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Development of Arkansas Net Energy Equation

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Development of Arkansas Net Energy Equation

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ABSTRACT

The modern broiler is growing at a rapid rate generating tremendous amounts of heat. A sensitive Net Energy (NE) system is needed to measure body heat production (HP) generated primarily by daily maintenance and synthesis and degradation of myofibrillar and sarcoplasmic protein. The first two chapters present evaluation of the classic way to calculate NE versus a new methodology; the Arkansas NE (Ark NE) system, with birds from two genetic lines fed diets with different AA content or different ME content in two different environmental temperatures. Utilizing together the Net Energy maintenance (NEm), determined from indirect calorimetry, and Net Energy gain (NEg), evaluated through DEXA, provide valuable information about type of gain and current broiler genetics. This combination provides a deeper understanding of diet NE, rather than the small indigestible fraction differences which have been only measured through heat increment (HI). Taking advantage of understanding the genetics and appropriate environment is an advantage of NE formulation. In addition, protein, the source and type of fat (fat vs. starch vs. protein) makes a difference in how energy is metabolized by broilers. Research in feeding broilers exogenous composite enzyme, either alone or in combination with exogenous amylase, showed protein is primarily going to go into retained energy while energy coming from carbohydrate is going to be in a functional form, i.e. fuel for metabolic processes. Therefore, providing energy in the appropriate amount but also in the correct metabolic form will manipulate the amount of protein or fat deposited and ultimately the retained energy (NEg). Net energy calculations proved to be a sensitive way to evaluate enzyme addition to broiler diets. Lastly, utilizing digestible amino acids and other nutrient contents of the ingredients, even undesirable qualities, can be used to understand net energy calculations.

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I. INTRODUCTION

INTRODUCTION

The major cost of poultry production is the cost of feed. This cost accounts for about 70% of the total cost of broiler production with energy making up the majority of this cost. Gross energy (GE) of feed is not completely utilized by birds. As dietary energy passes through the gastrointestinal tract a portion of the calories will be lost. Some of this is lost as fecal and urinary energy. The portion left is known as metabolizable energy (ME) and is currently used to formulate poultry diets due to its relative ease of calculation. ME of feeds can be further refined to net energy (NE) that takes into account the energy loss known as the heat increment. Heat increment (HI) is a term used to encompass energy lost during ingestion, digestion, metabolism, and excretion and is difficult to assess. The benefit of refining the flow of energy to net energy (NE) is because the dietary energy remaining is the net energy of maintenance and production. The dietary NE is a precise energy value that the bird uses for production, whether the energy is for eggs or meat, and the unseen costs of maintenance.

Modern broilers are very efficient in transforming ingredients into more valuable protein for human consumption and with the increase in demand for poultry products, formulating diets that meet the needs of the broilers is critical. Modern broilers also grow at a rapid rate which generate a tremendous amount of heat. Formulating diets on a NE basis is advantageous as this system accounts for energy lost as heat and more accurately predicts body weight gain (BWG) and feed conversion ratio (FCR) (Wu et al., 2018). A sensitive NE system is needed to measure the heat production primarily caused by maintenance and protein accretion by optimizing protein intake of digestible AA and energy. The classic way to calculate NE of feed is to determine ME and subtract heat increment (HI). This classic method to calculate NE only assesses the value of HI which only accounts for a small portion of dietary energy that is lost from ME (Farrell, 1974).

The classic way of analyzing NE can be misleading as more calorie efficiency (NE/ME) is given to fat deposition than to lean mass deposition. This current system does not take into consideration the type of production or gain that is occurring in the animal and mainly penalized protein accretion because of HI generated from nitrogen and carbon loss through uric acid production. Protein calories should be more important than fat calories for meat production.

A new NE system is proposed called the Arkansas NE (Ark NE), where indirect calorimetry is utilized to understand net energy for maintenance (NEm) and the use of dual energy X-ray absorptiometry (DEXA) is utilized to calculate protein and fat gain. These tools in combination are powerful techniques to study feed and feed ingredients for broilers that can improve the understanding of energy utilization in today's broiler.

II. LITERATURE REVIEW

INTRODUCTION

Proper nutrition is important to every aspect of poultry production. Due to the slim margins in animal agriculture, feed efficiency is a key metric for various levels in the highly vertically integrated US poultry industry as an indicator of overall performance and economic feasibility. Management, genetics and environment all are contributing factors to the success of global poultry meat and egg production. Nutrition influences not only the rate of gain but also the efficiency of which the bird utilizes feed for protein accretion in the case of broilers or the production of table and hatching eggs in the case of layers and breeders, respectively. Furthermore, nutrition has been shown to influence fertility, immunity, and the quality of the final products of poultry production. Since feed cost represents 70 percent of live production costs (Skinner et al., 1992), optimizing feed efficiency is becoming more of a priority. Due to the rapid changes in poultry genetics improving bird performance and shifting carcass composition to promote production of lean and wholesome protein, nutritionists must adapt to provide diets which contain optimal nutrient profiles at economically feasible diet costs. In addition, feed ingredients also shift, creating new challenges and potential tools (e.g. bi- and coproducts or exogenous enzymes).

There are at least 20 compounds essential for poultry (Pesti, 2005). A nutrient is considered essential if when it is removed from the diet there is impairment in the performance of the animal (NRC, 1994). Traditionally, there are six main nutrients essential for life: water, protein, carbohydrates, fats, vitamins and minerals, but an honorary seventh, energy, is sometimes included. For the best performance, nutrients need to be available in the balance with each other in the diet. Furthermore, some nutrient classes are comprised of subunits. Protein can be further divided into individual amino acids, the building blocks of protein (NRC, 1994),

which must be available in the correct biological form, D versus L, to allow for protein accretion. All seven of the nutrient classes must be present for growth to occur but it is a nutritionist's job to supply them in adequate quantities for growth while simultaneously doing it as economically as possible.

BASIC NUTRIENTS OF POULTRY

Water

Water consumption is essential for poultry production because birds consume almost twice as much water as feed (Fairchild and Ritz, 2006; Cox, 2017; Pesti, 2005). The quality of water impacts productivity of the bird (Cox, 2017). Water is a vital nutrient that plays a major role in almost every physiological function in the body (Kleyn, 2013a). Water is a major component in transportation of nutrients, gases, waste and hormones. Chickens receive water from three sources: drinking water, water within feeds, and metabolic water. Up to 70 to 80 percent of a chickens body weight is water (Kleyn, 2013a). Of this, 70 percent is inside the cells and 30 percent is surrounding the cells. Bound water is the water available within broiler diets, but due to potential negative effects of high levels of bound water (i.e. feed spoilage, aflatoxin production in grain, nutrient availability); this is not a preferred method of water procurement.

Minerals

Minerals are the elemental part of feeds. The classification of these minerals by nutritionist is based on the amount required in the diet, macro or micro. Macro minerals include calcium, phosphorus, potassium, sodium, chlorine, sulfur and magnesium (Spears, 1999). The micro minerals include; iron, iodine, copper, manganese, zinc, selenium and chromium (Spears, 1999). Minerals are vital and required for skeletal formation, cofactors of enzymes and for maintenance of the osmotic balance (NRC, 1994).

Calcium and phosphorus are mainly for bone formation and if laying, for eggshell formation. In addition an excess of calcium can interfere with other minerals ability to be absorbed. Majority of the trace minerals are provided in a premix, while macro minerals are provided in feed ingredients.

Vitamins

The definition of a vitamin according to Leeson and Summers (2001), is an organic compound which is essential for growth, maintenance and health. Vitamins are classified in two categories, fat soluble (A, D, E, K) and water-soluble which include B vitamins and vitamin C (NRC, 1994). Units for vitamins are given in international units (IU) or per kilogram of diet.

In today's poultry diets majority of vitamins are assembled within a premix. These dietary supplements usually contain vitamins in excess for safety purposes. If deficiencies are present, especially in young chicks or embryos, in general the water soluble vitamins are first detected, as the fat soluble vitamins are stored within fat deposits (Leeson and Summers, 2001). In the early twentieth century an epidemic was occurring for the disease rickets. This sparked an increase in research on this disease and it was discovered it could be cured by vitamin D, the sunshine vitamin. Due to the fact that the majority of commercial poultry are grown indoors, ultraviolet light or supplemented vitamin D helped eradicate this disease (Leeson and Summers, 2001).

Protein

There is no requirement for protein *per se* although it is an essential nutrient class. Protein is however, the second most costly component of the diet (Kleyn, 2013a). Poultry, and all living organisms, require the subunits of protein, amino acids. During the 19th century the chemistry of amino acids was discovered setting the stage for rapid developments in nutrition

(Elwinger et al., 2016). There are 22 amino acids, commonly found in animal protein, used to make thousands of different proteins. Meeting the amino acid needs of the chicken is one of the most important tasks. Not only is it expensive, but it is a very complex nutritional aspect with many interactions.

In the US, soybean meal provides the majority of the amino acids broilers use for protein synthesis, which have to be metabolized into individual amino acids for latter assimilation into body proteins (Camara, et. al., 2018). This type of protein is commonly referred to as "Intact Protein". Synthetic amino acids have been used in animal diets since the 1950s (Kidd et al., 2013; Liu et al., 2019). These products provide protein to the bird and do not require to be metabolized or broken down, therefore making less metabolic waste in the process. Excess amino acids cannot be stored in the body as amino acids, they are burned for energy, converted into fat, or de-animated and is excreted as uric acid (Kleyn, 2013a). In addition, the excess protein that is de-animated is burned as either a carbohydrate or a fat which results in heat production and has major seasonal effects (MacLeod, 1997, 2000, 2005). In the summer, high temperature can depress the appetite however can be useful in the winter to keep the bird warm but is expensive way to heat.

Proteins are polymers of amino acids. Amino acids are made of an amino group and carboxyl group attached to the same alpha-carbon. A protein is then a combination of these different amino acids linked together by peptide bonds. Proteins are then folded into a three-dimensional structure which is the first challenge in the digestion of protein (Kleyn, 2013a). Ratio and the manner in which the amino acids bond together changes the physical, chemical and biological properties of the protein. Each different scenario has a major impact on digestibility. There are several anti-nutritive factors within different grains that impact the digestibility of the

amino acids from that grain. These can include trypsin inhibitors and non-starch polysaccharides (NSPs).

This structure must be broken down or de-natured to allow the enzymes to break the peptide bonds. Endogenous enzymes are secreted to break these peptide bonds throughout the digestive tract. Hydrochloric acid is able to easily break down the weaker hydrogen bonds. However, the disulfide bonds are very strong and require heat and or pressure to breakdown. Heat processing protein has some limitations as it can cause the protein to form other bonds, for example free carboxyl or free amine group can combine with fatty acids or carbohydrates and therefore not be available as a protein source.

Digestion of proteins in chickens has three major steps, all of which activate a chain reaction of events. First protein denaturation occurs by hydrochloric acid (HCL) secretion from the proventriculus. Hydrochloric acid (HCL) breaks the hydrogen and hydrophobic bonds, this step will also activate pepsinogen to pepsin. Pepsin is a non-specific enzyme that attacks the large protein and breaks into smaller units. Next, the pancreas produces three major protein digesting enzymes, trypsinogen, chymotrypsinogen and procarxypeptidase. Chymotrypsinogen and procarboxypeptidase are activated by trypsin and converted to chymotrypsin and carboxypeptidase (Leeson and Summers, 2001).

Trypsin and chymotrypsin break similar bonds within the protein, however chymotrypsin is present in larger quantities and carboxypeptidase specifically attacks peptide bonds on the carboxyl end of a peptide chain releasing free amino acids. Lastly, as these smaller pieces of protein travel down the intestinal tract, villi produce specific enzymes to finalize the digestion. These include aminopeptidase, which breaks the N-terminal bond, and dipeptidases break

linkages between specific pairs of amino acids. The end result from these three major steps is free amino acids that can be absorbed (Leeson and Summers, 2001; Kleyn, 2013a).

Absorption occurs through specific sites on the villi, through active transport against the concentration gradient (Leeson and Summers, 2001) carried to the liver through the hepatic portal vein. This type of transport requires a large amount of energy and shows specificity for the L-form. There can be competition between amino acids for these absorption sites, hence the term amino acid antagonism. In the liver the amino acids that go here to be absorbed are used in the blood and various tissues. Anabolism is the term used for these amino acids that go to the various tissues for re-assembling into protein. Blood serum and muscles have short term free amino acids that are typically depleted in 1-3 days. There is no protein storage for protein needs in excess of the current demand. Meaning there is no reserve for protein should there be a shortage or inadequate amount diet provided or an imbalance. If an imbalance occurs between individual amino acids then excessive amino acids may trans-animate to synthesize other amino acids, while others are de-animated to be metabolized for energy via a glycogenic (carbohydrate) or ketogenic (fat) pathway. The extra amine from this type of metabolism is lost in the urine as uric acid.

The 22 amino acids are divided into essential and non-essential amino acids. Essential means indispensable and cannot be made through metabolic process throughout the body and therefore must be included in the diet (NRC, 1994). Non-essential are dispensable and can be made through metabolic processes and not necessarily needed to be an ingredient in the diet. This is why nutritionists are concerned with protein quality. This can mean the total amino acids present, Crude Protein (%CP), the portion of essential and non-essential amino acids, and the digestibility of these amino acids. The making of synthetic amino acids in the 1950s has aided in

providing supplementation when lower quality protein ingredients are being utilized (Kidd et al., 2013). The main synthetic amino acids available are methionine and lysine with threonine and tryptophan more recently added. In the US, poultry diets are mainly made up of corn and soybean meal. When using corn and soybean meal based diets, these ingredients are both low in available methionine which is a major concern as methionine is the first limiting amino acid in poultry production. There are two types of synthetic methionine available on the market. DL-methionine and a hydroxyl analogue. This has caused much debate in the industry on the effectiveness of these two forms. DL-methionine is a mixture of both D and L form, with the chick being able to readily absorb the L form and having to convert the D to L form, while the analogue has a hydroxyl group in place of the NH_3 therefore trans-amination coverts to the active form of methionine (Kidd et al., 2013). The analogue is readily absorbed with little cost to energy. Its metabolic process is converted to keto-acid in the liver and animated to methionine.

Energy

While energy is not one of the six traditional nutrient classes, carbohydrates and fats are added to diets to provide energy to the bird. Energy is needed not only for mechanical function but also metabolism and the conversion of nutrients into the body. In addition, when using least cost formulation, energy accounts for 70% of the total feed cost (Skinner et al., 1992).

Carbohydrates

Carbohydrates are the main source of energy for poultry, provided mainly by cereal grains in the form of starch (Cowieson and Adeola, 2005). Starch is the major component of energy with remaining carbohydrates including free sugars, oligosaccharides, pectin's, hemicellulose and cellulose (Annison, 1974). The small intestine is the main sight of carbohydrate digestion and absorption. Starch is readily digestible in the presence of endogenous

digestive enzymes (Moran, 1985) and any carbohydrates that do escape digestion in the small intestine are available for the microbial population in the cecum and large intestine. Amylase is the main digestive enzyme responsible for starch digestion (Moran, 1985), and digestion of starch can improve with the amount of starch available in the diet (Mateos et. al., 1982). In addition, the rate of passage through the digestive tract may also influence the amount energy that can be derived from starch.

Carbohydrates metabolically stimulate hepatic *de novo* fatty acid synthesis whereas dietary lipids can be stored in tissues. Fat or lipids are a more expensive source of energy. Lipids provide the essential fatty acids and aid in fat-soluble vitamin absorption. Common sources in poultry diets include animal fats and vegetable fats. However, nutritionists try to avoid addition of these fats through least cost formulation because of expense (Kleyn, 2013b). The natural fats consists primarily of triglycerides. Triglyceride digestion depends mainly on the fatty acid chain length and amount of saturation. Digestion of fat must start with emulsification with a bile salt to allow pancreatic lipase to break down into individual fatty acids, glycerol and monoglycerides (Annison, 1974; Kleyn, 2013b). Glycerol is directly absorbed in the intestine and can be re-synthesized at a later time while fatty acids are made into micelles. The ability of the bird to digest and absorb these fatty acids depends on several extra caloric factors including level of inclusion in the diet (Annison, 1974), chain length and age of the bird. In addition, Mateos and others (1982) found palatability, increased pellet quality and increased retention time all play a huge role in the extra caloric cost of the addition of fats as an energy source.

HISTORY OF ENERGY AND IMPLEMENTATION IN POULTRY PRODUCTION

Energy is not a nutrient *per se*, however the amount of energy in a diet in relation to all other nutrients must be balanced in order for a bird to reach its maximum genetic potential. All

diets and feedstuffs can be analyzed for gross energy (GE) however this is not the form of energy in which the bird utilizes for growth and production. Because of this, multiple bioassays throughout the history of poultry energy research have been developed.

There are multiple definitions for energy all of which depend on if referring to the physical properties or the biological properties (Scott, 1969). If considering the first, it's referred to plainly as work, however if it is the latter energy is the metabolic processes to create chemical reactions. With any chemical reaction, heat is produced and direct measurement of this heat was first determined in a calorimeter, an oxygen-bomb calorimeter (Young, 1969).

Energy is measured by the unit of heat that is emitted or absorbed during a metabolic reaction in kilocalories. By definition, one kilocalorie (kcal) of energy is the amount of heat needed to raise the temperature of 1000 g of water by 1°C (Young, 1969) at one atmosphere pressure. However in nutrition, it is impractical to measure all the net kcals of all metabolic reactions, therefore total heat produced on complete oxidation is the acceptable form of measurement (Young, 1969).

In a bomb calorimeter, energy can be measured in the form of gross energy (GE). The amount of GE that the bird can utilize for growth and performance depends on how easily this feedstuff or diet is digested. Once GE is consumed the energy must go through multiple digestive metabolic processes to breakdown the large molecules of nutrients into smaller absorbable nutrients is termed digestible energy. Further in the digestive process energy is further lost in the excreta and once energy is corrected for these losses the energy is now in the form of metabolizable energy (ME). Again, energy is lost once more in the form of heat production and the energy finally reaches its final form of net energy (NE). NE is the form of energy that the bird utilizes for maintenance and production (figure 1).

Energy sources from carbohydrates, fats and protein provided in poultry diets are the sources for how the bird is able to obtain the energy required for growth, egg production and normal maintenance of body temperature. The efficiency of the bird to reach its genetic potential is all dependent on the metabolizable energy content of the complete diet.

METABOLIZABLE ENERGY

Currently, poultry diets are formulated on a metabolizable energy (ME) basis, mainly due to the ease of measurement. However, this simple and straightforward measurement has caused much debate on the method of analysis (Sibbald, 1982). ME is determined by correcting the gross energy (GE) by subtracting the energy in excreta (Lopez and Leeson, 2005, 2008). In poultry, feces and uric acid are both voided together as excreta therefore digestible energy cannot be quantified without surgical methods (Sibbald, 1979).

Substitution method

The determination of ME was first developed by Hill at Cornell University in the 1950's (Scott, 1969; Leeson and Summers, 2001). Hill developed the assay in order to determine the ME of feed ingredients by feeding two diets to two different sets of birds from age 14 d to 28 d. One diet was referred to as the reference diet which contained a high amount of glucose, ~45%, and vitamins and minerals. While the test diet contained a very low amount of glucose, ~5%, vitamins and minerals and ~40% of test ingredient. This method will be referenced later as the substitution method. On the last four days, excreta was collected. Additionally, these diets contained chromic oxide (Cr_2O_3) as an indigestible marker. Excreta samples were homogenized, dried and finely ground. Moisture, combustible energy (GE) of diet and excreta, nitrogen and chromic oxide is analyzed in both excreta and diet. Calculations are then made to determine the ME per gram of diet:

ME/g diet= GE/g diet – (Excreta GE/g diet + 8.22 x g N retained/g diet)

Energy per gram of diet was determined by bomb calorimeter.

Excreta energy per g diet= Energy per g excreta x Cr_2O_3 in diet/Cr_2O_3 in excreta

Gram N retained per g diet= N/g diet – N/g excreta x Cr_2O_3 in diet/Cr_2O_3 in excreta

To measure the ME of test ingredient substituted for glucose:

ME per g substitute= 3.64 – ME per g reference diet – ME per g diet with substitution/ proportion of substitute

The use of 3.64 was experimentally determined ME per gram of glucose dry matter. The faults in this method are due to the fact that differences are calculated between reference and test diet the ME must be calculated accurately. In addition the test ingredient needs to be included in the diet at the right percentage (high as possible) to reduce increases in errors.

AME, TME and nitrogen correction

Apparent metabolizable energy (AME) was studied by Farrell in 1978. When excreta is collected during ME analysis it is 'apparent' that there may be some endogenous losses from normal body turnover and not just undigested nutrients included in the excreta (Lopez and Leeson, 2008). The correction for these endogenous losses yields true metabolizable energy (TME). TME and AME are similar in the way value are obtained for feedstuffs. TME was developed by Guillaume and Summers in 1970 and made popular by Sibbald in the early 1980's. This method is described to make a correction for fecal and endogenous urinary energy. This rapid bioassay utilizes adult roosters which are fasted for 24h then force fed dietary treatments and total excreta collected and weighed for 24h. Criticism of this method was feed ingredients are fed alone without the synergism that occurs between other nutrients provided by other ingredients (Leeson and Summers, 2001).

Hill and Anderson in 1958 assumed that if nitrogen is not retained it will appear as uric acid (Leeson and Summers, 2001) and proposed a nitrogen correction factor of 8.22 kcal/g. Correcting to zero nitrogen retention simplifies the calculations stating that the feedstuff being evaluated is used entirely as a source of energy (McNab and Boorman, 2002). This correction factor was justified by the ability to compare all data from birds that are under different states of nitrogen retention (Lopez and Leeson, 2008).

NET ENERGY

Net energy (NE) is the value for calories from ME that is utilized by the bird. By definition, NE is made up of the energy used for production (NEg); eggs or meat, and the NE for maintenance (NEm, Figure 2). Measurement of NE is difficult because the necessity to measure heat production, therefore many systems were invented to help navigate the NE system in broiler chickens.

Armsby and Fries, in 1915, are the first to define net energy (NE) value as NE= ME – HI, was termed the ‘Armsby net energy system (Emmans, 1994). This system was based on there being no relationship between amount of food consumed and the HI. Armsby believed that NE values for could be tabulated just like ME values. High variability associated with HI. However in 1928, Forbes and others discovered that heat production in relation to feed consumption is not a linear relationship and it must depend on the plane of nutrition within the diet being fed. Since this discovery it was known that it was impossible to tabulate HI and therefore NE values for feed ingredients. Additionally, the Armsby NE system the fasting heat production (FHP) and a positive energy retention are added together to form one variable. The question now was how to distinguish between the two.

Productive energy

Productive energy measures the net energy value of a feed. Frapps at Texas A&M did extensive work in this area in the 1940's (Leeson and Summers, 2001; McNab and Boorman, 2002). This is the amount of energy the bird uses toward maintenance and production. The basic principal behind productive energy is to collect data that can be utilized in the following equation:

WM + G = FX

W= Average chick weight for experimental period (usually 14 d)

M: Maintenance requirement

G: Gain in carcass energy during feeding period

F: Feed intake

X: Productive energy value of diet per unit weight

All are measured throughout the experimental days. However, the diet to be studied is analyzed in two planes, *ad libitum* feeding and feed restricted to ~60-70% of intake of the full-fed group. This equals to two sets of data being collected, one for each plane of feeding. By using the two sets of data the solution of the simultaneous equations allows the ability to solve for the unknown (X). However, the major drawback of this method is the assumption the maintenance energy for both sets of birds is equal. With productive energy, the ability to reproduce the energy value of a diet is highly variable and difficult to reproduce than the ME method.

AMEn nitrogen correction poses many questions. It underestimates the AME of high protein ingredients

Blaxter & Wainman (1961) decided to relate energy retention to feed intake. In their NE system two slopes were calculated that intersected at zero; one line is below maintenance and the other above. The one below maintenance was defined as zero energy retention. Overall, this

system showed that HI of one diet has one number for below maintenance and one above. In theory, this system gives one number to a positive energy retention and therefore tabulating NE for maintenance and one for energy retained means NE values for ingredients is possible to be calculated.

One important principal that was thought of by Kielanowski (1965) was as obvious as it was to include retained energy in the NE value, one must separate out energy from protein verse energy from fat. However, these energetic values for protein and fat are not equal and therefore indicated that Blaxter & Wainman, although on the right track, in fact could consider the retained energy as a single variable. Kielanowski (1965) proposes there should be three variables in animals performance that must be included; maintenance (now zero fat and protein retention), positive retention of fat and protein. Additionally, if all three of these could be calculated and rations appear to be similar then only a single number would work (Kielanowski, 1965).

However, in 1978 the work of Blaxter & Wainman was rejected by authors Blaxter and Boyne. The system developed by these authors goes on to become the official UK energy system for ruminants of the Agricultural Research Council in 1980. For this system, again there was no distinction between energy retained as fat or energy retained as protein, but became an exponential function of energy intake, which had to be adjusted to the fasting heat production. Lots of data sets of varying feed stuffs was utilized in developing this system. The proportion of gross energy (GE) was metabolized at maintenance level then two variables, Km and Kf, were considered linear (Blaxter, K.L., and Boyne, 1978). Additions to this system by Blaxter in 1989 have now expressed these two variables as a function of crude protein content of the feed. However there is not a difference in food classes.

The NE systems of Armsby, Forbes, Blaxter & Wainman, and Kielanowski all focused on retained energy and the effect of HI on feeding. While Kellner (1912) was working on calculating NE values based on nutritive components of the feed ingredient itself, specifically starch. With Kellner's system, there was no different values between energy retained as fat or protein, just total energy retained. In this system, maintenance energy is expressed in a term of an equivalent of starch and therefore energy retention. It was thought that this system would overcome the high variability that is associated with HI but still allow a single value be assigned to an ingredient. However, the downfall of this system is that the difference in energy retained as fat verse energy retained as protein was not of equal calorific value.

Effective Energy

Emmans (1993) attempted to account for the variability in HI. This presented two problems; the first was to compare fed and fasting of diets that resulted in positive energy retention and the second was to put a value on the "extra" HP when the retentions were positive. Again, there is no distinction between energy retained as protein versus fat.

Indirect Calorimetry

Calorimetry is the measurement of heat. There are two types of calorimetry; direct and indirect. The direct method is by quantitative measurement of chemical by-products of metabolism. In general, there are two approaches to indirect calorimetry. One approach is estimating the respiratory exchange to calculate heat. By knowing the O_2 and CO_2 concentration heat can be calculated and the oxidation of nutrients through Brouwer's equation (1965). The second approach is the estimation of retained energy. Several approaches have been utilized in the past for this estimation. These approaches include Blaxters measurement of carbon and

nitrogen balance, productive energy by Emmans, comparative slaughter, specific gravity of the carcass, isotope dilution method and the use of dual energy X-ray absorptiometry (DEXA).

The use of indirect calorimetry is useful in evaluating the patterns of metabolism. Understanding the different rates in which fat and carbohydrates are oxidized gives insight into the availability of energy in the form of ATP (Livesey and Elia, 1988). In the last decade, most of the NE research has been done through different teams in France; Noblet, and in Australia; Swick and Choct. Much of these research teams focus on the classical definition of net energy. This method is to subtract HI from AMEn (Noblet et al., 1994, 2010, 2015; Swick et al., 2013; Wu et al., 2018). A disadvantage of using indirect calorimetry is the expense of the chamber itself and it is labor intensive, giving reasoning to why other methodologies are utilized.

BODY COMPOSITION

High cost of nutrients and the efficiency of these nutrients to produce edible quality protein is just as important as performance and carcass composition (Jackson et al., 1982). In poultry nutrition the end goal is to produce protein products. The meat yield, and therefore the body composition, of the broiler is expected to increase by the year 2024 (OECD-FAO, 2015). In order to meet these needs, genetic selection has been improving the growth rate of broilers for over 40 years (Gous et al., 1999). Broilers are grown to a larger weight in a shorter amount of time and being able to predict or understand the effects of diet type on changes in body composition, in real time, is becoming more important in today's industry. There are several methodologies on ways to predict composition the main methodologies are comparative slaughter and dual energy X-ray absorptiometry (DEXA, Caldas et al., 2019).

Comparative Slaughter

One classical method to estimating changes in body composition is comparative slaughter with wet chemistry. Animals are selected at the beginning of an experiment and are slaughtered and analyzed for composition. Then, throughout the grow-out live weight measurements are taken and are used in conjunction with the analyzed carcass from the beginning (Wolynetz and Sibbald, 1984) to predict composition. One criticism of this method is birds that are selected of the same age and weight may have different composition and therefore may not accurately predict the composition (Wolynetz and Sibbald, 1984). The method in which the carcass is analyzed was by Sibbald and Fortin (1982, 1984) where whole birds were ground, homogenized and then freeze dried. Once freeze-dried, samples are measured for dry matter, nitrogen, crude fat and ash. This method, although could be useful, is very labor intensive, slow and requires sacrificing the bird (Salas, et. al., 2012).

Dual energy X-ray absorptiometry (DEXA)

Although the chemical analysis is the widely accepted methodology for assessing body composition, it is labor intense and requires the sacrifice of a bird. New techniques for carcass composition analysis are abundant. These new techniques all come with advantages and disadvantages as far as precision and accuracy. Dual energy X-ray absorptiometry (DEXA) is an alternative to chemical analysis and has the advantage of being non-invasive for real time studies. The time per scan is short compared to chemical analysis. DEXA is a measure of bone mineralization and bone density in humans (Salas, et. al., 2012). The DEXA can measure the four main components of the carcass, fat, lean and bone mineral mass, by measuring the attenuation of x-rays of two different energy levels by different materials. There have been few reports of utilizing DEXA to determine body composition in poultry until Salas and others (2012) published a paper with the objective of calibrating and validating the use of a GE Lunar

Prodigy DEXA for body composition in both broilers and broiler breeders. In addition, in a more recent paper by (Caldas et al., 2019) utilized the DEXA to provide carcass composition in the terms of fat, protein, mineral and gross energy.

The varying equations and methodologies for net energy research by previous authors, shows a need for a standardization that can be utilized for feed formulation. Research is needed to understand and estimate what is occurring metabolically in order to move the industry towards a net energy least cost formulation system.

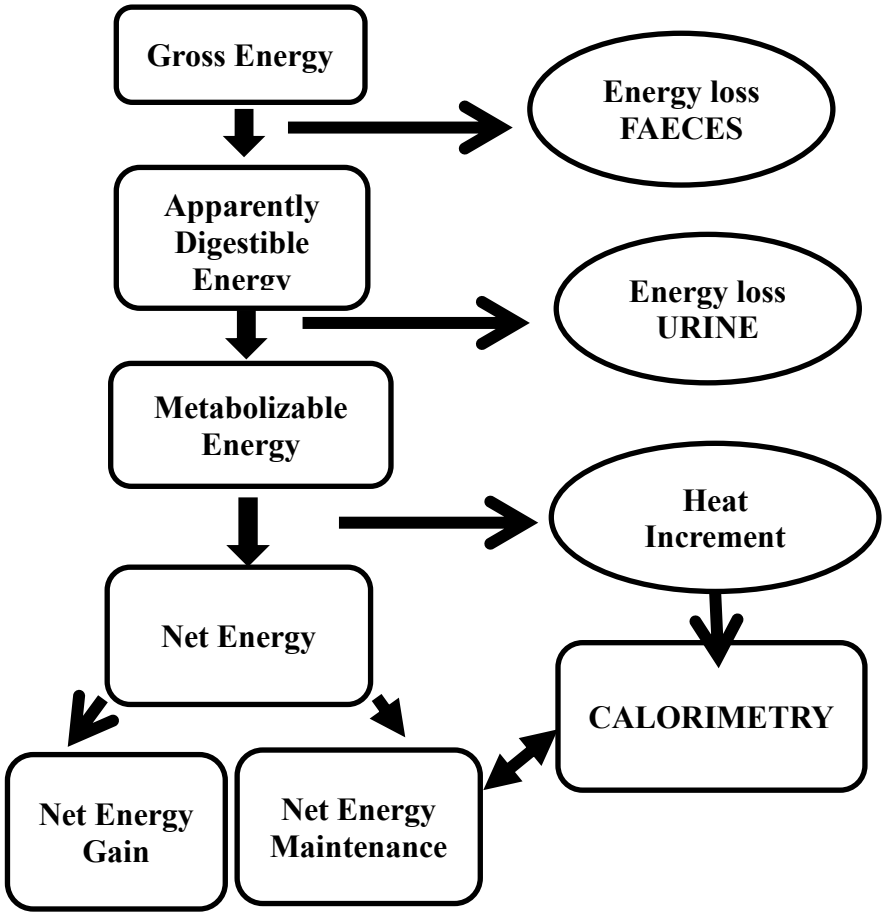

Figure 1. Energy utilization in poultry
Adapted from Farrell, 1974

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III. COMPARISON OF TWO NET ENERGY CALCULATIONS FOR TWO BROILER LINES FED VARYING LEVELS OF AMINO ACIDS AT TWO DIFFERENT TEMPERATURES

ABSTRACT

Two experiments with the same design were conducted under cool (Experiment 1; 15℃, Heat index=19℃) and hot (Experiment 2; 30℃, Heat index=44℃) climates. For each experiment, broilers from two commercial genetic lines (A and B) were fed common starter (d 0 to 10; 3,030 kcal/kg; 1.27% g digestible lysine (**dlys**), and grower (d 11 to 21; 3,080 kcal/kg; 1.09% dlys) diets (Table 1). Five experimental iso-caloric diets, formulated on a balanced ideal protein basis with increasing dlys concentrations (80, 90, 100, 110 and 120% of the AminoChick requirement), were fed from d 22 to 42. Birds were sampled at d 9, 38 and 42 and heat production (**HP**) measured for a 24 h period using respiratory chambers. After HP measurement, fasting heat production (**FHP**) was measured for 24 h and heat increment (HI) determined by difference: HI = HP – FHP. Body composition was measured on d 22 and d 42 by dual energy X-ray absorptiometry (**DEXA**) to determine Net Energy (**NE**) of gain (**NEg**) as NEg = protein (g) x 5.45 + fat gain (g) x 8.95 (Caldas et al., 2019). Classic NE and Arkansas NE (**Ark NE**) equations were compared: Classic NE (kcal/kg) = ME – HI; Ark NE (kcal/kg) = NEg + Net Energy of maintenance **(NEm)**, where NEm = HP – HI. Calorie efficiency (%) was calculated as NE/AMEn x 100, and calorie difference (**CD**) between the two equations as CD = Ark NE (kcal/kg) – Classic NE (kcal). No differences between diets were found for HP, HI and NEm. NEg was significantly ($P = 0.009$) different among diets, with increasing dlys producing more NEg (kcal). There was a significant increase ($P < 0.001$) in fat gain (g/bird) for birds fed decreased amino acid diets. The CD between Ark NE vs Classic NE increased from 700 kcal to approximately 1,200 kcal as dietary amino acids increased. Overall, the calorie efficiency was over 100% for Ark NE. The Ark NE system supports increasing protein calorie gain compared to the Classic NE system that favors fat calorie deposition.

INTRODUCTION

The modern broiler is growing at a rapid rate generating tremendous amounts of heat. A sensitive Net Energy (NE) system is needed to measure body heat production (HP) generated primarily by daily maintenance and synthesis and degradation of myofibrillar and sarcoplasmic protein. The advantage of formulating diets on a NE basis is the energy system accounts for energy lost as heat and more accurately predicts BWG and FCR better than other forms of dietary energy (Wu et al., 2018). The NE system is equivalent to formulating diets on a digestible amino acid (AA) basis compared to formulating with crude protein and total AA acids. Energy is a critical nutrient, as it is known to regulate body temperature and feed intake and is the most expensive component of poultry diets. Therefore, the continuous development of accurate and precise methods for measuring energy is vital in the modern industry. Obtaining precise energy values for various feeds will aid in minimizing feed formulation cost and ensure that the feed supply meets the energy requirements of animals (Noblet et al., 2010b). Gross energy (GE) of feed is not completely utilized by birds as an important percentage of the GE calories are lost as feed passes through the gastrointestinal tract. This leaves approximately 73% of the calories (Farrell, 1974) in a feedstuff in the portion known as metabolizable energy (ME). Metabolic utilization of ME calories is energy used for meat production plus the energy cost of maintenance, which is the definition of NE. The amount of energy that is utilized by the bird for maintenance and for gain is approximately 52-64% (Lopez and Leeson, 2005) of the ME calories. The NE system accounts for the energy lost as heat increment (HI) during ingestion, digestion, metabolism and excretion processes. In addition, NE predicts BWG and FCR with high precision (Noblet et al., 2010a), whereas dietary ME has not been shown to be a good predictor of performance. However, NE system is more labor intensive and expensive because

the heat expenditure from the test animal consuming diet has to be determined directly or indirectly. Additionally, as body composition is part of the general definition of NE, calorie values given to fat and protein must be considered due to higher calorie (8.95 kcal/g) value given to fat gain than to protein gain, (5.45 kcal/g, Caldas et al., 2019;Okumura and Mori, 1979). The objective of the study was to evaluate the classic way to calculate NE versus a new methodology; the Arkansas NE (Ark NE) system, with birds from two genetic lines fed diets with different AA content in two different environmental temperatures.

MATERIALS AND METHODS

Two experiments were conducted, one under cool (Experiment 1; 15℃, Heat index=19℃) climate and one under hot (Experiment 2; 30℃, Heat index=44℃) climate. The design of both experiments, except for the climate, was exactly the same. All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee #15048.

Birds and housing

Fertile eggs from two modern, high yielding genetic lines (genetic line A; genetic line B) were obtained from a commercial hatchery and incubated at the University of Arkansas hatchery. Once hatched, only male chicks were used.

4,050 male broilers (2,025 each genetic line) were randomly placed on new litter (softwood shavings) over 90 concrete floor pens (1.5 m x 3.0 m), 45 chicks per pen, distributed in two adjacent tunnel ventilated houses. Each pen had two hanging type feeders, providing 5.4cm of feeder space per chick, and 10 nipples. Minimum and maximum house temperatures

were recorded by an electronic environmental controller (Chore-Tronics, CTB Inc., Milford, Indiana). Lighting program was 23L:1D from d 0 to 7 and 18L:6D from d 8 to 42.

Diets and treatments

Diets were formulated based on standardized ileal digestible (**SID**) AA AMEn, according to AminoChick (Evonik Nutrition & Care GmbH) AA nutritional recommendations. All chicks were fed common starter (d 0 to 10; 3,030 kcal ME/kg; 1.27% dlys) and grower (d 11 to 21; 3,080 kcal ME/kg; 1.09% dlys) diets (Table 1). At d 21, one of five finisher (d 21 to 42) experimental diets (3,150 kcal ME/kg) formulated to contain 0.80, 0.90, 1.00, 1.10 and 1.20% dlys requirement with all other AA formulated in relationship to the dlys content (Table 2), were randomly assigned to the 45 pens. Each of the five experimental diets was fed to each genetic line, obtaining 10 treatments (5 diets and 2 genetic lines in a 5 x 2 factorial arrangement) with 9 replications each. Feed and water were provided *ad libitum*.

Chemical analysis of feed, ileal digesta, and excreta

At d 42, six broilers were selected from each treatment (5 diets x 2 genetic lines) within 1 SD of the mean treatment BW to determine the digestibility of the experimental diets. Additionally, 20 broilers (2 per treatment) were selected to determine the digestibility of the grower diet. All selected birds were placed in metabolic digestibility cages and for 2 d for an adaptation period to the cage. All diets had the addition of 0.5% titanium dioxide and fed *ad libitum*. For the grower phase, feed was removed on the evening of d 19 and replaced 8 h later for a fasting period. Birds were then sampled after 2 h of ad libitum feeding. For the finisher diets the same process was completed starting in the evening of 42 d. After eating for 2 h birds were immediately euthanized by CO_2 asphyxiation. Following euthanasia of the birds, ileal digesta was collected on these same days in order to determine nutrient digestibility. Clean

excreta (free from feathers and feed) was collected using plastic spatulas and placed in labeled plastic containers and frozen in liquid nitrogen immediately after collection.The digesta content of the ileum (between Meckel's diverticulum and the ileocecal junction collected from the birds of each cage were pooled to represent one replicate. Pooled samples were frozen in liquid nitrogen immediately after collection. All samples were lyophilized and fine ground (<2 mm) before analysis.

The analysis of AMEn (nitrogen-corrected apparent metabolizable energy) involved the analysis of gross energy (GE), dry matter (DM) and nitrogen, in feed and excreta. GE was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). DM was analyzed by method 934.01 (AOAC, 1990) and nitrogen levels were determined by the method 990.03 (AOAC, 1995). The marker, titanium dioxide (TiO_2), was measured on 96 well plates following the methodology of Myers (2004). In summary, 0.35 g of K_2SO_4, 0.04 g of $CuSO_4$, and 0.1 g of excreta, feed or ileal, were added to each glass test tube and diluted with 3 mL of 18M H_2SO_4 to be heated at 120°C for 24 h in a block digester. Contents of the digestion tube were allowed to cool for 15 min, after which 7 mL of distilled deionized water was added to the digested sample, gently mixed and transferred to new plastic test tubes. This step was repeated using 2 mL of distilled deionized water. Diluted, digested samples were centrifuged at 3,000 rpm for 22 min and the supernatant was recovered using filter paper. After mixing 1 mL of the supernatant with 0.20 mL of distiller deionized water and 0.13 mL of 30% H_2O_2, the absorbance was measured at 410 nm subsequent to the next 10 min after the addition of the last reagent.

The apparent ileal digestibility for each amino acid (AA) was calculated as follows: % DAA= ($AA_{diet} - AA_{ileal}$ x (TiO_{2diet} / TiO_{2ileal}))/ AA_{diet} x100). Amino acids were analyzed in triplicate

following the procedures: standard amino acid: AOAC 982.30 and Cystine/Methionine: AOAC 985.28 at the University of Missouri agriculture experiment station chemical laboratories. The standard AA method works under the principle of hydrolysis of the sample with HCl- 6N in the absence of oxygen to break down protein into individual amino acids. The samples are hydrolyzed in a drying oven at 120 ℃ for 16 hr. 2mL of norleucine (internal standard) is used and filtered through a #4 Whatman filter paper and then vacuum filtered through a 0.20 µm Gelman membrane filter. 1 mL of the stock sample is pipetted into a 50 ml borosilicate glass serum bottle and stored in freezer to cool. Glass bottles are placed in freeze drier to remove the HCl and pull a vacuum until no visible trace of liquid remains. 1 mL of 2.2 pH sodium diluent buffer is added to the dried residue, swirled to dissolve dried sample and phenol is added to the buffer for preservation longevity. Reconstituted sample is transferred to a 1.5 mL micro-centrifuge tube for holding for HPLC injection. For the sulfur amino acids, (cysteine/methionine) the methodology was AOAC 985.28. The principle of this method is that the protein is first oxidized with performic acid for 16 h. in an ice bath, neutralized with hydrogen bromide and hydrolyzed at 121 ℃ with 6N HCl for 18 hr. Cysteic acid and methionine sulfone standards are added to an additional bottle. After hydrolysis, samples are allowed to cool and filtered through #4 Whatman and the same steps for the previous standard AA is performed before loading the samples on HPLC.

Sampling

On d 19, 60 birds (30 per genetic line) were selected, weighed and transferred to respiratory chambers (5 birds per chamber) and given one day adaptation prior to HP measurement in order to establish a reference baseline. Thereafter, at d 38 and d 42, 24 birds were selected, weighed and transferred to the respiratory chambers (2 birds per chamber) and given one day adaptation prior to evaluation. Due to limited number of respiratory chambers, at d

38 and d 42 only those diets formulated to contain 80, 100 and 120% of the dlys nutritional requirement were used to determine HP.

For basal body protein and fat contents, 18 birds from each genetic line (total 36) were analyzed at d21, whereas 18 birds from each treatment (10 treatments; total 180 birds) were analyzed at d42. All birds were selected within one (± 1) SD of the treatment average BW.

Respiratory Chambers and HP Determination

Respiratory chambers (61 cm l x 51 cm w x 56 cm h) utilized were the same as described by Caldas (2018), with exception of the lighting program, temperature and air flow. The temperature inside the chambers was maintained at range 15℃ to 18℃ and 26℃ to 29℃ for the experiments under cool and hot temperatures, respectively. The room temperature was 10℃ to 12℃ and 21℃ to 23℃ for the experiments conducted under cool and hot climate, respectively; which is 10℃ lower than the temperature inside the chambers, which ensured that the temperature inside the respiratory chambers stayed within the expected temperature range. The indirect calorimetry system provided air flow of 20 to 25 L/min, depending on the size of the broilers in the chamber. Before each evaluation day, chambers were opened to determine individual BW and FI measurements and calibration of gas analyzers.

HP was determined for 24 h, followed by fasted heat production (**FHP**) for the next 24 h. Broilers were allowed *ad libitum* feed access during adaptation and fed periods.

Body Composition Analysis

Birds were humanely sacrificed by CO_2 asphyxiation before body composition was determined using dual energy X-ray absorptiometry (**DEXA**; General Electric Co., Madison, Wisconsin) with small animal body software module (Lunar Prodigy from General Electric Co.

encore version 12.2). DEXA results were adjusted to body chemical analyses performed by Caldas (2019).

NE calculations

Volumes of O_2 (VO_2) and CO_2 (VCO_2) within each chamber were averaged for each 24 h evaluation period. HP and FHP were calculated following the equation: HP kcal/d = 3.866 VO_2 L/d + 1.233 VCO_2 L/d (Brouwer, 1965) and normalized to kg of FI.

HP consists of the NE of maintenance (NEm) plus the heat increment (HI; see equation 1 below). HP and fasting heat production (FHP) were calculated using the Farrell (1974) equation (see equation 2 below). Classic NE was calculated according to Noblet et al. (2010) (see equation 3 below), where HI is defined as HP minus FHP (see equation 4 below). Net Energy of gain (NEg) was calculated based on body composition data (body protein and fat levels) from DEXA (see equation 5 below). Equation (6) was obtained rearranging equation (1), allowing NEm be evaluated with indirect calorimetry. This method is called the Arkansas NE Equation (Ark NE; 7), which encompasses both body composition and HP.

(1) HP = NEm + HI (Farrell, 1974)

(2) HP and FHP = 3.871 x VO_2 (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974)

(3) $NE_{classic}$ (kcal/kg) = ME (kcal/kg) – HI (Noblet et al., 2010b)

(4) HI = HP – FHP

(5) NEg = protein gain (g) x 5.66 (kcal/g) + fat gain (g) x 9.35 (kcal/g)

(6) NEm = HP – HI

(7) Ark NE = NEg + NEm

Statistical analysis

Chamber or pen was the experimental unit. Floor pen data were analyzed under a 5 (diets) x 2 (genetic lines) factorial arrangement. Calorimetry and body composition data for baseline information were analyzed under completely randomized designs with two treatments (genetic lines); however, subsequent data were analyzed under a 3 (diets) x 2 (genetic lines) factorial arrangement for calorimetry and a 5 (diets) x 2 (genetic lines) factorial arrangement for body composition. Data were analyzed by ANOVA of JMP Pro 13 (SAS Institute, 2017). When the means were significant ($P \leq 0.05$) student t-test was used. *P*-value was considered significant when ≤ 0.05.

ANOVA model:

$$Y_{ijk} = \mu + T_i + B_j + (TB)_{ij} + e_{ijk}$$

μ = mean

T_i = effect of i^{th} level of factor A

B_j = effect of j^{th} level of factor B

$(TB)_{ij}$ = effect of interaction between the i^{th} level of factor A and the j^{th} level of factor B

e_{ijk} = random error associated with the k^{th} replicate

For completely randomized designs, only one factor was present.

RESULTS

Body Composition

For body composition evaluation, a 2 x 5 factorial design provided differences in tissue gain between dietary treatments (Figure 1). No genetic line by diet interaction was found;

therefore, only main effects data are shown. Feeding iso-caloric diets with increasing AA changed broiler protein and fat body composition. At both hot and cool temperatures, increasing dietary AA concentration significantly ($P < 0.001$) produced higher body protein and fat depositions (Figure 1). The largest amounts of protein (g/bird) ($P < 0.001$) were found in broilers fed the 110% and 120% AA level diets during the cold temperature, 445g and 454g, respectively. The lowest amount of protein (g/bird) was found in broilers fed the 80% AA level diet ($P < 0.001$) during the hot temperature, 274g vs 297g, respectively. Differences in energy gain ($P < 0.001$) were found only at the cooler (Experiment 1; 15°C, Heat index=19°C) temperature, with broilers fed at the 110% AA level gaining 4,754 kcals, compared to other dietary treatments. A positive linear relationship was found between protein gain (g/bird) and increasing dietary dlys (%) in both cool ($P < 0.001$, $R^2 = 0.82$, slope = 1.5) and hot climates ($P < 0.001$, $R^2 = 0.68$, slope = 1.6).

Differences in body protein by genetic line followed the same trends at both hot and cool temperatures. In the hotter (~ 90 °F) climate, genetic line B had 41 g/bird more ($P < 0.001$) body protein than line A (Figure 2); whereas in the cooler temperature this difference ($P < 0.001$) was only 21 g/bird (Figure 2). Body fat within genetic line differed significantly ($P < 0.001$) between the two climates. At the hotter temperature, line B had 23 g/bird more fat compared to line A; however, during the cooler temperature, line A had 18 g/bird more ($P < 0.001$) fat than line B (Figure 2).

Calorimetry parameters

Heat expenditure was calculated by respiratory exchange in indirect calorimetry chambers: volume of oxygen consumption and carbon dioxide production. HP data showed no significant differences for diet or the interaction of genetic line and diet. However, the 2 x 5

factorial design indicated significant differences ($P < 0.001$) between genetic lines. Variances in HP (kcals) between genetic lines showed the same trend in both cool and hot temperatures during the finisher phase (Table 4). However, during the hot temperatures Line B had significantly ($P < 0.001$) higher + 21 kcals HP compared to Line A (Table 4). A similar trend was observed for NEm in hot temperature with Line B having +15 kcals more ($P < 0.001$) than line A. Lastly, when HP is expressed per metabolic BW ($kg^{0.70}$) a significant difference of 25 kcals between line A and B was observed.

Net Energy

As dietary AA levels increased, the Classic NE of the diet decreased in hot temperatures and the Ark NE value increased (Table 6). In addition, the Classic NE to ME (NE/ME) ratio declined 3%, and the Ark NE/ME ratio improved 18% (Table 6). However, during the cooler temperature as the dietary AA level increased the Classic NE of the diet varied little between the three dietary treatments (Table 5). While the Ark NE value during the cooler temperatures varied 448 kcals between the diets with the lowest AA and diets with the highest AA level (Table 5). Furthermore during the warm temperature the ratio of NE/AME for the Classic equation did not vary, while the Ark NE/AME ratio varied by 15% (Table 5).

The calorie difference between Classic NE and Ark NE rose as the AA to calorie ratio increased. Regarding genetic lines, line A had higher Ark NE value in both climates, 3,811 kcals vs. 3,471 kcals, for cool temperature and 3,811 kcals vs. 3,471 kcals in hot temperature. However for Classic NE, line A was numerically higher in the cool temperature (2,635 kcals vs. 2,602 kcals, Table 5) but numerically lower in the warm temperature 2,482 kcals vs. 2,539 kcals (Table 6). Overall, broilers in cooler temperature produced Ark NE values 9% higher when

compared to broilers housed in hotter temperature and Classic NE showed broilers in cooler temperature 1% higher compared to broilers housed in hotter temperature.

DISCUSSION

Body composition

Protein is said to be the building block of life. Protein is made up of peptide bonds between individual amino acids. Once consumed by the animal these bonds are hydrolyzed and individual amino acids are released. Then, once absorbed, the amino acids are reassembled to form body tissues which requires a tremendous amount of energy. In the present study, protein gain showed similar values for both genetic lines regardless of climate. As a consequence of having the least protein accretion, the highest fat gain (g/bird) was seen in the birds fed the 80% amino acid level diet and the lowest was found in those fed the 110% and 120% AA level diet. Increasing the dietary AA/AMEn ratio improved protein gain (g/bird) in the birds as reported by Deschepper and De Groote (1994), who fed iso-caloric diets supplemented with essential and non-essential AA and found that feeding lower ideal amino acid balance resulted in birds having higher carcass fat content. As the dietary AA/AME decreased, carcass fat (g/bird) increased regardless of genetic line. Veldkamp et al., (2017) fed iso-nitrogenous diets with increasing levels of ME, ultimately as energy was added to the diet the ratio of AA to energy decreased. This study indicated that more energy was utilized for protein deposition and therefore decreasing the body fat content. However, for the current study this was the opposite effect. The birds fed the lowest concentration of AA to energy ratio (80% AA diet) had the most fat gain. This is could be due to the changes in post absorption metabolism of the diets throughout the grow-out periods. Additionally, Caldas et al. (2019) found the caloric efficiency changes for feeding phases, indicating birds use less protein as an energy source during the finisher phase.

TEMPERATURE EFFECTS:

Since birds are homeotherms and because of this, energy is required to help maintain a normal body temperature. In order for birds to maintain normal body temperature, the environment provided needs to include adequate heat hence why chicks are brooded. In addition to this, provided nutrients that will increase the HP. Additionally, if birds are over-fed protein, and since chicks have no sweat glands and covered in feathers, they are more susceptible to heat stress causing a decrease in growth. In thermal neutral zones, energetic efficiency can be maximized because less energy is needed to maintain body temperature. However, this is not the case during heat stress or cold temperature. It has been proposed the addition of AA in warmer temperatures increases the HI and is not efficient form of combating high ambient temperatures (Waldroup, 2002).

Feed formulation used to be based on CP levels, but more recently, it has been shown that formulation needs to be on an ideal protein ratio in order to supply enough nitrogen to synthesize the indispensable amino acids (Waldroup, 2002). Amino acids, when fed in excess, are not stored in the tissues because the broiler has no amino acid storage pool. Thus, the carbon skeleton of the amino acids is used for energy and the nitrogen from the amine group is wasted in the form of uric acid thus causing an increase in metabolic heat.

HP and Net Energy

It has been shown that differences in genetic lines HP can be seen even at the embryonic development stage (Tona et al., 2010). The current trial also shows differences in the genetic lines during the finisher period, indicating these differences in HP start at the embryonic level. HP being higher shows a more advanced level of development and higher metabolic rate (Tona et al., 2010). In both experiments, the Classic NE/AME ratio decreased as the amino acids

increased. The classic NE/AME system decreases as the amino acid to energy ratio increases (Carré et al., 2014) consequently fat deposition increased relative to the ratio increase. This is because partial efficiency of ME utilization is explained by lipid deposition (Farrel, 1974). Likewise, Deschepper and De Groote (1994) found that increasing crude protein generally produced a lower NE/AME ratio. The current study supports these findings for the classical NE/AME ratio, but indicates the opposite effects using the Arkansas NE/AME ratio. This is explained to the fact that the Arkansas Net Energy Equation assigns greater caloric value to protein deposition and utilization than the classical NE, which highly values lipid deposition. In addition, lower amino acid to energy ratios suggest there is an inadequate amount of energy to provide for maintenance and therefore amino acids are being catabolized for protein synthesis (Classen, 2017).

Carré et al. (2014) and Noblet et al. (2010) found the classical NE/AME ratio average to be around 75%, regardless of the amino acid concentration of the diet. Although the current study showed a much higher Ark NE/AME ratio (114%), that finding was also confirmed in this study, suggesting that the types of utilization and gain (lipid vs. protein) have a large influence on the NE value of the diets.

The chemical composition of feed is an important consideration to classical NE discussions, especially the fiber content and its contribution to the HI and NE/AME ratios (Swick et al., 2013, Wu et al., 2018). Carré (2013) stated the range for NE/AME ratios varied in a limited range and attributed it towards very little of ME is transformed into HI. Additionally, McKinney and Teeter (2004) showed there was an increase in productivity due to bird behavior, by reducing the energy towards feeding and more time resting and utilizing energy for growth. However, these studies suggest that highly digestible diets (corn-soybean based) with low fiber

content require more than HI data in order to yield sufficient information about diet NE. In that regard, Wu et al. (2018) stated gross energy efficiency for AME is negatively dependent on crude protein. In addition, Emmans (1994) proposed a theoretical model showing that increasing crude protein decreased Classic NE, in contrast to the Ark NE further suggesting more evidence is needed to give more caloric value to protein and consequently amino acids.

The Classic NE method only assesses the value of HI which accounts for a small portion (Brouwer, 1965) of dietary energy that is lost from ME. This Classic NE can be misleading as more calorie efficiency (NE/ME) is given to fat deposition than lean mass deposition. Classic NE does not take into consideration the type of production or gain that is occurring in the animal and mainly penalizes protein accretion because of HI generated from nitrogen and carbon loss through uric acid production. Protein calories should be more important than fat calories for meat production and therefore be considered in the overall NE equation for predictive calorie value of ingredients. In conclusion, utilizing together the NEm, determined from indirect calorimetry, and NEg, evaluated through DEXA, provide valuable information about type of gain and current broiler genetics broiler genetics. This combination provides a deeper understanding of diet NE, rather than the small indigestible fraction differences. Taking advantage of understanding the genetics and appropriate environment is an advantage of NE formulation.

Table 1. Composition and nutrient calculations of the starter and grower diets.

Ingredient, %	Starter	Grower
Corn	54.58	58.44
Soybean meal, 48%	33.5	29.91
Corn gluten meal, 60%	4.70	5.00
Dicalcium phosphate 19%	2.21	1.94
Soybean oil	2.51	2.66
Limestone ($CaCO_3$)	0.84	0.78
Sodium bicarbonate	0.36	0.31
L-lysine-HCl	0.32	0.19
DL-methionine 99%[1]	0.30	0.19
Salt	0.21	0.25
Choline chloride 60%	0.12	0.11
L-threonine 98.5%[1]	0.10	0.02
L-valine 96.5%[1]	0.06	0.00
Vitamin and mineral premix[2]	0.22	0.22
Trace min premix[3]	0.10	0.10
Ethoxyquin[4]	0.02	0.02
Organic acid[5]	0.05	0.05
Calculated nutrient composition %, unless otherwise noted		
Crude protein	23.00	21.50
Crude fiber	2.24	2.20
Ether extract	5.52	5.78
Ash	6.72	6.20
Starch	38.06	40.43
Choline chloride, 60%	1.70	1.60
ME, kcal/kg	3030.00	3080.00
Ca	1.00	0.90
avP	0.50	0.45
Na	0.20	0.20
Cl	0.23	0.23
K	0.81	0.75
SID Lys	1.27	1.09
SID Met	0.62	0.50
SID Met+Cys	0.93	0.80
SID Thr	0.81	0.70
SID Trp	0.23	0.21
SID Arg	1.34	1.24
SID Ile	0.86	0.81
SID Val	1.00	0.89
Analyzed composition[5]		
AMEn		
Crude Protein	21.66	22.37

Table 1. Composition and nutrient calculations of the starter and grower diets (Cont.)

Analyzed composition[5]		
	Starter	Grower
Lys	1.30	1.27
Met	0.71	0.53
Met+Cys	1.05	0.89
Thr	0.90	0.86
Trp	0.24	0.26
Arg	1.30	1.41
lle	0.89	0.96
Val	1.04	1.05
SID Lys		1.21
SID Met		0.50
SID Met+Cys		0.81
SID Thr		0.77
SID Trp		0.23
SID Arg		1.20
SID Ile		0.90
SID Val		1.00

[1]DL-methionine (MetAmino), L-threonine (ThreAmino), L-valine (ValAmino) (Evonik Nutrition & Care GmbH).

[2]Vitamin premix: Vit A, 13227 IU/kg; Vit D3, 3968 IU/kg; Vit E, 66 IU/kg; Vit B12, 0.040 mg/kg; Biotin, 0.254 mg/kg; Menadione, 3.968 mg/kg; Thiamine, 3.968 mg/kg; Riboflavin, 13.228 mg/kg; Vit B6, 7.937 mg/kg; Niacin, 110.229 mg/kg; Folic acid, 2.205 mg/kg. Trace mineral premix: Mn, 60 mg/kg (manganese sulfate); Zn, 60 mg/kg (zinc sulfate); Fe, 40 mg/kg (ferrous sulfate); Cu, 5 mg/kg (copper sulfate); I, 1.25 mg/kg (calcium iodide); Co, 0.5 mg/kg (cobalt sulfate).

[3]Santoquin (Novus International, Inc).

[4]MycoCurb (Kemin Industries, Inc).

[5]Analysis on as is basis.

Table 2. Composition and nutrient calculations of the finisher experimental diets

Ingredient, %	80AA	90AA	100AA	110AA	120AA
Corn	75.03	73.33	69.82	67.7	64.3
Soybean meal, 48% CP	14.71	15.09	18.72	23.1	26.5
Corn gluten meal, 60% CP	5.29	6.31	5.34	1.9	1.0
Dicalcium phosphate	1.81	1.81	1.81	1.8	1.8
Soybean oil	1.00	1.00	1.68	2.6	3.2
Limestone	0.85	0.85	0.82	0.8	0.8
Sodium bicarbonate	0.35	0.40	0.41	0.4	0.4
Salt NaCl	0.23	0.19	0.18	0.2	0.2
L-lysine-HCl	0.24	0.35	0.38	0.4	0.4
DL-methionine 99%[1]	0.07	0.13	0.19	0.3	0.4
Choline chloride 60%	0.15	0.15	0.14	0.1	0.1
L-threonine 98.5%[6]	0.01	0.06	0.09	0.2	0.2
L-valine 96.5%	0.00	0.00	0.04	0.1	0.2
L-isoleucine	0.00	0.01	0.04	0.1	0.2
L-arginine	0.00	0.07	0.08	0.1	0.1
Vitamin premix[2]	0.10	0.10	0.10	0.1	0.1
Trace mineral premix[3]	0.10	0.10	0.10	0.1	0.1
Selenium premix 60%	0.02	0.02	0.02	0.0	0.0
Ethoxyquin[4]	0.02	0.02	0.02	0.0	0.0
Organic acid[5]	0.05	0.05	0.05	0.1	0.1
Calculated composition %, unless otherwise noted					
Crude protein	17.00	18.00	19.00	19.00	20.00
Crude fiber	2.24	2.24	2.33	2.46	2.54
Ether extract	4.05	4.05	4.60	5.29	5.81
Ash	5.45	5.48	5.64	5.84	5.99
Starch	48.44	47.57	45.39	43.71	41.62
AMEn, kcal/kg	3150.00	3150.00	3150.00	3150.00	3150.00
Ca	0.85	0.85	0.85	0.85	0.85
avP	0.42	0.42	0.42	0.42	0.42
Na	0.20	0.20	0.20	0.20	0.20
Cl	0.23	0.23	0.23	0.23	0.23
K	0.52	0.53	0.58	0.65	0.70
Analyzed composition %, unless otherwise noted[5]					

Table 2. Composition and nutrient calculations of the finisher experimental diets (Cont.)

	80AA	90AA	100AA	110AA	120AA
Analyzed composition %, unless otherwise noted[5]					
Dry matter	87.96	88.03	88.45	88.35	88.31
Crude protein	17.03	18.02	19.15	19.37	20.58
TMEn, kcal/kg	3306.00	3353.00	3324.00	3362.00	3355.00
ME, kcal/kg	3097.62	3094.30	3187.56	3091.27	3136.00
Lys	0.89	0.99	1.12	1.23	1.34
Met	0.37	0.44	0.51	0.59	0.63
M+C	0.66	0.75	0.82	0.90	0.95
Thr	0.63	0.70	0.77	0.83	0.90
Trp	0.17	0.17	0.20	0.21	0.23
Arg	0.96	1.05	1.18	1.31	1.43
Ile	0.67	0.71	0.80	0.87	0.95
Val	0.80	0.83	0.92	1.00	1.08
SID Lys	1.02	1.16	1.25	1.41	1.50
SID Met	0.41	0.49	0.52	0.65	0.71
SID M+C	0.72	0.81	0.84	0.98	1.03
SID Thr	0.68	0.76	0.82	0.90	0.96
SID Trp	0.19	0.23	0.27	0.25	0.25
SID Arg	1.02	1.12	1.21	1.39	1.50
SID Ile	0.79	0.85	0.92	1.02	1.05
SID Val	0.92	0.95	1.03	1.66	1.24

[1]DL-methionine (MetAmino), L-threonine (ThreAmino), L-valine (ValAmino) (Evonik Nutrition & Care GmbH).

[2]Vitamin premix: Vit A, 13227 IU/kg; Vit D3, 3968 IU/kg; Vit E, 66 IU/kg; Vit B12, 0.040 mg/kg; Biotin, 0.254 mg/kg; Menadione, 3.968 mg/kg; Thiamine, 3.968 mg/kg; Riboflavin, 13.228 mg/kg; Vit B6, 7.937 mg/kg; Niacin, 110.229 mg/kg; Folic acid, 2.205 mg/kg. Trace mineral premix: Mn, 60 mg/kg (manganese sulfate); Zn, 60 mg/kg (zinc sulfate); Fe, 40 mg/kg (ferrous sulfate); Cu, 5 mg/kg (copper sulfate); I, 1.25 mg/kg (calcium iodide); Co, 0.5 mg/kg (cobalt sulfate).

[3]Santoquin (Novus International, Inc).

[4]MycoCurb (Kemin Industries, Inc).

[5]Analysis on as is basis.

Table 3. Body composition gain of two genetic broiler lines fed different dietary amino acid levels at different temperatures.

	Experiment 1 (cool)			Experiment 2 (hot)		
	Protein g/bird	Fat g/bird	Energy kcals	Protein g/bird	Fat g/bird	Energy kcals
Amino acid level						
80AA	391[c]	333[a]	5110[a]	274[c]	277[a]	3927
90AA	429[b]	306[ab]	5075[a]	297[b]	262[ab]	3915
100AA	437[ab]	295[bc]	5022[ab]	311[b]	249[b]	3875
110AA	445[ab]	260[c]	4754[b]	333[a]	250[b]	3999
120AA	454[a]	265[c]	4848[ab]	337[a]	246[b]	3962
SEM	5.79	8.78	69.05	4.34	5.31	49.83
Strain						
Line A	421[b]	301[a]	4986	290[b]	245[b]	3722[b]
Line B	441[a]	283[b]	4937	331[a]	268[a]	4150[a]
SEM	3.66	5.55	43.67	2.75	3.36	31.44
P-Value						
Amino acid level	<0.001	<0.001	0.013	<0.001	0.003	0.419
Strain	0.002	0.026	0.434	<0.001	<0.001	<0.001

Factorial design 2 x 5. Levels (a,b,c) not connected by the same letter are significantly different, Tukey-HSD test $P < 0.05$.

Table 4. Indirect calorimetry results of two genetic broiler lines fed different dietary amino acid levels at different temperatures.

	Experiment 1 (cool)			Experiment 2 (hot)		
	Heat Production (kcals)	NEm (kcals)	Heat kcal/Kg$^{0.70}$	Heat Production (kcals)	NEm (kcals)	Heat kcal/Kg$^{0.70}$
AA x Strain						
A, 80 AA	204	162	115	217	151	143
A,100AA	216	168	129	210	157	144
A, 120 AA	205	159	118	228	153	148
B, 80AA	226	167	132	225	163	149
B,100 AA	227	161	122	241	166	169
B, 120 AA	240	192	152	250	178	169
SEM	12.9	8.9	15.7	8.0	4.7	149.9
AA level						
80	215	165	124	221	157	140
100	222	164	126	225	162	147
120	223	176	135	239	166	162
SEM	9.3	6.4	11.3	5.7	3.3	6.4
Strain						
Line A	208	163	121	218^{b}	154^{b}	137^{b}
Line B	231	173	136	239^{a}	169^{a}	162^{a}
SEM	7.7	5.3	9.3	4.6	2.7	5.3
P-Value						
AA Level	0.059	0.188	0.268	0.091	0.200	0.064
Strain	0.800	0.404	0.742	0.005	0.001	0.009
AA x Strain	0.723	0.146	0.499	0.348	0.218	0.789

Factorial design 2 x 5. Levels (a,b) not connected by the same letter are significantly different, Tukey-HSD test $P<0.05$.

Table 5. Comparison of Ark NE versus Classic NE values obtained

	Experiment 1 (cool)						Experiment 2 (hot)					
	AMEn	Classic NE	Ark NE	kcal diff.	Classic NE/AMEn	Ark NE/AMEn	AMEn	Classic NE	Ark NE	kcal diff.	Classic NE/AMEn	Ark NE/AMEn
	kcals/kg	kcals/kg	kcals/kg	kcals/kg	%	%	kcals/kg	kcals/kg	kcals/kg	kcals/kg	kcals/kg	kcals/kg
A, 80AA	3,137	2,835	3,087	251	86	93	3,137	2,575	2,789	213	78	84
A, 100AA	3,137	2,373	4,558	2,184	71	137	3,137	2,424	3,740	1,315	73	113
A, 120AA	3,137	2,698	3,789	1,090	80	113	3,137	2,447	3,035	587	73	90
B, 80AA	3,137	2,537	3,482	945	77	105	3,137	2,612	2,953	340	79	89
B, 100AA	3,137	2,601	3,054	452	72	92	3,137	2,374	3,249	874	71	98
B, 120AA	3,137	2,667	3,877	1210	79	116	3,137	2,631	2,982	351	78	89
SEM		185.1	474.0	616.7	0.06	0.14		162.6	282.7	423.5	0.1	0.1
AA Level												
80AA	3,137	2,685	3,284	598	81	99	3,137	2,594	2,871	277	78	87
100AA	3,137	2,486	3,806	1,318	75	114	3,137	2,399	3,495	1,095	72	105
120AA	3,137	2,682	3,832	1,150	80	114	3,137	2,539	3,008	469	75	90
SEM		141.1	341.4	444.4	0.04	0.10		115.0	199.9	299.5	0.03	0.06
Strain												
Line A	3,137	2,635	3,811	1,175	79	114	3,137	2,482	3,188	705	75	96
Line B	3,137	2,602	3,471	869	78	104	3,137	2,539	3,061	522	76	92
SEM		110.79	283.54	369.09	0.03	0.09		93.89	163.3	244.5	0.03	0.05
P-Value												
AA Level		0.54	0.42	0.48	0.53	0.47		0.48	0.10	0.16	0.45	0.10
Strain		0.83	0.41	0.57	0.83	0.41		93.89	163.3	244.50	0.03	0.05
AA Level x Strain		0.39	0.17	0.19	0.39	0.17		0.77	0.51	0.80	0.77	0.51

Table 6. Results comparison by temperature

Temperature	ME	Classic NE	Arkansas NE Equation	kcal Difference	Classic NE/ME	Arkansas Equation NE/ME
	kcal/kg	kcal/kg	kcal/kg	kcal/kg	%	%
Cool (Experiment 1)	3,137	2,640	3,551	911	79	107
Hot (Experiment 2)	3,137	2,511	3,125	614	75	94

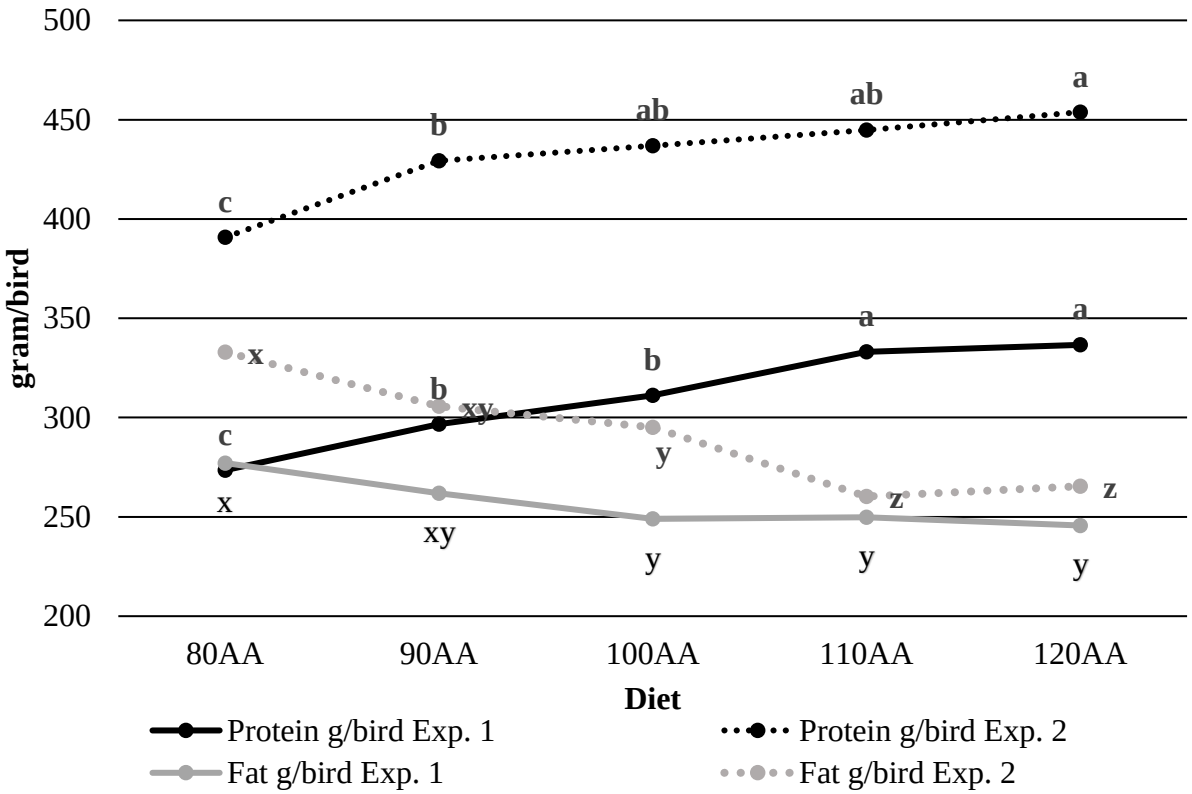

Figure 1. Body composition of broilers fed different dietary amino acid levels at different temperatures.
Factorial design 2 x 5. Body composition calculated on d 22 and d 42. Levels (a, b, c and x, y, z) not connected by same letter are significantly different among diets, Tukey-HSD test $P < 0.05$. Experiment 1, cool temperature; Experiment 2; hot temperature.

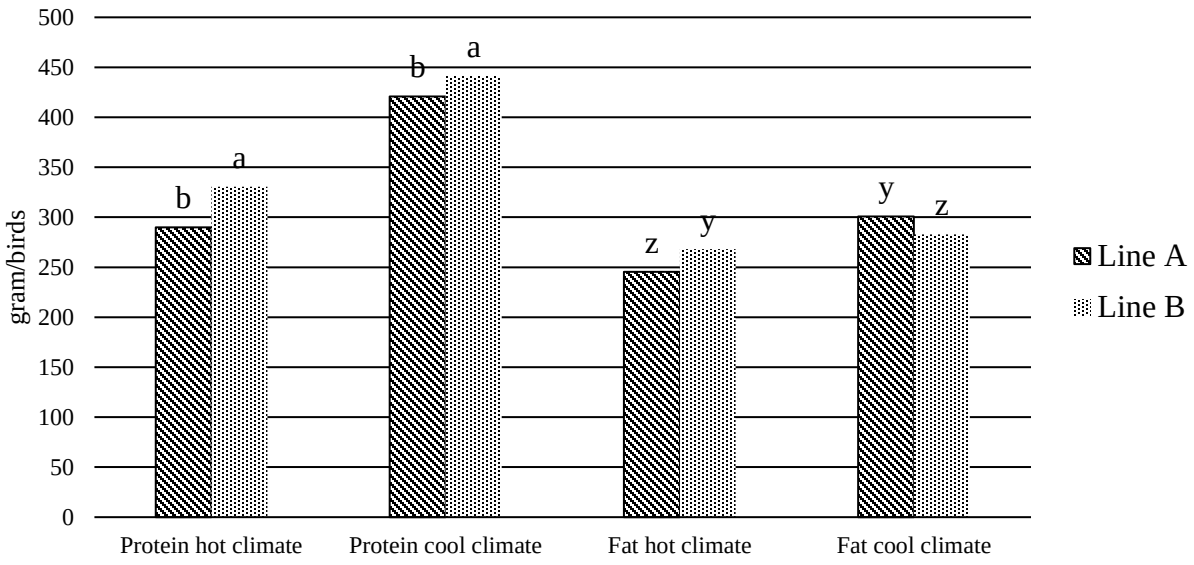

Figure 2. Body composition of broilers from two genetic lines at two different temperatures. Factorial design 2 x 5. Body composition calculated on d 22 and d 42. Levels (a, b, c and x, y, z) not connected by same letter are significantly different among diets, Tukey-HSD test $P < 0.05$.

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IV. COMPARISON OF TWO NET ENERGY CALCULATIONS FOR TWO BROILER LINES FED VARYING LEVELS OF METABOLIZABLE ENERGY AT TWO DIFFERENT TEMPERATURE

ABSTRACT

Two experiments with the same design were conducted under cool (Experiment 1; 19.5℃, Heat index=19℃) and one under hot (Experiment 2; 26℃, Heat index=28℃) climate. For each experiment, broilers from two commercial genetic lines (A and B) were fed common starter (d 0 to 10; 3,030 kcal/kg; 1.27% g digestible lysine (**dlys**), and grower (d 11 to 21; 3,080 kcal/kg; 1.09% dlys) diets (Table 1). Dietary amino acid and energy levels were formulated according to AminoChick nutritional recommendations (Evonik Nutrition & Care GmbH). At d 21, one of five finisher (d 21 to 42) experimental diets formulated to contain 19.5%CP, 1.0% dlys and five levels of AMEn; 2800, 2925, 3050, 3175 and 3300 kcal/kg. All other amino acids were formulated in relationship to the dLys level (Table 2). Birds were sampled at d 9, 38 and 42 and heat production (**HP**) measured for a 24 h period using respiratory chambers. After HP measurement, fasting heat production (**FHP**) was measured for 24 h and heat increment (HI) determined by difference: HI = HP – FHP. Body composition was measured on d 22 and d 42 by dual energy X-ray absorptiometry (**DEXA**) to determine Net Energy (**NE**) of gain (**NEg**) as NEg = protein (g) x 5.45 + fat gain (g) x 8.95 (Caldas et al., 2019). Classic NE and Arkansas NE (**Ark NE**) equations were compared: Classic NE (kcal/kg) = ME – HI; Ark NE (kcal/kg) = NEg + Net Energy of maintenance **(NEm)**, where NEm = HP – HI. Calorie efficiency (%) was calculated as NE/AMEn x 100, and calorie difference (**CD**) between the two equations as CD = Ark NE (kcal/kg) – Classic NE (kcal). No differences between diets were found for HI and NEm. NEg was significantly (P <0.001) different among diets, with increasing ME producing more NEg (kcal). There was a significant increase (P < 0.001) in fat gain (g/bird) for birds fed increased ME diets. The CD between Ark NE vs Classic NE decreased from 700 kcal to approximately 350 kcal as dietary ME increased. Overall, the calorie efficiency was over 100% for Ark NE.

The Ark NE system supports increasing protein calorie gain compared to the Classic NE system that favors fat calorie deposition.

INTRODUCTION

Energy is an expensive part of broiler diets but is a critical nutrient. Dietary energy has been shown to influence feed intake, body composition, and heat production (Leeson et al., 1996). However, it is the most expensive component of a poultry diet, representing approximately 70% of broiler feed costs (Skinner et al., 1992). Increases in demand for poultry meat has influenced the selection for increased growth rate and ultimately the improved efficiency seen with broilers (Carré et al., 2014). Nutrients such as protein, carbohydrates and fat yield energy when oxidized, that is important for broiler growth. However, if these important nutrients are not balanced or the amino acid to energy ratio is manipulated, differences in broiler performance and body composition can occur (Jackson et al., 1982; Latshaw and Moritz, 2009). As the balance of dietary nutrients is manipulated the chances of energy being lost in the form of heat production to increase is greater (Cerrate and Corzo, 2019b).

The continual change in broiler genetics results in a necessity to continually update nutrient requirements to allow broilers to achieve their genetic potential. These updates and improvements in broiler nutrition are indicative of changes in how and when the broiler deposits ingested nutrients. Carcass traits, such as breast meat yield, allow for evaluation of protein deposition regardless of broiler sex or strain, but these external factors can influence protein deposition and, therefore, body composition (Cerrate and Corzo, 2019a).

Therefore, the continuous development of accurate and precise methods of measuring energy is vital in the rapidly changing industry. In addition around the globe, broiler nutritionists are formulating different energy to amino acid rations based on the region and availability of ingredients. Hence, there is a push to move towards a net energy (NE) system as it better accounts for broiler genetics, energy to protein rations, body composition and temperature effects(Swick et al., 2013; Wu et al., 2018).

Evidence has shown that reducing the energy levels of the diets by 25 – 50 kcal/kg will not impact performance (Maynard et al., 2019), and some nutritionists are even going a step further in reducing energy and increasing the amino acid density of the diets to improve performance at the same diet cost and thus, reduce production cost per kg. Therefore, a renewed interest in the impact of energy on, not just performance, but also its impact on body composition is becoming apparent (Dozier and Gehring, 2014; Wang et al., 2015). However, many of the shortcomings reported by Classen (2013) are still evident: no *in-vivo* ME measurements of ingredients or diets, and small ranges in the energy levels of dietary treatments (i.e. 30 kcal/kg). Not only addressing the confounding factors reported by Classen (2013) but providing a new perspective beyond measuring productive performance and meat yield is needed. Studying wider ranges in dietary energy and amino acid levels as reported by these authors, and taking into consideration confounding factors such as *in-vivo* energy and amino acid level measurements, and physical quality of the feed could further contribute to our understanding on what drives feed intake. The objective of this study was to determine the interaction of dietary metabolizable energy and two modern broiler strains on weight gain, feed intake, FCR, carcass and parts yield, protein retention efficiency, amino acid retention efficiency, and %

protein and amino acid retention change, protein turnover, body composition, heat production and energy efficiency for two modern strains from d22 to d42.

MATERIALS AND METHODS

Two experiments were conducted, one under cool (Experiment 1; 19.5℃, Heat index=19℃) and one under hot (Experiment 2; 26℃, Heat index=28℃) climate. The design of both experiments, except for the climate temperature, was exactly the same. All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee #15048.

Birds and housing

Fertile eggs from two modern, high yielding genetic lines (genetic line A; genetic line B) were obtained from a commercial hatchery and incubated at the University of Arkansas hatchery. After hatching, broilers were vent sexed. Male broilers (2,025 each genetic line, 4,050) were randomly distributed in two adjacent tunnel ventilated houses in 90 concrete floor pens (1.5 m x 3.0 m; 45 chicks each). Each pen was equipped with fresh pine shavings, two hanging type feeders, and a water line with 10 nipples. Minimum and maximum house temperatures were recorded by an electronic environmental controller (Chore-Tronics, CTB Inc., Milford, Indiana). Lighting program was 23L:1D from d 0 to 7 and 18L:6D from d 8 to 42.

Diets and treatments

Dietary amino acid and energy levels were formulated according to AminoChick nutritional recommendations (Evonik Nutrition & Care GmbH). All chicks were fed common starter (d 0 to 10; 3,030 kcal/kg; 1.27% dlys) and grower (d 11 to 21; 3,080 kcal/kg; 1.09% dlys) diets (Table 1). At d 21, one of five finisher (d 21 to 42) experimental diets formulated to contain

19.5%CP, 1.0% dlys and five levels of AMEn; 2800, 2925, 3050, 3175 and 3300 kcal/kg. All other amino acids were formulated in relationship to the dLys level (Table 2). Broilers were randomly assigned to the 45 pens within each genetic line creating a 2 × 5 factorial (genetic line × diet) of 10 treatments with 9 replications each. Feed and water were provided *ad libitum*.

Chemical analysis of feed, ileal digesta, and excreta

At d 42, six broilers were selected from each treatment (5 diets x 2 genetic lines) within 1 SD of the mean treatment BW to determine the digestibility of the experimental diets. Additionally, 20 broilers (2 per treatment) were selected to determine the digestibility of the grower diet. All selected birds were placed in metabolic digestibility cages and for 2 d for an adaptation period to the cage. All diets had the addition of 0.5% titanium dioxide and fed ad libitum. For the grower phase, feed was removed on the evening of d 19 and replaced 8 h later for a fasting period. Birds were then sampled after 2 h of ad libitum feeding. For the finisher diets the same process was completed starting in the evening of 42 d. After eating for 2 h birds were immediately euthanized by CO_2 asphyxiation. Following euthanasia of the birds, ileal digesta was collected on these same days in order to determine nutrient digestibility. Clean excreta (free from feathers and feed) was collected using plastic spatulas and placed in labeled plastic containers and frozen in liquid nitrogen immediately after collection. The digesta content of the ileum (between Meckel's diverticulum and the ileocecal junction collected from the birds of each cage were pooled to represent one replicate. Pooled samples were frozen in liquid nitrogen immediately after collection. All samples were lyophilized and fine ground (<2 mm) before analysis.

The analysis of AMEn (nitrogen-corrected apparent metabolizable energy) involved the analysis of gross energy (GE), dry matter (DM) and nitrogen, in feed and excreta. GE was

determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). DM was analyzed by method 934.01 (AOAC, 1990) and nitrogen levels were determined by the method 990.03 (AOAC, 1995). The marker, titanium dioxide (TiO_2), was measured on 96 well plates following the methodology of Myers (2004). In summary, 0.35 g of K_2SO_4, 0.04 g of $CuSO_4$, and 0.1 g of excreta, feed or ileal, were added to each glass test tube and diluted with 3 mL of 18M H_2SO_4 to be heated at 120°C for 24 h in a block digester. Contents of the digestion tube were allowed to cool for 15 min, after which 7 mL of distilled deionized water was added to the digested sample, gently mixed and transferred to new plastic test tubes. This step was repeated using 2 mL of distilled deionized water. Diluted, digested samples were centrifuged at 3,000 rpm for 22 min and the supernatant was recovered using filter paper. After mixing 1 mL of the supernatant with 0.20 mL of distiller deionized water and 0.13 mL of 30% H_2O_2, the absorbance was measured at 410 nm subsequent to the next 10 min after the addition of the last reagent.

The apparent ileal digestibility for each amino acid (AA) was calculated as follows: % DAA= ($AA_{diet} - AA_{ileal}$ x (TiO_{2diet} / TiO_{2ileal}))/ AA_{diet} x100). Amino acids were analyzed in triplicate following the procedures: standard amino acid: AOAC 982.30 and Cystine/Methionine: AOAC 985.28 at the University of Missouri agriculture experiment station chemical laboratories. The standard AA method works under the principle of hydrolysis of the sample with HCl- 6N in the absence of oxygen to break down protein into individual amino acids. The samples are hydrolyzed in a drying oven at 120 °C for 16 hr. 2mL of norleucine (internal standard) is used and filtered through a #4 Whatman filter paper and then vacuum filtered through a 0.20 μm Gelman membrane filter. 1 mL of the stock sample is pipetted into a 50 ml borosilicate glass serum bottle and stored in freezer to cool. Glass bottles are placed in freeze drier to remove the HCl and pull a vacuum until no visible trace of liquid remains. 1 mL of 2.2 pH sodium diluent buffer is added to the dried residue, swirled to

dissolve dried sample and phenol is added to the buffer for preservation longevity. Reconstituted sample is transferred to a 1.5 mL micro-centrifuge tube for holding for HPLC injection. For the sulfur amino acids, (cysteine/methionine) the methodology was AOAC 985.28. The principle of this method is that the protein is first oxidized with performic acid for 16 h. in an ice bath, neutralized with hydrogen bromide and hydrolyzed at 121 °C with 6N HCl for 18 hr. Cysteic acid and methionine sulfone standards are added to an additional bottle. After hydrolysis, samples are allowed to cool and filtered through #4 Whatman and the same steps for the previous standard AA is performed before loading the samples on HPLC.

Sampling

On d 19, broilers were weighed, selected, and transferred (60 birds total; 30 per genetic line) to respiratory chambers (5 birds each one) and given one day adaptation prior to heat production (HP) measurement to establish baseline HP. Thereafter, at d 38 and d 42, broilers were weighed, selected, and transferred (24 birds total; 12 per line) to respiratory chambers (2 birds each) and given one day adaptation prior to evaluation. Due to limited number of respiratory chambers, at d 38 and d 42 only those diets formulated to contain 2800 kcal/kg, 3050 kcal/kg and 3300 kcal/kg of AME were used to determine heat production.

To establish basal body protein and fat composition, 18 broilers from each genetic line (36 total) and 18 birds from each treatment (180 total) were selected within one (± 1) SD of the treatment average BW at d 21 and 42, respectively. Selected broilers were killed via CO_2 inhalation prior to determination of body composition using dual energy X-ray absorptiometry (DEXA, General Electric Co., Madison, Wisconsin) with small animal body software module (Lunar Prodigy from General Electric Co. encore version 12.2). Obtained DEXA results were adjusted to body chemical analyses performed by Caldas et al., (2019).

Respiratory Chambers and Heat Production Determination

Respiratory chambers (61 cm l x 51 cm w x 56 cm h) utilized were the same as described by Caldas (2018), with exception of the lighting program, temperature and air flow. The temperature inside the chambers was maintained at range 60° F to 65° F and 80° F to 85° F for the experiments under cool and hot climate, respectively. The room temperature was 50° F to 55° F and 70° F to 75° F for the experiments under cool and hot climate, respectively; which is 10° F lower than the temperature inside the chambers, which ensured that the temperature inside the respiratory chambers stayed within the expected temperature range. The indirect calorimetry system provided air flow of 20 to 25 L/min, depending on the size of the broilers in the chamber. Before each evaluation day, chambers were opened for individual BW and feed intake (FI) measurements and calibration of gas analyzers. Fed heat production (HP) was determined for 24 h, followed by fasted heat production (FHP) for the next 24 h. Broilers were allowed *ad libitum* feed access during adaptation and fed periods.

Net Energy (NE) calculations

Volumes of O_2 (VO_2) and CO_2 (VCO_2) within each chamber were averaged for each 24 h evaluation period. HP and FHP were calculated following the equation: HP kcal/d = 3.866 VO_2 L/d + 1.233 VCO_2 L/d (Brouwer, 1965) and normalized to kg of FI.

Heat production (HP) consists of the Net Energy of maintenance (NEm) plus the heat increment (HI; see equation 1 below). HP and fasting heat production (FHP) were calculated using the Farrell (1974) equation (see equation 2 below). Classic NE was calculated according to Noblet et al. (2010) (see equation 3 below), where HI is defined as HP minus FHP (see equation 4 below). Net Energy of gain (NEg) was calculated based on body composition data (body protein and fat levels) from DEXA (see equation 5 below). Equation (6) was obtained rearranging equation (1),

allowing NEm be evaluated with indirect calorimetry. This method is called the Arkansas Net Energy Equation (Ark NE; 7), which encompasses both body composition and heat production.

(1) HP = NEm + HI (Farrell, 1974)

(2) HP and FHP = 3.871 x VO_2 (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974)

(3) $NE_{classic}$ (kcal/kg) = ME (kcal/kg) – HI (Noblet et al., 2010)

(4) HI = HP – FHP

(5) NEg = protein gain (g) x 5.66 (kcal/g) + fat gain (g) x 9.35 (kcal/g)

(6) NEm = HP – HI

(7) Ark NE = NEg + NEm

Statistical analysis

Chamber or pen was the experimental unit. Floor pen data were analyzed under a 5 (diets) x 2 (genetic lines) factorial arrangement. Calorimetry and body composition data for baseline information were analyzed under completely randomized designs with two treatments (genetic lines); however, subsequent data were analyzed under a 3 (diets) x 2 (genetic lines) factorial arrangement for calorimetry and a 5 (diets) x 2 (genetic lines) factorial arrangement for body composition. Data were analyzed by ANOVA of JMP Pro 13 (SAS Institute, 2017). When the means were significant ($P \leq 0.05$) student t-test was used. *P*-value was considered significant when ≤ 0.05.

ANOVA model:

$$Y_{ijk} = \mu + T_i + B_j + (TB)_{ij} + e_{ijk}$$

μ = mean

T_i = effect of i^{th} level of factor A

B_j = effect of j^{th} level of factor B

$(TB)_{ij}$ = effect of interaction between the i^{th} level of factor A and the j^{th} level of factor B

e_{ijk} = random error associated with the k^{th} replicate

For completely randomized designs, only one factor was present.

RESULTS

Body Composition

For body composition, a 2 x 5 factorial design provided differences in tissue gain between dietary treatments (Table 3, Figure 1). No genetic line by diet interactions were found; therefore, only main effects data are shown. Feeding iso-nitrogenous diets with increasing ME changed broiler protein and fat body composition. At both hot and cold temperature, increasing ME significantly ($P < 0.001$) produced higher body fat depositions (Figure 1). The largest amounts of fat gain (g/bird) ($P < 0.001$) was found in broilers fed the 3175 kcal/kg and 3300 kcal/kg ME level diets, 223g and 237g, respectively, during the cold temperature (Table 3). The lowest amount of fat gain (g/bird) was found in broilers fed the 2800 kcal/kg level diet ($P < 0.001$) during both hot and cold season (Table 3). Differences in energy gain ($P < 0.001$) were found at both hot and cold temperature, with broilers fed the lowest ME diet gaining 832 kcals less than the highest ME diet during the cold temperature and the opposite during the hot temperature. During the hot temperature birds on the lowest ME diet gained 275 kcals more ($P < 0.001$) energy than the 3300 kcal/kg diet. A positive linear relationship was found between energy gain (kcals/bird) and increasing ME (kcals/kg) in cool ($P < 0.001$, R^2=0.46, slope=1.6).

Differences in body protein by genetic line did not show any significant differences in both experiments. However, fat gain, within genetic line differed significantly ($P < 0.05$) only during the hot climate. At the hotter temperature, line A had 24 g/bird fatter compared to line B (Table 3).

Calorimetry parameters

Heat expenditure calculated by respiratory exchange in indirect calorimetry chambers: volume of oxygen consumption and carbon dioxide production. HP data showed no significant differences for diet alone. Variances in HP (kcals) between genetic lines showed line B having produced +45 kcals more ($P < 0.05$) than line A in experiment two during the finisher phase (Table 4). In addition, the 2 x 5 factorial design indicated significant interaction ($P < 0.05$) between genetic lines and diet during experiment one (Table 4). Here, line B fed 3050 kcal/kg diet produced +32 more kcals than line A on the same diet. Similar trend was seen for when HP is expressed per metabolic BW ($kg^{0.70}$) during experiment one. Line B on 3050 kcal/kg diet produced +28 kcals more ($P < 0.05$) heat per $BW^{0.70}$ than line A on the similar diet.

Net Energy

As dietary ME levels increased, the Classic NE of the diet increased in hot climate, and the Ark NE value decreased (Table 6). In addition, the Classic NE to ME (NE/ME) ratio improved 2%, and the Ark NE/ME ratio declined 13% (Table 5). However, during the cooler climate as the dietary ME level increased both the Classic NE and Ark NE of the diet varied little between the three dietary treatments (Table 5). While the Ark NE value during the cooler climate varied 237 kcals between the lowest ME level verse the highest ME level (Table 5). Furthermore during the warm temperature, the ratio of Classic NE/ME did not vary, while the Ark NE/ME ratio varied by 13% (Table 5).

The calorie difference between Classic NE and Ark NE declined as the amino acid to calorie ratio decreased. Regarding genetic lines, line B had higher Ark NE value in both climates, 3,000 kcals vs. 3,077 kcals, for cool temperature and 3,232 kcals vs. 3,615 kcals in hot temperature. However for Classic NE, line A significantly ($P < 0.05$) higher in the hot

temperature (2,596 kcals vs. 2,414 kcals, Table 6). Overall, broilers in cooler temperature showed Ark NE values 28% lower when compared to those in hotter climate and Classic NE showed broilers in cooler temperature 4% lower compared to those in hotter climate.

DISCUSSION

Relationships between energy and protein plays an important role in broiler responses. As protein to energy ratios increases, a sparing effect occurs and less protein is used to meet energy requirements, allowing for the additive effects of amino acids towards growth and maintenance (Classen, 2017). When broilers are fed enough energy to meet their maintenance requirement the leftover ME will be released as heat (Latshaw and Moritz, 2009). This alone indicates there is a specific dietary range energy to protein needs to stay within in order to maintain feed intake. If the intake of energy remains constant, the efficiency of production will also increase as long as all other nutrients are in balance with energy (Classen, 2017).

Body composition

The use of iso-nitrogenous diets with varying levels of ME, had differences in both experiments even though birds of similar weights were selected for body composition. In both experiments, there were no significant differences for protein gain, however both experiments showed the same linear trend (Figure 1) for fat gain. When dietary protein is not provided in adequate amounts, the broilers will gain more fat indicating the importance of maintaining an energy to protein ratio in adequate amounts (Farrell, 1974). Similar results can be seen in a study conducted by Jackson et al. (1982) in which a linear increase in fat and a linear decrease in protein deposition. Increasing increments of dietary ME decreases percent carcass protein. In Jackson (1982) and in current studies only carcass fat was impacted and no significant differences on carcass protein were detected. This classic body composition results is indicative

to the importance of protein to energy ratios. There typically is an inverse response of protein intake to carcass fat (Hilton et. al, 2019) but a direct response of ME intake to carcass fat (Table 3). If energy is not balanced with protein birds will still gain weight but it will be in the form of fat, which is lighter than protein at the detriment of feed efficiency (Nitsan et al., 1997a; Classen, 2017).

Soybean oil was utilized as the fat source in current experiments. It has been reported type of fat source and fat deposition may be correlated based on the nature of the fat (Nitsan et al., 1997a). These differences can be associated with the digestibility of the fat and its ability to release energy to the birds. The addition of soybean oil has also shown to increase the digestibility of total fat and reduce the digestibility of starch (Nitsan et al., 1997a).

HP and Net Energy

There is no question that high and low temperatures affect broiler performance, specifically feed intake (Classen, 2017). The NE system for energy evaluation is supposed to account for the differences in energy metabolism from dietary fat. The extra caloric effect from fat can be attributed to the lower heat increment and therefore the higher Classic NE (Classen, 2017). As in Figure 3, as diet ME increased broiler body fat increased the HP had a linear decrease (R^2=0.98). In a study conducted by Nitsan et al. (1997a), calculated HP was reduced by the addition of soybean oil diets, the same for experiment one. However, as temperature rises, past the thermo-neutral zone birds tend to decrease their feed intake to lower the metabolic heat production and try to lose this excess heat through panting. During this process, this mechanism removes heat that would have been available for growth (Oliveira et al., 2018; Zhang et al., 2018) seen in experiment two, as dietary energy increased HP (kcals/d) increased. It can be said the maintenance energy (NEm, Table 4) increased during the hotter climate due to the need to

increase the ability to dissipate heat. Thereby, reducing the amount of available energy for growth. As temperatures increase the ME requirement decreases, mainly due to a reduction in the energy put towards maintenance (Daghir, 2008). When looking at the cool temperature the average NEm was 53 kcals/d while in the hot temperature was 49 kcals/d (Table 4). Further demonstrating added ME by including more fat in the diet has more of an additive affect during hotter climates. However, for Ark NE calculations these added benefits of reduction of maintenance energy only has a benefit when addressing heat production alone. For the Ark NE equation broiler body composition plays a huge role. The added benefit of the addition of fat in the diet during hot climate only to lower the NEm is at the expense of protein gain. Whereas, the Classic NE showed a lower heat increment therefore allowing the Classic NE to be misinterpreted.

Broilers efficiency for digesting fat increases as the bird ages (Nitsan et al., 1997b). Dietary energy and protein ratio are very important to their associative effects on protein and energy metabolism. The characteristics between protein and energy determines the extent in which the uptake of other nutrients is conducted. (M. Abdel-Hafeez et al., 2016). When fat is added to the diet a portion of this fat is directly deposited to the body tissues. This direct transfer of fatty acids is more energy efficient as this process does not require synthesis from carbohydrates. This process reduces heat production and NE improves.

In a study done by Cerrate and Corzo (2019) where a meta-analysis was conducted to show trends over the past 16 years, it showed that body fat increased for every 100 kcal/kg of ME and increased for every 100 kcal ME/kg of dLys. In addition, this study showed that breast meat yield has drastically increased over the last 10 years thus altering the whole bird carcass protein of the modern broiler. This linear increase in lean mass is indicative that meeting the

birds requirements is needed. When broilers gain less fat there is less substrate for fatty acid synthesis, therefore at the expense of amino acids the balanced ratios between amino acids and energy is disrupted making for a decrease in efficiency.

In conclusion, these experiments demonstrated that like amino acids and protein, the source and type of fat (fat vs. starch vs. protein) makes a difference in how energy is metabolized by broilers. In addition utilizing together the NEm, determined from indirect calorimetry, and NEg, evaluated through DEXA, provide valuable information about type of gain and current broiler genetics broiler genetics. This combination provides a deeper understanding of diet NE, rather than the small indigestible fraction differences. Taking advantage of understanding the genetics and appropriate environment is an advantage of NE formulation.

Table 1. Composition and nutrient calculations of the starter and grower diets

Ingredient,%	Starter	Grower
Corn	60.40	62.31
Soybean meal	26.65	26.51
Corn gluten meal	6.54	5.00
Dicalcium phosphate	2.21	1.95
Soybean oil	1.21	2.02
Limestone	0.89	0.80
L-lysine HCl	0.47	0.25
Sodium bicarbonate	0.44	0.34
DL-methionine [1]	0.28	0.18
Vitamin and mineral premix[2]	0.22	0.22
Ethoxyquin[3]	0.02	0.02
Salt	0.15	0.23
Ethoxyquin	0.05	0.05
Choline chloride, 60%	0.15	0.13
L-threonine	0.13	0.04
L-valine	0.10	
L-arginine	0.09	
L-isoleucine	0.05	
Calculated composition %, unless otherwise noted		
Crude protein	23.14	21.50
Crude fiber	2.60	2.54
Ether extract	4.62	4.78
Ash	6.91	6.17
Starch	38.59	41.03
Choline chloride 60%	1.70	1.60
ME, kcal/kg	3030.00	3080.00
Ca	1.00	0.90
avP	0.50	0.45
Na	0.20	0.20
Cl	0.23	0.23
K	0.74	0.70
SID Lys	1.27	1.09
SID Met	0.63	0.51
SID M+C	0.93	0.80
SID Thr	0.81	0.70
SID Trp	0.22	0.20
SID Arg	1.31	1.20

Table 1. Composition and nutrient calculations of the starter and grower diets (Cont.)

	Starter	Grower
Calculated composition %, unless otherwise noted		
SID Ile	0.87	0.79
SID Val	1.01	0.88
Analyzed composition		
AMEn		2234
Crude Protein	22.43	20.86
Total Lys	1.37	1.16
Total Met	0.65	1.07
Total Met+Cys	1.00	1.41
Total Thr	0.92	0.8
Total Trp	0.23	0.23
Total Arg	1.40	1.27
Total Ile	0.93	0.84
Total Val	1.10	0.95
SID Lys		0.79
SID Met		1.42
SID Met+Cys		1.59
SID Thr		0.46
SID Trp		0.12
SID Arg		0.74
SID Ile		0.52
SID Val		0.51

Table 2. Composition and nutrient calculations of the finisher experimental diets

Ingredient, %	2800	2925	3050	3175	3300
Corn	37.79	41.52	51.51	58.23	61.09
Wheat middlings	20	20	7.44		
Sunflower meal, 27% CP	10	10	10	4.73	
Soybean meal, 48% CP	19.51	18.8	21.86	26.98	24
Corn gluten meal, 60% CP					3.93
Dicalcium phosphate	1.63	1.62	1.71	1.8	1.82
Soybean oil	4.53	4.77	5.11	6.05	6.72
Limestone	0.76	0.77	0.72	0.71	0.78
Sodium bicarbonate	0.39	0.39	0.35	0.28	0.32
Salt	0.21	0.2	0.23	0.28	0.25
L-lysine	0.22	0.23	0.17	0.09	0.19
L-Methionine[1]	0.22	0.21	0.2	0.21	0.19
Choline Chloride 60%	0.1	0.1	0.1	0.11	0.15
L-threonine[1]	0.09	0.09	0.06	0.04	0.06
L-valine[1]	0.02	0.02			
L-isoleucine[1]	0.04	0.05	0.02		0.01
Vitamin and mineral premix[2]	0.22	0.22	0.22	0.22	0.22
Ethoxyquin[3]	0.02	0.02	0.02	0.02	0.02
Organic acid[4]	0.05	0.05	0.05	0.05	0.05
Filler (sand/solka-floc)	4.2	0.94	0.23	0.2	0.2
Calculated composition %, unless otherwise noted					
Dry matter	90.49	90.17	89.92	89.66	89.69
Crude protein	19.5	19.5	19.5	19.5	19.5
Crude fiber	4.99	5.04	4.49	3.08	2.04
Ether extract	8.28	8.65	9.01	9.38	9.75
Ash	10.55	7.31	6.44	6.29	6.02
Starch	31.73	34.13	37.05	39.48	41.7
TMEn, kcal/kg	2,800	2,925	3,050	3,175	3,300
ME, kcal/kg	2,789	2,794	2,896	2,998	3,296
Ca	0.85	0.85	0.85	0.85	0.85
avP	0.42	0.42	0.42	0.42	0.42
Na	0.2	0.2	0.2	0.2	0.2
Cl	0.23	0.23	0.23	0.23	0.23
K	0.86	0.86	0.81	0.77	0.65
Analyzed composition %, unless otherwise noted[5]					
Dry matter	89.4	89.12	88.9	88.4	88.3
Crude protein	19.6	19.52	18.9	18.8	18.5
TMEn, kcal/kg	2,819	3,000	3,137	3,358	3,452
ME, kcal/kg					

Table 2. Composition and nutrient calculations of the finisher experimental diets (Cont.)

	2800	2925	3050	3175	3300
Analyzed composition %, unless otherwise noted[5]					
Lys	1.12	1.072	1.08	1.07	1.05
Met	0.51	0.494	0.5	0.5	0.48
M+C	0.84	0.816	0.8	0.8	0.79
Thr	0.8	0.773	0.77	0.77	0.76
Trp	0.24	0.245	0.24	0.24	0.22
Arg	1.36	1.297	1.32	1.3	1.15
Ile	0.82	0.816	0.81	0.82	0.79
Val	0.95	9.28	0.92	0.91	0.88
SID Lys	1.25	1.21	1.17	1.16	1.19
SID Met	0.54	0.64	0.54	0.49	0.48
SID M+C	0.89	0.98	0.84	0.80	0.79
SID Thr	0.83	0.79	0.79	0.74	0.80
SID Trp	0.26	0.22	0.22	0.23	0.22
SID Arg	1.42	1.37	1.31	1.32	1.35
SID Ile	0.89	0.85	0.83	0.83	0.88
SID Val	1.01	0.96	0.94	0.93	0.99

[1]DL-methionine (MetAmino), L-threonine (ThreAmino), L-valine (ValAmino) (Evonik Nutrition & Care GmbH).

[2]Vitamin premix: Vit A, 13227 IU/kg; Vit D3, 3968 IU/kg; Vit E, 66 IU/kg; Vit B12, 0.040 mg/kg; Biotin, 0.254 mg/kg; Menadione, 3.968 mg/kg; Thiamine, 3.968 mg/kg; Riboflavin, 13.228 mg/kg; Vit B6, 7.937 mg/kg; Niacin, 110.229 mg/kg; Folic acid, 2.205 mg/kg. Trace mineral premix: Mn, 60 mg/kg (manganese sulfate); Zn, 60 mg/kg (zinc sulfate); Fe, 40 mg/kg (ferrous sulfate); Cu, 5 mg/kg (copper sulfate); I, 1.25 mg/kg (calcium iodide); Co, 0.5 mg/kg (cobalt sulfate).

[3]Santoquin (Novus International, Inc).

[4]MycoCurb (Kemin Industries, Inc).

[5]Analysis on as is basis.

Table 3. Body composition gain of two genetic broiler lines fed different dietary metabolizable energy levels at different temperatures.

		Experiment 1 (cool)			Experiment 2 (hot)		
		Protein g/bird	Fat g/bird	Energy kcals	Protein g/bird	Fat g/bird	Energy kcals
Energy							
	2800	378	170^{c}	3510^{d}	393	147^{c}	5325^{a}
	2925	373	178^{c}	3564^{cd}	385	135^{c}	5288^{a}
	3050	377	206^{bc}	3840^{bc}	389	156^{bc}	5232^{ab}
	3175	378	223^{b}	4005^{b}	381	187^{ab}	4951^{b}
	3300	369	267^{a}	4342^{a}	388	198^{a}	5050^{ab}
SEM		4.80	10	74.68	6.11	9.03	72.15
Strain							
	Line A	374	218	3938^{a}	377^{b}	177^{a}	5194
	Line B	376	200	3766^{b}	397^{a}	153^{b}	5143
SEM		3.04	6.33	47.23	3.86	5.71	45.63
P-Value							
	Energy	0.60	<.0001	<.0001	0.74	<.0001	0.0012
	Strain	0.52	0.05	0.012	0.001	0.003	0.43

Factorial design 2 x 5. Levels (a,b,c) not connected by same letter are significantly different, Tukey-HSD test $P< 0.05$.

Table 4. Indirect calorimetry results of two genetic broiler lines fed different dietary metabolizable energy levels at different temperatures.

	Experiment 1 (cool)			Experiment 2 (hot)		
	Heat Production (kcals/d)	NEm (kcals/d)	Heat kcal/$Kg^{0.70}$	Heat Production (kcals/d)	NEm (kcals/d)	Heat kcal/$Kg^{0.70}$
Energy x Line						
A, 2800	265[ab]	64	180[ab]	269	48	178
A, 3050	240[b]	47	154[c]	267	47	165
A, 3300	249[ab]	54	157[bc]	247	44	156
B, 2800	237[b]	42	165[abc]	275	49	167
B, 3050	272[a]	67	183[a]	325	56	217
B, 3300	262[ab]	43	173[abc]	318	56	219
SEM	9.24	8.2	8.1	14.68	5.91	15.82
Energy						
2800	251	53	172	272	48	173
3050	256	57	168	296	51	191
3300	255	49	165	283	50	188
SEM	6.56	5.82	5.8	10.46	4.21	11.18
Strain						
Line A	251	55	163	261[b]	46	167[b]
Line B	257	51	174	306[a]	54	201[a]
SEM	5.43	4.82	4.7	8.53	3.36	9.13
P-Value						
Energy	0.845	0.572	0.88	0.307	0.881	0.485
Strain	0.457	0.518	0.144	0.002	0.127	0.016
Energy x Strain	0.03	0.07	0.04	0.099	0.682	0.067

Factorial design 2 x 5. Levels (a,b,c) not connected by same letter are significantly different, Tukey-HSD test $P< 0.05$.

Table 5. Comparison of Ark NE versus Classic NE values obtained

	Experiment 1 (cool)						Experiment 2 (hot)					
	AMEn kcals/kg	Classic NE kcals/kg	Ark NE kcals/kg	kcal difference kcals/kg	Classic NE/AMEn %	Ark NE/AMEn %	AMEn kcals/kg	Classic NE kcals/kg	Ark NE kcals/kg	kcal difference kcals/kg	Classic NE/AMEn kcals/kg	Ark NE/AMEn kcals/kg
						Energy x Strain						
A, 2800	3,345	2310	3049	739	70	92	2,968	2655	2859	204	90	96
A, 3050	3,512	2899	3066	167	84	88	2,870	2500	3335	835	90	120
A, 3300	3,493	3031	2885	-146	87	83	3,146	2634	3503	869	86	114
B, 2800	3,298	2697	2806	109	81	84	2,957	2389	4066	1677	81	137
B, 3050	3,425	2743	2983	240	79	86	2,674	2333	3482	1149	84	126
B, 3300	3,500	3087	3442	355	88	98	3,013	2520	3297	777	82	107
SEM		272.51	622.30	873.15	0.08	0.18		85.24	357.58	399.20	0.03	0.12
						Energy						
2800	3,322	2,503	2,927	424	75	88	2,963	2,522	3,462	941	85	117
3050	3,469	2,821	3,025	204	81	87	2,772	2,416	3,409	992	87	123
3300	3,496	3,059	3,164	105	87	90	3,080	2,577	3,400	823	84	110
SEM		192.69	440.10	617.40	0.06	0.13		60.28	252.85	282.3	0.02	0.085
						Strain						
Line A	3456	2,747	3,000	253	80	88	3004	2,596[a]	3,232	363	88[a]	110
Line B	3423	2,842	3,077	235	83	90	2904	2,414[b]	3,615	1201	82[b]	124
SEM		157.33	359.32	504.1	0.046	0.105		49.21	206.44	230.50	0.02	0.07
						P-Value						
Energy		0.152	0.930	0.933	0.344	0.983		0.188	0.982	0.91	0.488	0.594
Strain		0.673	0.881	0.980	0.663	0.891		0.02	0.21	0.10	0.02	0.20
Energy x Strain		0.6118	0.7961	0.8096	0.6071	0.7994		0.67	0.149	0.155	0.66	0.155

Factorial design 2 x 5. Levels (a,b,c) not connected by same letter are significantly different, Tukey-HSD test $P < 0.05$.

Table 7. Results comparison by temperature

Temperature	ME	Classic NE	Arkansas NE Equation	kcal Difference	Classic NE/ME	Arkansas Equation NE/ME
	kcal/kg	kcal/kg	kcal/kg	kcal/kg	%	%
Experiment 1	3,429	2,794	3,039	245	81	89
Experiment 2	2,938	2,505	3,424	919	85	117

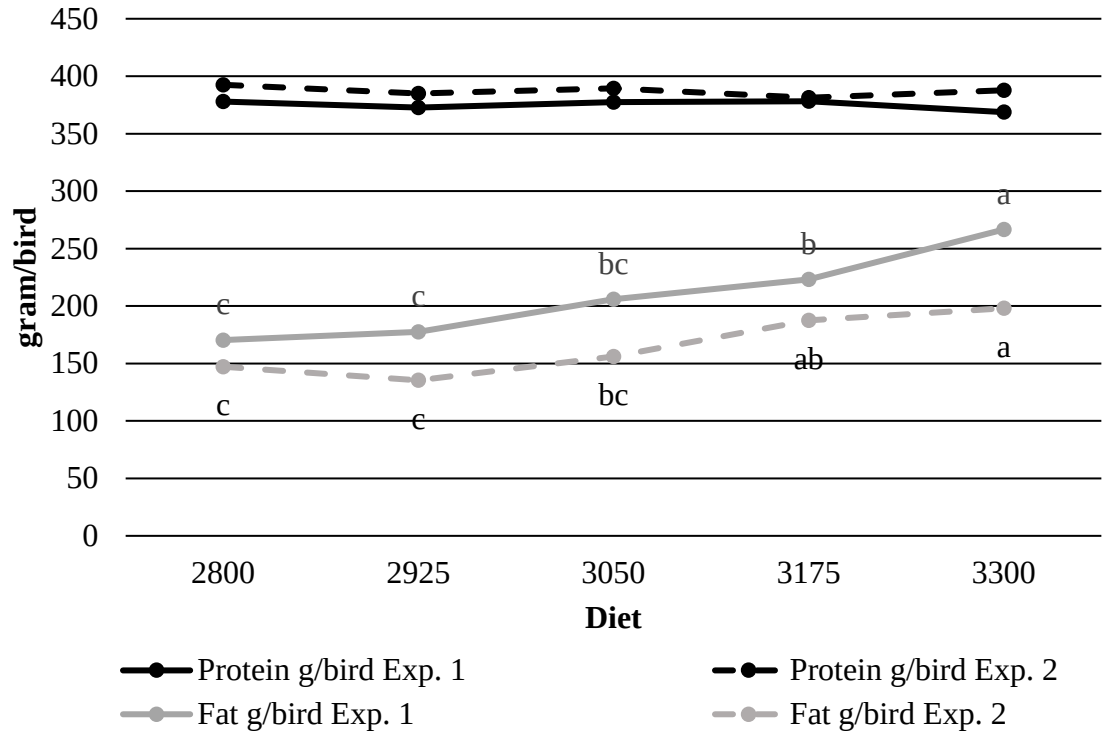

Figure 1. Protein and fat gain of broilers fed varying levels of metabolizable energy.

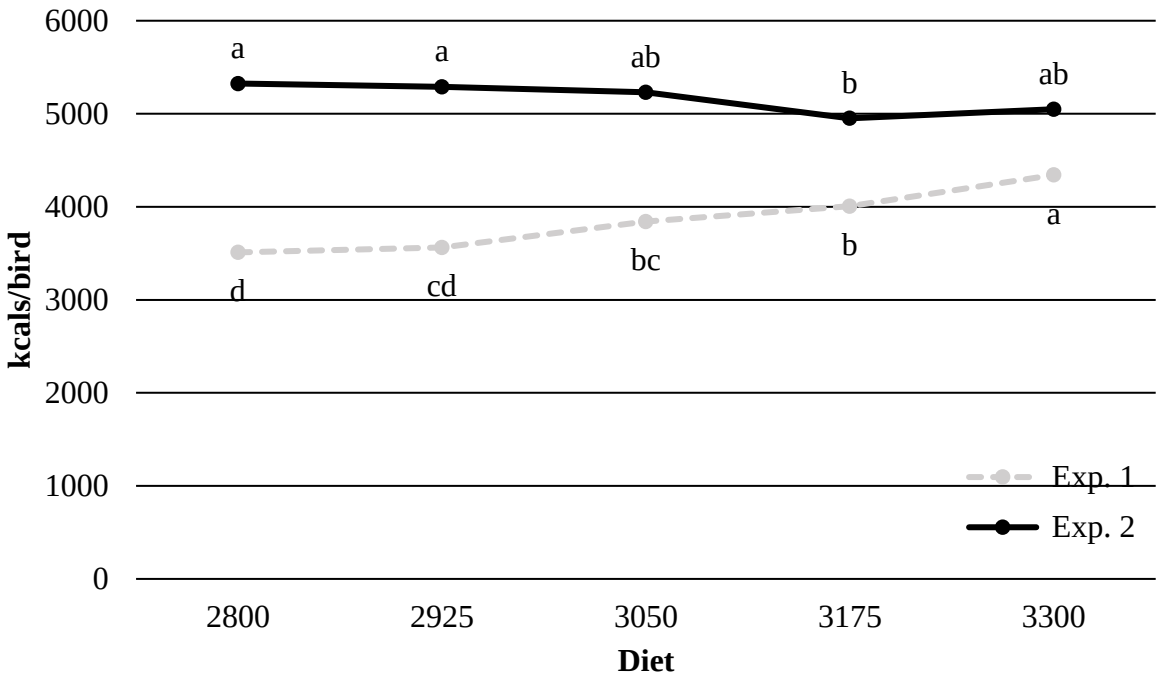

Figure 2. Energy gain of broilers fed varying levels of metabolizable energy.

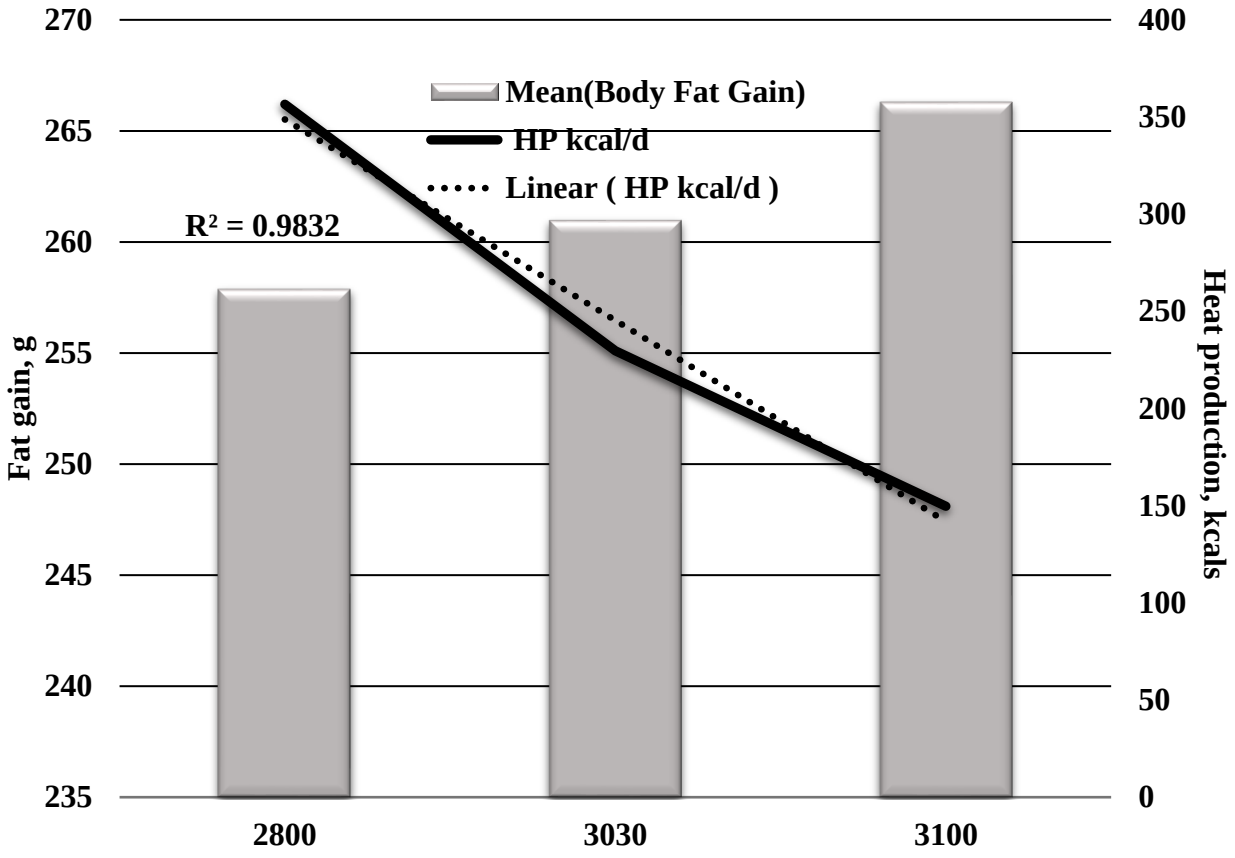

Figure 3. Correlation of fat gain and heat production**.**

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V. EFFECT OF COMPOSITE ENZYME ALONE OR IN COMBINATION WITH AN EXOGENOUS AMYLASE ON BROILER PERFORMANCE, NUTRIENT DIGESTIBILITY, BODY COMPOSITION AND NET ENERGY

ABSTRACT

Two trials were conducted to evaluate the effect of a composite enzyme alone or in combination with an alpha-amylase on broiler performance, nutrient digestibility, energy utilization and body composition during the starter (0 to14d), grower (15 to 28d) and finisher (29 to 49d) phases. In experiment 1 (floor pens), 612 Cobb broilers were randomly allotted into 3 treatments (4 pen replicates; 51 birds/pen). Dietary treatments consisted of an NC (negative control), NC + Composite (NC+V), and NC + Composite + Amylase (NC+V+A). The NC diet was reduced according to enzyme recommendations (Table 1). Bird performance and body composition were evaluated on days 14, 28, and 49. For overall performance (0-49d), feed conversion ratio (FCR) was improved by 18 points (P <0.05) with the addition of composite enzyme and amylase (Table 4). During the grower phase, body weight gain (BWG) was increased by 130g (P <0.05) for the NC+V treatment, compared to NC+V+A (Table 4). Finally, in the finisher phase, NC+V+A had improved FCR compared to NC, 1.39 verse 1.66, respectively (P <0.05). In trial 2 (digestibility cages), 432 Cobb broilers were randomly assigned to 6 treatments (12 cage replicates; 10 birds/cage). Dietary treatments included the same treatments evaluated in trial 1 plus a positive control (PC) and 0.50 or 1.50 times the recommended level of alpha-amylase (NC+V+0.5A and NC+V+1.5A, respectively) (Table 2).Growth performance, body composition, nutrient digestibility and AMEn were evaluated. Feed intake (FI), BWG and FCR were evaluated on day 14, 28 and 49 (Table 7). Excreta and ileal digesta were collected on d 5, 19 and 40 for the determination of nutrient utilization (Table 8). For BW there was a significant (P <0.05) improvement for d 14, 28 and 49. BW indicated the addition of composite enzyme either alone or in combination with alpha-amylase showed improvement when compared to the NC. For the starter phase FCR was improved (P <0.05,

Table 7) by 18 points for NC+V+1.5A. During the grower phase, 15-28d, birds fed NC+V diet had 132g more BWG than NC and birds fed NC+V+A had 130g more BWG than NC (P <0.05). During the starter phase, supplementation of composite enzyme alone (NC+V) improved (P <0.05) ileal digestibility (ID) of starch (IDs) by 3.8% and 5.3% compared to NC and PC, respectively. For the grower phase, composite enzyme alone (N+V) elicited an improvement (P <0.05) of 2.9% in IDs compared with NC+V+A0.5. During the finisher period, birds fed NC+V showed an improvement (P <0.05) in IDs of 2.2% compared to NC. In the starter phase, the AMEn was improved due composite enzyme supplementation single or in combination with the alpha-amylase compared to PC. During the grower phase, NC+V+A0.5 increased (P <0.05) AMEn compared with NC and PC by 151 kcal/kg 155 kcal/kg, respectively. Finally, in the finisher phase, the single supplementation of composite enzyme alone (NC+V) improved the AMEn by 29 kcal/kg (Table 8). Addition of composite enzyme increased BWG and improved FCR, however the addition of alpha-amylase separated from composite enzyme did not enhance starch digestibility for ME when compared with composite enzyme alone.

INTRODUCTION

Corn and soybean meal have historically been the most common feed ingredient in U.S. commercial broiler diets. However, the concentrations of total non-starch polysaccharides (NSP) in these feedstuffs are relatively high (9% and 29%, respectively) and may vary due to factors such as plant cultivar, growing conditions, processing, and analytical techniques used to assess the constituents (Malathi V, 2001; Aftab, 2012). Higher dietary concentrations of NSP present challenges for broiler nutrition, such as reduced digestibility of nutrients (e.g., starch) and lower energy density (Romero et al., 2014). Furthermore, the increasing demand over the past decade has placed pressure on grain markets, within the last decade, has resulted in a trend for diets with

reduced grain content and an increased co-product content, such as distiller's dried grain with soluble (DDGS). In general, DDGS has greater concentrations of protein, fat, vitamins and minerals, but its NSP contents are also 3–3.5 times higher (25-35%, Pedersen, Dalsgaard, Knudsen, Yu, & Lærke, 2014) than that of the parent grain (Zijlstra et al, 2010; Pedersent et. al., 2015). In an effort to combat variable ingredient quality and increased use of co-products, and thereby increase the efficiency of digestion and improve the profitability of commercial poultry production (Bao et. al., 2013) the inclusion of exogenous enzymes in corn-soybean meal based diets has become increasingly prevalent.

A quick and sensitive *in vivo* evaluation system can be established to aid in determining the benefits of adding exogenous enzymes to poultry feed. Body composition and heat production need to be assessed in order to determine the effects of enzymes on nutrient and energy utilization. Dual energy X-ray absorptiometry (DEXA) is a non-invasive, easy-to-operate, precise and relatively cheap technology, which can be used in combination with indirect calorimetry (IC), a sensitive tool for measuring heat production, to determine carcass composition and lean meat yield and account for the energy in the meat of the chicken. Since protein accretion (lean mass) requires 380% more oxygen intake to produce compared to fat accretion (Salas, et. al., 2012; Soladoye, et. al., 2016). The objective of this research is to determine the effects that exogenous composite enzyme and composite enzyme + amylase have on broiler performance, body composition, nutrient digestibility, heat production and net energy (NE) when included in a common corn-soybean meal diet.

MATERIALS AND METHODS

Two experiments were conducted, one in floor pens (Experiment 1) and the other nutrient digestibility (Experiment 2). All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC) #16078.

Birds and Housing

One thousand-four hundred and sixty-four Cobb-500 chicks were obtained from a local commercial hatchery (Cobb hatchery, Fayetteville, AR). For Experiment 1, 600 chicks were randomly placed on new litter (softwood shavings) over 12 concrete floor pens (1.99 m^2), 51 chicks each pen. Each pen had one hanging type feeder and 10 nipples. In Experiment 2, 864 chicks were placed in metabolic cages for nutrient digestibility, 12 chicks per cage. Each wire metabolic cage provided two nipple drinkers and a line feeder of dimensions 20" d x 24" w x 14" h. For both experiments lighting program was 23L: 1D from d 0 to 7 and 18L:6D d 8 to 49.

Diets and Treatments

A negative control (NC) basal diet, consisting of corn-soybean meal, was formulated to provide the Cobb 500 nutrient specs (Cobb Vantress, 2015), decreased by DSM recommendations for the composite enzyme (Table 1). Diets were formulated using Brill Formulation software (Feed Management Systems, Hopkins, MN). For Experiment 1, three dietary treatments were evaluated, consisting of NC, NC plus composite enzyme (NC+V) and NC plus composite enzyme with 120g/ton units of amylase (NC+V+A). In Experiment 2, six dietary treatments were evaluated, three from experiment one and three additional treatments. Additional treatments included: NC plus composite enzyme with 50% of the recommended level of amylase (NC+V+0.5A), NC plus composite enzyme with 1.5 times the recommended level of amylase (NC+V+1.5A) and a positive control (PC). The PC diet was made up of corn-soybean meal, formulated to provide the Cobb 500

nutrient specs (Cobb Vantress, 2015) with no nutrients decreased. All diets were fed *ad libitum* in mash form, with enzymes added on top. Composite enzyme composition and contributed enzyme activity can be found in Table 3. Recommended, half and 1.5 doses of amylase were included at 120g/ton, 60g/ton and 180g/ton providing 780, 396, and 11880 units/kg of complete feed, respectively. Feeding phases evaluated were starter (d0-14), grower (d15-28) and finisher (d29-49) for both experiments. Samples of each diet were sent to an appropriate laboratory for enzyme analysis (Novozymes A/S, Bagsvaerd, Denmark).

Sampling-Experiment 1

On d 12, 144 birds (48 per treatment), were selected, weighed and transferred to respiratory chambers (12 birds each one) Thereafter, at d 26, 60 birds (20 per treatment, 5 birds each one) and on d 48, 24 (8 per treatment, 2 birds each one) birds were selected. All birds were selected within one (±1) SD of the treatment average BW and given one day adaptation prior to heat production (HP) measurement.

For basal body protein and fat contents, 12 birds were analyzed at d 0, whereas 12 birds from each treatment (3 treatment; total 36) were analyzed at d 7, 14, 21, 28, 35, 42 and 49. All birds were selected within one (± 1) SD of the treatment average BW. Additionally, on d 7, 14, 21, 28, 35, 42 and 49 average BW, feed intake (FI), bodyweight gain (BWG) and feed conversion ratio (FCR) was calculated.

Respiratory Chambers and Heat Production Determination

Respiratory chambers (61 cm l x 51 cm w x 56 cm h) utilized were the same as described by Caldas et al. (2018), with exception of the lighting program, temperature and air flow. The temperature inside the chambers was maintained within a 2°F range. The room temperature was kept 10°F lower than the temperature inside the chambers, which ensured that the temperature

inside the respiratory chambers stayed within Cobb 500 recommendations. The indirect calorimetry system provided air flow of 20 to 25 L/min, depending on the size of the broilers in the chamber. Before each evaluation day, chambers were opened for individual BW and FI measurements and calibration of gas analyzers.

Fed heat production (HP) was determined for 24 h, followed by fasted heat production (FHP) for the next 24 h. Broilers were allowed ad libitum feed access during adaption and fed periods.

Body Composition Analysis

Birds were humanely sacrificed by CO_2 inhalation before body composition was determined using dual energy X-ray absorptiometry (DEXA; General Electric Co., Madison, Wisconsin) with small animal body software module (Lunar Prodigy, General Electric Co., encore version 12.2). DEXA results were adjusted to body chemical analyses performed by Caldas (2019).

Measurements and Calculations

DEXA body composition was used to determine the type of gain (protein versus fat) occurring in the broilers (Okumura and Mori, 1979). Energy efficiency (%) was calculated as EE = broiler body energy/ energy intake (kcal of gross energy) x 100, and Protein efficiency (%) was calculated as PE = body protein gain (g)/ protein intake (g) x 100.

Net Energy (NE) calculations

Volumes of O_2 (VO_2) and CO_2 (VCO_2) within each chamber were averaged for each 24 h evaluation period. HP and FHP were calculated following the equation: HP kcal/d = 3.866 VO_2 L/d + 1.233 VCO_2 L/d (Brouwer, 1965) and normalized to the mass of feed intake (in kg).

Heat production (HP) consists of the Net Energy of maintenance (NEm) plus the heat increment (HI; see equation 1 below). HP and fasting heat production (FHP) were calculated using the Farrell (1974) equation (see equation 2 below). Classic NE was calculated according to Noblet et al. (2010) (see equation 3 below), where HI is defined as HP minus FHP (see equation 4 below). Net Energy of gain (NEg) was calculated based on body composition data (body protein and fat levels) from DEXA (see equation 5 below). Equation (6) was obtained rearranging equation (1), allowing NEm be evaluated with indirect calorimetry. This method is called the Arkansas Net Energy Equation (Ark NE; 7), which encompasses both body composition and heat production.

(1) HP = NEm + HI (Farrell, 1974)

(2) HP and FHP = 3.871 x VO_2 (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974)

(3) $NE_{classic}$ (kcal/kg) = ME (kcal/kg) – HI (Noblet et al., 2010)

(4) HI = HP – FHP

(5) NEg = protein gain (g) x 5.66 (kcal/g) + fat gain (g) x 9.35 (kcal/g)

(6) NEm = HP – HI

(7) Ark NE = NEg + NEm

Sampling—Experiment 2

On d 7, 14, 21, 28, 35, 42 and 49, birds were group-weighed and average BW, FI, BWG and FCR were calculated. On d 5, 19 and 40, clean excreta (free from feathers and feed) was collected using plastic spatulas and placed in labeled plastic containers and frozen in liquid nitrogen immediately after collection. Following euthanasia of the birds, by CO_2 asphyxiation, ileal digesta was collected on these same days in order to determine nutrient digestibility. The digesta content of the ileum (between Meckel's diverticulum and the ileocecal junction)

collected from the birds of each cage were pooled to represent one replicate (12 replicates per treatment). Pooled samples were frozen in liquid nitrogen immediately after collection.
All samples were lyophilized and fine ground (<2 mm) before analysis. At the end of the digestibility study, the remaining birds were humanely sacrificed by CO_2 inhalation and scanned for body composition.

Chemical analysis of feed, ileal digesta, and excreta

The analysis of AMEn (nitrogen-corrected apparent metabolizable energy) involved the analysis of gross energy (GE), dry matter (DM) and nitrogen, in feed and excreta. GE was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). DM was analyzed by method 934.01 (AOAC, 1990) and nitrogen levels were determined by the method 990.03 (AOAC, 1995). The marker, titanium dioxide (TiO_2), was measured on 96 well plates following the methodology of Myers (2004). In summary, 0.35 g of K_2SO_4, 0.04 g of $CuSO_4$, and 0.1 g of excreta, feed or ileal, were added to each glass test tube and diluted with 3 mL of 18M H_2SO_4 to be heated at 120°C for 24 h in a block digester. Contents of the digestion tube were allowed to cool for 15 min, after which 7 mL of distilled deionized water was added to the digested sample, gently mixed and transferred to new plastic test tubes. This step was repeated using 2 mL of distilled deionized water. Diluted, digested samples were centrifuged at 3,000 rpm for 22 min and the supernatant was recovered using filter paper. After mixing 1 mL of the supernatant with 0.20 mL of distiller deionized water and 0.13 mL of 30% H_2O_2, the absorbance was measured at 410 nm subsequent to the next 10 min after the addition of the last reagent.

Starch content of the experimental diets and ileal contents were determined, in duplicate, using the dinitrosalicylic acid (DNS) colorimetric method, adapted for microtiter plates. All chemicals used were analytical grade, purchased from Sigma-Aldrich. The DNS reagent contained

3, 5-dinitrosalicylic acid (10 g L^{-1}), sodium potassium tartrate (30 g L^{-1}) and NaOH (16 g L^{-1}), and was stored in darkness at room temperature. D-(+)-Glucose calibration curves were created covering appropriate ranges, prepared in 0.1 M sodium acetate buffer (pH 5.5) and replicated twice per plate. Sixty microliters of each standard or reaction hydrolysate was added to 120 mL DNS reagent in a 96-well PCR microplate (Axygen Scientific Inc. PCR-96) for a total reaction volume of 180 mL. DNS reactions were carried out in a PCR thermocycler (Biorad C1000 Touch™, Hercules, CA), heating at 95°C for 5 min followed by cooling to 4°C for 1 min, and holding at 20°C. Thirty-six microliters of the completed DNS reaction were then added to 160 mL of ddH2O in flat-bottom microplates (Corning Life Sciences 3370, Corning, NY), and absorbencies were measured at 540 nm. The analyzed AME was corrected to zero N retention, AMEn, using a factor of 8.22 kcal/g (Hill and Anderson, 1958). The ileal digestibility of crude protein (CP) and starch were calculated by the following formula, using the TiO_2 marker ratios in the diet and ileal digesta (Ravindran et al., 2009):

Apparent nutrient digestibility, % = $[((NT/T_i)_d - (NT/T_i)_i) / (NT/T_i)_d] * 100$

where $(NT/T_i)_d$ is the ratio of nutrient to T_i in the diet and $(NT/T_i)_i$ is the ratio of nutrient to T_i in the ileal digesta.

Statistical analysis

Chamber or pen was the experimental unit for statistical analysis. A complete randomized block design (CRD) with factorial arrangement (treatment x age) was performed evaluating starter, grower and finisher independently because of the different multi-enzyme composition in each phase. For growth performance, the mean of all the birds in each pen were treated as a single observation to conduct statistical analysis. All analysis were conducted in JMP Pro 13 (SAS Institute, 2017) and differences were considered significant at $P \leq 0.05$.

ANOVA model:

$Y_{ijk} = \mu + T_i + e_{ijk}$

μ = mean

T_i = effect of i^{th} level of factor A

e_{ijk} = random error associated with the k^{th} replicate

For completely randomized designs, only one factor was present.

A Gompertz 3P model was fitted between Y=BW vs X=Age; and Y=FI vs X=Age, and taking diet as group, so all treatments can be displayed in the graph. Equivalence test was performed to compare the NC+V verse NC+V+A, NC+V+0.5A and NC+V+1.5A at $P \leq 0.05$.

RESULTS

Experiment 1

The influence of dietary treatment on broiler performance are show in Table 4. There was no dietary differences for BW, BWG, FI and FCR during the starter phase (d 0-14). During the grower phase, d 15-28, dietary differences did exist for BW, BWG and FCR. For BW, d 14 birds fed NC+V+A were 37 g significantly (P <0.05) heavier than the NC birds. While on d 28 the heaviest (P <0.05) birds were NC+V, by 140g. A similar trend was seen for BWG, NC+V birds gained 107g more (P <0.05) than NC birds. FI was not affected by diet throughout the experiment, however FCR was significantly (P <0.05) impacted during the grower, finisher and overall feeding phases. From d 15-28 birds fed NC, NC+V and NC+V+A had FCR of 1.59, 1.49 and 1.63, respectively, with significant difference (P <0.05) between NC+V and NC+V+A. During the finisher phase, FCR was significantly (P <0.05) improved approximately 10 points (1.66 vs 1.39) points for birds fed NC+V+A diet when compared to birds fed NC. Overall, d 0-49, the addition of composite enzymes, either alone or in combination with amylase significantly (P <0.05) improved the FCR.

Body Composition

For body composition evaluation, a randomized design proved differences in tissue between the dietary treatments. Protein mass (g/bird) was significantly different on d 14 and 28 (Table 5). On d 14, protein mass values were 55, 57 and 54 g/bird (P <0.05) for NC, NC+V and NC+V+A, respectively. On d 28, NC+V fed birds had 20g/bird more (P <0.05) protein mass than birds fed NC+V+A. Differences in fat mass were significant for each sample period (Table 5). On d 14, NC+V+A had significantly (P <0.05) more fat mass than NC and NC+V by 10 g. However, on d 28 and 49, birds fed NC+V had significantly more (P <0.05) fat mass than birds fed NC by 56 g and 63 g, respectively.

Protein gain and fat gain is expressed as the type of gain (g), protein or fat, a bird obtained during each growing phase: starter (d 0-14), grower (d 15-28), finisher (d 29-49) and overall d 0-49. Protein gain was only significant during the starter phase (Table 5). During this phase, birds consuming NC+V gained 3 g more (P <0.05) than NC diet and NC+V+A. Fat gain was significant during the starter, grower and overall 0-49 d growth periods (Table 5). In the starter phase, the addition of exogenous amylase showed no significant additive factor, however the addition of exogenous composite enzymes alone had a significant (P <0.05) impact during the starter and grower periods when compared to the NC broilers. Starter phase broilers fed NC+V+A gained 16.4g of fat significantly (P <0.05) more than birds fed NC (11.7 g) and NC+V (15.5 g). However, during the grower phase birds consuming the NC+V gain 52.2 g more (P <0.05) fat than birds consuming NC diet. Additionally for overall growth 0-49 d similar trend was present with exogenous composite enzyme treatment and composite enzyme in combination gaining more (P <0.05) fat deposition.

Protein and Energy Efficiency

Protein efficiency (%) was calculated as PE = body protein gain (g)/ protein intake (g) x 100, while energy efficiency (EE) was calculated as EE = NEg (kcal)/ energy intake (kcal of AMEn) x 100. PE was only significant for the grower growth phase, d 15-28. Here, birds fed the NC diet had the highest (P <0.05) efficiency at 86.1%, compared to 73.6% and 70.2% of NC+V and NC+V+A, respectively. EE was significant for the starter, grower and overall feeding phases (P <0.05). For both the starter and the grower the addition of composite enzyme alone or in combination with amylase showed significant differences when compared to the NC diets. Both enzyme treatments showed approximately four percent more efficiency than NC fed broilers. Overall, from d 0-49, diet type had a significant impact on EE. Here, birds consuming NC+V+A had an energy efficiency of 44.4%, significantly (P <0.05) better than birds consuming the NC diet.

Heat production and Net Energy

For all feeding phases there was no significant differences for HP, HI or NEm (Table 6). The NEg calculated by, NEg = protein gain (g) x 5.66 (kcal/g) + fat gain (g) x 9.35 (kcal/g) showed significant difference in the starter and grower phases. During the starter phase broilers fed exogenous composite enzymes in combination with amylase or alone had similar NEg compared to the NC, with 62 kcals and 64 kcals more (P <0.05) than the NC birds respectively. However, during the grower phase, broilers fed NC+V had significantly (P <0.05) more NEg than the other two treatments.

During the starter, grower and finisher feeding phases, HP was numerically increased with composite enzyme addition (Table 6). The classic method for calculating Net Energy (NE) is to subtract the fasting heat production from the fed heat production to get the heat increment and then

find: NE = AMEn - HI. Overall, NE was increased with the addition of composite enzyme, compared to the NC diet. NE was further increased with the addition of composite enzyme and amylase. Additionally, during the starter phase ARK NE/AMEn was significantly different between the treatments. Here NC+V was 8% more efficient than the NC birds. Furthermore, when observing the Classic NE verse the Ark NE, regardless of feeding phase the Classic NE value decreases with the addition of exogenous enzymes, while the Ark NE value shows the opposite trend.

Experiment 2

The influence of dietary treatment on broiler performance are shown in Table 7. Enzyme supplementation elicited significant differences in BW on d 14, 28 and 49 (Table 7). On d 14, birds fed NC+V had the heaviest body weight (P <0.05) compared to NC diet birds. However, birds fed NC+V, NC+V+A, NC+V+0.5A, NC+V+1.5A and PC diet birds were statistically similar in BW. On days 28 and 49, birds fed PC and supplemented diets were statistically similar; however, they were statistically heavier (P <0.05) than birds fed the NC diet.

The BWG for each feeding phase is presented in Table 7. During the starter phase, birds fed the PC diet and diets containing enzymes gained significantly (P <0.05) more weight (g) than birds fed the NC diet. During this phase, birds fed NC+V+1.5A had a higher BW gain compared to birds fed NC (379.5g versus 334.2g). For the grower phase, 15-28d, there was a significant difference (P <0.05). This phase broilers fed PC showed the highest weight gain compared to birds fed NC (988 g versus 810 g). Birds fed NC+V, NC+V+A and NC+V+1.5A all gained statistically similar weight. Similarly, NC+V+0.5A and NC gained statistically similar weight during this feeding phase. During the finisher phase, there were no significant differences in BW gain. There were no significant differences for FI. However, FCR was significant for the starter phase. Birds

fed NC+V+1.5A had an FCR of 1.38 versus birds fed the NC diet, which had an FCR of 1.54. The rest of the treatments were statistically similar to one another in this phase. For the grower and finisher periods, there were no significant differences in FCR. However, numerically, during the finisher phase (29-43d), birds fed NC+V+0.5A and NC+V+1.5A had the highest FCR.

Nutrient digestibility and AMEn

Supplementation of the composite enzyme either alone or in combination with three increasing levels of amylase increased the percentage of starch digested ($P < 0.05$) compared to NC and PC, in the starter phase. A similar trend was seen during the grower phase. During the finisher phase, improvements in digestible starch were elicited by enzyme supplementation. Digestible starch percentage in birds fed NC+V and NC+V+A were 24 and 22.9% higher than NC, respectively ($P < 0.05$). Birds fed NC+V showed a significantly higher ($P < 0.05$) ileal starch digestibility, improved by 4.14 and 5.32% compared to NC and PC, respectively, in the starter phase. Similarly, the digestibility of the starch was increased ($P < 0.05$) by 2.22% in birds fed NC+V, compared to NC, in the finisher phase. On the other hand, NC+V elicited a higher ($P < 0.05$) digestibility of starch compared to PC (96.73 versus 94.63) in the grower phase (Table 8). Although no statistical differences were detected in CP digestibility percentage in any phase, there was a numerical increase due to the composite enzyme and amylase supplementation, regardless of the level, compared to PC in the starter phase. CP digestibility coefficients from birds fed NC+V+A were numerically higher by 2.94 and 2.30% in the grower and finisher phases, respectively. The composite enzyme alone or in combination with an amylase improved AMEn values of diets in the starter phase versus the positive control ($P < 0.05$). During the grower the phase, NC+V+0.5A increased AMEn values by 154.92 and 151.04 kcal/kg compared to PC and NC, respectively ($P < 0.05$). In the finisher phase, the supplementation of amylase (NC+V+0.5A)

elicited superior AMEn values ($P < 0.05$) by 103.86 and 106.76 kcal when compared to PC and to the supplementation of the composite enzyme alone (Table 8).

The classical Gompertz non-linear growth curve was fit for BW showed a difference between the additions of amylase in different doses compared to no addition of amylase. The asymptote, the line that approaches zero as it tends to infinity is higher for the treatment where no amylase was added, 6,298 g. However, between the treatments with the addition of amylase the model shows the recommended dose of added amylase as the higher than the other added amylase treatments. Additionally, the FI varied among the treatments where birds fed the highest addition of amylase had the lowest FI compared to other treatments.

DISCUSSION

The positive effect of the enzymes on performance parameters specifically FCR may be due to the additive effects of the exogenous enzymes. The addition of exogenous enzymes modifies the feed but also has the ability to alter the broilers perception of the nutrient matrix of the feed(Stefanello et al., 2015, Stefanello et al., 2019). There are numerous physiological factors that play a major role in enzyme activity. These factors include: broiler age, type of enzyme, content of feedstuffs and nutrient digestibility. The increase in FI of today's broiler may cause a cap on how much starch can be digested. Both energy and protein efficiency was improved by the addition of exogenous enzymes. Xylanase destroys plant cell walls allowing for alpha-amylase to have access to the starch fraction (Stefanello et al., 2015).

Similar to Gracia et al (2003) the improvement in BWG was observed in the starter phases for both experiments, however in Experiment 2 the addition of different inclusions of amylase did not show any additive effect like Gracia et al. (2003) observed for all feeding phases. The composite enzyme did contain amylase during the starter and grower feeding phases.

This could be the reasoning behind why the additive effect of the amylase dose during only the starter phase. Young birds gastrointestinal tracts are less developed and it is well known the addition of exogenous enzymes show an added benefit for broiler growth (Stefanello et al., 2015, 2019).

In general, broiler diets are formulated to increase ME and decrease protein as the bird ages. As a consequence of this form of diet formulation, the percent of inclusion of different substrates changes with each change of the diet, effecting the types of substrates available for the enzymes. Here there is a combined DDGS+SBM in the starter, grower and finisher, their values are 33.8, 31.8 and 28.6%, respectively (Table 1 and 2). There is more substrate for the composite enzyme to attack and with the addition xylanase which is known to release energy. This could attribute to the differences in broiler performance as the bird ages (Gracia et al., 2003).

The enzymes tested in the current study contained activities to break down cell walls in both corn and SBM and assist the endogenous enzymes in digestibility. The results showed an increase in digestibility of starch as amylase was added to the composite enzyme (Table 8). Amerah et al. (2017) also reported an increase in starch digestibility with exogenous protease addition. In addition to the increase in digestibility of starch the supplementation of amylase either alone or in combination improved AMEn values of the diets during all feeding phases. Likewise, the highest body protein gain can be seen during the starter and grower phases. This not only shows the activity of the exogenous enzymes but also showing the highest availability of protein due to the protease activity. Exogenous amylase acted on freeing more energy to allow the broiler to gain more protein during these phases. The increase in AMEn values during all phases and the increase in protein gain during the starter and grower phases indicates potential to

reduce maintenance energy and amino acid costs when diets are supplemented with exogenous enzymes (Cowieson et al., 2019).

Cowieson et al. (2019a) studied the effect of exogenous protease on broiler retained energy and overall classic NE, the authors concluded the increase in energy retained (NEg) and overall classic NE increases was attributed to the overall higher digestibility of nutrients. In the present work energy efficiency was increased during starter and grower phases when exogenous composite enzyme was added, which included protease. In addition, the highest NEg was also observed during these phases as well, indicating an increase in digestibility of the overall diet for the use of retained energy.

The NE system is one of the more precise method of formulation compared to the current ME system (Swick et al., 2013; Barekatain et al., 2014; Wu et al., 2018). Many classic NE studies have been conducted (Noblet et al., 1994; Pirgozliev ' et al., 1999; Carré et al., 2014) which conclude the ME system overestimates the value of the fibrous content of high protein feed ingredients often used in broiler diets. Heat production is known to increase with the ingestion of fiber while the addition of xylanse is shown to effectively breakdown the dietary fiber and lead to a reduction in the heat produced from fiber digestion (Nian et al., 2011; Barekatain et al., 2014; Cowieson et al., 2019a). The Ark NE system includes body composition into the overall calculation of NE, compared to the classic NE which only subtracts HI. In the present study the addition of enzymes played a significant role in NEg in the starter and grower phases. In Figure 1, HP is increased with the treatments with higher protein birds while higher fat content does not produce heat, therefore showing a better diet classic NE value. This is evident in the classic NE values (Table 6), here classic NE values for the diets with enzymes decreases while the Ark NE values increase. Furthermore, the NC diet is burning protein for an energy

source. Exogenous enzyme allows for an additional dietary carbohydrate calories to mitigate those needed from protein making the HI lower. This trend continues into the NC+V+A treatment due the added amylase but not as effective as the composite enzyme alone.

In a study conducted by Barekatain et al. (2014), retained energy was calculated and incorporated into a HP calculation and then compared with Classic NE. Authors stated NE values were higher when the calculation with retained energy was included, which provides evidence for a new NE system to be created to include retained energy. Classic NE:AME ratios are similar to those published by (Wu et al., 2018) however the Ark NE:AME ratios showed a trend of being higher in ME efficiency due to the combination of body composition incorporated into the NE equation. In addition, Barekatain et al. (2014) also concluded that the addition of higher fiber ingredients may repartition energy toward protein and away from fat. However, the current study indicates that although the addition of enzymes aids in the fiber digestion and ultimately the protein gain, Barekatain and others (2014) state this lowered the Classic NE of the diet.

Protein is primarily going to go into retained energy while energy coming from carbohydrate is going to be in a functional form, i.e. fuel for metabolic processes. Therefore, providing energy in the appropriate amount but also in the correct metabolic form will manipulate the amount of protein or fat deposited and ultimately the retained energy (NEg). The most obvious findings arising from this study is the additive affects exogenous composite enzyme alone or in combination with amylase had on broiler performance especially in the starter and grower feeding phases. NE calculations proved to be a sensitive way to evaluate enzyme addition to broiler diets. Additionally, body composition and energy partitioning play a huge role in diet NE and should be included in the overall NE calculation.

Table 1. Feed composition and calculated nutrient specification in experimental negative control (NC) diets offered to broiler chickens in Experiment 1 and Experiment 2[1]

Ingredient	**Starter 1-14d**	**Grower 15-28d**	**Finisher 29-49d**
	%	*%*	*%*
Yellow Corn (7.4% CP)	52.64	53.56	57.52
Soybean Meal	29.80	24.89	27.02
Wheat Midds	7.00	3.53	0.00
DDGS	4.00	7.00	1.62
Poultry Fat	2.47	4.08	4.57
Limestone	1.40	1.34	1.10
Solk-a-floc Cellulose	1.13	4.28	7.04
Methionine 98.5%	0.30	0.24	0.21
Salt	0.30	0.29	0.27
Lysine	0.25	0.26	0.12
Choline Chloride-60	0.20	0.20	0.20
Vitamin and mineral premix[2]	0.30	0.30	0.30
Dicalcium Phosphate	0.10	0.00	0.00
Threonine 98%	0.08	0.00	0.01
Monsanto Santoquine ethoxiquin	0.02	0.02	0.02
Ronozyme Hiphos Phytase[3]	0.0045	0.0045	0.0045
Calculated composition			
ME, kcal/kg	2,959	3,020	3,026
Crude protein, %	21.09	19.00	18.00
Calcium[3]	0.75	0.69	0.59
Non-phytate phosphorus	0.28	0.25	0.22
Dig. Lysine	1.15	1.03	0.93
Dig. TSAA	0.87	0.77	0.71
Dig. Threonine	0.74	0.69	0.59
Dig. Arginine	1.30	1.13	1.12
Analyzed composition			
AMEn	2,962	3,209	3,359
Crude Protein, %	20.4	18.3	18.2

[1]Treatments with composite enzyme or amylase, enzyme was added on top of diet

[2]Supplies per kilogram of diet: antioxi 200 mg; retinyl acetate, 21 mg; cholecalciferol, 110 μg; D-α-tocopherol acetate, 132 mg; menadione, 6 mg; riboflavin, 15.6 mg; D-calcium pantothenate, 23.8 mg; niacin, 92.6 mg; folic acid, 7.1 mg; cyanocobalamin, 0.032 mg; pyridoxine, 22 mg; biotin, 0.66 mg; thiamine, 3.7 mg; choline chlorine, 1200 mg; Mn, 100 mg; Mg, 27 mg; Zn, 100 mg; Fe, 50 mg; Cu, 10 mg; I, 1 mg; Se, 200 μg.

[3]Ronozyme HiPhos, DSM, Nutritional Products LLC, Parsippany, NJ. Included at a rate of 50g/MT to the basal diet to supply a guaranteed minimum of 500 FTY/kg of feed.

Table 2. Feed composition and calculated nutrient specification in positive control (PC) experimental diets offered to broiler chickens in Experiment 2[1]

Ingredient (% as-is)	**Starter 1-14d**	**Grower 15-28d**	**Finisher 29-51d**
Yellow Corn	55.83	61.75	62.52
Soybean meal	31.48	26.09	25.10
DDGS[1]	3.50	3.50	3.50
Poultry Fat	2.91	3.04	2.98
Wheat midds	2.45	2.00	2.96
Limestone	1.16	1.12	1.00
Dicalcium phosphate	1.11	0.98	0.77
Salt	0.36	0.36	0.30
DL-Methionine, 98.5%	0.30	0.27	0.20
L-Lysine-HCl	0.26	0.26	0.20
Choline Chloride, 60%	0.20	0.20	0.20
Vitamin and mineral premix[2]	0.30	0.30	0.30
Mineral Premix[2]	0.10	0.10	0.10
L-Threonine, 98%	0.10	0.09	0.00
Monsanto Santoquine ethoxiquin	0.02	0.02	0.02
Ronozyme Hiphos Phytase[3]	0.05	0.05	0.05
Calculated composition, % unless otherwise noted			
ME, kcal/kg	3,036	3,108	3,125
Crude protein	21.26	19.00	18.50
Calcium	0.90	0.84	0.74
Non-phytate phosphorus	0.45	0.42	0.38
Dig. Lysine	1.18	1.05	0.93
Dig. TSAA	0.88	0.80	0.73
Dig. Threonine	0.77	0.69	0.59
Dig. Arginine	1.31	1.14	1.12
Analyzed composition			
AMEn, kcal/kg	2,836	3,223	3,291
Crude protein	20.5	18.3	17.8

[1]DDGS=Dried Distillers Grain with Solubles

Treatments with composite enzyme or amylase, enzyme was added on top of diet. See Table 1, for NC diets.

[2]Supplies per kilogram of diet: antioxidant, 200 mg; retinyl acetate, 21 mg; cholecalciferol, 110 μg; D-α-tocopherol acetate, 132 mg; menadione, 6 mg; riboflavin, 15.6 mg; D-calcium pantothenate, 23.8 mg; niacin, 92.6 mg; folic acid, 7.1 mg; cyanocobalamin, 0.032 mg; pyridoxine, 22 mg; biotin, 0.66 mg; thiamine, 3.7 mg; choline chlorine, 1200 mg; Mn, 100 mg; Mg, 27 mg; Zn, 100 mg; Fe, 50 mg; Cu, 10 mg; I, 1 mg; Se, 200 μg.

[3]Ronozyme HiPhos, DSM, Nutritional Products LLC, Parsippany, NJ. Included at a rate of 50g/MT to the basal diet to supply a guaranteed minimum of 500 FTY/kg of feed.

Table 3. Composition of enzyme cocktail added on top[1] to experimental diets in Experiments 1 and 2 and U of enzymes supplied per kg of finished diet

Enzyme, U/kg	Starter (1-14 d)	Grower (15-28 d)	Finisher (29-51 d)
Phytase	1,653	1,653	1,653
Xylanase	297	297	372
Cellulase	88	88	110
Beta - glucanase	3	3	
Protease	6,198	4,132	

[1]Added 375 g of enzyme cocktail to 907.2 kg of diet

Table 4. Influence of dietary treatments on broiler performance in Experiment 1.

	Dietary Treatments			
	NC	NC+V[1]	NC+V+A[2]	SEM
BW, g/bird				
0 d	43	43	43	0.001
7 d	125	129	131	2.698
14 d	365[b]	398[ab]	402[a]	0.009
21 d	668	700	703	14.550
28 d	1,074[b]	1,214[a]	1,089[ab]	0.032
35 d	1648	1754	1657	0.448
42 d	2268	2398	2306	0.848
49 d	2770	3006	2923	0.104
BW gain, g				
0-14 d	321.50[b]	353.75[ab]	358.75[a]	0.009
15-28 d	709.50[ab]	816.75[a]	686.25[b]	0.029
29-48 d	1696	1791.5	1834.23	0.093
0-49 d	2727.3	2962.3	2879.5	0.104
Feed intake, g				
0-14 d	0.29	0.26	0.30	0.021
15-28 d	1.27	1.41	1.31	0.043
29-48 d	3.99	3.65	3.49	0.073
0-49 d	4.15	4.14	4.04	0.108
FCR, g:g[3]				
0-14 d	1.08	0.91	1.04	0.062
15-28 d	1.59[ab]	1.49[b]	1.63[a]	0.037
29-48 d	1.66[a]	1.50[ab]	1.39[b]	0.053
0-49 d	1.86[a]	1.70[b]	1.68[b]	0.038

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$)

[1]V=Enzyme composite: glucanse+xylanase+protease+phytase

[2]A= recommended dose of amylase 120g/ton

[3]FCR = Feed conversion ratio; FCR corrected for mortality adjusted to the weight of the pen average.

Table 5. Influence of dietary treatments on broiler body composition and nutrient utilization in Experiment 1.

	Dietary Treatments			
	NC	NC+V[1]	NC+V+A[2]	SEM
Protein mass g/bird				
0 d	4.25	4.25	4.25	--
14 d	55.00[ab]	57.30[a]	54.81[b]	0.698
28 d	193.40[ab]	198.30[a]	178.15[b]	5.017
49 d	567.30	558.00	547.60	13.307
Fat mass g/bird				
0 d	6.02	6.02	6.02	--
14 d	12.20[b]	21.50[a]	22.40[a]	0.974
28 d	51.00[b]	107.00[a]	96.00[a]	4.478
49 d	270.90[b]	333.20[a]	327.80[a]	12.372
Protein accretion g/bird				
0-14 d	50.95[ab]	53.24[a]	50.26[b]	0.762
15-28 d	139.40	141.00	131.16	5.769
29-48 d	373.90	359.70	369.30	12.791
0-49 d	563.00	553.70	543.40	16.896
Fat accretion g/bird				
0-14 d	6.15[b]	15.52[a]	15.26[a]	0.774
15-28 d	39.18[b]	85.48[a]	80.18[a]	6.983
29-48 d	219.60	226.10	238.10	20.104
0-49 d	264.90[b]	327.10[a]	333.60[a]	15.560
Protein efficiency, %[3]				
0-14 d	65.80	64.90	61.60	0.019
15-28 d	86.10[a]	73.60[b]	70.20[b]	0.024
29-48 d	97.40	101.00	108.00	0.054
0-49 d	89.07	87.20	89.70	2.704
Energy efficiency, %[4]				
0-14 d	25.90[b]	29.40a	29.60a	0.006
15-28 d	41.90[b]	48.90[ab]	49.60[a]	0.018
29-48 d	47.80	48.20	52.80	0.017
0-49 d	40.10[b]	42.00[ab]	44.40[a]	0.008

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$)

[1]V=Enzyme composite: glucanse+xylanase+protease+phytase

[2]A= recommended dose of amylase 120g/ton

[3]Protein efficiency= (Body protein gain g/ Protein intake, g)*100

[4]Energy efficiency=(Energy gain, kcal / AMEn intake, kcal)*100

Table 6. Effect of exogenous enzymes on indirect calorimetry and Net Energy

	HP kcals/kg	HI kcals/kg	NEm kcals/kg	NEg kcals/kg	Classic NE kcals/kg	Ark NE kcals/kg	Classic NE/AME kcals/kg	Ark NE/AME kcals/kg
Stater 0-14 d								
NC	1789	788	1000	462[b]	2175	2149	74	73[b]
NC+V	1729	574	1154	531[a]	2354	2375	81	81[a]
NC+V+A	1542	536	1007	526[a]	2347	2245	82	78[ab]
SEM	108.41	108.63	58.65	7.27	108.63	58.65	0.04	0.02
Grower 15-28 d								
NC	1947	374	1574	1205[b]	2836	2913	88	91
NC+V	1962	474	1489	1602[a]	2770	3043	86	94
NC+V+A	1887	530	1356	1336[b]	2669	2759	83.5	86.3
SEM	213.88	85.39	204.73	46.74	85.39	204.70	0.03	0.06
Finisher 29-49 d								
NC	2083	390	1693	4046	2969	3608	88	108
NC+V	2357	410	1947	4019	2951	3904	88	116
NC+V+A	2199	373	1826	4,033	2986	3917	89	116
SEM	264.33	50.47	218.21	133.77	50.47	218.21	0.02	0.07

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$)

Table 7. Influence of dietary treatments on broiler performance in Experiment 2.

	Dietary Treatments						
	NC	PC	NC+V[1]	NC+V+A0.5	NC+V+A[2]	NC+V+A1.5	SEM
BW, g							
0d	44.0	44.1	44.2	43.9	44.1	44.0	0.008
14d	378.2[b]	416.2[a]	420.8[a]	415.8[a]	413.8[a]	415.5[a]	7.254
28d	1,188.3[b]	1,404.3[a]	1,362.6[a]	1,304.7[a]	1,353.9[a]	1,330.1[a]	24.350
49d	2,361.9[b]	2,234.4[ab]	2,664.6[a]	2,423.3[ab]	2,519.0[ab]	2,468.3[ab]	122.460
Body weight gain, g							
0-14d	334.3[b]	372.1[a]	376.7[a]	371.9[a]	369.7[a]	379.6[a]	6.656
15-28d	810.1[c]	988.1[a]	941.8[ab]	888.9[bc]	940.1[ab]	914.7[abc]	21.810
29-49d	1,166.3	1,164.6	1,301.9	1,218.5	1,278.7	1,138.2	48.812
Feed intake, g							
0-14d	439.3	466.1	463.1	466.5	455.5	445.6	0.011
15-28d	1,108.0	1,338.9	1,303.7	1,286.0	1,268.8	1,347.7	0.004
29-49d	1,605.0	1,930.8	1,932.9	1,871.6	1,850.0	1,772.8	0.102
FCR, g:g							
0-14d	1.57[a]	1.43[ab]	1.41[b]	1.43[ab]	1.48[ab]	1.39[b]	0.018
15-28d	1.7	1.49	1.55	1.57	1.51	1.51	0.025
29-49d	1.52	1.54	1.55	1.55	1.45	1.55	0.057

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$)

[1]V=Enzyme composite: glucanse+xylanase+protease+phytase

[2]A= recommended dose of amylase 120g/ton, 0.5A 60g/ton, 1.5A 180g/ton

Table 8. Effect of an exogenous amylase and a composite enzyme on digestible starch (DS), ileal digestibility of starch (IDs), AMEn and crude protein digestibility (CPD)

	Dietary Treatments						
	NC	PC	NC+V[1]	NC+V+0.5A	NC+V+A[2]	NC+V+A1.5	SEM
AMEn, kcals/kg							
0-14d	2963	2837	2929	2938	2882	2821	43.4
15-28d	3209	3223	3243	3285	3199	3161	30.2
29-49d*	3359^{a}	3292^{b}	3361^{a}	3366^{a}	3359^{a}	3315^{ab}	12.4
CPD, %							
0-14d	74.23	74.59	75.42	75.84	74.63	75.25	1.43
15-28d	83.81	81.9	82.58	81.1	84.31	82.05	1.14
29-49d	88.21	87.89	88.28	89.11	89.91	88.56	0.59
DS, %							
0-14d*	28.43^{b}	27.76^{b}	34.20^{a}	33.54^{a}	34.31^{a}	33.26^{a}	0.27
15-28d*	39.17^{a}	38.14^{bc}	38.61^{ab}	37.53^{c}	38.68^{ab}	38.67^{ab}	0.24
29-49d*	42.58^{d}	50.56^{c}	52.81^{a}	51.77^{b}	52.31^{ab}	51.74^{b}	0.22
IDs, %							
0-14d	89.64^{b}	88.34^{b}	93.04^{a}	91.25^{ab}	90.83^{ab}	90.48^{a}b	0.74
15-28d	93.27$^{a}_{bc}$	93.27^{abc}	94.4^{ab}	91.75^{c}	92.09^{bc}	94.54^{a}	0.59
29-49d	94.63^{b}	95.86^{ab}	96.73^{a}	94.81^{b}	95.81^{ab}	94.77^{b}	0.42

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$, $P<0.001$*)
[1]V=Enzyme composite: glucanse+xylanase+protease+phytase
[2]A= recommended dose of amylase 120g/ton, 0.5A 60g/ton, 1.5A 180g/ton

Table 9. The effect of the addition of amylase in three doses on body weight and feed intake utilizing a Gompertz 3P model.

Diet	Asymptote	Growth Rate	Inflection Point
	BW, g		
NC+V	6298[a]	0.041	38.4
NC+V+.05A	5760[b]	0.042	37.5
NC+V+A	5905[c]	0.043	37.1
NC+V+1.5A	4948[d]	0.046	34.0
	FI, kg		
NC+V	1.84[a]	0.076[a]	18.8[a]
NC+V+.05A	1.74[b]	0.079[b]	18.1[bc]
NC+V+A	1.72[c]	0.080[c]	18.2[c]
NC+V+1.5A	1.56[d]	0.092[d]	16.6[d]

[a-d]means not sharing a common superscript differ based on a parameter equivalent test based on 25% difference criterion.

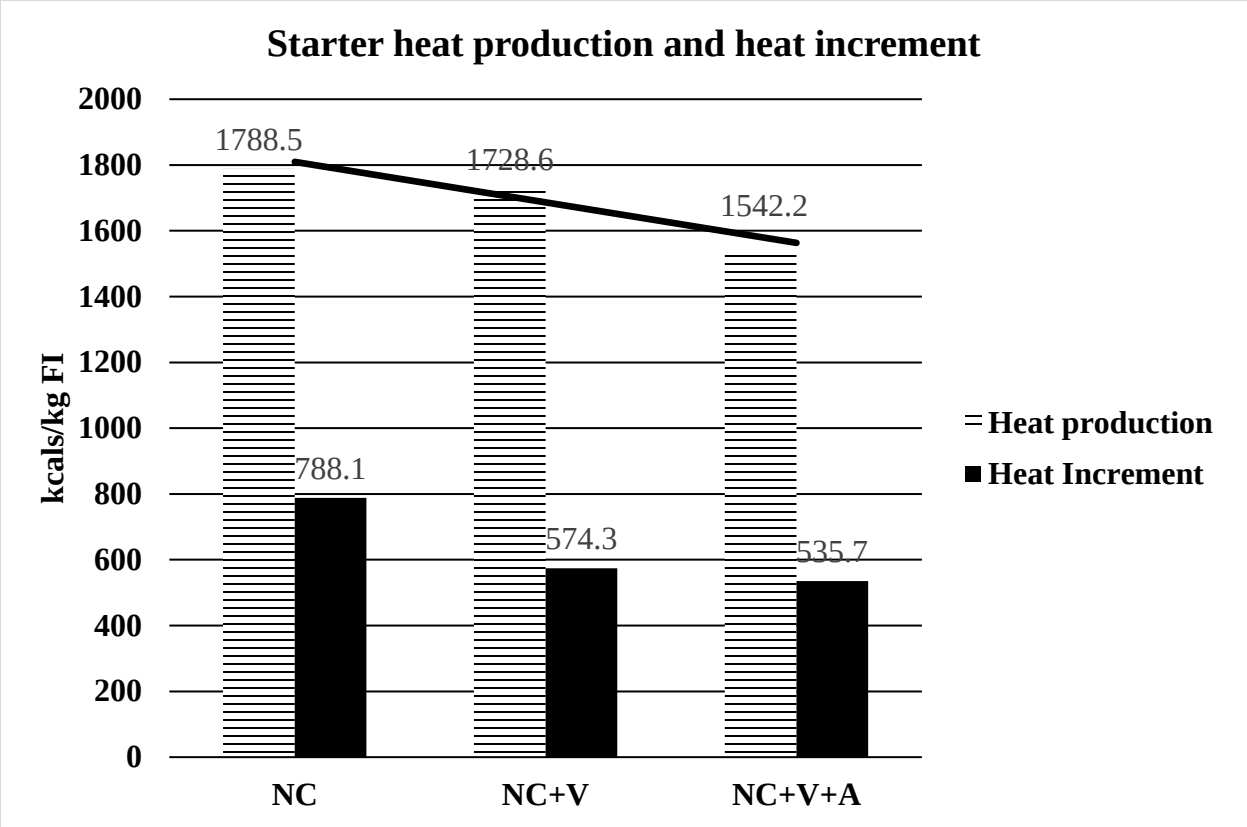

Figure 1. Starter phase heat production and heat increment.

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VI. EFFECT OF SOYBEAN MEAL AND CORN INGREDIENT QUALITY ON ARK NE

ABSTRACT

The continuous development of accurate and precise methods for measuring energy is vital in the modern poultry industry. The classical method of net energy (**NE**) assesses the value of metabolizable energy (**ME**) minus heat increment (**HI**). Previous research shows increasing amino acid levels increases body protein but also heat production (**HP**), while increasing ME increases body fat but lowers heat production. Classic NE can be misleading as more calorie efficiency is given to fat deposition than lean mass deposition. Therefore, it is desirable to provide an improved process of calculating NE based on body composition and HP (Ark NE). The objective of this study is to determine the effect of ingredient quality (digestible nutrient content) on NEg and HP (NE maintenance) and compare sensitivity of Classic NE and Ark NE systems based on diets varying SBM and corn qualities and inclusions. Three test diets were developed for each feeding phase, with two different samples of soybean meal **(SBM)** or corn. Diets were evaluated for starter (0-10 d, 3,008 kcal ME/kg, 21% CP), grower (10-22 d, 3,100 kcal ME/kg, 19% CP) and finisher (22-42 d, 3,200 kcal ME/kg, 18% CP). The highest concentration of lysine/CP/kcal for each ingredient was utilized as control ingredient. The control ingredient was formulated in a corn soybean diet to provide 80, 100 and 120% AA requirements for each phase with both AA levels and ME set according to the broiler recommendation. Each of the SBM or corn samples were fed equally on a percentage basis as determined for the control SBM and corn. Heat production (**HP**; kcal) = 3.872*VO_2 (L/d) + 1.195 VCO_2 (L/d; Farrell, 1974) was measured for one d. Fasting heat production (**FHP**) was also measured for one d and HI determined as HI = HP – FHP (Farrell, 1974). Body composition was measured throughout the experiments by dual energy X-ray absorptiometry (**DEXA**) to determine net energy gain (**NEg**). The Ark NE did show an interaction between AA levels by

variety during the starter phase. Birds aged 0-10 d and fed variety A at 100% AA level had the highest Ark NE value, 1,781 kcal/kg FI, while broilers fed variety B at the same AA level had the lowest, 1,379. Experiment 2, showed a significant ($P < 0.05$) interaction between AA level and corn variety for both Classic NE and Ark NE during starter phase (0-10 d). Classic NE was significant higher for broilers fed: variety A 100% AA level, variety A 120% AA level, and variety B at these same AA levels. These experiments indicate utilizing digestible amino acids and other nutrient quality of the ingredients, even undesirable qualities, can be used to understand net energy calculations.

INTRODUCTION

Feed ingredients are the highest cost of poultry production, with energy being the major component of this cost (Van der Kils and Kwakernaak, 2008). Gross energy (GE) of feed is not completely utilized by birds as some calories will be lost as fecal and urinary energy. The portion left is known as metabolizable energy (ME) and is currently used to formulate poultry diets due to its relative ease of calculation. Metabolizable energy of feeds can be further refined to net energy (NE) that takes into account the energy loss known as the heat increment (HI). Heat increment is a term used to encompass energy lost during ingestion, digestion, metabolism, and excretion and is difficult to assess. The benefit of refining the flow of energy to NE is because the dietary energy remaining is the NE of maintenance and production. The dietary NE is a precise energy value that the bird uses for production, whether the energy is for eggs or meat, and the unseen costs of maintenance.

The advantage of formulating diets on a NE basis is the energy system accounts for energy lost as heat and more accurately predicts body weight gain and feed conversion ratios better than other forms of dietary energy. The NE system is equivalent to formulating diets on a

digestible amino acid (AA) basis compared to formulating with crude protein and total amino acids. The modern broiler is growing at a rapid rate generating tremendous amounts of heat; consequently, a sensitive NE energy system is needed to measure body heat production primarily caused by maintenance and accretion of myofibrillar and sarcoplasmic protein by optimizing intake of digestible amino acids and energy. The classic way to calculate NE of feed is to determine ME and subtract the HI. That method only assesses the value of HI which accounts for a small portion (Farrell, 1974) of dietary energy that is lost from ME and can be misleading as more calorie efficiency (NE/ME) is given to fat deposition than lean mass deposition. Classic NE does not take into consideration the type of production or gain that is occurring in the animal and mainly penalizes protein accretion because of HI generated from nitrogen and carbon loss through uric acid production. Due to genetic selection to promote lean muscle accretion, lessened emphasis should be placed on fat and more on protein calories and will be considered in the overall NE equation for predictive calorie value of ingredients. The objective of this study is to determine the effect of ingredient quality (digestible nutrient content) on NEg and HP (NE maintenance) and compare sensitivity of Classic NE and Ark NE systems based on diets varying SBM and corn qualities and inclusions.

MATERIALS AND METHODS

Two experiments were conducted, one with two different soybean meals (SBM, 47.1% CP, 44.8% CP; Table 4) and one with two different corn qualities (8.92% CP, 8.01% CP; Table 4). The design of both experiments, except for the tested ingredient, was exactly the same. All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee #18024.

Birds and housing

For each experiment a total of 2,784 Cobb-500 (Cobb-Vantress, Inc.) were reared in 48 pens, with 58 chicks per pen. Broilers were randomly placed on new litter (softwood shavings) over concrete floor pens. Pens were in tunnel ventilated houses (1.5 m x 3.0 m) and each pen was equipped with two hanging type feeders and a nipple drinker (10 nipples per line). The lighting program was 23L:1D from d 0 to d 7 and 18L:6D from d 8 to d 42.

Diets and treatments

A total of three different amino acid levels was fed in each feeding phase. For Experiment 1, each of the amino acid levels also utilized two different SBM (SBM A, SBM B), and for Experiment 2 each amino acid level also utilized two different corns (Corn A, Corn B) for a total of six experimental diets (Tables 1-4). Diets were formulated based on standardized ileal digestible (SID) amino acids recommendations. The diets were formulated to either provide 20% above or below recommended level, to make 80%, 100% and 120% AA levels. Each treatment used a different SBM or corn source, and the SBM and corn with the highest concentration of lysine or CP was used to formulate the diets. Feeding phases evaluated were starter d 0-10, grower d 10-21, and finisher d 21-42. Each experimental diet had the same inclusion level of SBM or corn regardless of the source.

Sampling

On d 8, 120 birds (20 per treatment) were selected, weighed and transferred to respiratory chambers (10 birds each one). Thereafter, at d 19, 60 birds (10 per treatment, 5 birds each one) and on d 40, 24 birds (4 per treatment, 2 birds each one) were selected. All birds were given one day adaptation prior to heat production (HP) measurement.

For basal body protein and fat contents, 12 birds were analyzed at d 0, whereas 12 birds from each treatment (6 treatment; total 72) were analyzed at d 7, 10, 17, 21, 39 and 42. Additionally, on d 10, 21 and 42 average BW, feed intake (FI), bodyweight gain (BWG) and feed conversion ratio (FCR) were calculated.

Respiratory Chambers and Heat Production Determination

Respiratory chambers (61 cm l x 51 cm w x 56 cm h) utilized were the same as described by Caldas et al. (2018), with exception of the lighting program, temperature and air flow. The temperature inside the chambers was maintained within a 2°F range. The room temperature was kept 10°F lower than the temperature inside the chambers, which ensured that the temperature inside the respiratory chambers stayed within Cobb 500 recommendations. The indirect calorimetry system provided air flow of 20 to 25 L/min, depending on the size of the broilers in the chamber. Before each evaluation day, chambers were opened for individual BW and FI measurements and calibration of gas analyzers.

Fed heat production (HP) was determined for 24 h, followed by fasted heat production (FHP) for the next 24 h. Broilers were allowed ad libitum feed access during adaption and fed periods.

Body Composition Analysis

Birds were humanely sacrificed by CO_2 inhalation before body composition was determined using dual energy X-ray absorptiometry (DEXA; General Electric Co., Madison, Wisconsin) with small animal body software module (Lunar Prodigy, General Electric Co., encore version 12.2). DEXA results were adjusted to body chemical analyses performed by Caldas et al. (2019).

Net Energy (NE) calculations

Volumes of O_2 (VO_2) and CO_2 (VCO_2) within each chamber were averaged for each 24 h evaluation period. HP and FHP were calculated following the equation: HP kcal/d = 3.866 VO_2 L/d + 1.233 VCO_2 L/d (Brouwer, 1965) and normalized to kg of FI.

Heat production (HP) consists of the Net Energy of maintenance (NEm) plus the heat increment (HI; see equation 1 below). HP and fasting heat production (FHP) were calculated using the Farrell (1974) equation (see equation 2 below). Classic NE was calculated according to Noblet et al. (2010) (see equation 3 below), where HI is defined as HP minus FHP (see equation 4 below). Net Energy of gain (NEg) was calculated based on body composition data (body protein and fat levels) from DEXA (see equation 5 below). Equation (6) was obtained rearranging equation (1), allowing NEm be evaluated with indirect calorimetry. This method is called the Arkansas Net Energy Equation (Ark NE; 7), which encompasses both body composition and heat production.

(1) HP = NEm + HI (Farrell, 1974)

(2) HP and FHP = 3.871 x VO_2 (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974)

(3) $NE_{classic}$ (kcal/kg) = ME (kcal/kg) – HI (Noblet et al., 2010)

(4) HI = HP – FHP

(5) NEg = protein gain (g) x 5.66 (kcal/g) + fat gain (g) x 9.35 (kcal/g)

(6) NEm = HP – HI

(7) Ark NE = NEg + NEm

Chemical analysis of feed, ingredients, ileal digesta, and excreta

At d 0, ten chicks were placed in metabolic digestibility cages and fed one of the six experimental test diets. All diets had the addition of 0.5% titanium dioxide and were fed ad libitum. Feed was removed on the evening of d 4, 14 and 35, replaced 8 h later for a fasting period. Birds were then sampled after 2 h of ad libitum feeding. After eating for 2 h birds were immediately euthanized by CO_2 asphyxiation. Following euthanasia of the birds, ileal digesta was collected on these same days in order to determine nutrient digestibility. Clean excreta (free from feathers and feed) was collected using plastic spatulas and placed in labeled plastic containers and frozen in liquid nitrogen immediately after collection. The digesta content of the ileum (between Meckel's diverticulum and the ileocecal junction) collected from the birds of each cage were pooled to represent one replicate. Pooled samples were frozen in liquid nitrogen immediately after collection. All samples were lyophilized and fine ground (<2 mm) before analysis.

The analysis of AMEn (nitrogen-corrected apparent metabolizable energy) involved the analysis of gross energy (GE), dry matter (DM) and nitrogen, in feed and excreta. GE was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). DM was analyzed by method 934.01 (AOAC, 1990) and nitrogen levels were determined by the method 990.03 (AOAC, 1995). The marker, titanium dioxide (TiO_2), was measured on 96 well plates following the methodology of Myers (2004). In summary, 0.35 g of K_2SO_4, 0.04 g of $CuSO_4$, and 0.1 g of excreta, feed or ileal, were added to each glass test tube and diluted with 3 mL of 18M H_2SO_4 to be heated at 120°C for 24 h in a block digester. Contents of the digestion tube were allowed to cool for 15 min, after which 7 mL of distilled deionized water was added to the digested sample, gently mixed and transferred to new plastic test tubes. This step was

repeated using 2 mL of distilled deionized water. Diluted, digested samples were centrifuged at 3,000 rpm for 22 min and the supernatant was recovered using filter paper. After mixing 1 mL of the supernatant with 0.20 mL of distiller deionized water and 0.13 mL of 30% H_2O_2, the absorbance was measured at 410 nm subsequent to the next 10 min after the addition of the last reagent.

Each test ingredient (SBM A, SBM B, Corn A, Corn B) was subjected to starch, non-starch polysaccharide (NSP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) analysis (Table 4). Starch content was determined with a modified starch extraction using the hydrolysis method of Varns and Sowokinos (1974) and a glucose assay of (Miller (1959). Briefly, 20 mg of excreta or feed were added to a plastic tube and diluted with 1 ml of 80% ethanol and placed in a heated water bath at 90°C for 3 min. Tube contents were centrifuged at 10,000 x g for 3 min. and the supernatant discarded. The ethanol procedure was repeated two additional times. The starch was extracted with water and NaOH by adding 1 ml of distilled deionized water, placed in hot water bath at 96°C for 5 min, and centrifuged at 15,000 x g for 3 min. in order to recover the supernatant. Tube pellet contents were resuspended with 1 ml of 0.5 N NaOH and treated similar to the water extraction and centrifuged at 27,000 g. The starch hydrolysis was performed by adding 0.36 ml of 6 N HCl and placed in a hot water bath at 96°C for 2.5 h. This solution was neutralized with 0.3 ml of 10 N NaOH by determining glucose using the dinitrosalicylic acid method (Cerrate et al., 2019). NSP analysis was done according to the procedure by Maharjan et al. (2019). Sequentially for neutral-detergent fiber (NDF), acid-detergent fiber (ADF), by the batch procedures outlined by ANKOM Technology Corp. (Fairport, NY).

Statistical analysis

Chamber or pen was the experimental unit. All data were analyzed under a 6 (diets) x 2 (ingredient source) factorial arrangement. Data were analyzed by ANOVA of JMP Pro 13 (SAS Institute, 2017). When the means were significant ($P \leq 0.05$) student t-test was used. *P*-value was considered significant when ≤ 0.05.

ANOVA model:

$$Y_{ijk} = \mu + T_i + B_j + (TB)_{ij} + e_{ijk}$$

μ = mean

T_i = effect of i^{th} level of factor A

B_j = effect of j^{th} level of factor B

$(TB)_{ij}$ = effect of interaction between the i^{th} level of factor A and the j^{th} level of factor B

e_{ijk} = random error associated with the k^{th} replicate

RESULTS

Calculated and analyzed values for experimental diets fed in Experiment 1 and 2 and ingredient analysis can be found in Table 1, 2, 3, 4, 5 and 6 respectively.

Performance

Experiment 1

The influence of dietary treatment indicated no significant interaction between SBM variety and amino acid level on AME and AMEn (Table 4 and 5), therefore the main effects will only be discussed for these variables. For all feeding phases, amino acid level significantly ($P < 0.05$) affected the AMEn value, with the higher amino acid level diet having the most ($P < 0.05$) kcal/kg and the lowest amino acid level having the least ($P < 0.05$).

Tables 7 and 8 summarizes the production data. During the starter phase (d 0-10), there was a significant interaction between amino acid level and SBM variety. Broilers fed amino acid level 80, both SBM A and SBM B, 100 amino acid level SBM A and 120 amino acid level SBM B, all had significantly lower ($P < 0.05$) FCR than treatment SBM B 100 amino acid level which had an FCR of 1.523.

The main effect of SBM variety was only significant for BWG during the starter phase, d 0-10. Soybean meal A had 0.24 kg more ($P < 0.05$) gain. During grower and finisher phases, the main effect of amino acid level was significantly ($P < 0.05$) different with the 120 and 100 amino acid level gaining significantly more than the 80 amino acid level.

There were no differences in feed consumption between treatments until d 22-42, where a significant interaction between amino acid level and SBM variety was observed. Feed was consumed more 4.66 kg/bird ($P < 0.05$) by SBM A at the 100 amino acid level with treatments SBM A 80AA level and SBM B 80AA level consuming the least at 3.96 kg/bird and 4.04 kg/bird, respectively.

The main effect of amino acid level was significant ($P < 0.05$) for FCR throughout the broiler grow-out. The grower and finisher feeding phases showed the lowest FCR, 1.249 and 1.811 respectively, for the 120 amino acid level.

Experiment 2

In experiment 2, there was a significant interaction ($P < 0.05$) between corn variety and amino acid level in grower phase (Table 5) for AMEn. The grower diet with the highest AMEn was Corn A at the highest amino acid level.

For performance parameters, a 2 x 3 factorial design provided differences in BWG, FI and FCR. No variety by amino acid level interaction were found; therefore, only main effects

data are shown. During the starter, grower and finisher phases, birds fed the lowest amino acid level (80 AA) had the least ($P < 0.05$) BWG. Feed intake was significant ($P < 0.05$) only during the starter feeding phase, 100 amino acid level fed broilers consumed 0.218 kg/bird while 120 amino acid birds consumed 0.198 kg/bird.

Body Composition

Experiment 1

No variety by amino acid level interaction was found (Table 9); therefore, only main effects data will be shown. Feeding increasing levels of AA changed broiler protein and fat body composition. At grower and finisher feeding phases, increasing dietary AA concentration significantly ($P < 0.001$) produced higher body protein depositions (Figure 1). The largest amount of protein (g/bird) ($P < 0.001$) were found in broilers feed the 100% and 120% AA level diets during the grower and finisher phases, 106 g/bird and 366 g/bird, respectively. The lowest amount of protein (g/bird) was found in broilers fed the 80% AA level diet ($P < 0.001$) during the grower and finisher periods, 84 g/bird verse 338 g/bird, respectively. Differences in energy ($P < 0.001$) were found only in the starter and finisher period, with broilers fed at the 120% AA level gaining 226 kcal/bird in the starter period but broilers fed 80% AA level gain 4,262 kcal/bird in the finisher period (Table 9, Figure 3). In addition, during the finisher period significant differences ($P < 0.001$) in fat gain were observed with birds fed 80% AA diet gaining 276 g/bird verse 120% AA level gaining 187 g/bird (Table 9).

Differences in body protein by SBM variety showed increased ($P < 0.001$) protein gain in the grower and finisher phases. Birds fed SBM A gained more than SBM B, 5 g/bird verse 16 g/bird, respectively (Table 9, Figure 1)

Experiment 2

Differences in tissue gain between dietary treatments are presented in Table 10 and Figure 4, 5 and 6. Variety by amino acid level interaction was found during the grower and finisher phases; therefore, only these results will be discussed. As expected, broilers fed the 120% AA level with either Corn A and Corn B showed the highest ($P < 0.001$) protein gain during the grower phase. However, during the finisher phase 120% AA level Corn A broilers had the highest ($P < 0.001$), 385 g/bird, compared to Corn A at the 80%AA level, 295 g/bird.

Significant interaction ($P < 0.05$) for energy gain were found only in the finisher period, with broilers fed 80% AA Corn B level gaining 699 kcal/bird more than broilers fed 120% AA level Corn B and 573 kcal/bird more than 100% AA level Corn A (Table 10, Figure 3).

Calorimetry parameters

Heat expenditure was calculated by respiratory exchange in indirect calorimetry chambers: volume of oxygen consumption and carbon dioxide production. For both experiment 1 and 2, HP data showed no significant differences for AA level or variety of ingredient (Table 11, 12). However, in experiment 2 (corn), HI indicated a significant ($P < 0.001$) interaction during the starter phase. Here birds fed 120% AA level Corn A had 11 kcal more ($P < 0.001$) than birds fed 120% AA level Corn B. In addition to this interaction, experiment 2 also had significant ($P < 0.05$) for FHP for amino acid level. Here, broilers fed 120% AA level had 38 more kcal/d than broilers fed 80% AA level.

Net Energy

Experiment 1

In this experiment, Classic NE showed no AA level by variety interaction. For the main effect of AA level significant differences in both the starter and grower phases (Table 10). Broilers fed 120% AA level had 392 kcal/kg FI and 247 kcal/kg FI more than the 80% AA fed

birds. During the starter phase Classic NE recovered more calories (NE/ME) in comparison to Ark NE (Table 11). While in the grower and finisher phases Ark NE recovered more (NE/ME).

Experiment 2

This experiment showed a significant ($P < 0.05$) interaction between AA level and corn variety for both Classic NE and Ark NE during starter phase (0-10 d). Classic NE was significant higher for broilers fed: Corn A 100% AA level, Corn A 120% AA level, and Corn B at these same AA levels (Table 14). While the lowest was for Corn B at 80% AA level. Ark NE values however showed that the lowest Ark NE value was for Corn B 120%AA level with only 698 kcal/kg FI verse the highest Corn B at 100% AA level at 1404 kcal/kg FI. During the grower phase 11-22 d, no interactions were discovered, but the main effect of AA level showed significant differences between the Classic NE and Ark NE. Both variables showed the highest NE values for 120% AA level and the lowest for the 80% AA level. Classic NE recovered more calories (NE/ME) in comparison to Ark NE (Table 11). While in the grower and finisher phases Ark NE recovered more (NE/ME).

DISCUSSION

In the US, poultry diets mainly consist of corn and SBM. Soybean meal represents the majority of the protein source in the diets, while corn primarily functions as the energy source. The ratio in which these two ingredients are included in the diet affects the protein to energy ratios birds can utilize. Quality of ingredients play a role in bird performance and equations used to predict energy values are variable in their accuracy (Mateos et al., 2019), ultimately affecting not just rate of gain but also meat quality and intestinal integrity (Sakkas et al., 2019). Protein and energy ratios, along with the relationship between protein quality and energy plays a vital

role in broiler responses. Classen (2017) shows that increasing the protein to calorie ratio had a sparing effect where protein is no longer used for energy needs but can be utilized as an additive effect towards growth and maintenance. The gradual improvement in performance of birds fed on diets that increased in dietary energy concentration may be due to an increasing portion of energy stored in gain and less used for maintenance as birds grew more rapidly (Table 5). In the present study, although all treatment diets were formulated to contain the same calculated ME values, protein and energy values for the test ingredients were different (Table 6). The differences in AME and AMEn between the treatments, especially in experiment 2, played a major role in FI and ultimately affected BWG and FCR. Broilers fed corn B, which had lower protein but more AMEn, were lower in performance parameters. Maynard et al. (2019) also found differences in FI based on energy concentrations of diets. However, the difference in diet AMEn was approximately 100 kcal/kg in which Plumstead et al. (2007), found no differences. These differences in performance for experiment 2 could be due to the addition of oil as an energy source making the ratio of energy to protein increase. The extra caloric benefits of added oil has been shown to increase ileal amino acid digestibility (Cowieson et al., 2006; Cowieson and Bedford, 2010). Furthermore, Cerrate et al. (2019) found protein digestibility coefficient decreased as NDF increased. Additionally, the type of fiber can affect protein digestibility such as non-starch polysaccharides (NSP) (Choct et al., 2010). In the current study, NSP values (Table 6) are lower than others cited in the literature (Maharjan et al., 2019), due to a few sugars being undetected making for values to be 0.5%-1% lower than previous work done in the same lab. However, synergism between protein quality, fiber content and type of fiber played an important role in digestibility of the overall diets (Maharjan et al., 2019).

The literature has shown that a linear relationship exists between the metabolizable energy of a diet and broiler retained energy (NEg) as said diet is converted into body protein (Nitsan et al., 1997a; Liu et al., 2017; Classen, 2017). For experiment 1, there was no difference in grams of fat gain until the finisher period, however fat gain was significant throughout the grow-out period in experiment 2, indicating the decrease in protein to energy has an effect on body composition, specifically fat gain, as previously reported (Hilton et al., 2019). Variety differences between SBM and corn could be attributed to the amount of energy concentration in the diet, in addition to the oil content in the corn varieties available for the bird to utilize. Nitsan et al. (1997b) conducted an experiment increasing ME through inclusion levels of soybean oil and observed similar increases in NEg as a result of increased fat deposition. In addition, Hilton et al. (2019) also showed increasing energy in the diet increases fat gain and therefore increases NEg.

Furthermore, traditional diet energy values used for formulation, AME and TME, are derived from a catabolic standpoint, whereas, Ark NE estimates result in values derived from an anabolic standpoint due to the emphasis on increases in body mass (Kleiber, 1961). However, in experiment 2 (corn), HI indicated a significant ($P < 0.001$) interaction during the starter phase. Here birds fed 120% AA level Corn A had more HI ($P < 0.001$) than birds fed 120% AA level Corn B because broilers were in an anabolic state to gain more protein. When evaluating ingredient nutrients, Corn A had more amino acids and more amino acids produce heat (Hilton et al., 2019). Protein deposition rate is 5.2 kcal/g, however ME only gives credit for protein deposition at 4 kcal/kg (Kleiber, 1961; Pesti, 2005). Therefore, as long as the energy by protein deposition exceeds that lost by HI, the use of catabolic Atwater (1905) values, 4 kcal/kg of protein deposition, underestimates dietary metabolizable energy, which when evaluating Ark NE

value in comparison with Classic NE equation. For example, SBM A NE/AME was 90% vs 78%, respectively. Differences in the Ark NE value during the starter phase was highest at 100% SBM A lowest was 100% SBM B. This could be due to the differences in protein quality of the SBM utilized. The protein content in SBM B (Table 6) is lower than in A; therefore, less heat is produced and less protein gain, and ultimately a lower NEg value in determined. These experiments indicate utilizing digestible amino acids and other nutrient contents of the ingredients, even undesirable qualities, can be used to understand net energy calculations. In addition, protein inclusion and protein quality have major effects on broiler performance and body composition.

Table 1. Composition and nutrient calculation of the experimental test diets.

	Starter			Grower			Finisher		
AA Level	80%	100%	120%	80%	100%	120%	80%	100%	120%
Ingredient (%as-is)									
Corn[1]	67.02	53.36	39.69	72.27	61.68	49.79	73.64	66.66	55.82
SBM[1]	27.83	39.61	51.39	22.94	32.04	42.29	21.99	27.89	37.3
Dicalcium Phosphate	1.87	1.76	1.65	1.76	1.67	1.58	1.55	1.49	1.4
Limestone	0.96	0.94	0.92	0.91	0.89	0.88	0.83	0.82	0.81
Corn oil	1.13	3.1	5.07	1.00	2.52	4.23	1.00	1.96	3.56
salt	0.38	0.38	0.38	0.38	0.38	0.38	0.35	0.35	0.35
DL-Methionine, 98.5%	0.21	0.29	0.37	0.16	0.24	0.32	0.13	0.23	0.2
L-lysine	0.17	0.12	0.07	0.14	0.13	0.09	0.08	0.15	0.11
L-Threonine 98%	-	-	-	-	-	0.01	-	0.01	0.01
Vitamin and mineral premix[2]	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Organic acid[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Ethoxyquin[4]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Calculated values									
ME	3,030	3,030	3,030	3,080	3,080	3,080	3,100	3,100	3,100
CP	18.78	23.24	27.71	16.84	20.33	24.22	16.42	18.77	22.29
Dig Lysine	1.11	1.27	1.52	0.87	1.09	1.31	0.8	1.00	1.2
Dig Methionine	0.47	0.6	0.73	0.4	0.53	0.64	0.37	0.49	0.5
Dig C+M	0.74	0.92	1.1	0.65	0.81	0.97	0.61	0.76	0.81
Dig Threonine	0.64	0.8	0.96	0.57	0.7	0.84	0.56	0.65	0.78

[1]Source A and B inclusion was constant in both experiments.
[2]Vitamin premix: Vit A, 13227 IU/kg; Vit D3, 3968 IU/kg; Vit E, 66 IU/kg; Vit B12, 0.040 mg/kg; Biotin, 0.254 mg/kg; Menadione, 3.968 mg/kg; Thiamine, 3.968 mg/kg; Riboflavin, 13.228 mg/kg; Vit B6, 7.937 mg/kg; Niacin, 110.229 mg/kg; Folic acid, 2.205 mg/kg. Trace mineral premix: Mn, 60 mg/kg (manganese sulfate); Zn, 60 mg/kg (zinc sulfate); Fe, 40 mg/kg (ferrous sulfate); Cu, 5 mg/kg (copper sulfate); I, 1.25 mg/kg (calcium iodide); Co, 0.5 mg/kg (cobalt sulfate).
[3]MycoCurb (Kemin Industries, Inc).
[4]Santoquin (Novus International, Inc.).

Table 2. Analyzed composition of experimental test diets in experiment 1.

	Starter 0-10 d					
AA Level	80%	80%	100%	100%	120%	120%
Ingredient Variety	SBM A	SBM B	SBM A	SBM B	SBM A	SBM B
DM[1]	91.0	91.2	91.9	92.0	92.8	92.9
GE, kcal/kg[2]	3978	3955	4122	4108	4278	4317
AMEn kcal/kg[3]	3372	3548	3697	3355	3511	3743
CP	20.7	19.9	24.1	22.4	26.1	26.4
Ash	5.71	5.28	6.04	5.46	6.83	6.05
NDF[4]	9.77	9.94	10.03	11.03	12.23	10.24
ADF[4]	2.62	2.58	2.91	2.77	3.35	2.80
	Grower 11-22 d					
AA Level	80%	80%	100%	100%	120%	120%
Ingredient Variety	SBM A	SBM B	SBM A	SBM B	SBM A	SBM B
DM[1]	91.0	91.4	91.6	91.6	91.6	91.6
GE, kcal/kg[2]	3965	3961	3996	4021	4159	4197
AMEn kcal/kg[3]	3375	3444	3611	3417	3476	3615
CP	17.0	17.5	21.9	19.5	22.0	23.4
Ash	5.12	5.07	5.66	5.33	5.99	5.74
NDF[4]	9.38	9.64	10.58	9.44	12.70	12.85
ADF[4]	2.38	3.05	2.79	2.55	3.61	3.45
	Finisher 23-42 d					
AA Level	80%	80%	100%	100%	120%	120%
Ingredient Variety	SBM A	SBM B	SBM A	SBM B	SBM A	SBM B
DM[1]	91.5	91.1	91.5	91.3	91.5	91.6
GE, kcal/kg[2]	3978	3955	4122	4108	4278	4317
AMEn kcal/kg[3]	3430	3563	3689	3411	3551	3745
CP	17.0	15.7	19.8	19.3	21.9	22.8
Ash	5.31	4.84	5.63	5.13	6.11	5.62
NDF[4]	8.67	8.69	10.86	10.06	10.97	11.52
ADF[4]	2.49	2.51	3.22	2.88	3.69	3.47

[1] DM was analyzed by method 934.01 (AOAC, 1990)

[2]GE analyzed by bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.).

[3]AMEn (nitrogen-corrected apparent metabolizable energy) nitrogen, in feed and excreta analyzed by method 990.03 (AOAC, 1995). Titanium dioxide (TiO_2) marker and digestibility analyzed by Meyers (2004).

[4]NDF neutral-detergent fiber, acid-detergent fiber (ADF) by batch procedures outlined by ANKOM Technology Corp. (Fairport, NY).

Table 3. Analyzed composition of experimental test diets in experiment 2.

	Starter 0-10 d					
AA Level	80%	80%	100%	100%	120%	120%
Ingredient Variety	Corn A	Corn B	Corn A	Corn B	Corn A	Corn B
DM[1]	92.7	91.6	92.7	92.9	93.3	93.4
GE, kcal/kg[2]	3964	3929	4152	4154	4304	4175
AMEn kcal/kg[3]	3406	3645	3704	3293	3556	3604
CP	19.5	19.6	23.9	24.3	29.2	27.9
Ash	5.86	5.83	5.54	5.71	6.47	7.26
NDF[4]	10.22	11.40	10.80	9.39	11.20	11.29
ADF[4]	2.82	3.32	3.30	2.73	3.44	3.87
AA Level	80%	80%	100%	100%	120%	120%
Ingredient Variety	Corn A	Corn B	Corn A	Corn B	Corn A	Corn B
	Grower 11-22 d					
AA Level	80%	80%	100%	100%	120%	120%
Ingredient Variety	Corn A	Corn B	Corn A	Corn B	Corn A	Corn B
DM[1]	92.0	91.9	92.0	92.4	92.3	92.4
GE, kcal/kg[2]	3938	3846	4011	4040	4157	4138
AMEn kcal/kg[3]	3341	3369	3497	3254	3421	3480
CP	17.8	17.5	21.7	21.7	24.5	25.4
Ash	5.15	5.29	5.53	5.50	6.04	6.26
NDF[4]	8.54	8.93	9.37	10.61	9.05	10.80
ADF[4]	2.43	2.62	2.93	3.02	3.06	3.36
	Finisher 23-42 d					
AA Level	80%	80%	100%	100%	120%	120%
Ingredient Variety	Corn A	Corn B	Corn A	Corn B	Corn A	Corn B
DM[1]	90.1	89.9	90.0	89.9	89.8	90.2
GE, kcal/kg[2]	3948	3907	3992	4001	4105	4123
AMEn kcal/kg[3]	3206	3274	3310	3231	3240	3356
CP	16.7	15.6	19.1	19.2	22.7	23.9
Ash	5.14	5.23	5.36	5.11	5.80	5.48
NDF[4]	8.44	8.12	8.22	8.95	9.38	9.33
ADF[4]	2.21	2.03	2.45	2.51	3.08	3.10

[1] DM was analyzed by method 934.01 (AOAC, 1990)

[2]GE analyzed by bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.).

[3]AMEn (nitrogen-corrected apparent metabolizable energy) nitrogen, in feed and excreta analyzed by method 990.03 (AOAC, 1995). Titanium dioxide (TiO_2) marker and digestibility analyzed by Meyers (2004).

[4]NDF neutral-detergent fiber, acid-detergent fiber (ADF) by batch procedures outlined by ANKOM Technology Corp. (Fairport, NY).

Table 4. Analyzed metabolizable energy of experimental test diets in experiment 1.

	Starter (0-10 d)		Grower (11-22 d)		Finisher (22-42 d)	
	AME[1]	AMEn[1]	AME	AMEn	AME	AMEn
Amino acid level						
80AA	3388[c]	3364[c]	3421[c]	3396[c]	3445[c]	3420[c]
100AA	3557[b]	3530[b]	3489[b]	3460[b]	3586[b]	3557[b]
120AA	3751[a]	3720[a]	3645[a]	3613[a]	3748[a]	3717[a]
SEM	12.79	12.64	8.72	8.64	11.95	11.86
Ingredient Variety						
A	3567	3539[b]	3506[b]	3477[b]	3589	3561
B	3564	3537[a]	3531[a]	3503[a]	3597	3569
SEM	10.44	10.33	7.13	7.06	9.76	9.69
Amino acid level x Variety						
80,A	3397	3372	3400	3375	3455	3430
100,A	3577	3548	3474	3444	3593	3563
120,A	3728	3697	3643	3611	3720	3689
80, B	3379	3355	3442	3417	3435	3411
100,B	3538	3511	3504	3476	3579	3551
120,B	3775	3743	3647	3615	3776	3745
SEM	18.08	17.89	12.32	12.20	16.91	16.78
P- Value						
AA Level	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Variety	0.82	0.86	0.02	0.02	0.60	0.56
AA Level x Variety	0.07	0.07	0.32	0.28	0.06	0.07

[1]Analysis on as is basis, kcal/kg

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$)

Table 5. Analyzed metabolizable energy of experimental test diets in experiment 4.

	Starter (0-10 d)		Grower (11-22 d)		Finisher (22-42 d)	
	AME[1]	AMEn[1]	AME	AMEn	AME	AMEn
Amino acid level						
80AA	3375[c]	3350[c]	3320[c]	3297[c]	3240[b]	3219[b]
100AA	3632[b]	3600[b]	3423[b]	3395[b]	3282[b]	3257[b]
120AA	3691[a]	3654[a]	3520[a]	3489[a]	3363[a]	3333[a]
SEM	10.16	10.06	18.53	18.32	20.99	20.79
Ingredient Variety						
A	3616[a]	3585[a]	3430	3403	3289	3264
B	3515[b]	3484[b]	3412	3385	3301	3276
SEM	8.30	8.22	15.13	14.96	17.13	16.98
Amino acid level x Variety						
80,A	3431	3406	3364[bc]	3341[cd]	3228[b]	3206
100,A	3677	3645	3397[b]	3369[bc]	3299[ab]	3274
120,A	3741	3704	3528[a]	3497[a]	3339[ab]	3310
80, B	3319	3293	3276[c]	3254[d]	3252[b]	3231
100,B	3588	3556	3448[ab]	3421[abc]	3265[ab]	3240
120,B	3640	3604	3512[a]	3480[ab]	3387[a]	3356
SEM	14.37	14.23	26.20	25.91	29.68	29.41
P- Value						
AA Level	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Variety	<0.001	<0.001	0.42	0.41	0.61	0.62
AA Level x Variety	0.71	0.69	0.05	0.04	0.38	0.39

[1]Analysis on as is basis, kcal/kg

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$)

Table 6. Analyzed composition of experimental ingredients

	Experiment 1		
	SBM A	SBM B	Corn
DM	90.4	91.6	88.9
CP	49.6	47.8	8.66
GE	4305	4342	3957
NDF	11.1	11.01	7.49
ADF	4.30	4.12	1.59
Starch	1.08	1.25	56.9
NSP, soluble	0.3	0.3	ND
NSP, insoluble	13.6	11.7	8.1
AMEn, kcal	2376	2389	3277
Dig AA[1], % Essential AA			
Lysine	2.66	2.60	0.24
Methionine	0.574	0.57	0.17
Threonine	1.54	1.50	0.31
Tryptophan	0.59	0.58	0.06
Arginine	3.293	3.18	0.40
Valine	2.03	1.98	0.44
Leucine	3.269	3.16	1.20
Isoleucine	1.95	1.80	0.35
Histidine	1.15	1.10	0.26
Phenylalanine	2.22	2.10	0.48
Total TDEAA	19.28	18.57	3.91
	Experiment 2		
	Corn A	Corn B	SBM
DM	90.4	91.2	92.8
CP	7.83	7.77	47.3
GE	3827	3864	4219
NDF	11.3	12.8	15.1
ADF	2.1	2.5	4.4
Starch	60.1	58.0	5.52
NSP, soluble	0.7	0.8	ND
NSP, insoluble	8.5	7.2	8.1
Oil	2.57	1.66	1.88
AMEn	3305	3219	2382
Dig AA[1], % Essential AA			
Lysine	0.29	0.28	2.63
Methionine	0.17	0.17	0.57
Threonine	0.27	0.27	1.52
Tryptophan	0.00	0.05	0.59
Arginine	0.39	0.38	3.24
Valine	0.38	0.37	2.00
Leucine	0.96	0.97	3.22

Table 6. Analyzed composition of experimental ingredients (Cont.)

	Corn A	Corn B	SBM
Isoleucine	0.30	0.29	1.88
Histidine	0.23	0.23	1.13
Phenylalanine	0.40	0.40	2.16
Total TDEAA	3.40	3.42	18.92

[1]Digestible amino acids determined by Ravindran et al. (2014)
ND= non-detectable

Table 7. Influence of dietary treatments on broiler performance experiment 1.

	Starter (0-10 d)			Grower (11-22 d)			Finisher (22-42 d)		
	BWG[1], kg	FI, kg	FCR[2]	BWG, kg	FI, kg	FCR	BWG, kg	FI, kg	FCR
Amino acid level									
80AA	0.140	0.216	1.402[a]	0.473[b]	0.798	1.540[a]	1.703[b]	4.236	2.079[a]
100AA	0.147	0.215	1.331[ab]	0.559[a]	0.794	1.331[b]	1.956[a]	4.353	1.824[b]
120AA	0.158	0.228	1.261[a]	0.560[a]	0.804	1.249[c]	2.035[a]	4.121	1.811[b]
SEM	0.006	0.007	0.032	0.009	0.014	0.018	0.054	0.077	0.044
Ingredient Variety									
A	0.168[a]	0.224	1.289[b]	0.531	0.799	1.375	1.924	4.263	1.875
B	0.128[b]	0.215	1.375[a]	0.530	0.798	1.372	1.871	4.210	1.935
SEM	0.004	0.005	0.021	0.008	0.012	0.015	0.044	0.063	0.036
Amino acid level x Variety									
80,A	0.170	0.226	1.282[b]	0.474	0.801	1.553	1.694	4.167[ab]	2.101
100,A	0.165	0.225	1.312[b]	0.572	0.779	1.322	1.988	4.663[a]	1.820
120,A	0.169	0.221	1.272[b]	0.548	0.818	1.252	2.092	3.960[b]	1.704
80, B	0.110	0.206	1.523[a]	0.472	0.794	1.528	1.712	4.305[ab]	2.058
100,B	0.128	0.204	1.349[ab]	0.546	0.809	1.341	1.924	4.044[b]	1.828
120,B	0.146	0.234	1.251[b]	0.572	0.791	1.247	1.979	4.282[ab]	1.918
SEM	0.007	0.008	0.036	0.011	0.018	0.022	0.076	0.108	0.061
P- Value									
AA Level	0.1468	0.451	0.016	<0.001	0.884	<0.001	0.004	0.884	0.003
Variety	<0.001	0.273	0.026	0.903	0.948	0.860	0.419	0.566	0.269
AA Level x Variety	0.111	0.227	0.015	0.165	0.363	0.654	0.696	0.004	0.122

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$)

[1]BWG=body weight gain

[2]FCR= Feed conversion ratio; FCR corrected for mortality adjusted to the weight of the pen average.

Table 8. Influence of dietary treatments on broiler performance experiment 2.

	Starter (0-10 d)			Grower (11-22 d)			Finisher (22-42 d)		
	BWG[1], kg	FI, kg	FCR[2]	BWG, kg	FI, kg	FCR	BWG, kg	FI, kg	FCR
Amino acid level									
80AA	0.158[b]	0.209[ab]	1.329[a]	0.533[b]	0.834	1.567	1.830[b]	3.324	1.818[a]
100AA	0.180[a]	0.218[a]	1.216[b]	0.652[a]	0.880	1.360	2.023[a]	3.362	1.663[ab]
120AA	0.170[ab]	0.198[b]	1.250[ab]	0.670[a]	0.853	1.274	2.085[a]	3.242	1.623[b]
SEM	0.005	0.005	0.031	0.012	0.016	0.017	0.034	0.077	0.047
Ingredient Variety									
A	0.174	0.213	1.286	0.634[a]	0.871	1.386[a]	1.937	3.366	1.786[a]
B	0.165	0.204	1.244	0.603[b]	0.840	1.415[b]	2.021	3.253	1.616[b]
SEM	0.005	0.004	0.026	0.010	0.013	0.014	0.027	0.064	0.0385
Amino acid level x Variety									
80,A	0.157	0.210	1.350	0.561	0.870	1.557	1.788	3.276	1.833
100,A	0.180	0.224	1.253	0.657	0.885	1.348	1.976	3.504	1.771
120,A	0.184	0.203	1.254	0.685	0.857	1.252	2.047	3.317	1.752
80, B	0.159	0.208	1.308	0.506	0.798	1.578	1.871	3.371	1.802
100,B	0.179	0.211	1.178	0.648	0.874	1.372	2.070	3.219	1.554
120,B	0.157	0.192	1.246	0.655	0.848	1.295	2.123	3.168	1.493
SEM	0.006	0.005	0.035	0.015	0.019	0.020	0.047	0.108	0.066
P- Value									
AA Level	0.013	0.014	0.032	<0.001	0.130	<0.001	0.001	0.542	0.0359
Variety	0.194	0.119	0.268	0.033	0.110	0.152	0.057	0.237	0.0112
AA Level x Variety	0.221	0.696	0.739	0.350	0.329	0.889	0.984	0.286	0.2421

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$)

[1]BWG=body weight gain

[2]FCR= Feed conversion ratio; FCR corrected for mortality adjusted to the weight of the pen average.

Table 9. Effect of dietary treatments on broiler body composition in experiment 1.

	Starter (0-10 d)			Grower (11-22 d)			Finisher (22-42 d)		
	Protein gain, g	Fat gain, g	NEg, kcal	Protein gain, g	Fat gain, g	NEg, kcal	Protein gain, g	Fat gain, g	NEg, kcal
Amino acid level									
80AA	22	3	133[c]	84[c]	53	952	338[b]	276[a]	4262[a]
100AA	22	5	173[b]	97[b]	56	1031	366[a]	235[b]	4103[a]
120AA	24	1	226[a]	106[a]	49	1013	357[ab]	187[c]	3623[b]
SEM	0.78	1.26	10.89	1.47	3.93	34.22	5.62	9.28	78.17
Ingredient Variety									
A	23	3	183	98[a]	55	1027	362[a]	223	3965
B	23	2	172	93[b]	50	971	346[b]	243	4026
SEM	0.64	1.04	8.91	1.79	3.22	28.06	4.60	7.60	64.10
AA level x Variety									
80,A	23	3	147	85	59	994	336	252	4082
100,A	24	5	175	98	57	1045	379	226	4086
120,A	23	1	227	110	49	1041	371	191	3729
80, B	22	3	120	83	46	911	341	301	4443
100,B	21	5	171	96	55	1016	353	245	4119
120,B	25	0	224	102	48	985	344	183	3517
SEM	1.10	1.78	15.36	2.52	5.54	48.26	7.92	13.09	110.27
P- Value									
AA Level	0.243	0.070	<0.001	<0.001	0.399	0.260	0.003	<0.001	<0.001
Variety	0.357	0.690	0.366	0.034	0.255	0.160	0.019	0.060	0.510
AA Level x variety	0.094	0.928	0.721	0.390	0.489	0.860	0.095	0.120	0.050

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$). All values are per bird.

Table 10. Effect of dietary treatments on broiler body composition in experiment 2.

	Starter (0-10 d)			Grower (11-21 d)			Finisher (22-49 d)		
	Protein gain, g	Fat gain, g	NEg, kcal/b	Protein gain, g	Fat gain, g	NEg, kcal	Protein gain, g	Fat gain, g	NEg, kcal
Amino acid level									
80AA	23[b]	7[a]	190[a]	95[b]	67[ab]	1115[b]	305[c]	308[a]	4419[a]
100AA	26[a]	5[a]	169[b]	118[a]	72[ab]	1301[a]	330[b]	260[b]	4124[ab]
120AA	28[a]	-2[b]	74[b]	121[a]	61[b]	1260[a]	370[a]	218[c]	3965[b]
SEM	0.62	1.37	20.40	1.66	2.69	27.51	5.18	9.11	88.46
Ingredient Variety									
A	26	4	168[a]	112	67	1223	329[b]	260	4121
B	25	2	121[b]	110	66	1228	341[a]	263	4216
SEM	0.51	1.11	16.65	1.36	2.20	22.47	4.23	7.44	72.24
AA level x Variety									
80,A	22	7	187	98[b]	68	1139	295[c]	302	4310[ab]
100,A	27	6	202	121[a]	74	1317	307[c]	255	3954[b]
120,A	29	-1	115	118[a]	60	1212	385[a]	224	4101[ab]
80, B	23	7	193	92[b]	66	1090	315[c]	314	4527[a]
100,B	26	3	137	115[a]	70	1286	354[b]	264	4294[ab]
120,B	27	-4	33	123[a]	62	1308	354[b]	212	3828[b]
SEM	0.88	1.93	28.84	2.35	3.81	38.87	7.33	12.88	125.04
P- Value									
AA Level	<0.001	<0.001	0.003	<0.001	0.020	<0.001	<0.001	<0.001	0.002
Variety	0.311	0.221	0.049	0.325	0.714	0.873	0.045	0.764	0.356
AA Level x Variety	0.230	0.655	0.274	0.034	0.747	0.130	<0.001	0.617	0.042

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$). All values are per bird.

Table 11. NE results comparison, Experiment 1.

Treatment	AMEn	Classic NE	Ark NE Equation	Classic NE/ME	Ark Equation NE/ME
	kcals	kcals/kg	kcals/kg	%	%
Starter					
80 AA	2655	2407	1443	72	54
100 AA	3007	2500	1580	72	52
120 AA	2936	2799	1600	76	44
P-value		0.059	0.394	0.1686	0.8302
SBM A	2970	2507	1580	71	45
SBM B	2762	2631	1502	74	43
P-value		0.1301	0.4415	0.0865	0.4758
Grower					
80 AA	2947	2305[b]	2881[c]	78[b]	97
100 AA	3165	2599[a]	2979[b]	82[a]	94
120 AA	3245	2782[a]	3281[a]	85a	101
P-value		0.001	<0.001	0.0296	0.2808
SBM A	3198	2662[a]	3075[b]	83[a]	96
SBM B	3118	2462[b]	2976[a]	78[b]	95
P-value		0.0146	0.0029	0.0133	0.2623
Finisher					
80 AA	3126	2560	2973	82	95
100 AA	3309	2807	3215	84	97
120 AA	3331	2774	3186	83	115
P-value		0.298	0.8643	0.5971	0.9498
SBM A	3312	2612	3474	78	104
SBM B	3296	2815	3276	85	99
P-value		0.1575	0.116	0.1554	0.7983

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$). All values are per bird.

Table 12. NE results comparison, Experiment 2.

Treatment	AMEn	Classic NE	Ark NE Equation	Classic NE/ME	Ark Equation NE/ME
	kcals	kcals/kg	kcals/kg	%	%
Starter					
80 AA	2655	3287	1338	93	38
100 AA	3007	3299	1331	94	38
120 AA	2936	3359	1257	94	35
P-value		0.8166	0.8472	0.7084	0.6287
Corn A	3090	3318	1279	107	41
Corn B	3104	3312	1338	106	43
P-value		0.9523	0.6529	0.1991	0.551
Grower					
80 AA	2988	2345[b]	2623[b]	78	88
100 AA	3052	2684[a]	2758[a]	87	90
120 AA	3119	2718[a]	2827[a]	87	90
P-value		0.0019	0.0018	0.0701	0.0094
Corn A	3061	2606	2729	85	89
Corn B	3045	2559	2743	84	107
P-value		0.6357	0.4972	0.3418	0.6507
Finisher					
80 AA	3240	2725	3740	84	115
100 AA	3270	2654	4100	81	125
120 AA	3298	2467	4200	74	127
P-value		0.4153	0.8713	0.2526	0.569
Corn A	3156	2514	4358	79	138
Corn B	3166	2719	3906	85	123
P-value		0.2236	0.7457	0.232	0.6798

[a,b]Means within a row not sharing a common superscript differ ($P \leq 0.05$). All values are per bird.

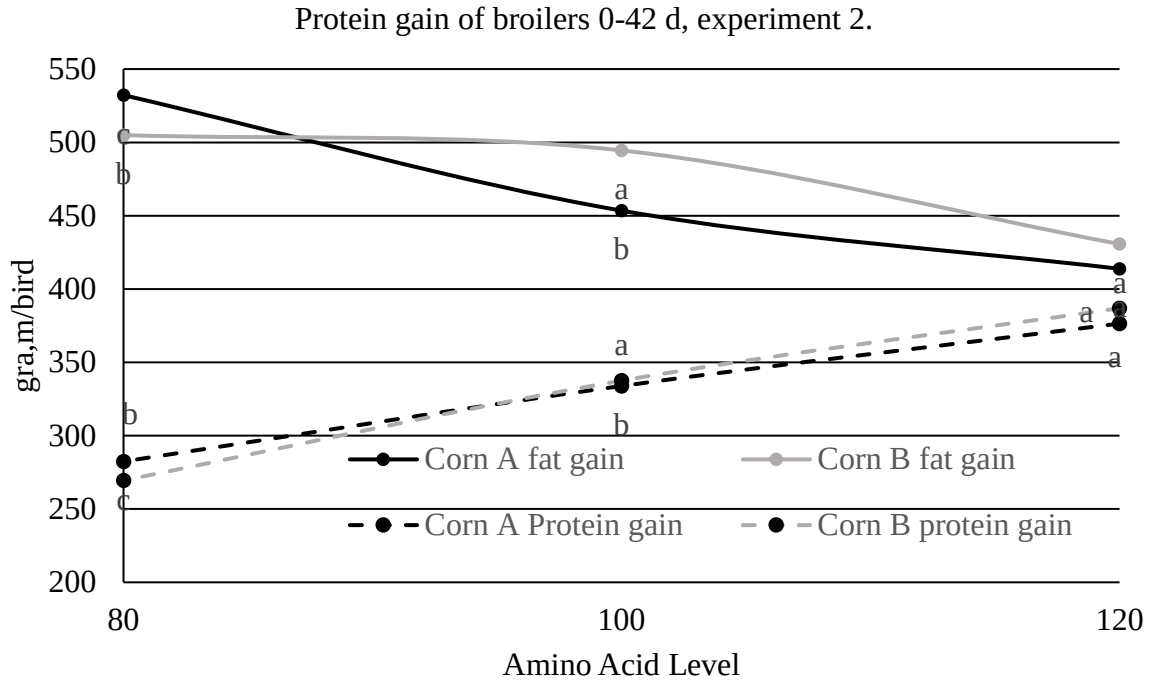

Figure 3. Protein gain of broilers 0-42d, experiment 2.

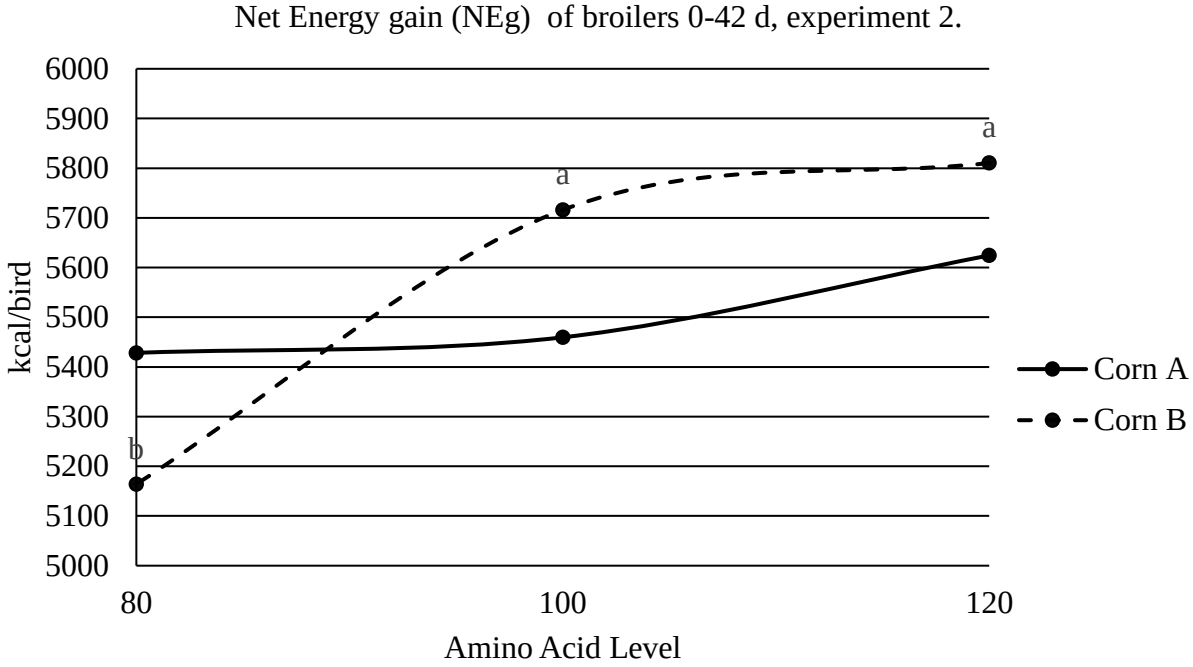

Figure 4. Net Energy of gain (NEg) of broilers 0-42 d, experiment 2.

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CONCLUSION

The overall results of these experiments indicate that dietary protein is primarily retained by the body and incorporated into body proteins, whereas dietary energy from carbohydrate is used as functional energy (i.e. fuel for metabolic processes). Therefore, properly addressing dietary energy needs encompasses not only providing the fuel needed for metabolism to occur, but also must supply the metabolic building blocks to allow for protein deposition and ultimately retained energy (NEg). As demand for poultry production increases, formulation based on NE values allows nutritionists to take advantage of protein metabolism, genetics and environmental conditions. The development of the Ark NE question, which utilizes body composition and energy lost as heat production, showed a more accurate method for diet formulation. Utilizing both NEm, determined from indirect calorimetry, and NEg, evaluated through DEXA, provide valuable information about not only how dietary energy is catabolized but also its deposition. This combination provides a deeper understanding of diet NE, rather than the small indigestible fraction differences. Additionally, the additive effects of exogenous enzymes on broiler performance carried over NE calculations, providing another sensitive method for determining the effects of exogenous enzymes on energy partitioning and body composition in addition to performance responses.

In conclusion, these experiments demonstrated that Classic NE gives less calorie value to protein deposition even though this is the ultimate endpoint of broiler production. Heat increment is a very small portion of the energy lost through metabolic processes, while protein deposition is the major cause of energy loss as heat. In addition, these studies show that quality of protein, and therefore amino acid to energy concentration, should be priority when formulating broiler diets. Different nutrient contents within ingredients (i.e. amino acids), source and type of fat and starch

content makes a difference in how energy is metabolized by broilers. Utilizing a NE equation, like Ark NE, showed to have more impact in the overall understanding of broiler genetics, environment and the additive effect of exogenous enzymes.