IMPACTS OF VARIABLE AMINO ACID DENSITIES DURING WITHDRAWAL PHASE DIETS ON GROWTH PERFORMANCE, YIELD, AND MEAT QUALITY OF BROILERS

A Thesis

by

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ABSTRACT

Two experiments were conducted to investigate the impact of variable AA densities on broiler live performance, processing yield and meat quality parameters. The objective in experiment I was to evaluate 4 diets of increasing AA density in 35 d male broilers. All broilers were fed a common diet until 12 d of age and then switched to dietary treatments from d 13 to 35. On d 13, broilers were fed 1 of 4 dietary treatments of increasing AA density (0.95/0.82, 1.00/0.87, 1.05/0.92, and 1.10/0.97% dig lysine for d 13 to 25/25 to 35). Evaluated parameters included average body weight (BW), body weight gain (BWG), mortality corrected feed conversion ratio (FCR), feed consumption (FC) and processing yields. Increasing the dig lysine level improved growth performance as incremental improvements were observed in BW, BWG, FCR, and processing weights were observed. However, whole carcass yield percentage was not impacted. In general, increasing dig lysine increased breast meat yield and decreased abdominal fat pad yield.

In experiment II, a similar approach was taken with 4 levels of increasing dig lysine fed in the final 2 diets of grow-out. However, the age of the birds evaluated increased to 48 d of age and all broilers were fed a common diet through 28 d of age. The 4 treatment diets had increasing AA densities and were fed as WD 1 from d 28 to 38 and WD 2 from d 38 to 48 (0.91/0.85, 0.96/0.89, 1.00/0.94, and 1.05/0.98% dig lysine for WD 1 and WD 2, respectively). All measured parameters were similar to that of experiment I however, breast fillets were observed for the presence and severity of woody breast and white striping. Similar to experiment I, increased dietary AA density improved growth performance parameters to include increased BW and decreased FCR through both phases of the experimental period. Reducing the dig lysine level increased FC. Whole carcass yield percentage increased when broilers were fed the highest AA density diet compared to those fed the lowest AA density diet. Breast weight and yield percentage increased in broilers fed the diet with a higher AA density as compared to the other diets. As AA density increased, the severity of woody breast also increased. In conclusion, dietary AA content will impact growth performance and can be used to improve profitability dependent upon ingredient prices.

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NOMENCLATURE

AA	Amino acid
AAs	Amino acids
BW	Body weight
BWG	Body weight gain
FCR	Feed conversion ratio
FC	Feed Consumption
WB	Woody breast
WS	White striping
WD	Withdrawal
Ph	Potential of hydrogen
h	hour
d	day
Kg	Kilograms
g	grams

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CHAPTER I

INTRODUCTION

Each poultry industry nutritionist is tasked with the goal of maximizing profitability. This can be done in a variety of strategies to include least cost feed formulation and achieving maximal growth. These two strategies are not necessarily complementary of each other as growth performance is driven by dietary nutrient density. Diets containing increased levels of nutrients such as AAs, minerals, and energy are more expensive but will result in improved growth performance. Fancher and Jensen (1987) reported the relationship between AA levels on observed body weight gain and the curvilinear increased in feed efficiency as crude protein increased.

Least cost feed formulations aim to reduce cost through inclusion of alternative type ingredients and potentially lower nutrient density. Therefore, nutritionists must decide if an increase in dietary cost can be offset with the improvements in performance such as increased body weight and improved feed conversion ratio. The only way for nutritionists to make these decisions is to have credible data to use for economic models dependent upon ingredient pricing. In the research described in this document, multiple experiments were conducted to evaluate the impact of increasing the digestible lysine level in broiler diets. The poultry industry uses digestible AAs levels for all dietary formulations. Digestible lysine is used as a base for setting the minimums or maximums of the other AAs in the diets under the Ideal Protein concept. In the current experiments, four different densities of AAs in a corn and soy base diet were evaluated when fed to broilers in the last two phases with one experiment utilizing a young bird (35 d of age) and the other experiment utilizing an older bird (48 d of age) for performance and processing evaluation. More recently, breast meat myopathies are becoming more common and thus experiments aimed at improving meat yield must also evaluate the functionality and quality of the filets.

According to the experiment conducted by Lilly et al. (2011), feeding broilers a high AA density in the diets during a finishing phase produced chickens with an excellent meat quality and carcass traits and maximizes growth. Additionally, the authors reported that feeding broilers high AA density diets increases breast meat yield and improves feed conversion efficiency. Moreover, the evaluation of lysine and other AAs impacts on meat quality have been widely explored. In an experiment by Zhai et al. (2016), lysine supplementation increased the amount of soluble protein concentration, increased water holding capacity and pH in the breast filet of broilers resulting in increased meat tenderness. In addition, Si et al. (2001) reported that lysine density has been shown to directly impact breast meat which is associated with the high concentration of lysine in breast tissue. However, according to Kuttapan et al. (2015) breast muscle myopathies such as woody breast and white striping are increasing in severity and incidence which is gaining the attention of poultry producers worldwide. The occurrence of these meat quality issues has been associated with the higher growth rate in birds and thus increasing AA density may impact the occurrence and severity of breast meat myopathies.

CHAPTER II

LITERATURE REVIEW

Amino Acid Description

In the poultry industry, nutrient density of feed is an important factor in the productive response of market birds. One such macronutrient important to the ultimate performance of the bird as well as the cost of the diet is protein. Protein is essential for the proper growth and development of the animal's body and has a significant effect on performance in broiler chickens (Farkhoy et al., 2012). This macronutrient is made of long molecules consisting of building blocks in their structure called AAs. The normal structure of an AA consists of a carboxyl group, an amine group, and a side chain. The carboxyl group is composed of one carbon, two oxygens, and also a hydrogen atom. The amine group has one nitrogen atom and it contain two hydrogen atoms attached to it structure. All these building blocks have a different composition when it comes to the side chain which is what differentiates all AAs.

Normally AAs are classified in two different groups, essential which cannot be synthesized by the animal itself and must be ingested from the diet, and non-essential AAs which can be produced by the bird and thus a requirement in the diet is not necessary. However, it is vitally important to include substantial non-essential AAs in the diet to prevent the use of essential AAs to supplement the deficiency of the non-essential. Furthermore, AAs must be supplied in the correct ratio in relation to the demands of the animal, or they will be deaminated and used as a source of energy instead of being used for biosynthesis of protein, and excess AAs will be excreted as nitrogen into the environment (Applegate, 2008).

Amino Acid Density

The poultry industry has been growing at high rates over the last 50 years and nutritionists are continuing to evaluate strategies to improve growth performance and processing yield. Broilers are fed high protein diets with the exact nutrient concentration dependent on the growth phase as nutritional requirement to maximize the genetic potential of high yielding broilers vary by age. Increasing AA density in the diet improves feed conversion and increases weight gain in broilers (Pereira de Oliveira et al., 2013). Furthermore, the dietary formulation containing high AA levels may increase economic return if it is fed during periods when growth rate is high and consumption is still low (Kidd, 2004). Requirements for AAs cannot be apply to all market broilers in the same concentration as they vary dependent on strain, age and sex which is the reason AA requirements are expressed as ideal ratios to lysine. (Schutte, J.B and De Jong, J., 1999).

The ratios of AAs in broiler diets typically fed in the poultry industry have changed over the last few years mostly associated with ever changing genetic potential and growth rate of market broilers. The NRC (1994) AA recommendations for broilers were based on previous research at the time of publication. Over the past 20 years, continued genetic selection has resulted in vast differences between the current commercial broiler and the commercial broiler when the NRC (1994) was published.

The growth response and conversion ratios of broilers fed a diet from 1991 was much improved when compared to birds fed a diet from 1957 (Havenstein, 1994), and thus the AA requirements for modern broilers is different as compared to 1991 in response to genetic selection. To accurately meet the nutrient requirements of broilers, the broiler industry uses feeding regimens divided into phases that are shorter as to more effectively supplement the nutrient needs of the birds (Applegate, 2008).

The constant increasing demand of breast fillets and similar products derived from chicken has pushed the industry to continue to increase the genetic potential of broilers to reach target weights in fewer days and with less feed input. The decrease in total feed consumption and the ever grow nutrient need for rapidly growing broilers results in high AA density diets to improve performance and quality. The AA density in poultry diets in the US has increased every year between 2001 and 2005 for the broilers that are 2.7 kg and larger (Dozier. et al., 2008).

When nutritionists formulate broiler diets, variation of protein content due to geographical differences in the ingredients used can impact the utilization of nutrients and ideal growth of broilers (Latham et al., 2016). Therefore, the inclusion of synthetic AAs in diets is widely used in animal feed formulation to assist in balancing and meeting their digestible AA requirements. Synthetic AAs can be added as liquid and powder and is used to meet the requirement of essential AAs, reduce costs, and pollution from nitrogen excretion as they increase the digestibility of AAs and promote growth (Han and Lee, 2000).

The addition of synthetic AAs is widely used in the poultry industry as a tool for balancing feed cost while supplying limiting AA levels (Guaiume, 2007). Synthetic AAs are useful ingredients that can be helpful for digestibility improvement as the birds are able to have a higher rate of absorption, and expend less energy in digestion due to high solubility.

Dozier (2008) conducted an experiment in which birds were fed a common diet high in AA density from d 1 to d 47. From d 48 to 59, broilers were subjected to dietary treatments which were high (H), medium (M), low (L) and suboptimum (S) AAs densities. Broilers provided the H diet had 2, 4 and 5 points cumulative FCR improvement when compared to birds fed the M, L and S diets, respectively, thus indicating the benefits of higher AA densities.

Primary breeders are continually selecting broilers for genetic potential, specifically, increased growth rate and tissue deposition. Dietary protein is identified as the most important nutrient for maximizing weight gain which is the main focus for broiler producers (Suida, 2001). This rapid growth rate of the current market broiler demands increased amounts of nutrients. However, the proportions have changed as compared to historical proportions such as those included in the NRC (1994). The AA requirement increases faster than the energy requirement which make it necessary to have a higher AA to energy ratio in rapidly growing broiler strains. This concept is outlined in the results of Vieira and Angel (2012) in which providing dietary protein above the concentrations used by the US poultry industry improved FCR in different chicken strains.

The rate of breast growth is an important factor for poultry producers. Allometry is the study of tissue deposition and how this tissue is proportional or different in sizes during the different growth phases of the bird (Swatland, 1994). Throughout the different life periods, birds show a high allometric growth ratio for breast muscles when compared with thigh and drumsticks in broilers (Govaerts., et al. 2000). Schmidt and colleagues (2009) indicated after evaluating two different broiler strains, a current and historical strain, that the breast muscle grows at higher rates than other tissues of the chicken at late ages. The demand for AAs is high for breast muscle growth while the remaining is used for maintenance in late stages. However, the importance of reducing feed cost in the poultry industry during the late grower stages in response to high feed intake, force nutritionists to reduce AA density in commercial feeds (Vieira and Angel, 2012).

Ideal Protein Concept

This conceptualization refers to the ratios of AAs that mutually interact in order to meet the animal requirement for protein and in most cases uses lysine as base to ratio the rest of the essential AAs. Lysine is the second limiting AA for chickens and is used as a reference for the inclusion of other AAs in the diet formulation (Pesti, 2008). Knowing about this concept is vitally important for nutritionists when it comes to diet formulation, because it makes possible to match the requirements of essential AAs in the bird and in that way not causing deficiencies or over supplementation of protein which is going to result in a negative economic impact for the producer. Ideal AA balance is a concept that refers AAs as organic compounds that are required to biosynthesize proteins

(Pesti, 2008). Moreover, an important idea of this concept is that birds have the need for AAs in a specific balance depending on their phase of production for an optimum performance. The AAs that are included in the diet in excess to the first limiting AA will be excreted as nitrogen which results in economic loses and may also affect the environment (Lemme, 2003).

Digestible Methionine

All essential AAs are important but methionine has a basic role in protein synthesis for broilers fed corn and soy based diets. Methionine is a sulfur AA which plays an essential role in growth and feather formation (Garcia and Batal, 2005). Amino acids such as methionine exist in two different forms which are D or L isomers. This sulfur AA occurs in tissues typically in the L form because the D form is not biologically active; however, broilers have the capacity to utilize both. Nutritionists usually use the form of dry D,L-methionine which is 99% pure or the liquid form which is derived from an acid analog and is equivalent to 88% methionine after its conversion to the active form. This AA is essential for the health of the broiler and also helps to increase the productivity in the industry (Jacob, 2013). Methionine supplementation improves the immune system response. Supplementation helps with leukocyte migration inhibition, cellular and humoral immune response (Maroufyan et al., 2010). Moreover, broilers react to AA deficiencies in a short period of time by reducing their feed intake mainly caused by a nutritional imbalance. When there is an imbalance, chickens lose the potential to adjust feed intake to meet their AA requirements, and thus the one of the most important impacts of methionine supplementation is the improvement of feed intake via the AA balance (Bunchansak, 2009). Meireless and colleagues (2003) conducted an experiment in which the performance of broilers fed different levels of methionine hydroxyl analogue and DL- methionine was evaluated. The inclusion of methionine was provided in four different levels per phase. Inclusion levels for methionine in the starter phase were 0.41, 0.47, 0.53, and 0.59%, grower phase was 0.35, 0.41, 0.47, and 0.53, and finisher phase was 0.30, 0.36, 0.42, and 0.48. For the starter phase (d1 to d21) DL- methionine was more effective in bird growth when compared to the diets containing the hydroxyl analogue methionine. However, feed intake and feed conversion was not affected by either of the methionine sources. During the grower phase (d22 to d 42), weight gain increased as methionine level increased. A weight gain increase of 4.65 % was observed from birds fed the diet highest in methionine (0.53%) when compared to the birds fed with the lowest methionine level (0.35%). Feed conversion in the grower phase was different between the different sources; birds fed the DL-methionine had an improved feed conversion than the liquid type of methionine. For the finisher phase (d43 to d47), no differences were observed for weight gain caused by sources or levels even though there was an increasing trend with the higher methionine levels (Meireless et al. 2003).

A study by Corzo and colleagues (2005) evaluated the effect of dietary AA density varying in a low and high density diet on growth and carcass yield of broilers differing in strain cross and sex from d 1 to d 56 of age. Regardless of sex, broiler chickens positively responded to increased AA density. In general, increased AA density diets above levels used by most of the US poultry integrators, had an improved performance level and white meat yield. Diets containing a decreased AA density reduced the total breast meat yield by 0.6 and 0.5 % for d 42 and d 56 of age. The authors conclude that benefits in growth and yield are observed when feeding higher AA density levels, however, the economic benefits related with the different diets associated with ingredients and meat prices can vary depending in the final market value.

Digestible Lysine

In the poultry industry, lysine has been used as the reference for the remaining AAs requirements because it is one of the first two limiting AAs. As intake of lysine increases, the body composition in the broilers is affected by increasing white meat yield (Leclercq, 1998).

Kidd and Kerr (1998) conducted an experiment in which they evaluated different lysine levels in starter, grower and finisher phases and the impact on growth and carcass traits. The authors sought to evaluate the interaction between phases with diets containing varying levels of lysine in response to live performance and breast meat yield. Increasing the level of lysine in all dietary phases, improved performance but also increased mortality. Breast meat weight and yield increased as the level of lysine in the diet increased.

An experiment was conducted by Si and colleagues (2001) investigating the relationship of dietary lysine and the concentration of all essential AAs in broiler diets. No differences or interactions between the level of lysine and other essential AAs for live performance were observed. However, for the measurements at d 21 and 42 the addition of 0.1 % lysine when compared to a diet following the NRC (1994) parameters had an improvement in BW.

It has been shown that lysine content in broiler breast tissue is higher when compared with any other amino acid as the content of lysine represents about 7% of the total protein in chicken breast. Also, birds fed with an inadequate amount of lysine in their diets have shown a reduction in meat yield in comparison with other muscles (Tesseraud et al. 2007). These facts outline the importance of providing adequate levels of dietary lysine to fast growing high yielding birds.

Meat Yield

The building blocks of proteins better known as AAs are critical for muscle tissue development. Kidd and colleagues (2004) reported that the inclusion of high AA density in the early age of birds especially from d 1 to 28 increased meat yield. Interestingly, continuing to feed high levels of AAs following 28 d only increased meat yield at 49 d of age by 1%. During their experiment, they reported that males have a higher requirement than females to maximize yield.

Dozier and colleagues (2006) conducted an experiment evaluating growth performance, meat yield and economic responses of broilers to diets varying in AA density from 36 to 59 d of age. Broilers were fed 4 diet phases throughout the trial varying in AA density following a common diet that was high in AA density during the starter and grower phase. Following the common diet period, broilers were fed diets varying in density during two withdrawal phases varying in high (H), moderate (M), and low (L) AA density. Decreasing the density from M to L in the experiment did not impact carcass yield and breast meat yield, however, providing the H regimen increased economic gross margin. After all, the conclusions had the H feed regimen as the only one that increased breast fillet, breast tender yield, and total breast meat yield when compared with the broilers subjected to the L regimen. Varying the density in the final withdrawal phase, did not result in differences in any measured parameter, indicating the importance of high AA density diets in the early ages of growth. Similar results have been reported such as those of Moran and Bilgili (1989) who evaluated meat yield of broilers receiving diets marginally deficient to adequate in lysine (0.95 to 1.05%). At the conclusion of the experiment, the proportions of breast and thigh increased on a raw and cooked basis even though no differences were observed in live performance parameters due to lysine content in the diets.

Toledo and colleagues (2004) evaluated the impact on yield and carcass composition of broilers fed diets based on the concept of either crude protein content or the ideal protein concept in two different strains. No interaction in feed consumption, weight gain and feed conversion were observed. Data from this study confirmed that diets formulated on an ideal protein concept outperformed birds fed diets formulated on a crude protein basis, indicating that not only the amount of protein in the diet but the ratio of AAs present are important drivers to the ultimate yield of broilers.

Diet Cost

The poultry industry's main concern is to produce a cost effective final product. Prices of breast meat influence the feeding margin more than the cost of the ingredients caused by the constant changes in a broiler production complex. Also the ingredients and breast meat price have to be taken in consideration for establishing the AAs inclusions in the formulated diets (Dozier et al., 2008). Another area of concern for the poultry industry is waste management. Excess of nitrogen in poultry diets can be excreted which could negatively impact the environment depending upon a variety of factors. However, nutritionists can use synthetic AAs to assist with more accurately feeding closer to the requirement of the bird (Toledo et al. 2004). Not only is the industry concerned about not providing the birds with enough protein and its impact on diet cost, but agriculture has become more responsive to public concern about the environment and potential waste management problems. The excretion of high amounts of nitrogen can be useful for crop growth, however, an excess of nitrogen is also detrimental and becomes a pollutant (Summers, 1993). Meeting the AA requirements represents a large portion of the final diet cost. Moreover, the biological need of the bird can be costly and may mean economic losses for the industry, however; not meeting the requirement can negatively impact economic return (Kidd et al. 2005).

Guaiume (2007) evaluated the impacts of reducing crude protein level while supplementing AA into the diet on economic performance of broilers. Reducing protein of the diet decreased feed costs as expected. Reducing the crude protein by 1.5% with supplementing adequate levels of essential AAs, did not have a direct effect on performance or meat yield of Ross broilers at 8 weeks of age, but did result in greater economic savings.

Feed costs represent almost 70 percent of the total cost of animal production. Improving the efficiency of protein utilization by using the correct amount of AAs is very important to keep feed costs as low as possible while maximizing meat production. Proteins are one of the most expensive nutrients in animal diets, and thus the use of synthetic AAs can serve as a helpful option in formulating diets. The amount of synthetic AA use in animal feed has increased by 4.5% during the last few years. The amount of AAs used for animal feed purposes represents more than 50% of the total synthetic AA production in the world (Han and Lee, 2000).

Meat Quality

The quality of poultry meat is an important aspect for consumers. The increased demand of poultry meat has resulted in increased growth rate and improved feed conversion but the industry must continue to focus on producing a high quality product. One of the causes for biochemical modifications of the broilers meat can be the stress that genetic progress has placed on the bird which can affect the acceptance of the product once in the market (Petracci, 2012).

In recent years, the presence of white striping (WS, White striation parallel to muscle fibers) and woody breast (WB, hardness of raw fillet) has increased in prevalence and severity to a point that consumers are rejecting the product.

White Striping

Kuttapan and colleagues (2013) define white striping as a condition in broiler chickens which is characterized mostly by the occurrence of white striations that appear parallel to muscle fibers. This detrimental factor has been associated with body weight and broiler age; prevalence of white striping increases as the bird ages (Ferreira, 2014).

It is possible that the intensive genetic selection for an increase in performance may result in this abnormality becoming more prevalent (Petracci and Cavani, 2012).

The prevalence of white striping in the US is increasing however; there is very little research about a relation between varying AA densities in broiler diets and this issue. Lilly and colleagues (2011) evaluated the effects of dietary AA density on meat quality characteristics. Their findings indicate that diets with different AA densities during the finisher phase had a minimal effect in quality and acceptability of the breast meat. However, breast meat that was provided to a sensory panel coming from broilers fed diets with a low and high AA density were more accepted by a greater percentage of consumers than breast meat from diets that were under or over the AAs requirements for broilers.

The severe levels of white striping are related to heavier birds, older birds, and thicker breast fillets. The occurrence of this recent poultry issue can affect raw meat quality factors, mainly color and water holding capacity (Tijare et al. 2015). Kuttappan and colleagues (2012) studied the relation between vitamin E and white striping severity, but were not able to identify a correlation; however, there was an effect on the fillet weight and the occurrence of white striping. Fatty acid content of breast meat with high white striping values is higher in monounsaturated fatty acids than in polyunsaturated fatty acids. The increase of EPA and DHA content in the broiler feed decreased the white striping occurrence. However it has not been proven that supplementing diets with vitamin E, selenium and sulfur- containing AAs will cause an effect in white striping (Rutz, 2015).

Woody Breast

Woody breast is considered an inflammatory condition that turns the breast muscle a pale color and has a very hard texture. When this abnormality is present the capillaries in the tissue surrounding the muscles bundles are constricted by hypertrophic muscle fibers resulting in a low vascularity and wider diffusion distances. However, there is no bacterial activity in the inflammatory process which is caused by hypoxia. Some evidence that exists showed that increasing arginine content in diets might decrease the woody breast occurrence in broilers (Rutz, 2015).

An experiment was conducted to evaluate dietary AA density and broiler breast myopathies in broiler chickens. As expected the performance parameters such as growth rate, feed conversion and meat yield were improved when birds were fed a high AA density over a low AA density. However, the increase in meat yield associated with high AA density increased the level of white striping and woody breast indicating a direct influence of AA to the meat quality factors (Bilgili et al. 2014). In a study conducted by Cemin and colleagues (2016), the occurrence of woody breast in broilers fed increasing levels of lysine in a grower (d12 to d28) and finisher (d28 to d42) phases increased breast weight and the occurrence of woody breast. The authors concluded that myopathies such as woody breast can be associated with increasing levels of digestible lysine as it increases the breast weight of broiler chickens. Tijare et al. (2016), report that severe degrees of woody breast negatively affect water holding capacity, cook loss, and marination. The causes of abnormalities such as woody breast are not completely understood. In an experiment by Mudalal and colleagues (2015), woody breast found in chicken meat was related to fillet thickness. In addition, pH is increased in fillets affected by white striping and woody breast. Therefore, this research program aims to evaluate growth and carcass composition of male broilers fed diets varying in AA density to identify the growth potential with elevated AAs in current industry strain broilers. Additionally, with increases in breast muscle myopathies and previous correlations to growth rate by other authors, the presence and severity of woody breast and white striping will be evaluated. These data will aid industry producers in determining proper level of AAs to maximize performance, meat quality and profitability.

CHAPTER III

EXPERIMENT I: IMPACTS OF VARIABLE AMINO ACID DENSITIES DURING THE FINISHER AND WITHDRAWAL PHASE ON GROWTH PERFORMANCE AND CARCASS YIELD IN YOUNG BROILERS

Introduction

An experiment was conducted to evaluate the effect of different AA densities during the final two phases of production with diets being fed at d 13 of age following a common starter diet. Broilers must have an adequate amount of AA in the diets they are fed in order to maximize their genetic potential. Moreover, previous literature has confirmed that increasing AA density improves broiler performance. The objective of this experiment was to identify the appropriate AA density required to maximize broilers growth performance through 35 days of age. It is important for producers to know the final market prices for the final products in order to establish the correct AA density that can be included in the diet to maximize feed efficiency and profits.

Materials and Methods

Table 1. Amino acid ratios used for feed formulation during study.

Amino acid	**Starter (d 0-10)**	**Grower (d 11-22)**	**Fin 1 (d 23-42)**	**Fin 2 (d 43)**
Lysine*	100	100	100	100
Methionine+Cysteine	75	76	78	78
Tryptophan	16	16	18	18
Threonine	65	66	68	68
Arginine	105	105	108	108
Valine	75	76	77	77
Isoleucine	67	67	68	68

*In the profile Lysine is always the reference amino acid, and is shown at 100%.
Adapted from Cobb 500 Broiler performance and nutrition supplement. By Cobb Vantress. 2015.

Experimental Design

The objective of experiment I was to evaluate the impact of varying amino acid (AA) densities in finisher and withdrawal phase diets on growth performance and carcass yield in young broilers. The experiment was conducted using a complete randomized block design with 4 dietary phases during a 35 d grow out.

Experimental Procedure

Birds were weighed on the evening of d 34 and following an 8 h feed withdrawal time, 6 birds per pen were processed for the determination of carcass and parts yield following emersion chilling for 1 h at 3 C. All animal husbandry procedures were conducted in accordance with an approved animal use protocol (IACUC).

Experimental Diets

Diets were corn and soybean meal based and formulated using the Ideal Protein Concept in which all essential amino acids are ratio to lysine in order to meet the bird requirement. For diet manufacturing, composite samples were collected and analyzed for total AA content. All diets varying in AA density were formulated based on ratios to lysine (Table 1).

Male broilers were fed four treatments in which number 2 was the one following the industry standards. All broilers were fed a common diet as crumble until 12 d of age (Table 2), from d 13 to 35 birds received 1 of 4 diets varying in AA concentration (Table 3 and 4). The diets varied in AA density and broilers were fed a finisher diet from d 13 to 26 and a withdrawal diet from d 27 to 35 both diets being fed as pelleted feed. The digestible lysine level of the finisher diets ranged from 0.95 to 1.10% while the digestible lysine level of the withdrawal ranged from 0.82 to 0.97%.

Animals and Management Practice

On d of hatch, Cobb 500 male broilers chicks were randomly allotted to floor pens and dietary treatments based on initial body weight. The experiment consisted of a total of 60 pens with 15 blocks of 4 contiguous pens, with a floor space of 0.8 ft^2/bird each. The pens contained 46 birds at d 1 of age then were reduced to 43 birds per pen at d 12. Feed and water was available ad libitum. Chicks were provided appropriate temperature for their age and a commercial style lighting program. All broilers and the remaining feed was weighed by pen at d 12, 25 and 35 corresponding to dietary phase changes to determine average body weight (BW), body weight gain (BWG), mortality corrected feed conversion ratio (FCR), and feed consumption (FC).

Table 2. Common starter diet formulation fed to male broilers (0 to12 days of age).

Ingredient Name	**Units**	**Value**
Corn	%	61.08
Soybean Meal	%	28.04
Corn DDGS	%	4.51
Meat & Bone	%	4.04
Limestone	%	0.79
Salt	%	0.36
DL Methionine	%	0.28
Fat, An./Veg blend	%	0.25
L-Lysine HCl	%	0.23
Choline Cl	%	0.09
L-Threonine	%	0.09
Trace Mineral Premix[1]	%	0.08
Copper Sulfate	%	0.05
Coccidiostat[2]	%	0.05
Vitamin Premix[3]	%	0.03
Enzyme Premix[4]	%	0.03
Nutrient name		
Calculated nutrient Content	**Units**	**Value**
AME, Poultry	kcal/kg	3003
Moisture	%	11.71
Crude Protein	%	21.48
Crude Fat	%	3.11
Linoleic Acid	%	1.51
Ash	%	4.45
Calcium	%	0.88
Total Phosphorus	%	0.54
Avail Phosphorus	%	0.44
Salt	%	0.39

Table 2. Continued.

Nutrient Name **Calculated Nutrient Content**	**Units**	**Value**
Dig Arginine	%	1.26
Lysine	%	1.31
Dig Lysine	%	1.18
Methionine	%	0.62
Dig Methionine	%	0.59
Met + Cys	%	0.97
Dig Met + Cys	%	0.88
Tryptophan	%	0.24
Dig Tryptophan	%	0.21
Threonine	%	0.87
Dig Threonine	%	0.77
Isoleucine	%	0.87
Dig Isoleucine	%	0.79
Valine	%	1.02
Dig Valine	%	0.91
Cystine	%	0.35
Dig Cystine	%	0.29
Analyzed Nutrient Content	**Units**	**Value**
Threonine	%	0.86
Cystine	%	0.32
Valine	%	1.02
Methionine	%	0.55
Leucine	%	1.77
Isoleucine	%	0.86
Lysine	%	1.31
Arginine	%	1.34
Tryptophan	%	0.25
Threonine	%	0.09
Fat	%	4.5
Ash	%	4.9
Dry matter	%	89.2
Calcium	mg/kg	8446

[1] Trace mineral premix added in the starter yields 180 mg of manganese, 108 mg of total zinc, 5.1 mg of copper, 3.51 mg of iodine, 0.3 mg of total selenium, 0.013 g of *bacillus*.
[2]Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella*, *Eimeria necatrix*, *Eimeria acervulina*, *Eimeria maxima*, *Eimeria brunetti and Eimeria mivati.*
[3] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet.
[4]Enzyme blend provides 1800U/kg of xylanase and 750 FTU/kg of phytase.

Table 3. Finisher dietary formulations varying in amino acid density fed to male broilers (d13 to 25).

Ingredient Name (%)	**TRT 1**	**TRT 2**	**TRT 3**	**TRT 4**
Corn	66.23	63.99	61.47	58.95
Soybean Meal	17.92	19.85	21.84	23.82
Corn DDGS	10.00	10.00	10.00	10.00
Meat & Bone	3.51	3.53	3.53	3.53
Limestone	1.00	0.90	0.91	0.91
Salt	0.34	0.34	0.33	0.33
DL Methionine	0.16	0.19	0.21	0.24
Fat, An./Veg blend	0.25	0.62	1.12	1.62
L-Lysine HCl	0.25	0.26	0.26	0.26
Choline Cl	0.08	0.07	0.07	0.06
L-Threonine	0.05	0.06	0.07	0.07
Trace Mineral Premix[1]	0.06	0.06	0.06	0.06
Copper Sulfate	0.05	0.05	0.05	0.05
Coccidiostat[2]	0.05	0.05	0.05	0.05
Vitamin Premix[3]	0.02	0.02	0.02	0.02
Enzyme Premix[4]	0.03	0.03	0.03	0.03
Nutrient Name				
Calculated Nutrient Content (%)				
AME,Poultry kcal/kg	3058	3058	3058	3058
Crude Protein	18.29	19.02	19.75	20.47
Crude Fat	3.27	3.59	4.02	4.44
Linoleic Acid	1.79	1.83	1.89	1.95
Crude Fiber	3.19	3.21	3.22	3.23
Ash	4.26	4.21	4.318	4.43
Calcium	0.88	0.85	0.861	0.87
Nutrient Name				
Calculated Nutrient Content (%)				
Total Phosphorus	0.51	0.52	0.527	0.54
Avail Phosphorus	0.42	0.43	0.43	0.44
Salt	0.45	0.39	0.392	0.39
Sodium	0.20	0.18	0.18	0.18
Potassium	0.76	0.80	0.84	0.88
Na+K-Cl mEq/kg	184.76	195.27	205.88	216.50

Table 3. Continued.

Nutrient Name				
Calculated Nutrient Content (%)	**TRT 1**	**TRT 2**	**TRT 3**	**TRT 4**
Arginine	1.08	1.14	1.20	1.25
Dig Arginine	0.99	1.00	1.10	1.16
Lysine	1.07	1.12	1.18	1.23
Dig Lysine	0.95	1.00	1.05	1.10
Methionine	0.47	0.50	0.53	0.57
Dig Methionine	0.44	0.47	0.505	0.54
Met + Cys	0.79	0.83	.87	0.91
Dig Met + Cys	0.71	0.75	0.79	0.82
Tryptophan	0.19	0.20	0.21	0.22
Dig Tryptophan	0.16	0.17	0.18	0.19
Threonine	0.71	0.75	0.78	0.82
Dig Threonine	0.62	0.65	0.68	0.72
Isoleucine	0.72	0.75	0.79	0.82
Dig Isoleucine	0.65	0.68	0.71	0.74
Valine	0.88	0.91	0.94	0.98
Dig Valine	0.77	0.80	0.84	0.87
Cystine	0.32	0.33	0.34	0.35
Dig Cystine	0.27	0.27	0.28	0.29
Analyzed Nutrient Content				
Threonine	0.75	0.71	0.79	0.85
Cystine	0.29	0.30	0.30	0.29
Valine	0.88	0.89	0.96	0.98
Methionine	0.46	0.46	0.48	0.50
Leucine	1.61	1.62	1.73	1.75
Isoleucine	0.72	0.74	0.80	0.83
Lysine	1.07	1.10	1.18	1.22
Arginine	1.10	1.11	1.24	1.27
Analyzed Nutrient Content				
Tryptophan	0.20	0.21	0.23	0.23
Threonine	0.06	0.06	0.06	0.08
Fat	4.7	5.1	5.5	6.2
Ash	4.5	5.2	4.8	5.0

Table 3. Continued.

Analyzed Nutrient Content				
Calcium	7968 mg/kg	10370 mg/kg	8325 mg/kg	8613 mg/kg
Phosphorus	5472 mg/kg	5726 mg/kg	5533 mg/kg	5721 mg/kg
Sodium	1678 mg/kg	1782 mg/kg	1604 mg/kg	1665 mg/kg

[1]Trace mineral premix added in the grower yields 150 mg of manganese, 90 mg of total zinc, 4.25 mg of copper, 2.92 mg of iodine, 0.25 mg of total selenium, 0.011 g of *bacillus subtilis*
[2]Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.*
[3]Vitamin premix added at this rate yields 6160 IU vitamin A, 4400 ICU vitamin D_3, 44 IU vitamin E, 1.2 mg vitamin K-3, 0.008 mg B_{12}, 5.28 mg riboflavin, 30.8 mg niacin, 7.92 mg d-pantothenic acid, 0.704 mg folic acid, 2.2 mg pyroxidine, 1.232 mg thiamine, 0.064 mg biotin per kg diet
[4]Enzyme blend provides 1800U/kg of xylanase and 750 FTU/kg of phytase.

Table 4. Withdrawal dietary formulations varying in amino acid density fed to male broilers (d 26 to 35).

Ingredient Name (%)	**TRT 1**	**TRT 2**	**TRT 3**	**TRT 4**
Corn	69.48	66.97	64.43	61.91
Soybean Meal	12.91	14.89	16.87	18.86
Corn DDGS	12.50	12.5	12.5	12.5
Meat & Bone	3.02	3.03	3.03	3.03
Limestone	0.95	0.95	0.96	0.97
Salt	0.33	0.33	0.33	0.33
DL Methionine	0.12	0.14	0.17	0.19
Fat, An./Veg blend	0.26	0.76	1.26	1.76
L-Lysine HCl	0.25	0.25	0.25	0.26
Choline Cl	0.02	0.02	0.01	0.003
L-Threonine	0.40	0.05	0.05	0.06
Trace Mineral Premix[1]	0.04	0.04	0.04	0.04
Coccidiostat[2]	0.05	0.05	0.05	0.05
Ingredient Name (%)				
Vitamin Premix[3]	0.01	0.01	0.01	0.01
Enzyme Premix[4]	0.03	0.03	0.03	0.03

Table 4. Continued.

Nutrient Name				
Calculated Nutrient Content (%)				
AME, Poultry kcal/kg	3102	3102	3102	3102
Moisture	11.68	11.65	11.62	11.59
Crude Protein	16.59	17.32	18.04	18.77
Crude Fat	3.35	3.78	4.21	4.64
Linoleic Acid	1.93	1.99	2.05	2.11
Crude Fiber	3.34	3.35	3.37	3.38
Ash	3.88	3.99	4.10	4.21
Calcium	0.80	0.81	0.82	0.83
Total Phosphorus	0.48	0.49	0.50	0.51
Avail Phosphorus	0.40	0.41	0.41	0.42
Salt	0.39	0.39	0.39	0.39
Sodium	0.18	0.18	0.18	0.18
Na+K-Cl mEq/kg	164.78	175.36	185.97	196.59
Arginine	0.94	0.99	1.05	1.11
Dig Arginine	0.86	0.91	0.97	1.02
Lysine	0.93	0.98	1.04	1.09
Dig Lysine	0.82	0.87	0.92	0.97
Methionine	0.41	0.44	0.47	0.51
Dig Methionine	0.38	0.41	0.45	0.48
Met + Cys	0.72	0.76	0.80	0.84
Dig Met + Cys	0.64	0.68	0.71	0.75
Tryptophan	0.16	0.18	0.19	0.20
Dig Tryptophan	0.14	0.15	0.16	0.17
Threonine	0.64	0.68	0.71	0.75
Dig Threonine	0.55	0.58	0.62	0.65
Isoleucine	0.64	0.67	0.71	0.74
Dig Isoleucine	0.57	0.60	0.63	0.67
Valine	0.80	0.83	0.87	0.90
Dig Valine	0.70	0.73	0.76	0.79
Cystine	0.31	0.32	0.32	0.33
Dig Cystine	0.25	0.26	0.27	0.27
Analyzed Nutrient Content				
Threonine	0.65	0.70	0.72	0.76

Table 4. Continued.

Analyzed Nutrient Content				
Cystine	0.27	0.29	0.29	0.31
Valine	0.80	0.86	0.87	0.91
Methionine	0.38	0.43	0.46	0.50
Leucine	1.53	1.61	1.65	1.72
Isoleucine	0.65	0.69	0.73	0.76
Lysine	0.95	1.02	1.04	1.12
Arginine	0.96	1.05	1.08	1.14
Tryptophan	0.18	0.19	0.20	0.21
Threonine	0.04	0.05	0.05	0.07
Fat	5.5	6.1	5.9	6.0
Ash	4.3	4.4	4.4	4.7
Dry matter	88.8	89.5	89.0	89.2
Calcium	7529 mg/kg	8025 mg/kg	7565 mg/kg	7316 mg/kg
Phosphorus	5090 mg/kg	5513 mg/kg	5399 mg/kg	5213 mg/kg
Sodium	1509 mg/kg	1528 mg/kg	1641 mg/kg	1697 mg/kg

[1] Trace mineral premix added in the finisher yields 90 mg of manganese, 54 mg of total zinc, 2.55 mg of copper, 1.75 mg of iodine, 0.15 mg of total selenium, 0.007 g of *bacillus subtilis*
[2]Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.*
[3]Vitamin premix added at this rate yields 4620 IU vitamin A, 3300 ICU vitamin D_3, 33 IU vitamin E, 0.9 mg vitamin K-3, 0.006 mg B_{12}, 3.96 mg riboflavin, 23.1 mg niacin, 5.94 mg d-pantothenic acid, 0.528 mg folic acid, 1.65 mg pyroxidine, 0.924 mg thiamine, 0.048 mg biotin per kg diet.
[4]Enzyme blend provides 1800U/kg of xylanase and 750 FTU/kg of phytase.

Statistical Analysis

All data were subjected to an Analysis of Variance (ANOVA) using the General Linear Model Procedure (SPSS V22.0) with treatment effects deemed significantly different at $P \leq 0.05$ and separated using Duncan's Multiple Range Test when appropriate.

Results

On d 12 following randomization and the beginning of the experimental period, all BW were similar after feeding the male broilers a common diet (Table 5). After the finisher phase (d25), male broilers fed with the two treatments highest in AA density (TRT 3 and 4) had a significantly higher BW when compared to broilers fed the two lowest levels of digestible Lysine (TRT 1 and 2). At the conclusion of the experiment (d35), significant incremental increases were observed in BW as AA density increased in the experimental diets. Broilers fed the treatment with the highest AA density (1.100/0.970 dLys) yielded the highest BW when compared to the rest of the diets fed during the experiment while broilers fed the lowest level of AA (0.950/0.820 dLys) had the lowest average BW at the conclusion of the experiment. The differences observed in BW can be attributed to the differences observed in body weight gain (BWG) during the two experimental phases (Table 6). During the finisher phase of production, broilers fed the two highest levels of AA density gained significantly more BW as compared to the two lowest levels of AA. During both the withdrawal phase and for the entire period of production, significant incremental increases in BWG were observed with each increased in AA density.

Table 5. Body weight (kg) of male broilers fed diets with varying levels of amino acid densities in the finisher and withdrawal phase diets.

TRT	Amino Acid density	d 12	d 25	d 35
1	0.95/0.82 dig Lysine	0.357	1.364[b]	2.210[d]
2	1.00/0.87 dig Lysine	0.357	1.380[b]	2.270[c]
3	1.05/0.92 dig Lysine	0.356	1.416[a]	2.339[b]
4	1.10/0.97 dig Lysine	0.358	1.428[a]	2.400[a]
	P-value	0.282	<0.001	<0.001
	Pooled SEM	0.001	0.005	0.013
	Pooled CV	1.3	2.6	4.2

a-d means within columns with different superscripts differ significantly at P≤0.05.
n = Treatments contained 15 replicates of 43 birds each.

Table 6. Body weight gain (kg) of male broilers fed diets with varying amino acid densities during the finisher and withdrawal phase diets.

TRT	Amino Acid density	Finisher (d 13 to 25)	WD (d 25 to 35)	Total Period (d 13 to 35)
1	0.95/0.82 dig Lysine	1.007[b]	0.846[d]	1.853[d]
2	1.00/0.87 dig Lysine	1.023[b]	0.890[c]	1.913[c]
3	1.05/0.92 dig Lysine	1.060[a]	0.923[b]	1.983[b]
4	1.10/0.97 dig Lysine	1.070[a]	0.971[a]	2.041[a]
	P-value	<0.001	<0.001	<0.001
	Pooled SEM	0.005	0.009	0.013
	Pooled CV	3.5	7.7	5.0

a-d means within columns with different superscripts differ significantly at P≤0.05.
n = Treatments contained 15 replicates of 43 birds each.

An inverse relationship for mortality corrected feed conversion ratio (FCR) was observed as compared to BWG (Table 7). For the finisher phase (d13 to 35), broilers fed the highest AA density had a significantly lower FCR when compared to all other diets.

Incremental increases in FCR were observed with each decrease in AA density. This relationship continued for all evaluated time periods for FCR to include the withdrawal phase of production, the experimental period (d12 to 35), and cumulatively for the entire grow-out period (d1 to 35). To calculate total FCR from d 1 to 35, total flock data during the common period was used. The impact of AA density on observed FCR was consistent with a decrease of 4 to 5 points (0.04 to 0.05) with each increase in AA density.

Table 7. Mortality corrected feed conversion ratio (FCR) of male broilers fed diets with varying amino acid densities during the finisher and withdrawal phase diets.

TRT	Amino Acid density	Finisher (d 13 to 25)	WD (d 25 to 35)	d 12 to 35	d 1 to 35	d 1 to 35 weight corrected*
1	0.95/0.82 dig Lysine	1.528[a]	1.921[a]	1.708[a]	1.633[a]	1.662[a]
2	1.00/0.87 dig Lysine	1.500[b]	1.827[b]	1.654[b]	1.590[b]	1.599[b]
3	1.05/0.92 dig Lysine	1.456[c]	1.775[c]	1.609[c]	1.552[c]	1.540[c]
4	1.10/0.97 dig Lysine	1.415[d]	1.701[d]	1.556[d]	1.507[d]	1.475[d]
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001
	Pooled SEM	0.006	0.012	0.008	0.006	0.010
	Pooled CV	3.3	5.3	3.7	3.2	5.0

a-d means within columns with different superscripts differ significantly at $P \leq 0.05$.
*** Adjusted to common body weight of 2.3 kg using 32g of BW equal to 1 point of feed conversion.**
n = Treatments contained 15 replicates of 43 birds each.

Feed consumption was less impacted than other performance measures during the experimental period (Table 8). For the finisher phase (d13-25), broilers fed the highest AA density had the lowest FC when compared with all the other diets. An inverse relationship was observed during the WD phase of production with the highest

AA density consuming significantly more feed when compared to the two lowest AA dense diets. No differences (P=0.614) were observed in FC for the total period.

Table 8. Feed consumption (FC - g/bird/day) of male broilers fed diets with varying amino acid densities during the finisher and withdrawal phase diets.

TRT	Amino Acid density	Finisher (d 13 to 25)	WD (d 25 to 35)	Total Period (d 13 to 35)
1	0.95/0.82 dig Lysine	117.9[a]	161.8[b]	137.1
2	1.00/0.87 dig Lysine	117.9[a]	162.6[b]	137.4
3	1.05/0.92 dig Lysine	118.1[a]	164.1[ab]	138.2
4	1.10/0.97 dig Lysine	116.0[b]	165.7[a]	137.7
	P-value	0.012	0.048	0.614
	Pooled SEM	0.314	0.772	0.462
	Pooled CV	2.1	3.7	2.6

a-b means within columns with different superscripts differ significantly at P≤0.05.
n = Treatments contained 15 replicates of 43 birds each.

No differences in mortality were observed during the experiment (Table 9).

Table 9. Mortality (%) of male broilers fed diets with varying amino acid densities during the finisher and withdrawal phase diets.

TRT	Amino Acid density	Total Experimental Period (d 13 to 35)
1	0.95/0.82 dig Lysine	0.93
2	1.00/0.87 dig Lysine	0.31
3	1.05/0.92 dig Lysine	1.55
4	1.10/0.97 dig Lysine	1.24
	P-value	0.226
	Pooled SEM	0.22

a-c means within columns with different superscripts differ significantly at P≤0.05.
n = Treatments contained 15 replicates of 43 birds each.

As expected, processing parameters and weights followed a similar trend to average BW. For live weight of the 6 birds per pen processed, broilers fed with the highest AA density had greater weight when compared to birds fed the two lowest levels of AA. The lowest level of AA density yielded a processed live weight significantly less than all other diets (Table 10). Carcass weight followed the same trend as live weight with the highest AA density yielding results significantly higher than the two lowest AA levels. This trend was the same for weights of breast, drumstick, thigh, tenderloin, and wing. For fat pad weight, broilers fed the diet with the highest AA density had the lowest fat pad weight when compared with all other diets.

Table 10. Processing weights of male broilers fed diets with varying amino acid densities during the finisher and withdrawal phases to 35 d of age.

TRT	Amino Acid density	Live Wt. (kg)	Carcass (WOG) (g)	Fat pad (g)	Breast (g)	Drums (g)	Frame (g)	Thigh (g)	Wing (g)	Tenderloin (g)
1	0.95/0.82 dig Lysine	2.290[c]	1688.8[c]	29.6[a]	393.4[c]	234.9[b]	500.7	257.8[c]	176.3[c]	78.5[c]
2	1.00/0.87 dig Lysine	2.343[b]	1732.5[b]	26.2[b]	417.0[b]	239.4[b]	510.4	263.8[bc]	181.0[b]	81.3[b]
3	1.05/0.92 dig Lysine	2.377[ab]	1760.0[ab]	24.7[b]	427.4[ab]	241.2[ab]	514.8	269.8[ab]	184.0[ab]	85.3[a]
4	1.10/0.97 dig Lysine	2.403[a]	1777.7[a]	22.0[c]	434.4[a]	246.6[a]	515.8	272.9[a]	185.9[a]	86.8[a]
	P-value	<0.001	<0.001	<0.001	<0.001	0.005	0.151	<0.001	<0.001	<0.001
	Pooled SEM	0.011	8.2	0.5	3.1	1.3	2.8	1.4	0.9	0.6
	Pooled CV	3.7	3.6	14.9	5.8	4.3	4.2	4.2	3.8	5.5

a-c means within columns with different superscripts differ significantly at P≤0.05.
n = 6 birds per pen.

Overall carcass, drumstick, thigh, wing, or frame yield percentage was not influenced by AA density (Table 11). Fat pad yield was influenced by AA density as a decrease in AA density yielded greater fat pad percentage. Broilers fed the diet with the highest AA density had the lowest observed fat pad yield which was significantly lower than all other AA densities. Broilers fed the lowest AA density had a significantly higher fat pad yield compared to all other densities. Breast yield was significantly reduced in the lowest AA density diet as compared to all other treatment groups.

Table 11. Processing yield percentage (%) of male broilers fed diets with varying amino acid densities during the finisher and withdrawal phase diets.

TRT	Amino Acid density	Carcass (WOG)	Frame	Fat Pad	Breast	Drums	Thighs	Wings	Tenderloin
1	0.95/0.82 dig Lysine	73.76	29.64	1.76^{a}	23.29^{b}	13.91	15.27	10.44	4.65^{b}
2	1.00/0.87 dig Lysine	73.94	29.47	1.51^{b}	24.06^{a}	13.82	15.23	10.45	4.70^{b}
3	1.05/0.92 dig Lysine	74.05	29.25	1.41^{b}	24.29^{a}	13.71	15.33	10.46	4.85^{a}
4	1.10/0.97 dig Lysine	73.99	29.00	1.24^{c}	24.44^{a}	13.88	15.35	10.46	4.89^{a}
	P-value	0.651	0.145	<0.001	<0.001	0.459	0.684	0.994	<0.001
	Pooled SEM	0.09	0.10	0.03	0.11	0.06	0.04	0.2	0.02
	Pooled CV	0.9	2.6	16.2	3.4	3.1	2.1	1.7	3.5

a-c means within columns with different superscripts differ significantly at $P \leq 0.05$.

n = Treatments contained 15 replicates of 43 birds each.

Discussion

These data show the importance and potential influence that the AA density as adjusted by the level of dig lysine can have in the poultry industry. Amino acid density has a direct correlation with growth performance as evidenced by the positive relationship between AA density and BW, BWG, FCR, FC, and processing yields. Previous authors have shown that a decrease in the AA supply can result in a reduction in feed consumption (Kiraz and Sengul, 2005). However, in the present experiment during the finisher phase, broilers had a higher rate of consumption when they were provided the diet with the lowest AA density. Interestingly, this observation was inversed during the withdrawal phase as broilers fed the diet with the highest AA density consumed feed at the highest rate which follows the observation of (Kiraz and Sengul, 2005). This inverse relationship between the two phases resulted in no differences between all diets when evaluating the entire experiment period (d12 to 35). The inverse in observation may be related to birds' possibly increasing consumption during the finisher phase on low AA density diets in an effort to increased AA intake and meet their growth potential. However, BWG during this period was not similar compared to highest level of AA. Thus, the different in BW may have been the driver for consumption during the withdrawal phase as larger birds consume elevated levels of feed.

Ebling et al. (2013) had a similar approach to the current study in which the authors evaluated the effect of different dietary concentrations of AAs on the performance of two different broiler strains. After feeding different nutritional programs representing varying AA densities and classifying them as low, regular, high and mixed, the authors concluded that the nutritional program with low AA density resulted in the poorest overall performance (Ebling et al., 2013). These observations agree with current study in which the lowest AA density resulted in the lowest BW, highest FCR, and lowest breast meat yield.

At the conclusion of the experiment, the data confirmed results of previously published literature when evaluating varying levels of AA density. Farkhoy et al. (2012) reported that increasing the level of CP in broiler diets resulted in a significant improvement in broiler performance. Those authors reported that the optimal inclusion of synthetic lysine level was 1.5% which was their highest level included in all of the diets (Farkhoy et al., 2012). Data reported from the current experiment overwhelmingly support the hypothesis that an increase in AA density improves live performance parameters and processing yields in 35 d old broiler chickens. However, there were some parameters that when evaluating the entire experimental period were not impacted such as feed consumption, mortality, and dark meat yield.

Corzo and colleagues (2005) evaluated the impact of AA levels for birds grown to d 35 of age. In this experiment, Ross and Cobb 500 broilers were fed either a high AA diet program that included from d 1 to 7 a lysine content of 1.49 %, d 8 to 20 of 1.33%, d 21 to 28 a 1.24 % and from d 29 to 35 a 1.10 % and a low AA that contained 1.35 % lysine from d 1 to 7,1.35 lysine from d 8 to 20, 1.10 % lysine from d 21 to 28, and 0.95% lysine from d 28 to 35 of age. Increasing the AA density in the diet improved flock uniformity and decreased FCR from 1.54 to 1.49 as compared to the low AA diet. The authors did report that differences were observed between broiler strains as well. However, the authors did not report any differences in processing yield observed by varying the AA level. In the current experiment, AA level did influence breast meat yield. Perhaps the reason for this difference between the current study and the reports of Corzo et al., (2005) is the continued increase in genetic potential of the bird and increase in breast meat yield over the past decade.

The results reported in the current experiment are in agreement with Kidd et al., (2005) who reported that broilers fed diets with lower AA density had reduced growth rate, reduced carcass yields, and a higher FCR. However, formulating diets with high or perhaps even excessive levels of AA will impact the economic returns due to increasing diet cost. Moreover, Cabel and Waldroup (1991) report that feeding excessive CP levels beyond that required for maximal BW gain can result in less fat deposition and improve FCR. These observations were also observed in the current experiment with improvements in FCR observed with increasing dietary AA content and reduction in fat pad weight and yield.

In conclusion, these data support the concept of higher AA density diets positively impacting performance parameters and yield in young broiler chickens. Incremental increases in BW and FCR of 60 g and 0.04, respectively. Additionally breast yield was increased with higher levels of AA density. However, producers may choose not to feed the highest levels of AA dependent on diet cost and the commodity price of meat. Regardless, these data will assist integrators in making decisions based on their internal economic models for AA levels of diets in their production systems.

CHAPTER IV

EXPERIMENT II: IMPACTS OF VARIABLE AMINO ACID DENSITIES DURING THE WITHDRAWAL PHASE ON GROWTH PERFORMANCE, MEAT YIELD, AND MEAT QUALITY

Introduction

Experiment II was conducted to evaluate the effect of varying AA densities during the final 2 phases of growth which were provided to the broilers at d 28 of age following a common starter and finisher diet. According to many studies, the inclusion of a higher AA density in the diet will improve broiler performance. However, there is not an exact amount of dig lysine inclusion for a bird response in which their maximum potential is expressed. The main idea of this experiment was finding the AA density content that make broiler chickens obtain the best performance.

Materials and Methods

The experiment was conducted in a complete randomized block design with 4 dietary treatments during a 49 d grow out. The experimental treatments varied in the level of digestible AA density which was adjusted by increasing the level of digestible lysine as all diets were formulated based in the ideal protein concept using lysine as the ratio AA. Diets were corn and soybean meal based and also included meat and bone meal and dried distillers' grains with solubles. All broilers were fed a common starter and finisher diet from d 1 to d 28 of age. The starter diet was fed from d 1 to 14 (Table

12) and the finisher diet fed from d 14 to 28 of age (Table 12). The experimental diets were fed beginning on 28 d of age. The digestible lysine in the withdrawal 1 (WD1) diet ranged from 0.910 to 1.047% which was fed from d 28 to 37 of age and the withdrawal 2 (WD2) diet ranged from 0.850 and 0.978% in digestible lysine and was fed from d 38 to 48 (Table 13). Following diet manufacturing, composite samples were collected and analyzed for total AA content (Table 14 and 15).

Animals and Management Practice

On day of hatch, Cobb 500 male broiler chicks were randomly allotted to floor pens and dietary treatments based on initial body weight. The experiment consist of a total of 60 pens with 15 blocks of 4 contiguous pens, each containing 43 birds at d 1 of age that was reduced to 36 birds per pen at d 28 at which time bird were fed experimental diets. Following the reduction of birds to 36 at d 28, all treatments contained statistically equivalent average bird weights. Feed and water were available *ad libitum.* Chicks were housed at appropriate temperature for their age. All broilers and the remaining feed were weighed by pen at d 14, 28, 38, and 48, which corresponded to dietary phase changes. Average body weight (BW), body weight gain (BWG), mortality corrected feed conversion ratio (FCR) and feed consumption (FC) were determined for each feeding phase. Birds were weighed on the evening of d 48, and following an 8 h feed withdrawal time, 7 birds per pen were processed for the determination of carcass and breast yield, and presence of breast muscle myopathies to include woody breast and white striping. The fillets were scored individually for WS and WB based on severity.

White striping was recorded on a 4 point scale of severity, a score of 0 represents no striping, score 1 (mild) which is a noticeable striping, score 2 (moderate) is a noticeable striping covering the breast surface extensively and a score 3 (severe) is very thick stripes extended over the breast surface (R. Bailey et al. 2015). Similarly, for woody breast the scoring method started with 0-being normal, 1-moderate light, 2-moderate and 3-severe (Cruz et al. 2016). All animal husbandry procedures were conducted in accordance with an approved animal use protocol (IACUC).

Diets

Table 12. Ingredient profiles of the common diets supplemented during the starter (d 1 to 14), and finisher (d 14 to 28) dietary phases.

Ingredient Profile	**Starter (%)**	**Finisher (%)**
Corn	58.79	64.90
Soybean Meal	28.05	21.81
Meat and bone meal	5.19	4.89
Corn DDGS	5.00	5.00
Fat, An /Veg blend	1.18	1.62
Limestone	0.59	0.61
Salt	0.35	0.36
DL Methionine	0.30	0.25
L-Lysine HCl	0.17	0.21
Choline Cl	0.10	0.09
Trace minerals Premix[1]	0.08	0.06
L-Threonine	0.06	0.06
Copper Sulfate	0.05	0.05

Table 12. Continued.

Ingredient Profile	**Starter (%)**	**Finisher (%)**
Coccidiostat[2]	0.05	0.05
Vitamin Premix[3]	0.03	0.02
Enzyme Premix[4]	0.03	0.03
Calculated Nutrient Content	**Starter (%)**	**Finisher (%)**
Protein	22.62	19.96
AME, Poultry (kcal/kg)	3025	3113
Crude Fiber	2.88	2.79
Calcium	0.88	0.84
Digestible Phosphorus	0.44	0.42
Digestible Methionine	0.617	0.537
Digestible Lysine	1.18	1.04
Digestible Threonine	0.767	0.679
Crude Fat	4.057	4.516
Sodium	0.18	0.18

[1] Trace mineral premix added in the starter yields 180 mg of manganese, 108 mg of total zinc, 5.1 mg of copper, 3.51 mg of iodine, 0.3 mg of total selenium, 0.013 g of *bacillus*

[1]Trace mineral premix added in the grower yields 150 mg of manganese, 90 mg of total zinc, 4.25 mg of copper, 2.92 mg of iodine, 0.25 mg of total selenium, 0.011 g of *bacillus subtilis*

[2]Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina*, *Eimeria maxima*, *Eimeria brunetti and Eimeria mivati*.

[3] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet.

[3] Vitamin premix added at this rate yields 6160 IU vitamin A, 4400 ICU vitamin D_3, 44 IU vitamin E, 1.2 mg vitamin K-3, 0.008 mg B_{12}, 5.28 mg riboflavin, 30.8 mg niacin, 7.92 mg d-pantothenic acid, 0.704 mg folic acid, 2.2 mg pyroxidine, 1.232 mg thiamine, 0.064 mg biotin per kg diet.

[4]Enzyme blend provides 1800U/kg of xylanase and 750 FTU/kg of phytase.

Table 13. Ingredient profiles and calculated nutrient content of the different treatments for withdrawal 1 (d 28 to 38), and withdrawal 2 (d 38 to 48) supplemented during the trial.

Ingredients	**Withdrawal 1 (%)**				**Withdrawal 2 (%)**			
	Trt 1	Trt 2	Trt 3	Trt 4	Trt 1	Trt 2	Trt 3	Trt 4
Corn	70.70	67.86	65.08	62.24	74.53	71.87	69.28	66.63
Soybean Meal	18.71	21.16	23.56	26.01	15.49	17.79	20.03	22.32
Meat and bone meal	2.53	2.44	2.36	2.27	2.64	2.56	2.48	2.40
Corn DDGS	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fat, An /Veg blend	1.40	1.89	2.37	2.86	0.72	1.18	1.62	2.08
Limestone	0.67	0.67	0.66	0.66	0.68	0.68	0.67	0.67
Salt	0.40	0.40	0.40	0.40	0.39	0.40	0.40	0.40
DL Methionine	0.17	0.19	0.21	0.22	0.15	0.16	0.18	0.19
L-Lysine HCl	0.20	0.18	0.16	0.15	0.22	0.21	0.19	0.17
Choline Cl	0.04	0.03	0.02	0.01	0.05	0.04	0.03	0.02
Trace Mineral Premix[1]	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03
L-Threonine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Copper Sulfate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coccidiostat[2]	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00
Vitamin Premix[3]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Enzyme Premix[4]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated Nutrient Content								
Protein	17.58	18.48	19.35	20.25	16.41	17.25	18.07	18.91
AME, Poultry kcal/kg	3157	3157	3157	3157	3157	3157	3157	3157
Crude Fiber	2.77	2.80	2.82	2.84	2.75	2.77	2.79	2.81

Table 13. Continued.

Calculated Nutrient Content								
Calcium	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Digestible Phosphorus	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Digestible Methionine	0.44	0.46	0.49	0.51	0.40	0.42	0.45	0.47
Digestible Lysine	0.91	0.96	1.00	1.05	0.85	0.89	0.94	0.98
Digestible Threonine	0.61	0.64	0.67	0.70	0.57	0.60	0.63	0.66
Crude Fat	4.18	4.59	4.98	5.39	3.61	3.99	4.36	4.74
Sodium	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[1] Trace mineral premix added in the finisher yields 90 mg of manganese, 54 mg of total zinc, 2.55 mg of copper, 1.75 mg of iodine, 0.15 mg of total selenium, 0.007 g of *bacillus subtilis*

[2]Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella*, *Eimeria necatrix*, *Eimeria acervulina*, *Eimeria maxima*, *Eimeria brunetti and Eimeria mivati.*

[3]Vitamin premix added at this rate yields 4620 IU vitamin A, 3300 ICU vitamin D_3, 33 IU vitamin E, 0.9 mg vitamin K-3, 0.006 mg B_{12}, 3.96 mg riboflavin, 23.1 mg niacin, 5.94 mg d-pantothenic acid, 0.528 mg folic acid, 1.65 mg pyroxidine, 0.924 mg thiamine, 0.048 mg biotin per kg diet

[4]Enzyme blend provides 1800U/kg of xylanase and 750 FTU/kg of phytase.

Table 14. Analyzed amino acid content of the different treatments for withdrawal 1 (d 28 to 38) supplemented during the trial.

Amino Acid (%)	**Trt 1**	**Trt 2**	**Trt 3**	**Trt 4**
Threonine	0.66	0.72	0.71	0.79
Proline	1.11	1.16	1.16	1.23
Cysteine	0.25	0.26	0.27	0.28
Valine	0.81	0.85	0.90	0.99
Methionine	0.38	0.43	0.45	0.46
Isoleucine	0.69	0.73	0.78	0.88
Leucine	1.54	1.61	1.64	1.78
Phenylalanine	0.80	0.84	0.88	0.98
Lysine	1.07	1.12	1.13	1.25
Arginine	1.01	1.07	1.12	1.26

Table 14. Continued.

Amino Acid (%)	Trt 1	Trt 2	Trt 3	Trt 4
Tryptophan	0.21	0.24	0.23	0.26
Total	16.52	17.51	17.95	19.68
Crude Protein	17.72	18.74	19.26	20.80

* Percentage N X 6.25. W/W%= grams per 100 grams of sample.
Results are expressed on an "as is" basis unless otherwise indicated.

Table 15. Analyzed amino acid content of the different treatments for withdrawal 2 (d 38 to 48) supplemented during the trial.

Amino Acid (%)	Trt 1	Trt 2	Trt 3	Trt 4
Threonine	0.64	0.69	0.68	0.75
Proline	1.07	1.12	1.12	1.16
Cysteine	0.25	0.27	0.27	0.28
Valine	0.78	0.83	0.84	0.91
Methionine	0.39	0.40	0.42	0.45
Isoleucine	0.66	0.71	0.72	0.79
Leucine	1.48	1.57	1.56	1.68
Phenylalanine	0.76	0.81	0.82	0.90
Lysine	0.98	1.08	1.06	1.15
Arginine	0.96	1.03	1.04	1.17
Tryptophan	0.20	0.20	0.20	0.21
Total	15.84	16.80	16.86	18.35
Crude Protein	17.06	17.70	18.04	18.66

* Percentage N X 6.25. W/W%= grams per 100 grams of sample.
Results are expressed on an "as is" basis unless otherwise indicated.

Statistical Analysis

All data were subjected to an Analysis of Variance (ANOVA) using the General Linear Model Procedure (SPSS V22.0) with means deemed significantly different at $P \leq 0.05$ and separated using Duncan's Multiple Range Test when appropriate.

Results

On d 28 following randomization, BW were similar across all treatments as birds were fed a common starter and finisher diet (Table 16). Following the WD1 phase (d38), broilers fed the diet with the highest AA density (1.047% dLys) had a significantly heavier BW when compared to the broilers fed the diet with the lowest AA density (0.910% dLys) during this phase with 0.956% and 1.001% dLys being intermediate. On d48 following the WD2 phase, broilers that were fed the highest AA density diet (1.047/0.978% digestible Lysine) resulted in the heaviest final BW which was significantly higher than the broilers fed the two lowest AA density diets which were 0.910/0.850 dLys and 0.956/0.893 dLys.

Table 16. Body weight (kg) of male broilers fed diets with different amino acid densities in four treatments during withdrawal 1 and 2 phase diets (d 28 to 48).

TRT	Amino Acid density	28 d	38 d	48 d
1	0.910/0.850 dig Lysine	1.640	2.673[b]	3.504[b]
2	0.956/0.893 dig Lysine	1.631	2.696[ab]	3.518[b]
3	1.001/0.935 dig Lysine	1.642	2.696[ab]	3.532[ab]
4	1.047/0.978 dig Lysine	1.632	2.725[a]	3.578[a]
	P-value	0.257	0.022	0.052
	Pooled SEM	0.006	0.010	0.020
	Pooled CV	1.1	1.8	4.3

a-c means within columns with different superscripts differ significantly at P≤0.05.
n = Treatments contained 15 replicates of 43 birds each.

The differences observed in average BW were as a result of differences observed in BWG during the experimental period (Table 17). During the WD1 phase (d28-38), broilers fed the diet with the highest AA density (1.047% dLys) had an increased BWG when compared to broilers fed the lowest AA density diet (0.910% dLys) and trt 3 (1.001% dLys). No differences (P≤0.05) were observed in BWG for the WD2 phase (d38-48). Cumulatively for the WD phases (d28-48), broilers fed the diet with the highest AA density had an increased BWG when compared to broilers fed all other diets.

Table 17. Body weight gain of male broilers fed d with different amino acid densities in four treatments during withdrawal 1 and 2 phase diets (d 28 to 48).

TRT	Amino Acid density	WD 1 (d 28 to 38)	WD 2 (d 38 to 48)	Total Period (d 28 to 48)
1	0.910/0.850 dig Lysine	1.033^{c}	0.831	1.864^{b}
2	0.956/0.893 dig Lysine	1.065^{ab}	0.823	1.881^{b}
3	1.001/0.935 dig Lysine	1.054^{bc}	0.824	1.888^{b}
4	1.047/0.978 dig Lysine	1.093^{a}	0.854	1.946^{a}
	P-value	0.003	0.416	0.027
	Pooled SEM	0.010	0.013	0.020
	Pooled CV	5.0	11.0	6.6

a-c means within columns with different superscripts differ significantly at P≤0.05.
n = Treatments contained 15 replicates of 43 birds each.

During the WD1 phase (d28-38), broilers fed the diet with the highest AA density had a significantly lower FCR when compared to all other diets while broilers fed the lowest AA density diet has a significantly higher FCR compared to all other treatments (Table 18). During the WD2 phase (d38 to 48), broilers fed the diet with the highest AA density had a significantly lower FCR when compared to broilers fed the two lowest AA densities. When combining the two WD phases for evaluation of the entire experimental period (d28 to 48), broilers fed the highest AA density diet had a significantly lower FCR as compared to broilers fed all other diets. Additionally, broilers fed the lowest AA density diet had a significantly higher FCR compared to all other diets. Cumulative FCR was calculated using total flock data for d 1 to 28 as all broilers were fed a common starter and finisher diet. Cumulative FCR for d1 to 38 was significantly reduced as the AA density increased in the diet. At the conclusion of the experiment, the highest AA density diet significantly decreased FCR compared to all other treatments while the lowest AA dense diet had a significantly higher FCR compared to all other treatments.

Table 18. Mortality corrected feed conversion ratio (FCR) of male broilers fed with different amino acid densities in four treatments during withdrawal 1 and 2 phase diets (d 28 to 48).

TRT	Amino Acid density	WD 1 (d 28 to 38)	WD 2 (d 38 to 48)	Total Period (d 28 to 48)	d 1 to 38	d 1 to 48	d 1 to 48 weight corrected *
1	0.910/0.850 dig Lysine	1.745[a]	2.341[a]	2.005[a]	1.660[a]	1.749[a]	1.748[a]
2	0.956/0.893 dig Lysine	1.677[b]	2.342[a]	1.958[b]	1.649[ab]	1.725[b]	1.719[b]
3	1.001/0.935 dig Lysine	1.661[b]	2.318[ab]	1.935[b]	1.622[bc]	1.714[b]	1.706[b]
4	1.047/0.978 dig Lysine	1.593[c]	2.233[b]	1.867[c]	1.615[c]	1.678[c]	1.654[c]
	P-value	<0.001	0.051	<0.001	0.021	<0.001	<0.001
	Pooled SEM	0.010	0.023	0.011	0.010	0.005	0.010
	Pooled CV	4.5	7.3	4.1	4.7	2.2	4.7

a-c means within columns with different superscripts differ significantly at P≤0.05.
*** Corrected using 32 g (0.07 lbs) of BW equal to 1 point (0.010) of FCR.**
n = Treatments contained 15 replicates of 43 birds each.

During the WD 1 phase (d28-38) of production, broilers fed diets with the two lowest AA densities consumed feed at a significantly higher rate as compared to the two treatments with the highest AA density (Table 19). No differences in feed consumption (P≤0.05) were observed during the WD 2 phase of production (d38-48). Cumulatively for the entire experimental period, broilers fed the lowest AA density diet consumed feed at a significantly higher rate as compared to broilers fed the two diets with the highest AA density diets.

Table 19. Feed consumption (FC - g/bird/day) of male broilers fed with different amino acid densities in four treatments during withdrawal 1 and 2 phase diets (d 28 to 48).

TRT	Amino Acid density	WD 1 (d 28 to 38)	WD 2 (d 38 to 48)	Total Period (d 28 to 48)
1	0.910/0.850 dig Lysine	180.5[a]	188.8	125.1[a]
2	0.956/0.893 dig Lysine	177.8[a]	188.3	124.2[ab]
3	1.001/0.935 dig Lysine	174.6[b]	184.5	123.1[b]
4	1.047/0.978 dig Lysine	172.6[b]	186.6	122.8[b]
	P-value	<0.001	0.319	0.018
	Pooled SEM	0.9	1.6	0.5
	Pooled CV	2.6	6.4	3.2

a-c means within columns with different superscripts differ significantly at P≤0.05.
n = Treatments contained 15 replicates of 43 birds each.

No differences were observed in live weight of sampled broilers or chilled carcass weight at the time of or following processing (Table 20). Carcass yield percentage was positively influenced with increased AA density with the highest AA dense diet have a significant increase in carcass yield compared to the lowest AA diet Similar to carcass yield, breast weight and breast meat yield were influenced by AA content of the diet. Broilers fed the diet with the highest AA density had a significant increase in breast weight when compared to broilers fed all other diets. This resulted in the highest AA level fed broilers yielding a significant increase in breast meat yield compared to all other treatments. Broilers fed the second highest AA density diet had a significant increase in breast yield when compared to broilers fed the two lowest AA density diets.

Table 20. Processing weights (kg) and yield (%) of male broilers fed with different amino acid densities in four treatments during withdrawal 1 and 2 phase diets (d 28 to 48).

TRT	Amino Acid density	Live Wt	Carcass Wt	Carcass Yield (%)	Breast Weight	Breast Yield (%)
1	0.910/0.850 dig Lysine	3.463	2.733	78.94^{b}	0.726^{b}	26.41^{c}
2	0.956/0.893 dig Lysine	3.461	2.742	79.28^{ab}	0.728^{b}	26.51^{c}
3	1.001/0.935 dig Lysine	3.450	2.744	79.57^{ab}	0.745^{b}	27.10^{b}
4	1.047/0.978 dig Lysine	3.500	2.789	79.71^{a}	0.773^{a}	27.71^{a}
	P-value	0.503	0.151	0.048	<0.001	<0.001
	Pooled SEM	0.020	0.015	0.12	0.007	0.15
	Pooled CV	4.5	4.0	1.1	6.7	4.0

a-c means within columns with different superscripts differ significantly at P≤0.05.
*** Corrected using 32 g (0.07 lbs) of BW equal to 1 point of FCR.**
n = Treatments contained 15 replicates of 43 birds each.

Amino acid density did not influence the average white striping score observed in the breast tissue following processing (Table 21). However, AA density was related to average score associated with woody breast. Broilers fed the diet with the highest AA density had a significant increase in average woody breast score as compared to broilers fed the two lowest levels of AA.

Table 21. White striping and woody breast incidence of male broilers fed with different amino acid densities in four treatments during withdrawal 1 and 2 phase diets (d 28 to 48).

TRT	Amino Acid density	White Striping	Woody Breast
1	0.910/0.850 dig Lysine	1.19	1.07^{c}
2	0.956/0.893 dig Lysine	1.15	1.13^{bc}
3	1.001/0.935 dig Lysine	1.16	1.29^{ab}
4	1.047/0.978 dig Lysine	1.23	1.45^{a}
	P-value	0.612	0.002
	Pooled SEM	0.03	0.05
	Pooled CV	16.8	21.1

a-c means within columns with different superscripts differ significantly at P≤0.05.
The score was from 0 to 3 as severity increased.

Variation in AA density did not impact WS percentage profiles in broilers raised through d 48 of age (Table 22). However, the different AA densities showed that broilers presented a wide-ranged scoring percentage for the evaluation of WB in the trial which influenced the final differences in WB values (Table 23).

Table 22. Distribution of percentages by treatment for scores on white striping of broilers fed different amino acid density diets.

TRT	Amino Acid density	Score	White striping
1	0.910/0.850 dig Lysine	0	5.15
		1	51.54
		1.5	27.83
		2	10.30
		2.5	5.15
2	0.956/0.893 dig Lysine	0	9.47
		1	54.73
		1.5	16.84
		2	13.68
		2.5	5.26
3	1.001/0.935 dig Lysine	0	7
		1	55
		1.5	24
		2	12
		2.5	2
4	1.047/0.978 dig Lysine	0	7.14
		1	48.97
		1.5	23.46
		2	13.26
		2.5	7.14

Table 23. Distribution of percentages by treatment for scores on woody breast of broilers fed different amino acid density diets.

TRT	Amino acid density	Score	Woody breast
1	0.910/0.850 dig Lysine	0	27.6
		1	42.9
		1.5	0
		2	22.9
		3	6.7
2	0.956/0.893 dig Lysine	0	23.8
		1	44.8
		1.5	1.9
		2	21.9
		3	7.6
3	1.001/0.935 dig Lysine	0	18.1
		1	46.7
		1.5	0
		2	22.9
		3	12.4
4	1.047/0.978 dig Lysine	0	16.2
		1	35.2
		1.5	0
		2	35.2
		3	13.3

Discussion

The objective of the current experiment was to determine the magnitude of the improvement in growth parameters and potential impact on breast meat yield with increasing levels of AA. It is well documented that AA content of the diet will influence growth performance, however increasing AA density is an expensive dietary alteration. Additionally, increased growth rate has been correlated with increased incidence of breast muscle myopathies. Therefore, data must be generated to quantify the potential improvement that can be observed with increasing AA density to allow for producers to evaluate economic models based on ingredient cost and product prices to determine to most economical strategy in raising meat type broilers. Additionally, the correlation between growth rate and breast muscle myopathy must be evaluated and considered as an increased severity could potentially decrease meat value.

The impact of increased protein and AA density and the benefits on performance was reported by Azarnik et al. (2010) who fed two different proteins levels and four feed restriction levels in isoenergetic diets and reported that feed restriction reduced BWG and FC significantly. Moreover, it was not found any difference in the rest of the measured parameters for performance in birds fed with diets containing different protein levels. Corzo et al. (2010) reported that live performance and breast meat yield of Cobb 500 broilers were enhanced when feeding diets higher in AA density, particularly in the late phases of production. These same observations were noted during the current experiment in which AA density was varied in the two late phases of production (d 28 to 48) as increases in BW, improvements in FCR and increases in breast meat yield were observed with increasing AA density. Broilers fed diets containing moderate AA density in the current experiment were intermediate in comparison with the low and high AA density diets for many of the performance parameters measured. Evaluated parameters in the current trial that were impacted due to varying the AA density of the WD1 and WD2 phases included BWG, FCR, FC, carcass yield, and breast yield.

In an experiment conducted by Corzo et al. 2010, the response of Cobb 500 x Cobb 500 broilers with different AA regimens was evaluated. Three dietary treatments included a high, moderate, and low AA density. Diets were fed during three phases consisting of a starter (d0 to 14), grower (d14 to 28), and finisher (d28 to 42). In the starter phase, digestible lysine levels were 1.25, 1.16, and 1.07 respectively, for the high, moderate, and low density diets. Furthermore, during the grower phase, digestible lysine values were 1.12 (high), 1.03 (moderate), and 0.95 (low). During the last phase of the trial (d 28-42), digestible lysine levels were 1.05, 0.97, and 0.88, respectively, for the high, moderate, and low AA density diets. The AA densities in this experiment were similar to diets fed in the current study. Moreover, the dramatic response to increased AA density in the final stages of production by Cobb x Cobb 500 broilers indicate that providing high AA density diets early during growout may not be justified. This agrees with the data obtained from the current experiment as performance and breast yield were highly sensitive and positively influenced with increased AA density during the final two stages of production. Not having to feed increased AA density during the starter phase and still be able to achieve maximal growth and yield would be a cost savings to the industry as high protein starter diets already represent the highest priced diet consumed during poultry production. Additional research should be conducted to determine if the inverse relationship is possible. Feeding increased AA density during the first two phases of production could result in similar final responses as targeting the final two stages; it can be a potential strategy to cut cost as feed consumption is much lower during the first two stages of growout as compared to the final two stages.

Recently, the increased incidence and severity of breast muscle myopathies have warranted significant interest from customers, producers, and the scientific community. The potential exists that such myopathies could result in a reduced price to the producer for breast muscle tissue due to decreased consumer preference. No impact of increased AA density was observed on white striping of breast tissue in the current experiment, however, Bilgili et al., (2014) reported that a reduction in AA density reduced the incidence of sever white striping in filets at 42 and 56 d of age. While the current data does not agree with Bilgili et al., (2014) regarding white striping, similar impacts on woody breast were observed between their experiment and the current experiment. A reduction of 5% of severe woody breast was reported with a reduction in 20 % below the requirement in AA density (Bigili et al., 2014). In the current study, a difference of 7% was observed in filets showing severe woody breast between the lowest and highest AA density diets. This separate was responsible for the reduction in average woody breast score observed in the current experiment.

White striping and woody breast are two breast tissue myopathies that have recently impacted the industry in a negative manner and have been related to increased growth rate broilers. Aside from characterizing the functional properties and correlating these myopathies with growth rate, little research has been conducted to determine factors that either cause or mitigate these myopathies. According to Vasquez Anon (2015), fast growing birds and heavier final weights compromise the integrity of the breast muscle in birds. There is a higher correlation between these myopathies and some minerals and vitamins more specifically vitamin E when compared to other nutrients. Prior to this experiment, little research had been conducted to study the increased AA density and breast tissue myopathies. However, it is common for white striping and woody breast to occur in breast tissue from broilers with larger breasts (Kuttapan et al., 2013). Thus, since AA density influences breast meat yield, the assumption had been made linking the two prior to this experiment.

These data confirm that the modern commercial broiler is still very sensitive to variations in AA density as evidenced by the 70 g difference in BW and the 7 point (0.070) separation in FCR observed in this experiment. These data can be used by poultry producers to determine potential return on investment of increasing the AA density based on the improvement in performance. Dependent upon ingredient costs and product prices, it may not be economical for producers to feed elevated levels of AA. Additionally, if in fact increasing AA density does increase the percentage of breast filets showing severe signs of woody breast, producers may decide not to feed the highest level of AA evaluated in this experiment. Continued research needs to be conducted to determine if AA density is in fact responsible for elevated levels of woody breast or if another factor is responsible.

CHAPTER V

CONCLUSION

Poultry nutritionists focus on formulating diets that are adequate in nutrient level to support growth and development of the bird. Each individual may chose different strategies to achieve the same end goal of a healthy, productive broiler chicken. Strategies are focused on the economics of production cost and profitability associated with product pricing. Two of the main sectors of the industry are a small fast food bird used in many restaurants and a big bird aimed at further processing market.

Amino acids are the building blocks of protein and thus breast tissue in broiler chickens. Amino acids represent a costly portion of a poultry diet, and one potential strategy to reduce diet cost is the reduction of AA density. Historically, broilers have responded positively with higher levels of AA evidenced by increased growth rate, improved feed efficiency, and elevated breast meat yield. However, the vast majority of research in the area of growth performance and AA concentration is approaching or is more than a decade old. Genetic advancement of birds has continued in this period, warranting an investigation into the impact of AA density in the modern commercial broiler. In addition, the recent increase in the incidence of breast muscle myopathies, specifically woody breast and white striping, in the large commercial broilers has become an issue. This issue has been connected with increased breast weights, and thus should be included in any evaluation that is targeting increased breast yield in large broilers. Therefore, this research program investigated the impacts of varied AA density in the final two dietary phases in small and large broilers and the impact on growth performance and processing yields.

In experiment I, four dietary AA densities were evaluated in 35 d of age broilers with the birds provided the experimental diets from d 12 to 35 of age. During the experiment, the highest AA dense diet (1.10/0.97 dLys) resulted in the greatest performance level Body weight and BWG was influenced linearly with AA density. An inverse relationship was observed with AA density and FCR, with the highest AA content diet yielding the lowest FCR. The elevated BW led to increases in carcass part weights including breast weight. At the conclusion of the experiment, the separation in performance between the highest (1.10/0.97 dLys) and lowest (0.95/0.82 dLys) AA density treatments was 190 g of BW, 13 points of FCR (0.13), and 1.2% breast yield.

Experiment II followed a similar approach to experiment 1 as multiple AA densities were fed to broilers during the final two dietary phases of growout. However, growout was extended from 35 d of age to 48 d of age and the common diet was fed for a longer period of time until day 28 of age. Regardless of the differences between the two experiments, a similar response was observed as increasing AA density increased BW, improved FCR, and increased breast meat yield. However, the increased breast meat yield that was observed in Experiment 2 was also associated with an increased in woody breast score. This is not to say that breast yield drives the presence of woody breast, however, this response was observed. However, reports that increasing incidence of white striping is related to increases in woody breast were not observed in this experiment. However, this breast tissue myopathy is currently garnering a significant amount of interest and could potentially lead to discounts of afflicted breast tissue. If in fact, woody breast is related to increases or an imbalance of AA in the diet, consideration must be given, especially, if product showing severe signs will be discounted.

In conclusion, continued genetic selection has not reduced the sensitivity of the modern commercial broiler to adjustments to AA concentration in the diet, and in fact may have increased the potential impact with these high yielding strains. However, the improvements in growth and yield do come with a cost and at times may not warrant consideration. The costs that are attributed are increases in diet cost driven by the addition of more soybean meal content in the diet which is the main source of protein in the feed and the potential for reduced meat quality in large broilers. These data can be used by nutritionists to gauge improvements that can be expected with incremental increases in AA density and inserted into economical models to determine the proper AA density needed to maximize profitability.

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