (Black *et al.*, 2005; Bryden *et al.*, 2009). Some researchers had found that the nutritional value is usually considered to have about 5% less feeding value than corn (Rooney, 1990; Dowling *et al.*, 2002; Leeson and Summers, 2005). Starch is the major proximate component (63 to 74%) and the major energy supplier in sorghum grain (Perez-Maldonado and Rodriguez, 2007). It's important to consider that sorghum has several anti-nutritional factors that could limit its inclusion in poultry diets. In addition, there are issues with sorghum as a feedstuff for chicken-meat production including kafirin proteins, phenolic compounds, and phytate. The likelihood is that both kafirin and phenolic compounds are compromising efficiency of energy utilization in broilers offered sorghum-based diets (Selle *et al*., 2006). Sorghum's predominant protein fraction in the grain is kafirin. Kafirin is a poor quality source of protein. This may not be crucial as kafirin only constitutes in the order of 15% of total protein in a sorghum-based broiler diet. Nevertheless, sorghum is potentially an attractive energy source for poultry industry (Selle, 2011).

Table I.1. Previous studies of corn replacement by sorghum

% Replacement of corn	Commercial strain and days of rearing	Live performance results	Reference
Up to 45%	Hubbard (49 d)	Similar results as corn on BW, BW gain, feed intake, FCR	Mohamed *et al.*, 2015
Up to 100%	Cobb 500 (42 d)	NS differences for BW gain, feed intake, FCR	Torres *et al.*, 2013
Up to 100%	From commercial company (63 d)	NS differences for BW, BW gain and FCR	Kwari *et al.*, 2012
Up to 100%	Broiler Lohman (49 d)	Similar results as corn on BW, BW gain, feed intake, FCR	Pour-Reza and Edriss, 1997
Up to 50%	Multi-colored broilers (56 d)	No differences for BW, BW gain and FCR	Kumar *et al.*, 2005
Up to 100%	Cobb 500 (56 d)	NS differences for BW and FCR with low tannin sorghum variety	Tandiang *et al.*, 2014
Up to 40%	Hybrid broiler (56 d)	NS differences for BW gain, feed intake, FCR	Dhliwayo, 2011
Up to 100%	Ross 308 (21 d)	NS differences for BW, BW gain and gain: weight	Nyannor *et al.*, 2015

Previous studies (Selle *et al.*, 2006; Torres *et al.*, 2013; Mohamed *et al.*, 2015) showed that partial or total replacement of corn-based diets with sorghum did not affect broiler live performance in different commercial strains. Most of these experiments used low-tannin sorghum. According to Hamman *et al.*, 2002, the processing method used for sorghum plays a major role in its effectiveness as a feed. The chemical components of sorghum and corn are similar and suggest that the two grains should have comparable feeding values. Consequently, a total replacement of corn with sorghum with no negative impacts on broiler production could be achieved.

Literature suggested that old varieties of sorghum grain contained relatively high amounts of an anti-nutritional compound called tannins (Hancock, 2000; Ravindran *et al.*, 2005; Selle *et al.*, 2010). The interest in looking at tannin content is because of its capacity to bind protein, phytate, and minerals to form, indigestible complexes (Van Soest, 1994). Several authors have reported (Price and Butler, 1980; Butler and Rogler, 1992; Hancock, 2000; Selle *et al.*, 2010) that the linkage between tannin and protein could affect sorghum's nutrient composition and availability to poultry. A research conducted with conventional brown colored sorghum (2.69% of tannins) and white colored grain low tannin content (0.03%) sorghum showed that broiler live performance could be affected when using conventional sorghum with high tannin content (Tandiang *et al.*, 2014). Sorghum can have similar protein content as corn or it can even be higher, but the individual digestibility of amino acids is affected by tannin concentration (Ebadi *et al.*, 2005). Despite identical sulfur amino acid contents in sorghum and corn, these amino acids seem to have a lower availability in sorghum as compared to those in corn (Diygkas *et al.*, 1991). Other researchers (Elkin *et al.*, 1990) had reported a negative and significant correlation between tannin content and amino acid availability ($P < 0.001$; r= -0.97). According to Ebadi *et al.* (2005), when analyzing amino acids profile and availability in low, medium, and high (0.09, 0.19, and 0.37%) tannin sorghum varieties, they were affected by tannin content in the grain. Digestible arginine of high-tannin sorghum was decreased 15% in comparison with low and medium-tannin sorghum varieties. Arginine and glycine are the amino acids from which GAA and creatine can be formed (Lemme *et al.*, 2007; Ringel *et al.*, 2007; Michiels *et al.*,

2012), therefore the *de novo* synthesis of this compounds involved in muscle metabolism can be compromised.

Investigations have shown that tannin preferentially binds to peptide bonds adjacent to proline (Damron *et al.*, 1968; Hagerman and Butler, 1981). Stephenson *et al.* (1970) also found the greatest negative effect of tannin on proline. In contrast to mammals, birds have low arginase activity in tissues and therefore, a limited ability to convert arginine into proline (Austic and Nesheim, 1972). Therefore, proline is a nutritionally essential AA for avian species, including chickens (Graber and Baker, 1973). Proline and its metabolite (hydroxyproline) are unique amino acids (AA) both chemically and biochemically (Hu *et al.* 2008; Kaul *et al.* 2008). They constitute one-third of AA in the collagen proteins which comprise approximately 30% of body proteins. Although hydroxyproline has been traditionally considered to have little nutritional significance, it is now recognized as a substrate for the synthesis of glycine (an essential AA for chickens). Furthermore, hydroxyproline may greatly impact the nutrition of birds which cannot sufficiently synthesize glycine from other AA (Wu *et al.*, 2011). Thus, creatine and GAA formation could be affected by tannin content in sorghum-based diets not only because of its impact on arginine availability, but also for the negative effect on proline availability which is important in the synthesis of glycine. Consequently, the use of feed additives that promotes creatine or GAA synthesis could be used as nutritional alternative to ameliorate the negative effects of tannins in metabolism.

Poultry by-products

Poultry slaughter and processing has intensified and centralized during the last decades (Urlings, 1993). The intensive and large-scale production in poultry industry generates large amount of by-products. These by-products include feet, intestines, heads, and blood and processing wastes (Jayathilakan *et al*., 2012). After being subjected to cooking, these by-products are converted to alternative feedstuffs in broiler diets, reducing feed costs (Caires *et al.*, 2010). Poultry by-product meal is one of the most important sources of animal protein in animal feed and it's made by combining the by-products coming from poultry slaughterhouses or poultry processing plants (Bhargava and O' Neil, 1975; Meeker *et al.*, 2006).

The AAFCO (2011) defines poultry by-product meal as the ground, rendered, clean parts of the carcass of slaughtered poultry such as necks, heads, feet, undeveloped eggs, gizzards and intestines (provided their content is removed), exclusive of feathers, except in such amounts as might occur unavoidably in good processing practices. Poultry by-product meal is golden to medium brown color with a fresh poultry odor (Hertrampf and Piedad-Pascual, 2000). It is generally a palatable and high-quality feed ingredient due to its composition of essential amino acids, fatty acids, vitamins and minerals. According to Naber *et al*. (1961), poultry by-products have a better amino acid balance and could be used with less limitations than feather meal or blood meal. However, the nutrient content of poultry by-product meal can be quite variable and depends on the substrate that is being processed (Watson, 2006; Dale *et al.*, 1993). Due to its high protein content level, this

feedstuff can partially replace soybean meal. Broiler performance results when animal byproducts are fed can be very variable as a function of raw material type and quality, processing temperature, use of antioxidants to maintain their quality, contamination by pathogenic microorganisms, high polyamine content, and storage conditions (Bellaver, 2001). In addition to its use in livestock, poultry by-products are in high demand in the pet food and aquaculture industries (Meeker *et al.*, 2006). Escalona, and Pesti (1987), concluded that an inclusion of poultry by-products in the diet higher than 5% could depress growth on broilers fed corn-SBM diets. Moreover, the use of poultry by-product meal in livestock feeding was banned in 2002 in the European Union (European Community, 2000).

According to Li *et al.* (2011), in an experiment where they analyzed the composition of amino acids in feed ingredients used in poultry diets, they observed that the values for arginine and glycine (Table I.2), were higher in poultry by-products meal than in corn, sorghum and soybean meal.

Table I.2. Total amino acid content in corn, sorghum, DDGs, soybean meal and poultry by-products

	Ingredients				
Nutrient Name	**Corn**	**Sorghum**	**Distillers dried grain w/solubles**	**Soybean meal**	**Poultry by-products**
Dry matter, %	86.00	89.00	92.00	90.00	94.00
Crude protein, %	7.50	11.00	27.00	44.00	57.00
Total amino acids					
Methionine[1], %	0.18	0.10	0.51	0.65	0.91
Cysteine[1], %	0.18	0.20	0.50	0.67	0.90
Lysine[1], %	0.24	0.27	0.80	2.70	2.25
Threonine[1], %	0.29	0.27	0.92	1.70	1.88
Isoleucine[1], %	0.29	0.6	1.00	2.50	2.10
Valine[1], %	0.42	0.53	1.33	2.40	2.32
Leucine[1], %	1.00	1.40	2.80	3.40	3.95
Arginine[1], %	0.40	0.40	1.10	3.40	3.50
Arginine[2], %	0.38	0.41	-	3.18	4.63
Histidine[1], %	0.25	0.27	0.65	1.10	1.42
Phenylalanine[1], %	0.42	0.45	1.20	2.20	1.60
Serine[2], %	0.45	0.46		2.12	2.67
Proline[2], %	1.06	0.96		3.05	6.72
Alanine[2], %	0.71	0.96		1.95	4.91
Aspartic acid[2], %	0.43	0.36		3.14	4.10
Glutamic acid[2], %	0.64	1.18		4.17	4.89
Glycine[2], %	0.40	0.39		2.30	9.42

[1]Feedstuffs Ingredient Analysis Table: 2014 edition
[2]Composition of amino acids in feed ingredients for animal diets (Li, 2010)

Considering the importance of these amino acids in muscle metabolism, the objective of evaluating the effects of GAA in diets containing corn, sorghum, and poultry by-products become of interest due to potential improvements on broiler live performance and meat quality. This is important when the market is moving towards using only vegetable ingredients in feed and also to validate the sparing effects of GAA on nutrients like arginine and valine that are considered to be rich in animal protein sources. Since the

feed ingredients previously discussed may affect meat quality, it is relevant to review current knowledge about meat quality, muscle development, and myopathies.

Meat quality

Poultry meat quality has been widely studied (Fletcher, 2002; Owens *et al.*, 2004; Petracci and Cavani, 2012), and has become a growing demand for the international market (FAO, 2016). Parameters that affect meat quality are complex, and occur throughout the production chain (Baracho *et al.*, 2006; Mead, 2004; Petracci *et al.*, 2010). The constant concern with meat quality by the exporting sectors is a response to consumer's demands and is achieved by increasing efficiency as well as investments in personnel training on quality. Understanding where critical points are in the poultry meat production chain, and investing in solving critical problems may lead to better control and management, and consequently less losses. Production and management practices from farm to processing plant, play an important role in meat quality. Additionally, the use of technologies to reduce risk factors throughout the production chain will allow the production of better quality poultry meat not only for exports, but also for the domestic market (Baracho *et al.*, 2006).

In general, the consumers judge meat quality based on appearance, texture, juiciness, water holding capacity, firmness, tenderness, odor and flavor. According to Cross *et al.* (1986), those meat quality features are among the most important and perceptible that influences the initial and final quality judgment by consumers. Furthermore, the quality of poultry meat gathers quantifiable properties of meat such as water holding capacity, shear

force, drip loss, cooking loss, pH, shelf life, collagen content, protein solubility, cohesiveness, and fat binding capacity, which are indispensable for processors involved in the manufacture of value-added meat products (Allen *et al.*, 1998). According to Gaya and Ferraz (2006), the main attributes related to poultry meat that may determine its quality are water holding capacity and color.

Water holding capacity is a parameter used to describe the ability of the meat products to maintain water within its fibers (Fennema, 1990). Mendes (2001) state that water holding capacity can be measured by the difference in weight of the *Pectoralis* muscle before and after cooking. According to Bowker and Zhuang (2015), muscle pH and protein denaturation are considered to be the main determinants of water holding capacity in meat. As muscle pH decreases with the progression of post-mortem metabolism, the net charges of the muscle proteins are reduced. This decrease in net protein charge results in diminished water holding capacity due to the availability of less charged protein sites for binding water and because the lack of repulsive charges allows muscle proteins to become more closely packed, which forces more of the immobilized water into the free water compartment (Bowker and Zhuang, 2015). Due to the dynamic nature of post-mortem muscle, water holding capacity characteristics in chicken meat have been shown to change during the first 24h after slaughter. Young *et al.* (2004) observed that, a combined dietary supplementation of creatine with glucose, decreased pH, increased the lightness, and reduced water holding capacity. Therefore, the evaluation of GAA as precursor of creatine and its effects on meat quality becomes of interest.

Meat color is a valuable quality attribute as it is one of the first aspects to be evaluated by consumers on the shelves of supermarkets. Color is an indication of meat freshness and directly influences the consumer's final purchase decision (Garcia *et al*., 2005). Muscle color and texture are always the two most important factors that influence meat quality. Fletcher *et al.* (2000) stated that cooked product appearance was significantly associated with raw meat color.

When meat quality is assessed, objective criteria such as pH, water holding capacity, tenderness, skin color, and meat color are taken into account. Subjective tests with panels of tasters are performed to estimate meat sensorial characteristics (Komiyama *et al.*, 2009). The causes of variation in fresh meat color is associated with differences in muscle morphology and pH, and some color defects may be caused by pre-slaughter changes. The most common causes of meat color change are dietary factors, and stress caused by management during rearing up to slaughter. Occasionally, meat color loss may also be caused by inadequate meat storage and further processing (Garcia *et al.*, 2005). According to Gaya and Ferraz (2006), the main attributes related to poultry meat that may determine its quality are color and water holding capacity.

Tenderness. Poole *et al.* (1999) suggested that shear force values change due to broiler aging. These authors determined the following categories when testing for shear force using a Warner-Bratzler device. Owens (2004), mentioned that when using the Warner-Bratzler shear device, recording the peak load on poultry meat tests in the instrumental parameter of choice, and it's recorded in kg. When using a Warner-Bratzler, Lyon and Lyon

(1991) reported the following categories of tenderness showed in Table I.3. Is important to mention that we chose this method because it was standardized by the USDA (Wheeler *et al.*, 1997), and also, this method is considered reliable and it has been shown to be correlated with instrumental analyses (Owens, 2004).

pH. Tougan *et al.* (2013) concluded that chicken meat quality is affected by several factors such as genotype, age, sex, breeding systems, feed composition, muscle pH, type of muscle, live weight, *post mortem* aging, feed withdrawal, and pre-slaughter stress. These factors influence not only meat quality but also muscle metabolic capabilities. The functional properties of meat are primarily dependent on glycolytic reactions that occur during *rigor mortis* that directly affect meat pH (Garcia *et al.*, 2013). Therefore, pH is an important factor in chicken meat quality assessment. For instance, low meat pH while meat temperature is still high, results in chicken breast meat pale color and reduced water holding capacity (Komiyama *et al.,* 2009). According to Fletcher *et al.* (2000), the ultimate pH (pH after 24 h of processing) of normal chicken breast meat ranges between 5.7 and 5.96 and between 6.10 and 6.20 for normal chicken thigh meat.

Meat pH, tenderness and water holding capacity are attributes of muscle texture that have been studied most extensively. Meat pH is determined by muscle glycogen content and its degradation rate. A rapid pH drop results in earlier onset of rigor and greater degree of rigor shortening, which determines the tenderness of meat (Khan, 1974). Muscle contraction is also controlled by the glycogen reserves ante-mortem and their breakdown rate postmortem. Meanwhile, rapid glycogen breakdown results in increased acidification, and at

this pH value, usually the isoelectric point of muscle, most proteins in the myofibril become denatured and can precipitate. The relaxed proteins decrease their capacity to hold water which is characterized by high water loss rate. In avian pectoral muscle, a fast pH drop is associated with poor slaughter management conditions. In addition to genetics, there are many rearing and slaughter conditions that can affect meat pH and quality. Some of them are likely to alter animal energy status with consequences on muscle post-mortem metabolism. This is the case for stress and bird activity before slaughter that alter post-mortem rate of glycolysis (Chabault *et al.*, 2012) or nutrition during the final phase of rearing that can modify bird intermediate metabolism and the energy amount stored in muscle cells (Berri *et al.*, 2014) due to the glycolytic metabolism and the type of fibers (Type IIB), which causes protein denaturation in the early post-mortem stages, with the result of poor water retention and less texture (Barbut *et al.*, 2008). In addition, Janisch *et al.* (2011) reported that the electrical conductivity, lightness, grill loss, and shear force values increased but the drip loss and a* (redness) values decreased with broiler aging.

The nutritional practices, specifically those related to the glycogen content prior to slaughter could be a short-term strategic approach to improve the nutritional quality of poultry meat. It was shown that cereal type, which the main starch source for birds, affected pH and color of meat probably through the increase or decrease of glycogen metabolism. A previous study (Del Puerto *et al.*, 2016) suggested that sorghum could modulate the pH drop after processing due to the action of tannins and its possible effect on starch and protein digestion. As meat quality is affected by glycogen metabolism, consequently the use of

creatine or GAA may have a beneficial effect due to its characteristic of maintain energy homeostasis (Nabuurs *et al.*, 2013), and their capacity to spare arginine (Dilger *et al.*, 2013; DeGroot, 2014), which is an amino acid that its availability is affected by tannin content in the grain (Ebadi *et al.*, 2005).

Table I.3. Meat quality parameters considered normal for breast samples

Parameter	**Values**	**Reference**
Humidity, %	74.1	Soglia *et al.*, (2016)
Protein, %	22.8	
Fat, %	0.87	
Ash, %	1.37	
Collagen, %	1.09	
Drip Loss, %	0.93; 0.87	Soglia *et al.*, (2016); Van Laack *et al.*, (2000)
Cook Loss, %	21.6; 28.2	Soglia *et al.*, (2016); Tijare *et al.*, (2016)
pH (ultimate pH after 24 h)	5.7 to 5.96; 5.96	Fletcher *et al.* (2000); Van Laack *et al.*, (2000)
Shear force (Warner-Bratzler), kg		Lyon and Lyon, (1991)
Very tender	< 3.82	
Moderately to slightly tender	3.62 to 6.61	
Slightly tender to slightly tough	6.62 to 9.60	
Slightly to moderately tough	9.61 to 12.60	
Very tough	> 12.60	
Color		Fletcher *et al.* (2000); Van Laack *et al.*, (2000)
Lightness (L*)	47.6; 55.1	
Redness (a*)	3.7; 2.2	
Yellowness (b*)	6.8; 9.6	

Since GAA supplementation could affects muscle metabolism, it is important to review the current knowledge about muscle development.

Biochemistry of meat development and growth

On the basis of their biochemical and functional properties, some researchers (Brook and Kaiser, 1970, McKee, 2002) classified the muscle fibers into different types. Types I slow-contracting red fibers, type IIA and IIB fast-contracting white fibers, and a type IIIA and IIIB which are slow, tonic "intermediate" fibers. In chickens, type I muscle fibers are required to sustain activities such as walking and standing (Hnik *et al.*, 1985). Type IIA fibers are found in muscle that is fast-moving and repetitive in action; therefore, they do not fatigue as easily as type IIB glycolytic. Type IIB muscle fibers are fast-contracting but are more easily fatigued in comparison to both type I and type IIA muscle fibers. Type IIB fibers have higher levels of ATP and glycogen and are found primarily in pectoral muscle (McKee, 2002). Dark muscles contain predominantly slow-contracting, while light muscles contain primarily fast-contracting glycolytic type of fiber (Brook and Kaiser, 1970). In fast-contracting skeletal muscles, like breast muscle of chickens, a large pool of phosphocreatine is available for immediate regeneration of ATP hydrolyzed during short periods of intense work (Wyss and Kaddurah-Daouk, 2000).

Like hemoglobin in the blood, myoglobin is a molecule that readily binds to oxygen. Oxygenated blood courses through muscles and oxygen is transferred from the hemoglobin in the red blood cells to the myoglobin molecules in the muscle fibers. The more myoglobin

within the muscle fibers of a muscle, the darker the muscle appears. Dark fibers are great for activities involving endurance. Therefore, a group of birds that spends much time in the air would be expected to have flight muscles designed for endurance (Clamann, 1993). White fibers, also called fast-contracting fibers or anaerobic fibers, are used for rapid, short-term activities like flight in avian species. These fast-twitch muscles are able to contract more quickly than the dark, slow-twitch muscles. However, they fatigue very quickly (Clamann, 1993; Mckee, 2002). These fibers operate in an anaerobic mode, a mode not requiring the continuous input of oxygen. To fuel their contraction, white fibers take up the starch glycogen, stored in the muscle fibers. The glycogen stores are quickly depleted so the rapid contraction of the white fibers are necessarily limited in duration (Mckee, 2002). Birds that migrate long distances have breast muscles made mostly of dark muscle fibers to enable long bouts of strenuous flight (Clamann, 1993). Ducks and geese have breast muscles made of aerobic fibers and are dark when cooked. Wild turkeys do not fly for great distances. These birds have breast muscles that contain fewer dark fibers than a duck but more dark fibers than a domesticated turkey. Broiler chickens and turkeys, on the other hand, have proportionally more type IIB fast-contracting white fibers in the pectoral muscle (Mckee, 2002).

The conversion of chicken muscle to meat results from the overall biochemical and mechanical changes of the muscles after slaughtering process. This biochemical and mechanical evolution of the muscle after slaughtering that comes progressively to its conversion to meat occurs in two phases: the dissipation of the energy reserves of the muscle during the installation of the *rigor mortis*, and the modification of the organization of

muscular proteins structure during the maturation of meat (Santé *et al.,* 2001; Maltin *et al.,* 2003). The transformation of the muscle in meat is a control point in the determination of meat quality. Stunning and bleeding modify potentially the muscular metabolism (El Rammouz *et al.,* 2003).

The interruption of blood circulation suppresses oxygen and exogenous energy substrate (glucose, amino acids and fatty acids) supplies. However, the mechanisms of homeostasis maintenance continue to function in the cell during a short time. The deprivation of oxygen decreases very quickly the oxidation power of cells, and only the anaerobic reactions persist, essentially the glycolysis (Lawrie, 1966; Bendall, 1973). The muscle which deprived of oxygen, becomes anoxic. The maintenance of the muscular homeostasis requires the synthesis of compound rich in energy such as ATP (Santé *et al.,* 2001). The reactions of ATP synthesis are assured by creatine-phosphate utilization and essentially by glycogenolysis and anaerobic glycolysis. Anaerobic glycolysis generates lactate that accumulates, lowering the intracellular pH, so that by 24 h *post mortem*, the pH falls to an ultimate pH of about 5.4 to 5.7. Muscle is highly sensitive to both ATP and Ca^{2+}, which are both involved in the contraction–relaxation process.

Consequently, as ATP levels are reduced and Ca^{2+} levels increase in post mortem, irreversible link between myosin and actin, and *rigor mortis* occurs in the tissue (Maltin *et al.,* 2003). The reactions of ATP utilization and that of glycogen have been described in detail by Bendall (1973). The fall in post mortem pH is characterized by its speed and its amplitude. The speed of the fall is mainly determined by the ATPase activity, whereas the amplitude of fall in post mortem pH depends mainly on glycogen reserves of the muscle at

the time of slaughtering (Santé *et al.*, 2001). According to Stewart *et al.* (1984), the *post mortem* biochemical reactions of broiler breast (*Pectoralis major*) meat of 7 to 9 weeks of age submitted to refrigeration stops between six and eight hours after slaughtering. The breast and thigh muscles of chicken represent the highest proportion of chicken carcass and differ in their chemical composition and technological and sensory quality (Oluyemi and Roberts, 2000).

Bearing in mind that GAA could affect pH, and consequently water holding capacity, it is important to review current problems in the industry related to pectoral myopathies.

Breast Muscle Myopathies

In recent years, the market demand for a large amount of cheap poultry meat has pushed for an intensive genetic selection for growth, feed conversion, and white meat yield by primary breeders has led to tremendous gains for the poultry industry (Russo *et al.*, 2015). It has been reported that 85 to 90% of the differences between today's bird and the average bird of 60 yr ago is due to genetic selection (Havenstein *et al.*, 1994 a,b, 2003a,b). Meat-type chickens are actually 2 times heavier at half the age than they were 50 years ago (Russo *et al.*, 2015). This fast growth rate is associated with the onset of idiopathic and stress-induced myopathies such as deep pectoral myopathy (Petracci and Cavani, 2012). Unfortunately, in recent years, studies indicate an increasing occurrence of new quality abnormalities in the breast muscles of broiler chickens (Mazzoni *et al.*, 2015). The specific etiologies of these muscle defects are not well-known, even if it is thought there is an involvement of the rapid

growth rate of broilers achieved in the modern strains by genetics (Petracci and Cavani, 2012)

Sihvo *et al.* (2014) have signaled a novel myopathy, referred to as "wooden breast" (WB). This myopathy shows pale expansive areas of substantial hardness companied with white striation. In addition, wooden breast has been associated with polyphasic myodegeneration with regeneration and accumulation of interstitial connective tissue or fibrosis (Sihvo *et al.*, 2014), causing negative changes in technological properties of breast fillets (Mudalal *et al.*, 2015).

Recently, some studies indicate an increasing occurrence of new quality abnormalities in the breast muscles of broiler chickens. A white striping (WS) defect was first pointed out by Bauermeister *et al.* (2009) and has been described as the appearance of white striation parallel to muscle fiber on the surface of *Pectoralis major* muscles. The quantity and thickness of the white stripes can vary from bird to bird (Figure I.4). Usually, WS is more evident on breast and thighs, but it can be observed also on tenders and drumsticks. Along a breast fillet it appears first in the cranial part, and then develops caudally (Kuttappan *et al.*, 2013b). This condition is becoming increasingly important in meat-type chickens, and occurs mainly in heavier birds (Kuttappan *et al.*, 2013a). A recent survey estimated that the total incidence of white striped breast fillets in medium-size birds was around 12% (Petracci *et al.*, 2013), but observations on heavy birds indicate that the occurrence can reach also higher rates (Kuttappan *et al.*, 2012a).

According to Mendes (2001) appearance is part of the consumer's selection of a product at the shelf, while product texture is valued by sensorial perception. Therefore, the

selection of carcass quality remains usually based on visual criteria. Previous studies reported that the white striping abnormality affect visual appearance of breast fillets. Additionally, other studies observed that white striping had an impact on chemical composition (Kuttappan *et al.*, 2012a; Petracci *et al.*, 2014a) and reduced ability to absorb and retain liquids of the meat during processing (Petracci *et al.*, 2013).

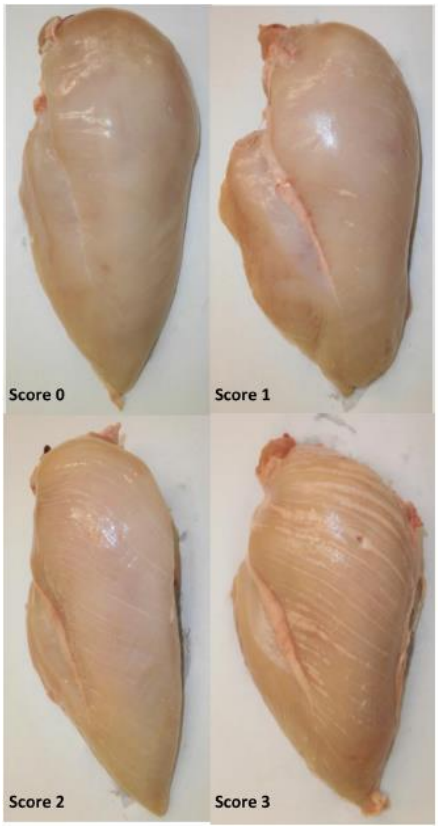

Figure I.4. Breast fillets displaying different degrees of white striping. Score 0 indicates no white striping, score 1 indicates low, score 2 medium, and score 3 severe white striping (Bailey *et al.*, 2015).

Hematology is the common clinical tool used for the diagnosis of various disease conditions. There could be differences in the peripheral blood profile, mainly in total leukocyte count and leukocyte differential count, during conditions like inflammation or stress (Mitchell and Johns, 2008; Huff *et al.*, 2010). In chickens, an inflammatory condition could cause a pronounced increase in heterophil count (Kuttapan *et al.*, 2013a). This damage occurring in the muscle tissue could be reflected in plasma or serum biochemical profiles. The condition could disrupt the integrity of the sarcolemma resulting in the leaking of various enzymes such as creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) into the plasma or serum (Hochleithner, 1994; Kuttapan *et al.*, 2013a). Considering that GAA could affect blood and enzymes involved in muscle metabolism, the objective of our study was to evaluate its effects on these parameters.

It has been reported (Bories *et al.*, 2009) that GAA supplementation reduced abdominal fat content in chicken meat. Hence, we could assume that GAA supplementation may affect lipid metabolism. Lipid metabolism in poultry differs from that in mammals with the liver being the main organ involved in its metabolic activity (compared to adipose tissue in mammals). The synthesis and decomposition of fatty acids is an important part of lipid metabolism; the catabolism of fatty acids (β-oxidation) is carried out primarily in the mitochondria, whereas fatty acids are synthesized in a variety of tissues, with the cytoplasm of the hepatocyte being the principle location in poultry. The analysis of gene expression and pathway by RNA-sequencing reasonably support the hypothesis that localized hypoxia, oxidative stress, increased intracellular calcium, and the presence of muscle fiber-type

switching may be responsible for wooden breast (Mutryn *et al.*, 2015). Impaired energy metabolism may trigger proapoptotic signaling (programmed cell death), oxidative damage to lipids, protein and DNA, and impair mitochondrial DNA repair (Kley *et al.*, 2007; Pathi *et al.*, 2012). In the muscle, ATP is tightly coupled with creatine and phosphocreatine levels. Studies show that the creatine may have a protective role in certain neuromuscular and neuro-degenerative diseases (Kolling *et al.*, 2013).

Kolling *et al.* (2013) explains the effect of creatine and associations of creatine per se of creatine with homocysteine altered glucose oxidation and protect muscle from imbalances in rats. Impaired energy metabolism may trigger proapoptotic signalling (programmed cell death), oxidative damage to lipids, protein and DNA, and impair mitochondrial DNA repair. It has been demonstrated that alterations in energy metabolism seem to be implicated in the pathogenesis of a number of muscle and neurological complications, metabolic disorders, aging and neuromuscular diseases. Nabuurs *et al.* (2013) also observed similar effects reversing muscular dystrophy effects of creatine deficiency by providing creatine in diets to rats.

As pectoral myopathies could be associated with lipid peroxidation, and considering the involvement of GAA and creatine in muscle metabolism and its beneficial effects in muscle diseases the aim of the present study in to evaluate the effects of GAA in vegetable diets and diets containing poultry by-products, this considering that the supply of creatine can be compromised due to availability of amino acids, such as arginine and glycine in the feed.

In conclusion, GAA may be an additive with potential to be used in broiler feed to improve live performance, meat yields, and minimize issues related to meat quality and myopathies. This GAA additive may fit within the current trends in feed formulation of all vegetable diets and use of alternative grains like sorghum.

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CHAPTER II

Growth performance, carcass and cut up yield, meat quality and pectoral myopathies of male broilers fed corn or sorghum diets supplemented with guanidinoacetic acid

2.1. ABSTRACT

This experiment was conducted to evaluate the effects of guanidinoacetic acid (GAA) supplementation in broilers fed corn or sorghum based diets on broiler live performance up to 50 d. Carcass and cut up yields, meat quality and pectoral myopathies were also evaluated at 51 and 55 d. Treatments consisted of corn or sorghum-based diets with or without the addition "on top" of GAA (600 g/ton) as CreAMINO®. A total of 800 male Ross 708 chicks were randomly placed in 40 floor pens with 10 replicates per treatment combination. At hatch, 14, 35, and 50d of age, BW and feed intake were recorded. BW gain and FCR adjusted by mortality weight were calculated at the end of each phase and flock uniformity (CV%) at 50 d. At 51 and 55 d, 4 birds per pen were processed. Carcass and cut up weights and yields were obtained and calculated. Breast fillets were evaluated for drip and cook loss, shear force, color, and pH was measured at 1, 4 and 24 h post-slaughter. Pectoral myopathies, such as white striping (WS) and wooden breast (WB), were scored. Data were analyzed as a randomized complete block design in a 2 x 2 factorial arrangement with grain type and GAA supplementation as main effects. At 50 d, diets containing GAA improved ($P < 0.01$) FCR (1.682 *vs*. 1.724 g:g) independently of grain type. At 55 d, an interaction effect ($P < 0.05$) was detected on breast meat yield. Drip loss, cook loss, and shear force were not affected (*P*

> 0.05) by GAA supplementation. However, GAA decreased ($P < 0.05$) the pH after 24 h of slaughter at 51 and 55 d in samples from broilers fed supplemented diets. At 51 d, broilers supplemented with GAA had ($P < 0.05$) double breast meat without WB (score 1) compared with broilers fed non-supplemented diets, therefore reducing the severity of this myopathy. In conclusion, GAA supplementation improved broiler live performance on broilers raised up to 50 d and increased breast meat yield in corn-based diets.

Key Words: Guanidinoacetic acid, broiler, live performance, corn, sorghum, myopathy.

2.2. INTRODUCTION

Creatine and its phosphorylated form phospho-creatine are naturally occurring components in the animal's body tissue and play a major role in energy metabolism (Wyss and Kaddurah-Daouk, 2000). The creatine/phospho-creatine system functions as a backup to the adenosine tri-phosphate (ATP)/adenosine di-phosphate (ADP) system in order to store and mobilize energy when required on short notice, particularly in muscle cells (Lemme *et al*., 2007a). Creatine can be produced naturally in the body from guanidine acetic acid (GAA), which in turn is synthesized from the amino acids arginine and glycine (Wyss and Kaddurah-Daouk, 2000). GAA is a compound synthesized in the avian kidney and liver (Dilger *et al*., 2013). A commercial form of guanidinoacetic acid GAA (CreAMINO®) was proved to act as precursor of creatine. The GAA was efficiently transformed to creatine in the liver which

subsequently was transported to the muscles (Ringel *et al,* 2007). Previous studies reported that GAA supplementation improved FCR in male broilers fed diets containing corn and wheat-based diets at 41 d of age (Lemme, *et al.*, 2007b). Likewise, Ringel *et al.* (2007) suggested that GAA may also have growth and feed efficiency promoting properties when added to practical corn-soybean meal diets. Other researchers found that GAA supplementation have a spare effect on arginine and it could be an efficient replacement for young chicks (Dilger *et al.*, 2013; DeGroot, 2014).

It has been reported that as long as animal by-products, which are rich in creatine, formed a certain part of poultry diets then, no signs of a creatine deficiency should be detected. However, the use of "all vegetable" diets based only on plant ingredients are increasing. As a result, the possibility of creatine production in the body and a deficiency has increased (Lemme *et al.*, 2007a). Vegetable diets generally contain corn, wheat, and sorghum as the main energy sources, and a source of protein like soybean meal, corn gluten meal, distilled dried grain with solubles (DDG), and sunflower meal (FAO, 2014; Beski *et al.*, 2015; Hossain *et al.*, 2015). Corn is a major source of dietary energy in poultry nutrition. Although it is produced throughout the world, there is a stiff competition for corn among human consumption and feed industry. This is because corn is high in energy as compared to other cereal grains (Mohamed *et al.*, 2015).

Some low-tannin sorghum varieties had been assessed to have the potential to replace corn as an alternative poultry feed ingredient. Its nutritional value is only slightly lower than corn (Douglas *et al.*, 1990). Low-tannin sorghum has been shown to substitute corn in

poultry feeds without affecting live performance (Garcia *et al.*, 2005; Campos, 2006; Bozutti, 2009).

In contrast, some other authors had found that high-tannin sorghum negatively affected live performance (Pour-Reza and Edriss, 1997). Kumar *et al.* (2005) concluded that cut up yields, especially breast meat yield, was not affected by different tannin levels in red sorghum. Therefore, the use of sorghum with low tannin content could be an alternative for corn in poultry diets (Tandiang *et al.*, 2014). Lemme *et al.* (2007a) observed that dietary inclusion of 20% sorghum and GAA supplementation improved FCR and breast meat yield, compared with broilers fed a negative control corn based-diet without GAA and with 20% of sorghum, when broilers were raised up to 42 d of age. According to Li *et al.* (2011) sorghum showed similar total amino acid content in arginine and glycine (main sources for the synthesis of creatine) when compared to corn. However, Ebadi *et al.* (2005) suggested that availability of amino acids can be affected by the tannin content in the grain. Methionine, cysteine, lysine, arginine, and proline availability were affected up to 23, 44, 32, 54, and 75%, respectively due to medium (0.19%) or high (0.37%) tannin content in the grain. Therefore, a better response in live performance could be hypothesized in sorghum-based diets.

An increasing demand for white chicken meat has made the poultry industry focus on the selection of genotypes exhibiting faster growth rates with higher breast yields. Concurrently, pectoral myopathies, such as wooden breast and white striping, are emerging as an increasing problem (Tasoniero *et al.*, 2016). Based on mice trials, creatine may have a protective role in certain neuromuscular (Tarnopolsky, 2007; Chung *et al.*, 2007) and neuro-

degenerative diseases (Bender *et al.*, 2006; Kolling and Wise, 2010; Beal, 2011), and could potentially reverse muscular dystrophy (Nabuurs *et al.*, 2013). These results are highlighted due to the capacity of creatine to prevent the inhibition of energy metabolism and lipid peroxidation.

Even though most of the studies of GAA had evaluated live performance, there has been no previous data about the possible effects of supplementation of GAA on pectoral myopathies in chickens. Therefore, the aim of the present trial was to evaluate the effects of GAA supplementation in corn or sorghum based-diets on broiler performance, carcass and cut up yields, meat quality and pectoral myopathies.

2.3. MATERIALS AND METHODS

Treatments and Birds Husbandry

All the procedures involving the birds used in the present experiment were approved by the North Carolina State University Institutional Animal Care and Use Committee. Four treatments from a 2 x 2 factorial arrangement of treatments with 2 grain based diets (corn or sorghum) and 2 levels of inclusion of GAA as CreAMINO® at 96% concentration (0 and 0.06%) as main factors to have a total of 4 treatments. This study was conducted from April 19th to June 8th, 2014 at North Carolina State University, Raleigh, NC, USA, in a solid-side wall house with negative pressure ventilation, tunnel capabilities and evaporative cooling. A

total of 800 Ross-708 day-old male chicks were placed in 40 floor pens (1.21 x 1.82 m) with 20 chicks per pen (9.18 broilers/m^2 at placement) for a final stocking density of 37.5 kg/m^2 at 50d of age. Broilers were raised on used litter. Chickens were exposed to continuous light on a 23L:1D (30 lux intensity) program during the first 7 d of age. Day length was then gradually reduced to 17L:7D (10 lux) up to 28 d of age. From 28 d until the end of the experiment at 50 d, light program was maintained at 17L:7D with an intensity of 5 lux. Brooding house temperature was set at 33.6°C at placement and gradually reduced until 20.6°C at 21 days of age. Chicks founded dead during the first 10 d of age were replaced with extra birds from same treatments and similar BW.

Diets

Experimental diets were mixed at the NCSU Feed Mill Research Facility. All diets were formulated to represent typical U.S. broiler industry practices, and digestible amino acid levels were based on AminoData® 5.0 (Evonik, 2015) recommendations (Table II.2). Macro ingredients (corn, sorghum, soybean meal, and distilled dried grain with solubles) were analyzed for total amino acid and metabolizable energy (ME) content prior to diet formulation. Digestible amino acid content was calculated from the total amino acid content and using table values for digestibility coefficients (Evonik, 2015). ME values (kcal/kg) were obtained from an *in vivo* trial with roosters (Dr. Nick Dale, University of Georgia). Diets were formulated to contain either corn or sorghum as the main grain source, soybean meal (SBM), and distilled dried grain with solubles (DDGs) as macro ingredients (Table II.1). Starter, grower, finisher and withdrawal diets were fed from 0-14, 14-35, 35-42, and 42-55

days of age, respectively. Starter was fed in crumbles and all other diets in pellets. For the pelleting process, a temperature between 82 and 85°C in the conditioner was used for 30 seconds. The steam pressure was 32 psi, and the pellet die was 11/64" x 1" 3/8" (4.4 x 34.9 mm) for an L/D ratio of 8. The capacity of pelleting used was 2 to 5 ton/hour to improve pellet quality. After being crumbled or pelleted, representative samples of each manufactured diet were analyzed for amino acid content, GAA concentration and macro minerals (Tables A.2, A.3). Experimental diets were formulated either from corn or sorghum basal diets to ensure that chickens had similar nutrient intake depending on grain source. GAA was added "on top" of the basal diets (600 g/ton) in the corresponding treatments. For each one of the dietary phases 0.85, 2.90, and 2.48 kg of starter, grower and finisher, respectively, were offered for each bird alive during each phase. The withdrawal was offered *ad libitum*. Water was provided for *ad libitum* consumption. Feeders were shook twice daily in order to stimulate uniform feed intake.

Data collection

Live performance

At hatch, 14, 35 and 50 days of age, body weight and feed intake were measured and body weight gain and feed conversion ratio calculated at the end of each phase. Mortality was monitored and recorded daily, and FCR was adjusted for mortality. Partial and cumulative results for every phase were also calculated. At 50 d, individual BW were obtained to calculate flock uniformity using the CV%.

Selection of birds for processing, meat quality and pectoral myopathies

At 50 d of age, individual and group BW were obtained. Average for each pen was calculated. Four broilers per pen were selected for processing, meat quality, and pectoral myopathies evaluation. Selected broilers had BW within two standard deviations above or under the corresponding average for each pen. Two processing days were performed at 51 and 55 d.

Carcass and cut up yields

Chickens were subjected to 12-h of feed withdrawal in each processing day (51 and 55d). Broilers were slaughtered at the NCSU pilot processing plant. Broilers were weighed, electrically stunned for 11 s, killed by exsanguination, and allowed to bleed for 90 s. Broilers were then scalded at 55°C for 90 s, picked for 30 s, and manually eviscerated. Carcasses were dressed by removing liver, gizzard, heart, oil gland, crop, proventriculus, lungs, and viscera. Carcasses were then air-chilled for 6 h, and manually deboned on stationary cones. Parts of the leg quarters, breast fillets (*Pectoralis major*), breast tenders (*Pectoralis minor*), wings, and rack with skin were weighed. The carcass yield was calculated for the chilled carcass as a percentage of the fasted live weight. Parts yield was expressed as a percentage of the chilled absolute carcass weight.

Meat quality evaluation

To determine cook loss, the breast fillets (*Pectoralis major*) were weighed, placed on aluminum pans, and cooked in a forced air oven (SilverStar Southbend, Model SLES/10sc, gas type, NC, USA). Fillets were cooked to an internal temperature of 75°C (approximately 35 min), as measured by a Therma Plus thermocouple with a 10-cm needle temperature probe (ThermoWorks Model 221-071, UT, USA). The cooked fillets were cooled to room temperature and re-weighed to determine cook yield as a percentage of the cooked weight relative to the raw weight. The water-holding capacity of meat was estimated by measuring drip loss of the fillets after storage. The *Pectoralis major* muscle was weighed 24 h postmortem and immediately placed in a plastic bag, hung from a hook, and stored between 4-6°C for 1 d. After hanging, the sample was wiped with absorbent paper and weighed again. The difference in weight corresponded to the drip loss and was expressed as the percentage of the initial muscle weight.

To determine shear force: cooked breast fillets samples were tested for texture using a Warner-Bratzler shear device (Warner-Bratzler meat shear, Bodine Electric Company, Chicago, USA). Two samples per breast fillets (2×2×2 cm) were sheared in a direction perpendicular to the muscle fiber. Crosshead speed was 4 mm/sec. The maximum force measured when cutting the samples was expressed in kg/cm^2.

For pH determination, pH was measured using a portable pH meter (Oakton waterproof pH Tester 30) on the carcasses at 1, 6 and 24 h after processing. Skin was removed from breast fillets and color was measured on the surface of raw breast fillets. Color

was measured by the CIE L*a*b* system using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Japan). A measuring area of 10 mm and illuminant D65 and 2° standard observer were used. The colorimeter was calibrated using a white tile (reference number 13033071; Y = 93.9, x = 0.3156, y = 0.3318). Triplicate measurements were taken for each sample. CIE lightness (L*), redness (a*), and yellowness (b*) were measured.

Pectoral myopathies

After breast fillets were obtained, methods were used to detect the phenotypic variation in severity of the conditions seen at the respective breast fillet. Sensorial analysis was done by experts in the field. Spaghetti muscle was recorded as present or absent, whereas white striping (WS) and (WB) were scored based on severity. Wooden breast severity was based on a four-point scale, where score 1 is a normal fillet with no wooden breast signs, score 2 was considered a low severity, score 3 a medium and score 4 a severe severity associated with WB. Similarly, white striping was recorded on a four-point based scale of severity. Score 1 was considered a breast fillet with no white striations on the surface. Score 2 were the fillets with white striations less than 1 mm of thickness and easily observed in the surface, score 3 was represented by the white striation more than 1 mm of thickness and covering less than 50% of the breast's fillet area, and score 4 was considered the fillets with white striations with more than 1 mm of thickness and covering an area more than the 50% of the breast's fillet surface.

Statistical analysis

Data were analyzed as completely randomized block design with a 2 x 2 factorial arrangement of treatments. Factor A corresponded to the type of grain based of the diet (corn or sorghum), and factor B corresponded to the supplementation or not of GAA (0 *vs.* 0.06%), for a total of 4 treatments. Two blocks (location of pens within the house), 4 treatments per block, and 5 replicates per treatment within each block, for a total of 40 replicates (pen). Location of the pen was considered random effect. Data were analyzed in JMP 12 (SAS Inst. Inc., Cary, NC, 2016) using ANOVA in a mixed model. Differences between means were separated using Tukey's or t-student test at a level of significance of alpha = 0.05.

In addition, the individual data of each selected broiler within each pen for carcass and cut up yields, meat quality, and pectoral myopathies analyses, was analyzed including a nested effect of pen inside every corresponding treatment and considered as random effect. For carcass and cut up yields, the cutter was also included in the mixed model and considered also as random effect.

2.4. RESULTS AND DISCUSSION

Live performance

Results of live performance are shown in Tables II.3 to II.6. No significant ($P > 0.05$) interactions were observed on BW or BW gain in any of the phases evaluated. An interaction effect ($P < 0.05$) on feed intake and FCR was observed only in the starter phase (0-14 d). In diets without GAA supplementation, chickens fed corn diets ate more than chickens fed

sorghum diets, while no difference was observed when GAA was added. For the interactions results of FCR, no difference was observed when chickens were fed diets without GAA, but when GAA was added FCR of chickens fed corn diets was improved (1.242 *vs*. 1.333 g:g) as compared to broilers fed sorghum diets (Table II.3). At 35 d, feed intake was affected by grain source. Chickens fed corn diets had higher (P <0.01) feed intake than broilers fed sorghum diets. Feed intake was lower (P < 0.001) for chickens fed sorghum between 14 to 35 days of age (Table II.4). No differences (P > 0.05) between treatments on feed intake were detected at 50 d, due to GAA supplementation. However, birds fed corn diets ate more ($P \leq$ 0.05) than chickens fed sorghum diets, regardless of GAA supplementation.

BW gain was higher (P <0.05) in broilers fed corn-based diets throughout the whole experimental period than the ones fed sorghum diets. Consequently, chickens fed corn were heavier (P <0.0001) than broilers fed sorghum diets at 14d, and remained heavier (P <0.05) at 35 (2,570 *vs*. 2,479 g), and 50 d (4,172 *vs*. 4,056 g) respectively. The GAA improved BW, BWG (P < 0.05), and FCR (P < 0.01) up to 35 d of age and from 0 to 50 days of age. No improvements (P > 0.05) in BW gain or FCR were observed between 35 to 50 days of age due to GAA (Table II.5). Improvements (P < 0.05) up to 4 points in FCR at 35 (1.46 *vs.* 1.50 g:g), and 50 d (1.68 *vs.* 1.72 g:g) of age respectively were attributed to GAA supplementation. No differences (P > 0.05) were detected on FCR at the end of each experimental phases due to grain source. Flock uniformity at 50 days of age (Table II.6) was improved (P = 0.0706) by GAA (7.33 *vs*. 8.44%) addition, while no interaction (P > 0.05) or effect of grain source was observed. No effect (P>0.05) of treatments was observed on mortality (Table II.6) throughout the whole experimental period.

Torres *et al.* (2013) reported similar results on FCR, where no differences were observed at 42 d of age when broilers were fed diets replacing 100% of corn with sorghum. Contrary to our findings, BW gain and feed intake were not affected ($P > 0.05$) by 100% of corn replacement with sorghum at this age. Tandiag *et al.* (2014) concluded that 100% of replacement of corn with sorghum did not affect broiler live performance when low tannins (0.03%) content-sorghum variety was used. Likewise, Garcia *et al.* (2013) found no significant differences on BW gain, feed intake, FCR, and mortality attributed to different levels of replacement of corn with sorghum on each phase up to 41 d of age. According to Torres *et al.* (2013) chickens fed sorghum diets with 100% of replacement were not different ($P > 0.05$) as compared to corn on FCR up to 42 d when using low tannin sorghum. Likewise, BW gain and feed intake were not affected ($P > 0.05$) by corn replacement up to 100% with sorghum. Considering these previous studies, sorghum can be used up to 100% of replacement of corn without affecting live performance. Although FCR was not affected by grain source, there was a lower feed intake on broilers fed sorghum-based diets that impaired BW gain and consequently BW. This could be attributed to the effects of tannins in the grain. It has been reported that tannins lowered protein and starch digestion (Kumar *et al*., 2005; Del Puerto *et al.*, 2016). Not finding differences on FCR due to grain source in our study suggest that the values used for digestible amino acids and available nutrients in the formulation were adequate.

It has been reported a greater ($P < 0.05$) BW gain up to 70.2 g, when male Ross 308 broilers where fed corn-based supplemented diets with GAA and raised up to 39 d of age

(Michiels *et al.*, 2012). Several investigators have observed that GAA supplementation reduces FCR ($P < 0.05$), suggesting improvements up to 5 points of FCR (Lemme *et al.*, 2007a; Mousavi *et al.*, 2013) on broilers fed corn-based or partially replaced with sorghum (20%) supplemented with GAA diets raised up to 42 d. Ringel *et al.* (2007) observed improvements up to 7 points in FCR with the same level of GAA (0.06%) inclusion, as compared to a negative control diet that did not contain any animal by-product ("all vegetable diet") without GAA supplementation. Findings in these studies concluded that GAA improved live performance in corn-based diets. In the present study, the improvement in BW gain was up to 55 g at 35 d, and FCR was improved by 7, 4, and 4 points at 14, 35 and 50 d, respectively, regardless of the type of grain source in the diet. Therefore, GAA could improve live performance in "all-vegetable" diets with either corn or sorghum as the base grain. The lack of an interaction effect of GAA with grain source dismisses our hypothesis that GAA will have greater improvements on sorghum diets.

Carcass and cut up parts weights and yields

Results for the effect of grain source (corn or sorghum) on carcass and parts weights and yield are presented in Tables II.7 to II.10. Chickens were processed twice (51 and 55 d) to observe muscle development in two common stages in the industry and meet experimental processing capabilities to evaluate all parameters**.** No differences ($P > 0.05$) on live BW of chickens selected for processing were detected due to either grain source or GAA supplementation in both processing days. No interaction effects ($P > 0.05$) were observed at

51 d of age on carcass and cut up yields. However, at 55 d an interaction effect ($P < 0.05$) of grain source and GAA was detected on breast meat yield. Differences due to GAA supplementation were detected only in corn diets. In broilers fed corn diets, the addition of GAA improved breast meat yield (39.15 *vs.* 38.19%), as compared to chickens fed corn non-supplemented diets, which attributed to an improvement of 96 grams in breast meat per bird at this age and weight.

At 51 days of age (Table II.7), the addition of GAA only improved ($P < 0.05$) leg quarter yield. Broilers fed GAA-supplemented diets had higher ($P < 0.05$) leg quarter yield. No effects ($P > 0.05$) of GAA addition were observed on carcass or other cut up yields. At this age, corn-based diet improved carcass and breast meat yield ($P < 0.05$). Broilers fed sorghum diets had less carcass yield (78.44% *vs*. 78.08%) and breast meat yield (38.42 *vs*. 37.83%) than broilers fed corn feed. No other differences in cut up yields were detected at 51 d attributed to grain type.

At 55 d of age, carcass and cut up yields (leg quarters, breast meat) were not affected either by GAA addition or grain source, except for wing yield. Broilers fed corn diets had greater wings ($P < 0.05$) yield than broilers fed sorghum diets (9.52 *vs*. 9.28 %).

According to Torres *et al.* (2013), carcass and cut up yields were not affected by 50 or 100% of replacement of corn with low tannin sorghum in broilers raised up to 42 d of age. Tandiang *et al.* (2014) observed that 100% replacement of corn with low-tannin sorghum enhanced broiler live weight of birds selected for processing, and also carcass weight as compared to broilers fed corn-based diets. However, carcass yield was not affected when

Cobb 500 mixed sex broilers were raised up to 6 wk of age. According to Kwari *et al.* (2012), weights and yields of carcass and cut ups were not affected when corn was gradually replaced by low tannin sorghum up to 100% in broilers raised up to 63 d. Similarly results were observed by Garcia *et al*. (2013) when corn was replaced up to 100% with sorghum. Results in this study indicated that sorghum can be effectively used in diets by replacing 100% the inclusion of corn without compromising carcass and cut up yields. The data at 51 d indicated a possible effect of tannins on carcass and breast meat yield and this effect may be associated with the adverse effect on feed intake by dietary inclusion of sorghum. Rodrigues *et al.* (2007) reported in a trial up to 42 d, that even though the relationships were not significant, increasing tannin levels still tended to depress breast meat yields ($r = -0.513$; $P < 0.07$). Nasr and Kheiri (2012) concluded that lysine availability in the diet affected carcass and breast meat yield. In agreement, Ebadi *et al*. (2005) observed that sorghum containing medium levels of tannins could adversely affect lysine availability. Therefore, our results of carcass and breast meat yield at this age could be explained by the possible effect of tannins on dietary lysine bioavailability.

Previous studies (Lemme *et al.*, 2007a; Michiels *et al.*, 2012) concluded that the use of GAA improved breast meat yield in vegetable-based diets. This response was observed even when corn was partially replaced by sorghum up to 20% (Lemme *et al.*, 2007a). However, no improvements on whole carcass, and other cut ups (upper leg, lower leg, wings) yields were observed. Our interaction effect observed only in corn diets suggest that the

improvements on breast meat yield could be dependent mainly on the availability of amino acids such as methionine, lysine, arginine, and proline, due to tannin content in sorghum.

Meat quality

Results from meat quality parameters are shown from Table II.11 to Table II.13. No interactions effects were observed on drip and cook loss at any of the processing days ($P > 0.05$). Drip and cook loss were not affected ($P > 0.05$) by GAA supplementation or grain type main effects. Shear force was not affected ($P > 0.05$) by grain source or GAA supplementation in the diet in any of the processing days (Table II.13).

The pH of breast muscle was evaluated 1, 4, and 24 h post-slaughter. No interaction effects ($P > 0.05$) were detected for both processing days. Breast meat's pH at 1 h post-processing was not affected either by grain type or GAA supplementation. In contrast, grain source affected ($P < 0.05$) pH after 24 h of processing at 55 d only. Samples from chickens fed corn diets had lower pH than samples from broilers fed sorghum diets (5.95 *vs.* 5.99). On the other hand, GAA supplementation lowered ($P < 0.05$) pH of breast meat after 24 h of slaughter at 51 d (6.01 *vs.* 6.08) as compared to samples from chickens fed non-supplemented diets. Similarly, GAA supplementation reduced ($P < 0.05$) pH after 4 (5.88 *vs.* 5.92) and 24 (5.95 *vs.* 5.99) h post-slaughter at 55 d of age respectively.

An interaction effect was observed on redness (a*) value at 51 d only. Differences were detected only in the supplemented diets with GAA, where chickens fed sorghum-

supplemented diets had higher ($P < 0.05$) a* value than samples from chickens fed corn-supplemented diets (4.91 *vs.* 3.78), samples of broilers fed corn or sorghum non-supplemented diets had intermediate results. At 51 d only, an interaction effect ($P < 0.05$) was found on yellowness (b*) value. Samples from broilers fed corn non-supplemented diets had the highest b* value. At 55 d of processing the b* value was affected ($P < 0.0001$) by grain type. Breast fillets from broilers fed corn diets had greater b* values (9.28 *vs.* 5.50) than samples from broilers fed sorghum diets. Opposite response was observed for lightness (L*), where no differences ($P > 0.05$) between treatments were detected due to grain or GAA supplementation at any processing day.

Related to drip and cook loss, Del Puerto *et al.* (2016) observed no effects on drip loss when corn was partially replaced (30%) by sorghum. Garcia *et al.* (2005) reported that cook loss was not affected ($P > 0.05$) by total replacement of corn diets with sorghum. Similarly Michiels *et al.* (2012) reported that drip loss was not affected ($P > 0.05$) by GAA supplementation. However, cook loss was affected ($P < 0.05$) when GAA was added to corn-based diets. Breast meat samples from chickens fed supplemented diets had higher % cooking loss than those fed non-supplemented diets.

Garcia *et al.* (2013) observed that breast meat pH was not affected ($P > 0.05$) by the substitution (50 and 100%) of corn by sorghum. Similarly to results obtained in our study, breast meat pH after 1, 4 and 24 h of processing were not affected by grain type at 51 d of age only. In contrast, Garcia *et al*. (2005) reported that the replacement of 100% of corn by sorghum lowered ($P < 0.05$) breast meat pH. According to Fletcher *et al*. (2000), the ultimate

pH (after 24 h of slaughter) of normal chicken breast meat ranges between 5.7 and 5.96. Therefore, the pH obtained in our study at 55 d of age could be considered as normal. Del Puerto *et al.* (2016) found no differences ($P > 0.05$) in ultimate pH when comparing samples of *Pectoralis major* from chickens fed corn or sorghum-based diets. However, they observed differences on pH after 45 and 90 min of slaughter, where samples from broilers fed corn had lower ($P < 0.05$) pH values than samples from chickens fed sorghum. These researchers suggested that the effect of sorghum to reduce the pH drop in the breast muscle as compared to corn, could be due to the difference in starch digestibility. Corn has higher digestibility than sorghum because anti-nutritional factors contained in sorghum grain such as tannins and kafirin proteins reduce the availability of amino acids and starch (Garcia *et al*., 2005). A previous study conducted by Michiels *et al.* (2012) found that GAA supplementation lowered ($P < 0.05$) breast meat pH of broilers fed supplemented diets in comparison with chickens fed sorghum diets.

Bories *et al.* (2009) also reported that breast meat pH after 4 h of slaughter was reduced ($P < 0.05$) in chickens fed diets containing GAA as compared to broilers fed non-supplemented diets (6.05 *vs*. 5.92). Considering that normal values of ultimate pH in breast meat ranges between 5.7 and 5.96 (Fletcher *et al*., 2000), our findings would suggest that GAA supplementation lowered the pH of breast meat to values close to what is considered normal, which could be beneficial for water holding capacity and therefore tenderness of breast meat. However, no differences were observed on drip losses, cook loss, and shear

force in both processing days. The water holding capacity of marinates was not evaluated in the present study.

Michiels *et al.* (2012) reported that when chickens were fed diets containing GAA, the ratios of phosphocreatine:ATP were higher than those fed a negative control without GAA supplementation, indicating the buffer capacity of ATP for hydrolysis by phosphocreatine which represent higher energy availability for muscle development. Moreover, intramuscular phosphocreatine can attract water into the muscle cell and increase the cell volume (Hultman *et al.*, 1996). Haussinger (1996) found that a superhydrated muscle may trigger protein synthesis, minimize protein breakdown, and increase glycogen synthesis, as partly demonstrated by Young *et al.* (2007). Abasht *et al.* (2016) observed that breast meat affected with wooden breast myopathy had lower ($P < 0.001$) glycogen content in muscle than samples from unaffected chickens. A significant higher ultimate pH (24 h after slaughter) observed in affected tissues may also be related to glycogen depletion and reduced glycolytic potential within affected muscle.

Shear force (Warner-Bratzler) was not affected either by grain source or GAA supplementation in the present experiment. Generally, results ranged between 4.27 to 4.70 kg, which according to different authors (Owens *et al.*, 2000; Schilling *et al.*, 2003; Corzo *et al.*, 2009) these values are considered as low tender, resulting in less acceptability by the consumer. However, Schilling *et al.* (2003) concluded that independently of the shear force value in samples considered as low tender, not many consumers would find these samples as unacceptable. The values obtained in our experiment showed similar results as observed by

Poole *et al.* (1999), who observed that broilers at 7 wk old had an average of 4.64 ± 0.18 kg shear force values (Warner-Bratzler). These authors concluded that shear force values of cooked breast meat changes, depending on age of the broilers. They suggested that shear force values of breast fillets in the scale from 3.46 to 6.41 kg (Warner-Bratzler) are considered "moderately tender". According to Garcia *et al.* (2005), no differences ($P > 0.05$) in shear force were observed when corn was supplemented up to 100% in the diet. In agreement with our findings, Michiels *et al.* (2012) reported that shear force was not affected ($P > 0.05$) by up to 1.2% GAA dietary supplementation. Therefore, the shear force values obtained in our experiment were more likely due to age of the broilers than related to dietary grain source or GAA supplementation. Zotte *et al.* (2017) found no differences in shear force when comparing breast fillets affected by wooden breast myopathy with non-affected breast fillets. According to Fletcher (2002), differences in tenderness can be due to the fact that older birds are more mature at the time of harvest and have more cross-linking of collagen. Considering the age of birds at processing in this trial, the incidence of wooden breast is related to increased cross-linked collagen in the pectoral muscle.

Results from our study related to color (lightness, redness and yellowness) in breast meat, are consistent with those detected by Garcia *et al*. (2013) and Harder *et al.* (2010), who evaluated the effects of sorghum replacing corn in the diet for broilers, these authors concluded that the lightness L* value of broiler breast meat depends on the carotenoid content in the diet. In the same study, meat b* value increased as dietary carotenoid content increased in the diets. Additionally, b* value decreased gradually as replacement of corn with

sorghum increased. According to Beyer (2010) and Garcia *et al*. (2013), grain sorghum is deficient in carotene and contains less yellow xanthophylls than corn. Therefore, the findings of b* value in our study may be due to carotenoid content in the diets considering its grain-based origin; thus, breast meat from chickens fed corn diets had higher b* values at both processing days. Garcia *et al*. (2013) also concluded that meat redness (a*) decreased, and lightness (L*) increased when corn was replaced by sorghum at 50 and 100% level. However, results from our experiment showed no effect (P >0.05) of grain source or GAA supplementation on L* value at both processing days, and a* value was affected by grain type only at 51 d. Garcia *et al.* (2005) also observed similar results as our findings. Breast meat L* values were not affected when corn was replaced 100% by sorghum. Michiels *et al.* (2012) observed that L* and b* values were increased (P < 0.05) by GAA supplementation of corn-based diets. However, Bories *et al.* (2009) found that breast meat L* value of broilers was not affected by GAA supplementation of corn-based diets. In contrast, we observed that dietary GAA supplementation reduced (P < 0.05) b* values. Zotte *et al.* (2017) found differences (P < 0.01) between breast meat of chickens affected with wooden breast and non-affected broilers.

Breast meat with wooden breast abnormality had higher (P < 0.01) L*, a*, and b* values, than those without the abnormality. In the project described herein, at 51 d only, an effect of GAA supplementation was observed on b* value (Table II.18) and also on wooden breast score (Table II.11). These findings could support the results obtained by Zotte *et al.* (2017) in which wooden breast was associated with higher b* values.

Myopathies

Breast meat was evaluated for white striping and wooden breast myopathies (Tables II.14 to II.18). White striping was evaluated by two different groups. The first evaluator used a score range from 1 to 3 and the second evaluator from 1 to 4. Data was evaluated by severity or average value of scores (Table II.14), individual distribution of scores (Table II.15 and II.16) and grouping scores. Myopathy severity (wooden breast or white striping) was evaluated by grouping score 1 and 2 to consider low severity and scores 3 and 4 were grouped and considered high severity (Table II.14). Incidence was evaluated by the presence (average of grouping scores 2, 3, and 4) or absence (score 1) of the myopathies in the breast meat samples (Table II.15). These analyses were done for both processing days. The supplementation of GAA reduced ($P < 0.05$) the incidence of wooden breast at 51 days of age (Table II.15), and increased ($P < 0.05$) the severity of white striping at 55 days of age (Table II.16). Only the first evaluator observed ($P < 0.05$) a reduction on the severity of white striping at 51 d, but not at 55 d due to grain source (Table II.14). Dietary supplementation of GAA decreased ($P < 0.05$) severity of wooden breast at 51 d of age, but not at 55 d. The addition of GAA duplicated the percentage or probability to have normal (score 1) breast meat without wooden breast (Table II.17). Broilers fed sorghum-based diets had a lower severity of wooden breast only at 55 d than broilers fed corn-based diets (Table II.18). Broilers fed sorghum had fewer breast meat with a score 3 for wooden breast than broilers fed corn only at 55 d (Table II.16). The severity of "*spaghetti muscle*" was evaluated, but no treatment effects ($P > 0.05$) were detected.

Previous studies suggested that these abnormalities might be related to a reduced levels of glycogen in the muscle (Mudalal *et al.*, 2014). Additionally, Zotte *et al.* (2017) reported higher ($P < 0.01$) ultimate pH values of breast meat affected by wooden breast than meat from non-affected broilers (6.30 *vs*. 5.92). Soglia *et al.* (2016) observed that ultimate pH of breast meat affected by woody breast was higher ($P < 0.01$) than those considered normal pH (5.87 *vs*. 5.82). Considering our results at 51 d of age, dietary GAA supplementation reduced ($P < 0.05$) the ultimate pH (Table II.22) and doubled the incidence of having breast meat considered as normal or with no signs of WB (Table II.15). Evidently, dietary GAA supplementation helped to prevent myopathies in broilers fed vegetable-based diets or ameliorates the severity of wooden breast. Apparently, the increased muscle creatine by dietary GAA supplementation may have a supportive effect on muscle energy metabolism (Kolling *et al.*, 2013; Nabuurs *et al*., 2013) as observed in rats with muscle dystrophies (Pearlman and Fielding, 2006; Chung *et al.*, 2007; Tarnopolsky, 2007). Kolling *et al.* (2013) explained the effect of creatine and associated it with homocysteine-altered glucose oxidation that protects muscle from imbalances in rats. An impaired energy metabolism may trigger proapoptotic signalling (programmed cell death), oxidative damage to lipids, protein and DNA, and impair mitochondrial DNA repair. It has been demonstrated that alterations in energy metabolism seem to be implicated in the pathogenesis of a number of muscle and neurological complications, metabolic disorders, aging and neuromuscular diseases. Nabuurs *et al.* (2013) also observed similar effect reversing muscular dystrophy effects of creatine deficiency by providing creatine in diets to rats.

2.5. CONCLUSION

Results of the current study indicated that dietary supplementation of GAA (600g/ton) improved BW gain and FCR of broilers fed corn and sorghum-based diets throughout their productive life to market age. An improvement in breast meat yield was observed when adding GAA to corn diets, but not when added to sorghum diets. GAA supplementation reduced the incidence and severity of wooden breast and reduce the pH of breast meat 24 hours post-processing. Findings associated with b* value and wooden breast suggest that GAA as source of creatine may have a potential benefit in lowering the severity of this myopathy.

2.6. REFERENCES

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Table II.1. Ingredient composition of starter, grower, finisher and withdrawal diets for males Ross-708 broilers.

Ingredient	Starter		Grower		Finisher		Withdrawal	
	Corn	Sorghum	Corn	Sorghum	Corn	Sorghum	Corn	Sorghum
	----------%----------							
Corn	52.509	-	56.573	-	60.911	-	64.344	-
Soybean meal, 46%	33.973	32.097	29.998	27.990	25.784	23.667	22.866	20.568
Sorghum	-	54.263	-	58.460	-	62.934	-	66.492
DDGS	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Poultry fat	4.302	4.112	4.849	4.643	5.114	4.885	4.847	4.614
Limestone fine	1.366	1.414	1.166	1.218	1.095	1.151	1.109	1.168
Dicalcium phosphate, 18.5%	1.151	1.232	0.915	1.001	0.736	0.828	0.431	0.530
Salt (NaCl)	0.303	0.246	0.312	0.252	0.281	0.216	0.251	0.182
DL- Methionine, 99%	0.286	0.315	0.239	0.271	0.197	0.231	0.197	0.234
L-Lysine-HCl, 78.8%	0.218	0.304	0.160	0.252	0.123	0.220	0.171	0.276
Mineral premix[2]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Sodium bicarbonate	0.183	0.256	0.134	0.212	0.177	0.261	0.185	0.274
Choline chloride, 60%	0.180	0.180	0.180	0.180	0.180	0.180	0.180	0.180
Vitamin premix[3]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
L-Threonine, 98%	0.081	0.103	0.055	0.078	0.035	0.059	0.052	0.079
None or GAA[5]	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Coccidiostat[1]	0.050	0.050	0.050	0.050	-	-	-	-
L-Valine, 96.5%	0.031	0.060	0.001	0.025	0.001	0.001	-	0.035
Phytase[4]	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Coban® 90 (Monensin), Elanco Animal Health, Greenfield, IN, at 500 g/ton in the starter and grower diets.

[2] Trace minerals provided per kg of premix: manganese (Mn SO_4), 60 g; zinc ($ZnSO_4$), 60 g; iron (FeSO4), 40 g; copper ($CuSO_4$), 5 g; iodine ($Ca(IO_3)_2$),1.25 g.

[3] Vitamins provided per kg of premix: vitamin A, 13,227,513 IU; vitamin D3, 3,968,253 IU; vitamin E, 66,137 IU; vitamin B12, 39.6 mg; riboflavin, 13,227 mg; niacin, 110,229 mg; d-pantothenic acid, 22,045 mg; menadione, 3,968 mg; folic acid, 2,204 mg; vitamin B6, 7,936 mg; thiamine, 3,968 mg; biotin, 253.5 mg

[4] Quantum Blue 5G® at 0.176 lbs/ton (80 g/ton) to provide 500 FYT (AB Vista) delivering 0.13% of available P, 0.06% of calcium and 0.03% of sodium.

[5] CreAMINO: Guanidinoacetic acid (GAA) with 96% of concentration, Lot number 3/29/16

Table II.2. Calculated nutrient content of starter, grower, finisher, and withdrawal diets for males Ross-708 broilers.

	Starter		Grower		Finisher		Withdrawal	
Nutrient	Corn	Sorghum	Corn	Sorghum	Corn	Sorghum	Corn	Sorghum
Metabolizable Energy, kcal/kg	3000.00	3000.00	3085.00	3085.00	3150.00	3150.00	3175.00	3175.00
Crude protein, %	22.109	22.109	20.410	20.410	18.684	18.684	17.600	17.600
Calcium, %	1.020	1.020	0.900	0.900	0.820	0.820	0.760	0.760
Total phosphorus, %	0.565	0.610	0.507	0.555	0.458	0.510	0.392	0.446
Available phosphorus, %	0.500	0.500	0.450	0.450	0.410	0.410	0.350	0.350
Total Glycine, %	0.908	0.865	0.842	0.796	0.773	0.723	0.725	0.672
Digestible lysine, %	1.220	1.220	1.080	1.080	0.950	0.950	0.920	0.920
Digestible methionine, %	0.589	0.603	0.527	0.542	0.468	0.484	0.457	0.475
Digestible total sulfur amino acids, %	0.890	0.890	0.810	0.810	0.732	0.732	0.708	0.708
Digestible threonine, %	0.780	0.780	0.702	0.702	0.627	0.627	0.607	0.607
Digestible tryptophan, %	0.232	0.238	0.212	0.218	0.190	0.198	0.175	0.183
Digestible valine, %	0.970	0.970	0.879	0.871	0.812	0.810	0.767	0.766
Digestible arginine, %	1.354	1.255	1.240	1.134	1.121	1.007	1.037	0.917
Sodium, %	0.200	0.200	0.190	0.190	0.190	0.190	0.181	0.181
Potassium, %	0.935	0.913	0.867	0.844	0.796	0.771	0.747	0.720
Chloride, %	0.280	0.280	0.277	0.277	0.236	0.236	0.245	0.245
Dietary electrolyte balance, mEq/100 g	263.916	261.933	240.575	238.484	232.316	230.038	215.500	213.077

Table II.3. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on live performance at 14 d

Additive	Grain	BW		BWG	Feed intake	FCR
		1d	14d	0 – 14	0 – 14d	0 – 14d
		---------- (g) ----------				--- (g:g) ---
GAA		39.91	468.48[a]	428.57[a]	551.16	1.288[b]
None		40.04	452.33[b]	412.29[b]	559.43	1.357[a]
SEM		0.12	3.06	3.07	6.68	0.016
	Corn	40.04	470.43[a]	430.40[a]	561.51	1.306
	Sorghum	39.91	450.37[b]	410.46[b]	548.97	1.339
	SEM	0.13	3.06	3.07	6.68	0.016
GAA	Corn	39.91	479.08	439.17	545.38[ab]	1.242[b]
	Sorghum	39.91	457.87	417.97	556.94[ab]	1.333[a]
None	Corn	40.16	461.79	421.63	577.85[a]	1.370[a]
	Sorghum	39.92	442.87	402.95	541.00[b]	1.344[a]
	SEM	0.18	4.32	4.34	9.45	0.023
CV%		1.39	3.01	3.32	5.46	5.71
Source of variation		---------- *P* – values ----------				
Additive		0.4706	0.0006	0.0006	0.3874	0.0057
Grain		0.4877	<0.0001	<0.0001	0.1893	0.1736
Additive*grain		0.5052	0.7925	0.7727	0.0147	0.0167

Values are means ± SEM of 10 pens per treatment combination with 20 male broiler chickens per pen.

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

Table II.4. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on live performance at 35 d

Additive	Grain	BW	BWG		Feed intake		FCR	
		35d	14 –35d	0 – 35d	14 – 35d	0 – 35d	14 _35d	0 – 35d
		---------- (g) ----------					---------- (g:g) --------	
GAA		2,550[a]	2,080[a]	2,509[a]	3,116	3,669	1.497[b]	1.461[b]
None		2,498[b]	2,042[b]	2,454[b]	3,129	3,693	1.528[a]	1.499[a]
SEM		12	10	11	21	25	0.006	0.007
	Corn	2,570[a]	2,098[a]	2,528[a]	3,178[a]	3,741[a]	1.514	1.478
	Sorghum	2,479[b]	2,024[b]	2,435[b]	3,067[b]	3,621[b]	1.511	1.482
	SEM	12	10	11	21	25	0.006	0.007
GAA	Corn	2,590	2,111	2,550	3,159	3,704	1.497	1.453
	Sorghum	2,510	2,049	2,467	3,073	3,633	1.497	1.469
None	Corn	2,549	2,085	2,506	3,197	3,778	1.531	1.504
	Sorghum	2,447	2,000	2,403	3,062	3,608	1.525	1.495
	SEM	16	14	16	32	38	0.010	0.011
CV%		1.96	2.10	1.99	3.44	3.55	2.49	2.77
Source of variation		---------- *P* – values ----------						
Additive		0.0022	0.0090	0.0014	0.6956	0.5608	0.0128	0.0057
Grain		<.0001	<.0001	<.0001	0.0025	0.0063	0.8189	0.7948
Additive*grain		0.4956	0.3888	0.4983	0.4738	0.2443	0.8208	0.3392

Values are means ± SEM of 10 pens per treatment combination with 20 male broiler chickens per pen.

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

Table II.5. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on live performance at 50 d

Additive	Grain	BW	BWG		Feed intake		FCR		FCR adj*	
		50d	35 - 50d	0 – 50d	35 – 50d	0 – 50d	35 – 50d	0 – 50d	35 – 50d	0 - 50d
		---------- (g) ----------					---------- (g:g) ----------			
GAA		4,167[a]	1,603	4,111	3,364	7,030	2.093	1.700[b]	2.067	1.682[b]
None		4,061[b]	1,563	4,022	3,322	7,022	2.123	1.739[a]	2.091	1.724[a]
SEM		51	49	57	29	43	0.043	0.012	0.034	0.009
	Corn	4,172[a]	1,588	4,117[a]	3,353	7,105[a]	2.109	1.718	2.063	1.700
	Sorghum	4,056[b]	1,578	4,016[b]	3,332	6,947[b]	2.107	1.720	2.095	1.706
	SEM	51	49	5.72	29	43	0.043	0.012	0.034	0.009
GAA	Corn	4,237	1,619	4,168	3,371	7,076	2.090	1.696	2.038	1.675
	Sorghum	4,096	1,586	4,054	3,356	6,984	2.097	1.737	2.096	1.689
None	Corn	4,106	1,557	4,065	3,336	7,134	2.128	1.740	2.088	1.725
	Sorghum	4,016	1,569	3,978	3,308	6,911	2.118	1.703	2.094	1.723
	SEM	60	59	66	48	70	0.054	0.017	0.045	0.014
CV%		3.45	9.34	3.62	5.01	3.44	6.92	3.02	6.42	2.71
Source of variation		---------- *P* – values ----------								
Additive		0.0267	0.4028	0.0628	0.4483	0.9212	0.5305	0.0262	0.5694	0.0073
Grain		0.0160	0.8269	0.0379	0.6940	0.0504	0.9720	0.8949	0.4515	0.6806
Additive*grain		0.5687	0.6288	0.7736	0.9054	0.4048	0.8595	0.7563	0.5490	0.6044

Values are means ± SEM of 10 pens per treatment combination with 20 male broiler chickens per pen.

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

*Adjusted value with body weight of mortality for this period.

Table II.6. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on mortality and body and weight coefficient of variation at 50 d

Additive	Grain	50d		Mortality			
		Individual BW	Flock Uniformity (CV)	0 – 14d[1]	14 – 35d	35 – 50d	Total
		--- (g) ---	--- (%) ---	---------- (%) ----------			
GAA		4,167[a]	7.23	-	0.25	3.52	3.77
None		4,061[b]	8.44	-	0.75	3.03	3.78
SEM		51	0.35	-	0.35	0.65	0.67
	Corn	4,172[a]	7.89	-	0.25	3.50	3.75
	Sorghum	4,056[b]	7.77	-	0.75	3.05	3.80
	SEM	51	0.35	-	0.35	0.65	0.67
GAA	Corn	4,237	6.92	-	0.00	5.00	5.00
	Sorghum	4,096	7.54	-	0.50	2.03	2.53
None	Corn	4,106	8.86	-	0.50	2.00	2.50
	Sorghum	4,016	8.01	-	1.00	4.06	5.06
	SEM	60	0.57	-	0.49	1.07	1.17
CV%		3.45	24.70	-	307.06[2]	108.68[2]	104.91[2]
Source of variation		---------- *P* – values ----------					
Additive		0.0267	0.0706	-	0.3101	0.7317	0.9977
Grain		0.0160	0.8538	-	0.3101	0.9399	0.9930
Additive*grain		0.5687	0.2522	-	1.0000	0.0862	0.1456

Values are means ± SEM of 10 pens per treatment combination with 20 male broiler chickens per pen.

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

[1]No mortality was observed between 1 to 14 days; [2]Very high variability in mortality data since several pens did not have any (0) mortality.

Table II.7. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on carcass, breast meat, and cut up yields at 51 d

Additive	Grain	Carcass yield	Cut – up parts relative to carcass weight					
			Wings	Leg Quarters	*Pectoralis major*	*Pectoralis minor*	Breast meat	Rack
		%						
GAA		78.28	9.50	30.63[a]	31.82	6.32	38.09	21.63
None		78.24	9.45	30.15[b]	31.93	6.30	38.15	21.91
SEM		0.12	0.09	0.17	0.21	0.07	0.20	0.23
	Corn	78.44[a]	9.43	30.23	32.11	6.37	38.42[a]	21.68
	Sorghum	78.08[b]	9.52	30.54	31.63	6.25	37.83[b]	21.86
	SEM	0.12	0.09	0.17	0.21	0.07	0.20	0.23
GAA	Corn	78.33	9.49	30.42	31.93	6.36	38.21	21.61
	Sorghum	78.33	9.50	30.83	31.71	6.28	37.98	21.65
None	Corn	78.54	9.37	30.05	32.30	6.37	38.63	21.75
	Sorghum	77.94	9.54	30.25	31.56	6.22	37.67	22.07
	SEM	0.16	0.11	0.23	0.27	0.08	0.28	0.28
CV%		1.30	6.15	4.98	5.89	6.34	5.06	5.84
Source of variation		*P* – values						
Additive		0.7809	0.3251	0.0434	0.7097	0.7562	0.8386	0.2360
Grain		0.0248	0.6346	0.1704	0.0907	0.1115	0.0443	0.4352
Additive*grain		0.1044	0.3975	0.6248	0.3527	0.6220	0.2195	0.5373

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

Table II.8. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on carcass, breast meat, and cut up yields at 55 d

Additive	Grain	Carcass yield	Cut – up parts relative to carcass weight					
			Wings	Leg Quarters	*Pectoralis major*	*Pectoralis minor*	Breast meat	Rack
		%						
GAA		78.97	9.41	30.70	32.24	6.49	38.76	20.94
None		78.72	9.39	30.94	32.02	6.45	38.43	21.94
SEM		0.18	0.10	0.19	0.28	0.06	0.30	0.35
	Corn	78.77	9.52[a]	30.77	32.21	6.45	38.67	20.95
	Sorghum	78.92	9.28[b]	30.87	32.05	6.48	38.52	21.22
	SEM	0.18	0.10	0.19	0.27	0.06	0.30	0.35
GAA	Corn	79.02	9.48	30.47	32.59	6.51	39.15[a]	20.69
	Sorghum	78.93	9.34	30.93	31.88	6.47	38.37[ab]	21.18
None	Corn	78.52	9.56	31.06	31.82	6.40	38.19[b]	21.21
	Sorghum	78.92	9.22	30.82	32.21	6.50	38.68[ab]	21.27
	SEM	0.21	0.13	0.24	0.34	0.08	0.37	0.37
CV%		1.18	5.53	4.45	5.63	7.39	5.34	5.35
Source of variation		*P* – values						
Additive		0.1228	0.8650	0.2357	0.4351	0.5701	0.2737	0.0846
Grain		0.3524	0.0352	0.5937	0.5732	0.6616	0.6271	0.1234
Additive*grain		0.1572	0.3860	0.0927	0.0649	0.3350	0.0432	0.2246

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

Table II.9. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on carcass, breast meat, and cut up weights at 51 d.

Additive	Grain	Live Weight	Carcass yield	Cut – up parts					
				Wings	Leg Quarters	*Pectoralis major*	*Pectoralis minor*	Breast meat	Rack
		---------- g ----------							
GAA		4,148	3,243	305.02	984.73	1,029	201.75	1,232	693.99
None		4,069	3,179	298.85	956.61	1,013	198.73	1,210	696.09
SEM		50	26	3.68	11.82	12	2.86	13	8.74
	Corn	4,156	3,255[a]	304.69	981.72	1,040[a]	204.89[a]	1,244[a]	700.98
	Sorghum	4,060	3,167[b]	299.18	959.63	1,002[b]	195.59[b]	1,197[b]	689.10
	SEM	50	26	3.67	11.76	12	2.84	13	8.69
GAA	Corn	4,217	3297	309.81	997.60	1,048	205.66	1,254	704.80
	Sorghum	4,078	3188	300.22	971.87	1,010	197.83	1,209	683.18
None	Corn	4,095	3213	299.57	965.83	1,032	204.11	1,234	697.17
	Sorghum	4,042	3145	298.14	947.39	994	193.34	1,185	695.02
	SEM	62	37	4.50	15.64	17	3.85	18	11.98
CV%		5.32	5.25	6.81	7.05	9.33	8.64	8.54	7.81
Source of variation		---------- *P* – values ----------							
Additive		0.1358	0.0983	0.1080	0.0635	0.3362	0.4210	0.2574	0.8592
Grain		0.0717	0.0236	0.1415	0.1362	0.0295	0.0154	0.0175	0.3132
Additive*grain		0.4124	0.5798	0.2737	0.8029	0.9871	0.6900	0.9122	0.4078

Values are means of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.

[ab] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's *t* or Tukey's test.

Table II.10. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on carcass, breast meat, and cut up weights at 55 d

Additive	Grain	Live Weight	Carcass yield	Cut – up parts					
				Wings	Leg Quarters	*Pectoralis major*	*Pectoralis minor*	Breast meat	Rack
		---------- g ----------							
GAA		4,548	3,592	337.10[a]	1,104[a]	1,156	233.51[a]	1,389	751.35
None		4,464	3,491	325.10[b]	1,073[b]	1,115	223.00[b]	1,337	737.29
SEM		48	35	3.65	11	17	3.46	19	15.07
	Corn	4,541	3,565	337.16[a]	1,095	1,149	229.72	1,379	743.32
	Sorghum	4,470	3,518	325.05[b]	1,082	1,122	226.78	1,348	745.32
	SEM	48	35	3.66	11	17	3.47	19	15.08
GAA	Corn	4,597	3,638	343.67	1,109	1,186	237.81	1,423	751.24
	Sorghum	4,500	3,546	330.53	1,100	1,127	229.20	1,356	751.47
None	Corn	4,486	3,491	330.65	1,082	1,113	221.63	1,335	735.41
	Sorghum	4,441	3,490	319.56	1,064	1,116	224.36	1,340	739.17
	SEM	63	51	4.53	14	24	4.58	27.29	17.15
CV%		4.81	4.39	5.69	5.62	8.04	9.56	7.75	6.62
Source of variation		---------- *P* – values ----------							
Additive		0.1577	0.0526	0.0027	0.0141	0.0883	0.0161	0.0650	0.2270
Grain		0.2354	0.3758	0.0026	0.2782	0.2618	0.4886	0.2705	0.8630
Additive*grain		0.6597	0.4087	0.7909	0.7212	0.2088	0.1923	0.2028	0.8806

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

Table II.11. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on *Pectoralis major*'s cook and drip loss, and post-mortem pH (1, 4, and 24 h) at 51 d

Additive	Grain	pH post - processing			Cook loss	Drip loss
		1h	4h	24h		
		---------- pH ----------			---------- % ----------	
GAA		6.30	5.91	6.01[b]	28.67	1.24
None		6.30	5.94	6.08[a]	28.57	1.28
SEM		0.02	0.01	0.02	0.46	0.06
	Corn	6.32	5.92	6.05	28.30	1.28
	Sorghum	6.28	5.93	6.04	28.94	1.24
	SEM	0.02	0.01	0.02	0.46	0.06
GAA	Corn	6.35	5.90	6.03	28.14	1.28
	Sorghum	6.26	5.91	5.99	29.19	1.20
None	Corn	6.30	5.94	6.07	28.47	1.29
	Sorghum	6.30	5.95	6.09	28.68	1.27
	SEM	0.02	0.02	0.03	0.65	0.09
CV%		2.46	2.13	3.04	14.32	45.19
Source of variation		---------- *P* – values ----------				
Additive		0.9966	0.0995	0.0153	0.8866	0.6737
Grain		0.0579	0.6259	0.7189	0.3325	0.6302
Additive*grain		0.0815	0.9415	0.2561	0.5207	0.7410

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

Table II.12. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on *Pectoralis major*'s cook and drip loss, and post-mortem pH (1, 4, and 24 h) at 55 d

Additive	Grain	pH post - processing			Cook loss	Drip loss
		1h	4h	24h		
		---------- pH ----------			---------- % ----------	
GAA		6.35	5.88[b]	5.95[b]	31.01	1.31
None		6.32	5.92[a]	5.99[a]	30.52	1.19
SEM		0.01	0.01	0.01	0.51	0.05
	Corn	6.33	5.88	5.95[b]	30.97	1.26
	Sorghum	6.34	5.91	5.99[a]	30.57	1.24
	SEM	0.01	0.01	0.01	0.51	0.05
GAA	Corn	6.34	5.87	5.95	30.98	1.31
	Sorghum	6.35	5.88	5.96	31.04	1.31
None	Corn	6.32	5.90	5.96	30.96	1.21
	Sorghum	6.32	5.95	6.03	30.09	1.17
	SEM	0.02	0.02	0.02	0.72	0.07
CV%		2.23	1.80	1.71	14.73	33.39
Source of variation		---------- *P* – values ----------				
Additive		0.1768	0.0079	0.0094	0.4992	0.0777
Grain		0.7700	0.0676	0.0196	0.5786	0.7279
Additive*grain		0.8408	0.3300	0.1387	0.5157	0.7845

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

Table II.13. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on shear force and color at 51 and 55 d

Additive	Grain	Shear force[1]		Color					
		51d	55d	51d			55d		
				L*	a*	b*	L*	a*	b*
		---------- (kg) ----------							
GAA		4.60	4.58	58.98	4.34	7.03^{b}	59.49	3.90	7.23
None		4.35	4.67	58.83	4.47	7.90^{a}	58.57	3.81	7.55
SEM		0.18	0.14	0.33	0.17	0.23	0.34	0.17	0.22
	Corn	4.50	4.59	58.61	4.17^{b}	8.78^{a}	59.23	3.89	9.28^{a}
	Sorghum	4.45	4.66	59.21	4.64^{a}	6.15^{b}	58.82	3.82	5.50^{b}
	SEM	0.18	0.14	0.34	0.17	0.23	0.35	0.17	0.22
GAA	Corn	4.57	4.54	58.50	3.78^{b}	7.92^{b}	59.85	4.02	9.34
	Sorghum	4.62	4.62	59.46	4.91^{a}	6.14^{c}	59.13	3.78	5.13
None	Corn	4.43	4.64	58.71	4.56^{ab}	9.64^{a}	58.62	3.76	9.23
	Sorghum	4.27	4.70	58.95	4.38^{ab}	6.17^{c}	58.52	3.85	5.87
	SEM	0.22	0.17	0.48	0.23	0.33	0.50	0.24	0.31
CV%		22.68	23.65	5.11	34.04	27.74	5.36	38.90	26.66
Source of variation		---------- *P* – values ----------							
Additive		0.1818	0.6369	0.7527	0.5917	0.0089	0.0684	0.6914	0.3104
Grain		0.6922	0.6369	0.2107	0.0491	<.0001	0.4113	0.7526	<.0001
Additive*grain		0.5436	0.9648	0.4548	0.0066	0.0109	0.5389	0.4784	0.1732

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.
Color: a* = redness; b* = yellowness; L* = lightness. [1] Warner-Bratzler shear device.

Table II.14. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on pectoral myopathies overall average scores at 51 and 55 d.

Additive	Grain	White striping scores				Wooden breast score		Spaghetti muscle score	
		First evaluator		Second evaluator					
		51d	55d	51d	55d	51d	55d	51d	55d
		-------- (1 – 3) -------		-------- (1 – 4) -------		-------- (1 – 4) -------		-------- (1 – 2) -------	
GAA		2.16	2.54	2.24	2.30	2.33[b]	2.37	1.14	1.02
None		2.13	2.51	2.17	2.21	2.76[a]	2.41	1.09	1.04
SEM		0.06	0.06	0.09	0.08	0.10	0.08	0.03	0.02
	Corn	2.05[b]	2.55	2.16	2.29	2.51	2.58[a]	1.10	1.03
	Sorghum	2.24[a]	2.50	2.25	2.23	2.57	2.19[b]	1.14	1.03
	SEM	0.06	0.06	0.08	0.08	0.10	0.08	0.03	0.02
GAA	Corn	2.00	2.53	2.20	2.30	2.18	2.58	1.13	1.02
	Sorghum	2.33	2.55	2.27	2.31	2.48	2.15	1.16	1.03
None	Corn	2.11	2.57	2.13	2.27	2.85	2.59	1.08	1.05
	Sorghum	2.15	2.44	2.22	2.15	2.67	2.23	1.11	1.04
	SEM	0.08	0.09	0.11	0.11	0.14	0.11	0.04	0.03
CV%		26.85	19.11	33.17	33.07	45.36	36.95	30.26	17.31
Source of variation		---------- *P* – values ----------							
Additive		0.7248	0.7288	0.5734	0.4168	0.0032	0.7226	0.2786	0.4113
Grain		0.0327	0.5845	0.4467	0.6103	0.6666	0.0013	0.4358	0.9733
Additive*grain		0.7248	0.3876	0.9123	0.5648	0.0846	0.7447	0.9820	0.6208

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

Table II.15. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on severity probability distribution (expressed as a percentage[1]; 0.00-1.00) of pectoral myopathies scores (1-4) at 51 d

Additive	Grain	Wooden Breast				White Striping			
		1	2	3	4	1	2	3	4
		---------- (0.0 – 1.0) ----------							
GAA		0.2993[a]	0.2883	0.1942	0.2183	0.1299	0.6104	0.2123	0.0505
None		0.1425[b]	0.2698	0.2808	0.3067	0.1551	0.4901	0.2988	0.0522
SEM		0.0634	0.0645	0.0245	0.0253	0.0430	0.0548	0.0396	0.0293
	Corn	0.2250	0.2750	0.2625	0.2375	0.1500	0.5500	0.2250	0.0750
	Sorghum	0.2168	0.2831	0.2124	0.2876	0.1350	0.5505	0.2861	0.0277
	SEM	0.0633	0.0645	0.0337	0.0348	0.0430	0.0546	0.0392	0.0293
GAA	Corn	0.3000	0.3750[a]	0.1750	0.1500	0.1000	0.6750	0.1250	0.1000
	Sorghum	0.2986	0.2017[b]	0.2133	0.2867	0.1599	0.5457	0.2997	0.0010
None	Corn	0.1500	0.1750[b]	0.3500	0.3250	0.2000	0.4250	0.3250	0.0500
	Sorghum	0.1351	0.3646[a]	0.2115	0.2885	0.1101	0.5552	0.2726	0.0543
	SEM	0.0785	0.0813	0.0469	0.0485	0.0584	0.0781	0.0631	0.0381
Source of variation		---------- *P* – values ----------							
Additive		0.0183	0.7920	0.3150	0.0993	0.6549	0.1302	0.2196	0.9622
Grain		0.9009	0.9074	0.4628	0.4055	0.7890	0.9952	0.3778	0.1708
Additive*grain		0.9183	0.0106	0.1444	0.2555	0.1842	0.1026	0.1076	0.1366

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

[1]Each value represent the probability (0-1) of developing each severity score according to main factors or factorial arrangement of treatments, $n = 10$; based on a 4-point scale (4 = severe, 3 = medium, 2 = low, 1 = normal).

Table II.16. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on severity probability distribution (expressed as a percentage[1]; 0.00-1.00) of pectoral myopathies scores (1-4) at 55 d.

Additive	Grain	Wooden Breast				White Striping			
		1	2	3	4	1	2	3	4
		---------- (0.0 – 1.0) ----------							
GAA		0.1712	0.3856	0.3442	0.0948	0.1277	0.5315	0.2900	0.0489
None		0.1565	0.4105	0.2950	0.1444	0.1344	0.5374	0.2765	0.0536
SEM		0.0272	0.0790	0.0970	0.0230	0.0376	0.1077	0.0633	0.0219
	Corn	0.0922^{b}	0.3618	0.4024^{a}	0.1426	0.1083	0.5667	0.2667	0.0583
	Sorghum	0.2355^{a}	0.4342	0.2368^{b}	0.0965	0.1538	0.5023	0.2998	0.0442
	SEM	0.0270	0.0790	0.0970	0.0225	0.0375	0.1077	0.0633	0.0218
GAA	Corn	0.0833	0.3667	0.4333	0.1167	0.1000	0.5500	0.2833	0.0667
	Sorghum	0.2590	0.4045	0.2552	0.0729	0.1554	0.5131	0.2967	0.0312
None	Corn	0.1011	0.3569	0.3715	0.1686	0.1167	0.5833	0.2500	0.0500
	Sorghum	0.2119	0.4640	0.2185	0.1201	0.1522	0.4915	0.3030	0.0572
	SEM	0.0436	0.0909	0.1056	0.0381	0.0489	0.1169	0.0758	0.0299
Source of variation		---------- *P* – values ----------							
Additive		0.7625	0.6959	0.4063	0.2552	0.8793	0.9273	0.8190	0.8710
Grain		0.0031	0.2548	0.0055	0.2780	0.3040	0.3163	0.5731	0.6238
Additive*grain		0.5056	0.5868	0.8318	0.9571	0.8228	0.6694	0.7370	0.4604

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

[1]Each value represent the probability (0-1) of developing each severity score according to main factors or factorial arrangement of treatments, $n = 10$; based on a 4-point scale (4 = severe, 3 = medium, 2 = low, 1 = normal).

Table II.17. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on pectoral myopathies incidence and severity at 51 d

Additive	Grain	Wooden Breast				White Striping			
		Grouped		Incidence		Grouped		Incidence	
		None to mild[1]	Severe[2]	None[3]	Yes[4]	None to mild[1]	Severe[2]	None[3]	Yes[4]
		(%)							
GAA		0.5875[a]	0.4125[b]	0.2994[a]	0.7006[b]	0.7273	0.2727	0.0823	0.9177
None		0.4125[b]	0.5875[a]	0.1424[b]	0.8576[a]	0.7611	0.2389	0.1467	0.8533
SEM		0.0459	0.0459	0.0627	0.0627	0.0561	0.0561	0.0576	0.0576
	Corn	0.5000	0.5000	0.2250	0.7750	0.7750	0.2250	0.1250	0.8750
	Sorghum	0.5000	0.5000	0.2168	0.7832	0.7134	0.2866	0.1039	0.8961
	SEM	0.0459	0.0459	0.0626	0.0626	0.0561	0.0561	0.0576	0.0576
GAA	Corn	0.6750[a]	0.3250[b]	0.3000	0.7000	0.7500	0.2500	0.1000	0.9000
	Sorghum	0.5000[ab]	0.5000[ab]	0.2987	0.7013	0.7045	0.2955	0.0645	0.9355
None	Corn	0.3250[b]	0.6750[a]	0.1500	0.8500	0.8000	0.2000	0.1500	0.8500
	Sorghum	0.5000[ab]	0.5000[ab]	0.1349	0.8651	0.7222	0.2778	0.1434	0.8566
	SEM	0.0649	0.0649	0.0768	0.0768	0.0793	0.0793	0.0673	0.0673
Source of variation		*P* – values							
Additive		0.0107	0.0107	0.0176	0.0176	0.6725	0.6725	0.1997	0.1997
Grain		1.0000	1.0000	0.8970	0.8970	0.4428	0.4428	0.6712	0.6712
Additive*grain		0.0107	0.0107	0.9132	0.9132	0.8398	0.8398	0.7714	0.7714

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

[1] Each value represents the probability (0-1) of developing each severity score according to main factors or factorial arrangement of treatments [1] None to mild = grouped by scores 1 and 2, [2]Severe = grouped by scores 3 and 4; [3]None = score 1; [4]Yes = grouped by score

Table II.18. Effect of supplementation of guanidinoacetic acid (GAA) in corn or sorghum diets for Ross 708 broilers on pectoral myopathies incidence and severity at 55 d

Additive	Grain	Wooden breast				White Striping			
		Grouped		Incidence		Grouped		Incidence	
		None to mild[1]	Severe[2]	None[3]	Yes[4]	None to mild[1]	Severe[2]	None[3]	Yes[4]
		---------- (0.0-1.0) ----------							
GAA		0.5556	0.4444	0.1720	0.8280	0.6555	0.3445	0.0979[b]	0.9021[a]
None		0.5697	0.4303	0.1552	0.8448	0.6084	0.3916	0.2158[a]	0.7842[b]
SEM		0.0535	0.0535	0.0238	0.0238	0.0546	0.0546	0.0358	0.0358
	Corn	0.4547[b]	0.5453[a]	0.0927[a]	0.9073[a]	0.6145	0.3855	0.1512	0.8488
	Sorghum	0.6706[a]	0.3294[b]	0.2346[b]	0.7654[b]	0.6494	0.3506	0.1625	0.8375
	SEM	0.0535	0.0535	0.0237	0.0237	0.0546	0.0546	0.0358	0.0358
GAA	Corn	0.4500	0.5500	0.0833	0.9167	0.6333	0.3667	0.1333	0.8667
	Sorghum	0.6612	0.3388	0.2608	0.7392	0.5956	0.4044	0.0624	0.9376
None	Corn	0.4594	0.5406	0.1020	0.8980	0.6776	0.3224	0.1691	0.8309
	Sorghum	0.6799	0.3201	0.2083	0.7917	0.6212	0.3788	0.2626	0.7374
	SEM	0.0756	0.0756	0.0368	0.0368	0.0772	0.0772	0.0477	0.0477
Source of variation		---------- *P* – values ----------							
Additive		0.8537	0.8537	0.6747	0.6747	0.5464	0.5464	0.0124	0.0124
Grain		0.0071	0.0071	0.0010	0.0010	0.6543	0.6543	0.8019	0.8019
Additive*grain		0.9514	0.9514	0.3805	0.3805	0.9043	0.9043	0.0747	0.0747

Values are means ± SEM of 10 pens per treatment combination with 4 male broiler chickens per pen selected for processing.

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

[1] Each value represent the probability (0-1) of developing each severity score according to main factors or factorial arrangement of treatments [1]

None to mild = grouped by scores 1 and 2, Severe = grouped by scores 3 and 4; [3]None = score 1; [4]Yes = grouped by score

CHAPTER III

Growth performance, carcass and cut up yield, meat quality and pectoral myopathies of male broilers fed corn-based diets with or without poultry by-products and supplemented with guanidinoacetic acid

3.1. ABSTRACT

This experiment was conducted to evaluate the effects of guanidinoacetic acid (GAA) supplementation in broilers fed corn-based diets with or without the inclusion of poultry by-products (PBP) on live performance, carcass and cut up yields, blood metabolites, liver enzymes, meat quality and pectoral myopathies of broilers raised up to 55 d. The treatments consisted in corn-based diets with or without PBP, supplemented or not supplemented with GAA (600 g/ton) as CreAMINO®. A total of 1,280 Ross 708 chicks were randomly placed in 64 floor pens with 16 replicates per treatment combination. At hatch, 14, 35, 48 and 55 d, BW and feed intake were recorded. BW gain and FCR adjusted by mortality weight were calculated at the end of each phase. At 55 d, chickens were selected for processing and for blood collection. Flock uniformity (CV%) was also calculated at this age. After processing breast fillets were obtained to analyze meat quality and pectoral myopathies. Data was analyzed as a randomized complete block design in a 2 x 2 factorial arrangement with PBP and GAA supplementation as main effects. BW gain at 48 and 55 d of age was enhanced ($P < 0.05$) by GAA supplementation. An improvement ($P < 0.05$) in FCR was detected at 55 d

due to GAA supplementation (1.65 *vs*. 1.67). An interaction effect ($P < 0.05$) was observed on cook loss and breast meat yield. GAA supplementation increased breast meat yield in diets with poultry by products only. No differences ($P > 0.05$) were detected on white striping due to PBP or GAA supplementation. However, the probability of having breast score with low severity of wooden breast was increased ($P < 0.05$) by GAA in diets without PBP. Treatment interaction effects ($P < 0.05$) was detected on GAA concentration levels in blood. In conclusion, GAA supplementation improved FCR, regardless of the inclusion of PBP in the diets. Generally, meat quality parameters were not affected by GAA. However, it reduced the severity of wooden breast in broilers fed corn-based diets without PBP inclusion.

Key words: Guanidinoacetic acid, poultry by-product, performance, pectoral myopathy, broiler.

3.2. INTRODUCTION

Creatine and its phosphorylated form phospho-creatine are naturally occurring components in the animal's body tissue and it plays a major role in energy metabolism (Wyss and Kaddurah-Daouk, 2000). In general, about 1.7% of the creatine and phospho-creatine pool is irreversibly converted to creatinine each day and excreted in the urine (Wyss and Kaddurah-Daouk, 2000). Consequently, creatine must be continually replaced (Lemme *et al.*, 2007). The demand for creatine can be supplied either directly from animal protein in the diet or by endogenous synthesis (Wyss and Kaddurah-Daouk, 2000). Creatine

can be produced naturally in the body from guanidine acetic acid (GAA), which in turn is synthesized from the amino acids arginine and glycine (Wyss and Kaddurah-Daouk, 2000). These amino acids are highly involved in muscle metabolism (Lemme *et al.*, 2007). The marked growth-promoting effect of creatine may be most simply explained on an arginine-sparing basis (Dilger *et al.* 2013; DeGroot, 2014). Arginine and glycine are normally found in higher concentrations in feedstuffs such as animal by-products meal (Baker, 2009). Poultry by-product meal is one of the most important sources of animal protein in animal feed and it's made by combining the by-products coming from poultry slaughterhouses or poultry processing plants (Bhargava and O'Neil, 1975; Meeker and Hamilton, 2006). The AAFCO (2011) defines poultry by-product meal as the ground, rendered, clean parts of the carcass of slaughtered poultry such as necks, heads, feet, undeveloped eggs, gizzards and intestines (provided their content is removed), exclusive of feathers, except in such amounts as might occur unavoidably in good processing practices. According to Li *et al.* (2011) the values for arginine and glycine, were higher in poultry by-products meal than in corn and soybean meal. It has been reported that if animal by-products, which are rich in creatine were included in poultry diets then no signs of a creatine deficiency should be detected. As a result of the ban of meat and bone meal in 2001, european poultry producers observed a certain drop in performance (European Community, 2000). This could have been caused by the lack or insufficient supply of creatine because vegetable based feed ingredients do not contain high concentration of this semi-essential nutrient (Ringel *et al.*, 2007). Generally, corn and soybean meal represent up to 75% of the poultry diets, and approximately 90% of diets for poultry in the U.S. is comprised of corn and soybean meal (Baker, 2009).

Consequently, broilers fed vegetable-based diets could face the possibility of a deficiency in creatine supply and therefore depress broiler live performance. According to Petracci *et al.* (2015), novel pectoral myopathies, such as white striping and wooden breast, are characterized by a reduced supply of oxygen and nutrients to muscle cell. In addition, this could also be associated with inadequate elimination of metabolic intermediate products leading to a membrane dysfunction and increased plasma concentration of enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH) (Mitchell, 1999; Sandercock and Mitchell, 2003; MacRae *et al.*, 2006). Several management and nutritional solutions has been proposed to minimize and solve these myopathies and meat quality issues.

Among these techniques the reduction of growth rate by using feed restriction programs or lowering the energy value of the diets (Kuttappan *et al.*, 2012a; Trocino *et al.*, 2015; Radaelli *et al.*, 2017), higher dietary vitamin E and selenium (Guetchom *et al.*, 2012; Kuttappan *et al.*, 2012b), had been tested and showing some benefits to improve meat quality and reduce myopathies occurrence. However, the reduction of growth rate can be prejudicial for productivity. Therefore, our study evaluated a possible beneficial effect of GAA supplementation on pectoral myopathies without reducing the genetic potential growing rate of broilers. Based on mice trials, it has been proposed that creatine may have a protective effect on certain neuromuscular (Tarnopolsky, 2007; Chung *et al.*, 2007), and neuro-degenerative (Bender *et al.*, 2006; Kolling and Wise, 2010; Beal, 2011) diseases, and it also may reverse muscular dystrophy (Nabuurs *et al.*, 2013). These results may be due to the capacity of creatine to prevent the inhibition of energy metabolism and lipid peroxidation.

Previous research of blood parameters was done in young chickens (Michiels *et al.*, 2012; DeGroot, 2014), and muscle metabolism could vary with age of broilers. Therefore, the present experiment focused on metabolites in heavy broilers of 8 wk of age. In the U.S. chickens are grown for more than 56 d (Agristats, 2016). Previous studies showed that GAA supplementation lowered pH of the breast meat. Consequently, affecting the water uptake capacity of meat in muscle could improve the tenderness of the meat. According to Petracci *et al.,* (2015) wooden breast and white striping pectoral myopathies are highly correlated with ultimate pH, drip loss, etc. Therefore, as it has been reported that dietary supplementation of GAA affects ultimate pH (Michiels *et al.*, 2012), its use could have a beneficial effect on meat quality and the occurrence of breast meat myopathy. It is important to mention that previous studies did not analyze the effect of GAA supplementation on pectoral myopathies. Consequently, the objective of this study was to evaluate the effects of GAA on live performance, meat quality, energy metabolites concentration and pectoral myopathies in diets with or without poultry by-products.

3.2 MATERIALS AND METHODS

Treatments and Birds Husbandry

All the procedures involving the birds used in the present experiment were approved by the North Carolina State University Institutional Animal Care and Use Committee. Four treatments from a 2 x 2 factorial arrangement with 2 corn-SBM diets with (5%) or without inclusion of poultry by-products and 2 levels (0 and 0.06%) of supplementation of

guanidinoacetic acid (GAA) as CreAMINO® at 96% of concentration as main factors were considered. This study was conducted from September 27th to November 23th, 2017 at North Carolina State University, Raleigh, NC, USA, in an open-sided house with clear curtains with negative pressure ventilation. A total of 1,280 Ross-708 day-old male chicks were placed in 64 floor pens (3.81 x 1.19 m) with 20 chicks per pen (4.42 broilers/m^2 at placement) for a final stocking density of 22.31 kg/m^2 at 55d of age. Chickens were raised on used litter. Chickens were exposed to continuous light on a 23L:1D (30 lux light intensity) program during the first 7 d of age. Day length was then gradually reduced to 17L:7D (10 lux) up to 28 d of age. From 28 d until the end of the experiment, light program was maintained at 17L:7D with an intensity of 5 lux. Brooding house temperature was set at 33.6°C and gradually reduced until 20.6°C at 21 d. Chicks founded dead during the first 10 d of age were replaced with extra birds from same treatments and similar BW.

Diets

Experimental diets were mixed at the NCSU Feed Mill Research Facility. All diets were formulated to represent typical U.S. broiler industry practices, and digestible amino acid levels were based on AminoData® 5.0 (Evonik, 2015) recommendations. Macro ingredients (corn, soybean meal, and distilled dried grain with solubles) were analyzed for total amino acid and metabolizable energy (ME) content prior to diet formulation. Digestible amino acid content was calculated from the total amino acid content and using table values for digestibility coefficients (Evonik, 2015). ME values (kcal/kg) were obtained from an *in vivo* trial with roosters (Dr. Nick Dale, University of Georgia). GAA was added “on top” of

the basal diets (600 g/ton) in the corresponding treatments. Diets were considered to either contain 5% poultry by-products or not (Table III.1). Experimental feeds were produced from basal diets to ensure that broilers had similar nutrient intake Starter, grower, finisher and withdrawal diets were fed from 0-14, 14-35, 35-42, and 42-55 days of age, respectively. Starter was fed in crumbles and all other diets in pellets. For the pelleting process, a temperature between 82 and 85°C in the conditioner was used for 30 seconds. The steam pressure was 32 psi, and the pellet die was 11/64" x 1" 3/8" (4.4 x 34.9 mm) for an L/D ratio of 8. The capacity of pelleting used was 2 to 5 ton/hour to improve pellet quality. Final diets after being crumbled or pelleted, representative samples of each manufactured diet were analyzed for amino acid content, GAA concentration and macro minerals (Table III.10). For each one of the dietary phases 0.85, 2.90, and 2.48 kg of starter, grower and finisher, respectively, were offered for each bird alive during each phase. The withdrawal was offered *ad libitum*. Water was provided for *ad libitum* consumption. Feeders were shook twice daily in order to stimulate uniform feed intake. This experiment was conducted following animal management and production protocols.

Live performance and blood collection

At hatch, 14, 35, 48 and 55 days of age body weight (BW) and feed intake were measured and BW gain (BWG) and feed conversion ratio (FCR) calculated at the end of each phase. Mortality was monitored and recorded daily. Partial and cumulative results for every phase were also calculated. At 55 d, individual BW were obtained to estimate flock

uniformity using the CV%. Blood serum samples were collected from non-fasting chickens (1 per pen) for hematologic profile with differential for white blood cells count (WBC), red blood cells count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), count of heterophils, thrombocytes, monocytes, lymphocytes, T-cell, B-cell. Also, serum enzyme activity of creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were analyzed. Other metabolites involved in metabolism were also analyzed (glucose, uric acid, creatinine, homocysteine, creatine, guanidinoacetic acid). Blood mineral (phosphorus, calcium, sodium, potassium, and chloride), protein-total, albumin, and cholesterol content were also analyzed.

Selection of birds for processing, meat quality and pectoral myopathies

At 55 days of age, individual and group BW were obtained and the average for each pen was calculated. Four broilers per pen from 16 pens/treatment combination were selected for processing, meat quality, and pectoral myopathies evaluation. BW of selected broilers remained within two standard deviations above or under their corresponding average for each pen.

Carcass and cut up yields

At 55 d, feed was withdrawn for 12-h. Broilers were slaughtered at the NCSU pilot processing plant. Broilers were weighed, electrically stunned for 11 s, killed by exsanguination, and allowed to bleed for 90 s. Broilers were then scalded at 55°C for 90 s,

picked for 30 s, and manually eviscerated. Carcasses were dressed by removing liver, gizzard, heart, oil gland, crop, proventriculus, lungs, and viscera. Carcasses were then air-chilled for 6 h, and manually deboned on stationary cones. Parts of the leg quarters, breast fillets (*Pectoralis major*), breast tenders (*Pectoralis minor*), wings, and rack with skin were weighed. The carcass yield was calculated for the chilled carcass as a percentage of the fasted live BW. Parts yield was expressed as a percentage of the chilled absolute carcass BW.

Meat quality evaluation

To determine cook loss, the breast fillets (*Pectoralis major*) were weighed, placed on aluminum pans, and cooked in a forced air oven (SilverStar Southbend, Model SLES/10sc, gas type, NC, USA). Fillets were cooked to an internal temperature of 75°C (approximately 35 min), as measured by a Therma Plus thermocouple with a 10-cm needle temperature probe (ThermoWorks Model 221-071, UT, USA). The cooked fillets were cooled to room temperature, wiped with absorbent paper and re-weighed to determine cook yield as a percentage of the cooked weight relative to the raw weight. The water-holding capacity of meat was estimated by measuring drip loss of the fillets after storage. The *Pectoralis major* muscle was weighed 24 h postmortem and immediately placed in a plastic bag, hung from a hook, and stored between 4-6°C for 1 d. After hanging, the sample was wiped with absorbent paper and weighed again. The difference in weight corresponded to the drip loss and was expressed as the percentage of the initial muscle weight. To define shear force: cooked breast fillets samples were tested for texture using a Warner-Bratzler shear device (Warner-Bratzler

meat shear, Bodine Electric Company, Chicago, USA). Two samples per breast fillets (2×2×2 cm) were sheared in a direction perpendicular to the muscle fiber.

Crosshead speed was 4 mm/sec. The maximum force measured when cutting the samples was expressed in kg. For pH determination, pH was measured using a portable pH meter (Oakton waterproof pH Tester 30) on the carcasses at 6 and 24 h after processing. Skin was removed from breast fillets and color was measured on the surface of raw breast fillets. Color was measured by the CIE L*a*b* system using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Japan). A measuring area of 10 mm and illuminant D65 and 2° standard observer were used. The colorimeter was calibrated using a white tile (reference number 13033071; Y = 93.9, x = 0.3156, y = 0.3318). Triplicate measurements were taken for each sample. CIE lightness (L*), redness (a*), and yellowness (b*) were measured.

Pectoral myopathies

After breast fillets were obtained, methods were used to detect the phenotypic variation in severity of the conditions seen at the respectively breast fillet. Sensorial analysis was done by experts in the field. Spaghetti muscle was recorded as present or absent, whereas white striping (WS) and (WB) were scored based on severity. Wooden breast severity was based on a four-point scale, where score 1 is a normal fillet with no wooden breast signs, score 2 was considered a low severity, score 3 a medium and score 4 a severe severity associated with WB. Similarly, white striping was recorded on a four-point based scale of severity. Score 1 was considered a breast fillet with no white striations on the

surface. Score 2 were the fillets with white striations less than 1 mm of thickness and easily observed in the surface, score 3 was represented by the white striation more than 1 mm of thickness and covering less than 50% of the breast's fillet area, and score 4 was considered the fillets with white striations with more than 1 mm of thickness and covering an area more than the 50% of the breast's fillet surface.

Statistical analysis

Data were analyzed as completely randomized block design with a 2 x 2 factorial arrangement of treatments. Factor A corresponded to the inclusion of poultry by-products or not (5 *vs*. 0%), and factor B corresponded to the supplementation or not of GAA (0 *vs.* 0.06%), to have a total of 4 treatments. Four blocks (location of pens within the house), 4 treatments per block, and 4 replicates per treatment within each block, for a total of 64 replicates (pens). Location of the pen was considered random effect. Data were analyzed in JMP 12 (SAS Inst. Inc., Cary, NC, 2016) using ANOVA in a mixed model. Differences between means were separated using Tukey's or t-student test at a level of significance of alpha = 0.05.

In addition, for blood, carcass and cut up yields, meat quality and pectoral myopathies analyses, the individual data for each selected broiler within each pen was analyzed including a nested effect of pen inside every corresponding treatment and considered as random effect. For carcass and cut up yields, the cutter was also included in the mixed model and considered as random effect.

3.4. RESULTS AND DISCUSSION

Live performance

No interaction (P>0.05) effects of treatments were observed on BW or BW gain, feed intake, and FCR in any of the phases evaluated (Tables III.6). Feed intake was not affected (P>0.05) by poultry by-products inclusion in the diet nor GAA addition in any of the experimental phases. At 0-14, 0-48 and 0-55 d, dietary inclusion of poultry by-products decreased (P < 0.05) BW and BWG. Broilers that were fed corn-based diets without poultry by-products were heavier and gained more weight than broilers that were fed corn-based diets with poultry-byproducts. At 0-48 and 0-55 d, FCR was affected (P < 0.05) by dietary poultry by-products inclusion. Chickens fed diets with poultry by-products had worse FCR than broilers fed diets with no by-products. FCR was improved (P < 0.05) by GAA supplementation at 0-48 and 0-55 d of age (Tables III.5 and III.6). Broilers fed diets supplemented with GAA had lower FCR up to 2 points than treatments with no GAA, regardless of the dietary poultry by-product inclusion. Diets without poultry by-product and with GAA supplementation had slightly worse (P < 0.05) flock uniformity with higher CV% of individual BW. However, dietary GAA supplementation reduced (P < 0.05) mortality by 2 fold (3.59% *vs*. 6.09%) from 48 to 55d of age, and dietary poultry by-product inclusion increased (P < 0.05) mortality from 14 to 35d of age by 2 fold (2.34% vs. 0.94%). Escalona and Pesti (1987) concluded that an inclusion of poultry by-products in the diet of more than 5% could depress growth in broilers fed corn-SBM diets. On the other hand, Caires *et al.* (2010) found no negative impacts (P > 0.05) on BW, BW gain and FCR when including

poultry by products up to 5% in corn-based diets. Unexpectedly, in the present experiment, the dietary inclusion of poultry by-products (5%), resulted in reduced performance for an unknown reason, since the quality and laboratory analyses of nutrient content conducted resulted in normal characteristics of this ingredient. Total amino acid content in the final diets for each phase showed similar values among treatments (Table A.6). Results of mortality from 14 to 35 d could suggest an unknown problem in the poultry by-products.

Bories *et al.* (2009) found an improvement ($P < 0.05$) on FCR when GAA was included at a level of 0.09%. However, no effects on lower levels of supplementation (0.06%) were detected, mainly because treatments were also compared with a positive control that contained fish meal in the feed and when chickens were raised up to 42 d. Likewise, Michiels *et al.* (2012) found no improvements on live performance attributed to GAA supplementation when two levels of inclusion of GAA (0.06 and 0.09%) were compared to a positive control that contained fish meal and broilers were raised up to 39 d. However, these levels of inclusion enhanced live performance when treatments were compared with corn-SBM based diets.

Bories *et al.* (2009) showed that signs of intolerance to GAA could occur when the level of dietary inclusion of GAA was about 10 times the recommended dose; these negative effects include depression of feed intake and lower weight gain. Previous studies validated the efficacy of dietary GAA inclusion on improving live performance in chickens that were fed diets containing only vegetable ingredients (Lemme *et al.*, 2007; Ringel *et al.*, 2007; Michiels *et al.*, 2012), inclusion of animal by-products (Bories *et al.*, 2009), and even in

arginine-deficient diets up to 22 d (Dilger, 2013; DeGroot, 2014). Therefore, dietary GAA supplementation improved live performance even in cases where arginine supply in feed was deficient. As poultry by-products and generally all animal products contain a more balanced amino acid profile, we hypothesized that its inclusion will improve FCR. However, in our study this was not observed, although there were no differences between treatments up to 35 d, from 0 – 48, and 0 – 55 the FCR or broilers fed diets containing poultry by-products were affected up to 2 points. Part of these findings are related to a consequence of a better BW gain obtained in broilers fed corn-based diets without poultry-by products inclusion. As GAA supplementation improved FCR, regardless of poultry by-products inclusion in the feed, this supports previous findings that the use of GAA in vegetable-based diets promotes broiler live performance even in diets (all-vegetable) where the supply of amino acids could be limited.

Carcass yield and cut up parts

At 56d of age birds were processed (Tables III.13 and III.14). Interaction effects ($P<0.05$) were observed on *Pectoralis major* and breast meat yield. Supplementation with GAA improved *Pectoralis major* and breast meat yield only in diets with inclusion of poultry by products. Broilers fed diets containing poultry by-products and supplemented with GAA had higher yield than chickens fed non-supplemented diets with inclusion of poultry by-products. Carcass, wings, and leg quarters yields were not affected ($P > 0.05$) either by poultry by-products in the diet or GAA supplementation. Caires *et al.* (2010) observed no differences ($P > 0.05$) in carcass and breast meat yields when corn-SBM diets were

compared to diets containing poultry by-products (5%). Michiels *et al.* (2012) observed improvements ($P < 0.05$) in breast meat yield by up to 4% when GAA was added (0.06% and 1.2%) in corn-based diets.

However, when treatments were compared with a positive control containing fish meal in the diet, this effect was not observed ($P > 0.05$). Moreover, dietary GAA supplementation increased ($P < 0.05$) leg yield, as compared to the positive control. According to Bories *et al.* (2009), GAA supplementation showed no improvements ($P > 0.05$) either on carcass or breast muscle yield when GAA was supplemented at a level of 0.06%. As breast meat is more glycolytic than other cut up parts (McKee, 2002), the results of our experiment suggest that GAA improved ($P < 0.05$) breast meat yield only in diets containing poultry by-products (Table III.8). Interestingly, the treatment that had the highest breast meat yield (diet containing poultry by-products and GAA supplementation) also had the greatest GAA concentration in blood ($P < 0.05$). However, creatine concentration showed no interaction effects due to poultry by-products in the diet and GAA inclusion. Therefore, this could explain breast meat's intermediate results of the rest of the treatments. We can assume that as the supply of creatine was not limited in the chickens that ate diets containing poultry by-products and GAA, thus there was more opportunity to promote muscle growth which translates into a greater breast meat yield.

Meat quality

No effect of treatments (P>0.05) was observed on drip loss (Table III.10). However, an interaction effect (P<0.05) was observed on cook loss. Chickens fed diets with poultry by-products and not supplemented with GAA had lower cook loss than the chickens fed diets without poultry by-products and not supplemented with GAA (16.76 *vs*. 19.95%). The pH of breast muscle was evaluated at 6 and 24 h post-slaughter. No significant effects (P>0.05) were observed among treatments due to GAA supplementation or poultry by-products inclusion in the diet (Table III.10). Shear force (Table III.10) was not affected (P>0.05) by GAA supplementation or poultry by-product inclusion. Poultry by-product inclusion in the diet affected (P<0.05) the b* (yellowness) color value. Chickens fed diets with poultry by-product had higher b* value than the ones fed diets with no poultry by-products (10.06 *vs*. 9.27).

Similar results on drip loss were detected in a trial conducted by Michiels *et al.* (2012) in which GAA addition did not affect (P>0.05) breast drip loss when supplemented diets were compared with treatments with or without fish meal. However, cooking loss was greater (P<0.05) in supplemented diets than the non-supplemented corn-based diet. Bories *et al.* (2009) also found no differences among treatment on drip loss due to GAA addition in samples of chickens fed corn-based diets and raised up to 42 d. In the present experiment, the addition of GAA resulted in an intermediate response, consequently dietary GAA supplementation did not improved cook loss. Shear force was not affected (P>0.05) either by dietary GAA supplementation or PBP inclusion.

Michiels *et al.* (2012) reported that ultimate pH was lowered ($P<0.05$) by GAA supplementation in corn-based diets. However, when treatments with GAA addition were compared with a positive control containing fish meal in the diet, this effect was not observed ($P>0.05$). Bories *et al.* (2009) observed that the ultimate pH of breast muscle was lowered ($P<0.05$) by GAA addition as compared to samples from chickens that ate non-supplemented diets (5.92 vs. 6.05). According to Mudalal *et al.* (2014), pH of breast meat affected with either white striping, wooden breast or both, presented higher ($P<0.001$) pH values on breast's fillets affected by these myopathies compared with samples that were not affected by any of these myopathies. pH values of affected breast meat were higher than 5.8. Even though the present results showed pH values between 5.98 and 6.03, the severity of wooden breast or white striping was not affected by treatments. According to Khan (1974), meat pH is determined by muscle glycogen content and its degradation rate. Mudalal *et al.*, (2014) suggested that GAA supplementation could avoid glycogen depletion and improve meat quality. According to Fletcher *et al.* (2000), the ultimate pH (after 24 h of slaughter) of normal chicken breast meat ranges between 5.7 and 5.96. Even though no differences ($P>0.05$) were observed in our experiment, our results of ultimate pH ranged from 5.99 to 6.03. Interestingly, no effects on shear force were also observed ($P>0.05$) due to GAA supplementation or poultry by-products in the diets, and values remained over the scale that Poole *et al.* (1999) considered as "moderate tender".

Michiels *et al.* (2012) found no differences on shear force due to GAA supplementation. Generally, our measurements of shear force (Warner-Bratzler) were from 4.30 to 4.47 kg, which are values that can be considered as "moderately to slightly tender"

category based on Lyon and Lyon (1991) sensory ranges. These values also can be expressed as 42.17 to 43.84 [N], which are high. According to some reports (Owens *et al.*, 2000; Schilling *et al.*, 2003; Corzo *et al.*, 2009) shear force values lower than 30 N are considered to be highly accepted by the consumer. However, Schilling *et al.* (2003) concluded that independently of the value of the shear force value, not an overwhelming proportion of consumers would find samples unacceptable. In addition, Poole *et al.* (1999) found that broilers around 56 d of age should have an average value of shear force (Warner-Bratzler) of about 4.64 kg ± 0.18 kg. They suggested that breast fillets in the scale from 3.46 to 6.41 kg are considered "moderate tender" which are similar values to the ones observed in this experiment. Fletcher (2002) concluded that differences in tenderness depend on age of the chickens and can be due to the fact that older birds are more mature at the time of harvest and have more cross-linking of collagen. Zotte *et al.* (2017) found no differences on shear force when compared breast fillets affected by wooden breast myopathy and non-affected breast fillets. Likewise, other authors (Mudalal *et al.*, 2014; Tijare *et al.*, 2016), found no differences ($P>0.05$) among the scores of severities within wooden breast and white striping on shear force when breast fillets were not marinated. Therefore, results of our trial suggested that the values of shear force obtained were related to age of the birds at processing.

Similarly to our findings, Michiels *et al.* (2012) observed that a* value was not affected ($P>0.05$) by GAA supplementation, even though when supplemented diets were compared with a negative corn-based feed and a positive control containing fish meal in the diet. The same experiment found that GAA supplementation increased ($P<0.05$) L*, and b*

values in breast meat. However, L* values were not affected by dietary GAA inclusion in another experiment conducted by Bories *et al.* (2009). The b* value results of our study could be explained by the level of inclusion of corn in the diet, and the theoretically higher carotenoid content in diets with poultry by products (Table III.2). All experimental final feeds containing poultry by-products had higher inclusion of corn, compared with diets that did not contain poultry by-products throughout the whole experiment. Consequently, they contained a greater content of carotenoids in the feed that was later deposited in breast muscle.

Pectoral myopathies

Breast meat was evaluated for pectoral myopathies (Tables III.11 to III.13). Samples for histopathology analysis were also collected and analyzed (Table III.11). White striping considered 3 levels of severity (mild, moderate, and severe 1, 2, 3), and wooden breast included 4 levels of severity (normal, low, moderate and severe; 1-4). Data was analyzed by severity or average value of scores (Table III.11), individual distribution of scores (Table III.12) and grouping scores (Table III.13) to determine differences between normal and none to mild *vs*. moderate and severe. No interaction effects ($P>0.05$) were observed in either white striping or wooden breast on the average of scores (Table III.11). Likewise, white striping and wooden breast average scores were not affected ($P>0.05$) by dietary GAA supplementation or poultry by-products inclusion. When data was analyzed by individual

scores, an interaction effect ($P<0.05$) was observed on wooden breast for score #2. Broilers fed diets with no dietary inclusion of poultry by-products and GAA supplementation had more (0.53%) fillets with low wooden breast severity than the chickens (0.26%) fed diets with no inclusion of poultry by-products and no supplementation of GAA (Table III.12). The severity of "*spaghetti muscle*" was evaluated, but not enough breast fillets were affected with this myopathy to have replicates to run the statistical model. Previous studies (Ringel *et al.*, 2007; Michiels *et al.*, 2012; DeGroot, 2014) showed that GAA supplementation increased concentration of creatine in breast muscle. According to Hultman *et al.* (1996), intramuscular phospho-creatine can attract water into the muscle cell and increase the cell volume. Haussinger (1996) found that a super-hydrated muscle may trigger protein synthesis, minimize protein breakdown, and increase glycogen synthesis, as partly illustrated by Young *et al*. (2007). According to Soglia *et al.* (2016), glycogen phosphorylase was lower ($P<0.05$) in broilers with wooden breast myopathies compared with samples considered normal.

Results of our experiment suggest that GAA could reduce wooden breast myopathy in corn-based diets (Table III.13). The explanation for this also can be observed in the protective effects of creatine found in muscle energy metabolism as reported by Kolling *et al.* (2013) and Nabuurs *et al.* (2013) in rats and other studies about muscle dystrophies (Pearlman, *et al.*, 2006; Chung *et al.*, 2007; Tarnopolsky, 2007). Nabuurs *et al.* (2013) concluded that muscular dystrophy could be reversed by supplementing creatine in the diet of rats. Kolling *et al.* (2013) suggested that the effect of creatine and its interaction with homocysteine altered glucose oxidation and protected muscle from energy imbalances in rats.

It has been demonstrated that alterations in energy metabolism seem to be implicated in the pathogenesis of several muscle and neurological complications, metabolic disorders, aging and neuromuscular diseases. Considering the intermediate results obtained in diets containing poultry by-products for wooden breast severity (Table III.13), it could be suggested that dietary precursors of creatine contained in poultry by products (arginine and glycine) or in the feed additive (GAA), supplied enough creatine avoiding glycogen depletion and therefore preventing muscle damage.

Blood hematology, chemical chemistry and GAA metabolites

Blood results showed interaction effects of treatments on MCV and GAA serum concentration only. Chickens fed diets containing poultry by-products without GAA supplementation had the lowest ($P < 0.05$) MCV's blood concentration. For GAA serum concentration, broilers fed diets with poultry by-products and GAA addition showed greater ($P < 0.05$) GAA concentration up to 12.4-fold as compared to samples from chickens fed non-supplemented diets regardless of poultry by-products inclusion in the feed. Bories *et al.* (2009) detected no differences ($P > 0.05$) in MCV attributed to GAA when the level of supplementation was 0.06%. However, gradual increment in the level of supplementation up to 0.6% showed higher ($P \leq 0.05$) values of MCV.

Hematological results of blood cells count were not affected ($P > 0.05$) by poultry by-product inclusion nor GAA supplementation (Table III.10). Similar response ($P > 0.05$) was

detected by Bories *et al.* (2009) for leukocytes concentration, when GAA was gradually included in the feed (0.06, 0.15, and 0.3%). Likewise, DeGroot (2014) found no effects on blood cell count (leukocytes, heterophils, lymphocytes, monocytes, eosinophils, basophils) due to GAA supplementation in broilers fed both arginine-deficient and arginine-adequate diets. However, differential cell proportions (based on total leukocytes detected) of heterophils were reduced ($P < 0.05$) and increased ($P < 0.05$) in lymphocytes due to GAA supplementation.

Serum cholesterol concentration was affected ($P \leq 0.05$) by poultry by-product inclusion. Chickens fed diets containing poultry by-products showed higher (140.34 mg/dl) serum cholesterol concentration than the chickens (133.15 mg/dl) fed diets without this ingredient (Table III.9). However, other substrates (albumin, protein, glucose, uric acid) and enzymes ALT, AST, GGT, LD, and CK were not affected ($P > 0.05$) either by poultry by-products inclusion in the diet or GAA supplementation. Bories *et al.* (2009) also showed that serum protein, albumin, cholesterol, glucose, urea, uric acid and enzymes ALT, AST, and GGT remained essentially unchanged ($P > 0.05$) even when dietary GAA inclusion was up to 0.6%. Likewise, Michiels *et al*. (2012) found no differences on serum glucose and uric acid in broilers fed corn-based diets and raised up to 26 d. According to DeGroot (2014), no differences ($P > 0.05$) were detected on AST and CK serum concentrations attributed to GAA supplementation. Tossenberger *et al.* (2016) also concluded that serum glucose, total protein, urea, uric acid, and cholesterol were not affected by GAA supplementation in corn-based diets in chickens raised up to 35 d. Interestingly, in our study a response close to be

significant (P = 0.0693) was observed on GGT serum concentration. According to Abasht *et al.* (2016), higher levels of gamma-glutamyl amino acid catabolite 5-oxoproline were observed in tissue of samples affected by wooden breast myopathy up to 1.57-fold as compared to un-affected samples. They concluded that these findings strongly suggest that affected chickens possess biological markers of muscle degradation and oxidative stress. Moreover, Sweiry *et al.* (1995) suggested that GGT is involved in the transport of gamma-glutamyl amino acids. Considering our findings, we could propose that GAA reduced wooden breast severity by managing GGT serum blood concentration and therefore the transport of gamma-glutamyl amino acids.

Creatine concentration in blood was higher (P < 0.01) in broilers fed supplemented diets than chickens fed non-supplemented diets (65.87 vs. 41.46 µM) due to GAA addition (Table III.11). A previous research found that GAA supplementation increased (P < 0.01) creatine and GAA serum concentration due to GAA supplementation at a level of inclusion of 0.06 and 0.12% in the feed (DeGroot, 2014). HBE and MCH were not affected (P > 0.05) either by poultry by-products inclusion or GAA supplementation. Similar response on MCH was observed by Bories *et al.* (2009) when GAA was included at a 0.06% level. However, MCH was increased (P < 0.05) when GAA was supplemented at 0.3 and 0.6% in the feed.

Neither poultry by-products or GAA supplementation affected (P > 0.05) homocysteine serum concentration. Bories *et al.* (2009) found no differences among treatments when GAA was included in the diet up to 0.3% level of inclusion in the feed. However, homocysteine serum concentration was increased (P < 0.05) at a 0.6% level of

inclusion in the diet, leading to a hyper-homocysteinemia status due to deficient methyl donors to complete the conversion from GAA to creatine. A trial conducted on rats support this findings and hypothesis (Setoue *et al.*, 2008). The same response on homocysteine ($P < 0.05$) was observed by Tossenberger *et al.* (2016), when GAA was supplemented in corn-based diets at a level of 0.6% in the feed.

Minerals (P, Ca, Na, K, and Cl) in serum were not affected ($P > 0.05$) by poultry by-products inclusion in the diet or GAA supplementation. Likewise, the same response was observed in a trial conducted by DeGroot (2014) in which calcium and phosphorous in serum were not affected by GAA supplementation when GAA was included in the diet in different levels (0, 0.06, and 0.12%).

According to Petracci *et al.* (2015) the presences of pectoral myopathies may be explained by an increased concentration of enzymes, such as creatine kinase and lactate dehydrogenase. Even though, reductions in wooden breast severity was observed in the present experiment, no effects on serum concentration of creatine kinase and lactic acid dehydrogenase were detected due to GAA supplementation or poultry by-products inclusion.

Bories *et al.*, (2009) reported that improvements in broiler live performance due to GAA supplementation can be observed up to 0.12% level of inclusion in the diet. However, some results also suggest that the usage of levels of more than 0.15% of addition in the diet decreased BW gain (Bories *et al.*, 2009). These researchers concluded also that the increase in the mean corpuscular volume of erythrocytes (MCV) observed in levels of inclusion higher than 0.15% were considered as a sign of deficiency of vitamin B_{12}, and folic acid

and/or methyl donors (except methionine). They suggested that the high requirement for methyl groups and methyl transferases by endogenous creatine synthesis from GAA may have created this deficit. Considering that the formation of GAA requires methyl groups (from methionine, vitamins, etc.) to form creatine, and that the production of creatine and phospho-creatine in the muscle for growth is high-demanded in energy (Michiels *et al.*, 2012), this could explain the depression in growth. Moreover, in our study since serum homocysteine levels were not affected, we can assume that this was not the case and also explain the improvement on live performance due to GAA inclusion.

This study was designed to evaluate the efficacy of GAA to improve live performance, meat quality, and pectoral myopathies in diets with or without poultry by-products. Even though this study showed generally no effects on blood serum parameters, this research provides evidence that dietary GAA supplementation improved live performance, being an efficient nutritional alternative in a market where animal by-products inclusion in the diet are being reduced or banned without affecting carcass and cut ups yields while maintaining the fast-growing characteristic gained from genetic selection in the past decades. Also, the alleviation of wooden breast severity could be associated with the capacity of GAA to prevent glycogen depletion in the muscle by improving cell energy and metabolic intermediate products management, consequently decreasing the chances of membrane dysfunction and cellular damage.

3.5. CONCLUSION

In conclusion, addition of GAA improved broiler BW, BW gain, and FCR in corn diets regardless the inclusion of poultry by-products in the diets after 35 days of age and in the whole period from 0 to 55 d of age. An improvement in breast meat and carcass yield was observed when adding GAA mainly in diets with poultry by-product inclusion. The GAA reduced the incidence of moderate to severe wooden breast, suggesting a possible beneficial effect on preventing muscle damage. No significant effects of GAA were observed on blood hematology or chemistry except for an increment on serum GAA and creatine concentrations.

3.6. REFERENCES

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Table III.1. Ingredient composition of starter, grower, finisher and withdrawal basal diets for males Ross 708 broilers.

Ingredient	Starter		Grower		Finisher		Withdrawal	
	PBP	No PBP	PBP	No PBP	PBP	No PBP	PBP	No PBP
	----------%----------							
Corn	57.683	52.509	60.303	55.030	64.264	58.992	69.093	63.821
Soybean meal, 46%	25.899	33.973	23.114	31.316	19.219	27.421	15.047	23.249
Poultry By Products (PBP)	5.000	-	5.000	-	5.000	-	5.000	-
DDGS	5.000	5.000	5.000	5.048	5.000	5.000	5.000	5.000
Poultry fat	2.724	4.302	3.451	5.000	3.790	5.386	3.305	4.902
Limestone fine	1.212	1.366	1.013	1.167	0.942	1.096	0.955	1.109
Dicalcium phosphate, 18.5%	0.776	1.151	0.527	0.901	0.346	0.719	0.053	0.427
Salt (NaCl)	0.243	0.303	0.252	0.315	0.188	0.251	0.181	0.244
DL- Methionine, 99%	0.276	0.286	0.251	0.243	0.204	0.196	0.213	0.205
L-Lysine-HCl, 78.8%	0.270	0.218	0.236	0.170	0.183	0.118	0.264	0.198
Mineral premix[2]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Sodium bicarbonate	0.195	0.183	0.184	0.167	0.272	0.255	0.263	0.246
Choline chloride, 60%	0.180	0.180	0.180	0.180	0.180	0.180	0.180	0.180
Vitamin premix[3]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
L-Threonine, 98%	0.088	0.081	0.070	0.045	0.044	0.020	0.077	0.053
None or GAA	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Coccidiostat[1]	0.050	0.050	0.050	0.050	-	-	-	-
L-Valine, 96.5%	0.036	0.031	0.001	0.001	0.001	-	-	-
Phytase[4]	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Total	1000.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] Coban® 90 (Monensin), Elanco Animal Health, Greenfield, IN, at 500 g/ton in the starter and grower diets.

[2] Trace minerals provided per kg of premix: manganese (Mn SO_4), 60 g; zinc ($ZnSO_4$), 60 g; iron (FeSO4), 40 g; copper ($CuSO_4$), 5 g; iodine ($Ca(IO_3)_2$),1.25 g.

[3] Vitamins provided per kg of premix: vitamin A, 13,227,513 IU; vitamin D3, 3,968,253 IU; vitamin E, 66,137 IU; vitamin B12, 39.6 mg; riboflavin, 13,227 mg; niacin, 110,229 mg; d-pantothenic acid, 22,045 mg; menadione, 3,968 mg; folic acid, 2,204 mg; vitamin B6, 7,936 mg; thiamine, 3,968 mg; biotin, 253.5 mg.

[4] Quantum Blue 5G® at 0.176 lbs/ton (80 g/ton) to provide 500 FYT (AB Vista) delivering 0.13% of available P, 0.06% of calcium and 0.03% of sodium.

[5] CreAMINO: Guanidinoacetic acid (GAA) with 96% of concentration, Lot numbers: 3/29/16, 9/26/16.

Table III.2. Calculated nutrient content of starter, grower, finisher, and withdrawal basal diets for males Ross 708 broilers.

Nutrient	Starter		Grower		Finisher		Withdrawal	
	PBP	No PBP	PBP	No PBP	PBP	No PBP	PBP	No PBP
Metabolizable Energy, kcal/kg	3000.00	3000.00	3085.00	3085.00	3150.00	3150.00	3175.00	3175.00
Crude protein, %	22.109	22.109	20.950	20.950	19.307	19.307	17.800	17.800
Calcium, %	1.020	1.020	0.900	0.900	0.820	0.820	0.760	0.760
Total phosphorus, %	0.610	0.610	0.507	0.555	0.458	0.510	0.392	0.446
Available phosphorus, %	0.500	0.500	0.450	0.450	0.410	0.410	0.350	0.350
Total Glycine, %	0.792	0.908	0.718	0.865	0.653	0.800	0.585	0.733
Digestible lysine, %	1.220	1.220	1.080	1.080	0.950	0.950	0.920	0.920
Digestible methionine, %	0.595	0.589	0.555	0.534	0.493	0.472	0.485	0.465
Digestible total sulfur amino acids, %	0.890	0.890	0.810	0.810	0.732	0.732	0.708	0.708
Digestible threonine, %	0.780	0.780	0.702	0.702	0.627	0.627	0.607	0.607
Digestible tryptophan, %	0.218	0.232	0.201	0.219	0.181	0.199	0.159	0.178
Digestible valine, %	0.970	0.970	0.863	0.892	0.801	0.829	0.737	0.766
Digestible arginine, %	1.317	1.354	1.188	1.249	1.081	1.141	0.967	1.028
Sodium, %	0.201	0.200	0.200	0.200	0.200	0.200	0.195	0.195
Potassium, %	0.838	0.935	0.767	0.891	0.700	0.824	0.631	0.754
Chloride, %	0.280	0.280	0.280	0.280	0.236	0.236	0.245	0.245
Dietary electrolyte balance, mEq/100 g	241.29	263.916	221.732	250.728	214.741	243.740	195.743	224.740

Table III.3. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on live performance at 14 d

PBP	Additive	BW		BWG	Feed intake	FCR	FCR adj*
		1d	14d	0 – 14d	0 – 14d	0 – 14d	0 – 14d
		---------- (g) ----------				-------- (g:g) ---------	
No PBP		46.1	509[a]	463[a]	555	1.199	1.195
PBP		46.0	502[b]	456[b]	550	1.206	1.199
SEM		0.07	2	2	2	0.005	0.004
	GAA	46.0	505	459	551	1.199	1.195
	None	46.0	506	460	554	1.205	1.200
	SEM	0.07	2	2	2	0.05	0.04
No PBP	GAA	46.1	506	460	551	1.197	1.197
	None	46.1	511	465	559	1.201	1.194
PBP	GAA	46.0	504	458	552	1.207	1.193
	None	46.0	500	454	549	1.210	1.206
	SEM	0.09	3	3	3	0.006	0.005
CV%		0.61	2.42	2.65	2.19	1.75	1.34
Source of variation		---------- *P* – values ----------					
Poultry By-Products		0.1517	0.0200	0.0218	0.1745	0.1766	0.3289
GAA		0.7724	0.8546	0.8382	0.4660	0.2507	0.2254
PBP*GAA		0.8066	0.1576	0.1453	0.0665	0.7109	0.0682

Values are means ± SEM of 16 pens per treatment combination with 20 male broiler chickens per pen

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

*Adjusted value with body weight of mortality for this period

Table III.4. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on live performance at 35 d

		BW	BWG		Feed intake		FCR		FCR adj*	
PBP	Additive	35d	14 -35d	0 – 35d	14-35d	0 – 35d	14-35d	0 – 35d	14-35d	0 - 35d
		---------- (g) ----------					---------- (g:g) ----------			
No PBP		2,673[a]	2,211	2,619	3,120	3,675	1.419	1.404	1.439	1.396
PBP		2,650[b]	2,195	2,605	3,132	3,683	1.427	1.414	1.446	1.403
SEM		11	1.12	8	16	16.43	0.005	0.004	0.004	0.003
	GAA	2,665	2,206	2,617	3,131	3,684	1.420	1.407	1.439	1.396
	None	2,659	2,200	2,608	3,121	3,674	1.425	1.411	1.446	1.403
	SEM	11	1.12	8	16	16.42	0.005	0.004	0.004	0.003
No PBP	GAA	2,679	2,219	2.627	3,134	3,686	1.414	1.399	1.436	1.394
	None	2,668	2,203	2.612	3,106	3,664	1.423	1.409	1.442	1.398
PBP	GAA	2,651	2,192	2,606	3,129	3,683	1.427	1.414	1.442	1.398
	None	2,650	2,198	2,605	3,135	3,683	1.426	1.414	1.450	1.408
	SEM	13	13	10	21	21	0.008	0.007	0.006	0.005
CV%		1.57	1.64	1.37	2.27	1.99	2.32	1.97	1.65	1.46
Source of variation		---------- *P* – values ----------								
Poultry by-Products		0.0363	0.0921	0.1266	0.5133	0.6820	0.3515	0.1631	0.2202	0.1837
GAA		0.5767	0.5555	0.3857	0.5533	0.5702	0.5942	0.5274	0.2633	0.1801
PBP*GAA		0.6199	0.2174	0.4640	0.3568	0.5702	0.5413	0.4715	0.8935	0.5714

Values are means ± SEM of 16 pens per treatment combination with 20 male broiler chickens per pen

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

*Adjusted value with body weight of mortality for this period

Table III.5. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on live performance at 48 d

		BW	BWG		Feed intake		FCR		FCR adj*	
PBP	Additive	48d	35 -48d	0 – 48d	35-48d	0 – 48d	35-48d	0 – 48d	35-48d	0 - 48d
		---------- (g) ----------					---------- (g:g) ----------			
No PBP		4,278[a]	1,606	4,237[a]	2,950	6,625	1.834[b]	1.569[b]	1.818[b]	1.552[b]
PBP		4,237[b]	1,587	4,200[b]	2,978	6,662	1.873[a]	1.591[a]	1.849[a]	1.565[a]
SEM		20	17	16	17	27	0.014	0.005	0.011	0.003
	GAA	4,272	1,606	4,235[a]	2,949	6,634	1.839[b]	1.572[b]	1.812[b]	1.551[b]
	None	4,243	1,587	4,200[b]	2,979	6,653	1.869[a]	1.587[a]	1.854[a]	1.566[a]
	SEM	20	17	20	17	27	0.014	0.005	0.011	0.003
No PBP	GAA	4,287	1,606	4,248	2,919	6,605	1.819	1.559	1.806	1.548
	None	4,270	1,605	4,227	2,981	6,646	1.849	1.579	1.829	1.557
PBP	GAA	4,257	1,605	4,222	2,979	6,663	1.858	1.586	1.819	1.554
	None	4,217	1,568	4,173	2,978	6,661	1.888	1.596	1.880	1.576
	SEM	23	20	23	23	35	0.017	0.006	0.013	0.005
CV%		1.48	3.57	1.48	2.82	1.81	2.82	1.54	2.12	1.25
Source of variation		---------- *P* – values ----------								
Poultry By-Products		0.0119	0.1985	0.0091	0.1887	0.2339	0.0053	0.0007	0.0023	0.0130
GAA		0.0807	0.1888	0.0214	0.1642	0.5295	0.0315	0.0172	<.0001	0.0031
PBP*GAA		0.4724	0.2186	0.3505	0.1466	0.4879	0.9877	0.4158	0.0672	0.2029

Values are means ± SEM of 16 pens per treatment combination with 20 male broiler chickens per pen

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

*Adjusted value with body weight of mortality for this period

Table III.6. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on live performance at 55 d

PBP	Additive	BW	BWG		Feed intake		FCR		FCR adj*	
		55d	48 -55d	0 – 55d	48 -55d	0 – 55d	48 -55d	0 – 55d	48 -55d	0 – 55d
		---------- (g) ----------					---------- (g:g) ----------			
No PBP		5,058[a]	779	5,012[a]	1,738	8,362	2.241	1.669[b]	2.273	1.650[b]
PBP		5,014[b]	776	4,966[b]	1,767	8,429	2.276	1.695[a]	2.337	1.673[a]
SEM		33	25	33	37	52.27	0.028	0.004	0.036	0.003
	GAA	5,056	784	5,012[a]	1,740	8,373	2.210[b]	1.670[b]	2.266[b]	1.652[b]
	None	5,016	771	4,966[b]	1,766	8,418	2.308[a]	1.694[a]	2.344[a]	1.671[a]
	SEM	33	25	33	37	52.25	0.028	0.004	0.036	0.003
No PBP	GAA	5,076	791	5,031	1,731	8,335	2.192	1.658	2.209	1.643
	None	5,041	767	4,994	1,746	8,389	2.291	1.681	2.338	1.658
PBP	GAA	5,036	777	4,993	1,748	8,411	2.228	1.683	2.323	1.661
	None	4,992	774	4,939	1,786	8,447	2.325	1.708	2.351	1.685
	SEM	36	28	35	41	61	0.04	0.006	0.045	0.005
CV%		1.66	9.57	1.67	5.71	2.03	6.60	1.41	6.42	1.29
Source of variation		---------- *P* – values ----------								
Poultry By-Products		0.0430	0.8548	0.0295	0.2584	0.1268	0.3655	<.0001	0.0954	0.0001
GAA		0.0675	0.4767	0.0340	0.3070	0.3036	0.0132	0.0002	0.0427	0.0009
PBP*GAA		0.8325	0.5675	0.6873	0.6437	0.8380	0.9836	0.9752	0.1832	0.3807

Values are means ± SEM of 16 pens per treatment combination with 20 male broiler chickens per pen

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

*Adjusted value with body weight of mortality for this period

Table III.7. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on flock uniformity, mortality and culls.

		55d		Mortality and culls				
PBP	Additive	Individual BW	Flock Uniformity (CV)	0 – 14d	14 – 35d	35 – 48d	48-55d	Total
		--- (g) ---	--- (%) ---	---------- (%) ----------				
No PBP		5,058[a]	5.05[a]	0.47	0.94[b]	3.44	5.00	9.84
PBP		5,014[b]	5.00[b]	0.94	2.34[a]	4.06	4.69	12.03
SEM		33.10	0.03	0.27	0.38	0.61	0.91	1.08
	GAA	5,056	5.05[a]	0.469	1.88	4.06	3.59[b]	10.00
	None	5,016	5.00[b]	0.938	1.41	3.44	6.09[a]	11.88
	SEM	33.10	0.03	0.27	0.38	0.61	0.91	1.08
No PBP	GAA	5,076	5.07	0.00	0.63	2.81	4.38	7.81
	None	5,041	5.03	0.94	1.25	4.06	5.63	11.88
PBP	GAA	5,036	5.04	0.94	3.13	5.31	2.81	12.19
	None	4,992	4.96	0.94	1.56	2.81	6.56	11.88
	SEM	36.33	0.03	0.41	0.60	0.95	1.24	1.61
CV%		1.66	1.68	251.69[1]	159.64[1]	109.40[1]	97.82[1]	61.71[1]
Source of variation			---------- *P* – values ----------					
Poultry By-Products		0.0430	0.0171	0.2938	0.0360	0.5447	0.7929	0.2001
GAA		0.0675	0.0117	0.2938	0.4770	0.5447	0.0392	0.2712
PBP*GAA		0.8325	0.3781	0.2938	0.1003	0.0728	0.2958	0.2001

Values are means ± SEM of 16 pens per treatment combination with 20 male broiler chickens per pen

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

[1]Very high variability in mortality data since several pens did not have any (0) mortality. These are percentages of 20 broilers per pen (2/20 = 10%; 3/20= 15% mortality). Main causes of mortality and culls were ascites and leg problems.

Table III.8. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on carcass, breast meat, and cut up yields at 56 d

PBP	Additive	Carcass yield	Cut – up parts relative to carcass weight					
			Wings	Leg Quarters	*Pectoralis major*	*Pectoralis minor*	Breast meat	Rack
		%						
No PBP		78.72	9.41	29.83	32.14	6.29	38.39	22.41
PBP		78.63	9.26	30.21	32.04	6.38	38.39	22.30
SEM		0.14	0.05	0.32	0.21	0.06	0.21	0.11
	GAA	78.62	9.34	29.92	32.13	6.36	38.44	22.43
	None	78.72	9.33	30.11	32.05	6.32	38.34	22.28
	SEM	0.14	0.05	0.32	0.21	0.06	0.21	0.11
No PBP	GAA	78.53	9.39	29.92	31.92^{ab}	6.29	38.20^{ab}	22.32
	None	78.72	9.43	29.73	32.36^{a}	6.30	38.59^{a}	22.27
PBP	GAA	78.73	9.30	29.92	32.35^{a}	6.43	38.68^{a}	22.55
	None	78.72	9.22	30.49	31.74^{b}	6.34	38.09^{b}	22.28
	SEM	0.18	0.08	0.35	0.26	0.07	0.27	0.18
CV%		5.58	6.42	4.39	5.45	7.21	4.88	6.28
Source of variation		*P* – values						
Poultry By-Products		0.5449	0.0847	0.0690	0.6232	0.1861	0.9680	0.5739
GAA		0.5115	0.8884	0.3662	0.6867	0.5501	0.6635	0.4367
PBP*GAA		0.5533	0.4761	0.0704	0.0133	0.4169	0.0343	0.6016

Values are means ± SEM of 16 pens per treatment combination with 4 male broiler chickens per pen selected for processing

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

Table III.9. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on carcass, breast meat, and cut up weights at 56 d

PBP	Additive	Live weight	Carcass yield	Cut – up parts weight					
				Wings	Leg quarters	*Pectoralis major*	*Pectoralis minor*	Breast meat	Rack
		---------- g ----------							
No PBP		4,975	3,915	367[a]	1,165	1,261	245.89	1,506	876
PBP		4,948	3,902	359[b]	1,172	1,241	248.12	1,487	866
SEM		28	27	3	11	15	1.85	16	7
	GAA	5,006[a]	3,950[a]	366	1,174	1,263[a]	250.07[a]	1,511[a]	880
	None	4,917[b]	3,867[b]	360	1,162	1,238[b]	243.94[b]	1,481[b]	862
	SEM	28	27	3	11	15	1.85	16	8
No PBP	GAA	5,016	3,968	370	1,180	1,267	248.53	1,516	890
	None	4,934	3,862	364	1,149	1,254	243.25	1,495	861
PBP	GAA	4,995	3,932	362	1,168	1,259	251.61	1,507	870
	None	4,900	3,873	356	1,175	1,222	244.62	1,467	863
	SEM	36	33	4	13.02	18	2.98	19	13
CV%		5.66	5.46	7.78	7.39	8.36	8.84	8.06	8.13
Source of variation		---------- *P* – values ----------							
Poultry By-Products		0.3940	0.6666	0.0443	0.5520	0.1074	0.4408	0.1730	0.4799
GAA		0.0094	0.0069	0.1324	0.3063	0.0443	0.0384	0.0309	0.2127
PBP*GAA		0.8455	0.4239	0.9794	0.1027	0.3323	0.7655	0.4807	0.4854

Values are means ± SEM of 16 pens per treatment combination with 4 male broiler chickens per pen selected for processing

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

Table III.10. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products on *Pectoralis major*'s post-mortem pH (6 and 24 h), drip and cook loss, shear force, and color at 56 d for Ross 708 broilers.

PBP	Additive	pH post – processing		Cook loss	Drip loss	Shear force[1]	L*	a*	b*
		6 h	24 h						
		---------- pH --------		---------- % -------		-----kg----			
No PBP		6.01	5.99	19.30[a]	1.08	4.40	55.59	6.11	9.27[b]
PBP		6.00	6.01	17.82[b]	1.14	4.38	55.37	6.10	10.06[a]
SEM		0.02	0.02	0.42	0.19	0.12	0.50	0.43	0.33
	GAA	6.02	6.00	18.76	1.19	4.42	55.58	6.18	9.66
	None	5.99	6.00	18.36	1.04	4.37	55.38	6.04	9.68
	SEM	0.02	0.02	0.42	0.19	0.12	0.50	0.43	0.33
No PBP	GAA	6.02	5.98	18.65[ab]	1.17	4.36	55.70	6.20	9.13
	None	6.00	6.00	19.95[a]	1.00	4.44	55.49	6.02	9.41
PBP	GAA	6.02	6.03	18.87[ab]	1.20	4.47	55.46	6.16	10.19
	None	5.98	5.99	16.76[b]	1.08	4.30	55.27	6.05	9.94
	SEM	0.03	0.03	0.62	0.21	0.16	0.60	0.48	0.42
CV%		3.65	2.63	23.41	61.54	27.69	5.54	33.57	30.45
Source of variation		----------		---------- *P* – values ----------					
Poultry By-Products		0.6876	0.3076	0.0271	0.6736	0.9512	0.6282	0.9778	0.0396
GAA		0.2746	0.7730	0.5401	0.2994	0.7566	0.6804	0.6500	0.9627
PBP*GAA		0.6822	0.1693	0.0118	0.8900	0.4351	0.9791	0.9195	0.4851

Values are means ± SEM of 16 pens per treatment combination with 4 male broiler chickens per pen selected for processing

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test. [1] Warner-Bratzler shear device

Table III.11. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products on overall pectoral myopathies average scores for Ross 708 broilers at 56 d.

PBP	Additive	Sensorial Scores		Histopathology[1]
		White striping 1-3	Wooden Breast 1-4	Wooden Breast 1-4
No PBP		1.86	2.60	2.74
PBP		1.98	2.49	2.50
SEM		0.13	0.07	0.13
	GAA	1.94	2.49	2.69
	None	1.90	2.59	2.55
	SEM	0.13	0.07	0.13
No PBP	GAA	1.90	2.47	2.85
	None	1.81	2.72	2.64
PBP	GAA	1.97	2.51	2.53
	None	1.99	2.47	2.46
	SEM	0.15	0.09	0.20
CV%		32.68	25.13	33.85
Source of variation		---------- *P* – values ----------		
Poultry By-Products		0.2402	0.2565	0.2403
GAA		0.6940	0.2624	0.5001
PBP*GAA		0.5975	0.1166	0.7419

Values are means ± SEM of 16 pens per treatment combination with 4 male broiler chicken per pen selected

[1]Values are means of 16 pens per treatment combination with 1 male broiler chickens per pen selected

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test

Table III.12 Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products on severity probability distribution scores of pectoral myopathies for Ross 708 broilers at 56 d

PBP	Additive	Wooden Breast				White Striping		
		1	2	3	4	1	2	3
		---------- (0.0 – 1.0) ----------						
No PBP		0.03	0.39	0.52	0.06	0.29	0.57	0.14
PBP		0.04	0.46	0.47	0.03	0.26	0.49	0.24
SEM		0.02	0.05	0.06	0.02	0.08	0.06	0.06
	GAA	0.04	0.46	0.45	0.04	0.27	0.52	0.21
	None	0.03	0.38	0.54	0.05	0.28	0.55	0.17
	SEM	0.02	0.05	0.06	0.02	0.08	0.06	0.06
No PBP	GAA	0.02	0.53^{a}	0.41	0.04	0.27	0.56	0.17
	None	0.05	0.26^{b}	0.63	0.07	0.30	0.59	0.11
PBP	GAA	0.06	0.40^{ab}	0.49	0.04	0.28	0.48	0.25
	None	0.02	0.51^{a}	0.44	0.02	0.25	0.51	0.24
	SEM	0.03	0.08	0.08	0.03	0.10	0.08	0.08
Source of variation		---------- *P* – values ----------						
Poultry By-Products		0.7482	0.4044	0.5339	0.4179	0.7760	0.3216	0.0970
GAA		0.7843	0.3290	0.2990	0.8672	0.9626	0.7369	0.5712
PBP*GAA		0.2328	0.0183	0.1036	0.3526	0.6768	0.9808	0.6893

[a-b] Means followed by different superscripts within a column are significantly different ($P < 0.05$)

Each value represents the probability (0-1) of developing each severity score according to main factors or factorial arrangement of treatments, $n = 16$; based on a 4-point scale (4 = severe, 3 = medium, 2 = low, 1 = normal).

Table III.13. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products on pectoral myopathies incidence of Ross 708 broilers at 56 d

PBP	Additive	Wooden breast incidence		White Striping	
		None to mild*	Severe**	None to Mild*	Severe **
		----------(0.0 – 1.0) ----------			
No PBP		0.42	0.58	0.29	0.71
PBP		0.49	0.51	0.26	0.74
SEM		0.05	0.05	0.08	0.08
	GAA	0.50	0.50	0.27	0.73
	None	0.42	0.58	0.28	0.72
	SEM	0.05	0.05	0.08	0.08
No PBP	GAA	0.54^{a}	0.46	0.27	0.73
	None	0.31^{b}	0.69	0.30	0.70
PBP	GAA	0.45^{ab}	0.55	0.28	0.72
	None	0.53^{ab}	0.47	0.25	0.75
	SEM	0.07	0.07	0.10	0.10
Source of variation		---------- *P* – values ----------			
Poultry By-Products		0.4122	0.4122	0.7760	0.7760
GAA		0.3572	0.3572	0.9626	0.9626
PBP*GAA		0.0548	0.0548	0.6768	0.6768

Values are means of 16 pens per treatment combination with 4 male broiler chickens selected for processing

[a-b] Means followed by different superscripts within a column are significantly different ($P < 0.05$)

[1] Each value represent the probability (0-1) of developing each severity score according to main factors or factorial arrangement of treatments grouped by scores 1 and 2 (None to mild*); and 3 and 4 (Severe*).

Table III.14. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on serum blood parameters at 55 d

PBP	Additive	Blood metabolites		Blood minerals				
		Glucose	Uric Acid	P	Ca	Na	K	Cl
		---------- (mg/dl) ----------					------- (mmol/L) ------	
No PBP		273.09	4.92	7.16	11.16	156.86	5.63	111.04
PBP		263.27	4.83	7.04	11.09	155.85	5.87	109.60
SEM		3.35	0.28	0.08	0.14	0.60	0.12	0.70
	GAA	267.78	5.05	7.14	11.15	156.01	5.69	110.07
	None	268.59	4.70	7.07	11.10	156.69	5.82	110.57
	SEM	3.35	0.28	0.08	0.14	0.60	0.12	0.70
	GAA	274.37	4.95	7.26	11.22	156.47	5.49	110.64
No PBP	None	271.81	4.90	7.07	11.10	157.25	5.78	111.44
	GAA	261.19	5.16	7.01	11.08	155.56	5.89	109.50
PBP	None	265.36	4.50	7.07	11.10	156.13	5.86	109.70
	SEM	4.91	0.39	0.12	0.18	0.84	0.17	0.90
CV%		7.40	29.96	6.46	4.90	2.12	11.94	2.86
Source of variation		---------- *P* – values ----------						
Poultry By-Products		0.0595	0.7949	0.3228	0.6029	0.2366	0.1845	0.0790
GAA		0.8744	0.3519	0.5911	0.7285	0.4263	0.4935	0.5351
PBP*GAA		0.5116	0.4227	0.2977	0.6221	0.9007	0.3702	0.7100

Values are means of 16 pens per treatment combination with 1 male broiler chicken per pen selected for blood collection

[a-b] Means followed by different superscripts within a column are significantly different ($P < 0.05$)

Table III.15. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on serum blood parameters and liver enzymes at 55 d

PBP	Additive	Blood enzymes						Proteins	
		Cholesterol	ALT	AST	GGT	LD	CK	Albumin	Protein
		(mg/dl)	----------(IU/L) ----------					-----(g/dl) -----	
No PBP		133.15[b]	6.53	651.96	13.57	2,562	64,885	1.39	3.10
PBP		140.34[a]	5.88	570.16	14.54	2,263	55,878	1.45	3.09
SEM		2.33	0.49	50.23	0.52	456	10,360	0.04	0.07
	GAA	136.05	6.43	639.38	13.19	2,432	63,536	1.43	3.13
	None	137.44	5.98	582.73	14.92	2,393	57,227	1.41	3.05
	SEM	2.33	0.49	50.23	0.52	456	10,360	0.04	0.07
No PBP	GAA	131.67	6.92	688.82	12.14	2,599	70,443	1.40	3.14
	None	134.63	6.14	615.09	15.00	2,525	59,326	1.39	3.05
PBP	GAA	140.44	5.94	589.94	14.25	2,265	56.628	1.47	3.13
	None	140.25	5.81	550.38	14.84	2,261	55,127	1.43	3.04
	SEM	3.44	0.57	66.05	0.83	554	11,976	0.05	0.08
CV%		10.41	25.89	40.13	25.93	72.50	55.62	10.36	8.99
Source of variation		---------- *P* – values ----------							
Poultry By-Products		0.0501	0.1152	0.1907	0.2996	0.5049	0.2940	0.1381	0.8911
GAA		0.7018	0.2746	0.3629	0.0693	0.9307	0.4611	0.5706	0.2521
PBP*GAA		0.6638	0.4283	0.7830	0.2269	0.9383	0.5740	0.6705	0.9997

Values are means of 16 pens per treatment combination with 1 male broiler chicken per pen selected for blood collection

[a-b] Means followed by different superscripts within a column are significantly different ($P < 0.05$)

ALT (alanine aminotransferase), AST (aspartate aminotransferase), GGT (gamma-glutamyl transpeptidase), LD (lactic acid dehydrogenase), CK (creatine kinase)

Table III.16. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on white blood cells count at 55 d

PBP	Additive	Blood leukocytes				Lymphocytes	
		Thrombocytes	Heterophils	Monocytes	Lymphocytes	T-cells	B-cells
		---------- (cells/µl of blood) ----------					
No PBP		40,861	11,841	949	16,424	14,198	2,193
PBP		38,988	10,552	724	15,593	13,363	2,215
SEM		6,517	1,799	222	1,672	1,468	304
	GAA	33,980	10,550	624	15,067	12,928	2,083
	None	45,869	11,843	1,050	16,950	14,633	2,325
	SEM	6,242	1,833	231	1,704	1,495	310
No PBP	GAA	36,914	11,321	583	16,455	14,045	2,333
	None	44,807	12,360	1,314	16,394	14,351	2,054
PBP	GAA	31,047	9,779	664	13,680	11,811	1,834
	None	46,930	11,326	785	17,507	14,914	2,596
	SEM	9,099	2,449	307	2,272	1,975	405
CV%		37.64	51.32	79.74	33.69	33,41	42.77
Source of variation		---------- *P* – values ----------					
Poultry By-Products		0.8575	0.5834	0.4377	0.7018	0.6536	0.9535
GAA		0.2642	0.5980	0.1813	0.4088	0.3835	0.5393
PBP*GAA		0.6913	0.9172	0.3320	0.3930	0.4729	0.1919

Values are means of 16 pens per treatment combination with 1 male broiler chicken per pen selected for blood collection

[a-b] Means followed by different superscripts within a column are significantly different ($P < 0.05$)

Table III.17. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on red blood cell count and chemical chemistry at 55 d

PBP	Additive	Hematological parameters					
		Erythrocytes[1]	Hemoglobin[1]	Hematocrit[2]	MCV[1]	HBE (MCH)[2]	MCHC[2]
		(T/l)	(g/l)	(%)	Fl	pg	g/dl
No PBP		2.38	98.27	0.28	105.67	41.58	35.88
PBP		2.50	102.22	0.29	104.84	40.77	35.04
SEM		0.04	4.71	0.01	0.49	1.43	1.28
	GAA	2.43	100.11	0.28	105.36	41.16	35.50
	None	2.44	100.37	0.28	105.15	41.19	35.43
	SEM	0.04	4.71	0.01	0.48	1.43	1.28
No PBP	GAA	2.39	96.65	0.27	104.79^{ab}	40.56	35.38
	None	2.36	99.89	0.28	106.54^{a}	42.59	36.39
PBP	GAA	2.47	103.58	0.29	105.92^{ab}	41.57	35.62
	None	2.52	100.86	0.29	103.75^{b}	39.79	34.47
	SEM	0.06	5.63	0.01	0.82	1.77	1.59
CV%		9.37	15.82	10.16	3.17	13.05	13,70
Source of variation			---------- *P* – values ----------				
Poultry By-Products		0.0575	0.3680	0.0633	0.3740	0.5863	0.5310
GAA		0.8936	0.9527	0.8232	0.8199	0.9810	0.9593
PBP*GAA		0.4965	0.5001	0.9687	0.0433	0.1864	0.4284

Values are means of 16 pens per treatment combination with 1 male broiler chicken per pen selected for blood collection

[a-b] Means followed by different superscripts within a column are significantly different ($P < 0.05$)

MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration)

[1] Flow cytometry method; [2] Results calculated

Table III.18. Effect of supplementation of guanidinoacetic acid (GAA) in diets with or without poultry by-products for Ross 708 broilers on serum GAA metabolites at 55 d

PBP	Additive	Plasma metabolites			
		Creatinine	Homocysteine	Creatine	GAA
		--(µg/ml) --	----------(µM) ----------		
No PBP		0.36[b]	15.14	50.12	3.34[b]
PBP		0.79[a]	16.59	57.21	4.53[a]
SEM		0.05	1.01	5.06	0.33
	GAA	0.65	16.65	65.87[a]	7.09[a]
	None	0.50	15.07	41.46[b]	0.77[b]
	SEM	0.05	1.01	5.06	0.33
No PBP	GAA	0.36	16.14	58.25	6.01[b]
	None	0.37	14.13	41.98	0.66[c]
PBP	GAA	0.95	17.17	73.48	8.18[a]
	None	0.64	16.01	40.94	0.88[c]
	SEM	0.08	1.46	6.65	0.45
CV%		60.35	37.54	45.05	43.19
Source of variation		---------- *P* – values ----------			
Poultry By-Products		<.0001	0.3372	0.2503	0.0069
GAA		0.0966	0.2973	0.0002	<.0001
PBP*GAA		0.0717	0.7785	0.1885	0.0248

Values are means of 16 pens per treatment combination with 1 male broiler chicken per pen selected for blood collection
[a-c] Means followed by different superscripts within a column are significantly different ($P < 0.05$)

CHAPTER IV

FINAL CONCLUSIONS

Live performance and carcass and cut up yields

The results obtained in both experiments supports previous findings indicating that the supplementation with guanidinoacetic acid improved live performance at a level of 0.06%, regardless of the inclusion of poultry by-products and in diets where corn has been replaced up to 100% by sorghum. Overall, cut up yields were not improved by the supplementation with GAA. However, breast meat yield was improved in corn-based diets and in diets with poultry by product inclusion.

Meat quality

Drip loss and shear force for both experiments conducted showed no effects due to the inclusion of guanidinoacetic acid. In contrast, ultimate pH was lowered due to the inclusion of this feed additive especially when broilers consumed all vegetable-based diets.

Pectoral myopathies

Spaghetti muscle and white striping incidence and severity were not affected either by GAA supplementation, type of grain, or inclusion of poultry by-products in the diet. However, wooden breast myopathy severity was reduced due to the inclusion of GAA. This response

was observed mainly due to the reduction on the probability of observing medium and severe wooden breast.

Blood hematology, clinical chemistry, and GAA metabolites

Significant effects of GAA supplementation were only observed on serum GAA concentration and creatine. Even though, no other significant responses were observed on blood hematology and chemistry, a response close to be significant in GGT (gamma-glutamyl transpeptidase), may partially explain the reduction response observed on wooden breast severity.

The use of feed additives with metabolites directly involved in muscle development, growth, and metabolism could be used not only as live performance enhancers due to its sparing effects on nutrients, but also to prevent or reduce the wooden breast severity by improving the management of nutrients involved in these processes.

APPENDICES

Appendix A. Nutrient and amino acid composition of feed ingredients of the first experiment

Nutrient Name	Corn	Sorghum	DDGs	Soybean meal
Dry matter, %	87.17	88.53	90.86	88.53
Metabolizable energy[1], kcal/kg	3,250.00	3,243.00	2,506.00	2,253.00
Crude protein, %	7.97	9.12	24.42	47.84
Total amino acids[2]				
Methionine, %	0.182	0.178	0.471	0.643
Cysteine, %	0.169	0.173	0.459	0.742
TSAA, %	0.351	0.351	0.930	1.385
Lysine, %	0.262	0.222	0.803	2.915
Threonine, %	0.299	0.314	0.932	1.847
Arginine, %	0.416	0.348	1.181	3.504
Isoleucine, %	0.281	0.382	0.894	2.167
Leucine, %	0.954	1.245	2.748	3.652
Valine, %	0.386	0.470	1.207	2.271
Histidine, %	0.229	0.204	0.667	1.238
Phenylalanine, %	0.400	0.521	1.197	2.482
Glycine, %	0.315	0.315	1.003	2.005
Serine, %	0.374	0.430	1.191	2.405
Proline, %	0.699	0.769	1.912	2.382
Alanine, %	0.581	0.842	1.748	2.080
Aspartic acid, %	0.527	0.640	1.645	5.351
Glutamic acid, %	1.457	1.891	4.128	8.493
Digestible amino acids[2]				
Methionine, %	0.171	0.158	0.405	0.585
Cysteine, %	0.147	0.137	0.353	0.608
SAA, %	0.318	0.295	0.763	1.191
Lysine, %	0.241	0.200	0.602	2.624
Threonine, %	0.254	0.261	0.671	1.570
Arginine, %	0.387	0.306	0.862	3.259
Isoleucine, %	0.267	0.344	0.751	1.929
Leucine, %	1.096	1.096	2.446	3.250
Valine, %	0.405	0.409	0.978	1.998
Histidine, %	0.171	0.171	0.534	1.139
Phenylalanine, %	0.464	0.464	1.053	2.209

[1]Values obtained from *in vivo* trial in roosters at the University of Georgia (Dr. Nick Dale).
[2]Analyzed by Evonik Degussa GmbH, Hanau-Wolfgang, Germany

Appendix B. Results of corn or sorghum-based diets with or without guanidinoacetic acid (GAA) supplementation

Grain	Additive	Feed Form	Creatine	Guanidino acetic acid[1]	CreAMINO
Starter				----------(mg/kg, as is) ----------	
Corn	Sand	Mash	<20	11	11
Corn	GAA	Mash	<20	489	509
Corn	Sand	Pellet/Crumble	<20	27	28
Corn	GAA	Pellet/Crumble	<20	504	525
Sorghum	Sand	Mash	<20	<1	<1
Sorghum	GAA	Mash	<20	693	722
Sorghum	Sand	Pellet/Crumble	<20	1	1
Sorghum	GAA	Pellet/Crumble	<20	464	483
Grower					
Corn	Sand	Mash	<20	2	2
Corn	GAA	Mash	<20	633	659
Corn	Sand	Pellet	<20	14	15
Corn	GAA	Pellet	<20	594	619
Sorghum	Sand	Mash	<20	3	3
Sorghum	GAA	Mash	<20	656	683
Sorghum	Sand	Pellet	<20	4	4
Sorghum	GAA	Pellet	<20	513	534
Finisher					
Corn	Sand	Mash	<20	<20	<21
Corn	GAA	Mash	<20	541	563
Corn	Sand	Pellet	<20	<20	<21
Corn	GAA	Pellet	<20	528	550
Sorghum	Sand	Mash	<20	<20	<21
Sorghum	GAA	Mash	<20	591	616
Sorghum	Sand	Pellet	<20	<20	<21
Sorghum	GAA	Pellet	<20	474	494
Withdrawal					
Corn	Sand	Mash	<20	<20	<21
Corn	GAA	Mash	<20	697	726
Corn	Sand	Pellet	<20	<20	<21
Corn	GAA	Pellet	<20	526	548
Sorghum	Sand	Mash	<20	<20	<21
Sorghum	GAA	Mash	<20	636	662
Sorghum	Sand	Pellet	<20	<20	<21
Sorghum	GAA	Pellet	<20	406	423

[1]Guanidinoacetic acid (GAA) was administered by adding the feed additive CreAMINO® (>96% GAA; Evonik Degussa GmbH, Hanau-Wolfgang, Germany). [2]CreAMINO®: lot numbers: 3/29/16.

Appendix C. Amino acid content of corn or sorghum-based diets with or without guanidinoacetic acid (GAA) supplementation

	Experimental diets															
	Starter				Grower				Finisher				Withdrawal			
	Corn		Sorghum		Corn		Sorghum		Corn		Sorghum		Corn		Sorghum	
Nutrient Name	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**
Dry matter, %	87.32	87.7	88.23	87.16	88.91	88.63	89.46	89.75	89.97	88.05	88.26	88.30	86.99	87.64	88.20	88.16
Crude protein, %	23.23	23.6	20.57	22.18	22.51	21.43	19.51	21.7	19.32	19.01	18.78	19.33	17.46	17.25	18.56	19.08
Total amino acids																
Methionine, %	0.534	0.546	0.505	0.527	0.506	0.51	0.49	0.527	0.467	0.463	0.472	0.493	0.419	0.441	0.469	0.508
Cystine, %	0.361	0.359	0.312	0.336	0.35	0.337	0.298	0.316	0.322	0.314	0.295	0.312	0.291	0.283	0.287	0.293
Lysine, %	1.368	1.366	1.165	1.294	1.269	1.22	1.117	1.234	1.083	1.044	1.019	1.084	1.001	1.027	1.032	1.055
Threonine, %	0.932	0.933	0.823	0.888	0.863	0.842	0.794	0.851	0.764	0.756	0.741	0.773	0.679	0.689	0.727	0.737
Isoleucine, %	0.986	0.98	0.866	0.941	0.902	0.866	0.801	0.858	0.815	0.782	0.774	0.824	0.698	0.711	0.769	0.773
Valine, %	1.109	1.105	0.993	1.074	1.007	0.968	0.912	0.975	0.917	0.895	0.885	0.960	0.823	0.812	0.907	0.926
Leucine, %	1.982	1.957	1.839	1.935	1.916	1.845	1.771	1.901	1.736	1.672	1.723	1.783	1.518	1.515	1.781	1.785
Arginine, %	1.55	1.517	1.226	1.364	1.442	1.372	1.17	1.297	1.266	1.208	1.106	1.159	1.117	1.089	1.072	1.079
TSAA, %	0.895	0.905	0.817	0.863	0.856	0.847	0.788	0.843	0.789	0.777	0.767	0.805	0.710	0.724	0.756	0.801
Histidine, %	0.599	0.595	0.495	0.539	0.563	0.542	0.47	0.512	0.503	0.485	0.447	0.473	0.469	0.467	0.462	0.472
Phenylalanine, %	1.17	1.146	1.028	1.1	1.103	1.062	0.983	1.074	0.993	0.922	0.968	0.968	0.847	0.844	0.941	0.925
Serine, %	1.14	1.121	0.963	1.047	1.085	1.048	0.941	1.039	0.957	0.913	0.910	0.926	0.852	0.831	0.869	0.875
Proline, %	1.335	1.317	1.185	1.235	1.23	1.205	1.133	1.214	1.166	1.159	1.117	1.175	1.065	1.047	1.155	1.131
Alanine, %	1.158	1.148	1.12	1.17	1.119	1.081	1.08	1.153	1.030	1.013	1.070	1.117	0.894	0.893	1.099	1.113
Aspartic acid, %	2.363	2.333	1.969	2.173	2.177	2.087	1.853	2.047	1.916	1.823	1.785	1.866	1.693	1.669	1.680	1.702
Glutamic acid, %	4.136	4.079	3.663	3.941	3.893	3.732	3.479	3.78	3.450	3.290	3.335	3.445	3.025	2.963	3.327	3.337
Glycine, %	0.957	0.949	0.795	0.869	0.903	0.864	0.757	0.83	0.804	0.768	0.730	0.750	0.723	0.720	0.709	0.721

Appendix C. Continued

Nutrient Name	Experimental diets															
	Starter				Grower				Finisher				Withdrawal			
	Corn		Sorghum		Corn		Sorghum		Corn		Sorghum		Corn		Sorghum	
	None	GAA	None	GAA	None	GAA	None	GAA	None	GAA	None	GAA	None	GAA	None	GAA
Minerals[1]																
Calcium, %	-	-	-	-	0.620	0.620	0.850	0.850	0.770	0.770	0.720	0.720	-	-	-	-
Phosphorous, %	-	-	-	-	0.530	0.530	0.590	0.590	0.590	0.590	0.550	0.550	-	-	-	-
Magnesium, %	-	-	-	-	0.160	0.160	0.190	0.190	0.170	0.170	0.180	0.180	-	-	-	-
Potassium, %	-	-	-	-	0.880	0.880	0.850	0.850	0.960	0.960	0.830	0.830	-	-	-	-
Sodium, %	-	-	-	-	0.142	0.142	0.163	0.163	0.164	0.164	0.157	0.157	-	-	-	-
Chloride Ion, %	-	-	-	-	0.250	0.250	0.280	0.280	0.310	0.310	0.230	0.230	-	-	-	-

[1]Analyzed in samples from basal diets of grower and finisher only

Appendix D. Nutrient and amino acid composition of feed ingredients of the second experiment

Nutrient Name	Corn	DDGs	Soybean meal	Poultry by-products
Dry matter, %	87.17	90.20	88.80	95.61
Metabolizable energy[1], kcal/kg	3,250.00	2,506.00	2,261.90	3,100.00
Crude protein, %	7.97	24.99	47.84	68.39
Total amino acids				
Methionine, %	0.182	0.473	0.644	1.334
Cysteine, %	0.169	0.475	0.676	0.617
TSAA, %	0.351	0.948	1.320	1.951
Lysine, %	0.262	0.746	2.832	4.004
Threonine, %	0.299	0.947	1.863	2.518
Arginine, %	0.416	1.121	3.439	4.501
Isoleucine, %	0.281	0.911	2.189	2.518
Leucine, %	0.954	2.760	3.661	4.595
Valine, %	0.386	1.195	2.281	3.021
Histidine, %	0.229	0.654	1.216	1.485
Phenylalanine, %	0.400	1.177	2.432	2.533
Glycine, %	0.315	1.003	1.995	6.504
Serine, %	0.374	1.218	2.413	2.624
Proline, %	0.699	1.915	2.260	4.004
Alanine, %	0.581	1.763	2.094	4.495
Aspartic acid, %	0.527	1.649	5.426	5.427
Glutamic acid, %	1.457	4.130	8.502	8.607
Digestible amino acids				
Methionine, %	0.171	0.407	0.580	1.027
Cysteine, %	0.147	0.390	0.534	0.346
SAA, %	0.318	0.806	1.109	1.366
Lysine, %	0.241	0.485	2.520	2.843
Threonine, %	0.254	0.682	1.546	1.788
Arginine, %	0.387	0.919	3.164	3.556
Isoleucine, %	0.267	0.729	1.904	1.889
Leucine, %	1.096	2.374	3.222	3.538
Valine, %	0.405	0.932	1.984	2.236
Histidine, %	0.171	0.484	1.094	1.099
Phenylalanine, %	0.464	0.942	2.164	1.950

[1]Values obtained from *in vivo* trial in roosters at the University of Georgia
[2]Analyzed by Evonik

Appendix E. Results of diets containing or not poultry by-products with or without guanidinoacetic acid (GAA) supplementation

PBP	Additive	Feed Form	Creatine	Guanidino acetic acid[1]	CreAMINO
Starter				----------(mg/kg, as is) ----------	
PBP	None	Mash	<20	<20	<21
No PBP	None	Mash	<20	<20	<21
PBP	GAA	Mash	<20	550	573
No PBP	GAA	Mash	<20	599	624
PBP	None	Pellet/Crumbled	<20	33	34
No PBP	None	Pellet/Crumbled	<20	<20	<21
PBP	GAA	Pellet/Crumbled	<20	486	506
No PBP	GAA	Pellet/Crumbled	<20	447	466
Grower					
PBP	None	Mash	<20	<20	<21
No PBP	None	Mash	<20	<20	<21
PBP	GAA	Mash	<20	681	709
No PBP	GAA	Mash	<20	601	626
PBP	None	Pellet	<20	<20	<21
No PBP	None	Pellet	<20	<20	<21
PBP	GAA	Pellet	<20	578	602
No PBP	GAA	Pellet	<20	611	636
Finisher					
PBP	None	Mash	<20	<20	<21
No PBP	None	Mash	<20	<20	<21
PBP	GAA	Mash	<20	593	618
No PBP	GAA	Mash	<20	672	700
PBP	None	Pellet	<20	<20	<21
No PBP	None	Pellet	<20	<20	<21
PBP	GAA	Pellet	<20	532	554
No PBP	GAA	Pellet	<20	534	556
Withdrawal					
PBP	None	Mash	<20	36	38
No PBP	None	Mash	<20	<20	<21
PBP	GAA	Mash	<20	622	648
No PBP	GAA	Mash	<20	614	640
PBP	None	Pellet	<20	<20	<21
No PBP	None	Pellet	<20	<20	<21
PBP	GAA	Pellet	<20	520	542
No PBP	GAA	Pellet	<20	516	538

[1]Guanidinoacetic acid (GAA) was administered by adding the feed additive CreAMINO® (>96% GAA; Evonik Degussa GmbH, Hanau-Wolfgang, Germany). [2]CreAMINO®: lot numbers: 3/29/16, 9/26/16.

Appendix F. Amino acid content of corn-based containing or not poultry by-products (PBP) with or without GAA.

	Experimental diets															
	Starter				Grower				Finisher				Withdrawal			
	No PBP		PBP		No PBP		PBP		No PBP		PBP		No PBP		PBP	
Nutrient Name	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**
Dry matter, %	88.34	88.23	88.65	88.81	88.99	88.80	89.27	89.08	88.68	89.11	88.59	88.71	88.00	88.00	88.00	88.00
Crude protein, %	21.29	22.40	21.50	22.33	19.53	20.38	20.55	20.49	18.50	18.75	18.83	18.80	18.02	18.81	18.68	18.85
Total amino acids																
Methionine, %	0.557	0.578	0.615	0.600	0.503	0.545	0.569	0.567	0.460	0.486	0.498	0.500	0.465	0.485	0.522	0.531
Cystine, %	0.334	0.339	0.314	0.331	0.317	0.321	0.311	0.310	0.301	0.299	0.286	0.284	0.300	0.303	0.303	0.302
Lysine, %	1.320	1.332	1.328	1.352	1.175	1.205	1.228	1.227	1.029	1.043	1.073	1.063	1.072	1.115	1.158	1.134
Threonine, %	0.872	0.890	0.869	0.893	0.795	0.833	0.815	0.811	0.703	0.704	0.725	0.714	0.737	0.745	0.758	0.787
Isoleucine, %	0.894	0.912	0.861	0.898	0.813	0.854	0.810	0.813	0.765	0.762	0.735	0.729	0.723	0.764	0.732	0.722
Valine, %	1.023	1.048	1.025	1.061	0.918	0.959	0.940	0.948	0.869	0.866	0.855	0.854	0.825	0.864	0.864	0.852
Leucine, %	1.811	1.832	1.783	1.867	1.727	1.751	1.740	1.756	1.636	1.609	1.623	1.611	1.578	1.624	1.640	1.623
Arginine, %	1.414	1.444	1.382	1.440	1.309	1.348	1.320	1.326	1.190	1.188	1.171	1.158	1.150	1.205	1.212	1.172
TSAA, %	0.891	0.918	0.929	0.931	0.821	0.866	0.879	0.877	0.761	0.785	0.784	0.784	0.765	0.788	0.825	0.833
Histidine, %	0.564	0.575	0.551	0.573	0.528	0.546	0.527	0.531	0.480	0.477	0.460	0.459	0.491	0.508	0.510	0.506
Phenylalanine, %	1.056	1.069	1.005	1.050	0.976	1.001	0.961	0.968	0.897	0.890	0.859	0.858	0.867	0.901	0.867	0.852
Serine, %	1.063	1.071	1.005	1.048	0.998	1.002	0.969	0.977	0.903	0.895	0.879	0.869	0.869	0.896	0.881	0.866
Proline, %	1.210	1.246	1.272	1.334	1.189	1.184	1.283	1.287	1.107	1.103	1.163	1.186	1.042	1.076	1.147	1.131
Alanine, %	1.071	1.085	1.150	1.199	1.025	1.027	1.125	1.137	0.971	0.955	1.051	1.049	0.939	0.965	1.065	1.061
Aspartic acid, %	2.178	2.206	2.025	2.119	1.991	2.047	1.926	1.930	1.789	1.780	1.683	1.666	1.709	1.788	1.683	1.651
Glutamic acid, %	3.805	3.857	3.604	3.782	3.553	3.577	3.477	3.488	3.274	3.240	3.132	3.108	3.14	3.244	3.139	3.084
Glycine, %	0.893	0.914	1.049	1.108	0.828	0.858	1.030	1.038	0.769	0.767	0.943	0.947	0.754	0.784	0.960	0.962

Appendix F. Continued

	Experimental diets															
	Starter				Grower				Finisher				Withdrawal			
	No PBP		PBP		No PBP		PBP		No PBP		PBP		No PBP		PBP	
Nutrient Name	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**	**None**	**GAA**
Minerals[1]																
Calcium, %	-	-	-	-	0.63	0.63	0.71	0.71	-	-	-	-	0.69	0.69	0.58	0.58
Phosphorous, %	-	-	-	-	0.53	0.53	0.54	0.54	-	-	-	-	0.46	0.46	0.45	0.45
Magnesium, %	-	-	-	-	0.17	0.17	0.15	0.15	-	-	-	-	0.15	0.15	0.14	0.14
Potassium, %	-	-	-	-	0.88	0.88	0.77	0.77	-	-	-	-	0.73	0.73	0.65	0.65
Sodium, %	-	-	-	-	0.155	0.155	0.160	0.160	-	-	-	-	0.170	0.170	0.174	0.174
Chloride Ion, %	-	-	-	-	0.23	0.23	0.27	0.27	-	-	-	-	0.32	0.32	0.26	0.26

[1]Analyzed in samples from basal diets of grower and withdrawal only