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# Calorimetry and Body Composition Research in Broilers and Broiler Breeders

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Calorimetry and Body Composition Research in Broilers and Broiler Breeders

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**ABSTRACT**

Indirect calorimetry to study heat production (HP) and dual energy X-ray absorptiometry (DEXA) for body composition (BC) are powerful techniques to study the dynamics of energy and protein utilization in poultry. The first two chapters present the BC (dry matter, lean, protein, and fat, bone mineral, calcium and phosphorus) of modern broilers from 1 – 60 d of age analyzed by chemical analysis and DEXA. DEXA has been validated for precision, standardized for position, and equations and validations developed for chickens under two different feeding levels. These equations are unique to the machine and software in use. Research in broilers fed exogenous enzymes added alone or mixed as a multi-enzyme composite (protease + glucanase + xylanase + phytase) has shown lower HP and higher protein deposition when protease was evaluated alone or in combination. An unexpected change from protein to fat deposition was seen in the grower phase (around 22d), with a switch back to more protein synthesis in the finisher (>29d). The lower HP let to believe exogenous enzymes reducing the energy for maintenance, the next study resulted in enzymes reducing 6.6% the MEm (metabolizable energy for maintenance) evaluated in retained energy from the body of chicks (16 -27 d). The study was conducted increasing feeding levels and fit by linear regression. The maintenance experiment also showed that under feed deprivation conditions, body directs nutrients to protein synthesis before fat synthesis occurred. Research with broiler breeders resulted in HP increasing continuously along egg production and age from 26 - 59 wk. HP was the highest at 59 wk when the lean tissue was the highest. Respiratory exchange ratio (RER = $VO_2/VCO_2$) showed the lowest value at 43 wk suggesting fat utilization is higher at this point of egg production compared to the beginning (26 wk). Lean mass was the lowest at 37, and 50 wk and increased after 50 wk suggesting lean mass being more important than fat during

egg production. Hens increased lean tissue after 50 wk suggesting preparation for next clutch as it happens in the wild, so fat is used as fuel for maintenance energy.

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## DEDICATION

This work is dedicated to Juan Kalinowski[†], a mentor, and friend, who encouraged me to pursue my dreams of higher education. He left this world, but his legacy remains with me.

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# INTRODUCTION

Energy and protein represent the major cost in poultry feed, and feed accounts for about 70% of the total cost of broiler production. Modern broilers are very efficient in transforming vegetable ingredients into more valuable protein for the humankind as it is meat; however there is still opportunity to improve the energy and protein utilization from ingredients to an excellent quality, and inexpensive protein source to feed the world. Therefore, the study of energy and protein are important to understand the mechanisms by which modern broilers and broiler breeders utilize these nutrients. Calorimetry is a technique to measure heat production (HP) and it has been used in animals for more than 200 years to study nutrient utilization (McLean, and Tobin, 1987). Indirect calorimetry measures the heat generation when carbohydrates, proteins, and fats are oxidized to release the energy to fuel metabolic processes; however in a production system, HP is the inefficiency of nutrients being metabolized, so reducing this HP will increase the efficiency of energy utilization towards meat. On the other hand, dual energy X-ray absorptiometry (DEXA) is a non-invasive technique used to analyze body composition (lean mass, fat mass, and mineral content) faster than chemical analysis but validations are needed to standardize the technique and create equations exclusive to a machine and software for future analysis (Mitchel, *et al.* 1997, Swennen, *et al.* 2004). These two powerful techniques have been utilized in research for the present manuscript. DEXA resulted to be a precise methodology but equations were needed to make it accurate. Chemical analysis was utilized to create two sets of equations for broiler under fasted and fed conditions for future DEXA adjustments for soft tissue and mineral content, including Calcium and Phosphorus. Calorimetry and DEXA have been proved to account for small energy and protein differences when exogenous enzymes were added to diets as individual (protease, glucanase)

or as multi-enzyme composite (protease + glucanase + xylanase + phytase). Lower HP with enzymes in a range of 52 – 257 kcal/kg feed, suggested enzymes reducing the energy for maintenance. Maintenance energy requirement accounts for 42-44% of the energy intake, being a major requirement in broilers (Lopez, and Leeson, 2008b). Linear regression and logarithm models were used to evaluate the maintenance energy with and without enzymes. Retained energy ((fat gain, g x 9.35 kcal/g + protein gain, g x 5.66 kcal/g)/ $kg^{0.70}$) was regressed on MEI kcal/$kg^{0.70}$ (metabolizable energy intake). Enzymes reduced significantly, the energy for maintenance by 6.6% in chicks from 16 – 27 d of age sparing the system energy that could be used for growth, the energy retention for protein was higher with enzymes, while the energy retention for fat was lower. The dynamic of nutrient utilization in broiler breeders during production is a complex process. Hens have shown to utilize glucose at the beginning of egg production and fat by the end of production (Salas, 2011) and higher protein degradation rate occurs during peak production (33 - 37 wk) (Vignale, 2014). Calorimetry and DEXA played a big role to understand these dynamics of nutrient utilization in broiler breeders during egg production 26 – 59 wk. In fact, RER (respiratory exchange ratio) changed along egg production showing different nutrients being utilized by the hens. Body lean mass composition decreased with age until 50 wk while body fat composition increased. After 50 wk, the opposite occurred suggesting hens using more fat for energy to allow lean mass increment for next clutch as occurs in the wild. Calorimetry and DEXA both are powerful techniques to study feeding strategies for broilers and breeders that can improve desirable traits for the poultry industry.

# I. LITERATURE REVIEW

## CALORIMETRY SYSTEM

Calorimetry is the measurement of heat (McLean and Tobin, 1987) and it has been used for over 200 years to account for heat production: directly by physical methods (direct calorimetry) or by a quantitative measurement of the chemical by-products of metabolism (indirect calorimetry). Indirect calorimetry is the most common system used in animals and it measures the oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$) over a period of time when the animals are consuming (or not consuming) a certain diet to calculate heat production. Many authors have developed coefficients from gas exchange measurements ($VO_2$ and $VCO_2$ coefficients) for estimating heat production in humans and animals. Even though, the values are very similar between species, Brouwer's equation (1965) is most commonly used for livestock: HP (kcal/d) = 3.87 $VO_2$ (L/d) + 1.23 $VCO_2$ (L/d). Calorimetry works under two main laws of thermodynamics. The conservation of energy is the first law which states that energy cannot be created or destroyed, only changed in form. The constant heat summation is the second law which means the heat released by a chain of reactions is independent of the biochemical pathways and dependent only on the end-products $CO_2$ and $H_2O$. This implies that it doesn't make a difference if the substrate is directly or completely oxidized or if the intermediate substrates such as lactic acid, fatty acids, ketone bodies, are produced because they will be transformed and oxidized at a later stage. Animal metabolism is complex, but over a period of time, calorimetry has shown consistency in results and in close agreement with direct calorimetry (McLean and Tobin, 1987). It is also used as a diagnostic tool in hospitals for the investigation of metabolic disorders. Calorimetry is used to assess nutritional requirements and evaluate feeds in humans and livestock. Calorimetry is a powerful research

tool to study fundamental nutrition and physiological processes under normal and stress conditions.

**Historical review**

The discovery that the air was a mixture of gases by John Mayow in 1674 was the opening for the use of indirect calorimetry to quantify substrate oxidation. However at the beginning direct calorimetry system was commonly used. Adair Crawford in Glasgow, Scotland and about the same time, Lavoisier in Paris, France (1783) were the pioneers in the use of calorimetry. While Crawford measured the raise of temperature of a water jacket surrounding and animal, Lavoisier measured heat output from a guinea pig in a chamber iced by recording the amount of ice melted inside an insulated chambers. It is still doubt who did the experiment first, but there is no doubt that Lavoisier gave the name oxygen to what it was believed to be "pure air" necessary to support both animal life and flame of candle (McLean and Tobin, 1987). Lavoisier is considered the founder of indirect calorimetry because he concluded that the respiration is a combustion. Sadly, Lavoisier was executed in 1794, so his work was stopped for nearly a century until a major discovery was made in animal calorimetry. The question remained if heat is due to carbon oxidation only, so to encourage more research in the area, the academie of science I Paris set a prize in which Despretz (1824) and Dulong (1842) competed. The calorimeters these two scientists built are considered to be true respiratory calorimeters. Both concluded that heat was produced from carbon and hydrogen. Another important contribution to calorimetry was made in 1842 when the German Robert Meyer formulated the first law of thermodynamics, the principle of conservation of energy. According to this law a complete balance must exist between the various categories as expresses in the equation: gross

energy of food = energy of faeces + energy of urine + energy of methane + energy retained + retrievable mechanical work + metabolic heat production (McLean and Tobin, 1987). In this equation the metabolic heat production is measured by indirect calorimetry. Another expression of the first law of thermodynamics provides the basis of direct calorimetry is expressed in the equation: heat elimination = heat lost by radiation + heat lost by convection + heat lost by conduction + heat lost by evaporation. Validation has been done on both, direct and indirect system with very close numbers. Another important law for the understanding calorimetric principles is the law of Constant Heat Summation postulated in 1838 by G.H. Hess (Hess law from Blaxter, 1989). During mid-19$^{th}$ and the 20$^{th}$ century, various scientists (Regnault & Reiset, 1849; Pettenkofer & Voit, 1862; Laulanie, 1894; Rubner, 1902; Armsby, 1904; Zuntz, 1905; Atwater & Benedict, 1905, Mϕllgard & Anderson, 1917; Marston, 1948; Charlet-Lery, 1958; Blaxter, Brockway & Boyne, 1972; Aulic, *et al.*, 1983 and more) have built different types of calorimeters and tested in humans and animals as dogs, ruminants, pigs, and birds. While most the calorimetry works has been done in Europe, there are also scientists in US who built calorimeters in that 20$^{th}$ century Benedict & Carpenter, 1910 in Boston; Williams, Lusk & Dubois at Cornell University, NY, 1928. After the Second World War, there were major advances in electronics and instrumentation increased the practice of animal calorimetry because of the controlled systems, computer and data processing; however the principles used are the same as the ones discovered more than 200 years ago. Acceptance of the law of thermodynamics and the advancements in technology over the two and a half centuries has led indirect calorimetry be accepted as a tool to measure heat production from the oxidation of nutrients (Gerrits *et al*., 2013). Nowadays, calorimetry research is combined with the use of stable isotopes are utilized to track, and study specific metabolic pathways in

humans and animals; for example, amino acid requirements in humans and animals with the technique called indirect amino acid oxidation developed in the research institute of the hospital for sick children in Toronto, Ontario, Canada by Ronald Ball.

**Indirect Calorimeters**

The difference of direct and indirect calorimeters is that the first measures the rate of heat dissipation of a subject and the second measures the heat generation. Over a period of time, both rates will be equal or very close, so indirect calorimetry can measure the heat as good as direct calorimetry, one cannot believe because of its name that indirect calorimetry is a second –rate means of measuring heat production. There are four classes of indirect calorimetry because of the operating system: confinement, closed-circuit, total collection, and open-circuit systems. Each of this classification have sub-classifications, for more details an elegant description of these methods are described by McLean and Tobin (1987). In a confinement system the subject is held in a sealed chamber and the rates gas change concentration in the chamber are recorded. In a closed-circuit system, the subject is held within or breathes into a sealed apparatus, the carbon dioxide and water vapor produced are measured as the weight gain of appropriate absorbers, and the amount of oxygen consumed is measured by metering the amount required to replenish the system. In total collection, all the air expired by the subject is accumulated in order to measure subsequently its volume and chemical composition. In the open-circuit systems there are two forms. The first one is a portable system in which the subject breathes directly from atmosphere and by means of a non-return vale system expires into a separate outlet line. In the second form, the ventilated flow-through system, the subject inspires from, expires to, a stream of air passing, by means of a pump or fan, across the face. In

both cases the flow of air is measured either on the inlet or outlet side of the subject. Air from the outlet is collected continuously or periodically for later analysis, or is sampled continuously for on-line analysis. There are several reports in the use of open-circuit chambers for humans (Jequier & Schuts, 1983), poultry (Bϕnsdorff Petersen, 1969; Misson, 1974). Using open-circuit systems, the heat production can be calculated with ± 1.2% accuracy (McLean and Tobin, 1987).

**Calorific equivalents**

There are two assumptions when indirect calorimetry is used for energy studies. The first one stands that the heat in the body is a result of the combustion or synthesis of carbohydrates, fats, and proteins. The second assumption is that there are fixed ratios between the quantities of oxygen consumed and carbon dioxide produced and heat produced. Even though these are oversimplifications because mineral metabolism is disregarded, the only justification for these assumptions is that indirect calorimetry is remarkably consistent and in close agreement with direct calorimetry (McLean and Tobin, 1987). The respiratory exchange ratio (RER) or respiratory quotient (RQ) is another term used to explain the oxidation of nutrients involved in the metabolism. This ratio is the volumetric (and molar) ratio of carbon dioxide produced to oxygen consumed. In carbohydrate oxidation, each atom of carbon combines with one molecule of oxygen to form one molecule of carbon dioxide; that is why RER is equal to 1 as illustrated in the chemical equation $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 2817$ kJ. In other terms, the amount of $O_2$ needed to oxidize 1 g of glucose is 0.746 L and the same amount of $CO_2$ is released, so the RER is 0.746/0.746 = 1. There is few variability in the amount of $O_2$ needed to oxidize other carbohydrates like fructose, galactose and polysaccharides. On the other hand,

fatty acids composition are extremely variable but the majority of animal fats include high proportions of palmitic acid (C16), stearic acid (C18) and monounsaturated oleic acid (C18:1), however the mean value is slightly increased with glycerol which is a minor constituent of all fats. Palmitic acid is used as an example: $C_{15}H_{31}COOH+23O_2 = 16CO_2 + 16H_2O + 10040$ kJ. The RER is 16/23 = 0.696. In terms of liters of $O_2$, 2.013 liters of $O_2$ are needed to oxidize 1 g of fat and 1.431 L of $CO_2$ is produced. In the case of protein, there are also high RER variations between different amino acids; however, as with fats, the effect of the mixture of amino acids in proteins, the variation is reduced and the amino acid alanine is used for the example: $4CH_3CH(NH_2)COOH + 12O_2 = 2(NH_2)_2CO+10CO_2+10H_2O+5223$ kJ. This means 0.957 liter of $O_2$ is needed to oxidize alanine and 0.774 of $CO_2$ is produced producing a RER = 0.833. The calorific values given above correspond mainly to mammals, but the values are the same in birds for carbohydrates and fats, the only difference is seen in RER for proteins where is reported from two different studies that RER is 0.72 - 0.74 (McLean and Tobin, 1987), close to the RER for fats. The heat or energy released from these reactions are 4.19 kcal/g for carbohydrates, 4.40 kcal/g for proteins and 9.50 kcal/g for fats (Gerrits *et al*., 2013). A brief summary for these calorific values produced by several researchers are summarized in Table 1. With the use of these calorific values the ME (metabolizable energy) of a diet can be estimated.

**ENERGY**

Energy is not a nutrient but a property of nutrients when they are oxidized during metabolism (NRC, 1994). Carbohydrates, proteins and fats of food, all have potential to yield energy to a common energy currency (Lawrence and Fowler, 1998). Life itself is an energy-consuming process that is why energy is usually referred as “fuel of life” and the principle currency of

nutrition (Scott, *et al*, 1969, Kleyn, 2013). The term energy is a combination of two Greek word: en, meaning "in" and ergon meaning "work", but work is only one of the many uses of energy in the biological sense. Oxidation or degradation of nutrients provides the energy for the continuation of life. Nutrients are reduced to building blocks at cellular level to produce energy for all metabolic pathways. All life forms use adenosine triphosphate (ATP) as common energy currency for metabolic releasing and requiring energy pathways. ATP acts a free energy donor in most energy requiring processes. Living organisms use free energy for many purposes being the most important process the mechanical work in muscle contraction and cellular movements, the active transport of molecules and ions, and the synthesis of macromolecules and other biomolecules from simple precursors (Berg *et al*., 2012). In essence, metabolism is a linked series of chemical reactions that begins with a particular molecule and converts it into some other in a unique way. The basic classification of metabolic process are pathways that convert energy from fuels into biologically useful forms and those pathways that require inputs of energy to proceed; therefore the terms catabolic reactions corresponds to degradation or releasing energy process and anabolic corresponds to synthesis or requiring energy process. Most metabolic pathways are either anabolic or catabolic but depending on the energy conditions in the cell, few pathways could be both, in that case they are called amphibolic. Most of the catabolism consists of reactions that extract energy from fuels such as carbohydrates and fats and convert it into ATP. ATP is not a long-term storage of free energy rather it serves as an immediate donor of free energy in biological systems. For example, in a typical cell, an ATP molecule is consumed within a minute of its formation. In a human body, the amount of ATP is about 100 g which is very limited, however the turnover of this small quantity of ATP is very high. In a resting human there is a 40 kg of ATP consumed in 1 day,

during strenuous exercise, the rate of utilization of ATP is 0.5 kg/min. Therefore, the mechanism of creating ATP is vital, so the primary role of the catabolism is the generation of ATP. The carbon in fuel molecules, such as glucose and fat is oxidized to $CO_2$. The resulting electrons are captured and used to regenerate ATP from ADP and $P_i$. In aerobic organisms, the ultimate electron acceptor in the oxidation of carbon is $O_2$ and the oxidation product is $CO_2$. The fuel molecules are more complex than a single-carbon compound such as glucose; however, the oxidation takes place one carbon at the time.

The energy from foodstuffs is extracted in three stages (Figure 1). The first stage is called digestion and it basically consists in large molecules of food broken down into smaller units. Proteins are hydrolyzed to their 20 different amino acids, polysaccharides are hydrolyzed to simple sugars, and fats are hydrolyzed to glycerol and fatty acids. These products are then absorbed by the intestinal cells and distributed throughout the body. This stage is only a preparation stage, no useful energy is captured in this phase. In the second stage, these molecules are degraded to a few simple units that play a central role in metabolism. Most of the sugars, fatty acids, glycerol and several amino acids are converted into the acetyl unit of acetyl CoA. Some ATP is generated in this stage, but the amount is small compared to the next stage. In third stage, ATP is produced from the complete oxidation of the acetyl unit of acetyl CoA. This stage consists of the citric acid cycle and oxidative phosphorylation, which are final common pathways in the oxidation of fuel molecules. Acetyl CoA carries acetyl units into the citric cycle or Krebs cycle, where they are completely oxidized to $CO_2$. Four pairs of electrons are transferred (three to $NAD^+$ and one to FAD) for each acetyl group that is oxidized. Then, a proton gradient is generated as electrons flow from the reduced forms of these carriers to $O_2$, and this gradient is used to synthesize ATP (Figure 2). All these energy machinery is located in

the mitochondria of biological organisms. Because of the importance of oxygen and carbon dioxide in the production of energy, these two gases are used to estimate heat production in human and animals.

**Energy terminology**

There are some units to describe amounts of energy in science reflecting that energy can be measured in terms of work of heat. The standard SI (*Systéme International*) unit of energy is the joule which is defined as a force of 1 Newton acting over 1 metre in the direction of action of the force (Lawrence and Fowler, 1998), so 1 joule = 1 kg ($m^{-2}s^{-2}$). However, historically the unit of heat to describe the energy-yielding capability in feedstuffs after a complete combustion was the calorie. The calorie (cal) is defined as the heat required to raise the temperature of 1 g of water from 16.5° to 17.5° (NRC, 1994). One calorie is equal to 4.184 joules. This interconversion has become confusing sometimes because the long-term association of the word calorie with the energy in human food. The calorie just defined lines above is called a "small" calorie to distinguish it from Calorie with a capital C, which is equivalent to 1000 small calories or 1 kilocalorie (kcal) (Lawrence and Fowler, 1998). This is a common unit for energy used in livestock including poultry feed. A megacalorie (Mcal) equals 1000 kcal and is commonly used as a basis for expressing requirements of other nutrients in relation to dietary energy. A joule (J) equals $10^7$ ergs (1 erg is the amount of energy expended to accelerate a mass of 1 g by 1 cm/s). The joule has been selected SI (International System of Units) and the U.S. National Bureau of Standards (1986) as the preferred unit for expressing all forms of energy. Many countries use joule as the unit for energy in nutritional work; however, calorie is

also popular because it is the standard energy terminology used in the U.S. poultry industry and there is no difference in accuracy between the two terms.

**Energy Systems in Poultry Diets**

The partitioning of energy in feed until it changes to meat (ex. broilers) is depicted in Figure 3. The first law of thermodynamics (energy is not created, no destroyed, only changes in form) allows us the measure the inputs and outputs of the process. The energy stored in the feed is called gross energy (GE), when the feed is ingested and then subjected to the enzymes in the gastrointestinal tract; about 30% of the GE is wasted in the feces and the energy available at this point is called apparent digestible energy (ADE). The next energy loss occurs in the urine and once this is measured the results are called apparent metabolizable energy (AME). It is apparent because the energy in the feces and urine contains the endogenous losses of the normal turnover of the body and not just the losses due to the diet. Once the endogenous losses are accounted for and added back to the calculations, the energy is called true metabolizable energy (TME). The ME is usually corrected for nitrogen (N) retention (AMEn, TMEn) to convert all data to a basis of N equilibrium for comparative purposes (Lopez and Leeson, 2008) based on the assumption that all N retained will be excreted as uric acid. These authors arise the controversy of using a correction for nitrogen corrections because species-age comparison are not critical for poultry nutritionists, and broilers are considered to be relatively uniform in protein accretion over time; however this nitrogen correction, which varies from 8.22 – 8.73 kcal/g N retained, reduced the bird to bird variation resulting in a more reliable AME value (Van der Klis and Kwakernaak, 2008). Metabolizable energy and its different variations (AMEn, AME) apparent metabolizable energy corrected and not corrected by

nitrogen, respectively, and TMEn and TME, true metabolizable energy corrected or not correct by nitrogen, respectively, are mostly used by the poultry industry around the world. Fast methods to determine AMEN, TME value of feedstuffs depend on the use of adult cockerels that are fasted for 36 hours, so this circumstances may affect the digestibility of nutrients in the gastrointestinal tract and consequently the ME; therefore, adult animals may have limited value for fast growing broilers. There is also the Dutch AME system which considers an additional correction factor to account for fat and carbohydrate energy utilization (adult poultry kj/kg = 19.03 dCP + 38.83 d C. Fat + 17.32 dNfE, and for broilers kj/kg = 15.56 dCP + 38.83 d C. Fat + 17.32 dNfE) (Van der Klis and Kwakernaak, 2008). However, ME is not the ultimate energy the bird uses for maintenance and production. Net energy (NE) is the "true energy content of the diet as well as a true indication of animal's requirement" (Kleyn, 2013). Net energy (NE) systems for feed formulation have been utilized in feed formulation for livestock for over 70 years. There are number of ways by which NE can be measured. These involve measurement of heat increment, which is difficult to quantify and expensive to perform. Indirect calorimetry is a tool that can be used to calculate heat production in animals. The metabolic nitrogen and gas energy losses are considered less important in non-ruminant animals, so they are not accounted for in the calculations of heat production, only $VO_2$ consumption and $VCO_2$ production are considered. The calculation of heat increment (HI) in the diets is the ultimate waste of energy before it is directed for maintenance and production. If the HI is measured and then subtracted from the ME, the energy will be called NE. The heat increment depends on the nutrient composition of a feedstuff. The net energy values for fat and protein per unit of ME are 20% higher and 20% lower respectively compared to carbohydrates (McLeod, 2002). This difference is the metabolic efficiency of proteins, fats and carbohydrates and it is considered in

the net energy system, so it is a preferred system (De Groote, 1999). For example, net energy system is used in pigs to improve the accuracy of the estimations of the utilizable energy content of feedstuffs. These NE system in pigs take into account lower energy value for protein and higher value for fat relative to carbohydrates. The need of a NE for system for poultry can be debated but NE is a topic of research in many countries (Van der Klis and Kwakernaak, 2008). The source of the debate in NE system is the many different approaches researchers have made to calculate the NE, the systems are different, and some are more acceptable than others. For instance, the original NE by Armsby and Fries (1918) take the classical equation NE kcal/kg = AMEn – HI. The productive energy (PE) from Fraps (1946) PE kcal/kg = NEg + NEm (Net energy of gain plus the net energy of maintenance). The NE system from Emmans (1994) called effective energy EE kcal/kg = 1.17 AMEn – (10x%CP) -580.

It is well known that feed cost are the major costs for poultry production. Three quarters of these feed cost are related to dietary energy (Van der Klis and Kwakernaak, 2008), therefore it's very important to understand and evaluate the energy for poultry to choose the system that best describes the efficiency of the modern broiler.

**POLYSACCHARIDES**

Polysaccharides are defined as condensation polymers of high molecular weight based on simple monosaccharide units (Aspinall, 1970). Two monosaccharides units are joined together by a glycosidic linkage involving elimination of water between the hemiacetal hydroxyl group of one unit and an available hydroxyl group of another. The linkages are the same as the ones found on oligosaccharides (disaccharides, tri-saccharides, etc.). The term polysaccharide is

usually used for compounds with more than ten residues; however, the majority of polysaccharides are substances of much higher molecular weight, several have molecular weights more than a million. The conformation of the individual monosaccharide units that form a polysaccharide can be studied from the likelihood of the groups interacting with one another, position of linkage, the branching between others. However the properties that makes a polysaccharide different is not only the summation and arrangement of the monosaccharides but also in the conformation of the polymer chain as a whole (Davidson, 1967).

Polysaccharides are found in living organisms as skeletal substances in the cell walls of higher land plants and seaweeds providing reserve food in plants, microorganisms, and animals. They also work as protective substances in plants in the form of exudate gums sealing off sites of injury, and in microorganisms as encapsulating substances (Aspinall, 1970). Polysaccharides of economic importance are mainly derived from the plant kingdom. Cellulose is by far the most abundant of all polysaccharides and it is used in the textile industry, plastics, paper-making between others. Starch is the main carbohydrate polymer for human consumption.

Polysaccharides can be broken down by acid or enzymatic hydrolysis to component sugars. The sugar constituents of polysaccharides are most commonly the pentose or hexose sugars derived from oxidation e.g. hydroxymethyl groups at $C_6$ to carboxylic acids (hexuronic acids), by reduction e.g. of hydroxyl groups to methyl groups (6-deoxyhexoses), by substitution, e.g. of hydroxyl by amino groups (hexoamines) or by the formation of methyl ethers from certain hydroxyl groups.

**Classification of polysaccharides**

Polysaccharides are classified in two main groups: homo-polysaccharides and hetero-polysaccharides. Aspinall (1970) and Davidson (1967) provide 2 sub-classification in each main group as linear and branched.

***Homo-polysaccharides***

Polysaccharides in this group contain carbohydrates of a single type although linkages and configurations can change. The most important examples in this groups are chitin, cellulose, glycogen, starch, and xylan. This group can be subdivided in two sets: the straight chain and the branched chain structures (Table 2). In general, these type of polysaccharides serve as structure of cell wall materials in plants and lower animals. For example, cell wall content of molds, fungi, crustaceans, and insects contain a large proportion of chitin, whereas many plant structures contain cellulose or similar glucans, as structural components. On the other hand, the branched homo-polysaccharides are usually used as energy reserve such as glycogen, they are subject of rapid enzymatic degradation. The homo-polysaccharides are known to have well-defined chemical structures.

***Hetero-polysaccharides***

Polysaccharides in this group contain two or more different carbohydrate units e.g. arabinoxylans and glucomannans. There is a possibility that covalently bound lipid or protein may also be present. This fact makes complicated the analysis of these structures. In this type again, there is a sub-classification of linear and branched hetero-polysaccharides. In the linear group hyaluronic acid, chondroitin, keratosulfate are cited as examples. It is of interest that the

majority of hetero-polysaccharides contain charged residues such as carboxyl, sulfate, or phosphate, whereas the majority of homo-polysaccharides are neutral sugars. The branched chain hetero-polysaccharides may contain as many as 6 different carbohydrate residues, and frequently combined with protein or lipid; few structures have been elucidated. Many are specific for microorganism or animals (blood group substances), others serve recognition functions for cell surfaces (Davidson, 1967) (Table 3). The complexity of these hetero-polysaccharides in the feedstuffs for animals could be beneficial in some species and disadvantageous in other such as poultry that have shorter large intestine where the polysaccharides can be utilized by the hot's microflora. Therefore, the understanding of this topic will become important to increase the utilization of these nutrients by extracting them or hydrolyzing with the use of exogenous enzymes.

**Non-Starch Polysaccharides (NSPs)**

A group of polysaccharides that is being of interested for animal feed are the non-starch polysaccharides. The term non-starch polysaccharides refers to polysaccharides molecules excluding α–glucans (starch) (Aspinall, 1970). Approximately 90% of the cell-wall material in human food can be defined chemically as NSP (Englyst, *et al.*, 1994). Non-starch polysaccharides are also the major components of dietary fiber in traditional ingredients used in poultry diets. NSPs include cellulose and non-cellulosic polysaccharides (Slominski, 2011). Classification of fiber made by differences in solubility is not precise in terms of chemical structures and biological functions (Choct, 1997). For instance, the methodology used to analyze NSP could change between laboratories suggesting the soluble and insoluble NSPs are subjected to the methodology in use which also assume the solubility of this NSPs will be the

same in the animal. Choct, (1997) provides a review on the terms and definitions used when fiber is described. For example, crude fiber refers to the fragments of plant material after extraction with acid and alkali and includes variable portions of the insoluble NSP. Neutral detergent fibre (NDF) is the insoluble portion of the NSP plus lignin, and the acid detergent fibre (ADF) refers to a portion of insoluble NSP comprised generally of cellulose and lignin. Therefore, a proper classification has been difficult. However, there is a basic classification proposed by Bailey (1973) where NSPs are classified in three main groups (Figure 4): cellulose, non-cellulosic polymers and pectic polysaccharides. Cellulose is the major component in nature because it is found in 50% of the carbon in vegetation (Choct, 1997), however the use of this NSP is limited in poultry because they lack the enzyme to break the linkages and release the glucose units of cellulose which are bond by 1, 4 - β-glucose units. The non-cellulosic polysaccharides comprised most of the NSPs where exogenous or commercial carbohydrases have potential to work on. In cereal grains, including corn, the non-cellulosic polysaccharides consist of arabinoxylans and β-glucans whereas in soybean and canola meals arabinans, arabinogalactans, galactans, galactomannans, mannans, and pectic polysaccharides predominate (Slominski, 2011). The water-soluble and viscous β-glucans and arabinoxylans present in barley, rye, and wheat interfere in the digestion of nutrients by the digestive enzymes avoiding the digesta to be hydrolyzed, and transported in the intestinal mucosa. Consequently, these effects may cause a decrease in animal performance (Graham and Aman, 1991). In addition, management problems related to sticky droppings have been indicated to be directly associated with the high water-holding capacity of β-glucans and arabinoxylans. Many commercial enzymes such as of β-glucanase and xylanase have been developed over the past 30 years (Slominski, 2011) to reduce viscosity problems. Moreover,

the use of effective combinations of NSP-degrading enzymes could reduce the nutrient encapsulating “cage effect” of cell walls which, in turn, could result in an increase in protein, starch, and energy utilization. It has been studied that corn and SBM do not pose a viscosity problem, and that an argument could be made for the use of a combination of much diversified carbohydrase activities to bring about effective cell wall degradation. Smits and Anisson (1996) provide a graphic list of the most important NSP in animal feed (Figure 5) and Bach Knudsen shows a model of cell wall (Figure 6) where the principal structures of the complex cell walls of a grain are illustrated. There a few laboratories that analyze the NSPs in the ingredients for animals, most of the research in fibers is directed to human diets. On table 4, a summary of the principal components of NSPs are summarized. Data from Bach Knudsen (1997), and Jaworski (2015), corresponds to the same laboratory technique, therefore the values are close for corn and wheat; however the values for wheat middlings are quite different, since wheat middlings is a by-product from the flour industry, denominations and variability of the products can vary. The soluble part of the NSPs is considered to cause an anti-nutritive effect in monogastric animals because it causes the increase of the viscosity in the gut digesta, and it affects the microflora; consequently affecting the transit time, modification of the intestinal mucosa, and changes in hormonal regulation because of change of rate of nutrient absorption (Choct, 1997). The anti-nutritive effect of insoluble NSPs is believed to be the increase in the bulkiness of the chyme and enhancement of the passage rate of digesta in the small and large intestines (Smits and Anisson, 1996) but they are believed to have little effect in nutrient utilization in monogastric animals (Carre, 1990). Exogenous enzymes, such as carbohydrases, work on the soluble part of the non-starch polysaccharides and have little effect on the insoluble part; enzyme companies are working on screening and

selecting a microorganism that can produce enzymes to solubilize the insoluble part of the polysaccharides (Pettersson, 2015, personal communication). This is the future challenge of enzyme companies due to the high concentrations of insoluble NSPs in animal ingredients. Exogenous enzymes are very popular but the knowledge on their substrates is still scarce (Angel, and Sorbara, 2014). Therefore, more emphasis on substrate availability is needed to consider when evaluating exogenous enzymes.

## EXOGENOUS ENZYMES FOR ANIMAL FEED

Exogenous enzymes are enzymes added to feed and differ from the enzymes produced by the animal which are called endogenous enzymes. Phytase is by far the most common enzyme. Most broiler companies add phytase to diets (Glitsø et. al., 2012) with the main objective of improving phosphorus and calcium digestibility (avP, Ca). Since the price of phytase has been reduced, broiler companies may add more phytase (super dosing) to improve energy availability by reducing the anti-nutritional factor –phytate. Carbohydrases are perhaps the next enzyme group of economic importance: xylanases and glucanases in combination with phytase [introduced in the mid-1980s (Cowieson and Bedford, 2009)] and pectinases, hemicellulases, mannanases are being added to poultry feed to improve nutrient utilization. Proteases are mostly used in diets with higher protein content such as broiler starter and turkey diets to release amino acids from poorly digested ingredients. Research is ongoing to understand the effect of individual enzymes and the combination of 2, 3 and more enzymes in a multi enzyme composite. The purpose of multi enzymes is to hydrolyze the various indigestible substrates, [phytate, non-starch polysaccharides (NSPs), and indigestible proteins] in the ingredients. The cocktail of multi- enzymes that will provide optimum utilization of dietary energy in the feed

will depend on the type of non-starch polysaccharide (NSPs) in the diets. Most of the research with enzymes has been focused on digestibility studies because of the immediate application of digestible nutrients that can be used in formulating economical poultry diets and the lower cost of the conducting digestibility and performance research compared to determining the net energy value of an enzyme composite. Even though carbohydrases are used to improve energy from the diets, the enzymes also have been reported to increase amino acid digestibility (Cowieson and Bedford, 2009). The majority of the exogenous enzymes in the market are derived from one organism and produced in another organism. For example, the gene encoding production of the protease Ronozyme ProAct (Novozymes, Bagsvaerd, Denmark) originates from *Nocardiopsis prasina* which is the donor microorganism. Then, this gene is moved into a *Bacillus licheniformis* to ensure a safe and efficient production of the protease in large scale. *Bacillus licheniformis* is the host or production organism.

**Protease**

Chickens have the ability to digest proteins because they can produce the enzymes such as pepsin, trypsin, chymotrypsin, elastase, carboxypeptidases, collagenase and peptidases (Leeson and Summers, 2001). However, there is still a fraction of the dietary protein that is not used by the bird and it is excreted (Glitsø, et al., 2012) causing environmental problems because more nitrogen is put to the environment, so commercial proteases have potential to work on this indigestible fraction. It has been some approach to produce proteases from *Aspergillus* or *Bacillus*; however the protease has not been the major enzyme produced by these microorganisms making difficult the evaluation of the protease as mono-component (Glitsø, et al., 2012). The process followed by some companies to obtain a newly efficacious protease for

poultry feed starts by defining the required action of the enzyme, the conditions at which the enzyme should be active, the ingredients where the enzyme will work. Then, a screening from a databank of isolated natural microorganism that will produced the desired enzyme is selected, the target is to break down the indigestible protein of the ingredient in use after it has been degraded by pepsin, trypsin, and by carbohydrases in an in –vitro setting. The next step is to test if the enzymes will support the gastrointestinal tract conditions; for example, if the enzyme will resist pH and the endogenous digestive proteases produced by the stomach and small intestine of the chick. The next steps include in vivo trials to evaluate the response of the animal, and also storage conditions, resistance of the enzyme through post-mixing conditions e.g. pelleting. The final step is the commercial production of the enzymes including fermentation, recovery, and formulation (Glitsø, et al., 2012)

**Carbohydrases**

The inability of animals to produce enzymes to digest cellulose, arabinoxylans, β-glucans, or pectins has encouraged further processing as pelleting, extruding with not positive outcome (Bedford, 1995). Carbohydrases available in the market for animal feed are xylanases that work on the arabinoxylans structures of rye and wheat particularly to decrease the gut viscosity created by these feedstuffs (Bedford, 1995); β-glucanases work on galactomannan and xyloglucan which are mostly present in cell walls of soybean and other dicotyledons, rapeseed meal, sunflower meal and canola meal can also contain high levels of the substrate for glucanases (Ravn, et al, 2015); β-mannanases work on β-mannans which are prevalent in soybean meal (Kleyn, 2013), guar meal, and other ingredients used in feed for animals; α-amylases work on the starch of mostly cereals; even though poultry has the capacity to produce

α-amylase, some resistant starch can still be degraded by the use of α-amylases (Svihus, 2014). Carbohydrases are produced as multi-component enzymes meaning that a microorganism can produce more than one enzyme, so providing a wide variety of enzyme activities; as a consequence, enzyme companies try to standardize to one or two enzymes and make it a consistent product; however a mono-component production is desirable (Pettersson, 2015, personal communication).

## BODY COMPOSITION IN POULTRY

The global meat consumption will grow 1.4% annually during the decade 2015-2024 [OECD-FAO]. According to OECD-FAO (2015), poultry meat will be half of this growth increasing from 111.9 Mt (million tons) in 2015 to 133.8 Mt in 2024. The expected increase in poultry meat production will demand producers provide poultry meat more efficiently in time and cost. Consumer demands less fat and leaner tissue. It is well known that body composition is affected not only by age and phenotypic but also by diets and feeding programs. Therefore; the body composition analysis is vital in feed strategies research because of the ability of some ingredients to change body composition in poultry. For example, low protein diets supplemented with crystalline amino acid diets increased retained body nitrogen improving feed efficiency (Bregendahl, et al., 2002). Eits *et al*. (2002) also showed that nutrition affects fat-free body composition. The authors showed a strong relationship between ash and protein when the plane of nutrition was changed. Geneticists continuously work to improve the efficiency of the modern broiler making them grow faster, and leaner (Deeb and Lamont, 2002). As a result, modern broiler reach market weights faster every year, so body composition of chicken just 10 years ago could be different of the current broilers. There is also research in

broiler breeders fed three different energy diets during the rearing period that show different carcass fat and protein at the time of first oviposition, about 25 wk of age (Bennett and Leeson, 1989). The authors found a strong relationships of body weight and body composition at 20 wk of age and first egg. It is a common practice in the broiler breeder industry to feed restrict pullets causing delays at first egg as well as many welfare issues with this practice; body composition research becomes important to understand the mechanism of the first oviposition and production in order to produce better feed strategies to support the body composition required for a successful flock production and animal welfare.

**Body composition analyzed by Dual Energy X-ray Absorptiometry (DEXA)**

Carcass analysis is laborious and time consuming, so the preparation of dry homogenates for chemical analysis are usually avoided (Sibbald and Fortin, 1982). Chemical analysis is the golden methodology for body composition analysis; however an accurate and fast methodology is needed to assess body composition to support nutrition research. Techniques available for carcass composition analysis are abundant. Mitchel *et al*., (1997) reports a review from Topel (1988) to be more than 30 different techniques. Within these techniques the most important are cited by Raffan *et al*., (2006), chemical analysis, morphometry, densitometry, total body water measurement, dual-energy x-ray absorptiometry, and advanced imaging techniques. Regardless of the method chosen, investigators need to be aware of the precision and accuracy of the method of choice. Laskey and Phil (1995) describe dual energy x-ray absorptiometry (DEXA) as a method with higher resolution images, precision and more rapid scan times. X-ray was discovered by Wilhelm Roentgen in November of 1895. X-rays are invisible to the eye, affect the photographic plate; which produce fluorescent phenomena and pass through wood, metal

and the human body (Münsterberg, 1896). X-rays are electromagnetic waves generated from the electron cloud orbiting the atomic nucleus, when the shell of the target atom has a vacancy, an electron from the outer orbit will fill the spot, so the energy difference is released as a form of X-ray. This X-ray penetrates solid barriers related to density producing image on photographic paper. X-rays have the capability to go through skin layers and deposit their energy in cells deep inside the body. X-rays also have enough energy to ionize atoms in deep tissue and break chemical bonds within comes critical biological molecules. The intensity of X-rays are measured as exposure rate in Roetgens, mR/hr (University of Arkansas, 2015). The old DEXA machines had the problem of time limitations because of the pencil-beam DEXA used about 20-30 minutes to scan a dog for example (Raffan, 2006); requiring more anesthesia to keep the animal asleep; however the new DEXA machines use fan-beam technology which reduces the timing dramatically that a dog can be scanned in 5 minutes. In poultry, when hens are scanned alive the DEXA machines takes about 3.5 minutes to scan one hen at production without a need of anesthesia (BW 4 kg) (Caldas *et al*. 2015, chapter 7). DEXA is considered by many as the method of choice to evaluate bone mineral density in humans because it allows rapid, noninvasive, and precise measurements. X-rays of two different energy levels that pass through the body are impeded differently by bone and soft tissue; therefore, the type and amount of tissue scanned can be distinguished (Zotti *et al*., 2003). A direct comparison of scan data with chemical analysis of body composition is not possible due to variations in the software and instrument used. Mitchel *et al*., (1997) conducted a study in chickens and found that chemical analysis is needed to develop prediction equations with the scan data for lean, fat and body mineral composition. This allowed for more accurate predictions of lean, fat and body mineral composition with future scans. Moreover, Swennen *et al*., (2004) mention that

regression equations are strictly limited to one particular instrument, software version, and applied methodology. Consequently, prediction equations are imperative for a new instrument or new software. In addition to the accuracy of the instrument; precision in methodology is also essential. It is important to be able to reproduce the same results in repeated measurements Zotti *et al*., (2001). Some research conditions might be different in some circumstances. For example, frozen chickens might be the sample for postmortem chickens. Swennen *et al*., 2004, reports scan values for frozen chickens, but there is lack of comparison between frozen and fresh chickens. Wähnert *et al*., (2009) report differences in bone mineral density (BMD) between frozen and thawed human femora. Finally, position of the animal being scanned might have an impact in the values obtained. Some trials indicate no differences in scanning positions (Swennen *et al*., 2004), while others show some evidence that indicates position is an important fact to consider (Raffan *et al*., 2006). There are several papers on the use of DEXA for body composition because the validation is unique to every software and machine.

**Efficiency of energy and protein deposition in the carcass of broilers**

Efficiency is commonly expressed as a ratio of output/input (Lawrence and Fowler, 1998). The energy recovery in the animal body from the energy supplied can be expressed as: total energy in the body gain/ total energy in feed, it can be converted to a percentage by multiplying x 100. This energy could be metabolizable energy (ME), meaning the energy available after the energy losses in fecal and urine have accounted; or it could also be the gross energy (GE). Another common expression of efficiency is related to protein gain; for example: gain of protein in the body/weight of protein provided in the feed. Other models such as multiple linear regression and logarithmic models have been used to obtain the efficiency of energy for

maintenance and gain, but also the efficiency for fat and protein gain (Sakomura, 2004; Lopez and Leeson, 2008b).

**Table 1. Calorific factors for oxidation of carbohydrates, proteins and fats**

| References | Heat of combustion kcal/g | $O_2$ consumed L/g | $CO_2$ produced L/g | Respiratory quotient ($CO_2/O_2$) |
|---|---|---|---|---|
| **Carbohydrates** | | | | |
| Magnus-Levy, 1907 (starch) | 4.11 | 0.829 | 0.829 | 1.00 |
| Lusk, 1928 (after Zuntz & Loewy) | 4.18 | 0.829 | 0.829 | 1.00 |
| Abramson, 1943 (after Benedict) | 4.21 | 0.829 | 0.829 | 1.00 |
| Kleiber, 1961 | 3.99 | 0.800 | 0.800 | 1.00 |
| Brouwer, 1965 | 4.21 | 0.829 | 0.829 | 1.00 |
| Elliot & Davison, 1975 (glucose) | 3.74 | 0.746 | 0.746 | 1.00 |
| **Proteins*** | | | | |
| Magnus-Levy, 1907 (after Rubner) (mammals) | 4.10 | 0.965 | 0.781 | 0.810 |
| Peters & van Slyke, 1931 (after Rubner) (mammals) | 4.26 | 0.950 | 0.761 | 0.801 |
| Lusk, 1928 (after Zuntz & Loewy) (mammals) | 4.24 | 0.950 | 0.762 | 0.802 |
| Abramson, 1943 (after Benedict) (mammals) | 4.24 | 0.919 | 0.743 | 0.809 |
| Kleiber, 1961 (mammals) | 4.82 | 1.072 | 0.866 | 0.808 |
| Brower, 1965 (mammals) | 4.39 | 0.957 | 0.774 | 0.809 |
| Dargol'tz, 1973 (birds) | 4.76 | 1.032 | 0.877 | 0.850 |
| | 4.38 | 0.955 | 0.707 | 0.740 |
| Braefield & Llewellyn, 1982 (birds) | 4.35 | 0.936 | 0.674 | 0.720 |
| **Fats** | | | | |
| Magnus-Levy, 1907 (after Rubner) | 9.30 | 2.019 | 1.433 | 0.710 |
| Lusk, 1928 (after Zuntz & Loewy) | 9.46 | 2.019 | 1.428 | 0.707 |
| Cathcart & Cuthbertson, 1931 (liver and muscle fat) | 9.18 | 1.937 | 1.391 | 0.718 |
| Cathcart & Cuthbertson, 1931 (adipose tissue) | 9.51 | 2.001 | 1.423 | 0.711 |
| Abramson, 1943 (after Benedict) (animal fat) | 9.51 | 2.013 | 1.431 | 0.711 |
| Abramson, 1943 (after Benedict) (human fat) | 9.54 | 1.992 | 1.420 | 0.713 |
| Brower, 1965 | 9.51 | 2.013 | 1.431 | 0.711 |
| Dargol'tz, 1973 (avian fat) | 9.30 | 2.030 | 1.441 | 0.710 |
| Ben-Porat et al, 1983 | 9.50 | 2.028 | 1.430 | 0.705 |

Source: Adapted from McLean and Tobin, 1987

*$O_2$ consumption of proteins is given in to L/g of protein, the original report was L/g of N, but it has been converted by dividing the value over 6.25 for comparison purposes with carbohydrates and fats

## Table 2. Principal homopolysaccharides

| Types of linkage | Types of chain | Common name (if any) | Sources |
|---|---|---|---|
| **Glucans** | | | |
| β- 1, 2- | linear | | Agrobacteria |
| α- 1, 3-, α-1, 4- | linear | nigeran | *Aspergillus niger* |
| α- 1, 3-, α-1, 4- | linear | isolichenan | Iceland Moss |
| β- 1, 3- | essentially linear | laminaran, callose, various | Brown seaweeds, higher plants, algae, fungi, yeasts |
| β- 1, 3-, β- 1, 4- | linear | lichenan | Iceland Moss |
| β- 1, 3-, β- 1, 4- | linear | | cereal grain |
| α-1, 4- | linear | amylose | starches of higher plants |
| α- 1, 4-, α-1, 6- | branched | amylopectin, glycogen | starches of higher plants, microorganisms |
| α- 1, 4-, α-1, 6- | linear | pullulan | Fungi (*Pullularia spp*.) |
| β- 1, 4- | linear | cellulose | cell walls of higher plants |
| α- 1, 6-, α-1, 3- | branched | dextran | Bacteria (especially *Leuconostoc spp*.) |
| β- 1, 6- | linear | pustulan | lichen *Umbilicaria pustulata* |
| **Fructans** | | | |
| β- 2, 1- | linear | inulin | dahlias, Jerusalem artichokes |
| β- 2, 6- | linear | grass levans | pasture grasses |
| β- 2, 6-, β- 2, 1- | branched | various | various plants |
| β- 2, 6-, β- 2, 1- | branched | bacterial levans | various bacteria |
| **Mannans** | | | |
| α- 1, 2-, α-1, 6- | branched | | yeast and other microorganisms |
| β- 1, 4- | linear | | certain land plants, seaweeds |
| **Galactans** | | | |
| β- 1, 3-, α- 1, 4- | linear | carrageenan | red seaweeds |
| β- 1, 3-, β- 1, 6-, β- 1, 4- | branched | | beef lung |
| β- 1, 4- | linear | | pectic substances of higher plants |
| β- 1, 5-* | linear | galactocarolose | mould *Penicullium charlessi* |
| **Arabinans** | | | |
| α- 1, 3-,* α-1,5-* | branched | | pectic substances of higher plants |
| **Xylans** | | | |
| β- 1, 3- | linear | | green seaweed *Caulerpa filiformis* |
| β- 1, 3-, β- 1, 4- | linear | rhodymenan | red seaweed *Rhodymenia palmata* |
| β- 1, 4- | linear | | cell walls of higher plants |
| **Fucan** | | | |
| α- 1, 2-, α-1, 4- | branched | fucoidan | Brown seaweeds (*Fucus spp*.) |
| **Galacturonan** | | | |
| α- 1, 4- | linear | pectic acid | pectic substances of higher plants |
| **Glucosaminan** | | | |
| β- 1, 4- | linear | chitin | lobster and crab shells, fungi |

Source: Aspinall, 1970

*Sugar residues in the furanose form

**Table 3. Principal heteropolysaccharides**

| Types of linkage | Types of chain | Common name (if any) | Sources |
|---|---|---|---|
| DL-galactan | branched | | snails |
| DL-galactans (O-sulphated or contain 3, 6 anhydro-galactose units) | Linear | agarose | red seaweeds |
| Arabinoxylans | branched | porphyran | plant cell walls |
| Glucuronoxylans | branched | | plant cell walls |
| Arabinogalactans | branched | | coniferous woods |
| Glucomannans | Linear | | coniferous woods |
| Galactomannans | branched | various | leguminous seeds |
| Galactomannans | branched | | pathogenic fungi |
| Glucoglucuronan | Linear | | Pneumococcus type III |
| Guluronomannuronan | Linear | alginic acid | brown seaweeds bacteria |
| Galactoglucosaminan (O-sulphated) | Linear | keratosulphate | Cornea |
| Galactosaminoglucuronan | Linear | Chondroitin | Cornea |
| Galactosaminoglucuronans (O-sulphated) | Linear | chondroitin sulphates A&C | cartilage |
| Galactosaminoiduronan (O-sulphated) | Linear | dermatan sulphate | Skin |
| Glucosaminoglucuronan | Linear | hyaluronic acid | Animal tissues |
| Glucosaminoglucuronan (N-and O-sulphated) | Linear | Heparin | blood anticoagulant from mammalian tissues |

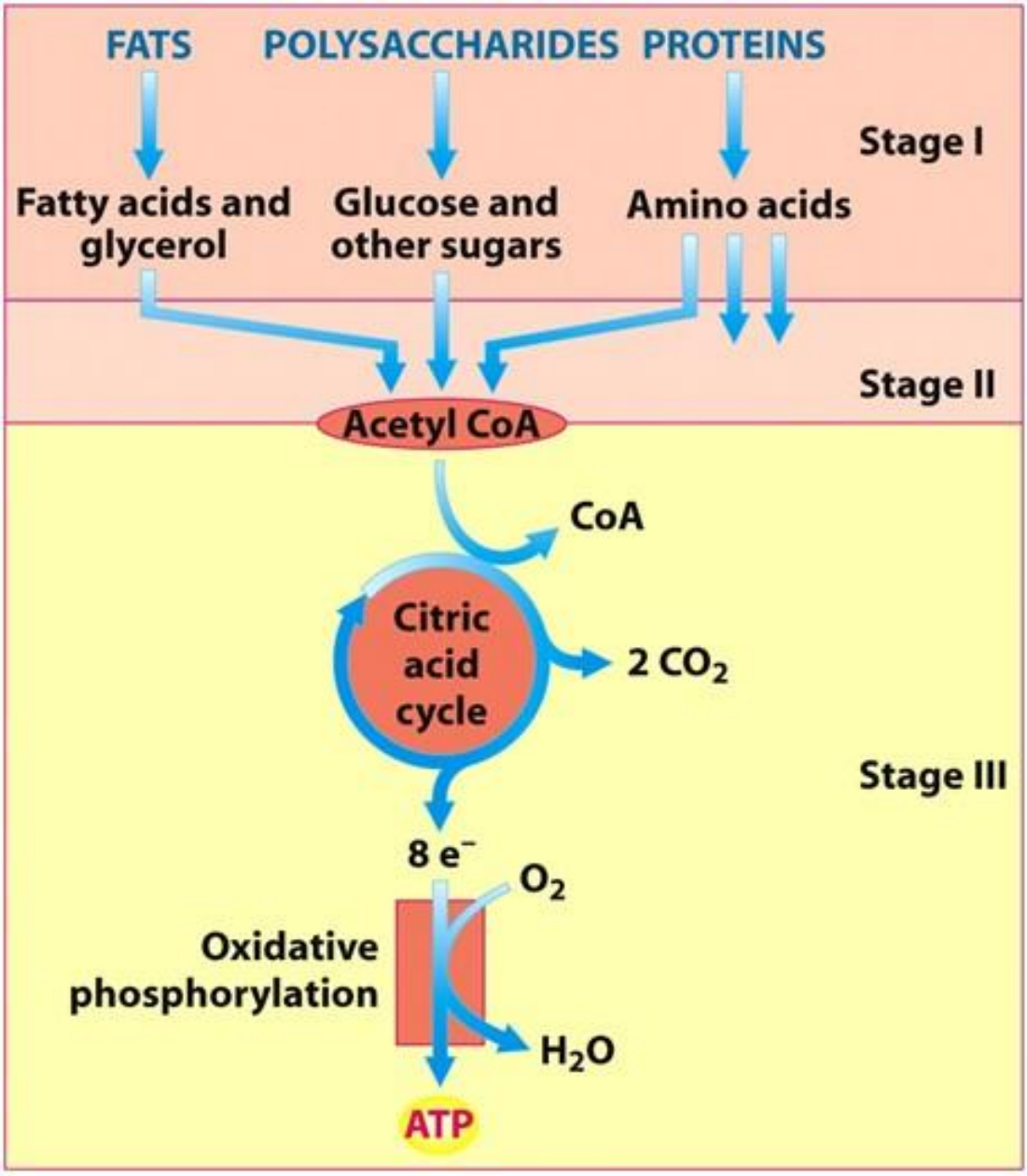
Source: Aspinall, 1970

**Table 4. Starch and Non-Starch polysaccharides composition in common poultry ingredients**

| | Corn | | Soybean meal | | Corn DDGS | Wheat | | | Wheat Middlings | |
|---|---|---|---|---|---|---|---|---|---|---|
| Carbohydrate, % | Jaworski *et al*., 2015 | Bach Knudsen, 1997 | Bach Knudsen, 1997 | Choct, 1997 | Jaworski *et al*., 2015 | Jaworski *et al*., 2015 | Bach Knudsen, 1997 | Englyst, 1989 | Jaworski *et al*., 2015 | Bach Knudsen, 1997 |
| Starch | 62 | 69 ± 1.8 | 2.7 ± 1.2 | 1.0 | 8.6 | 61.8 | 65.1 ± 2.7 | | 16.8 | 57.5 |
| S-NCP | 2.5 | 0.9 ± 0.7 | 6.3 ± 1.0 | | 2.4 | 1.9 | 2.5 ± 0.4 | | 1.2 | 7.1 |
| Arabinose | 0.5 | 0.3 ± 0.2 | 0.1 ± 0.1 | 0.5 | 0.9 | 0.6 | 0.7 ± 0.2 | 0.8 | 0.2 | 2.1 |
| Xylose | 0.4 | 0.2 ± 0.2 | 0.2 ± 0.1 | 0.1 | 0.9 | 0.7 | 0.9 ± 0.4 | 1.0 | 0.2 | 3.1 |
| Mannose | 0.1 | 0.2 ± 0.1 | 0.5 ± 0.1 | 0.2 | 0.7 | 0.1 | 0.2 ± 0.1 | t | ND | 0.2 |
| Galactose | 0.4 | 0.1 ± 0.1 | 1.6 ± 0.3 | 0.6 | 0.2 | 0.2 | 0.2 ± 0.1 | 0.1 | 0.2 | 0.3 |
| Glucose | 0.6 | 0.1 ± 0.1 | 0.6 ± 0.3 | 0.2 | 0.3 | 0.2 | 0.4 ± 0.3 | 0.4 | 0.2 | 1.1 |
| Uronic acids | 0.4 | 0.1 ± 0.1 | 2.4 ± 0.4 | 1.1 | 0.4 | ND | 0.1 ± 0.1 | t | 0.3 | 0.3 |
| I-NCP | 3.8 | 6.6 ± 1.1 | 9.2 ± 0.9 | | 15.8 | 6.2 | 7.4 ± 0.6 | | 22.7 | 10.1 |
| Arabinose | 1.2 | 1.9 ± 0.2 | 1.7 ± 0.2 | 2.4 | 4.3 | 1.7 | 2.2 ± 0.1 | 2.5 | 7 | 2.7 |
| Xylose | 1.7 | 2.8 ± 0.3 | 1.7 ± 0.3 | 1.7 | 6.2 | 2.9 | 3.8 ± 0.3 | 3.8 | 11.4 | 3.6 |
| Mannose | 0.1 | 0.1 ± 0.1 | 0.8 ± 0.2 | 0.7 | 1.2 | 0.2 | 0.1 ± 0.1 | t | 0.3 | 0.6 |
| Galactose | 0.4 | 0.4 ± 0.1 | 2.5 ± 0.3 | 3.9 | 1.1 | 0.2 | 0.2 ± 0.1 | 0.3 | 0.5 | 0.4 |
| Glucose | ND | 0.9 ± 0.4 | 0.1 ± 0.2 | 0.3 | 1.8 | 0.9 | 0.7 ± 0.3 | 0.4 | 2.3 | 2.1 |
| Uronic acids | 0.4 | 0.6 ± 0.1 | 2.3 ± 0.3 | 2.5 | 1.2 | 0.3 | 0.4 ± 0.1 | 0.2 | 1.2 | 0.7 |
| Cellulose | 1.7 | 2.2 ± 0.3 | 6.2 ± 1.8 | 4.4 | 5.8 | 1.3 | 2.0 ± 0.4 | 2.0 | 6.7 | 1.9 |
| Total NSP | 8.1 | 9.7 ± 0.2 | 21.7 ±2.7 | 19.2 | 25 | 9.5 | 11.9 ± 1.1 | | 30.7 | 19 |
| Klason lignin | 0.8 | 1.1 ± 0.2 | 1.6 ± 0.4 | | 3.9 | 1.8 | 1.9 ± 0.2 | | 7.3 | 1.1 |
| Soluble dietary fiber | 2.5 | | | | 3.4 | 1.9 | | | 1.2 | |
| Insoluble dietary fiber | 6.4 | | | | 25.5 | 9.3 | | | 36.9 | |

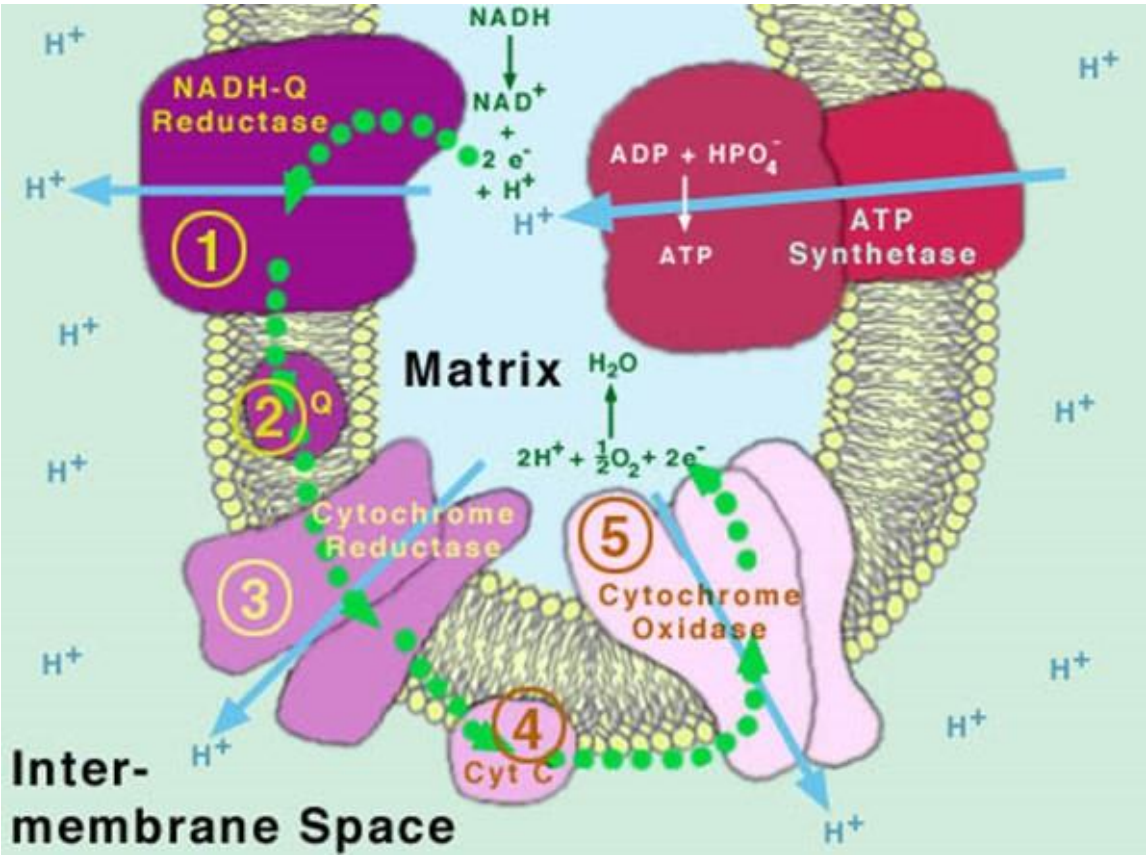
Source: Various (Jaworski *et al*., 2015; Bach Knudsen, 1997; Choct, 1997, Englyst, 1989)
S-NCP: Soluble- non cellulosic polysaccharides, I-NCP: Insoluble – non-cellulosic polysaccharides, ND: Non detectable, t: trace

**Figure 1. Stages of catabolism. The extraction of energy from fuels can be divided into three stages**



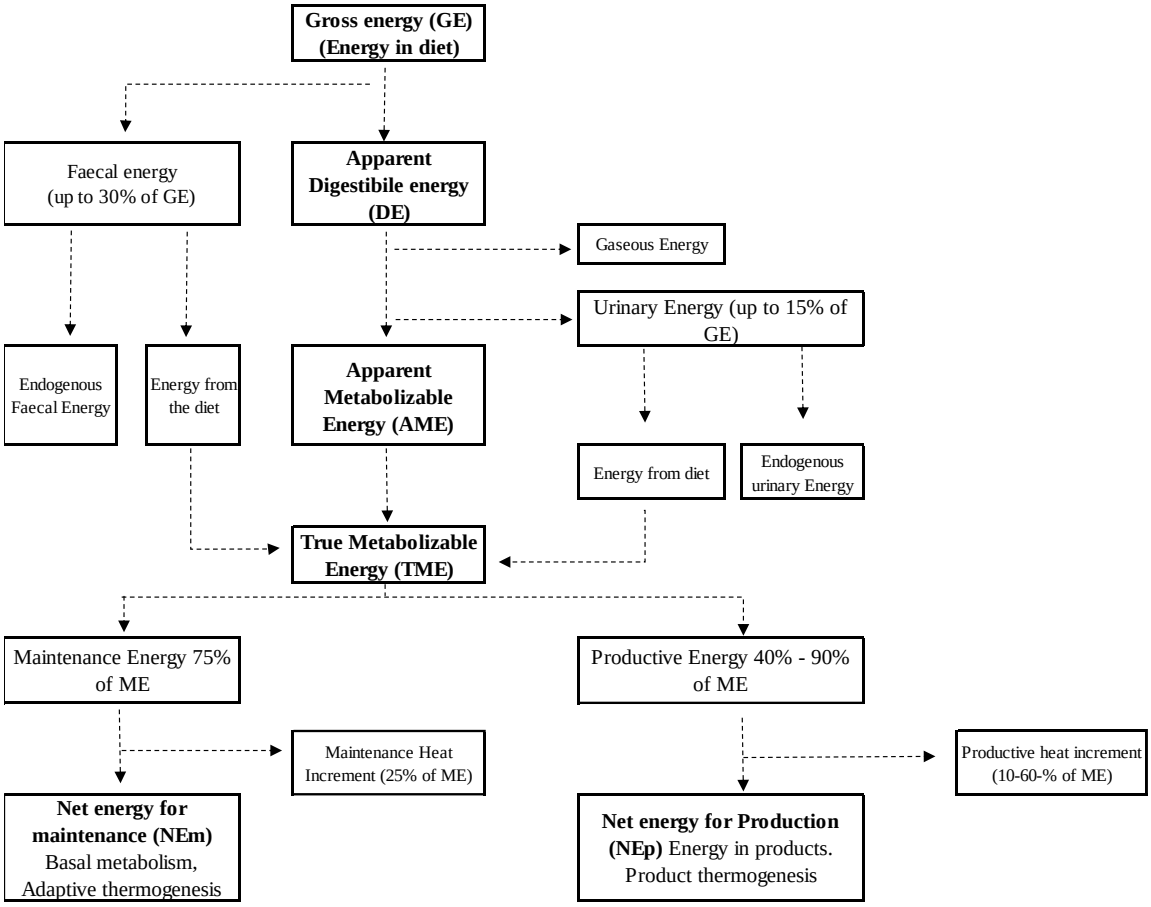

Source: Berg et al., 2012

**Figure 2. Oxidative phosphorylation**



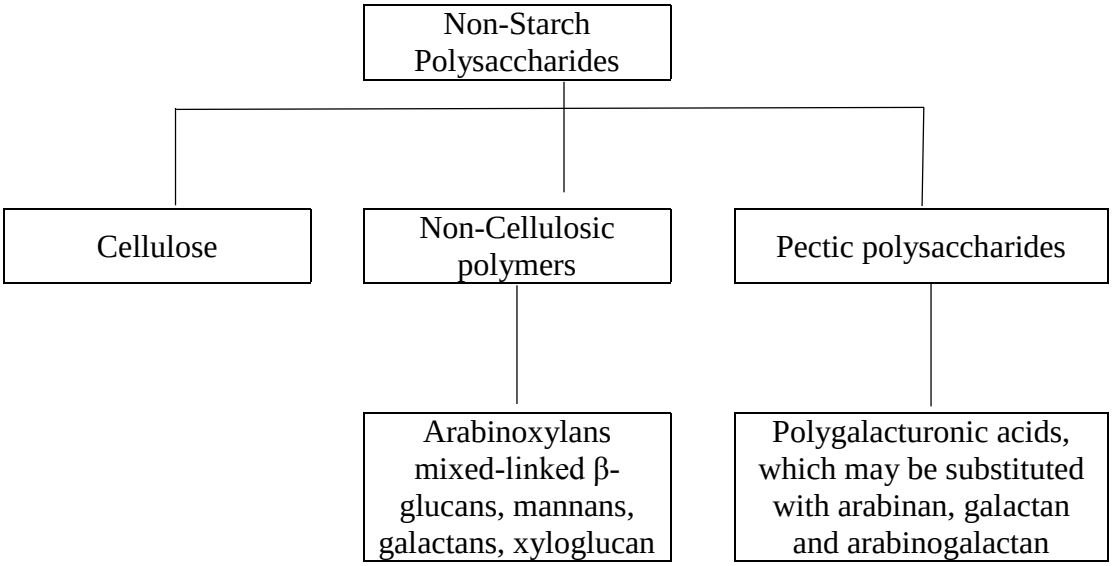

Source: Casidy *et al*., 1999

**Figure 3. Energy utilization in poultry**



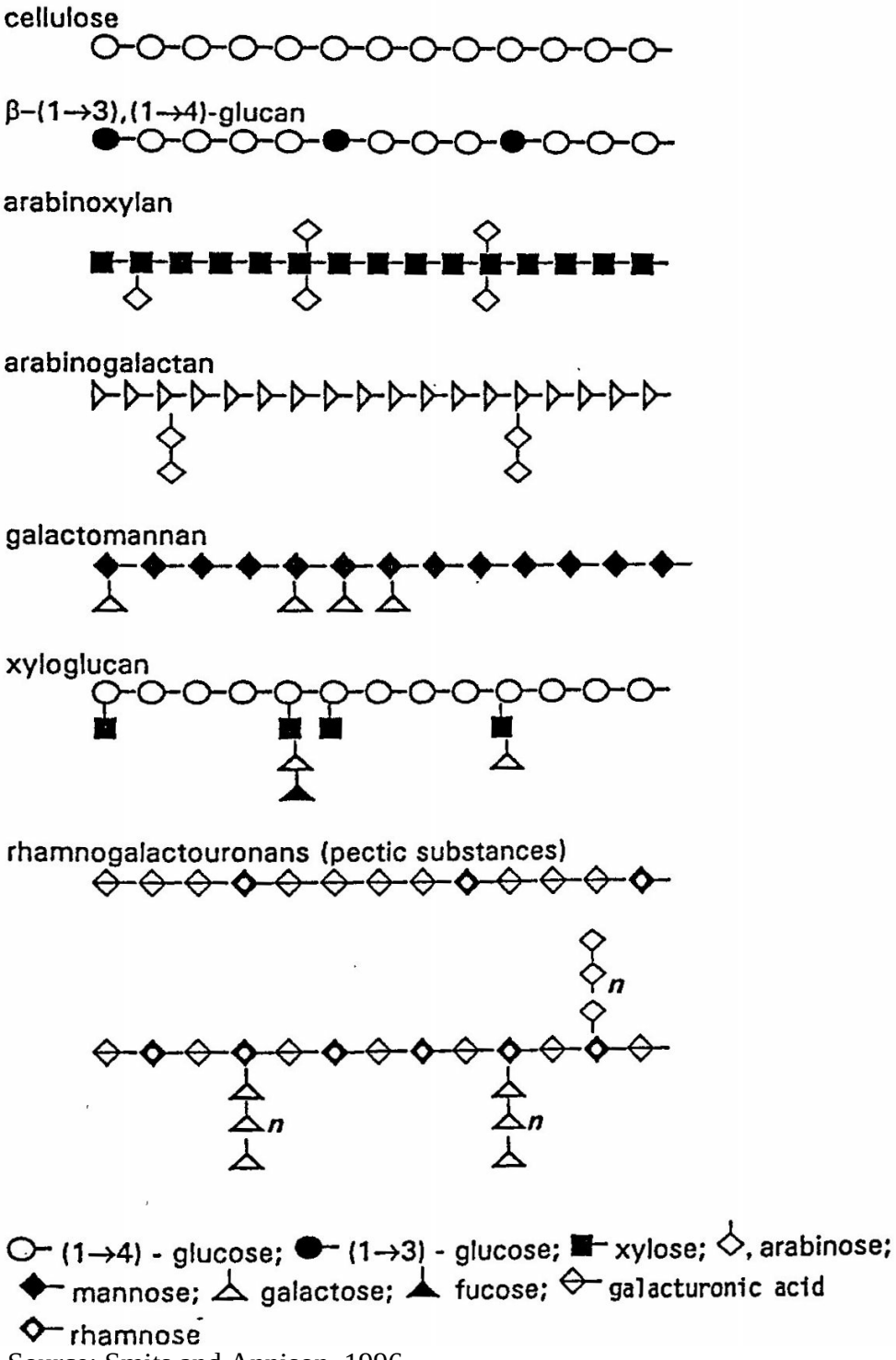

Source: Kleyn, 2013

**Figure 4. Non-Starch Polysaccharides classification**



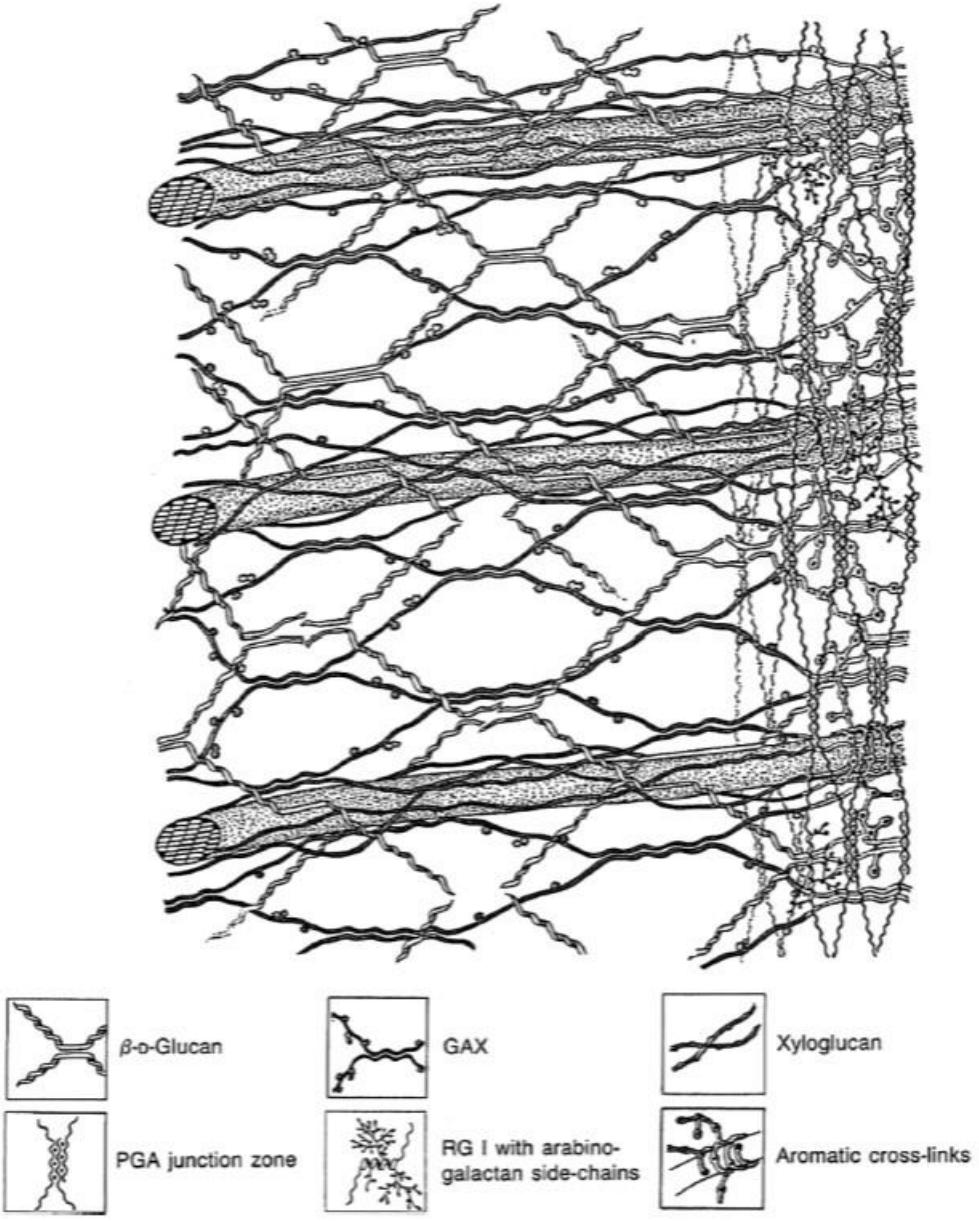

Source: Bailey, 1973

**Figure 5. Polysaccharide structures commonly found in feed ingredients of plant origin**




Source: Smits and Annison, 1996

**Figure 6. Cell wall model**




Source: Bach Knudsen, 2014

Cell wall model showing cellulose fibrils interlocked by glucuromo-arabinoxylans (GAX). Some of the GAX are wired onto the cellulose fibrils by phenolic linkages, whereas the substituted parts of GAX block hydrogen bonding. A small amount of pectic substances (PGA, RG1) are also present. Reprinted from Carpita and Gibeaut, 1993.

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# II. THE DYNAMICS OF BODY COMPOSITION AND BODY ENERGY IN BROILERS, ANALYZED BY CHEMICAL ANALYSIS

**ABSTRACT**

Consumer demands leaner meat, and chicken meat is one of the most efficient animal producing protein source for human consumption, so nutrition research needs to include not only performance but also body composition measurements to meet current market demands. Body composition by chemical analysis was achieved in 151 broilers from 1-60 d of age. Birds were fed mash diets *ad libitum* in four phases (starter 1-14d, grower 15-28d, finisher 19-42d, and withdrawal 43-60d). Chicks were selected at 12 points of evaluation (1, 4, 7, 12, 17, 22, 27, 33, 39, 47, 54 and 60 days of age). Gompertz 3P model, multiple linear regression and CRD with ANOVA analysis were used in the experimental design. The growth in terms of BW, protein, fat, minerals (calcium and phosphorus) follow a Gompertz 3P model with similar growth rates of about 4.9% per day and the maximum growth was obtained at about 34 days of age. Body weight ranged from 56 – 4184 g, water from 683 – 751 g/kg, protein from 154 – 182 g/kg, fat from 53 – 101 g/kg, minerals (ash) from 17.9 – 22.5 g/kg as expressed in fresh or as is basis. When data was expressed in dry matter basis protein ranged from 563 – 613 g/kg, fat from 197 – 317 g/kg, minerals from 65.2 – 86.6 g/kg, calcium from 11.7 – 18.9 g/kg, and phosphorus from 10.3 – 15.3 g/kg. The calorific coefficients for protein and fat were determined by multiple regression and resulted in 5.45 ± 0.09 kcal/g for protein and 8.95 ± 0.16 kcal/g for fat. These two coefficients are used to predict the body energy content or energy of gain. The protein: fat ratio was the highest at the beginning of growth and decreased gradually until d60. The body composition in terms of water, protein, and fat changes with age, water being reduced and increasing protein and fat towards market growth. Mineral composition remained constant at the end of growth but some

fluctuations occurred during the grower period. Calcium comprised about 22% of the mineral content and phosphorus 18%. Ca: P ratio ranged from 1.03 at the beginning of growth to 1.28 at the end of growth. The understanding of the dynamics of body composition will bring new opportunities to change feed strategies and increase the efficiency for meat while maintaining a healthy broiler.

Key words: Body composition, broilers, protein, fat, minerals

## INTRODUCTION

The evolution of the poultry meat production dates more than 3000 years ago, while pigeons, ducks, and geese were bred in China; at the same time, chickens were developed and domesticated from Asian jungle fowl (FAO, 2010). In the sixteen century, chickens were introduced into America from Europe, and the rest is history. Due to the increase in technological advancements and research, the poultry meat industry experienced a rapid development since the 1930's accounting for the 30% of the global meat production in 2010 (FAO, 2010). In the 1940's and 1950's, genetic companies arouse and focused in selecting chickens for meat consumption (Havenstein, 2003b) and continue to increase yield. Genetics and nutrition have contributed in 85-90% and 10-15%, respectively to development of the current broiler type chicken (Havenstein, 2003a). Certainly, the body composition of broilers have also been changed through the years and it could still be changed. Muscle growth is affected by intrinsic factors (e.g. genetic factors), and extrinsic factors such as nutrients, metabolism, sex, hormones, and activity. For example, birds from slower growing genotype had higher protein content; however lower breast and thigh muscle yield (Mikulski, 2011). This author also showed that free range chickens had a higher protein but less juicy and darker color compared to chickens raised indoors because of the increased activity when the birds are outdoors. Energy intake and diet composition can also change carcass composition of meat producing animals. A study by Boekholt (1997) shows that total energy retention consist of a daily basic protein retention and a variable additional energy retention mainly consisting of fat. The three major components when carcass composition is measured are protein, fat and mineral content because the amount of glycogen is usually small and it is measured by difference. Proteins are found

in throughout the body, with over 40% in skeletal muscle, over 25% in body organs, and the rest mostly found in the skin and blood (Gropper, 2009). Protein deposition is very rapid during early life and it is determined by the high fractional rate of protein synthesis in skeletal muscle (Davis and Fiorotto, 2005). Regarding fat composition, modern meat animal production requires minimal fat deposition; however many factors need to be controlled to achieve high protein and low fat content. For example, an animal that is raised under non-optimal weather conditions require fat depots for insulation, to provide oxidative substrates, and to produce metabolites that help regulate their metabolism (Mersmann and Smith, 2005). Mineral content in the carcass are mainly composed by calcium, and phosphorus. The genetic potential in broiler type chickens requires a fine work in nutrition, management and health to produce a good meat quality economically viable. The whole carcass composition of modern meat-type chickens is scarce and dates from Sibbald (1982) who standardized the methodology for evaluating carcass for chemical analysis. The carcass composition analysis needs to be evaluated under standard nutrition and management practices. The objective of this study is to provide carcass composition in terms of protein, fat, mineral (calcium and phosphorus), and energy of broiler at different points of the growth period from 1- 60 d fed a standard commercial diets.

## MATERIALS AND METHODS

All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC) # 12041.

**Birds and Housing**

Four hundred eighty, one day old Cobb male chicks of a commercial strain (Cobb Vantress, Siloam Springs, AR) were obtained from a local hatchery (Cobb hatchery, Fayetteville, AR) and reared in 4.5 $m^2$ floor pens of 40 chicks per pen. Each pen was equipped with 10 nipples per line, two hanging type feeders, with a round pan that provided 208 cm of feeder space per pen. Chicks from the same flock were placed in three different groups. Group 1 with 160 chicks for the starter period (1-14d), group 2 with 120 chicks for the grower period (15-28d), group 3 with 80 chicks for the finisher (29-42d), and group 4 with 120 chicks for the withdrawal period (43-60d). Chicks were selected at twelve points of the growth-out period with a body weight (BW) mean ± 1.6 SD at 1, 4, 7, 12, 17, 22, 27, 33, 39, 47, 54, 60 days of age for carcass composition evaluation. The amount of chicks selected at each point was 12 (range 9 to 16), the difference was the availability of the chicks in the range of the desired BW at each point of evaluation. The total number of chicks used was 151, out of 480. Regarding to the ventilation of the chicken house, the house was equipped with 4 tunnel fans in the far end wall. One of these fans was set to run as a minimum ventilation fan to keep the air-fresh and remove excess humidity. The side-walls were solid with 7 vent-boards on each wall. The vent-boards automatically opened prior to fans coming on and their opening is adjusted automatically based on desired static pressure. The 2 cool cells were covered with a curtain that is automatically lowered and raised based on desired temperature and to maintain a static pressure of .09 when any of the tunnel fans are running. This maintains the air velocity needed to keep the air fresh and to add a wind chill factor to the cooling of the birds during periods of hot temperatures. The cool cells themselves only

runs water when additional cooling is necessary. There were 4 Re-Verber-Ray radiant tube heaters (Detroit Radiant Products Company, Warren, MI) to provide heat during brooding or cold weather. All ventilation and heating equipment was controlled by a Chore-tronics Model 40 controller (ChoreTime). The controller was programmed to maintain specific temperature and ventilation curves based on the age of the bird. There were specific set points at different ages and the controller calculates what the set points are for every day in between, providing a gradual transition between ages. Temperatures in the chicken house were changed according to the genetic broiler management recommendations (Cobb 500, 2012) starting at 33°C and decreasing 3 degrees °C every week until 18°C at 42d from which it was maintained until the end of the study. The light program was 23 h. light: 1 h. dark for all feeding periods.

**Diets and feeding program**

Broilers were fed mash diets *ad libitum* from 1- 60 days of age. Four feeding programs were used, starter 1 – 14 d, grower 15 – 28d, finisher 29 – 42d, and withdrawal 43-60d. Diets consisted of a corn-soybean meal basal formulated to provide the Cobb 500 nutrient specs (Cobb Vantress, 2012) (Table 1). Major ingredients such as corn and soybean meal, and minor ingredients such as wheat middlings and distiller's dried grain with solubles (DDGS) were analyzed with NIR (Near Infrared Reflectance) (Bruker, MA, USA), the spectra sent to Precise nutrition evaluation program (PNE) (Adisseo, Antony, France) for analysis of AMEn, total and digestible amino acids, calcium and phosphorus. Diets were formulated using Brill Formulation software (Feed Management Systems, Hopkins, MN) using the values from analyzed ingredients.

**Body Composition Analysis**

At each point of evaluation from 1 – 60 d, chicks were selected in the morning after 5 hours of feed deprivation to emptying the gastrointestinal tract. Chicks were humanely sacrificed by $CO_2$ inhalation. Carcasses were frozen for further analysis. The preparation of the dry homogenates from the whole chickens was prepared in a similar way as described by Salas (2012). Briefly, carcasses were thawed for 24 - 36 hours and transferred to individual aluminum tubs, about 10% of water was added to avoid adhesion of the carcass to the container during the autoclave process. Chickens, tubes and water were weighed for initial weight and the container covered with aluminum foil and autoclaved at 121°C with 22 psi pressure. The time in the autoclave varied according to the size of the birds from 1 to 6 hours (1-60d). Once the cycle was finished, the carcasses were left in the autoclave for at least 2 hours to let them cool and wait until the pressure reached zero and the temperature normal laboratory conditions. Tubes were reweighed and if loss were observed, it was assumed to be water loss. The whole chicken which included feathers and visceral content were homogenized with a heavy duty blender (Waring laboratory, Blender LBC15, Model CB15). After homogenization, about 120 g sample was obtained and frozen for 48 hours before lyophilization for 2 wk. Dried samples were reweighed and ground for further analysis.

**Laboratory analysis**

Dry matter was determined by weighing the sample before and after lyophilization. The water content was determined by subtracting DM from 100, but it also accounted the

water loss in the autoclaving process. Nitrogen was analyzed by the method 990.03; AOAC (1995), carcass protein was defined as N x 6.25, the fat analyzed was prepared by method 920.39C; AOAC (1990), mineral content (ash) was analyzed by the method AOAC 923.03. The method for minerals, calcium and phosphorus was AOAC 968.08 adapted for an inductively coupled plasma, ICP. The gross energy (GE) was determined in a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). All analysis were conducted at the Central Analytical Laboratory, University of Arkansas, and Center of Excellence for Poultry Science.

**Calculations**

The body composition will be reported AS IS in absolute g or g/kg of body weight. Body composition will also be reported as DM taking the water out by dividing the components over the dry matter, g/kg. Water, protein, fat, minerals should amount 100; however there was a remaining part called rest and is assumed to consist mainly of glycogen (Boekholt, 1997); however it could also be the variability of the analysis, so the value will not be discussed further but it will be reported. Ratio of calcium and phosphorus in the body will also be given as Ca/P. The energy of the body is mainly due to the amounts of protein and fat, so multiple linear equation will be fitted to obtain the calorific coefficients for protein and fat. The slopes of the equation are considered to be the calorific values.

**Statistical analysis**

For carcass composition, a complete randomized design (CRD) was performed and one way (age) ANOVA analyzed and when ANOVA was significant, the means were separated using a Tukey HSD test. *P -value* was considered significant when ≤ 0.05. A Gompertz 3P growth model was chosen to fit the body growth and its components: water protein, fat, minerals (calcium and phosphorus) from the body of broiler chickens from 1-60 days of age. The Gompertz 3P model is = $a*e^{[-e^{[-b*[Age-c]}}$, where a = asymptote, b = growth rate, c = inflection point using the non-linear platform in JMP12 (SAS institute, 2015). A multiple linear regression model (method least squares) with two independent variables was fitted to obtain the calorific values for body protein and body fat. The model that describes this relationship is:

$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \epsilon$

Where:

Y = Body energy content, kcal

$\beta_0$ = Intercept

$\beta_1$ = Partial regression coefficient for body protein

$X_1$ = Body protein content, g

$\beta_2$ = Partial regression coefficient for body fat

$X_2$ = Body fat content, g

$\epsilon$ = Error term

(Douglas, 2013)

## RESULTS

### Growth curves

The Gompertz 3P growth curve for body weight and its components (water, protein, fat, minerals, calcium, and phosphorus) fitted against age are presented in Table 2, Figure 1, 2, 3, and 4. For every response variable (BW, water, protein, fat, minerals, calcium, and phosphorus contents) the interpretation of the data is similar. The asymptote means, the adult body weight or body component weight, growth rate is given relative % to the inflection point, which means the rate of growth in %/day, and inflection point is the age at which the growth rate is maximum. Adult BW, water, protein, and fat is 5465, 3676, 1001, and 526 g respectively; and adult mineral, calcium and phosphorus weight is 108, 27, and 21g respectively. The $R^2$ for the model ranged from 0.983 – 0.990, all high values meaning the model is explaining most the variation. The growth rate relative to the inflection point was very similar for all the parameters evaluated in the present experiment. The growth rate for BW, water, protein, and fat was 4.7, 4.8, 4.9 and 5.1% respectively, and 5.1, 4.7, and 4.8% for minerals, calcium and phosphorus, respectively. The age at which the growth rate was the maximum was very similar as well. BW, water, protein and fat has 33.5, 32.3, 34.5, and 35.1 days of age respectively, at which the growth rate was the maximum, and 31.7, 34.4 and 33.5 days of age for minerals, calcium and phosphorus, respectively. The RSEM (root mean square error) which measures how far the data are from the model's predicted values were relatively low (<4.0%) (Table 2).

**Body composition**

The growth curves explained lines above provide information about the rate at which the growth of each body component is growing, however when the data is needed to be expressed as g/kg of BW, more information can be drawn to understand the dynamics of the growth of each part of the body composition. The BW range in the present study was from 56 g (d1) – 4184 g (d60) (Table 3), the coefficient of variance (CV) varied from 6.6 – 9.4% which is considered a uniform flock (Cobb, 2012). The body water content g/kg was the highest at d4, d7, and d12 (741, 746, and 751 g/kg respectively) compared to other ages ($P \leq 0.001$). The water content was not the highest at day 1 (724 g/kg) ($P \leq 0.001$) (Figure 5). The body water was the same at d1, d17, 22 and d27. (724, 739, 724, and 719 g/kg respectively). The lowest body water was from 39 d – 60 d ($P \leq 0.001$). At day 33, the water content was the same as d27 and d39. The CV, % for body water ranged from 0.90 – 3.1%. Body protein as is was the highest at d60 (182 g/kg) but it was not different from d54 (180 g/kg), d47 (180 g/kg), d39 (177 g/kg), d33 (173 g/kg), d27 (172 g/kg) ($P \leq 0.001$). The lowest protein content (as is) was from d4 – d22 (146 g/kg – 155 g/kg) compared to the other ages. Body protein at d1 was higher than d4 –d17, but not different from d22 – d39. The body protein CV, % ranged from 2.6% at d33 to 7.0% at d39 (Table 3). Body fat was the highest at d60 (101 g/kg) ($P \leq 0.001$), not being different from d54 (95 g/kg), d47 (98 g/kg), d39 (100 g/kg), d33 (90 g/kg). The lowest body fat was found at d7 (53 g/kg); however, this was not significant to d1, d4, and d12 ($P \leq 0.001$). The CV for body fat was higher than the CV for protein and water content being the lowest 10.1% at 54d and the highest 35.2% at d7. The body mineral content was the highest at d27 (22.5 g/kg) ($P \leq 0.001$), this value was not significant to d12, d39,

d47, d54 and d60. The lowest mineral content was on d1 (17.9 g/kg); however this value was not different to d4 (18.5 g/kg). The CV for the mineral content ranged from 3.9% at d12 to 8.8% at d39. The rest of the body which was calculated to get to 100% and it is the highest on d1 (38.2 g/kg) ($P \leq 0.001$), followed by d4, d7, d12 and d22; after that the values were not different. The CV was quite variable (CV ranged from 17.4 – 89.5%). An overall comparison is shown in Figure 3, where water takes up the highest component of the body, followed by protein, fat and mineral contents. Once the water content was taken out of the calculation. The total dry matter of the body was the highest on d60 (317 g/kg) compared to other ages but not different from d54, d47, and d39 ($P \leq 0.001$). The lowest DM content was on d 12 (249 g/kg), this values was not different from d4, d7, and d17. The CV of the DM ranged from 3.6 – 6.9 %. The protein content in DM was the highest on d27 (613 g/kg) compared to d22 (563 g/kg) ($P \leq 0.001$) (Figure 6), but it was the same compared to other ages. The CV was lower than 8%. Moving the body fat content in DM, the amount was the highest on d39 (323 g/kg) compared to ages lower and including d27. The lowest amount of fat DM was on d7 (193 g/kg) compared to ages higher and including d12, so the values was not different from d1, and d4 ($P \leq 0.001$). The CV was also higher in fat DM content ranging from 7.2% to 29.2%. Mineral content was the highest on d12 (86.6 g/kg) compared to ages higher d17, except for d27. This value was also different from d1, and d4. The lowest values were found on d1, however not different to d4, and ages higher than d33. The CV for mineral component in DM ranged from 4.9 – 12%. The rest has little meaning since it is probable to be the error in analysis more than the glycogen portion of the body. This values happened to be the highest on d1 and d7 compared to other ages. The CV is quite variable as well as the mineral (AS IS).

Figure 5 depicts the major component of the body in dry matter basis. Protein comprises the highest amount, followed by fat and minerals at the end. Calcium and phosphorus are the major components of the skeleton, so both were analyzed and the values on DM are provided on Table 4. Calcium (DM) happens to be the highest on d12 (18.9 g/kg) compared to other ages but not different than d17, d22 and d27 ($P \leq 0.001$). The lowest amount of calcium was found on d1 (11.7 g/kg) compared to other ages except to d4 (13.3 g/kg) ($P \leq 0.001$). Phosphorus in the body (DM) was the highest on d12 (15.3 g/kg) compared to other ages but not to d7, d17, and d27. The lowest amount was also on d1 (10.3 g/kg) compared to other ages ($P \leq 0.001$). The ratio of Ca: P in the body happened to range from 1.03 – 1.28. The highest being on d60 compared only to d1, d4, d33 and d54. The lowest ratio was found on d4 (1.03) compared to other ages. Calcium and phosphorus in the body were also expressed as % over the mineral content. Calcium g/100 minerals was the lowest on d1 (15.9%) ($P \leq 0.001$) compared to other ages. The highest percentage was on d54 (18.8%) compared only to d33 (17.5%) and d1 ($P \leq 0.001$). The minerals, Ca and P contents are shown also in Figure 7. Calcium and P follow the pattern of the mineral content of the body.

**Energy content in the body**

The gross energy results from the total body (AS IS and DM) are shown in Table 6, as well as the ratio of protein: fat since these two components are the major contributors of the body energy. The body energy as is will be reported first. The highest amount of energy in body was on d60 (1889 kcal/kg) compared to other ages but d54, d47 and d39 ($P \leq 0.001$). The lowest energy was on d7 (1387 kcal/kg) compared to other ages but d4,

d12, and d17. The lowest CV was on d33 (2.7%) and the highest on d7 (11.3%). Moving to the report in dry matter, since the water content is taken out, then energy content kcal/kg is similar between ages. The highest value is found on d60 (6001 kcal/kg), but only compared to d1, d7, and d12 ($P \leq 0.001$). The lowest energy is again found on d7 (5545 kcal/kg) compared to other ages but d1 and d12 ($P \leq 0.001$). The CV of the body energy in dry matter basis is lower than the as is basis ranging from 1.8 – 5.4%. Protein: fat ratio is found to be the highest on d 1 (3.08) compared to other ages but d4, d7 and d12. The lowest ratio are found at the broiler ages from d17; except on d27 where the birds show a higher ratio (2.34) compared to d39 and d60 ($P \leq 0.001$). The ratio however shows higher variability from 8.7 to 34.7%. Figure 8, depicts the body energy (DM) and the protein: fat ratio along the age. As it was been mentioned, protein and fat are the major contributors of the energy in the body of the chickens and these components are usually used to predict the energy values of gain, so one way to obtain these calorific values is by fitting a multiple linear regression line, where the response variable Y = body energy, kcal and the predictors $X_1$ = protein, g, and $X_2$ = fat, g, the slopes of these values can be used to predict the calorific values (Table 7, Figure 9). The $R^2$ (0.99) and the p values of the estimates for protein ($P \leq 0.001$), and fat ($P \leq 0.001$) are high and acceptable for prediction; however since the VIF (variance inflation factor) is 24.2, when the acceptable number is lower than 10, this means protein and fat are highly collinear (r=0.979), so the interpretation of the estimates could result in errors. Therefore, the estimates (or slopes) can be used for prediction only. The calorific values are presented for each phase of feeding. The estimate for protein is the highest (6.03) in the starter (1-14d), and the lowest (4.59) during the finisher (29-42d); however the range during this

period is very high that these may not be significant different that previous phase. During the finisher and withdrawal period, the value gets higher (5.40). The overall coefficient for protein resulted in 5.45 ± 0.09 SE in a range (5.28 – 5.62). The calorific value for fat is highest in the grower phase (10.26) and the lowest in the starter phase (8.35). The overall coefficient is 8.95 ± 0.16 SE in a range (8.64 – 9.26).

## DISCUSION

### Growth curves

Growth is a characteristic of living organisms and the simplest definition would be getting bigger (Lawrence and Fowler. 1998); however this process is very complex. Logistics and Gompertz models are usually used to fit growth over a unit of time, each of them have different assumptions (Winsor, 1932). Gompertz model has assumptions which is that the quantity of growth is proportional to the organism weight, then the effectiveness of the growth decays with time according to first-order kinetics, substrate is no limiting, and growth is irreversible (France, 1996). Gompertz model parameters (asymptote, growth curve, and inflection point) have biological meaning, a starting weight while logistic model starts from zero, and mature body weight to obtain the rate of growth, the age at which maximum growth occurs at around third of the way to maturity which is considered normal. The model needs to reflect that the animal would grow when unconstrained by feed, environment or disease, which is met with Gompertz model, so these are the reasons why Gompertz is a chosen model for growth (Gous et al., 1999). As mentioned, Gompertz 3P produces 3 parameters: asymptote, growth rate and inflection

point (JMP, 2015) which will be discussed for each variable evaluated. The present study used a Gompertz model to explain the growth of broilers in terms of BW, water, protein, fat, minerals, calcium, and phosphorus and showed that the adult weight was reached for 5465 g with 4.7% growth rate and 33.5 days of age as maximum growth. Fitting Cobb, 2012 performance data for males, this adult weight was obtained at 6873 g, more than 1kg compared to the BW found in this study, 4.1% growth rate and 37.9 days for maximum growth. This result may be due to different management conditions, feed type (mash), used in the present study. Winsor (1932) shows that a typical inflection point of a Gompertz curve is reached when 37% of the final growth was been reached which is true for this study, the BW at 33 was 2044 g which is 37% of the maximum adult BW (5465g). Adult BW reached can be variable depending upon on many factors such as genetics, environmental, nutrition. For example Soares (2015) shows adult BW found in males and females of autochthonous breeds of chickens in Portugal (e.g. Amarela breed reached adult BW at 2851 g for males and 1952 g for females). The rate of maturing or the rate of BW growth shares the same potential as the body components (body water, protein, fat, minerals), as it is the case in the present study in which the growth rate of this components (4.8 – 5.1%) are very close to the rate of growth for BW (4.7%), meaning they share the same potential rate of maturing and are said to be allometrically related; therefore they can all be predicted from the weight of one of these components (Gous, 2015 personal communication). Some parts such as feathers may not follow the same growth potential of the parts evaluated on this study; however this is part of future investigation.

**Body composition**

Life cannot be sustained without water. Water makes up about half to two-thirds of the BW (Pond *et al*., 2005) which is exactly what the present study found. The water on the body of broilers ranged from 683 to 751 g/kg being higher at earliest stage of age compared to the later stages of age which was expected. The water however was not the highest on d1 compared. This value (724 g/kg) was different form the water at d4 (741 g/kg), d7 (746 g/kg), and d12 (751 g/kg). This lower water on d1 could be caused by the high yolk residual content in the body at hatch which is reported to be 10-15% of the chick's BW (Ding and Lilburn, 1996). Between 35 – 40% of this yolk are lipids which are the main source of energy after hatch (Noy & Sklan, 1998) or it could also be due to dehydration during transportation which seems unlikely in this experiment because the hatchery these chicks were hatch at are 2 miles away from our facilities and they were transported during early morning. The amount of water is decreased at the end of the growth out, in this case from d39 onwards because the protein and lipid increase taking away part of the water content; however water is still a big component of the body of the broilers, therefore supplying *ad libitum* and good quality of water will help to maintain a normal body composition of broilers. Protein in the body of chicks when is expressed as fresh or as is basis is higher from d27 to the end 60 d. This is because the water component in young birds is higher and taking the space compared to bigger birds that have less water. That's why when body protein is expressed in DM the values are not different between ages; however in the present experiment, the lowest protein in DM was found on d22 compared to d27; this low protein on d22 corresponds to a higher fat composition (when a contrast analysis performed between d22 and d27); if this

performance is real or not needs to be investigated further, because the cause of this behavior is unknown. Body protein is the biggest component when the body is expressed in dry matter basis comprising around 59% of the BW which has been cited in other reports when whole body was evaluated (Olukosi *et al*., 2008). In fact, genetic companies have worked for years to make this valuable part of the broiler being the highest since lean is more desirable to consumers. Lean comprises water and protein which is the edible component for human consumption. The fat or lipid component is the least desirable component as part of the meat; however it takes part of many important functions in the metabolism. Fat expressed as is as part of the whole body component accounts from 53 – 101 g/kg. It is important to understand that this amount includes the viscera's and gastrointestinal tract of the chicks, it is the fat over the whole content of the chick. The fat as expressed in dry matter is about 27%, about half of the protein content which is in accordance with the values reported by other research groups (Olukosi *et al*., 2008). Fat was the lowest during the first week of age which coincides with the highest water content. This is because fat is hydrophobic, so more water in the body will avoid fat to increase. The mineral component of the body is usually analyzed as ash and it comprises Ca, P, Mg, K, Na, Cu, I, Mn, Se, Ni, Cl, Zn between the most important minerals and minerals that are included in the diets. Mineral content in this experiment showed a range of 17.9 – 22.5 g/kg as is; but when expressed as DM the values ranged from about 65.2 – 86.6 g/kg. Ca and P, are the macro minerals with the highest requirement for poultry, so the importance of analyses in the whole body. Ca was 22% of the total mineral and P was 18% of the total body mineral content. Ca and P in the body were the highest close to the end of the starter period (12d), and grower period (27d),

maybe due to higher concentrations of Ca and P in the diets during starter, and grower, compared to finisher and withdrawal. Even thought, Ca and P are the major compounds of the skeletal, both comprise for 40% of the mineral content; so there are still other important minerals in the body of broilers. Sales (2014) analyzed the body mineral composition of whole quails and found that Ca was 20.7 %, P 17. 6% of the mineral content of the body, very similar of the values in chickens in the present study. This author also analyzed other minerals such as Mg (10.9%), K (11.7%), Na (7.5%), Cu (8.5%), Iron (11.8%), Mn (7.6%), Se (0.9%), and Zn (11.5%) showing the importance of minerals in birds. The mineral content was the lowest during early ages (d1, d4), however when expressed as DM, the values tended to be similar; however d12, d7, and d27 showed higher mineral content, the reason is unknown but may some diet related (grower changed on d14 and finisher on d29) because the amount of Ca and P in the diets is higher in the first diets and drops with age. Ca: P relation is 2:1 when diet is formulated; however the relation in the body can be different as found to be 1.03 – 1.28 in this study.

**Energy content in the body**

Protein and fat content are usually used to predict the energy value of the body. For example Okumura (1979) provides the coefficients numbers of 5.66 kcal/g for protein and 9.35 kcal/g for fat. Multiple linear regression can be a tool to obtain the calorific values. In the present study these values were obtained at different points of the feeding strategy (starter, grower, finisher, and withdrawal) but also an overall values are provided. The intercept of the equation is not of much value however, since it was significant for the overall data, it was included in the equation (Figure 9). The values of

the slopes or energetic coefficients for protein and fat varied between phases. The starter had higher calorific, and finisher the lowest maybe suggesting that protein is more important as source of energy for the starter than for the finisher. Withdrawal phase was expected to have the lowest coefficient for protein because of bigger birds contain usually more body fat content; however the coefficient was higher than finisher which matches up the high amount of protein found in birds at d60. The question then arises if the modern broilers are producing more protein at d60 than before? , so the calorific values may have changed. With regards to the fat coefficient, the highest value was not in the withdrawal phase but in the grower phase (15-28d) and the lowest in the starter which was expected. It seems unreasonably that the calorific values for protein and fat changes according to the age of the bird, 1 g of protein and 1g of fat should contain the same calorie values at any point of growth; however, if the fatty acid profile of the chicks varies with age, then the calorific values for fat would be different. The overall coefficients for protein was 5.45 kcal/g and for fat 8.95 kcal/g which are in close agreement with the values from Okumura (1979).

The understanding of the dynamics of body composition in the modern broiler will bring new opportunities to change feed strategies and increase the efficiency for meat while maintaining a healthy broiler.

**Table 1. Composition and nutrient calculations (g/100g as fed) of the diet**

| Ingredient, % | Starter 1-14 d | Grower 15-28 d | Finisher 29-42 d | Withdrawal 43 – 60 d |
|---|---|---|---|---|
| Yellow Corn (8.27% CP) | 52.71 | 57.24 | 57.72 | 60.16 |
| Soybean meal (47.4% CP) | 35.60 | 29.55 | 25.99 | 23.58 |
| Wheat middlings (16.7%CP) | 1.50 | 2.00 | 3.00 | 3.00 |
| Corn DDGS (29.4% CP) | 2.50 | 3.40 | 5.00 | 5.00 |
| Poultry Fat | 3.66 | 4.02 | 4.91 | 4.91 |
| DL-Methionine | 0.33 | 0.29 | 0.25 | 0.23 |
| L-Lysine HCl | 0.32 | 0.31 | 0.26 | 0.26 |
| L-Threonine | 0.15 | 0.14 | 0.13 | 0.11 |
| Calcium Carbonate | 0.95 | 0.94 | 0.91 | 0.93 |
| Dicalcium Phosphate | 1.37 | 1.19 | 0.93 | 0.93 |
| Sodium Chloride | 0.37 | 0.36 | 0.35 | 0.35 |
| Vitamin and mineral premix[1] | 0.54 | 0.54 | 0.54 | 0.54 |
| Propionic acid | 0.05 | 0.05 | 0.05 | 0.05 |
| Phytase[2] | | + | | |
| ***Calculated composition*** | | | | |
| ME, kcal/kg | 3035 | 3,108 | 3,180 | 3202 |
| Crude Protein | 22.9 | 20.7 | 19.6 | 18.6 |
| Calcium[3] | 0.90 | 0.84 | 0.76 | 0.76 |
| Non-phytate phosphorus | 0.45 | 0.42 | 0.38 | 0.38 |
| Digestible lysine | 1.18 | 1.05 | 0.95 | 0.90 |
| Digestible methionine + cysteine | 0.88 | 0.80 | 0.74 | 0.70 |
| Digestible threonine | 0.77 | 0.69 | 0.65 | 0.61 |
| Digestible arginine | 1.24 | 1.10 | 1.03 | 0.97 |
| **Analyzed composition** | | | | |
| AMEn, kcal/kg | 2827 | 2954 | 3174 | 3270 |
| Crude protein | 21.5 | 20.4 | 19.8 | 18.2 |

[1]Supplied 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.

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

[3]Includes contribution from phytase of 0.10% Ca and 0.10% digestible P.

**Table 2. Parameter estimates of the Gompertz 3P curve for BW, water, protein, fat, minerals, calcium, and phosphorus in the body of broiler chickens**

| Y = Response variable | [1]Parameter | Estimate | Standard Error | Lower 95% | Upper 95% | $R^2$ | RMSE |
|---|---|---|---|---|---|---|---|
| Body weight, g | Asymptote | 5465 | 169 | 5133 | 5797 | | |
| | Growth Rate | 0.047 | 0.002 | 0.044 | 0.051 | 0.983 | 138.5 |
| | Inflection Point | 33.5 | 0.82 | 31.9 | 35.1 | | |
| Water wt., g | Asymptote | 3676 | 115 | 3451 | 3901 | | |
| | Growth Rate | 0.048 | 0.002 | 0.044 | 0.052 | 0.987 | 109.9 |
| | Inflection Point | 32.3 | 0.82 | 30.7 | 34.0 | | |
| Protein, g | Asymptote | 1001 | 32 | 939 | 1064 | | |
| | Growth Rate | 0.049 | 0.002 | 0.045 | 0.053 | 0.989 | 27.1 |
| | Inflection Point | 34.5 | 0.82 | 32.9 | 36.1 | | |
| Fat, g | Asymptote | 526 | 30 | 468 | 585 | | |
| | Growth Rate | 0.051 | 0.004 | 0.043 | 0.058 | 0.966 | 25.0 |
| | Inflection Point | 35.1 | 1.42 | 32.3 | 37.9 | | |
| Minerals, g | Asymptote | 108 | 3 | 102 | 113 | | |
| | Growth Rate | 0.051 | 0.002 | 0.048 | 0.055 | 0.990 | 2.8 |
| | Inflection Point | 31.7 | 0.65 | 30.4 | 33.0 | | |
| Calcium, g | Asymptote | 27 | 1.1 | 25 | 29 | | |
| | Growth Rate | 0.047 | 0.002 | 0.042 | 0.052 | 0.983 | 0.9 |
| | Inflection Point | 34.4 | 1.10 | 32.3 | 36.6 | | |
| Phosphorus, g | Asymptote | 21 | 0.7 | 20 | 22 | | |
| | Growth Rate | 0.048 | 0.002 | 0.044 | 0.053 | 0.987 | 0.6 |
| | Inflection Point | 33.5 | 0.88 | 31.7 | 35.2 | | |

Gompertz 3P model: $a*e^{[-e^{[-b*[Age-c]}}$, where a = asymptote, b = growth rate, c = inflection point.

[1]Asymptote, adult body or body composition weight. Growth rate, it is relative at the inflection point, when multiplied by 100, it's the percentage of growth per unit of time (day). Inflection point, age (d) when the growth rate is maximum.

RMSE (root mean square error) means how far the data are from the model's predicted values.

**Table 3. Body weight and body composition of broilers 1-60d (AS IS basis)**

| | | | | | Body composition, AS IS | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BW, g | | | Water g/kg | | | Protein g/kg | | | Fat g/kg | | | Minerals g/kg | | | Rest g/kg | | |
| Age | N | Mean | [1]SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % |
| 1 | 10 | 56 | 3 | 5.4 | 724$^{bc}$ | 15.9 | 2.2 | 166$^{bc}$ | 8.6 | 5.2 | 55$^{ef}$ | 8.8 | 16.0 | 17.9$^{d}$ | 0.9 | 4.9 | 38.2$^{a}$ | 11.0 | 28.8 |
| 4 | 16 | 98 | 7 | 7.1 | 741$^{a}$ | 17.8 | 2.4 | 154$^{d}$ | 7.3 | 4.8 | 62$^{def}$ | 13.7 | 22.2 | 18.5$^{cd}$ | 1.2 | 6.6 | 24.9$^{b}$ | 4.3 | 17.4 |
| 7 | 9 | 154 | 14 | 9.1 | 746$^{a}$ | 15.2 | 2.0 | 154$^{d}$ | 7.5 | 4.9 | 53$^{f}$ | 18.8 | 35.2 | 20.1$^{bc}$ | 1.4 | 6.8 | 26.8$^{b}$ | 5.5 | 20.7 |
| 12 | 10 | 388 | 34 | 8.8 | 751$^{a}$ | 11.3 | 1.5 | 146$^{d}$ | 5.3 | 3.6 | 61$^{def}$ | 10.9 | 18.0 | 21.6$^{ab}$ | 0.8 | 3.9 | 21.2$^{bc}$ | 6.3 | 29.8 |
| 17 | 16 | 571 | 49 | 8.5 | 739$^{ab}$ | 6.6 | 0.9 | 154$^{d}$ | 10.3 | 6.7 | 71$^{cde}$ | 7.8 | 11.1 | 20.6$^{b}$ | 1.4 | 6.6 | 15.3$^{cd}$ | 9.9 | 64.6 |
| 22 | 15 | 985 | 64 | 6.5 | 724$^{bc}$ | 9.3 | 1.3 | 155$^{cd}$ | 6.1 | 4.0 | 82$^{bc}$ | 11.6 | 14.1 | 20.7$^{b}$ | 1.3 | 6.2 | 18.3$^{bcd}$ | 5.1 | 27.6 |
| 27 | 15 | 1402 | 74 | 5.3 | 719$^{cd}$ | 8.8 | 1.2 | 172$^{ab}$ | 10.9 | 6.3 | 75$^{cd}$ | 9.1 | 12.2 | 22.5$^{a}$ | 1.1 | 5.0 | 11.5$^{d}$ | 8.4 | 72.8 |
| 33 | 15 | 2044 | 155 | 7.6 | 707$^{de}$ | 6.7 | 1.0 | 173$^{ab}$ | 4.4 | 2.6 | 90$^{ab}$ | 13.0 | 14.5 | 20.8$^{b}$ | 1.4 | 6.6 | 10.3$^{d}$ | 9.2 | 89.5 |
| 39 | 10 | 2560 | 240 | 9.4 | 692$^{ef}$ | 21.1 | 3.1 | 177$^{ab}$ | 12.4 | 7.0 | 100$^{a}$ | 11.1 | 11.2 | 21.1$^{ab}$ | 1.9 | 8.8 | 10.8$^{cd}$ | 4.4 | 40.9 |
| 47 | 11 | 3094 | 229 | 7.4 | 686$^{f}$ | 14.8 | 2.2 | 180$^{a}$ | 8.3 | 4.6 | 98$^{a}$ | 12.6 | 12.8 | 21.8$^{ab}$ | 1.0 | 4.5 | 14.0$^{cd}$ | 5.3 | 38.1 |
| 54 | 12 | 3770 | 156 | 4.2 | 687$^{f}$ | 10.7 | 1.6 | 180$^{a}$ | 5.2 | 2.9 | 95$^{ab}$ | 9.6 | 10.1 | 20.9$^{ab}$ | 0.9 | 4.3 | 17.3$^{bcd}$ | 6.2 | 35.7 |
| 60 | 12 | 4184 | 328 | 7.8 | 683$^{f}$ | 15.6 | 2.3 | 182$^{a}$ | 8.7 | 4.8 | 101$^{a}$ | 15.1 | 15.0 | 21.0$^{ab}$ | 1.5 | 7.3 | 13.4$^{cd}$ | 3.4 | 25.3 |
| *[2]SEM* | | | | | *3.72* | | | *2.34* | | | *3.41* | | | *0.36* | | | *2.01* | | |
| *P-value* | | | | | *<0.001* | | | *<0.001* | | | *<0.001* | | | *<0.001* | | | *<0.001* | | |

Levels (a, b, c, d, e, f) not connected by same letter are significantly different
[1] SD, standard deviation
[2]SEM, standard error mean (pooled harmonic mean)

**Table 4. Body composition of broilers 1-60d (Dry matter basis)**

| | | | | | | | | Body Composition, dry matter | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BW, g (DM) | | | Dry matter g/kg | | | Protein g/kg | | | Fat g/kg | | | Minerals g/kg | | | Rest g/kg | | |
| Age | N | Mean | [1]SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % |
| 1 | 10 | 15 | 0.8 | 5.4 | $277^{cde}$ | 15.9 | 5.7 | $600^{ab}$ | 32.3 | 5.4 | $197^{f}$ | 22.5 | 11.4 | $65.2^{d}$ | 3.4 | 5.2 | $137.8^{a}$ | 38.9 | 28.3 |
| 4 | 16 | 25 | 1.8 | 7.1 | $259^{f}$ | 17.8 | 6.9 | $596^{ab}$ | 30.8 | 5.2 | $229^{ef}$ | 38.5 | 16.3 | $71.9^{cd}$ | 6.7 | 9.3 | $96.0^{bc}$ | 14.7 | 15.3 |
| 7 | 9 | 39 | 3.6 | 9.1 | $254^{f}$ | 15.2 | 6.0 | $607^{ab}$ | 48.3 | 8.0 | $193^{f}$ | 60.6 | 29.2 | $79.9^{ab}$ | 9.6 | 12.0 | $105.8^{ab}$ | 23.6 | 22.3 |
| 12 | 10 | 97 | 8.5 | 8.8 | $249^{f}$ | 11.2 | 4.5 | $587^{ab}$ | 27.2 | 4.6 | $242^{de}$ | 34.1 | 14.1 | $86.6^{a}$ | 7.1 | 8.2 | $85.6^{bcd}$ | 25.7 | 30.1 |
| 17 | 16 | 149 | 12.8 | 8.5 | $261^{ef}$ | 6.6 | 2.5 | $591^{ab}$ | 36.8 | 6.2 | $271^{bcd}$ | 24.3 | 9.0 | $79.1^{b}$ | 5.6 | 7.1 | $60.4^{def}$ | 38.8 | 65.7 |
| 22 | 15 | 272 | 17.7 | 6.5 | $276^{de}$ | 9.3 | 3.4 | $563^{b}$ | 35.3 | 6.3 | $296^{abc}$ | 32.1 | 10.9 | $75.2^{bc}$ | 6.1 | 8.1 | $66.1^{cde}$ | 17.3 | 26.2 |
| 27 | 15 | 394 | 20.7 | 5.3 | $281^{cd}$ | 8.8 | 3.1 | $613^{a}$ | 39.5 | 6.4 | $265^{cd}$ | 25.8 | 9.8 | $80.3^{ab}$ | 3.9 | 4.9 | $41.1^{ef}$ | 30.4 | 74.0 |
| 33 | 15 | 599 | 45.3 | 7.6 | $293^{bc}$ | 6.7 | 2.3 | $589^{ab}$ | 21.6 | 3.7 | $297^{abc}$ | 37.3 | 12.2 | $70.9^{cd}$ | 4.6 | 6.5 | $35.0^{f}$ | 31.1 | 88.8 |
| 39 | 10 | 788 | 73.7 | 9.4 | $308^{ab}$ | 21.1 | 6.9 | $573^{ab}$ | 20.4 | 3.6 | $323^{a}$ | 23.3 | 7.2 | $68.5^{cd}$ | 4.1 | 6.1 | $35.5^{ef}$ | 14.0 | 39.3 |
| 47 | 11 | 970 | 71.8 | 7.4 | $314^{a}$ | 14.8 | 4.7 | $574^{ab}$ | 25.9 | 4.5 | $312^{a}$ | 28.1 | 9.0 | $69.7^{cd}$ | 4.2 | 6.1 | $44.6^{ef}$ | 17.9 | 40.1 |
| 54 | 12 | 1181 | 49.0 | 4.2 | $313^{a}$ | 10.7 | 3.4 | $575^{ab}$ | 22.4 | 3.9 | $304^{ab}$ | 23.1 | 7.6 | $66.8^{d}$ | 3.6 | 5.4 | $54.4^{def}$ | 18.7 | 34.4 |
| 60 | 12 | 1326 | 104.0 | 7.8 | $317^{a}$ | 15.6 | 4.9 | $574^{ab}$ | 32.5 | 5.7 | $317^{a}$ | 36.9 | 11.6 | $66.4^{d}$ | 3.7 | 5.6 | $42.1^{ef}$ | 10.1 | 24.0 |
| *[1]SEM* | | | | | *3.86* | | | *9.09* | | | *8.2* | | | *1.54* | | | *7.18* | | |
| *P* | | | | | *<0.001* | | | *<0.001* | | | *<0.001* | | | *<0.001* | | | *<0.001* | | |

Levels (a, b, c, d, e, f) not connected by same letter are significantly different
[1]SD, standard deviation
[2]SEM, standard error mean (pooled harmonic mean)

**Table 5. Mineral composition of broilers 1-60d (Dry matter basis)**

| | Minerals, g/kg | | | Calcium g/kg | | | Phosphorus g/kg | | | Ca: P | | | Ca, g/100 minerals | | | P, g/100 minerals | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | **Mean** | **[1]SD** | **CV, %** | **Mean** | **SD** | **CV, %** | **Mean** | **SD** | **CV, %** | **Mean** | **SD** | **CV, %** | **Mean** | **SD** | **CV, %** | **Mean** | **SD** | **CV, %** |
| 1 | 65.2$^{d}$ | 3.4 | 5.2 | 11.7$^{f}$ | 0.84 | 7.2 | 10.3$^{e}$ | 0.70 | 6.7 | 1.13$^{cd}$ | 0.08 | 7.2 | 17.9$^{e}$ | 1.01 | 5.7 | 15.9$^{c}$ | 0.87 | 5.5 |
| 4 | 71.9$^{cd}$ | 6.7 | 9.3 | 13.3$^{ef}$ | 1.62 | 12.2 | 12.9$^{cd}$ | 0.93 | 7.2 | 1.03$^{e}$ | 0.06 | 5.9 | 18.5$^{de}$ | 1.22 | 6.6 | 18.0$^{ab}$ | 0.80 | 4.5 |
| 7 | 79.9$^{ab}$ | 9.6 | 12.0 | 16.2$^{bcd}$ | 2.30 | 14.2 | 14.5$^{ab}$ | 1.41 | 9.8 | 1.12$^{d}$ | 0.06 | 5.0 | 20.2$^{cd}$ | 1.01 | 5.0 | 18.1$^{ab}$ | 0.53 | 2.9 |
| 12 | 86.6$^{a}$ | 7.1 | 8.2 | 18.9$^{a}$ | 1.63 | 8.6 | 15.3$^{a}$ | 0.82 | 5.3 | 1.23$^{ab}$ | 0.05 | 3.9 | 22.2$^{abc}$ | 1.27 | 5.7 | 18.0$^{ab}$ | 0.75 | 4.2 |
| 17 | 79.1$^{b}$ | 5.6 | 7.1 | 17.4$^{abc}$ | 1.73 | 10.0 | 14.2$^{ab}$ | 0.91 | 6.4 | 1.22$^{ab}$ | 0.05 | 4.3 | 22.1$^{abc}$ | 1.44 | 6.5 | 18.1$^{ab}$ | 0.74 | 4.1 |
| 22 | 75.2$^{bc}$ | 6.1 | 8.1 | 16.9$^{abc}$ | 1.52 | 9.0 | 13.7$^{bc}$ | 0.91 | 6.7 | 1.23$^{ab}$ | 0.04 | 3.4 | 22.5$^{a}$ | 1.47 | 6.5 | 18.3$^{ab}$ | 0.86 | 4.7 |
| 27 | 80.3$^{ab}$ | 3.9 | 4.9 | 17.9$^{ab}$ | 1.14 | 6.3 | 14.2$^{ab}$ | 0.69 | 4.8 | 1.26$^{a}$ | 0.04 | 2.8 | 22.3$^{ab}$ | 1.05 | 4.7 | 17.7$^{ab}$ | 0.61 | 3.5 |
| 33 | 70.9$^{cd}$ | 4.6 | 6.5 | 14.7$^{de}$ | 1.06 | 7.2 | 12.4$^{d}$ | 0.70 | 5.7 | 1.19$^{bc}$ | 0.03 | 2.4 | 20.7$^{bc}$ | 1.01 | 4.9 | 17.5$^{b}$ | 0.82 | 4.7 |
| 39 | 68.5$^{cd}$ | 4.1 | 6.1 | 15.8$^{cd}$ | 1.36 | 8.6 | 12.7$^{cd}$ | 0.75 | 5.9 | 1.24$^{ab}$ | 0.05 | 3.8 | 22.9$^{ab}$ | 1.86 | 8.1 | 18.5$^{ab}$ | 0.98 | 5.3 |
| 47 | 69.7$^{cd}$ | 4.2 | 6.1 | 16.1$^{bcd}$ | 2.11 | 13.1 | 12.6$^{cd}$ | 1.56 | 12.3 | 1.27$^{a}$ | 0.04 | 3.2 | 23.1$^{a}$ | 2.22 | 9.6 | 18.1$^{ab}$ | 1.62 | 8.9 |
| 54 | 66.8$^{d}$ | 3.6 | 5.4 | 15.6$^{cd}$ | 1.34 | 8.6 | 12.5$^{cd}$ | 0.93 | 7.5 | 1.24$^{b}$ | 0.04 | 3.0 | 23.3$^{a}$ | 1.55 | 6.7 | 18.8$^{a}$ | 1.06 | 5.7 |
| 60 | 66.4$^{d}$ | 3.7 | 5.6 | 15.5$^{cd}$ | 1.08 | 7.0 | 12.2$^{d}$ | 0.69 | 5.7 | 1.28$^{a}$ | 0.05 | 3.6 | 23.4$^{a}$ | 1.00 | 4.3 | 18.4$^{ab}$ | 0.75 | 4.1 |
| *[2]SEM* | *1.54* | | | *0.431* | | | *0.267* | | | *0.014* | | | *0.393* | | | *0.254* | | |
| *P - value* | *<0.001* | | | *<0.001* | | | *<0.001* | | | *<0.001* | | | *<0.001* | | | *<0.001* | | |

Levels (a, b, c, d, e, f) not connected by same letter are significantly different

[1] SD, standard deviation

[2]SEM, standard error mean (pooled harmonic mean)

**Table 6. Energy and protein: fat ratio in the body of broilers (1-60d)**

| Age, d | Body Energy, kcal/kg (AS IS) | | | Body Energy, kcal/kg (DM) | | | Protein: Fat | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | [1]SD | CV, % | Mean | SD | CV, % | Mean | SD | CV, % |
| 1 | 1564[def] | 113 | 7.3 | 5545[bcd] | 117 | 2.1 | 3.08[a] | 0.39 | 12.7 |
| 4 | 1503[efg] | 141 | 9.4 | 5799[abc] | 204 | 3.5 | 2.59[ab] | 0.50 | 19.3 |
| 7 | 1387[g] | 156 | 11.3 | 5545[bcd] | 300 | 5.4 | 3.05[a] | 1.06 | 34.7 |
| 12 | 1398[g] | 138 | 9.7 | 5704[cd] | 258 | 4.5 | 2.58[ab] | 0.56 | 21.7 |
| 17 | 1495[fg] | 106 | 7.1 | 5796[abc] | 163 | 2.8 | 2.21[bcd] | 0.27 | 12.2 |
| 22 | 1626[cde] | 94 | 5.8 | 5883[ab] | 163 | 2.8 | 1.93[cd] | 0.31 | 16.1 |
| 27 | 1633[cd] | 87 | 5.3 | 5805[abc] | 151 | 2.6 | 2.34[bc] | 0.34 | 14.5 |
| 33 | 1734[bc] | 46 | 2.7 | 5932[a] | 88 | 1.5 | 1.95[cd] | 0.17 | 8.7 |
| 39 | 1809[ab] | 116 | 6.4 | 5965[a] | 106 | 1.8 | 1.78[d] | 0.18 | 10.1 |
| 47 | 1851[ab] | 111 | 6.0 | 5898[ab] | 136 | 2.3 | 1.88[cd] | 0.22 | 11.7 |
| 54 | 1848[a] | 93 | 5.0 | 5914[a] | 150 | 2.5 | 1.91[cd] | 0.21 | 11.0 |
| 60 | 1889[a] | 129 | 6.8 | 6001[a] | 188 | 3.1 | 1.84[d] | 0.33 | 17.9 |
| [2]*SEM* | *30.8* | | | *47.8* | | | *0.103* | | |
| *P - value* | *<0.001* | | | *<0.001* | | | *<0.001* | | |

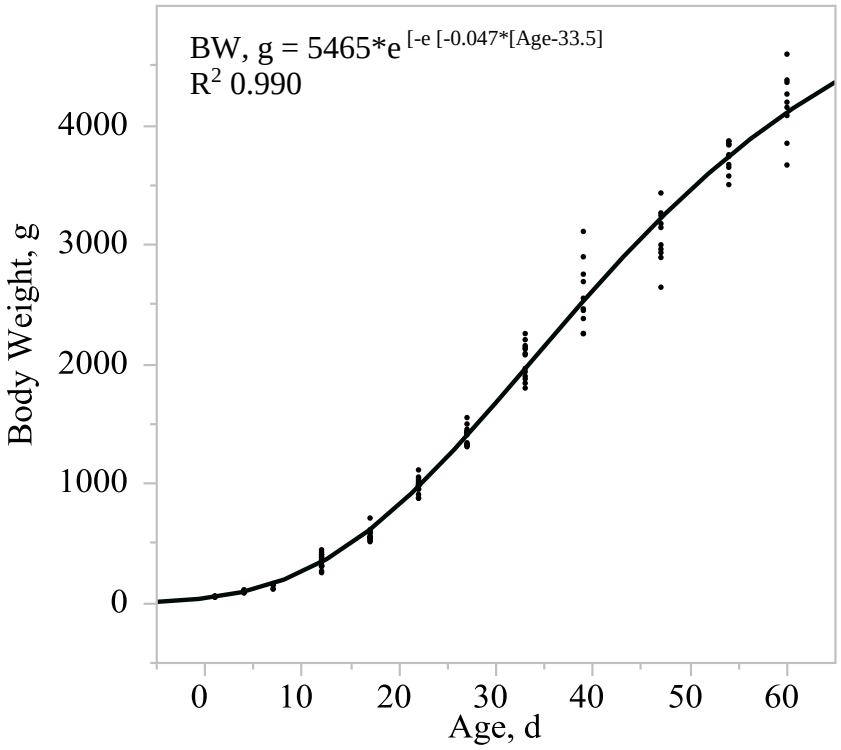
Levels (a, b, c, d, e, f, g) not connected by same letter are significantly different
[1]SD, standard deviation
[2]SEM, standard error mean (pooled harmonic mean)

**Table 7. Multiple regression analysis for Y= Body energy, kcal, X1= protein, g, X2 = fat, g (1-60d)**

| | | Starter (1-14d) | Grower (15-28d) | Finisher (29-42d) | Withdrawal (43- 60 d) | Overall data |
|---|---|---|---|---|---|---|
| Summary of Fit | N | 52 | 46 | 22 | 31 | 151 |
| | $R^2$ | 0.998 | 0.997 | 0.997 | 0.995 | 1.000 |
| | RMSE | 8.9 | 36.3 | 39.6 | 91.0 | 53.8 |
| Intercept | Estimate | 5.97 | 18.72 | 385.29 | 11.15 | 22.75 |
| | Std. Error | 2.26 | 14.74 | 66.96 | 110.08 | 6.35 |
| | Lower 95% | 1.44 | -11.01 | 245.14 | -214.34 | 10.20 |
| | Upper 95% | 10.51 | 48.45 | 525.43 | 236.65 | 35.31 |
| | P-value | 0.011 | 0.211 | <.0001 | 0.920 | 0.001 |
| Protein, g | Estimate | **6.03** | **4.94** | **4.59** | **5.40** | **5.45** |
| | Std. Error | 0.19 | 0.19 | 0.33 | 0.26 | 0.09 |
| | Lower 95% | 5.64 | 4.56 | 3.90 | 4.88 | 5.28 |
| | Upper 95% | 6.41 | 5.31 | 5.27 | 5.92 | 5.62 |
| | P-value | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Fat, g | Estimate | **8.35** | **10.26** | **8.78** | **9.09** | **8.95** |
| | Std. Error | 0.41 | 0.41 | 0.39 | 0.31 | 0.16 |
| | Lower 95% | 7.52 | 9.43 | 7.96 | 8.46 | 8.64 |
| | Upper 95% | 9.19 | 11.09 | 9.59 | 9.72 | 9.26 |
| | P-value | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |

VIF (variance inflation factor) = 24.2. Since this value is higher than 10 which is the maximum acceptable. The estimates for protein and fat are valid for prediction of body energy but not for interpretation.

**Figure 1. Gompertz growth curves for body weight and water content in broilers body 1-60d**



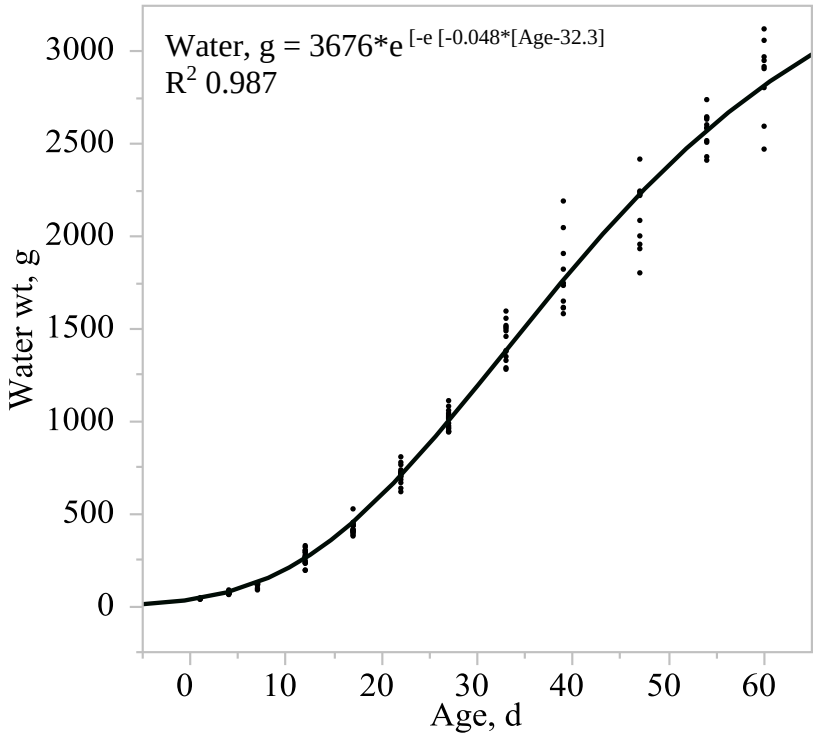



**Figure 2. Gompertz growth curves for protein and fat content in broilers body 1-60d**

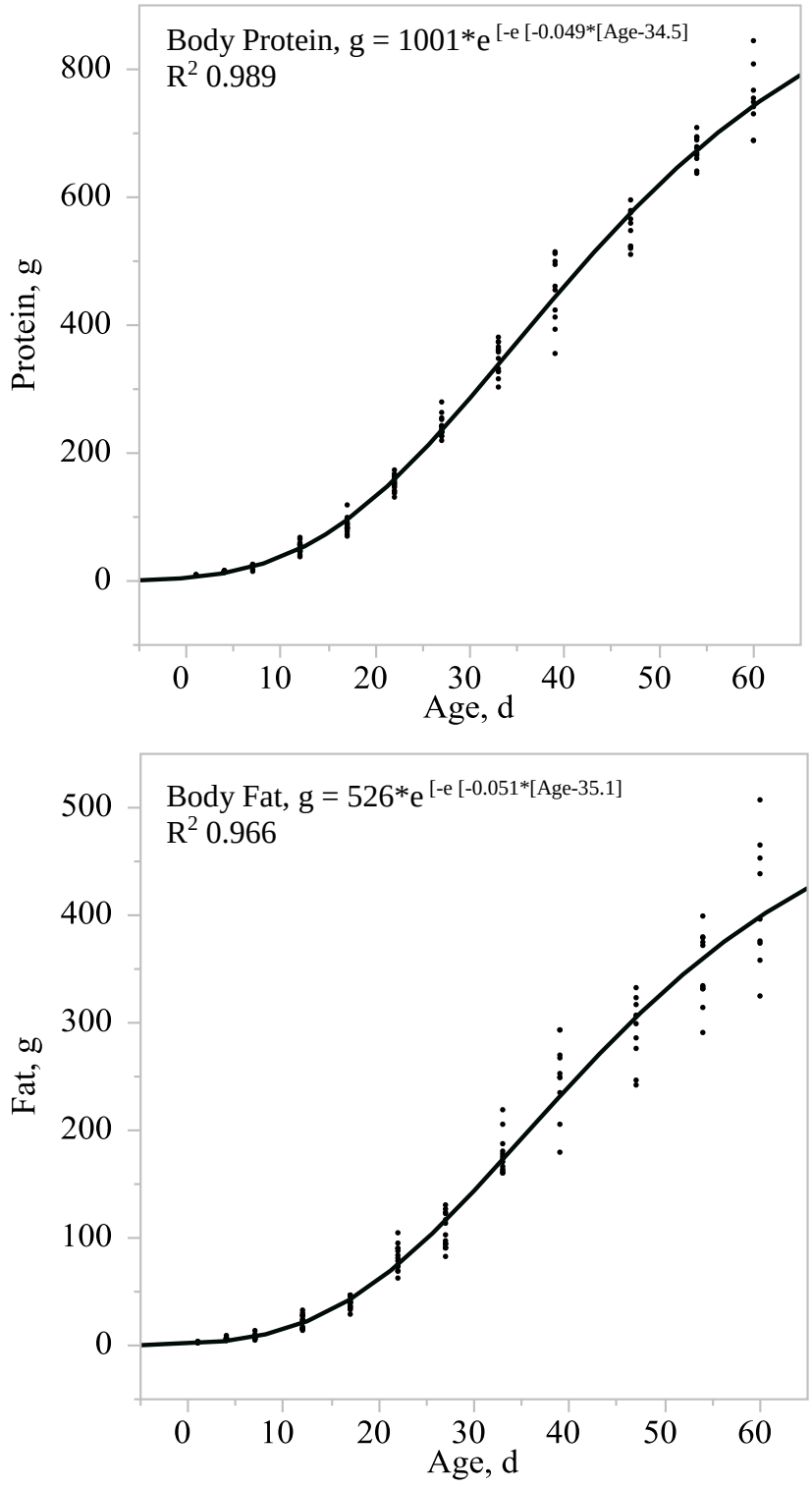


**Figure 3. Gompertz growth curves for mineral content in broilers body 1-60d**

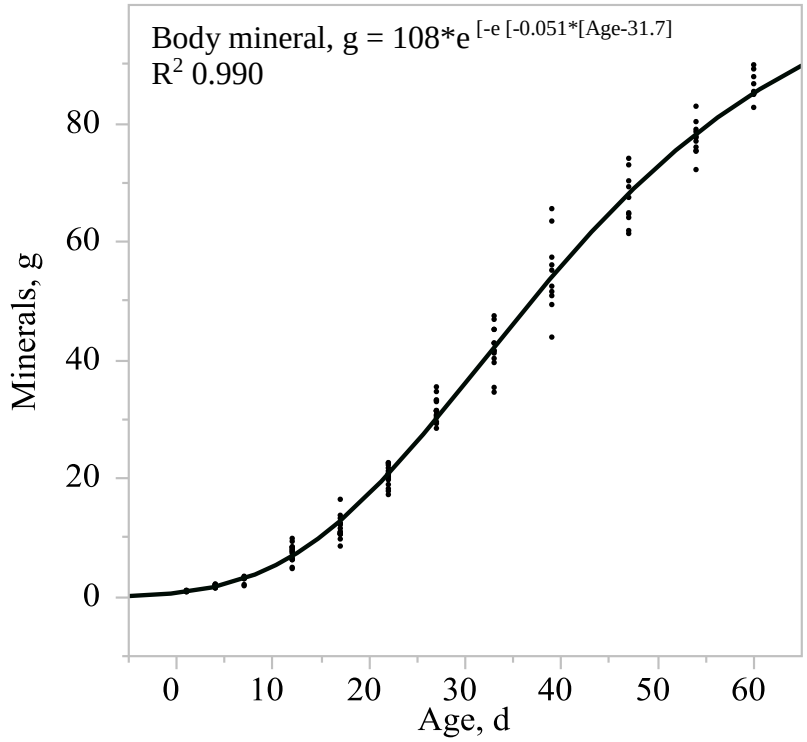
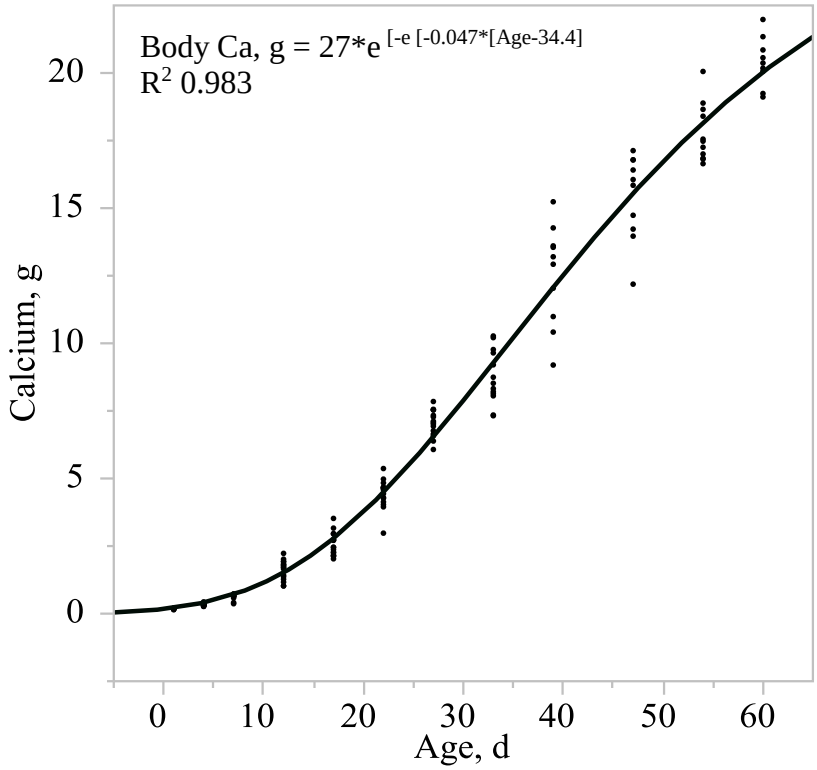


**Figure 4. Gompertz growth curves for calcium and phosphorus content in broilers body 1-60d**



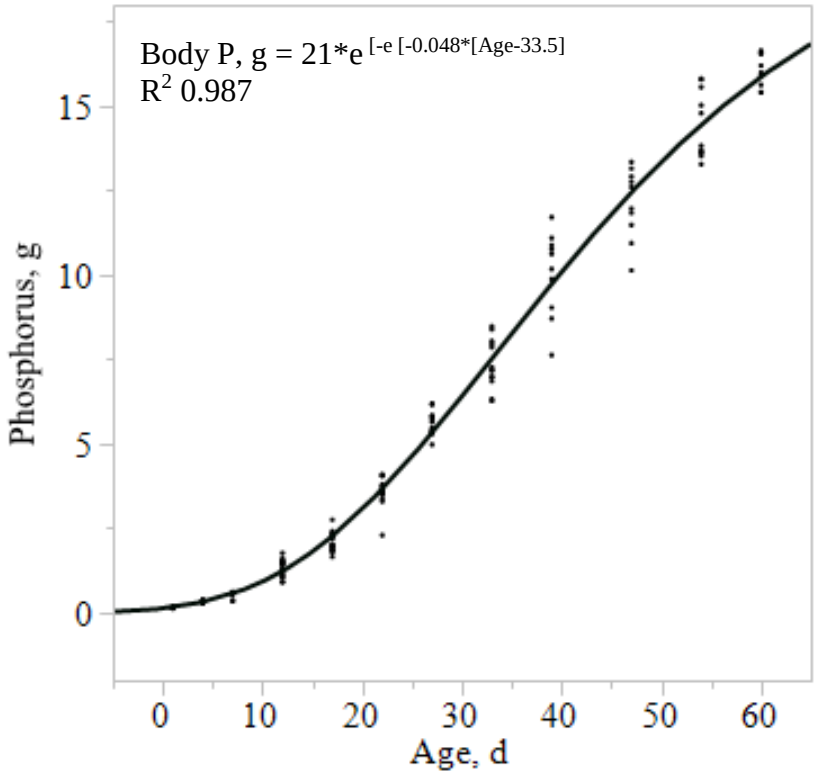
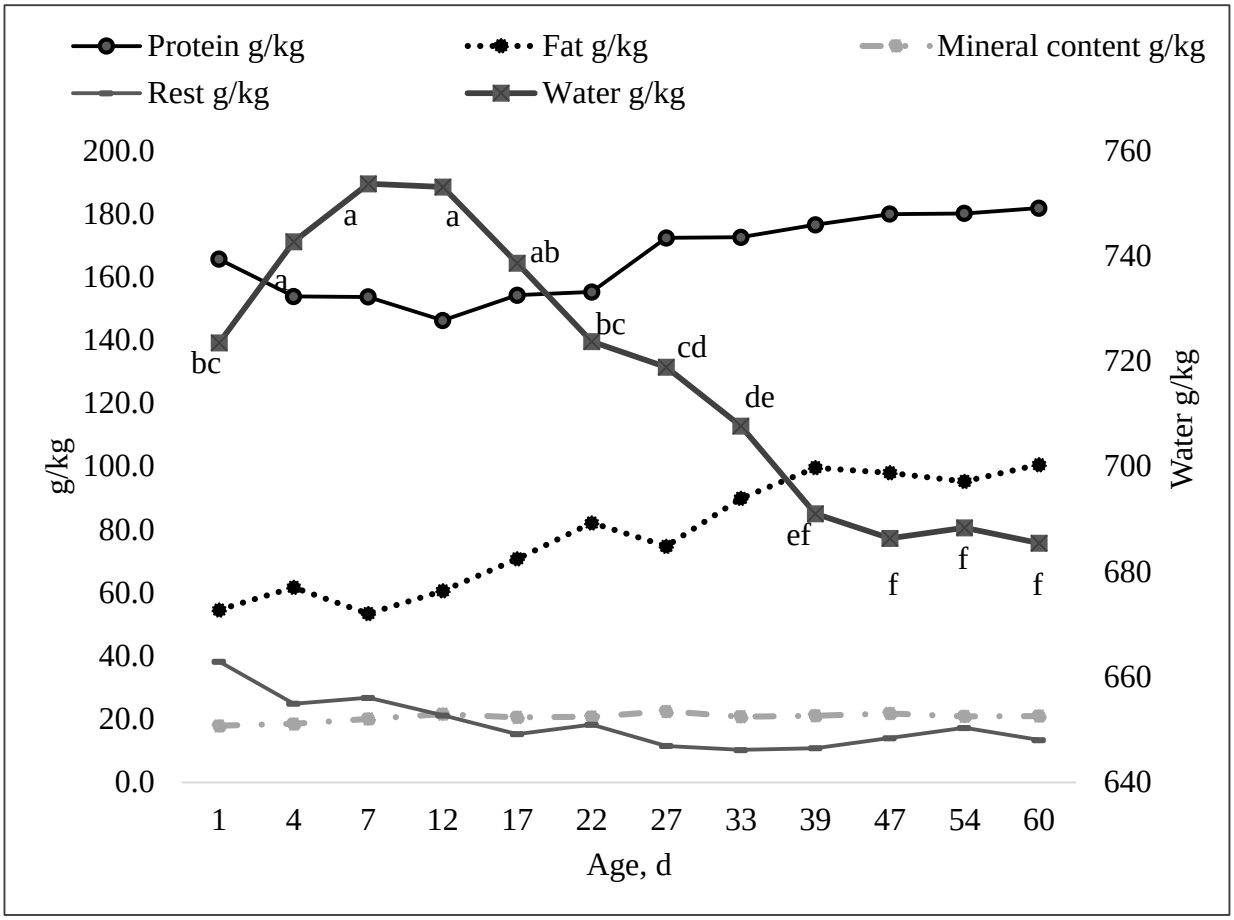
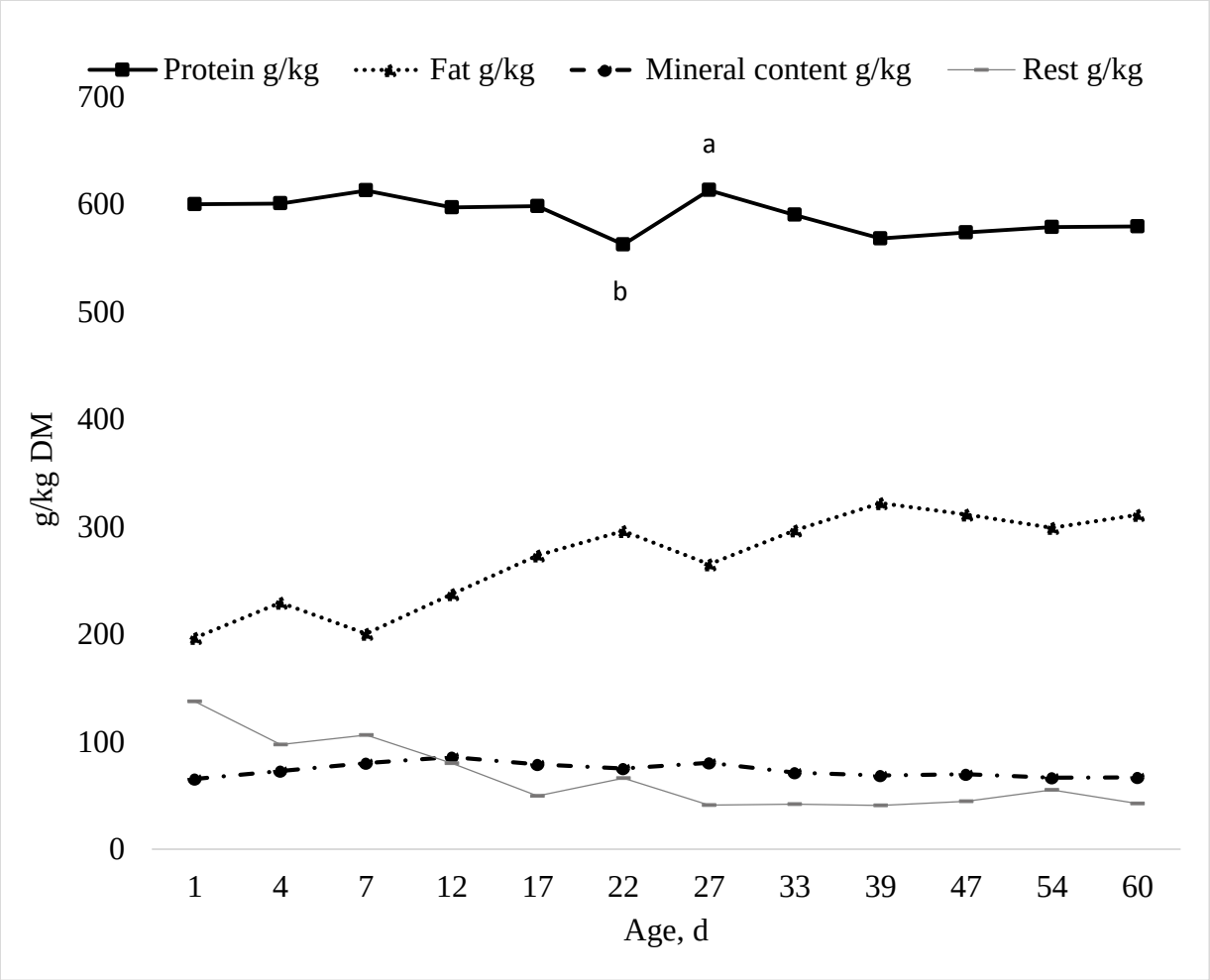



**Figure 5. Body composition in broilers (1-60d) in AS IS basis**




Levels (a, b, c, d, e, f) not connected by same letter are significantly different at $P<0.05$

**Figure 6. Body composition in broilers (1-60d) in Dry matter basis**




Levels (a, b) not connected by same letter are significantly different at $P<0.05$

**Figure 7. Total mineral, calcium and phosphorus contents (dry matter) in the body of broilers (1-60d)**

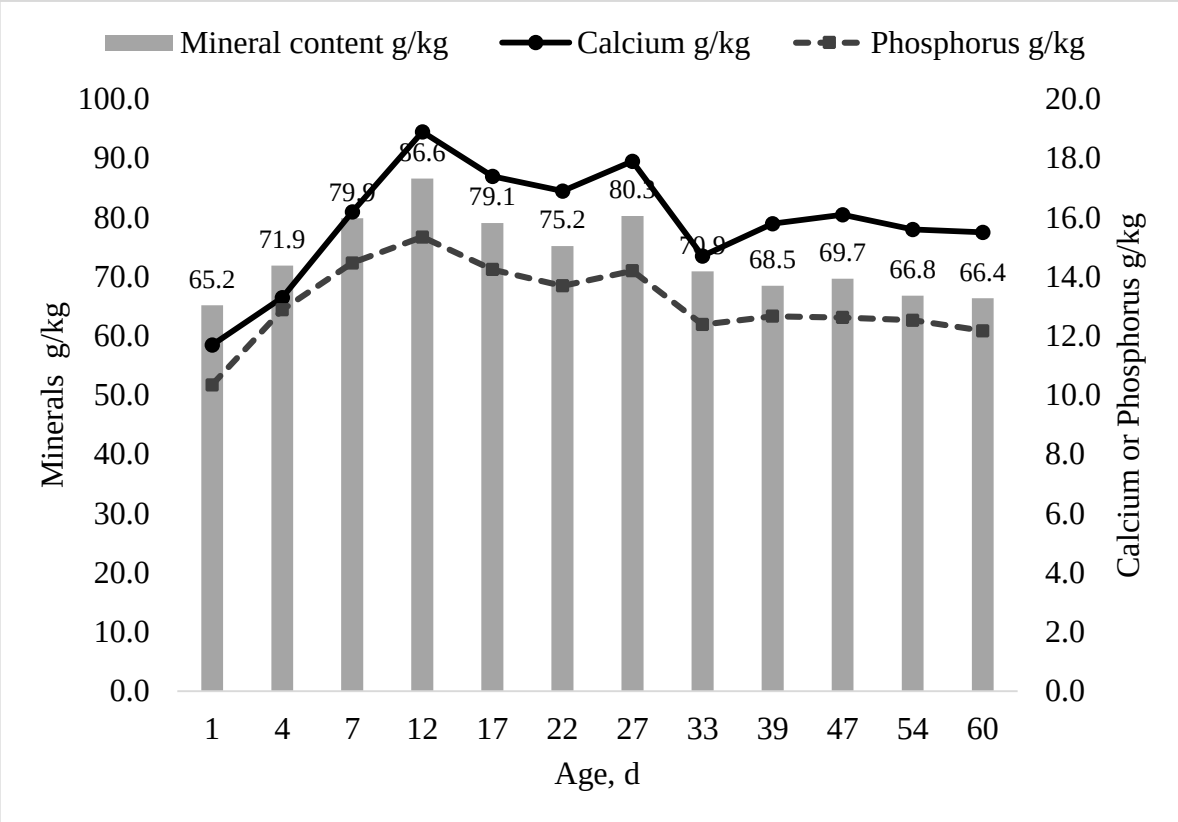


**Figure 8. Energy content and Protein: Fat ratio in the body of broilers (1-60d)**

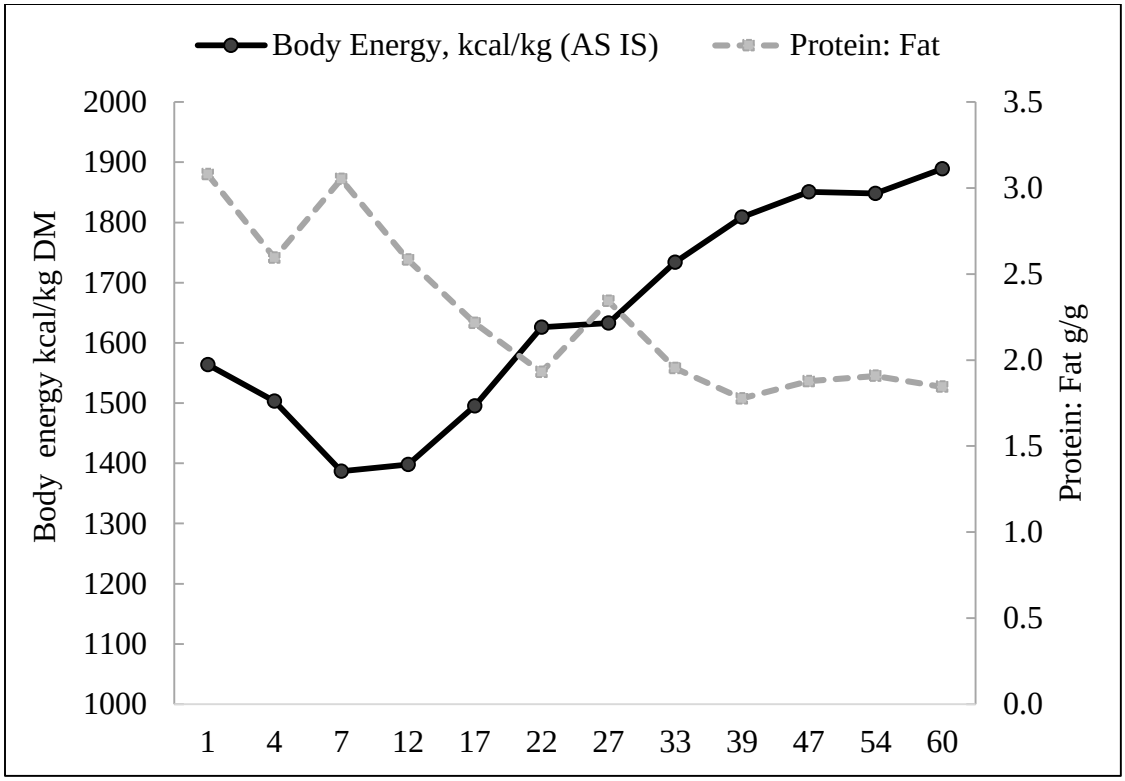
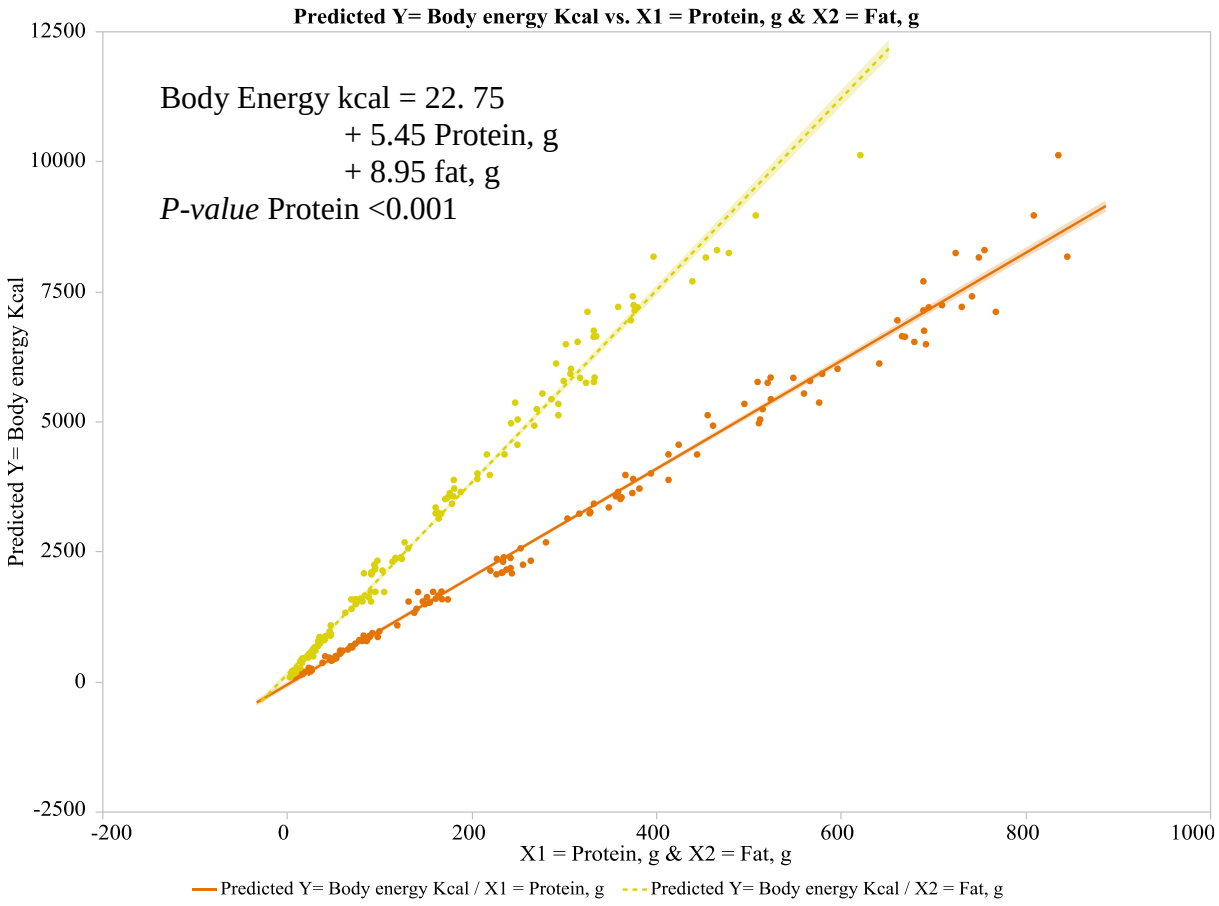


**Figure 9. Linear relationships of body energy (kcal) with protein and fat composition (g)**



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# III. VALIDATION OF DUAL X-RAY ENERGY ABSORPTIOMETRY (DEXA) IN FED RESTRICTED AND FED AD LIBITUM BROILERS

**ABSTRACT**

The purpose of the present research was to validate Dual Energy X-Ray absorptiometry (DEXA – Lunar Prodigy equipped with small animal software) for measuring body composition of fed restricted and fed ad libitum broilers. Four experiments were conducted using Cobb male broilers, 81 fed restricted chicks at 28 d of age (BW range 600 to 1300 g) and 160 fed ad libitum broilers from 1 – 60 d of age (BW range 51 – 4690 g). In experiment 1, precision was tested by scanning 21 birds 4 repeated times. The precision was highest for total body and lean mass followed by BMD (bone mineral density) and BMC (bone mineral content) (CV<2.5%) and was the lowest for fat mass (CV 4.5%). In experiment 2, positions lateral, dorsal and ventral recumbency were compared followed by correlations between DEXA and chemical analysis. Position affected mineral DEXA parameters in broiler fed 85%, and mineral, fat and lean tissue in chicks fed 55 and 35%. Ventral position showed better correlation for fat and lower variability between DEXA scans. In experiment 3, linear and non-linear equations were developed for fed restricted chickens by fitting DEXA body component by chemical analysis of the same chicken. After DEXA scan, broilers were autoclaved at 121 ºC, 22 psi, for 2 h. The carcasses were blended individually for 30 sec. The homogenate was lyophilized, grounded and analyzed for dry matter, crude protein, fat, and minerals (ash). The $R^2$ for the equations to predict total mass, dry matter, lean, protein, fat, and BMC were 0.99, 0.99, 0.99, 0.99, 0.75, and 0.98. All equations showed statistical significance ($P$<0.01). The validation of the equations showed good agreement of predicted vs analyzed by chemical analysis values. Experiment 4, equations were developed for fed *ad libitum* broilers from 1 – 60 d. Non-linear equations were developed for most of the body components, but linear equation for body energy was achieved. All equations showed high $R^2$ (>0.96) and significant parameters (intercept and independent variables) ($P$<0.01). Validation

of the equations in experiment 4 showed good agreement and soft tissue between predicted and analyzed values for soft tissue (lean, protein, fat tissue) and total BMC. It is concluded that after proper methodological standardization of positioning, and application of specifically determined regression equations DEXA can be used for estimating the body composition of fed restricted and fed *ad libitum* broilers.

Key words: DEXA, Body composition, broilers, feeding level

## INTRODUCTION

Meat production is a demanding global activity, and chicken meat is one of the most efficient meat that has evolved speedily over the past 50 years (Vieira, 2009). Consumer demands leaner meat and devalues fat composition. Therefore; an accurate and fast methodology is needed to assess body composition to support nutrition research. Chemical analysis is a cumbersome methodology, so other alternatives techniques are needed to be studied. Laskey and Phil (1995) describe dual energy x-ray absorptiometry (DEXA) as a practical tool for body composition analysis. These authors mention that DEXA is an improvement of dual-photon absorptiometry with the replacement of the radionuclide source that allows DEXA higher resolution images, precision and more rapid scan times than before. However, a direct use of scan data is not possible due to variations in the software and instrument used. The values are different when compared to chemical analysis but show high correlation Swennen *et al*., (2004). Mitchel *et al*., (1997) also suggests that chemical analysis is needed to develop prediction equations with the scan data for lean, fat and body mineral composition for future adjustments when DEXA is used to measure body components. Swennen *et al*., (2004) also mentions that regression equations are strictly restricted to one particular instrument, software version, and applied methodology. Consequently, prediction equations are imperative for a new instrument or new software. In addition to the accuracy of the instrument; precision in methodology is also important. It is important to be able to reproduce the same results in repeated measurements Zotti *et al.,* (2001). Position of the animal being scanned might also have an impact in the DEXA values. Some authors indicate no differences in scanning positions (Swennen *et al*., 2004), while others show some evidence that indicates position is an important fact to consider (Raffan *et al.,* 2006). The objectives of the present study is to validate the DEXA instrument in our laboratory at the Center of Excellence for

Poultry Science in the University of Arkansas, in terms of precision, position and finally build prediction equations for future adjustments of the raw data from DEXA scans. Body protein and body dry matter are not provided by the DEXA, lean (water + protein) is provided instead, so chemical analysis is vital to build the equation for protein and dry matter contents. Two groups of chickens will be used for 4 experiments designed for these experiment. The first group of chicks will be used for measuring precision (trial 1), position (experiment 2) and the equations and validation for fasted chicks (experiment 3).The second group will be used to build equations and validate them under *ad libitum* consumption with different set of chickens analyzed by DEXA and chemical analysis (experiment 4). The equations needed future adjustments to DEXA scans will be for soft tissue (total mass, lean, protein, and fat tissue, and body energy value) and mineral content (total mineral, calcium and phosphorus).

## MATERIALS AND METHODS

All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC) # 12041.

### Birds and Housing

Male chicks of a commercial strain (Cobb hatchery, Cobb Vantress, Siloam Springs, AR) were obtained from a local hatchery (Cobb hatchery, Fayetteville, AR) in two different sets to accomplish 4 experiments. The first set of broiler (81 chicks) were obtained from a trial were the birds were fed restricted from 12 – 28 d of age (85, 55, 35% feeding level from Cobb, 2012 feed intake). Twenty seven chicks from each restriction treatment were obtained to have a wide range of BW for development of equations. These chicks were raised in wire metabolic cages with

dimensions of 91 cm x 30 cm for 1 – 28 d old chicks. The metabolic cages provided 2 nipple drinkers and a line feeder of 85 cm. The second set of chicks were raised and reared in 4.5 $m^2$ floor pens of 40 chicks per pen under *ad libitum* feed conditions of a diet corn-soybean diet based. Each pen was equipped with 10 nipples per line, two hanging type feeders, with a round pan that provided 208 cm of feeder space per pen. Chicks from the same flock were placed in four different groups. Group 1 with 160 chicks for starter period (1-14d), group 2 with 120 chicks for the grower period (15-28d), group 3 with 80 chicks for finisher (29-42d), and group 4 with 120 chicks for withdrawal period (43-60d). Chicks were selected at twelve points of the growth-out period from body weight (BW) mean ± 1.6 SD at 1, 4, 7, 12, 17, 22, 27, 33, 39, 47, 54, 60 days of age for DEXA scan and carcass composition evaluation. The amount of chicks selected at each point was an average of 13. The total number of chicks used was 160, out of 480 initially placed. Temperatures in the chicken house were changed according to the genetic broiler management recommendations (Cobb 500, 2012) starting at 33°C and decreasing 3 degrees °C every week until 18°C at 42d and kept the same until the end of the study (60d). The light program was 23 h. light: 1 h. dark for all feeding periods.

***Experiment 1***

The purpose of this trial was to evaluate precision, so twenty one – 28d chicks were scanned consecutively four times in ventral position. Continuously repeated scans in one day were performed having 84 scans total (21 x 4). Because of the source of these chicks (fed restricted study) the BW range was 603 – 1299 g. The DEXA parameters evaluated were BMD (bone mineral density), BMC (bone mineral content), area, $cm^2$, total tissue, tissue fat, and tissue lean, g.

***Experiment 2***

Twenty one fed restricted chicks were scanned twice in lateral, supine (dorsal), and prone (ventral) positions, producing 63 scans total. In the lateral position, chicks were laid on their right side. In the supine or dorsal position the chicks were facing upwards, and in the prone or ventral position, the chicks were facing downwards towards the DEXA table. Seven chicks from 85%, seven from 55%, and seven from 35% restriction levels were analyzed separately. After DEXA scan, the same birds were analyzed by chemical analysis to evaluate correlation between DEXA and chemical analysis due to position. These chicks were obtained from the same source as in experiment 1.

***Experiment 3***

Eighty one fed restricted chicks were scanned by DEXA in prone (ventral) position and then analyzed by chemical analysis. Sixty birds were used to build equations for further adjustments of body composition from DEXA scans, and 21 birds were used to validate the equations by predicting using the equations developed with 60 chicks. The reason for doing equations for chicks under feed restricted conditions was the negative values DEXA provides when chicks are reared under feed restriction conditions for research purposes. In fed restriction conditions, the fat content in the body of the chicks is minimal, so DEXA scans result in non-real values for fat because it seems that DEXA accounts first for lean tissue, BMC and fat is analyzed by difference.

***Experiment 4***

One hundred sixty fed chicks from d1- d60 were scanned in prone (ventral) position and then analyzed by chemical analysis. These chicks were obtained from the second set of chickens placed fed *ad libitum*. The BW range was 51 – 4690 g. 120 chicks were used to developed equations and 40 extra chicks were used to validate the equations.

**Dual Energy X-ray absorptiometry (DEXA) scan procedure**

Birds were humanely sacrificed by $CO_2$ inhalation before body composition was determined using dual energy X-ray absorptiometry, DEXA scanner (GE, Madison, WI) with small animal body software module (Lunar Prodigy from GE encore version 12.2). Every morning, before chicks were scanned, a complete daily quality assurance procedure was performed, printed and archived. Then, the information of the chicks to be scanned was recorded in a proper database to start DEXA scan. After each scan, results were analyzed using custom platform. The region of interest (ROIs) were selected up to 10 (Figure 1). The results showed the following parameters: BMD (body mineral density g/$cm^2$), BMC (bone mineral content, g), area of the bird ($cm^2$), tissue, g (this reproduces BW), tissue fat, %, fat, g and lean, g. Lean has two components, water and protein that DEXA cannot separate, so chemical analysis is needed to obtain the equation for protein content estimation. The scanner moves along a table of 200 cm length and 60 cm wide. The chickens to be scanned can be arranged up to 10 per scan time and the dimensions adjusted before each scan. The scan time varied from 10 – 20 minutes for scan of 10 chickens depending on the age. Chicks more than 28d were scanned in groups of 6 because limitation in space in the table. Smaller chickens were scanned faster than big birds. Experiment 1 (precision) required

only scans with DEXA, while experiment 2 (position), experiment 3 (equation and validation of fed restricted chicks), and experiment 4 (equation and validation of fed *ad libitum* chicks) required scans and chemical analysis of the whole body.

**Chemical Analysis**

After DEXA scan, the chicks were frozen at -20 C before chemical analysis was performed. The preparation of the dry homogenates from the whole chickens was prepared in a similar way as described by Salas (2012) with small modifications. Briefly, carcasses were thawed for 24 - 36 hours and transferred to individual aluminum tubs, about 10% of water was added to avoid adhesion of the carcass to the container during the autoclave process. Chickens, tubes and water were weighed for initial weight and the container covered with aluminum foil and autoclaved at 121°C with 22 psi pressure. The time in the autoclave varied according to the size of the birds from 1 to 6 hours (1-60d). Once the cycle was finished, the carcasses were left in the autoclave for at least 2 hours to let them cool and wait until the pressure reached zero and the temperature to normal laboratory conditions. Tubes were reweighed and if loss was observed, it was assumed to be water loss. The whole chicken which included feathers and visceral content were homogenized with a heavy duty blender (Waring laboratory, Blender LBC15, Model CB15). After homogenization, about 120 g sample was obtained and frozen for 48 hours before lyophilization occurred for 2 weeks. Dried samples were reweighed and ground for further analysis. Dry matter was determined by weighing the sample before and after lyophilization. The water content was determined by subtracting DM from 100, plus the water loss in the autoclaving process. Nitrogen was analyzed by the method 990.03; AOAC (1995), carcass protein was defined as N x 6.25, the fat analyzed was prepared by method 920.39C; AOAC

(1990), mineral content (ash) was analyzed by the method AOAC 923.03. The method for the analysis of the macro-minerals, calcium and phosphorus was AOAC 968.08 adapted for an inductively coupled plasma, ICP. The gross energy (GE) for the whole body of the chicks was determined in a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). All analysis were conducted at the Central Analytical Laboratory, University of Arkansas, and Center of Excellence for Poultry Science.

***Statistical analysis***

Various tools and models were used to analyze data from 4 experiments. For experiment 1, precision, a quality process variability tool (JMP12, SAS institute, 2015). For experiment 2, position, data were analyzed in CRD for each type of feed restrictions by ANOVA, when the effects were significant, means were separated by t-student test for p-value $< 0.05$. Position data were analyzed separately and not by factorial design because feeding level was not of interest on this study, and also because the feeding level was not equidistant (35, 55, 85%), so non meaningful interactions could occur, as mentioned before, chicks were obtained from a previous study, so feeding level could not be monitored for the present study. For experiments 3 and 4, the independent variable were converted to natural logarithm before a Non-linear regression approached conducted with the exception of fat in experiment 3 in which a linear-model was developed, also a linear model was developed for body energy in experiment 4. Multiple linear regression analysis was fitted for fat mass content in fasted birds and body energy. For validation, a matched pair t-test was performed between the predicted and analyzed values at $P<0.05$, and correlations analyzed. The predictors were DEXA lean, DEXA fat, DEXA mineral, area $cm^2$. All analysis were achieved using JMP12 (SAS institute, 2015)

## RESULTS

### Precision (experiment 1)

The DEXA machine showed high repeatability for all DEXA parameters (98.1, %) because the replications variability analyzed by Bayesian test was low (1.88%). Total tissue, equivalent to scale body weight (BW), showed the smallest variation between repeated scans (CV 0.11%, range 0.0 – 0.22 %), and followed by lean tissue (CV 0.47%, range 0.16 – 0.96 %). Bone mineral content (BMC) had lower CV (0.91%, range 0.43 – 1.49 %), similar to the variation for BMD (bone mineral density) (CV 1.34%, range 0.29 – 2.67 %). BMD is the relation between BMC, g/ area, $cm^2$, so the variation of BMD will depend on the variability of BMC and area. Body area had (CV 2.04%, range 0.85 – 3.73 %). Finally, the biggest variation was found in fat tissue, both in g and % (CV 4.5%, range 0.94 – 12.9 %; and CV 4.6%, range 0.99 – 13.4 %). The range of CV is higher in fat tissue, this means, some chickens had very small variation of 0.94% and others 13% which makes the range big. Total, and lean tissue showed followed by BMC, BMD, area, and finally fat tissue (Table 1).

### Position (experiment 2)

DEXA technology was originally developed for human monitoring health and research when only one person can be scanned at the time; however when small animals such as chickens are scanned, positioning could affect results. The present study showed that total tissue was not different due to positions for any feeding levels, however total tissue is equivalent to body weight which can be measured by a scale, so it is not as important as other components that were

different due to positioning (Table 2). Lean tissue, g was higher when the chicks were scanned in ventral position compared to lateral position ($P$<0.024) for chicks at 55% of intake (BW 880 g) and a trend ($P$<0.091) was found at 35% of intake (BW 680 g). No differences were observed for bigger birds (BW 1188 g) at 85% of intake. Fat tissue, g was lower when ventral (16 g) and dorsal (25 g) positions were used to scan the birds respect to lateral position (50 g) for 55%, and 35% of intake at ($P$<0.001), and ($P$<0.001) respectively, no differences were observed in bigger birds. In small chicks (35% intake) fat values were even negative for ventral and dorsal positions but not for lateral positions. Bone mineral density (BMD) was different between positions for all the feeding levels. In 85% of feeding level, lateral position showed higher values of BMD (0.242 g/cm$^2$) compared to dorsal (0.198 g/cm$^2$) and ventral (0.205 g/cm$^2$) ($P$<0.001). In 55% of feeding level, lateral position showed again higher values of BMD (0.225 g/cm$^2$) compared to dorsal (0.183 g/cm$^2$) and ventral (0.188 g/cm$^2$) ($P$<0.001). In 35% of feeding level, lateral position showed higher values of BMD (0.208 g/cm$^2$) compared to dorsal (0.170 g/cm$^2$) and ventral (0.179 g/cm$^2$), and dorsal was lower significantly to ventral position ($P$<0.001). These differences in BMD are due to differences in amount of BMC and also positioning showed differences in the area of the chick for 85 and 35% of feeding level. Pairwise correlations of DEXA with chemical analysis showed similar correlations for total tissue, lean tissue and BMC; only fat was better correlated with ventral position (0.83) compare to lateral (0.80), and dorsal (0.76) positions. All correlations were significant ($P$<0.001) (Table 3). Two scans were performed in this positioning trial and ventral position showed lower standard deviations between the two scans.

**Equations and Validation in fed restricted chicks (experiment 3)**

The need of chemical analysis to adjust DEXA values is justified by differences between DEXA and chemical analysis are high (0.76 – 81%) (Table 4). DEXA underestimates fat and BMC and overestimates lean mass. However, the high significant correlations between these two methods (Table 4) allow research to develop equations to validate and adjust DEXA values for future scans. DEXA readings in fed restricted chicks resulted in negative values for fat, so fat tissue was the only parameter not transformed to Ln (natural logarithm), fat was fitted by a multiple linear regression analysis. All equations were significant ($P<0.05$) and most equations had higher coefficient of determination $R^2$ (>0.986) (Figure 2, 3); however fat tissue prediction was the one with the lowest $R^2$ (0.746) (Table 5, Figure 4). The parameters for the non-linear were developed with the column formula approach (JMP, 2015). The validation of these equations were used in another set of chickens for a matched pair comparison (Table 6). The range of BW in experiment 3 was from 604 – 1237 g. The predicted and analyzed mean, SD, and range are very similar, that's probably why the *P*-value between predicted and analyzed values were no significant which is expected since it is desired these values be the similar meaning the equations are good predictors. Correlations between predicted and analyzed values were all high (r >0.829) at ($P<0.001$).

**Equations and Validation in fed *ad libitum* conditions (experiment 4)**

Fed *ad libitum* broilers reared under normal conditions ranged in BW from 51 – 4690 g (1 – 60 d age) as showed in the present experiment which is in close agreement to the commercial line standards (Cobb, 2012). All body components were transformed to Ln (natural logarithm) before

non-linear equations were fitted (Figure 5, 6, 7, 8, and 9). Body energy equation were fitted by multiple linear regression (Table 7, Figure 7). The parameters a, b were developed in the same manner as experiment 3. The coefficient of determination ($R^2$) which expresses the % of variability that the model is explaining is very high ($R^2$>0.965). The reason of developing non-linear regression is because the conditions to develop linear regressions were not met. For example the lack of fit which represents the pure error of the model was significant which should not be, and the intercept in many cases was not significant. By fitting non-linear model, the $R^2$ for fat was increased to 0.965 compared to feed restricted broilers in experiment 3. The validation of the equations meaning the comparison between the predicted and analyzed values showed *P*-values no significant and high correlation values (Table 8) as needed.

## DISCUSION

### Precision (experiment 1)

Analysis of body composition is cumbersome, so DEXA is an available alternative to obtain body composition analysis quicker with no need to sacrifice the animal; however, precision, standardization, and validations are necessary before these type of machines can be used in research. Lean tissue had very low variability compared to fat tissue maybe because the amount of lean tissue compared to fat in the body is about 15 times more depending upon on age and hydration of the bird. Lauten (2001) reports similar values of CV for DEXA scans in normal dogs, such as 0.10% for lean tissue vs 0.47% in the present study, and 5.19% CV for fat vs 4.55 % in this study. Reproducibility was shown to vary according the age of individuals (Leonard, 2009), in the present experiment the chicks were the same age, however because of the fed

restricted condition, the BW ranged from 607 – 1237 g which is a wide range for the same age bird, and the reproducibility was high. Fat variability has been reported before not only in poultry but in other species (Swennen *et al*, 2004). Fat is considered the most difficult component of the body to analyze because it shows high variability (Mitchel, 1997). According to Swennen *et al*. (2004), this variation in fat tissue is due to variation in soft tissue, hydration, age, sex, and diet composition. Pietrobelli *et al.,* (1998) studied fat estimation errors due to hydration and found that systematic errors in DXA percent fat arise with fluid balance which can explain the negative values or errors found when fat tissue is changed due to hydration. Lean tissue includes water and protein mass, so when the water which is the biggest component of the body changes, will affect fat tissue content. Precision of the mineral content (BMD, and BMC) has shown to be high with CV 0.84 – 2.2 % in mice experiments (Nagy, 2000) similar to the CV found in the present study. Variability of <10 % measured as CV in biological systems is considered to be normal, the average of CV in DEXA components in the present experiments complies with CV < 5%; even though the range can vary more as shown for fat tissue (up to 13% CV). It is suggested that if dietary treatments been evaluated in body composition by DEXA, blocking the scan could be a good practice. This means set up chicks in DEXA table of all treatments per scan, so the variability would be the same for all treatments.

**Position (experiment 2)**

Positioning can affect absolute values of body components even though the correlations between DEXA measurements and chemical analysis are high. Standardization in positioning would allow less variability as showed in the present study with ventral recumbency being less variable than lateral and dorsal recumbency. It seems positioning in bigger chicks is less important than in

small birds as shown in this experiment when more body components were different between positioning in smaller chicks. Raffan *et al*., (2006) suggested a dorsal recumbency when this position was compared to lateral in dogs because it allowed lower variability, no ventral position was compared. The authors suggested also, to determine which position correlates best with chemical analysis. Analysis of chemical composition in chickens is fairly easier compared to chemical analysis of bigger animals, so the bigger correlation with ventral positioning for fat tissue analysis suggest the use of ventral positioning for future scans. In addition, the DEXA scan of live animals is more practical with ventral positioning because it provides more opportunity to have a steady position while the bird is being scanned. Correlations between DEXA and chemical analysis for all positions were significant, however the variability for soft tissue was lower with ventral position. The reason for ventral positioning being more accurate than lateral and dorsal could be the arrangement of the soft tissue in the body of the chicks that allows the X-rays to produce more accurate results.

**Equations and Validation in fed restricted broilers (experiment 3)**

Development of equation and validation of DEXA have been achieved before in chickens (Mitchel, *et al.* 1997; Swennen, *et al.* 2004, Salas, 2012); however as pointed out by Swennen *et al.* (2004), development of equations and validations must be done for a particular machine and software. Salas (2012) validated DEXA in chicks reared under normal conditions in this laboratory, however the non-linear equations could not be used when DEXA provides negative values for fat in fed restricted birds. The development of equation for fat in fed restricted chicks followed a linear relationship which has been validated to be used for future scans under these type of scenario. Equations for lean tissue, dry matter, protein, and BMC have been updated for

fed restricted chicks which provides equations for a particular type of research allowing more accurate measurements. Development of equations under fed restricted conditions are important because bias can occur when DEXA scan is used as showed by Williams (2006) when healthy and ill patients were scanned, the accuracy of DXA was different because of age, sex, size, and disease state. The variability in human body composition can be higher compared to a uniformity of chicken population, so the better the uniformity, less bias for DEXA scans.

**Equations and Validation in fed *ad libitum* broilers (experiment 4)**

When the birds are fed *ad libitum* the amount of body components, particularly fat, can change dramatically, most the research in this laboratory is done under these type of condition, so the equations and validations have been worked for chicks from 1 – 60 d covering most of the growth curve of a commercial broiler nowadays. Since the market age could vary from 35 – 56 d, these equations could be used for most part of the experiments. Mitchel, *et al.*, 1997 developed linear equations with CHEM values by DEXA values, however in this study non-linear equations were also included to fulfill with the assumptions of the model. Swennen, *et al*., (2004) validated the equations and reported extremely good agreement for total body mass, lean tissue and fat but not for BMC. These study showed good agreement for all body components including BMC, calcium and phosphorus. The equations for body calcium and body phosphorus components have not been reported before.

**Table 1. Precision of Dual energy X-ray absorptiometry (DEXA) (experiment 1)**

| Evaluation method | Total Tissue, g | Lean tissue, g | Fat tissue, g | Fat, % | [1]BMD, g/cm$^2$ | [2]BMC, g | Body area, cm$^2$ |
|---|---|---|---|---|---|---|---|
| [3]CV ± [4]SD (%) | 0.11 ± 0.06 | 0.47 ± 0.19 | 4.52 ± 3.39 | 4.55 ± 0.06 | 1.34 ± 0.65 | 0.91 ± 0.31 | 2.04 ± 0.78 |
| Range of CV, % | 0.00 - 0.22 | 0.16 - 0.96 | 0.94 - 12.91 | 0.99 - 13.4 | 0.29 - 2.67 | 0.43 - 1.49 | 0.85 - 3.73 |
| [5]Bayesian (replication variability, %) | 1.869 | 1.870 | 1.882 | 1.898 | 1.897 | 1.878 | 1.881 |
| [6]Repeatability, % | 98.13 | 98.13 | 98.12 | 98.10 | 98.10 | 98.12 | 98.12 |

[1] BMD bone mineral density
[2]BMC bone mineral content
[3]CV Coefficient of variance.
[4]SD, standard deviation
[5]The Bayesian provides the variability due to replications, obtained from variance component (Precision – Variability, JMP platform)
[6]Repeatability, obtained from Gauge R&R (Precision – Variability, JMP platform)

**Table 2. Comparison of DEXA values between lateral, dorsal and ventral position in fed restricted birds (experiment 2)**

| Feeding level | Position | Total Tissue, g | Lean tissue, g | Fat tissue, g | Fat tissue, % | [1]BMD, g/cm$^2$ | [2]BMC, g | Area, cm$^2$ |
|---|---|---|---|---|---|---|---|---|
| **85% (Big chickens)** | Lateral | 1190 | 1118 | 75.2 | 6.3 | 0.242$^{a}$ | 21.2$^{b}$ | 87$^{b}$ |
| | Supine (dorsal) | 1184 | 1104 | 89.4 | 7.6 | 0.198$^{b}$ | 22.1$^{ab}$ | 107$^{a}$ |
| | Prone (ventral) | 1188 | 1097 | 90.6 | 7.6 | 0.205$^{b}$ | 23.2$^{a}$ | 110$^{a}$ |
| | *[3]SEM* | *9.42* | *8.80* | *8.76* | *0.72* | *0.001* | *0.44* | *2.20* |
| | *P-value* | *0.903* | *0.306* | *0.420* | *0.385* | *<0.001** | *0.0197** | *<0.001** |
| **55% (Medium chickens)** | Lateral | 879 | 829$^{b}$ | 49.6 a | 5.6$^{a}$ | 0.225$^{a}$ | 14.8$^{a}$ | 66 |
| | Supine (dorsal) | 877 | 852$^{ab}$ | 25.1 b | 2.8$^{b}$ | 0.183$^{b}$ | 12.6$^{b}$ | 69 |
| | Prone (ventral) | 881 | 865$^{a}$ | 16.0 b | 1.8$^{b}$ | 0.188$^{b}$ | 11.8$^{b}$ | 63 |
| | *SEM* | *8.81* | *8.46* | *6.93* | *0.79* | *0.01* | *0.72* | *2.41* |
| | *P-value* | *0.942* | *0.024** | *<0.001** | *0.0094** | *<0.001** | *0.0217** | *0.288* |
| **35% (Small chickens)** | Lateral | 679 | 679 | 0.86$^{a}$ | 0.11$^{a}$ | 0.208$^{a}$ | 12.2$^{a}$ | 59$^{a}$ |
| | Supine (dorsal) | 677 | 688 | -10.64$^{a}$ | -1.64$^{ab}$ | 0.170$^{c}$ | 10.7$^{b}$ | 63$^{a}$ |
| | Prone (ventral) | 685 | 712 | -27.7$^{b}$ | -4.14$^{b}$ | 0.179$^{b}$ | 9.4$^{c}$ | 52$^{b}$ |
| | *SEM* | *12.28* | *10.35* | *5.53* | *0.84* | *0.001* | *0.32* | *1.40* |
| | *P-value* | *0.916* | *0.091* | *<0.001** | *<0.001** | *<0.001** | *<0.001** | *<0.001** |

Levels (a, b, c) not connected by same letter are significantly different
[1] BMD bone mineral density
[2]BMC bone mineral content
[3]SEM, standard error mean

**Table 3. Pairwise correlation between DEXA measurements and chemical analysis by position (experiment 2)**

DEXA scans Correlations with Chemical Analysis

| Position | Total Tissue, g | Lean tissue, g | Fat tissue, g | BMC, g |
|---|---|---|---|---|
| Lateral | 0.9980 | 0.9952 | 0.7990 | 0.9656 |
| Supine (dorsal) | 0.9996 | 0.9932 | 0.7622 | 0.9660 |
| Prone (ventral) | 0.9997 | 0.9956 | 0.8282 | 0.9622 |

[2]DEXA scan analysis - Standard deviations

| Position | Total Tissue, g | Lean tissue, g | Fat tissue, g | BMC, g |
|---|---|---|---|---|
| Lateral | 0.84 | 6.43 | 5.32 | 0.32 |
| Supine (dorsal) | 0.81 | 4.55 | 4.20 | 0.23 |
| Prone (ventral) | 0.57 | 2.83 | 2.69 | 0.23 |

[1]BMC bone mineral content
[2]Each position was scanned twice
All correlations were highly significant at *P*-value< 0.001**

**Table 4. Comparison between DEXA and chemical analysis parameters (experiment 3)**

| Parameter | DEXA | Chemical analysis | Absolute difference, g | Percentage difference, % | Correlation coefficient, r |
|---|---|---|---|---|---|
| Body mass, g | 926 ± 204 (613 - 1241) | 919 ± 204 (607 - 1237) | 7.0 | 0.76 | 0.9995 |
| Lean mass, g | 896 ± 179 (634 - 1208) | 840 ± 181 (563 - 1138) | 56.0 | 6.25 | 0.9866 |
| Fat mass, g | 29.4 ± 40.4 (-51 - 119) | 44.9 ± 24.3 (-12.6 - 108) | -15.5 | -52.7 | 0.7410 |
| Fat, % | 2.6 ± 4.5 (-5.6 - 10.1) | 4.7 ± 1.9 (1.52 - 10.1) | -2.1 | -80.8 | 0.6764 |
| [1]BMC, g | 14.7 ± 5.05 (6.9 - 24.5) | 22.1 ± 4.4 (14.6 - 29.3) | -7.4 | -50.3 | 0.9035 |

[1]BMC bone mineral content
All correlation coefficients were significant at *P*-value < 0.001**

**Table 5. Equations to estimate body components from DEXA in fed restricted broilers (607 – 1237 g BW) (Experiment 3)**

| Component - Whole Body | Parameter estimates | | | | $R^2$ | RMSE |
|---|---|---|---|---|---|---|
| Total mass (g) (BW) = $e^{(a+b*Ln(DEXA\ Tissue,\ g)}$ | **Parameter** | **Estimate** | **Lower** | **Upper** | 0.999 | 6.57 |
| | a | -0.05 | -0.11 | 0.01 | | |
| | b | 1.01 | 1.00 | 1.01 | | |
| Dry matter mass (g) = $e^{(a+b*Ln(DEXA\ lean,\ g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.999 | 18.5 |
| | a | -2.40 | -3.14 | -1.67 | | |
| | b | 1.15 | 1.05 | 1.26 | | |
| Lean mass (g) = $e^{(a+b*Ln(DEXA\ lean,\ g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.999 | 30.0 |
| | a | -0.45 | -0.78 | -0.12 | | |
| | b | 1.06 | 1.01 | 1.10 | | |
| Protein mass (g) = $a*DEXA\ lean^b$ | **Parameter** | **Estimate** | **Low** | **High** | 0.999 | 6.01 |
| | a | 0.15 | 0.10 | 0.21 | | |
| | b | 1.02 | 0.97 | 1.07 | | |
| Fat mass (g) = | **Parameter** | **Estimate** | **SE** | | 0.746 | 12.58 |
| -15.88 | Intercept | -15.88 | 8.94 | | | |
| + 0.09* DEXA tissue, g | DEXA Total mass, g | 0.09 | 0.01 | | | |
| + 0.28* DEXA fat, g | DEXA Fat, g | 0.28 | 0.05 | | | |
| - 0.47* area, $cm^2$ | Area, $cm^2$ | -0.47 | 0.14 | | | |
| Mineral, g = $e^{(a+b*Ln(DEXA\ BMC,g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.986 | 1.89 |
| | a | 1.73 | 1.54 | 1.91 | | |
| | b | 0.51 | 0.45 | 0.58 | | |

[1]RMSE = root mean square error, [2]VIF = variance inflation factor, expected to be <10, [3]SE = standard error [4]BMC bone mineral content. All equations were significant at *P*-value < 0.05

**Table 6. Comparison between predicted and analyzed body parameters in fed restricted broilers (Validation, experiment 3)**

| Parameter | Predicted, Mean ± SD (Range) | Analyzed, Mean ± SD (Range) | Difference, Mean ± SD (Range) | P-value | Correlation |
|---|---|---|---|---|---|
| Body mass, g | 877 ± 189 (633 - 1165) | 878 ± 190 (631 - 1165) | -1.0 ± 7 (-18 - 9) | 0.907 | 0.999 |
| Dry mass, g | 221 ± 46 (155- 294) | 219 ± 53 (146- 304) | 1.8 ± 11 (-18 - 22) | 0.485 | 0.981 |
| Lean mass, g | 801 ± 153 (578 - 1042) | 807 ± 168 (585 - 1062) | -5.1 ± 26 (-43 - 40) | 0.398 | 0.991 |
| Protein mass, g | 146 ± 27 (107 - 189) | 147 ± 30 (99 - 200) | -0.2 ± 6 (-16 - 8) | 0.912 | 0.984 |
| Fat mass, g | 41 ± 21 (0.4 - 82.4) | 36 ± 20 (10.3 - 69) | 4.6 ± 11 (-10 - 21) | 0.454 | 0.895 |
| BMC, g | 22 ± 4 (17 - 27) | 21 ± 4 (15 - 29) | 0.4 ± 2 (-7 - 4) | 0.457 | 0.829 |

[1]BMC bone mineral content

**Table 7. Equations to estimate body components from DEXA in fed *ad libitum* broilers 1 – 60 d (51 – 4690 g BW) (experiment 4).**

| Component - Whole Body | Parameter estimates | | | | R2 | RMSE |
|---|---|---|---|---|---|---|
| Total mass, g = $e^{(a+b*Ln(DEXA\ tissue,g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.998 | 15.6 |
| | a | -0.05 | -0.08 | -0.02 | | |
| | b | 1.01 | 1.00 | 1.01 | | |
| Dry matter mass (g) = $e^{(a+b*Ln(DEXA\ lean,\ g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.998 | 86.9 |
| | a | -1.74 | -2.29 | -1.22 | | |
| | b | 1.09 | 1.02 | 1.16 | | |
| Lean mass (g) = $e^{(a+b*Ln(DEXA\ lean,\ g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.998 | 40.4 |
| | a | -0.24 | -0.32 | -0.15 | | |
| | b | 1.03 | 1.02 | 1.04 | | |
| Protein mass (g) = a*DEXA lean$^b$ | **Parameter** | **Estimate** | **Low** | **High** | 0.998 | 18.9 |
| | a | 0.09 | 0.07 | 0.11 | | |
| | b | 1.11 | 1.08 | 1.13 | | |
| Fat mass (g) = $e^{(a+b*Ln(DEXA\ fat,\ g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.995 | 24.5 |
| | a | 0.38 | 0.06 | 0.69 | | |
| | b | 0.87 | 0.82 | 0.92 | | |
| Mineral, g = $e^{(a+b*Ln(BMC,g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.990 | 4.7 |
| | a | 0.53 | 0.33 | 0.73 | | |
| | b | 0.92 | 0.87 | 0.97 | | |
| Calcium, g = $e^{(a+b*Ln(DEXA\ BMC,g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.991 | 1.3 |
| | a | -1.16 | -1.44 | -0.90 | | |
| | b | 0.98 | 0.91 | 1.05 | | |
| Phosphorus, g = $e^{(a+b*Ln(DEXA\ BMC,g)}$ | **Parameter** | **Estimate** | **Low** | **High** | 0.965 | 0.2 |
| | a | -1.28 | -1.53 | -1.04 | | |
| | b | 0.95 | 0.89 | 1.01 | | |
| Body energy, kcal = -86.8 + 1.48 * DEXA lean + 4.20 * DEXA fat) | **Parameter** | **Estimate** | **SE** | **P-value** | 0.973 | 221.6 |
| | Intercept | -86.76 | 34.84 | 0.014 | | |
| | DEXA Lean, g | 1.48 | 0.07 | <.0001 | | |
| | DEXA Fat, g | 4.20 | 0.38 | <.0001 | | |

**Table 8. Comparison between predicted vs. analyzed body composition parameters in fed *ad libitum* broilers (Validation, experiment 4)**

| Parameter | Predicted, Mean ± SD (Range) | Analyzed, Mean ± SD (Range) | Difference, Mean ± SD (Range) | P-value | Correlation |
|---|---|---|---|---|---|
| Total body mass, g | 1443 ± 1424 (46 - 4581) | 1443 ± 1429 (51 - 4596) | 2.2 ± 16.9 (-48 - 37) | 0.417 | 0.999 |
| Dry mass, g | 437 ± 439 (10 - 1372) | 417 ± 448 (13 - 1620) | -20± 88 (-248 - 285) | 0.264 | 0.986 |
| Lean mass, g | 1260 ± 1222 (36 - 3820) | 1262 ± 1242 (46 - 3934) | -3 ± 45 (-114 - 69) | 0.950 | 0.999 |
| Protein mass, g | 252 ± 256 (5 - 801) | 253 ± 265 (9 - 845) | -0.5 ± 22 (-63 - 55) | 0.440 | 0.994 |
| Fat mass, g | 131 ± 149 (4 - 516) | 129 ± 141 (2 - 507) | 2.1 ± 17 (-48 - 49) | 0.254 | 0.972 |
| BMC, g | 30 ± 30 (0.4 - 90) | 30 ± 29 (0.9 - 89) | 0.10 ± 2 (-10 - 8) | 0.846 | 0.994 |
| Calcium, g | 6.7 ± 6.9 (0.07 - 21) | 6.7 ± 6.9 (0.14 - 22) | -0.02 ± 1 (-3 - 2) | 0.848 | 0.988 |
| Phosphorus, g | 5.4 ± 5.5 (0.06 - 17) | 5.4 ± 5.5 (0.13 - 17) | -0.01 ± 0.7 (-2.3 - 1.8) | 0.957 | 0.991 |
| Body energy, kcal | 2578 ± 2737 (-1 - 8977) | 2555 ± 2692 (73 - 8964) | 23 ± 182 (-663 - 533) | 0.432 | 0.998 |

SD Standard deviation
BMC body mineral content

**Figure 1. DEXA scan report for chicks at 28d of age**

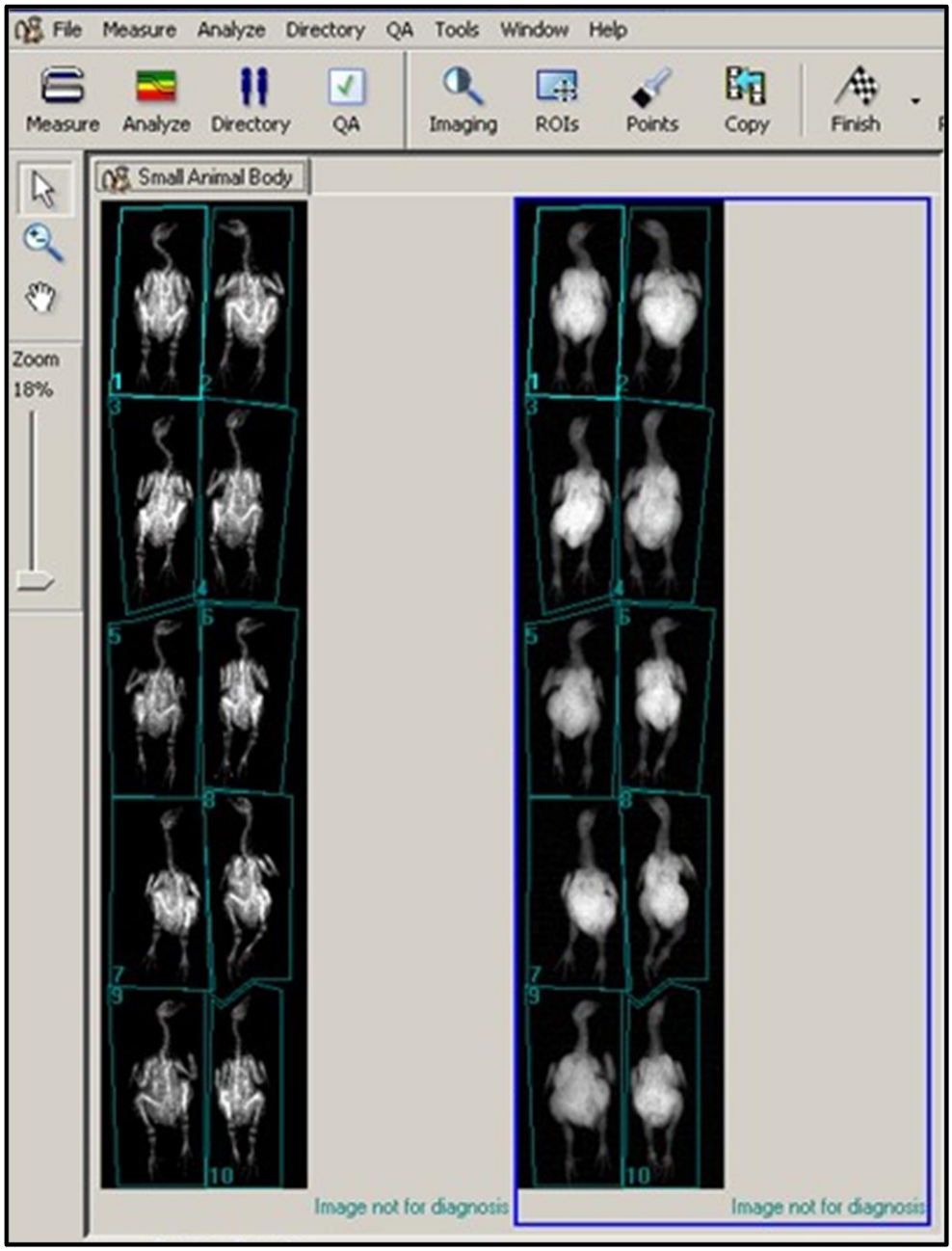
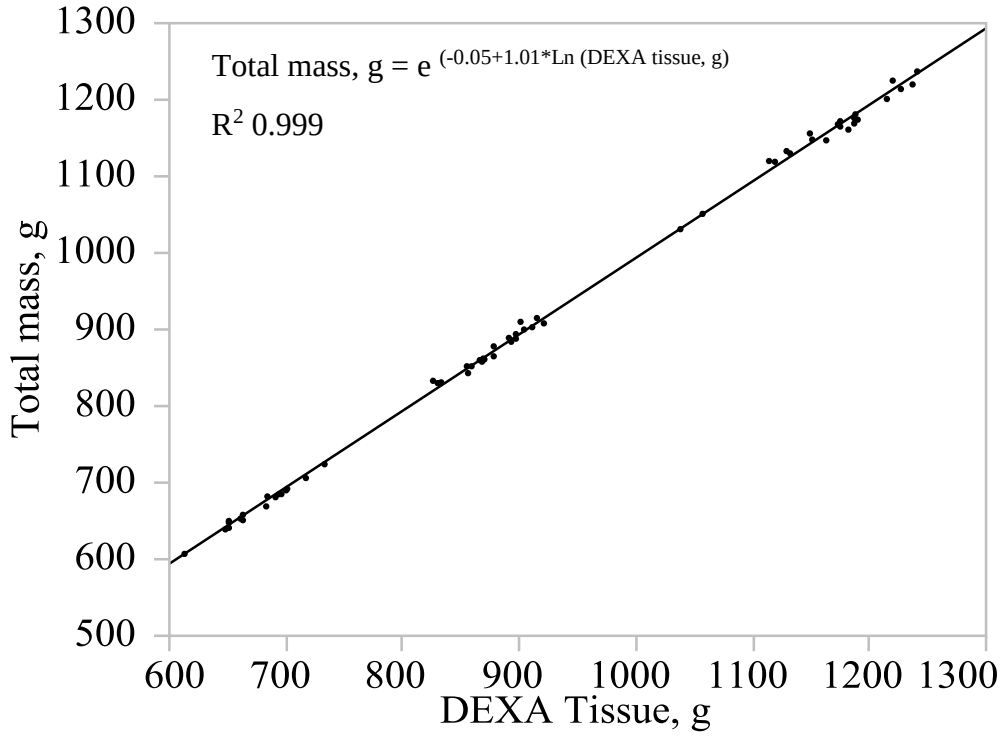


**Figure 2. Fit between Chemical and DEXA analysis for total mass and DM mass in fed restricted broilers 28d (BW 607 - 1237 g) (exp. 3)**



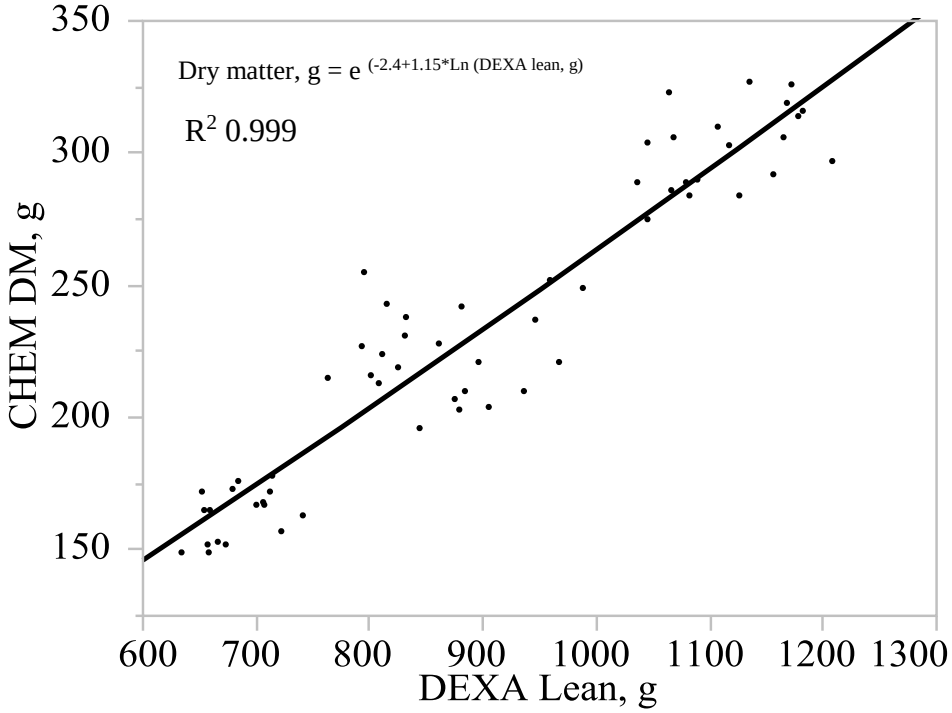
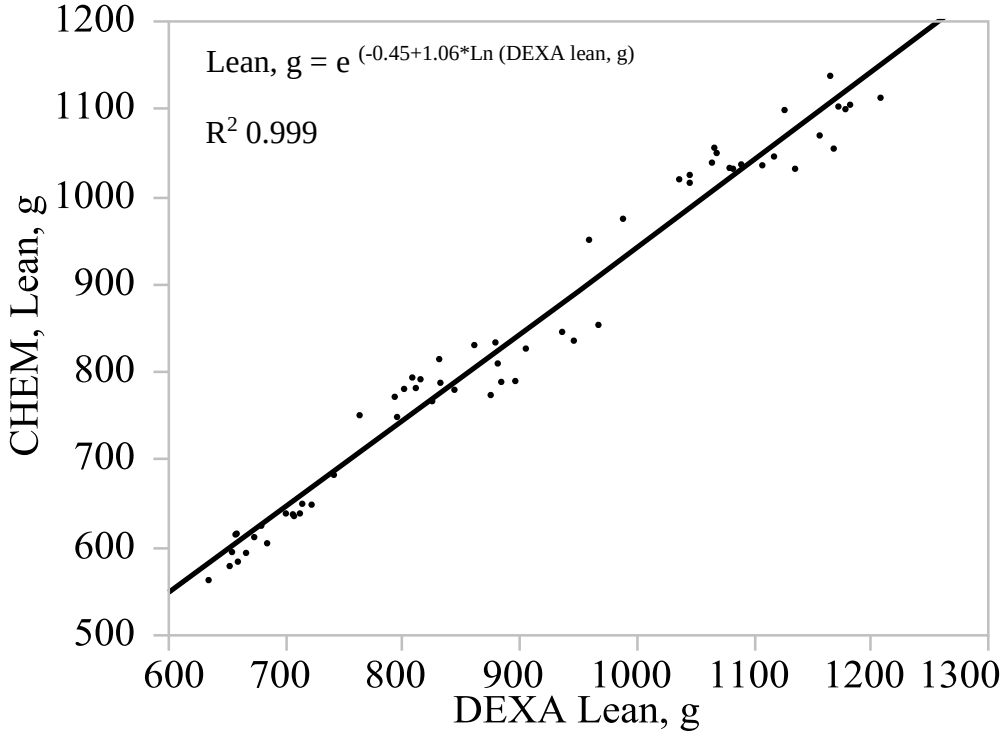



**Figure 3. Fit between Chemical and DEXA analysis for lean and protein mass in fed restricted broilers 28d (BW 607 - 1237 g) (exp. 3)**



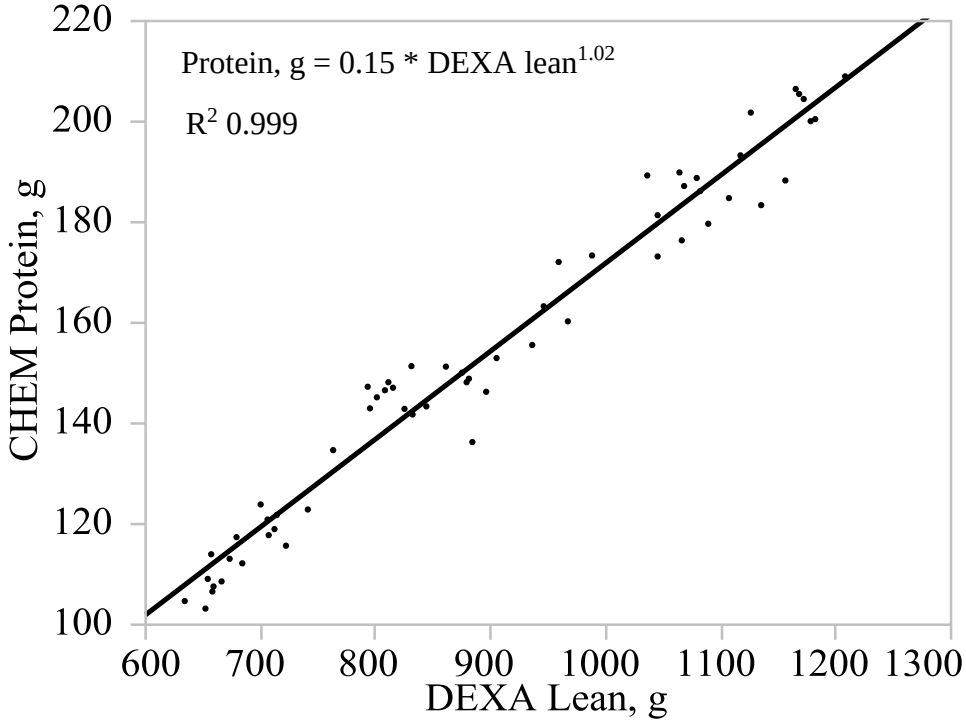
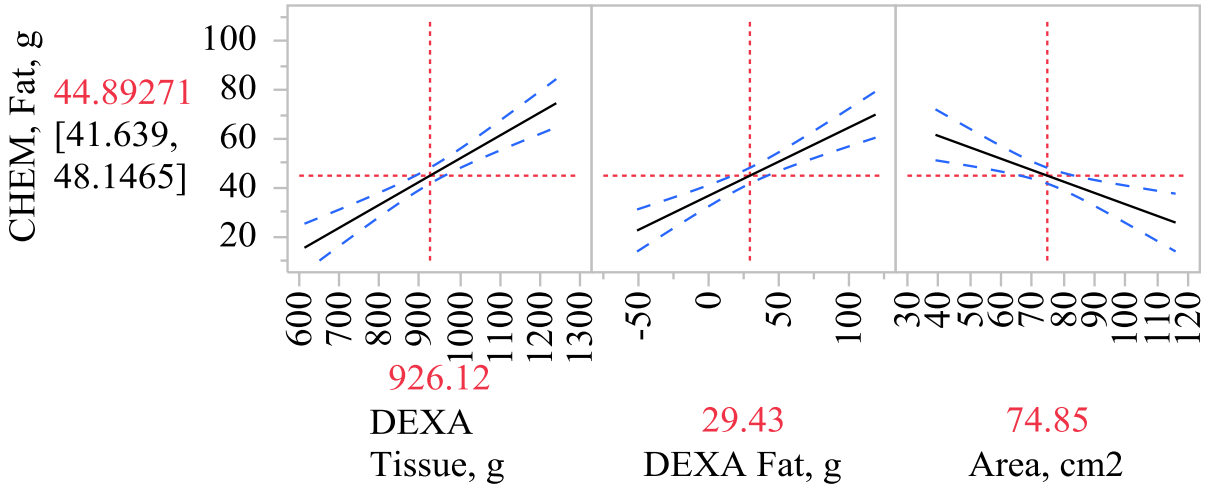



**Figure 4. Fit between Chemical and DEXA analysis for fat mass and mineral content in fed restricted broilers 28d (BW 607 - 1237 g) (exp. 3)**

Fat, g = -15.9 + 0.09* DEXA tissue + 0.28* DEXA fat - 0.47 * Body area, $cm^2$

$R^2$ 0.746



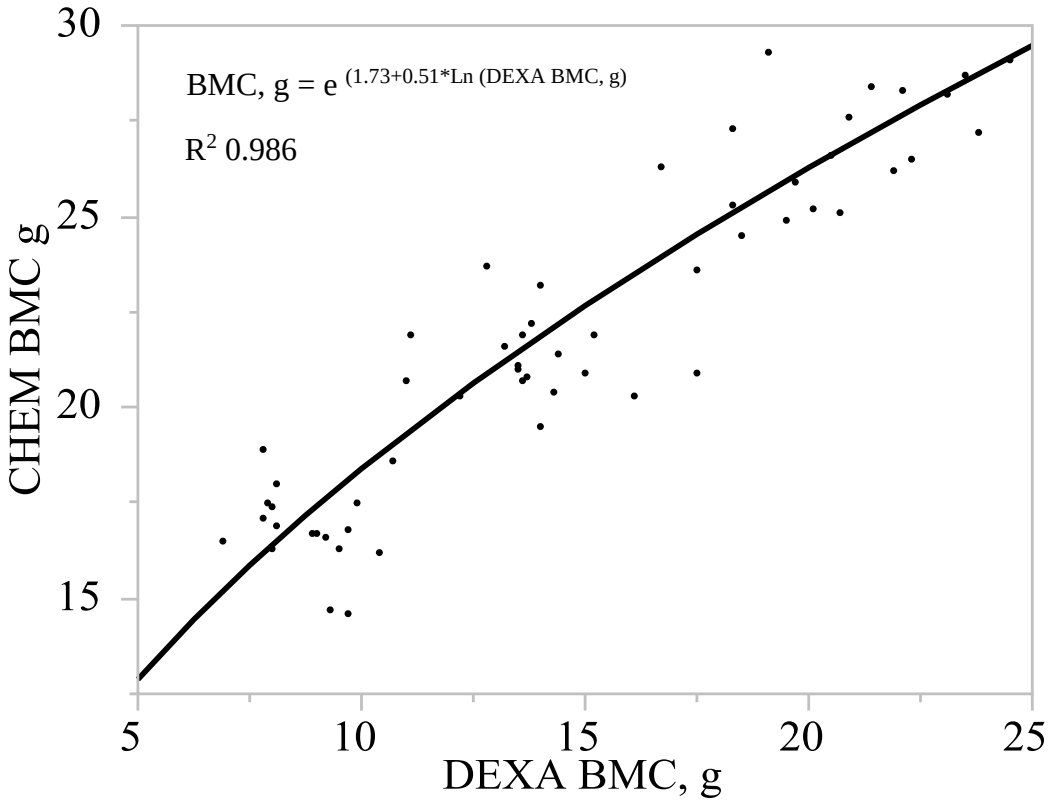



**Figure 5. Fit between Chemical and DEXA analysis for total mass and DM content in fed *ad libitum* broilers 1 – 60d (BW 51 – 4690) (exp. 4)**

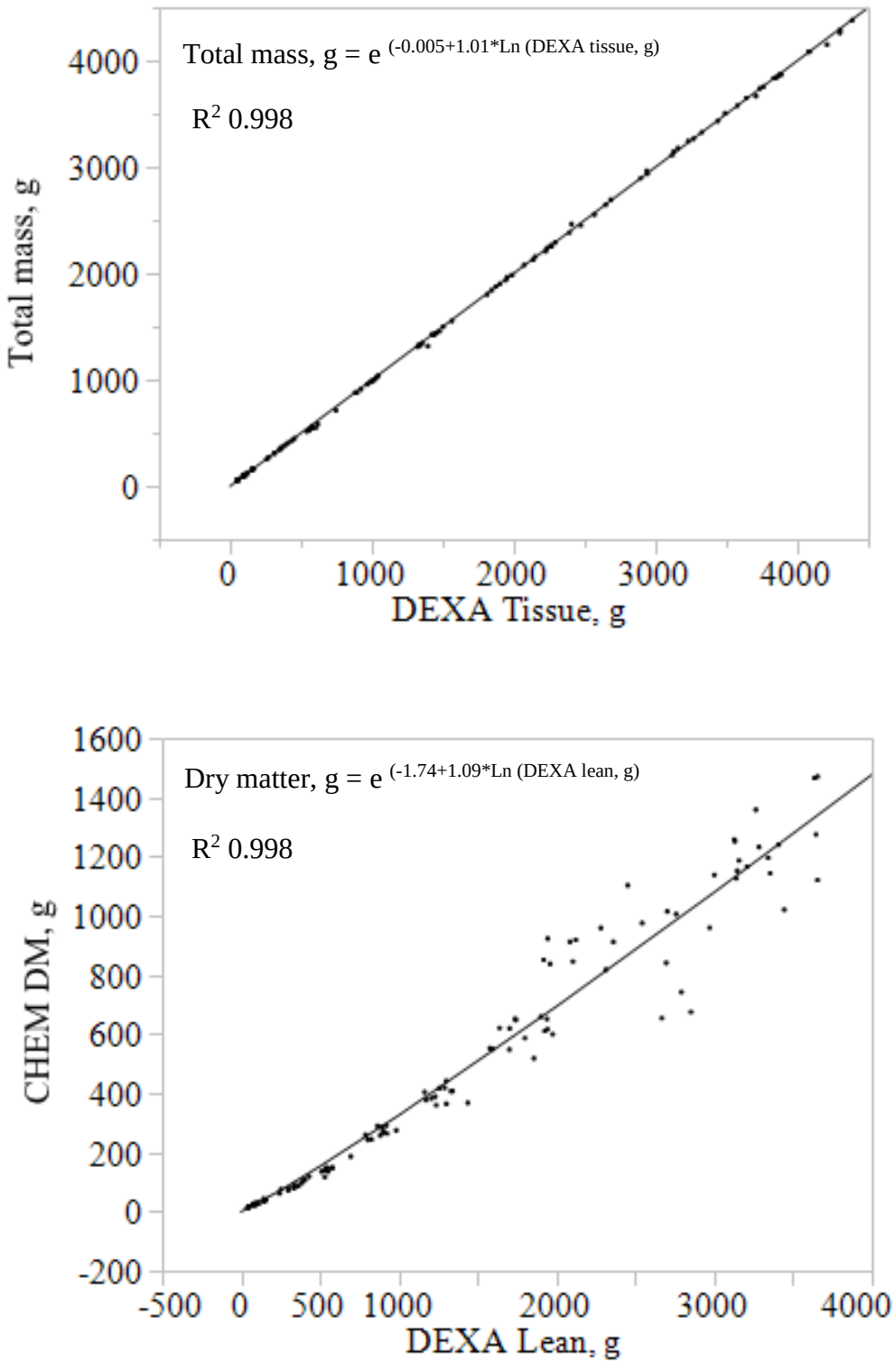
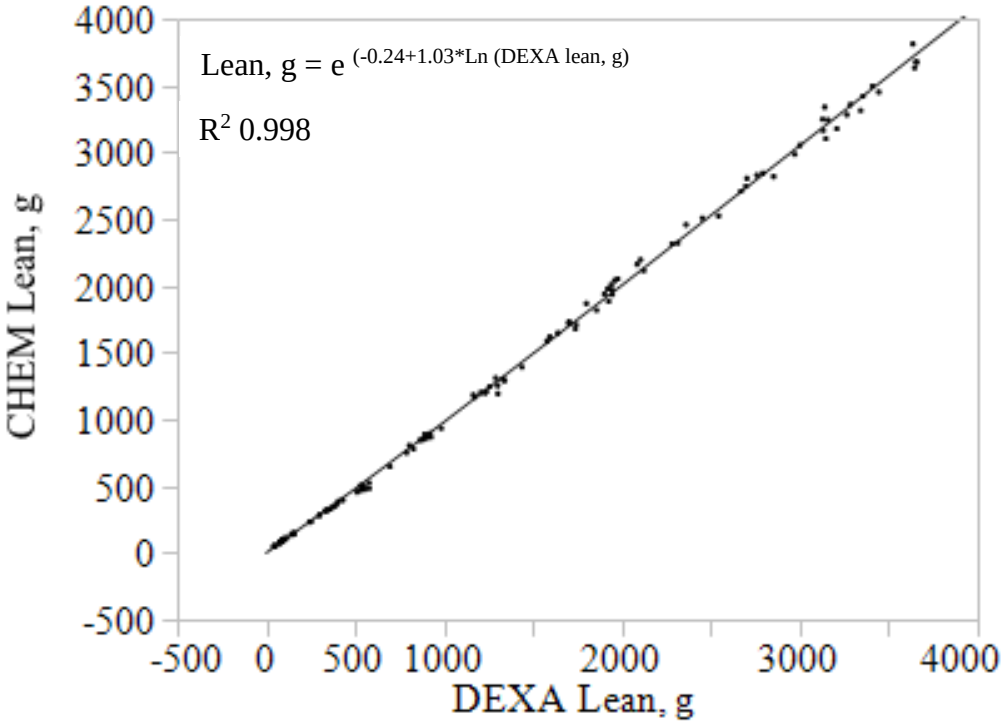


**Figure 6. Fit between Chemical and DEXA analysis for lean and protein mass fed *ad libitum* broilers 1 – 60d (BW 51 – 4690) (exp. 4)**



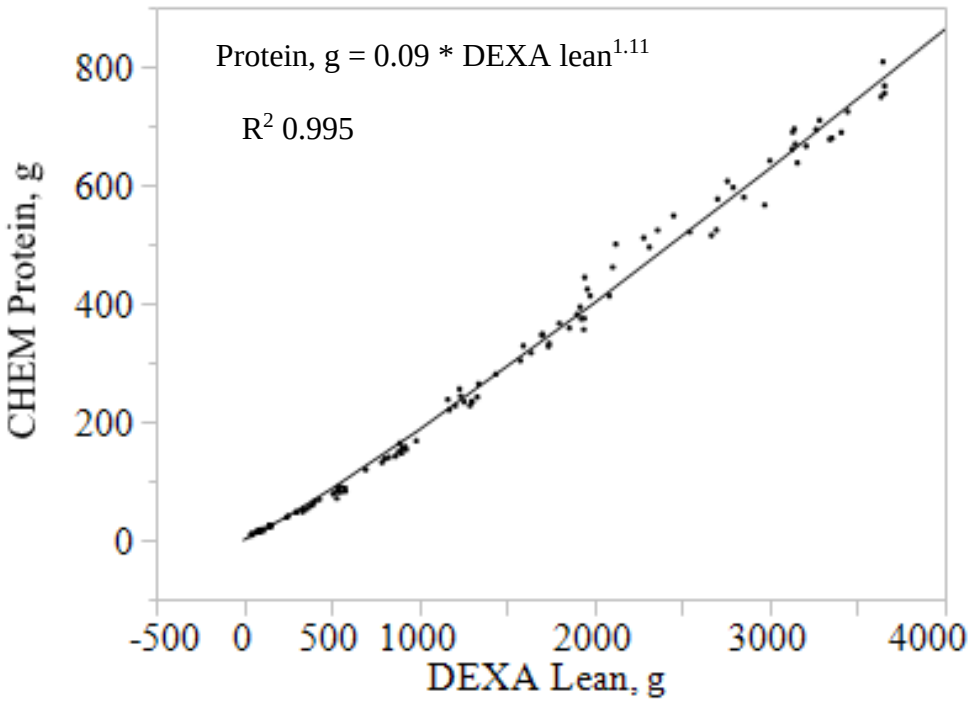
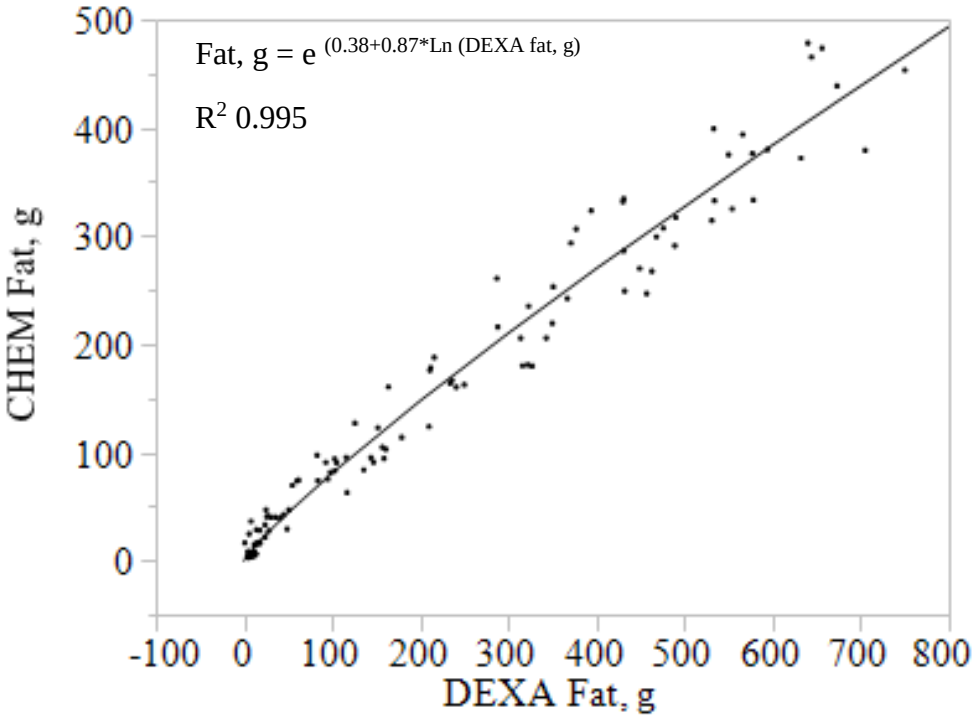



**Figure 7. Fit between Chemical and DEXA analysis for fat mass and body energy fed *ad libitum* broilers 1 – 60d (BW 51 – 4690) (exp. 4)**




Body energy, kcal = -86.8+ 1.48* DEXA lean + 4.20* DEXA fat

$R^2$ 0.973

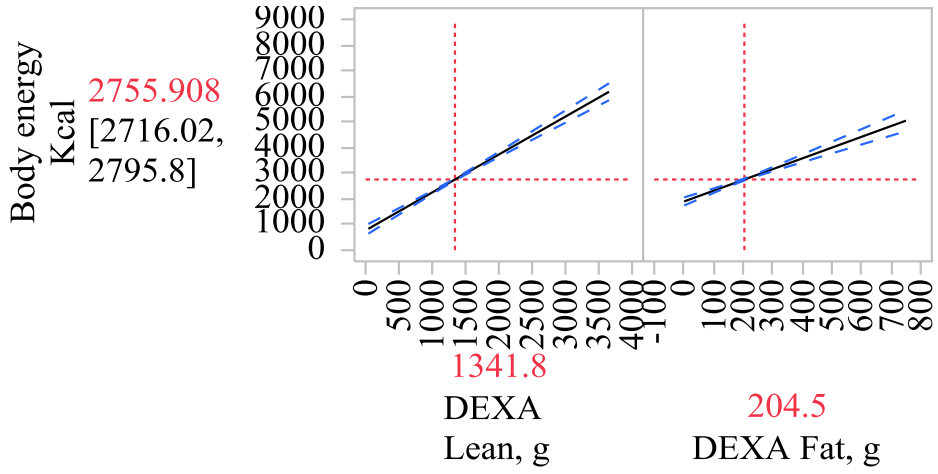


**Figure 8. Fit between Chemical and DEXA analysis for mineral content in fed *ad libitum* broilers 1 – 60d (BW 51 – 4690) (exp. 4)**

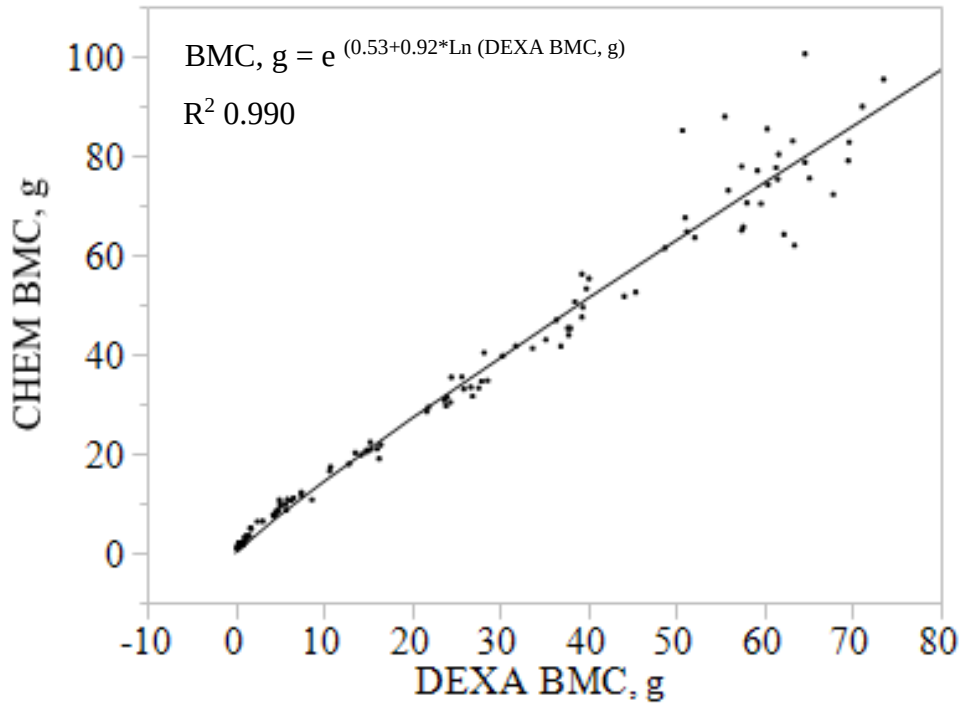
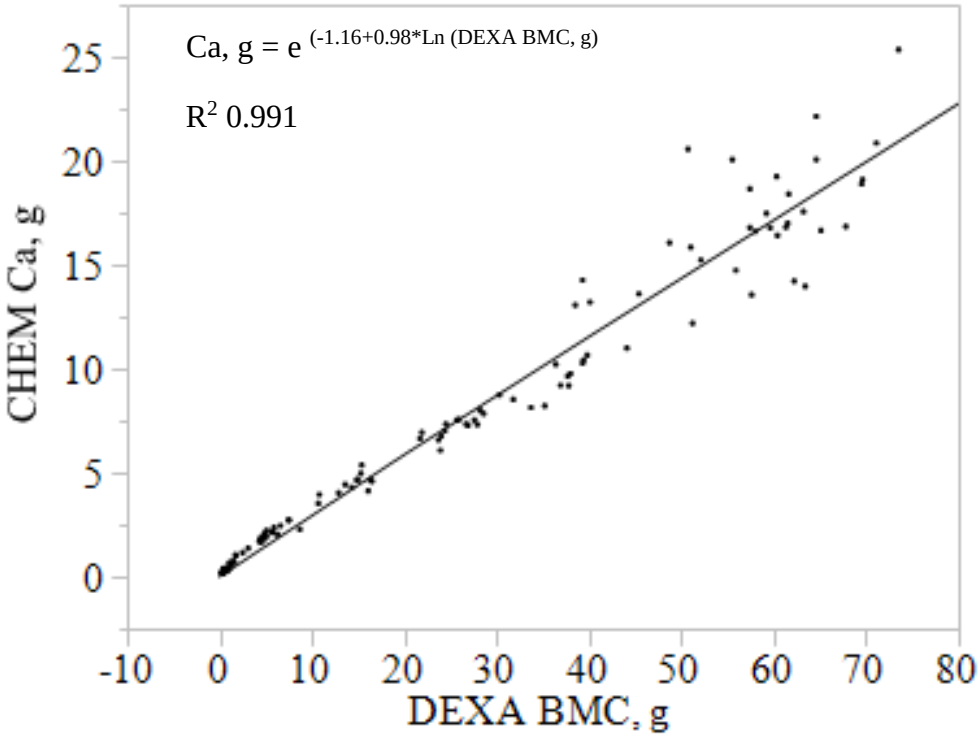
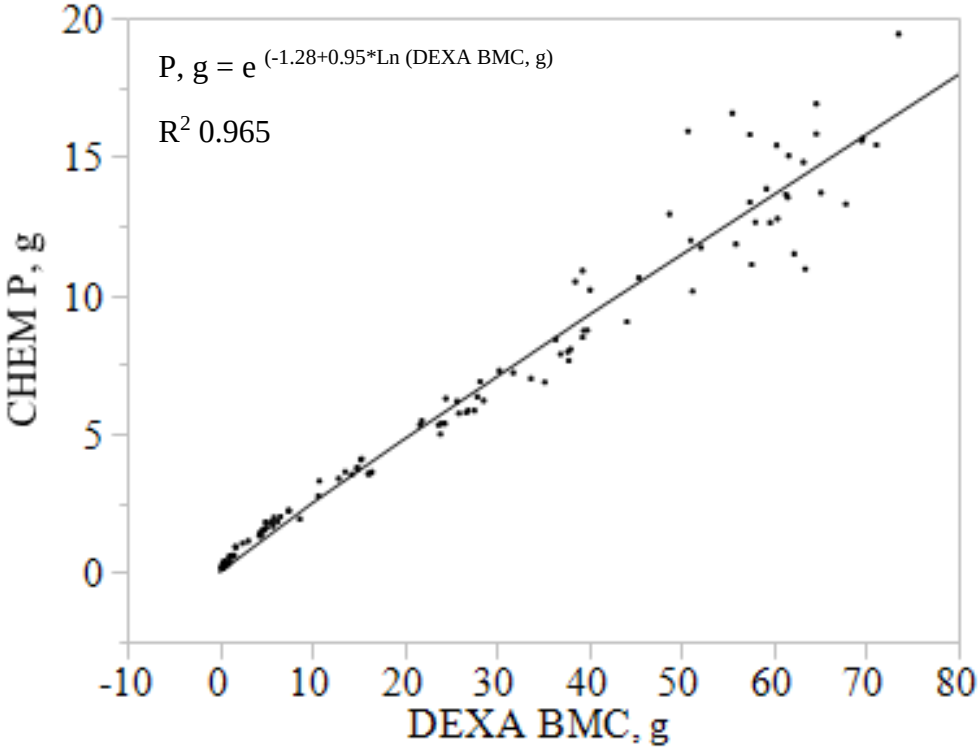


**Figure 9. Fit between Chemical and DEXA analysis for Ca and P content in fed *ad libitum* broilers 1 – 60d (BW 51 – 4690) (exp. 4)**



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# IV. THE EFFECT OF A CARBOHYDRASE AND A PROTEASE IN BROILER DIETS EVALUATED ON HEAT PRODUCTION AND BODY COMPOSITION

**ABSTRACT**

Exogenous enzymes are used in broiler diets to enhance nutrient availability from indigestible component of diets; however the mechanism by which exogenous enzymes work are still under study due to high variability when chemical analysis such as AMEn (apparent metabolizable energy corrected by nitrogen) are use. Three dietary treatments were evaluated in mash form. An exogenous carbohydrase with glucanase as main enzyme and a protease were added to a basal negative control (NC) for a grower broiler study 14 – 21d, in a corn-soybean based diet to study the heat production and body composition. The carbohydrase produced by fermentation of a wild type organism, *Aspergillus aculeatus*, was added in 50 g/MT and a serine protease with chymotrypsin specificity from *Nocardiopsis prasina* expressed in *Bacillus licheniformes* was added in a rate of 75 g/MT to the NC. A total of 600 male Cobb broilers were allocated in floor pens in three consecutive times to allow 6 replications per treatments in the respitatory chambers. Chicks of similar weight (CV 4-5%) were moved to 6 respiratory chambers on d15 and allocated in group of 8 chicks/chamber during the adaptation period (15 – 18d), and 4 chicks/chamber for the evaluation period (19-21d). Heat production (HP) was measured by indirect calorimetry, and body composition with dual energy X-ray absorptiometry (DEXA). Birds fed enzymes showed lower HP in -52 kcal/ kg when fed with the carbohydrase, and -75 kcal/kg when fed the protease ($P \leq 0.021$). Dry matter accretion (13 g/d) and protein accretion (7 g/d) were higher with protease compared to NC (11.5 DM, and 6.1 g/d protein accretion), and carbohydrase (11.5 DM, and 6.3 g/d protein accretion) ($P \leq 0.029$); as consequence the protein content g/kg was also higher with the protease (148.9 g/kg) compared to NC (144.3 g/kg) but not compared to Carbohydrase (146.7 g/kg). Protein efficiency was higher with protease (48.5%) compared to NC (44.5%) and carbohydrase (43.7%) ($P \leq 0.05$). This study shows that indirect calorimetry can be sensitive to

show statistical significance differences of energy savings from adding exogenous enzymes to poultry diets. DEXA as a tool to measure body composition has shown to account protein accretion differences due to addition of a protease. The mechanisms by which carbohydrases and proteases enzymes are reducing HP need further studies. It seems exogenous enzymes are reducing the maintenance energy requirement.

Key words: heat production, protein accretion, broilers, carbohydrase, protease

## INTRODUCTION

Avian species can digest nutrients from the feed to a certain degree. The main nutrients that yield energy for animals are carbohydrates, proteins and fats. Once feed is ingested, the anabolic system turns on the digestive enzymes to degrade the feed and release the nutrients needed for maintenance and growth; however poultry lacks of enzymes for complex carbohydrates (Leeson, and Summers, 2001), and may not produce endogenous enzymes in sufficient amount for the high feed intake of the modern broiler. Poultry diets are based in cereals and legumes such as corn, wheat, sorghum, soybean meal, canola meal, so the digestive enzymes in the chicken gastrointestinal tract should be able to break down nutrients; however nature has given cell walls to these cereals and legumes as a protection against the exterior disorders of weather conditions, insects, etc.…Cellular walls are composed mainly by indigestible carbohydrates for monogastric animals, these non-digestible carbohydrates are not only excreted but also cause problems in the gastrointestinal tract (Choct, 1997). After the phytase boom, carbohydrases and proteases seem to be in line of use for the poultry industry. The majority of the exogenous enzymes in the market are derived from one organism and produced in another organism. For example, the gene encoding production of the protease Ronozyme ProAct (Novozymes, Bagsvaerd, Denmark) originates from *Nocardiopsis prasina* which is the donor microorganism. Then, this gene is moved into a *Bacillus licheniformis* to ensure a safe and efficient production of the protease in large scale. *Bacillus licheniformis* is the host or production organism (Glitsϕ *et al*., 2012). Therefore, high variability can occur between exogenous enzymes depending upon on the donor and the host microorganism. There is also high variability of non-starch polysaccharides between cereals and legumes (Bach Knudsen, 2014) which can influence the response of exogenous enzymes due to enzyme-substrate response. Exogenous enzymes are proteins of high specificity

and work under certain conditions of temperature, pH, and solubility (Berg, 2012). Exogenous proteases have been designed to hydrolyze indigestible proteins; however some multi-component proteases may only increase solubility and not hydrolysis which make them less efficient (Glitsϕ *et al*., 2012). Carbohydrases on the other hand are designed to break down complex polysaccharides. It is important to find a methodology to account for small differences in nutrient utilization using exogenous enzymes to provide the poultry industry options depending upon on the ingredients used for poultry. The objective of the present study is to evaluate 2 exogenous enzymes, a multi-carbohydrase produced by a single microorganism and a protease on the energy and protein utilization during a grower period.

## MATERIALS AND METHODS

All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC) # 12041.

### Diets and Treatments

Three dietary treatments consisted of a negative control (NC), a multi-carbohydrase (C) and protease (P) (Table 1) were evaluated during the grower period 14-21 d in heat production, and body composition evaluation. The basal NC diet consisted of a corn-soybean meal formulated to provide decreased specifications by 100 kcal/kg and decreased amino acids to keep the same ratio as in Cobb 500 nutrient specs (Cobb Vantress, 2012) (Table 2). The multi-carbohydrase was produced by fermentation of a wild type organism (*Aspergillus aculeatus*), the same studied by Ravn *et al*. (2015). The guarantee value of this enzyme is for endo-glucanase with activity of

120 FBG/ml (fungal beta-glucanase units/ml), other enzymes such as hemicellulose, pectinase and mannanase activities have also been proven to occur (Ravn *et al*., 2015); however laboratory procedures are complex to analyzed them and offer a guarantee activity. Carbohydrase with glucanase activity was added in 50 g/MT to the basal diet to produce diet 2 and the protease (P) in 75 g/MT to produce diet 3 (Table 1). The protease is a serine protease with chymotrypsin specificity from *Nocardiopsis prasina* expressed in *Bacillus licheniformes*. The protease is a commercial product and contains 75,000 protease units (PROT/g). One PROT is defined as the amount of enzyme that releases 1 µmol of p-nitroaniline from 1µM of substrate (Suc-Ala-Ala-Pro-Phe-p-nitroaniline) per minute at pH 9.0 and 37°C. Major ingredients such as corn and soybean meal, and minor ingredients such as wheat middlings and distiller's dried grain with solubles (DDGS) were analyzed with NIR, Near Infrared Reflectance, (Bruker, MA, USA), the analyzed spectra was sent to precise nutrition evaluation program, PNE, (Antony, France) for AMEn, digestible amino acids, calcium and total phosphorus. Diets were formulated using Brill formulation software (Feed Management Systems, Hopkins, MN). Diets were fed in mash form and samples of each diet sent for enzyme recovery analysis to an appropriate laboratory (Technical marketing Analytical Services – TMAS- Belvidere, New Jersey). The commercial starter diet provided before the experiment (1 – 14 d) was based in the same ingredients as the grower. It is important to note that the enzyme either C in diet 2 or P in diet 3 were added on-top of the starter diet in order to adapt the microflora population in the chick to the enzymes from the beginning. Negative control chicks were not fed enzymes of feed prior to the evaluation period. However, chicks were selected to have the same average body weight (CV = 4-5 %) between treatments at the beginning of the experiment.

**Birds and Housing**

Six hundred - one day old Cobb male chicks of a commercial strain (Cobb Vantress, Siloam Springs, AR) were obtained from a local hatchery (Cobb hatchery, Fayetteville, AR) in three consecutive times of two hundred chicks per time. Chicks were raised in 4.5 $m^2$ floor pens of 50 chicks per pen from 1 – 14 days. Each pen was equipped with 10 nipples per line, two hanging type feeders, with a round pan that provided 208 cm of feeder space per pen before chicks were transferred to respiratory chambers for heat production (HP) and body composition studies. On day 15, forty eight chicks were moved to respiratory chambers and placed 8 chicks/ chamber; six chambers were available, so three consecutive studies were needed to fulfil at six replications per treatment. An additional ten chicks per treatment (total 30 chicks/ treatment per time) were selected and sacrificed with $CO_2$ inhalation for initial body composition on day 15. Chicks in the chambers were identified, labeling in the shank and kept for 4 d of adaptation to the new chamber environment (d15-d18), followed by 3 d (d19-d21) of evaluation. In the morning of day 19, four chicks of similar body weight (BW) remained in the chamber for evaluation. The other four chicks were discarded based in the body gain with the objective to have low coefficient of variance (CV) between the chicks in the chambers. The normal average CV in a commercial broiler flock is considered to be 10 % (Cobb, 2012), the present study more chicks were placed at the beginning of the study with the objective to have half or less of the average normal variation in a flock (4-5% CV), so BW between treatments were very similar at the beginning of the study.

**Respiratory chambers**

Respiratory chambers were made from polycarbonate plastic glass with 61 cm long x 51 cm wide x 56 cm high equipped with 1 feeder and 1 nipple drinker according to the specifications of FASS, 2010 (Champaign, IL). The room where the respiratory chambers were located was equipped with two heating and air conditioning units. These units were controlled by a Honeywell programmable thermostat that automatically switches between cooling and heating within a 2°C range. Minimum ventilation was provided by two ventilation fans that exhaust to the outside and draw fresh air from the hall. Each ventilation fan was controlled by a timer. The on/off cycle was adjusted as needed to maintain room air quality and desired $CO_2$ levels. To control humidity, the room was equipped with two de-humidifiers (GE, Madison, WI) running continuously in addition to the ventilation system of the room. Relative humidity (RH) ranged from 40-80% at the end of 72 h. evaluation period. Temperature (T) in the chicken house and respiratory chambers were changed according to the genetic broiler management recommendations (Cobb 500, 2012) starting at 33 °C and decreasing 3 degrees °C every week up to 24 °C at 21 days. The light program was 23 h light: 1 h dark.

**Indirect Calorimetry System**

The system is an Open-Circuit Calorimeter (Columbus Instruments, Columbus, OH). Heat is derived by assessment of the exchange of oxygen consumption ($VO_2$) and carbon dioxide ($VCO_2$) that occurs during the metabolic processes. The integrated instrumentation is designed to monitor $VO_2$ consumption and $VCO_2$ production. The system is a mass flow with pull or negative ventilation system. In a negative ventilation, fresh air travels from atmosphere through the vent

into the chamber; then through the air supply line to the ventilation pump and is returned back to the atmosphere. It is the air of the same quality that the animal would be subjected in floor pens or metabolic cages. A small sample drawn for gas analysis is dried to assure the readings are made in a sample that is not under the influence of water vapor air exiting the chamber and there is an ammonia filter in line. There are four filters in line in the system to avoid humid air and strange material get into the sensors. Carbon dioxide, $CO_2$, is measured by the principle of non-disperse infrared absorption (NDIR) with a working range of 0-0.9% and oxygen, $O_2$, is measured by a paramagnetic sensor with a range from 19.3 – 21.5%. The calorimeter has two calibration gases. An offset gas which is pure nitrogen, ultra-grade with certification accuracy <100 ppm total impurities and it is used to calibrate the offset or zero of the $O_2$ and $CO_2$ sensors. The second calibration gas is a set point or span gas mixture of oxygen (20.5%), carbon dioxide (0.50 %), and nitrogen (79%) that has been blended with great precision, after which exact contents are measured and certified by the supplier (Airgas, Springdale, AR). The set point gas is used to calibrate the span or gain of both the $O_2$ and $CO_2$ sensors. The calibration was performed at the beginning of every experiment. This system is fully automated utilizing a computer as a dedicated controller. The sensors are connected to a computer and appropriate software (Oxymax, Columbus, OH) provides volumes of $O_2$, $CO_2$, and RER (Respiratory exchange ratio, $RER = CO_2/O_2$) and finally heat production (HP) is obtained using the equation HP kcal/d = 3.866 $VO_2$ L/d + 1.233 $VCO_2$ L/d (Brouwer, 1965). The gas evaluation in each chamber was measured every 12 minutes, so every chamber unit provided 5 readings during one hour, 120 readings in a day and 360 for three days of evaluation, however the first hour of evaluation of each day after chambers were opened to measure feed intake was discarded while the machine was stabilized.

**Body Composition Analysis**

Birds were humanely sacrificed by $CO_2$ inhalation before body composition was determined by dual energy X-ray absorptiometry, DEXA scanner (GE, Madison, WI) with small animal body software module (Lunar Prodigy from GE encore version 12.2). Validation of DEXA with chemistry analysis was validated in chapter 3 of this manuscript. Birds that underwent gas evaluation in the chambers were scanned for body composition analysis at 21d. Ten birds/treatment were scanned on d15 as starting point for evaluation of fat and protein gain at d21.

**Measurements and Calculations**

The respiration chambers were opened every morning of the evaluation days for excreta collection, feed withdrawal and calibration of gas analyzers. These operations took 45-60 minutes. Volumes of $O_2$ and $CO_2$ were averaged within a day discarding the first hour of evaluation. As mentioned lines above, heat production (HP) was calculated with the equation HP kcal = 3.866 $VO_2$ L/d + 1.233 $VCO_2$ L/d (Brouwer, 1965); and normalized to kg of feed intake. The DEXA body composition was used to determine the type of gain that occurs for the broilers in terms of protein and fat. The type of gain was used to determine the feed value for net energy of gain ($NE_g$) following the equation NEg = fat gain (g) x 9.35 (kcal/g) + protein gain (g) x 5.66 (kcal/g) (Okumura, 1979) and normalized by feed intake. The period of gain accounted the time birds were in the respiratory chambers in each experiment. Energy efficiency (%) was calculated = NEg kcal/ energy intake (kcal of apparent metabolizable energy corrected by nitrogen, AMEn) x 100. Protein efficiency (%) was calculated = body protein gain (g)/ protein intake (g) x 100.

**Statistical analysis**

Every individual chick in the study was evaluated for body composition at the end of the study (N=72, meaning 24 chicks each time); however since 4 chicks were allocated per chamber, the results for body composition were pooled per chamber, having at the end N= 18. For heat production, since about 360 data points were obtained, data was pooled per chamber. A complete randomized block design (CRBD) was performed to account the differences of chicks coming from different flocks. The block was each consecutive study. When ANOVA analysis was significant, the means were separated using t-student test at $P \leq 0.05$. *P -value* was considered significant when $\leq 0.05$ and cite as tendency when $\leq 0.10$.

**RESULTS**

In feed analysis of the principal component of carbohydrase and protease used in the present experiment show recoveries from 100- 117% (Table 3) indicating the enzyme was in the mash diets fed to broilers.

**Heat production**

When exogenous enzymes were added to grower diets from 14-21 d, broilers consumed less oxygen ($VO_2$) per kg of feed. Volume $O_2$ L/kg was lower with carbohydrase (355 L/kg), and protease (351 L/kg) compared to negative control (368 L/kg). These differences accounted for 13 and 17 L/kg with carbohydrase - glucanase (C) and protease (P), respectively ($P \leq 0.031$) compared to a negative control. Volume of carbon dioxide production ($VCO_2$) however show no

significance. The effect of lower $VO_2$ kcal/kg feed, produced lower heat production kcal/kg feed with the enzymes in 52 and 75 kcal with C and P respectively ($P \leq 0.021$) compared to NC (Table 4).

**Body composition and productive parameters**

From the body composition results, broilers fed P had higher dry matter (DM) body composition P (0.259) ($P \leq 0.019$) compared to NC (0.253). Fat body content was lower with the enzymes C (0.263), and P (0.260) ($P \leq 0.022$) compared to NC (0.282). Dry matter body accretion was higher with the Protease in 1.5 g/d ($P \leq 0.025$) compared to Carbohydrase and NC. Protein body accretion was higher with P in 0.90 g/d ($P \leq 0.029$). As consequence of higher body protein accretion, treatment P showed higher body protein g/kg (148.9) ($P \leq 0.027$) compared to NC (144.3) but to carbohydrase (146.7 g/kg). Protease treatment also showed a tendency for lower body fat g/kg (67.3) ($P \leq 0.077$†) compared to NC (70.7). Energy efficiency was no different between treatments ($P > 0.05$) but protein efficiency was higher with protease (48.5%) compared to NC (44.5%) ($P \leq 0.066$†), and Carbohydrase (43.7%) (Table 4). Body gain tended to be higher with P compared to NC and C ($P < 0.09$), no difference in feed intake between treatments were seen ($P > 0.05$). Feed conversion ratio (FCR) was improved with P (1.49) compared to NC (1.62) and C (1.60) ($P < 0.025$) (Table 5). Coefficient of variance (CV) is also presented in Table 5 at 14 d, 18d, and 21d of age. CV is very similar at 14 d, and lower CV with enzymes are seen at 21d, however no significant ($P > 0.286$) maybe due to a short period of evaluation.

**DISCUSION**

It can be argued the fact that the CV of the chicks in the present study was fixed; however other researchers also prefer to work with very uniform chicks during calorimetry studies because body weight is correlated to the energy of maintenance which is included in the heat production analysis measured by indirect calorimetry and the objective of this work was to evaluate heat due to feed intake and no body weight in a short period of time. In addition, the same criterion was used for all treatments, so differences between treatments are valid. Heat production in a production system such as growth is an inefficiency of the system, so less HP is desirable in terms of growth. Heat production is composed of fasting heat production (FHP), physical activity, and thermic effect of feeding or heat increment (Lopez, and Lesson, 2008). Maintenance energy is considered to be composed by FHP as the major component + physical activity. Carbohydrase – glucanase and protease both decreased HP kcal/kg feed, so the enzymes may be decreasing the maintenance energy, and/or thermic effect of feeding. There is few published information using exogenous enzymes with calorimetry studies. Some preliminary abstracts show lower heat production and enzymes lowering HP and improving NE (net energy). For example, Toghyani et al. (2015) reports lower HP and higher NE when using the same carbohydrase – glucanase used in the present study, however with high inclusions of canola meal which is one of the legumes with high amounts of galactomannan, and xyloglucan which are the major substrates for glucanase (Ravn, *et al*., 2015). The calculation of thermic effect of feeding is needed to calculate NE, so it seems that carbohydrases are reducing heat increment according to Toghyani and group. Protease, on the other hand also reduced HP, maybe by providing extra amino acids, so sparing the gastrointestinal system to make more, as a consequence the maintenance energy is reduced. However, further studies are needed to confirm our theory on the

mechanisms of how exogenous enzymes are reducing HP. Protein accretion g/d and content g/kg was higher with protease but not with carbohydrase. Protein accretion was higher from 1-14 and 0-21 d when the same protease of the present study was combined with xylanase + phytase in a corn-soybean meal based diets (Olukosi *et al*., 2008), however no significant when protease was combined to xylanase only. The present study contained phytase in the basal diet, so the negative effect of phytate was already been blocked to allow the enzymes to work with the remaining substrates. Protease may be releasing more amino acids by increasing the digestibility of amino acids of the diets. Protease in combinations with carbohydrases has shown to increase the amino acids digestibility of a large number of amino acids when added in broiler and turkey diets (Barekatain *et al*., 2013, Adebiyi and Olukosi, 2015, Romero *et al*., 2014). Carbohydrases have shown to increase amino acids digestibility (Cowieson, and Bedford, 2009), however the present study show no difference in protein accretion with carbohydrase compared to NC neither protease dietary treatment, maybe due to a small inclusion level 50 g/MT that needed more than 7 days to show positive effects. The efficiency for energy utilization show no difference between treatments (P>0.134), however, the heat production was significantly lower with both enzymes compared to the control. It may be due to different methodology utilized to get the analysis of these two parameters. The energy efficiency is based on the net energy of gain as analyzed by body composition over the AMEn intake kcal, while the heat production was evaluated by analysis of $VO_2$ and $VCO_2$. Heat Production seems to be more sensitive to better explain the productive parameters of the birds in a short period of evaluation as 7 d in the present experiment. FCR was improved with protease which is a result of lower HP and more tissue gain. The protein efficiency was higher with protease because protein accretion was high when the same amount of protein was provided suggesting protease increasing the availability of

amino acids from the indigestible protein. There are few research showing FCR improvement with protease alone, but research when protease was combined with xylanase and amylase showed improved FCR in corn – soybean meal diets in broilers 7 -27 d (Liu *et al*., 2015), and corn-soybean-rapeseed-cotton mixed diets (Tang, et al., 2014).

**Table 1. Dietary treatment**

| N° | Treatments | Abbrev. | Enzyme source | Principal enzyme | Min. content (Units/kg feed) | Grower (14- 21 d) dose level, g/MT |
|---|---|---|---|---|---|---|
| 1 | Negative Control (NC) | NC | - | - | - | - |
| 2 | NC + Carbohydrase | C | *Aspergillus aculeatus* | β- glucanase | 2.5 FBG | 50 |
| 3 | NC + Protease | P | *Nocardiopsis prasina* | Serine protease | 5625 PROT | 75 |

**Table 2. Composition and nutrient calculations (g/100g as fed) of the basal diet**

| Ingredient, % | Grower (14-21 d) | Nutrient composition, % | |
|---|---|---|---|
| Yellow Corn (8.8 % CP) | 59.30 | ***Calculated*** | |
| Soybean meal (46.4% CP) | 25.18 | ME, kcal/kg | 3,008 |
| Wheat middlings | 5.00 | Crude Protein | 20.1 |
| Corn DDGS | 4.00 | Calcium[3] | 0.81 |
| Poultry Fat | 3.04 | Non-phytate phosphorus | 0.41 |
| DL-Methionine | 0.19 | Digestible lysine | 1.02 |
| L-Lysine HCl | 0.16 | Digestible methionine + cysteine | 0.77 |
| L-Threonine | 0.04 | Digestible threonine | 0.67 |
| Calcium Carbonate | 1.23 | Digestible arginine | 1.06 |
| Dicalcium Phosphate | 0.94 | **Analyzed composition** | |
| Sodium Chloride | 0.38 | AMEn, kcal/kg | 3036 |
| [1]Vitamin and mineral premix | 0.54 | Crude protein | 20.5 |
| Propionic acid | 0.05 | | |
| [2]Phytase | + | | |

[1]Supplied 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.
[2]Ronozyme HiPhos, DSM, Nutritional Products LLC, Parsippany, NJ. The Enzyme was included at a rate of 150 g/MT to the basal diet to supply a guaranteed minimum of 1500 FTY/kg of feed.
[3]Includes contribution from phytase of 0.10% Ca and 0.10% digestible P.

**Table 3. Enzyme activity analysis in feed[1]**

| Enzyme | Enzyme Source | Treatment | Enzyme analyzed units/Kg | Target, U/kg | % of Guarantee |
|---|---|---|---|---|---|
| Phytase FTY/kg | *Aspergillus oryzae* | NC | 1561 | 1500 | 104 |
| | | C | 1533 | 1500 | 102 |
| | | P | 1521 | 1500 | 101 |
| Carbohydrase (β-Glucanase[2]) | *Aspergillus aculeatus* | C | 270.3 | 270 | 100 |
| Protease<br><br>PROT/kg | *Bacillus licheniformis* | P | 6599 | 5625 | 117 |

[1]Samples from the diets were analyzed by laboratory of Technical marketing Analytical Services – TMAS- Belvidere, New Jersey.
[2]Analytics for multi-carbohydrases depend on the standard used which could or not have the same units as the guarantee values.

**Table 4. Calorimetry and Body Composition parameters**

| | | | Grower period (14-21 d) | | | | |
|---|---|---|---|---|---|---|---|
| **Evaluation** | **Item** | ***Units*** | **Negative Control (NC)** | **NC + C** | **NC + P** | ***SEM*** | ***P-value*** |
| **Calorimetry parameters** | $VO_2$[1] | *L/kg* | 368.0[a] | 355.0[b] | 351.0[b] | 8.4 | 0.031* |
| | $VCO_2$[2] | *L/kg* | 335.0 | 333.0 | 328.0 | 4.5 | 0.144 |
| | RER[3] | *ratio* | 0.927 | 0.937 | 0.917 | 0.01 | 0.197 |
| | Heat Production[4] | *kcal/kg* | 1824[a] | 1772[b] | 1749[b] | 32.0 | 0.021* |
| **Body composition parameters** | Dry matter, DM | *coef* | 0.253[a] | 0.257[ab] | 0.259[a] | *0.001* | 0.019* |
| | Protein, DM | *coef* | 0.571 | 0.571 | 0.572 | *0.0003* | 0.328 |
| | Fat, DM | *coef* | 0.282[a] | 0.263[b] | 0.260[b] | *0.004* | 0.022* |
| | Protein | *g/kg* | 144.3[b] | 146.7[ab] | 148.9[a] | 0.88 | 0.027* |
| | Fat | *g/kg* | 70.7 | 67.3 | 67.3 | 1.17 | 0.077† |
| | DM accretion | *g/d* | 11.5[b] | 11.5[b] | 13.0[a] | 0.38 | 0.025* |
| | Protein accretion | *g/d* | 6.1[b] | 6.3[b] | 7.0[a] | 0.22 | 0.029* |
| | Fat accretion | *g/d* | 3.9 | 3.5 | 4.0 | 0.20 | 0.292 |
| | Net Energy of gain[5] | *kcal/kg* | 1035 | 992 | 1081 | 28.4 | 0.3091 |
| **Nutrient Efficiency** | Energy Efficiency[6] | *%* | 34.8 | 32.5 | 35.3 | 0.97 | 0.134 |
| | Protein Efficiency[7] | *%* | 44.5[b] | 43.7[b] | 48.5[a] | 1.39 | 0.050 |

Levels (a, b) not connected by same letter are significantly different.
[1]$VO_2$ = Volume of oxygen consumption L/kg of feed/d
[2]$VCO_2$ = Volume of carbon dioxide production L/kg of feed/d
[3]RER = Respiratory exchange ratio $VCO_2/VO_2$
[4]Heat Production = 3.866 $VO_2$ L/d + 1.233 $VCO_2$ L/d (Brouwer, 1965) kcal/kg of feed
[5]Net energy of gain in kcal /kg feed, NEg = (fat gain (g) x 9.35 (kcal/g) + protein gain (g) x 5.66 (kcal/g)) feed intake
[6]Energy efficiency = (NEg kcal/ ME intake, kcal)*100, AMEn = Apparent metabolizable energy kcal
[7]Protein efficiency = (Body protein gain g/ Protein intake, g)*100, *P* –value ≤0.001**, ≤0.05*; ≤0.10†

**Table 5. Productive parameters 14 -21 d**

| Item | *Units* | Negative Control (NC) | NC + C | NC + P | *SEM* | *P-value* |
|---|---|---|---|---|---|---|
| **Body gain** | *g/d* | 58.0 | 58.7 | 65.3 | *2.3* | *0.09†* |
| **Feed intake** | *g/d* | 94.0 | 94.0 | 97.0 | *2.7* | *0.624* |
| **FCR** | *ratio* | 1.62[a] | 1.60[a] | 1.49[b] | *0.03* | *0.025** |
| **CV at 14 d** | *%* | 4.25 | 4.16 | 4.55 | *0.38* | *0.751* |
| **CV at 18 d** | *%* | 4. 98 | 5.26 | 5.52 | *1.14* | *0.943* |
| **CV at 21 d** | *%* | 6.93 | 5.68 | 4.89 | *0.91* | *0.286* |

Levels (a, b) not connected by same letter are significantly different.
FCR feed conversion ratio
CV coefficient of variance

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# V. HEAT PRODUCTION AND BODY COMPOSITION IN BROILERS FED EXOGENOUS MULTI-ENZYME COMPOSITE

**ABSTRACT**

A multi-enzyme composite (NC+EnzC) was fed to male broilers during starter (1-13d) and grower (14-28) (glucanase + xylanase + protease + phytase) and (xylanase + phytase) during the finisher period (29-47), compared to a negative control (NC) diet reduced in 100 kcal/and AA in the same ratio. A total of 1500 male Cobb broilers were allocated in floor pens at the same time and selected gradually for evaluation during starter 5-7 d, 10-12 d, grower 15-17 d, 20-22, 25-27d and finisher 37-39, 45-47 d. During these times, broilers were evaluated but the adaptation to the experimental diets began at d1, d13, d28 for starter, grower and finisher, respectively. Heat production was measured by indirect calorimetry, body composition with dual energy X-ray absorptiometry (DEXA), amino acid digestibility from the lower end of the ileal digest by HPLC. Birds fed enzymes showed higher protein accretion during the starter ($P$<0.079) and finisher ($P$<0.007). In the grower, it seems that a surge of fat accretion in broiler fed enzymes happens after 22d of age ($P$<0.019). Broiler fed enzymes during the first grower period 15-22d showed higher protein g/kg (0.099) and lower RER (respiratory exchange ratio) ($P$<0.079). During the later grower stage 25-27d, broiler fed enzymes showed higher fat deposition ($P$<0.019), and lower protein deposition ($P$<0.004). In the finisher broilers fed enzymes showed higher protein deposition ($P$<0.007), no differences in fat deposition. Heat production in the finisher was lower with the enzymes in 257 kcal/kg ($P$<0.015) because the $VO_2$ consumption $VCO_2$ production was lower as well. Protein efficiency was better with enzymes in the starter in 3.9% ($P$<0.017), no differences in energy efficiency. In the later stage of grower 25-28d, energy efficiency was better with enzymes in 6.4% ($P$<0.09) and lower protein efficiency 10.1 % ($P$<0.041). In the finisher the protein efficiency was better with the enzymes in 10.6% ($P$<0.037) and lower energy efficiency ($P$<0.075). Gompertz and exponential curves explain broiler fed

enzymes grow better than the control. Addition of enzyme composite improved protein accretion in starter and finisher, but the grower. Feed intake was lower in birds fed enzymes during the grower, so there is an opportunity for the enzymes during this period, understanding the protein and fat accretion in the modern bird, will provide tools for decision making using enzymes. Overall, the dynamics of body composition and heat expenditure studies using multi-enzymes bring a new era of research prospects for the future of enzyme utilization in broiler diets. The evaluation of individual enzymes first and then the design of the composites according to the type of diets will increase the opportunities in the use of exogenous enzymes for the poultry industry.

Key words: calorimetry, DEXA, broilers, multi-enzyme

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## INTRODUCTION

The feed industry for monogastric is experiencing the exogenous enzyme era. According to recent analysis, the global market for non-starch polysaccharide (NSP) and protease is around US$ 550 million, even larger than the US$450 million phytase market (Feeinfo, 2014). The enzyme market will continue to increase as more science and technology are being developed to account the value of the enzymes when added to animal diets. The beneficial effects of adding commercial carbohydrases for broiler diets have been known since the 1960's and established since the 1980's. Most of the initial research with carbohydrases was conducted with wheat or barley based diets (Moran and McGinnis, 1965, Classen et al., 1985). Xylanase and β-glucanase fed in wheat and barley based diets has been shown to produce significant improvements using conventional weight gain and FCR performance assays (Bedford, 2000, Slominski, 2011). During the past decade the broiler industry has started to utilize more commercial carbohydrases enzymes because of the high cost of feed energy. The beneficial effects of commercial carbohydrase enzymes added to corn and soybean based diets has produced inconsistent results using traditional assays (Bedford, 2000). Corn and soybean meal based diets with small inclusions of animal/poultry meal have historically been the main ingredients used by the US broiler industry as well as Brazil and other countries. Corn and soybean meal have different types and amounts of non-starch polysaccharides (NSPs) compared to other grains (Bach Knudsen, 1997) and this has created new challenges for enzyme companies to provide appropriate enzyme composites for corn soybean meal diets. The broiler industry is also including more alternate ingredients in their diets such as DDGS from 1 – 11% of inclusion, being in average 3.85%, 5.24% and 6.52% during the starter, grower and withdrawal, respectively in 71 companies over 139 in USA (Agristats, April 2015, Fort Wayne, IN, US).

Sorghum and wheat are also used in small amounts in the grower and withdrawal diets. More efficient evaluation methods are required to account for the beneficial effects of commercial enzymes. An *in vivo* evaluation system needs to be established that is extremely sensitive and can be used for short periods of time during the different phases of the grow-out period. A quick and sensitive assay can support the feed enzyme industry as they determine which enzymes are appropriate for different NSPs and also help clarify the interactive effects of enzyme composites. Traditionally, indirect calorimetry (IC) has been used to account for heat production (HP) from birds being fed different diets, but recently Caldas et al. (2014) has shown IC to be a powerful technique that can help explain metabolism of nutrients and provide a sensitive assay to measure the energy coming from enzymes. Body composition analysis using DEXA (dual X-ray absorptiometry) is also a potential tool (Salas et al., 2012, Caldas, 2015 chapter 3) that may be used together with IC to account for the energy in the meat of chickens fed exogenous enzymes. The IC, and DEXA system could also be useful to identify the mechanism on how carbohydrase enzymes work. Recently, Choct (2010) proposed net energy (NE), instead of metabolizable energy (ME), as the preferred method to account for energy provided by feed enzymes. Teeter et al. (1996) has previously suggested a need for an energy-requirement system that achieves maximum protein deposition with minimal fat accretion. Although lean meat is desired by the consumer, the production of lean meat (protein) requires 380% greater oxygen intake than for fat production (Teeter et al., 1996) which generates a corresponding higher heat increment. The objective of the present study is to study the dynamics of energy and protein utilization measured in heat production and body composition when broilers are fed exogenous enzymes. To the authors' knowledge, this is the first trial in which both heat production and body composition are measured with multi-enzymes in modern broilers.

## MATERIALS AND METHODS

All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC) # 12041.

### Diets and Treatments

Two dietary treatments consisting of a negative control (NC) and NC+ multi-enzyme composite were evaluated in 3 phases of broiler's growth out (starter, grower and finisher). The enzyme composite included the enzymes β-glucanase + β-xylanase+ protease + phytase in different inclusion levels according to feed phase (Table 1). While these 4 enzymes were in starter and grower, β-xylanase+ phytase were the 2 enzymes remaining in the finisher diet. Diets consisted of a corn-soybean meal basal formulated to provide the Cobb 500 nutrient specs (Cobb Vantress, 2012) decreased by 100 kcal energy/kg and decreased amino acids to keep the same ratio as in 2012 specifications (Table 2). Major ingredients such as corn and soybean meal, and minor ingredients such as wheat middlings and distiller's dried grain with solubles (DDGS) were analyzed with NIR (Near Infrared Reflectance) (Bruker, MA, USA). Diets were formulated using Brill Formulation software (Feed Management Systems, Hopkins, MN). Diets were given in mash and samples of each sent for enzyme analysis to an appropriate laboratory (TMAs, Belvidere, NJ). Prior to each period of evaluation for testing the enzyme composite, chicks were fed with enzymes added on-top of the NC diet to help develop the microflora. Negative control chicks were not fed enzymes of feed prior to the evaluation period. Chicks were selected from negative control group and enzyme treated groups, at the beginning of the evaluation period, to have the same starting body weight.

**Birds and Housing**

One day old Cobb male chicks of a commercial strain (Cobb Vantress, Siloam Springs, AR) were obtained from a local hatchery (Cobb hatchery, Fayetteville, AR) and reared in 4.5 $m^2$ floor pens of 40 chicks per pen. Each pen was equipped with 10 nipples per line, two hanging type feeders, with a round pan that provided 208 cm of feeder space per pen before the chicks were transferred for evaluation. Chicks were evaluated during starter from 5 – 7d and 10 – 12d; grower from 15 – 17d, 20 – 22d and 25 – 27d; and finisher from 37 – 39d and 45 – 47d of age in order to have six, nine and six replications during starter, grower and finisher, respectively. One thousand five hundred chicks from the same flock were assorted into two groups of chicks with the same BW at every point of evaluation. One group was moved to respiratory chambers for heat production (HP) measurements and the second group to metabolic cages for body composition and amino acid digestibility studies. This means 120 in the starter, 72 in the grower, and 24 chicks in the finisher were evaluated in the respiratory chambers and the same number of chicks were evaluated in metabolic cages. Additional chicks were taken at the beginning of each study for initial body composition analysis. The number of chicks placed was three times the number needed in order to have the opportunity to select birds from similar BW. All chicks evaluated at different ages were obtained from the same flock at the same time. Chicks were transferred to respiratory chambers and metabolic cages 3 d before each period of evaluation. Chicks were adapted to the experimental diets by feeding at 1, 14, and 28d for starter, grower and finisher diets, respectively. The chambers held ten, four and two chicks/chamber during the starter, grower and finisher respectively. The same number of chicks were placed in wired metabolic cages with dimensions of 91 cm x 30 cm for 1 – 28 d old chicks and 51 cm x 38 cm for 32 – 47 d old chicks. The metabolic cages provided 2 nipple drinkers and a line feeder of 85

cm and 45 cm for both sizes of cages, respectively. The density (chicks/chamber), feeder space and drinkers in the chambers and metabolic cages were set up to comply with the regulation of the Federation of Animal Science Societies (FASS, 2010).

***Respiratory chambers***

Respiratory chambers were made from polycarbonate plastic glass (61 cm long x 51 cm wide x 56 cm high) and equipped with 1 feeder and 1 nipple drinker according to the specifications of FASS, 2010 (Champaign, IL). The room for the respiratory chambers was equipped with two heating and air conditioning units. These units are controlled by a Honeywell programmable thermostat that automatically switches between cooling and heating within a 2°C range. Minimum ventilation was provided by two ventilation fans that exhaust to the outside and draw fresh air from the hall. Each ventilation fan is controlled by a timer. The on/off cycle can be adjusted as needed to maintain room air quality and desired $CO_2$ levels. To control humidity, the room was equipped with two de-humidifiers (GE, Madison, WI) running continuously. Relative humidity (RH) ranged from 40-80% at the end of 72 hr. evaluation period. Temperatures (T) in the room with chambers and respiratory chambers were changed according to the genetic broiler management recommendations (Cobb 500, 2012) starting at 33 °C and decreasing 3 degrees °C every week. The light program was 23 hr. light: 1 hr. dark for all feeding periods.

**Indirect Calorimetry System**

The system is an Open-Circuit Calorimeter (Columbus Instruments, Columbus, OH). Heat is derived by assessment of the exchange of oxygen and carbon dioxide that occurs during the

metabolic process. The integrated instrumentation is designed to monitor oxygen consumption and carbon dioxide production. The system is a mass flow with pull or negative ventilation system. In a negative ventilation system, fresh air travels from atmosphere through the vent into the chamber; then through the air supply line to the ventilation pump and is returned back to the atmosphere. It is the air of the same quality that the animal would be subjected in floor pens or metabolic cages. The flow used in this experiment was 2, 6 and 12 liters per minute LPM/bird for starter, grower and finisher, respectively. A small sample drawn for gas analysis is dried to assure that the readings are made in a sample that is not under the influence of water vapor air exiting the chamber and there is an ammonia filter in line. There are four filters in line in the system to avoid humid air and strange material getting into the sensors. Carbon dioxide, $CO_2$, is measured by the principle of non-disperse infrared absorption (NDIR) with a working range of 0-0.9% and oxygen, $O_2$, is measured by a paramagnetic sensor with a working range from 19.3 – 21.5%. The calorimeter has two calibration gases. An offset gas which is pure nitrogen, ultra-grade with certification accuracy <100 ppm total impurities and it is used to calibrate the offset or zero of the $O_2$ and $CO_2$ sensors. The second calibration gas is a set point or span gas mixture of oxygen (20.5%), carbon dioxide (0.50 %), and nitrogen (79%) that has been blended with great precision, after which exact contents are measured and certified by the supplier (Airgas, Springdale, AR). The set point gas is used to calibrate the span or gain of both the $O_2$ and $CO_2$ sensors. The calibration was performed at the beginning of every experiment. This system is fully automated utilizing a computer as a dedicated controller. The sensors are connected to a computer and appropriate software (Oxymax, Columbus, OH) that provides volumes of $O_2$, $CO_2$, and RER (Respiratory exchange ratio, RER = $CO_2/O_2$). Heat production (HP) is obtained using the equation: HP kcal/d = 3.866 $VO_2$ L/d + 1.233 $VCO_2$ L/d (Brouwer, 1965). The gas

evaluation in each chamber was measured every 12 minutes, so every chamber unit provided 5 readings during one hour, 120 readings in a day and 360 for three days of evaluation.

**Body Composition Analysis**

Birds were humanely sacrificed by $CO_2$ inhalation before body composition was determined by dual energy X-ray absorptiometry, DEXA scanner (GE, Madison, WI) with small animal body software module (Lunar Prodigy from GE encore version 12.2). Validation of DEXA with chemistry analysis was done by Caldas, 2015 (Chapter 3). Chicks from the chamber studies were scanned at end of every point of evaluation. Ten, ten and five chicks of same BW were selected per treatment at each point of evaluation, in the starter, grower and finisher, respectively, and were scanned at the beginning of every experiment to have a basal body protein and fat content. The stages evaluated were starter from 5 – 7d and 10 – 12d; grower from 15 – 17d, 20 – 22d, and 25 – 27d; and finisher from 37 – 39d and 45 – 47d of age.

**Measurements and Calculations**

The respiration chambers were opened every morning during the evaluation period for excreta collection, feed withdrawal and calibration of gas analyzers. The experimental operations took 45-60 minutes. Volumes of $O_2$ and $CO_2$ were averaged within a chamber for each period of evaluation. As mentioned before, heat production (HP) was calculated with the equation: HP kcal = 3.866 $VO_2$ L/d + 1.233 $VCO_2$ L/d (Brouwer, 1965). Heat production was normalized to kg of feed intake to express the heat due to the feed. DEXA body composition was used to determine the type of gain that occurs for the broilers in terms of protein and fat. The type of gain was used to determine the

feed value for net energy of gain ($NE_g$): NEg kcal = fat gain, g x 9.35 (kcal/g) + protein gain, g x 5.66 (kcal/g) (Okumura, 1979), then NEg was normalized by feed intake. The period of gain was three days, at each point of evaluation. Energy efficiency (%) was calculated as EE = NEg kcal/ energy intake (kcal of apparent metabolizable energy corrected by nitrogen, AMEn) x 100, and Protein efficiency (%) was calculated as PE = body protein gain (g)/ protein intake (g) x 100.

**Laboratory analysis**

The analysis of AMEn (apparent metabolizable energy corrected by nitrogen) involved analysis of gross energy, dry matter and nitrogen in feed and excreta. Gross energy (GE) was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Dry matter was analyzed by method 934.01 (AOAC, 1990) and nitrogen determined by the method 990.03 (AOAC, 1995). The AMEn assay was conducted by the classical total excreta collection method. The birds were adapted to the experimental diets from 3-10 d before excreta collection (3 d) in each period of evaluation. On the third day of collection, the excreta was pooled within a metabolic cage, mixed, and representative sample (120 g) was lyophilized in a freeze drier. The lyophilized excreta sample was ground with a commercial grinder to pass through a 0.5-mm sieve and sent to the Central Laboratory at the University of Arkansas for chemical analysis. Apparent ileal digestibility of amino acids was measured after the birds were scanned by DEXA. The ileal content was collected approximately 15 minutes after the birds were scanned. The ileum was defined as the portion of the small intestine extending from vitelline diverticulum to a point 40 mm proximal to the ileo-cecal junction. 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. 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. The marker, titanium dioxide ($TiO_2$) was measured on a UV spectrophotometer following the methodology of Myers (2004). Briefly, 0.35 g of $K_2SO_4$, 0.04 g of $CuSO_4$, and 0.1 g of excreta or feed was added to glass test tubes and diluted with 3 mL of 18M $H_2SO_4$ to be heated at 120°C for 24 hr. in a block digester. Contents of the digestion tube were allowed to cool for 15 min, after which 7mL of distilled deionized water was added to the digested sample, gently mixed and transferred to new plastic test tubes. This previous step was repeated using 2 mL of distilled deionized water. Diluted digested samples were centrifuged at 3000 rpm for 22 min to recover the supernatant using a filter paper. After mixing 0.20 mL of distiller

deionized water, 1mL of supernatant, 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.

**Statistical analysis**

A complete randomized block design (CRD) with a factorial arrangement (Treatment x Age) was performed evaluating starter, grower and finisher independently because of the different multi-enzyme composition in each phase. ANOVA was analyzed and when the means were significant, t-student test was used. *P -value* was considered significant when $\leq 0.05$ and cite as tendency when $\leq 0.10$. A Gompertz 3P model was fitted between Y = body protein gain vs X= age; and Y= body fat gain vs X =age, and taking diet as group, so both treatments can be displayed in the graph. Equivalence test was performed to compare the NC curve and the NC+EnzC curve at $P<0.05$. Gompertz model was used to fit all feed stages (starter, grower, and finisher); however, there is a behavior shown during the grower period, so an exponential 2P model was fitted for starter and grower for the same parameters as in Gompertz. All statistical analysis were performed using JMP 12 (SAS institute, 2015).

**RESULTS**

In feed analysis of the principal components of the multi enzymes used in the present experiment show recoveries from 91- 140% (Table 3) indicating the enzyme was present in the diets.

**Performance, body composition and calorimetry data**

Performance, besides the feed intake having a tendency ($P<0.063$) to be lower in broilers 15-22 d fed the multi-enzyme composite (NC+EnzC) compared to negative control (NC), was no different until the finisher period. The multi-enzyme composite treatment in the finisher showed better body gain in 28 g ($P<0.011$) and FCR improved 2.01 NC vs 1.61 NC+EnzC ($P<0.003$) (Table 4), no differences in feed intake was found. In the starter period, body composition as g/kg (5-13 d) showed higher body protein g/kg for NC+EnzC ($P<0.029$) but no difference was found when the protein was expressed in dry matter (DM). On the other hand, body fat g/kg was lower as expected ($P<0.007$) for the multi-enzyme treatment in both as is and DM basis ($P<0.008$). Body dry matter and body protein accretion g/d had a tendency to be higher with NC+EnzC ($P<0.059$) and ($P<0.079$) respectively, but fat accretion with the NC+EnzC was significantly lower 1.5 g/d compared to NC 1.8 g/d ($P<0.029$). There was no difference in heat production parameters during this period. Because of the higher protein accretion with the NC+EnzC, the protein efficiency was higher in + 3.9% ($P<0.017$). In the grower period, three points of evaluation 15-17, 20-22, and 25-27 were designed at the beginning of the experiment; however the first two points were separated from the last one (25-27) because the birds showed and interaction between age and treatment that was not expected. While the data show no significant differences between treatments for most of the variables in the periods 15-22 d, significant differences are shown from 25-27d. In the period 15-22 d, there is a tendency of higher body protein composition with the multi- enzyme 150.3 vs 146.8 g/kg ($P<0.090$), there is also a tendency of lower RER (respiratory exchange ratio) with the multi-enzyme treatment (0.945 vs 0.964 NC) ($P<0.079$) meaning more fat or protein or both being oxidized more compared to the broilers in the NC. The stage 25-27 d, show an opposite response of the

previous grower stage. The body protein was lower with multi-enzyme treatment (162.7 vs 169.0 NC) ($P$<0.007), however no difference when expressed in dry matter. The fat content on the other hand was higher for the multi-enzyme treatment (83.6 g/kg vs 74 g/kg NC) ($P$<0.007), the body fat expressed in DM was also higher significantly with the multi-enzyme ($P$<0.002). The body DM, protein accretion was lower for the multi-enzyme treatment ($P$<0.04) for both compared to NC, and fat accretion was higher ($P$<0.019). Due to a higher fat accretion, the NEg kcal/kg tended to be higher with the multi-enzyme ($P$<0.09). As mentioned in the methods, the calorific value for fat is higher (9.35 kcal/g) than protein's calorific value (5.66 kcal/g), so whenever, fat accretion is higher, there is a high possibility that the NEg kcal/kg will be higher, unless the feed intake difference is large between the treatments. The calorimetry data show no difference in this late stage of the grower. The energetic efficiency tended to be higher with the multi-enzyme in + 6.4 % ($P$<0.09) but lower protein efficiency in – 10.1% ($P$<0.041). (Table 4). In last period of evaluation, the finisher, where only two enzymes (xylanase + phytase) were part of the multi-enzyme, body protein composition g/kg were higher again with the enzymes as in the starter period. Broilers fed enzymes in the finisher showed more body dry matter coefficient (0.310 vs 0.305) ($P$≤0.039*) and less body fat in DM (0.307 vs 0.338 NC). Body protein g/kg resulted higher in broiler fed enzymes (179.9 vs 175.0) ($P$≤0.005*) and less body fat g/kg (94.5 vs 102.4) ($P$≤0.007*). Body dry matter accretion higher in broilers fed enzymes in 7.6 g/d more ($P$≤0.001*), body protein accretion was also higher in this chicks with 4.8 g/d more ($P$≤0.007). As a result of the higher protein accretion in broilers fed enzymes, protein efficiency was higher in 10.6% ($P$≤0.037), and energy efficiency tended to be lower in 5.8% ($P$≤0.075). Body fat accretion g/d was not different. Regarding the calorimetry parameters, birds fed enzymes showed lower $VO_2$ (55 L), $VCO_2$ (37 L) and as consequence lower HP (257 kcal) when expressed as kcal

per kg feed intake at ($P \leq 0.011$), ($P \leq 0.09$) and ($P \leq 0.015$) respectively. This means less waste of energy as heat was produced when broilers were fed NC+EnzC. The classical Gompertz non-linear, growth curve was fit for protein gain g/d (starter to finisher) showed that birds under NC+EnzC treatment differed from the NC curve ($P \leq 0.05$), $R^2$ 0.77 (Figure 1). The asymptote, meaning when the line that approaches zero as it tends to infinity is higher with the NC+EnzC treatment (18.73 g/d vs 14.12 g/d NC). This may be due to the higher protein gain in the finisher that pulls the curve higher than the curve of the NC. The growth rate, however was lower with the enzymes (0.08 vs 0.14) and the inflection point is higher with the NC+EnzC (14.97 g/d vs 11.91g/d NC) meaning the body protein gain in chicken fed the enzymes smooths at higher gain than the control group, probably because there is still genetic potential for growth and the enzymes are providing those extra nutrients for growth. The equivalence test performed to test equality between curves showed the curves are not equal ($P \leq 0.05$). Because the body protein gain is higher at the end, the starter and grower periods seems not to be noticed, so when only these two periods were fitted (Figure 2). An exponential curve provide a better explanation for protein gain during starter and grower. In these early stage it can be noted that the curves follow a different pattern. The growth rate for protein accretion is lower with the enzymes 5.3% vs 6.9% NC of age; however, the scale is higher for the NC+EnzC 2.93 vs. 2.20 which means the enzyme treatment is having more protein accretion at the starter period. The fit fat gain vs age (Figure 3) shows the opposite pattern as the body protein gain with enzymes. The point at which the fat gain in broilers fed enzymes flattens is at a lower fat gain compared to the control (7.01 g/d vs 9.15 g/d) respectively. In the same manner as body protein gain, the body fat gain was fitted only for starter and grower data (Figure 4) with an exponential fit. The growth rate for fat gain is higher with enzymes compared to NC (12% vs 7.9%) respectively. Before 22 d, broilers fed

enzymes show lower fat gain compared to the control but after 22 d, the line of fat gain becomes higher than the control. The scale for fat gain is lower with the enzymes, meaning chicks eating enzymes were coming with lower fat in the body, which matches the lower fat composition of the birds during the starter when fed enzymes.

### *Amino acid digestibility (AAD)*

During the starter period, broilers fed exogenous enzymes showed higher AAD for Cys, Thr, Arg, Phe, Ser, and Asp ($P \leq 0.05$*) and tendency to be higher in Lys, Met, Iso, Tyr, Gly, His, Ala, and Gly ($P \leq 0.097$†). The overall mean for AAD in broilers fed enzymes in the starter was 80.7 % vs 74.5 % ($P \leq 0.050$*) (Table 5). During the grower period, AAD with enzymes were higher for Lys, Arg, Leu, Phe, Ala ($P \leq 0.001$**) and tendency to be higher for Val, Tyr, Gly, His, and Asp. The overall AAD in the grower with the enzymes was 76.3 % vs 73.0% ($P \leq 0.044$*). In the finisher, the AAD in birds fed exogenous enzymes showed only a tendency to be higher in Lys and Tyr ($P \leq 0.095$†). The overall mean AAD was not significant between treatments in the finisher.

## DISCUSION

The positive effect of the enzymes in the performance of chicks at the end of the experiment, finisher period, may be due to an additive effect of the enzymes in the metabolism of the bird because these birds were fed the enzymes on top of their diet, even though the birds were selected to have the same weight at the beginning of each study. The birds were selected from > 60 quartile of the growth curve population for both treatments, so bigger birds were evaluated in

order to give better opportunity to the birds to show their genetic potential under experimental conditions. Studies of feed efficiency and mitochondrial function has shown differences in feed efficiency between small and big chicks from the same genetic line and fed the same diet (Bottje, et. al., 2006); therefore, reducing the coefficient of variance (CV) for the present study may have helped to see differences just due to the diets. Broilers fed exogenous enzymes had higher body protein composition g/kg and accretion g/d and less body fat content g/kg and less fat accretion g/d in the starter period resulting in higher protein efficiency in 3.9% from 5-13 d. At low feed intakes, the metabolism prioritizes protein synthesis (Boekholt, 1997) as in this case, the starter period has low feed intakes compared to later stages (37 g/d starter, 99 g/d grower, 140 g/d finisher), however this does not mean the bird is not synthesizing fat. The fat accretion is also occurring but in lower amounts. The multi-enzyme composite fed to broilers in the starter period may be releasing more amino acids which is supported by the increased amino acid digestibility evaluated in this study. The enzymes in the starter diet, increased the digestibility of essential and non-essential amino acids in different percentages. The average AAD was improved by 6.4% ($P \leq 0.05$). The highest improvement was in the digestibility of serine, cysteine, threonine, and glycine (>7% improvement). High amounts of these amino acids conform the principal proteins on the mucins of the intestine (Lobley *et al*., 1999). Heat production (HP) show no significance in the starter, it may be due to residual yolk being utilized during the first week of age that avoid to see differences in heat with enzymes, maybe more replications can help to show differences at this stage. In the grower period, the separation of the data in small chicks 15-22 d and big 25-27 d chicks could be a controversial decision; however, it explained better the dynamics of protein and fat gain in modern broilers fed enzymes in the grower and help elucidate what is happening with the body composition at this early stage that can compromise the protein

and fat gain at a later stage when the birds gets to the BW for commercialization. According to Boekholt (1997)'s experiment in which he showed that broilers direct nutrients to protein deposition before fat; therefore, as more nutrients are available, the bird will increase fat accretion relative to protein. In the first part of the grower 15-22d, broilers fed exogenous enzymes, show no protein or fat accretion; however in the next phase 25-27d, broilers fed enzymes showed higher fat accretion, and lower protein accretion with no difference in performance. Unlike Boekholt's study, in this case, feed intake increases in the finisher period in birds fed exogenous enzymes, the protein accretion increased relative to fat accretion. This may be due to the BW at which Boekholt finished the experiment was at 1500 g, but the present experiment, after 18 years, had broilers up to 47 d with 3160 g. In addition, the modern broilers are continuously selected for higher lean body gain than 18 years before. The higher fat and less protein accretion in the later stage of grower, 25-27d remains unclear. Until this point the birds fed enzymes received 4 different exogenous enzymes glucanase + xylanase + protease and phytase. The individual and mixed effect of these enzymes is suggested to be studied to explain more about this trend. A study by Olukosi *et al*. (2008) showed differences on protein and fat accretion when birds were fed a similar composite (phytase + xylanase + amylase + protease) than the present experiment, from 0-21 d compared to a NC. In these case both, protein and fat increased when the enzymes were fed to broilers. During the grower period 15-22d, the Respiratory exchange ratio (RER) was lower with enzymes. RER represents the type oxidation of nutrients. Fat oxidation corresponds a 0.70 ratio, starch oxidation 1.0 ratio; and protein oxidation 0.73 ratio (McLean & Tobin 1987), since the feed is a balanced diet, and the only difference of the treatments are the enzymes composite addition, a smaller number could mean even more fat and/or protein or both oxidation being happening when enzymes are added in

smaller birds during the grower. This smaller RER value explains why more body protein composition happens with enzymes at this stage, 15-22 d. The amino acid digestibility was higher in the birds fed enzymes during the grower. No differences was found in AAD between the three different points of grower evaluation, so the samples were pooled. During the grower period, less amino acids became significant in broiler fed enzymes compared to the starter period. Lysine, arginine, valine, leucine, phenylalanine, tyrosine, glycine, histidine and aspartic acid were higher than the NC. The overall grower AAD was 3.3% more for the enzyme treatment. On the other hand, because of a higher fat accretion in the grower 25-27 d, the energy efficiency was higher in broilers fed the multi-enzyme composite and lower protein efficiency because of the lower protein being retained. It was not expected to have higher protein accretion in the finisher period when more feed intake is seen; however, this experiment showed that broilers with higher feed intake fed the multi-enzyme composite had more protein accretion compared to fat accretion. This may be due to the birds had more room for the genetic potential to synthesize more protein and for feed intake to reach the highest point for fat accretion. The daily average feed intake from 39-47d in the present experiment was 153 g/d with the enzymes, however, this intake is still far from 228 g/d recommended by Cobb (2012), so the birds has still potential for higher protein accretion when the enzymes were added. In the finisher only phytase + xylanase were part of the composite, but the effect on more protein accretion maybe due to the fact that these birds were fed with exogenous enzymes even before of the point of evaluation. There was no difference in the amino acid digestibility. Many explanations can be found in for the lack of DDA in the finisher but the fact is higher variability was seen because the ileal digesta was collected from 2 chicks which is lower than the number of chicks that were used in the starter and grower study 10 and 4 respectively to make 1 sample or replication. More chicks

maybe be needed to evaluate AAD at later stages of growth. What is clear is that broilers grew better and ate more than the NC during the finisher period, so this change is beneficial in the broiler industry since meat is more desirable than fat at commercialization age. The heat production in the finisher was 257 kcal/kg lower in birds fed enzymes compared to NC. This means less waste of energy in the metabolism occurred because of the addition of enzymes in the finisher. The dynamics of body composition and heat expenditure studies using multi-enzymes bring a new era of research prospects for the future of enzyme utilization in broiler diets. The evaluation of individual enzymes first and then the design of the composites according to the type of diets used will increase the opportunities in the use of exogenous enzymes for the poultry industry.

**Table 1. Dietary treatments**

| N° | Treatments | Abbrev. | Enzyme source | Principal enzyme | Minimum content (Units/kg feed) | Inclusion g/MT | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Starter 1-13 d | Grower 14- 28 d | Finisher 32-47 d |
| 1 | Negative Control | NC | - | - | - | - | - | - |
| 2 | NC+ Multi-enzyme composite | NC+ EnzC | Carbohydrase from Aspergillus aculeatus | β- glucanase[1] | 2.5 FBG | 50 | 50 | - |
| | | | Carbohydrase from Trichoderma longibrachiatum | endo- 1,4 β xylanase[2] Cellulase | 23.6 U 70 – 87 U | 100 | 100 | 125 |
| | | | Protease from Nocardiopsis prasina | Serine protease[3] | 5625 - 3750 PROT | 75 | 50 | - |
| | | | Phytase from Aspergillus oryzae | Phytase[4] | 1500 FTY | 150 | 150 | 150 |

[1]This carbohydrase is a multicomponent enzyme produced by submerged fermentation of *Aspergillus aculeatus*; however it has been standardized only for β-glucanase (*endo-1, 3 (4)-β-glucanase)*, so it has a guarantee value when analyzed in the final feed. The product also has hemicellulose and pectinase activities (Ravn, et al., 2015). Only the principal enzyme is cited because of the difficulty in the analysis of the other components. The analysis of the principal enzyme will be reported in results.

[2]The carbohydrase Xylanase is produced from *Trichoderma longibrachiatum*. It contains xylanase, cellulase, and *-1, 4-β-glucanase, and endo-1, 3 (4)-β-glucanase*. However, only 1, 4 endo-xylanase and cellulose are cited as principal enzymes because it has been standardized for this enzyme and guarantee values are provided.

[3]The serine protease has chymotrypsin specificity from Nocardiopsis prasina expressed in *Bacillus licheniformes*.

[4]The phytase is produced from *Aspergillus Oryzae.* The activity is 1500 FTY/kg of feed. The phytase is included on top the basal diet (NC) that has already 500 FTY/kg

**Table 2. Composition and nutrient calculations (g/100g as fed) of the basal diet**

| Ingredient, % | Starter 1-13 d | Grower 14-28 d | Finisher 29-47 d |
|---|---|---|---|
| Yellow Corn (8.27% CP) | 56.63 | 61.54 | 61.09 |
| Soybean meal (47.4% CP) | 33.60 | 27.20 | 24.41 |
| Wheat middlings (16.7%CP) | 1.50 | 2.00 | 3.00 |
| Corn DDGS (29.4% CP) | 2.50 | 3.40 | 5.00 |
| Poultry Fat | 1.32 | 1.69 | 2.67 |
| DL-Methionine | 0.31 | 0.27 | 0.23 |
| L-Lysine HCl | 0.31 | 0.32 | 0.26 |
| L-Threonine | 0.14 | 0.13 | 0.12 |
| Calcium Carbonate | 0.92 | 0.92 | 0.91 |
| Dicalcium Phosphate | 1.31 | 1.13 | 0.87 |
| Sodium Chloride | 0.36 | 0.36 | 0.35 |
| Vitamin and mineral premix[1] | 0.54 | 0.54 | 0.54 |
| Propionic acid | 0.05 | 0.05 | 0.05 |
| Titanium dioxide | 0.50 | 0.50 | 0.50 |
| Phytase[2] | | + | |
| ***Calculated composition*** | | | |
| ME, kcal/kg | 2,935 | 3,008 | 3,080 |
| Crude Protein | 22.3 | 20.1 | 19.9 |
| Calcium[3] | 0.87 | 0.81 | 0.74 |
| Non-phytate phosphorus | 0.44 | 0.41 | 0.37 |
| Digestible lysine | 1.14 | 1.02 | 0.92 |
| Digestible methionine + cysteine | 0.85 | 0.77 | 0.72 |
| Digestible threonine | 0.74 | 0.67 | 0.63 |
| Digestible arginine | 1.20 | 1.06 | 1.00 |
| **Analyzed composition** | | | |
| AMEn, kcal/kg | 2827 | 2954 | 3174 |
| Crude protein | 21.5 | 20.4 | 19.8 |
| Digestible lysine | 1.32 | 1.22 | 1.15 |
| Digestible methionine + cysteine | 0.89 | 0.74 | 0.76 |
| Digestible threonine | 0.75 | 0.61 | 0.60 |
| Digestible arginine | 1.06 | 0.95 | 0.89 |

[1]Supplied 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.

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

[3]Includes contribution from phytase of 0.10% Ca and 0.10% digestible P

**Table 3. Enzyme activity analysis in feed[1]**

| Enzyme | Treatment | Diet | Enzyme analyzed units/Kg | Target, U/kg | % of Guarantee |
|---|---|---|---|---|---|
| β-Glucanase U/kg[2] | NC+EnzC | Starter | 85 | 80 | 106 |
| | | Grower | 89 | 80 | 111 |
| Xylanase, U/kg[3] | NC+EnzC | Starter | 315 | 270 | 117 |
| | | Grower | 273 | 270 | 101 |
| | | Finisher | 358 | 336 | 107 |
| Xylanase, FYT/kg[4] | NC+EnzC | Starter | 1788 | 1500 | 119 |
| | | Grower | 1801 | 1500 | 120 |
| | | Finisher | 1921 | 1500 | 128 |
| Cellulase, U/kg[5] | NC+EnzC | Starter | 85 | 70 | 121 |
| | | Grower | 98 | 70 | 140 |
| | | Finisher | 108 | 87 | 124 |
| Protease PROT/Kg | NC+EnzC | Starter | 6967 | 5625 | 124 |
| | | Grower | 3455 | 3750 | 92 |
| Phytase, FTY/Kg | NC | Starter | 440 | 500 | 88 |
| | | Grower | 455 | 500 | 91 |
| | | Finisher | 460 | 500 | 92 |
| | NC+EnzC | Starter | 2552 | 2000 | 128 |
| | | Grower | 2116 | 2000 | 106 |
| | | Finisher | 1921 | 2000 | 96 |

[1]Samples from the diets were analyzed by laboratory of Technical marketing Analytical Services – TMAS- Belvidere, New Jersey.
[2]When a multi-enzyme composite is added to one diet, the analytics can become cumbersome because β-Glucanase could be originated not only from the microorganism *A. aculeatus* but also from *Trichoderma longibrachiatum*. Each of these microorganisms produce multi-enzymes by themselves, so the analytical method measures in a different unit as the guarantee units but comparable to expected values U/kg.
[3, 4, 5] the same scenario as with the β-Glucanase happens for the Xylanase measurements, so 2 different analytics have been performed to achieve the guarantee values.

## Table 4. Body composition and Calorimetry informatioin

| Parameters | Item | Units | Starter (5-13 d) | | | | Grower (15-22 d) | | | | Grower (25-27 d) | | | | Finisher (39 – 47 d) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | NC | NC+ EnzC.[1] | SEM | P-value | NC | NC+ EnzC. | SEM | P-value | NC | NC+ EnzC. | SEM | P-value | NC | NC+ EnzC. | SEM | P-value |
| Performance | BW | g | 246 | 249 | 1.72 | 0.245 | 777 | 769 | 13.3 | 0.683 | 1428 | 1415 | 13.68 | 0.514 | 2722 | 2860 | 33.9 | 0.011* |
| | Body gain, | g/d | 26.7 | 28.1 | 1.64 | 0.525 | 777 | 63.4 | 60.0 | 0.341 | 83.3 | 78.8 | 2.37 | 0.249 | 79.03 | 106.7 | 6.41 | 0.011* |
| | Feed intake | g/d | 37.4 | 38.3 | 1.33 | 0.289 | 99.5 | 94.1 | 1.79 | 0.063† | 146 | 136 | 4.5 | 0.194 | 136 | 153 | 7.01 | 0.116 |
| | FCR | ratio | 1.44 | 1.38 | 0.043 | 0.391 | 1.56 | 1.59 | 0.079 | 0.807 | 1.76 | 1.73 | 0.06 | 0.768 | 2.01 | 1.61 | 0.07 | 0.003* |
| Body composition | Dry matter, DM | coef | 0.250 | 0.254 | 0.0009 | 0.027* | 0.254 | 0.260 | 0.004 | 0.361 | 0.288 | 0.280 | 0.002 | 0.056† | 0.305 | 0.310 | 0.001 | 0.039* |
| | Protein in DM | coef | 0.600 | 0.599 | 0.009 | 0.660 | 0.577 | 0.578 | 0.007 | 0.930 | 0.587 | 0.586 | 0.02 | 0.7170 | 0.576 | 0.581 | 0.002 | 0.172 |
| | Fat in DM | coef | 0.231 | 0.210 | 0.004 | 0.008* | 0.303 | 0.283 | 0.017 | 0.426 | 0.305 | 0.356 | 0.008 | 0.002 | 0.338 | 0.307 | 0.003 | 0.004* |
| | Protein | g/kg | 150.2 | 152.2 | 0.51 | 0.029* | 146.8 | 150.3 | 1.35 | 0.099† | 169.0 | 162.7 | 0.27 | 0.001** | 175.0 | 179.8 | 0.69 | 0.005* |
| | Fat | g/kg | 57.9 | 53.1 | 0.92 | 0.007* | 76.4 | 72.5 | 3.08 | 0.398 | 74.0 | 83.6 | 0.93 | 0.007* | 102.4 | 94.5 | 2.12 | 0.007* |
| | DM accretion | g/d | 6.9 | 7.3 | 0.10 | 0.059† | 17.5 | 17.8 | 1.41 | 0.875 | 24.7 | 19.1 | 0.51 | 0.004* | 20.7 | 28.3 | 1.71 | 0.010* |
| | Protein accretion | g/d | 4.1 | 4.3 | 0.06 | 0.079† | 9.3 | 9.5 | 0.59 | 0.810 | 13.9 | 10.7 | 0.29 | 0.004* | 12.4 | 17.2 | 1.01 | 0.007* |
| | Fat accretion | g/d | 1.8 | 1.5 | 0.08 | 0.029* | 5.9 | 4.5 | 0.79 | 0.231 | 8.3 | 12.6 | 0.67 | 0.019* | 8.9 | 6.1 | 1.26 | 0.129 |
| | NE of gain[2] | kcal/kg | 1012 | 955 | 33.2 | 0.260 | 1076 | 975 | 51.30 | 0.209 | 1075 | 1264 | 53.40 | 0.090† | 1386 | 1265 | 72.40 | 0.255 |
| Calorimetry | $VO_2$[3] | L/kg | 366.5 | 337.2 | 15.30 | 0.217 | 379.3 | 394.3 | 8.10 | 0.223 | 481.5 | 474.3 | 28.60 | 0.867 | 489.9 | 434.9 | 6.70 | 0.011* |
| | $VCO_2$[4] | L/kg | 376.7 | 353.5 | 13.20 | 0.243 | 365.4 | 372.9 | 6.20 | 0.419 | 434.6 | 422.2 | 22.49 | 0.716 | 452.7 | 415.7 | 10.20 | 0.090† |
| | RER[5] | ratio | 1.049 | 1.067 | 0.01 | 0.274 | 0.964 | 0.945 | 0.01 | 0.079† | 0.904 | 0.892 | 0.01 | 0.407 | 0.923 | 0.956 | 0.02 | 0.264 |
| | Heat Production[6] | kcal/kg | 1793 | 1728 | 90 | 0.640 | 1906 | 1973 | 38 | 0.251 | 2384.0 | 2341.0 | 137 | 0.836 | 2438.0 | 2181.0 | 36 | 0.015* |
| Nutrient efficiency | Energy Efficiency[7] | % | 35.8 | 33.8 | 1.2 | 0.259 | 36.4 | 34.7 | 1.97 | 0.539 | 36.4 | 42.8 | 1.85 | 0.090† | 43.7 | 37.9 | 2.11 | 0.075† |
| | Protein Efficiency[8] | % | 48.0 | 51.9 | 0.9 | 0.017* | 45.9 | 52.8 | 2.80 | 0.124 | 47.0 | 36.9 | 2.10 | 0.041* | 57.9 | 68.5 | 3.10 | 0.037* |

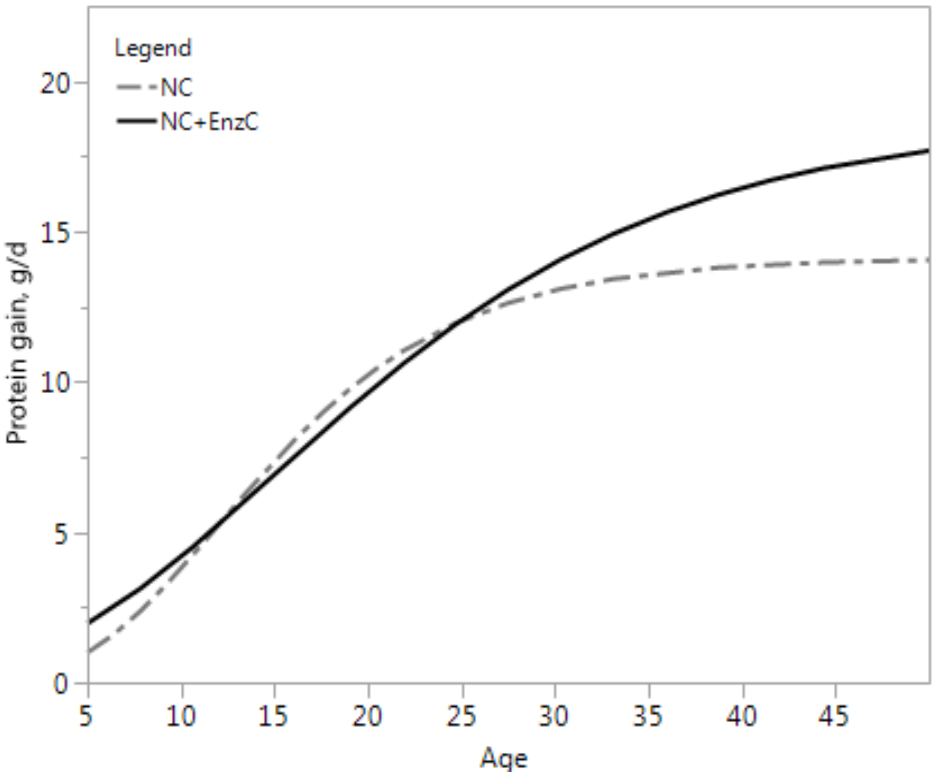
Means were obtained from 6 replications per treatment in the starter (5-7d, 10-12 d), 6 replications per treatment in the first phase of the grower which corresponds to 15-17d and 20-22d and 3 replications for the phase 25-27 d, 6 replications for the finisher (37-39, 45-47). [1]Enz = Enzyme composite: glucanase + xylanase + protease + phytase, [2]Net energy of gain in kcal /kg feed, NEg = (fat gain (g) x 9.35 (kcal/g) + protein gain (g) x 5.66 (kcal/g)) feed intake (Okumura, 1979), [3]$VO_2$ = Volume of oxygen consumption L/kg of feed/day, [4]$VCO_2$ = Volume of carbon dioxide production L/kg of feed/day, [5]RER = Respiratory exchange ratio $VCO_2/VO$, [6]Heat Production = 3.866 $VO_2$ L/d + 1.233 $VCO_2$ L/d (Brouwer, 1965) kcal/kg of feed, [7]Energy efficiency = (NEg kcal gain/ AMEn intake, kcal)*100, AMEn = Apparent metabolizable energy corrected by nitrogen, kcal, [8]Protein efficiency = (Body protein gain g/ Protein intake, g)*100. P –value ≤0.001**, ≤0.05*; ≤0.10†

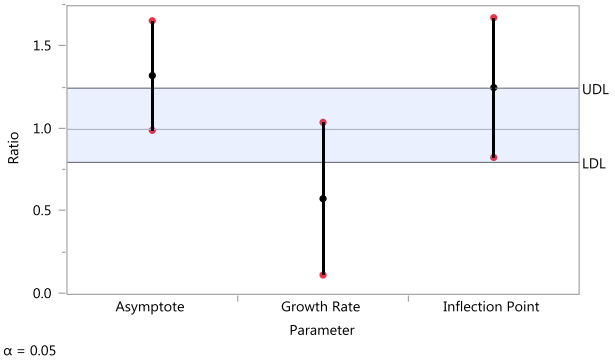
**Table 5. Effect of an enzyme composite on apparent ileal amino acid digestibility (%) of broiler fed corn/soy based diet[1]**

| | Starter[2] 5 -13 d | | | | | Grower[3]14-28 d | | | | | Finisher[4] 33-47 d | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AA** | **NC** | **NC + EnzC** | **Dif[5]** | **SEM[6]** | ***P-value*[7]** | **NC** | **NC + EnzC** | **Dif.** | **SEM** | ***P-value*** | **NC** | **NC + EnzC** | **Dif** | **SEM** | ***P-value*** |
| Lys | 76.1 | 81.9 | 5.8 | 2.2 | *0.099†* | 73.1 | 77.7 | 4.6 | 1.3 | *0.039** | 74.4 | 81.5 | 7.1 | 2.5 | *0.095†* |
| Met | 84.3 | 88.6 | 4.3 | 1.3 | *0.054†* | 82.1 | 85.0 | 2.9 | 0.5 | *0.197* | 82.9 | 87.2 | 4.4 | 2.2 | *0.219* |
| Cys | 65.3 | 73.4 | 8.1 | 2.1 | *0.029** | 66.1 | 71.0 | 4.9 | 2.2 | *0.148* | 72.9 | 76.7 | 3.8 | 3.0 | *0.405* |
| Thr | 68.1 | 75.8 | 7.8 | 2.2 | *0.043** | 65.6 | 68.2 | 2.6 | 1.2 | *0.179* | 66.9 | 74.7 | 7.9 | 4.4 | *0.289* |
| Arg | 80.5 | 86.5 | 6.1 | 1.4 | *0.012** | 81.0 | 85.1 | 4.2 | 0.4 | *0.001*** | 80.5 | 84.7 | 4.2 | 2.4 | *0.260* |
| Val | 74.2 | 79.8 | 5.6 | 2.2 | *0.106* | 70.4 | 73.5 | 3.1 | 1.1 | *0.089†* | 73.7 | 78.2 | 4.5 | 3.1 | *0.350* |
| Leu | 74.3 | 80.5 | 6.2 | 2.0 | *0.059** | 74.6 | 78.2 | 3.6 | 0.9 | *0.022** | 77.2 | 81.5 | 4.4 | 2.5 | *0.283* |
| Iso | 74.2 | 80.1 | 5.9 | 2.1 | *0.078†* | 71.6 | 74.2 | 2.6 | 1.2 | *0.138* | 74.0 | 78.7 | 4.7 | 3.0 | *0.304* |
| Phe | 75.6 | 81.5 | 5.9 | 1.8 | *0.047** | 74.7 | 78.2 | 3.5 | 0.9 | *0.031** | 76.9 | 81.0 | 4.1 | 2.6 | *0.310* |
| Tyr | 77.1 | 84.1 | 7.0 | 2.2 | *0.051†* | 74.7 | 78.0 | 3.3 | 0.8 | *0.060†* | 75.7 | 82.0 | 6.3 | 2.2 | *0.094†* |
| Gly | 68.9 | 76.1 | 7.2 | 2.2 | *0.051†* | 66.5 | 69.2 | 2.7 | 0.8 | *0.056†* | 69.5 | 74.0 | 4.5 | 3.7 | *0.429* |
| Ser | 68.6 | 77.0 | 8.4 | 2.3 | *0.039** | 66.9 | 70.5 | 3.6 | 1.4 | *0.130* | 72.5 | 80.1 | 7.6 | 3.6 | *0.183* |
| His | 74.9 | 81.7 | 6.8 | 2.3 | *0.062†* | 75.9 | 79.0 | 3.0 | 0.9 | *0.064†* | 78.8 | 81.8 | 3.0 | 2.6 | *0.442* |
| Ala | 72.0 | 78.2 | 6.2 | 2.3 | *0.097†* | 71.9 | 74.4 | 2.5 | 0.6 | *0.023** | 75.5 | 79.6 | 4.1 | 2.9 | *0.361* |
| Asp | 75.8 | 81.5 | 5.7 | 1.7 | *0.044** | 72.2 | 74.5 | 2.3 | 0.7 | *0.065†* | 71.7 | 77.9 | 6.2 | 4.6 | *0.392* |
| Glu | 81.0 | 85.0 | 4.1 | 1.4 | *0.090†* | 81.0 | 83.6 | 2.6 | 1.4 | *0.193* | 82.8 | 86.1 | 3.3 | 2.6 | *0.405* |
| Mean AA | 74.5 | 80.7 | 6.3 | 1.9 | *0.050** | 73.0 | 76.3 | 3.3 | 0.6 | *0.044** | 73.5 | 80.7 | 7.2 | 2.7 | *0.139* |

[1]Each value represents the mean of 6 replicates (10 birds per replicate in starter; 4 birds per replicate in the grower and 2 birds per replicate in the finisher), [2]Starter contains glucanase + xylanase + protease 5625 PROT units/Kg + phytase,
[3] Grower contains glucanase + xylanase + protease 3750 PROT/units/Kg+ phytase,
[4] Finisher contains xylanase + phytase,
[5]Value is the difference between (NC + EnzC) – NC,
[6]Pooled standard error of the mean, [7]Differences are significant when P-value ≤0.05 and it shows a tendency when P-value ≤ 0.10
P –value ≤0.001**, ≤0.05*; ≤0.10†

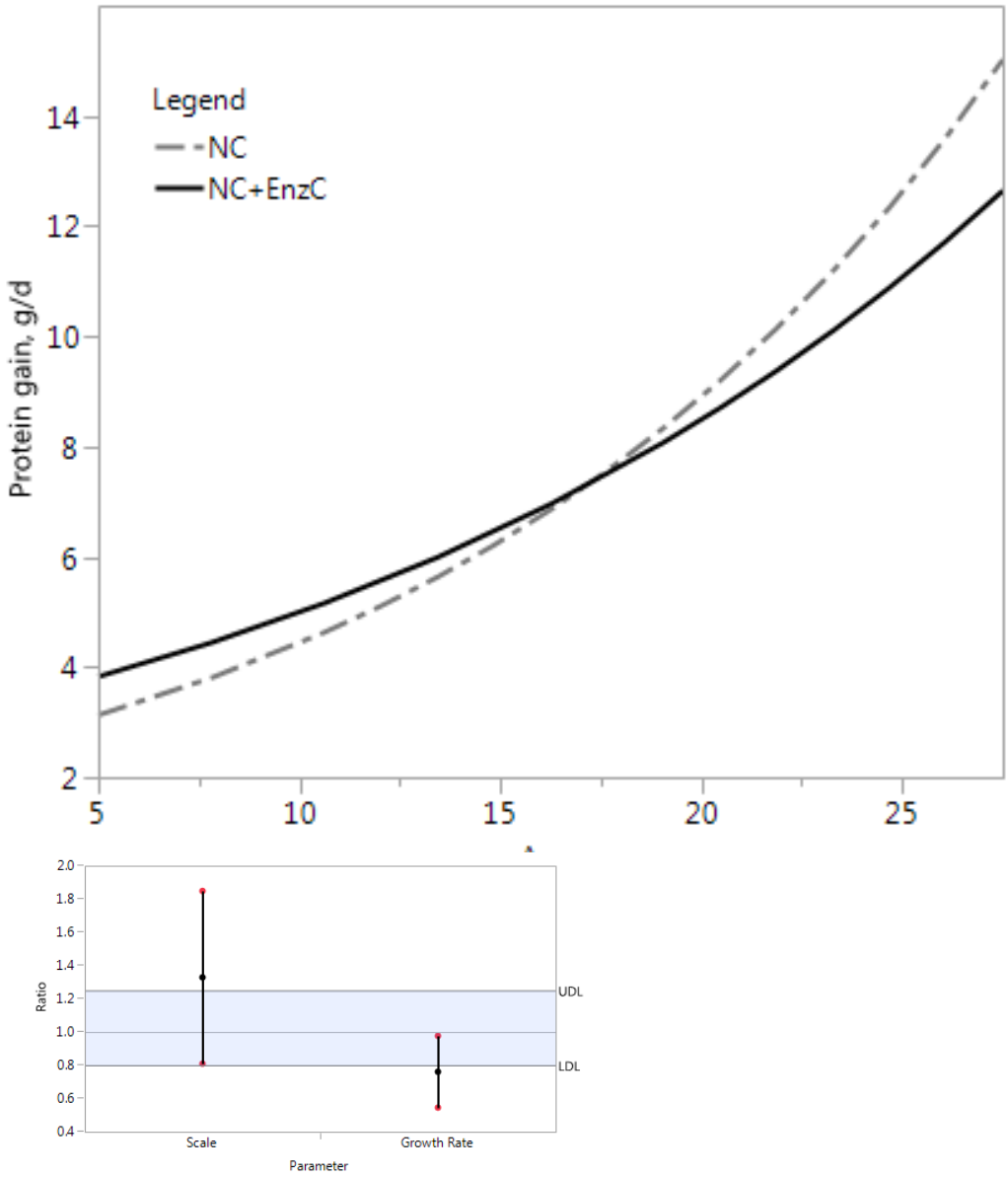
**Figure 1. Fit body protein gain by age[1] for starter, grower and finisher stages and equivalence test[2].**



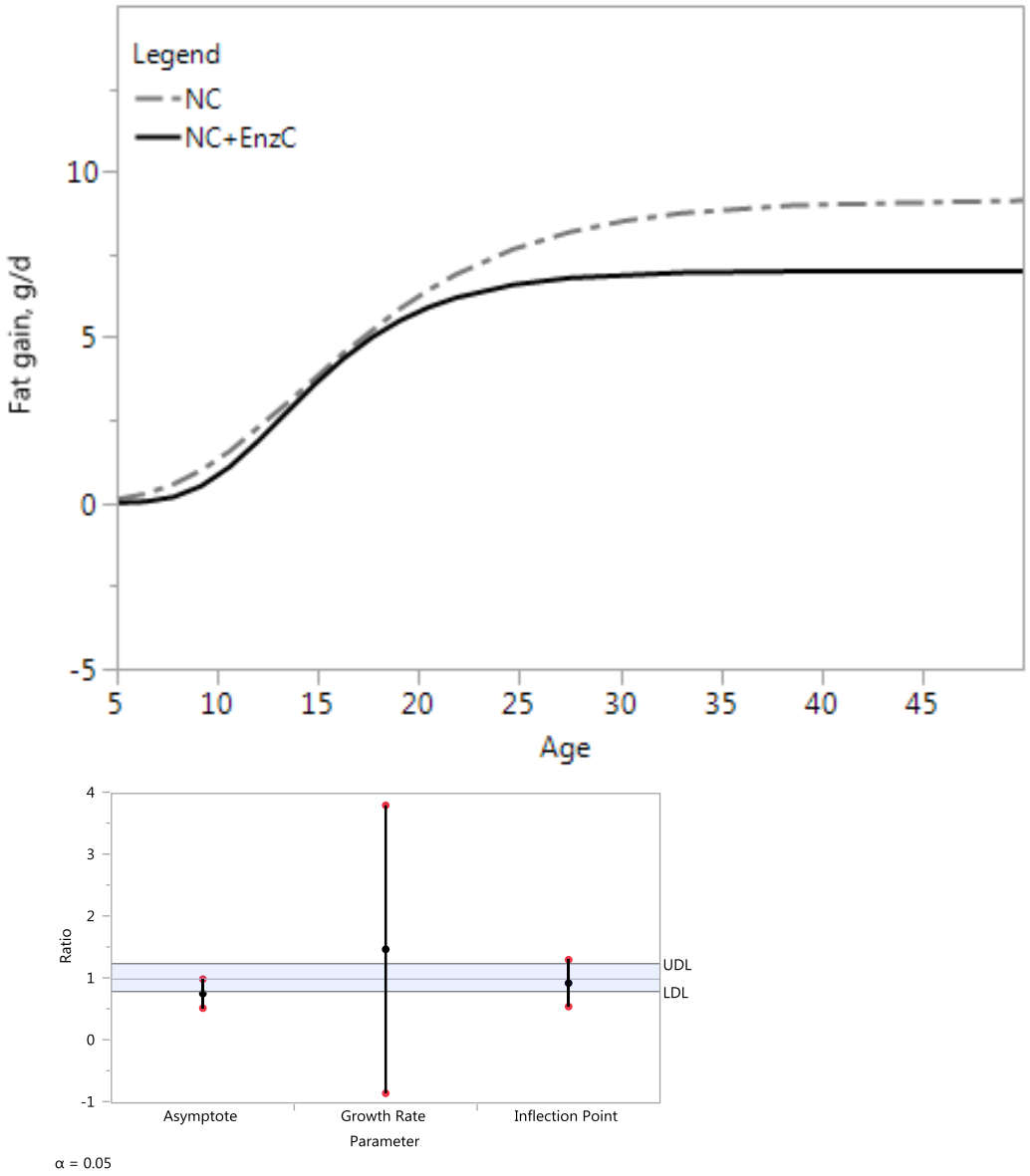

[1]Gompertz 3P non-linear fit body protein gain vs. age. Protein gain = a*e$^{[-e[-b*[Age-c],}$ where a = asymptote, b = growth rate, c = inflection point. Parameter estimates, NC: a = 14.12, b = 0.14, c = 11.91, and NC + EnzC. a = 18.73, b = 0.08, c = 14.97. $R^2$ 0.77, RMSE 2.76.
[2]The curves were subjected to an equivalence test for asymptote, growth rate parameter, inflection point. The equality of the parameters is tested by analyzing the ratio of the parameters. The default decision lines are placed at ratio values of 0.8 and 1.25 difference. If all confidence intervals are inside the decision lines, then the two treatments are practically equal. At least two confidence interval for all parameters are outside the decision lines, meaning the curves for NC, and NC+EnzC are not equal ($P$<0.05)
NC = Negative control, NC + EnzC = NC + Enzyme composite

**Figure 2. Fit body protein gain vs Age[1] during starter and grower stages and equivalence test[2]**



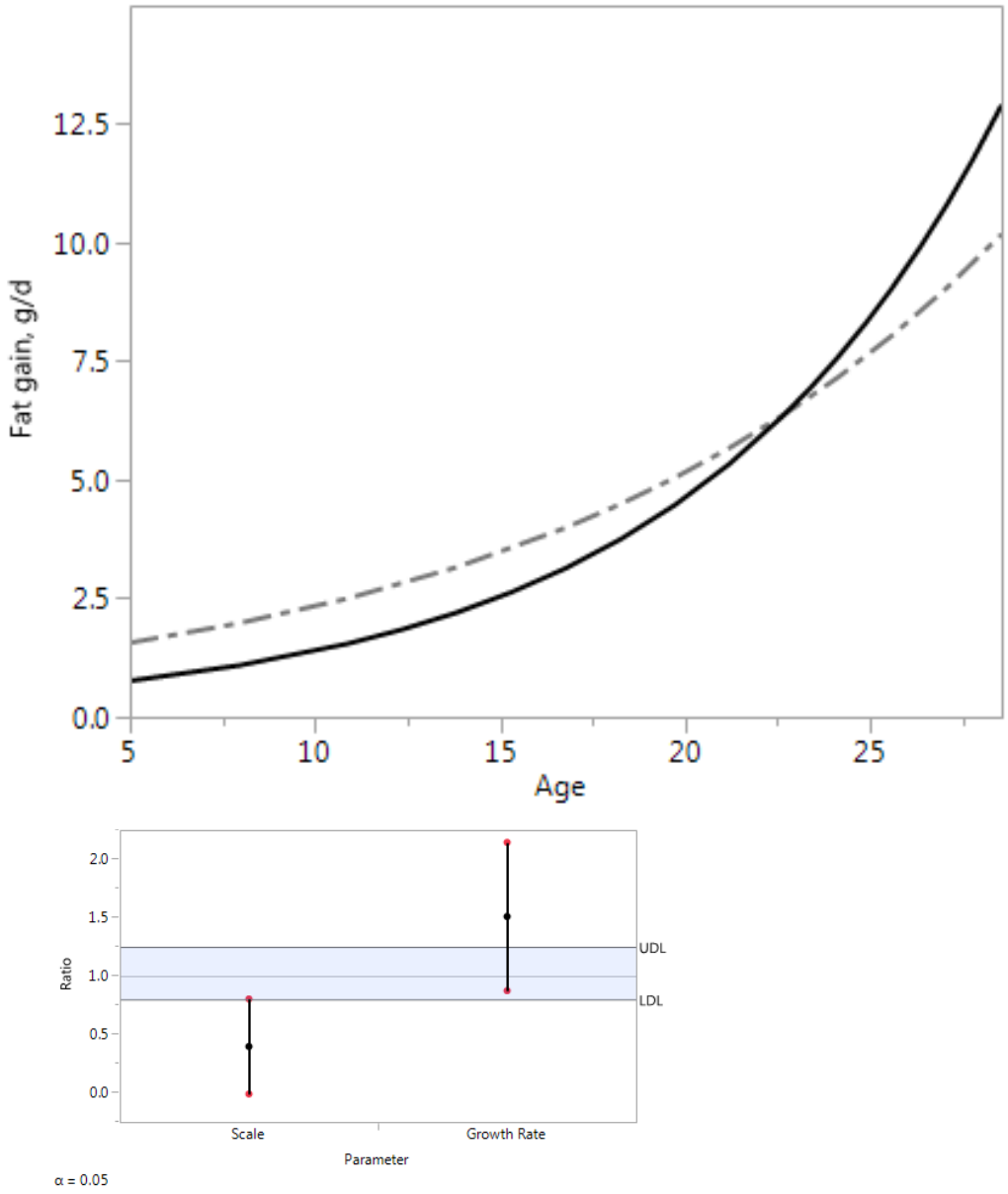

[1]Exponential 2P fit between body protein gain vs. age for starter and grower stages. Protein gain g/d = a*e$^{[b*Age]}$, where a = scale, b = growth rate. Parameter Estimates for NC: a = 2.20, b = 0.069, and for NC + EnzC. a = 2.93, b = 0.12, c = 0.053. $R^2$ 0.88, RMSE 0.88.
[2]The curves were subjected to an equivalence test for scale and growth rate parameter. The equality of the parameters is tested by analyzing the ratio of the parameters. The default decision lines are placed at ratio values of 0.8 and 1.25 difference. If all confidence intervals are inside the decision lines, then the two treatments are practically equal. In this case, both scale and growth rate confidence intervals are outside the decision lines, so the curves for NC, and NC+EnzC are not equal ($P$<0.05)
NC = Negative control, NC + EnzC = NC + Enzyme composite

**Figure 3. Fit fat gain vs Age[1] for starter, grower and finisher and equivalence test[2]**




[1]Gompertz 3P non-linear fit body fat gain vs. age. Fat gain = a*e$^{[-e[-b*[Age-c],}$ where a = asymptote, b = growth rate, c = inflection point. Parameter Estimates for NC: a = 9.15, b = 0.16, c = 14.09, and for NC + EnzC. a = 7.01, b = 0.24, c = 13.18. $R^2$ 0.51, RMSE 2.95.
[2]The curves were subjected to an equivalence test for asymptote, growth rate parameter, inflection point. The equality of the parameters is tested by analyzing the ratio of the parameters. The default decision lines are placed at ratio values of 0.8 and 1.25 difference. If all confidence intervals are inside the decision lines, then the two treatments are practically equal. The asymptote and the growth curve confidence interval are outside the decision lines means the curves for NC, and NC+EnzC are not equal ($P$<0.05), but the inflection point is the same.
NC = Negative control, NC + EnzC = NC + Enzyme composite

**Figure 4. Fit body fat gain vs Age for starter and grower and equivalence test[2]**




[1]Exponential 2P fit between body fat gain vs. age for starter and grower stages. Fat gain g/d = $a*e^{[b*Age]}$, where a = scale, b = growth rate. Parameter Estimates for NC: a = 1.05, b = 0.079, and for NC + EnzC. a = 0.41, b = 0.12. $R^2$ 0.81, RMSE 1.63

[2]The curves were subjected to an equivalence test for scale and growth rate parameter. The equality of the parameters is tested by analyzing the ratio of the parameters. The default decision lines are placed at ratio values of 0.8 and 1.25 difference. If all confidence intervals are inside the decision lines, then the two treatments are practically equal. In this case, both scale and growth rate confidence intervals are outside the decision lines, so the curves for NC, and NC+EnzC are not equal ($P$<0.05)

NC = Negative control, NC + EnzC = NC + Enzyme composite

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# VI. MAINTENANCE ENERGY REQUIREMENTS IN MODERN BROILERS FED EXOGENOUS ENZYMES

**ABSTRACT**

Maintenance energy requirement is the biggest component of the energy necessities for poultry (40 - 65%) that needs to be covered before tissue gain or production can occur. Exogenous enzymes have shown to decrease heat production (HP) in broilers. HP biggest component is maintenance energy suggesting enzymes are reducing the maintenance need. The present attempt to determine the maintenance needs of modern broilers with and without enzymes. Two trials with 100 and 360 Cobb male broilers were conducted with increasing feeding levels (10 – 50% trial 1 from 16 – 22 d, and 30 – 100% trial 2 from 16 – 27d). In the second trial a negative control (NC) and NC + Enz (multi-enzyme composite: glucanase + xylanase + protease + phytase) were studied. The enzymes were added to a basal corn-soybean mash diet and fed from 16 – 27d. The light program was 18 h light: 6 h dark and the temperature was kept constant for both trials and both treatments from 27 °C (16d) until 22 °C (27 d). Metabolizable energy kcal/kg was evaluated as the classical total collection in the ad libitum birds of trial 2. The retained energy was evaluated as protein gain g * 5.66 + fat gain * 9.35. The body composition of the birds was analyzed by dual X-ray absorptiometry (DEXA). JMP statistical software was used to fit the models. Linear regression (retention energy kcal/kg$^{0.70}$ by ME intake kcal/kg$^{0.70}$), and logarithmic model (HP kcal/kg$^{0.70}$ = a * e $^{(b * MEI)}$) were fitted. Metabolizable energy for maintenance (MEm) was determined as MEI at zero retention and net energy for maintenance (NEm) was determine from the logarithmic equation (a). The slope of the linear regression was considered to be the $\kappa_g$ (efficiency of energy utilization for gain), and the $\kappa_m$ (efficiency of energy utilization for maintenance) was determined as NEm/MEm. The MEm requirement was 152 ± 8 kcal/kg$^{0.70}$ ($R^2$, 0.91) and 128 ± 6 kcal/kg$^{0.70}$ ($R^2$, 0.98) for trial 1 and 2 (only NC) respectively ($P$<0.01). The NEm was 97.2 ± 8 kcal/kg$^{0.70}$ ($R^2$, 0.87) and 97.9 ± 6 kcal/kg$^{0.70}$ ($R^2$, 0.95) for trial

1 and 2 (only NC) respectively ($P$<0.01). The $\kappa_g$ and $\kappa_m$ were 0.62 and 0.64 for trial 1 and 0.55 and 0.79 for trial 2 (only NC) respectively. The NC + Enz showed lower MEm in 8.5 kcal/kg$^{0.70}$ which represents 6.6% of the maintenance energy requirement ($P$<0.01). When this value was expressed as kg of feed intake the energy savings ranged from 75 kcal at ad libitum up to 236 kcal at maintenance intake. The body composition as g/kg was not changed with the enzyme for protein and BMC (body mineral content) but showed a trend of more fat g/kg DM ($P$<0.09). The feeding level changed the body composition in broilers. Protein g/kg DM and BMC g/kg DM decreased while fat g/kg DM) increased as the feeding level increased to ad libitum consumption ($P$<0.01). The MEm for broilers at 22 °C in the second trial (128 kcal/kg$^{0.70}$) is in close agreement with other researchers (112 kcal/kg$^{0.70}$ Sakomura, 2004), the value MEm in the first trial (152 kcal/kg$^{0.70}$) resembles more to chickens under lower temperature 13 °C (158 kcal/kg$^{0.70}$ Sakomura, 2004) suggesting this birds were under temperature comfort condition because of the lower range of feeding levels (10 -50%) compared to trial 2 (30 -100%). This is the first work to the author knowledge that a maintenance energy by linear regression is evaluated with multi-enzyme composite. Further investigation is needed to understand the mechanism by which enzymes are decreasing the maintenance energy requirement for broilers.

Key words: maintenance, enzymes, broilers

## INTRODUCTION

The importance of energy in biological systems is well known since energy is the common currency of nutrition for metabolic reactions. Energy is the property of nutrients (carbohydrates, proteins, and fats) to generate usable energy in the body. Not only energy is important for physiological process, but it is also the most expensive component in poultry feed (Kleyn, 2013). Therefore, it is important to understand where the energy is used in the metabolism, so research can be directed to improve the efficiency of these processes. Energy needs is usually separated in two components. The first one, energy associated to maintenance which includes basal metabolism, adaptive thermogenesis, dietary thermogenesis, and physical activity. The second component, energy for production requirements, including energy within products (meat, eggs, milk, wool, etc.…) and thermogenesis associated with their synthesis. These two components have been studied in poultry. Maintenance requirement is defined as a requirement at zero gain meaning a balance of anabolism and catabolism are met (Sakomura, 2004). This definition applies to adult animals but for growing or producing animals this balance or equilibrium never occurs, so Chwalibog (1985) defines maintenance requirement as a dynamic equilibrium of protein and fat turnover to maintain temperature. Metabolizable energy is the most common system used in poultry, and it is important because other nutrients intake will be affected by provision of dietary energy (Lopez, and Leeson, 2008). The ME (metabolizable energy) maintenance requirement is determined from the intersection of the regression line with the zero energy retention line (Farrell, 1974). Maintenance energy requirement is expressed on a metabolic weigh basis ($BW^{0.70}$) (Noblet, et al., 2015). There are some approaches to determine maintenance energy. Increasing levels of a diet is fed to animals. The most commonly used is by linear regression of energy balance between retained energy and metabolizable energy intake

(MEI) (Sakomura, 2004). Tissue deposition occurs after the maintenance energy is met. Maintenance energy varies according to ambience temperature (Sakomura, 2003) and month of the year (Grimbergen, 1974), age (De Groote, 1974), type of chickens, broilers need more maintenance energy than breeders kg/kg$^{0.75}$ (Sakomura, 2004), microflora population (Eggum and Chwalibog, 1983). Energy for maintenance accounts 65% of the energy requirement for laying hens and it is more susceptible to environmental change (McLeod, 1988), for broilers of intermediate growth rate represents 42-44% (Lopez and Lesson, 2005). There is some evidence that exogenous enzymes reduce the heat production making more efficient feed utilization (Caldas, 2015, Choct, 2010); however, there is still a lack of understanding if the enzymes are working at maintenance level, heat increment of feeding or both. Two experiments were conducted with the objective to evaluate the maintenance energy for modern broilers and determine if exogenous enzymes are working at maintenance energy level.

## MATERIALS AND METHODS

All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC) # 12041.

### Birds and Housing

Experiment 1 (108 chicks), and experiment 2 (360 chicks) were raised for the present study. One day old Cobb male chicks of a commercial strain (Cobb Vantress, Siloam Springs, AR) were obtained from a local hatchery (Cobb hatchery, Fayetteville, AR) and reared in 4.5 m$^2$ floor pens of 40 chicks per pen. Each pen was equipped with 10 nipples per line, two hanging type feeders,

with a round pan that provided 208 cm of feeder space per pen. Chicks were reared under standard conditions and commercial diets until d15. On day 16, chicks were moved to wire metabolic cages with dimensions of 91 cm x 30 cm in the same chicken house. Fifty cages for experiment 1 (10 replications) and ninety cages for experiment 2 (6 replications for 30 – 80 % feeding level and 5 replications for 90, and 100% ad libitum, same replications for NC and NC + Enz) were used. Two chicks per cage were allocated in experiment 1 and four chicks per cage for experiment 2. The metabolic cages provided 2 nipple drinkers and a line feeder of 85 cm. The density (chicks/cage), feeder space and drinkers in metabolic cages were set up to comply with the regulation of the Federation of Animal Science Societies (FASS, 2010). Chicks were selected from a bigger population to have an initial BW432 g ± 28 SD, 6.4% CV (experiment 1), and 451 g ± 29 SD, 6.4% CV (experiment 2). Experiment 1 was evaluated from 16 – 22 d, and experiment 2 from 16 – 27 d of age. Regarding to ventilation management of the house, it is equipped with 4 tunnel fans in the far end wall. One of these fans is set to run as a minimum ventilation fan to keep the air-fresh and remove excess humidity. The side-walls are solid with 7 vent-boards on each wall. The vent-boards automatically open prior to fans coming on and their opening is adjusted automatically based on desired static pressure. The 2 cool cells are covered with a curtain that is automatically lowered and raised based on desired temperature and to maintain a static pressure of .09 when any of the tunnel fans are running. This maintains the air velocity needed to keep the air fresh and to add a wind chill factor to the cooling of the birds during periods of hot temperatures. The cool cells themselves only runs water when additional cooling is necessary. There are 4 Re-Verber-Ray radiant tube heaters (Detroit Radiant Products Company, Warren, MI) to provide heat during brooding or cold weather. All ventilation and heating equipment is controlled by a Chore-tronics Model 40 controller (ChoreTime). The

controller is programmed to maintain specific temperature and ventilation curves based on the age of the bird. There are specific set points at different ages and the controller calculates what the set points are for every day in between, providing a gradual transition between ages. Temperatures in the chicken house were changed according to the genetic broiler management recommendations (Cobb 500, 2012) starting (d1) at 33°C and decreasing 0.43 degrees °C every day until 22°C at 27d from which it was maintained until the end of the study. The light program was 18 hr. light: 6 hr. dark to decrease the stress of feed restriction in the birds.

**Diets and feeding program**

Broilers were fed mash diets *ad libitum*. In experiment 1, five feeding levels were fed (10, 20, 30, 40, and 50% from the *ad libitum* consumption of the d15). The amount of feed was fixed on d16 for each feeding level and the birds were fed for 6 days (16 -22d). In experiment 2, eight feeding levels were fed (30, 40, 50, 60, 80, 90, and 100% or *ad libitum*) with and without enzymes, so 16 (8 x 2) dietary treatments were evaluated. In experiment 2, the amount of feed was adjusted daily for 11d (16 – 27 d) according the intake of the *ad libitum* group), so feed intake was higher for every feeding level. Feed intake in experiment 1 does not correspond to feeding level in experiment 2 because feeding level was fixed to be the same during the 6 days with the *ad libitum* feed intake of on d15. On experiment 2, feed intake was adjusted daily during the trial based on the *ad libitum* intake. For example, 50% of feeding level is close to 30% of the feeding level in experiment 2 (Table 1). Chickens were weighed at 16, 20, 22d in experiment 1 and 16, 22, 24 and 27d in experiment 2. The basal diet consisted of a corn-soybean meal base formulated to provide the Cobb 500 nutrient specs (Cobb Vantress, 2012) decreased by 100 kcal energy/kg and decreased amino acids to keep the same ratio as in 2012 specifications (Table 2).

The multi-enzyme composite was added on-top of the basal diet or negative control (NC) to make treatment (NC + Enz.) (Table 3). The multi-enzyme was composed of a glucanase (50 g/MT) + xylanase (100 g/MT) + protease (50 g/MT) + phytase (150 g/MT). Samples of each diet in experiment 2 were sent for enzyme analysis to an appropriate laboratory (TMAs, Belvidere, NJ). Prior to the grower period, chicks were fed with enzymes added on-top of the NC diet to help develop the microflora. Negative control chicks were not fed enzymes of feed prior to the evaluation period. Chicks were selected from negative control group and enzyme treated groups, at the beginning of the evaluation period, to have the same starting body weight. The analysis of AMEn (apparent metabolizable energy corrected by nitrogen) was evaluated in the *ad libitum* group in experiment 2. AMEn involves analysis of gross energy, dry matter and nitrogen in feed and excreta. Gross energy (GE) was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Dry matter was analyzed by method 934.01 (AOAC, 1990) and nitrogen determined by the method 990.03 (AOAC, 1995). The AMEn assay was conducted by the classical total excreta collection method. The birds were adapted to the experimental diets from 16 – 20d before excreta collection (3 d). On the third day of collection, the excreta was pooled within a metabolic cage, mixed, and representative sample (120 g) was lyophilized in a freeze drier. The lyophilized excreta sample was ground with a commercial grinder to pass through a 0.5-mm sieve and sent to the Central Laboratory at the University of Arkansas for chemical analysis.

**Body Composition Analysis**

Chicks were humanely sacrificed by $CO_2$ inhalation at the end of the trials. Twenty chicks of the same initial weight as the birds placed for the experiments were scanned on day 16 to have the

initial body protein and body fat composition. Carcasses were scanned by dual energy X-ray absorptiometry (DEXA) for body composition analysis. DEXA values were adjusted using the equations (Caldas, 2015 – chapter 3) for fasted birds. The equations were: protein, g = 0.149 * DEXA Lean, g $^{0.02}$, and fat, g = -15.9 + 0.095* DEXA tissue, g + 0.28 * DEXA fat, g – 0.468 * DEXA area, $cm^2$.

**Calculations**

AMEn in the feed was calculated according the following equation

$$AME_n = \frac{(GE_d * FI) - ((GE_{exc.} * Exc.) + (N_d * FI, g - N_{exc.}\ g/g * exc.\ g) * 8.22\ kcal/g))}{FI}$$

Where: AMEn = apparent metabolizable energy corrected by nitrogen, $GE_d$ = gross energy in the diet (kcal/kg), FI = feed intake (kg), Exc = excreta output (kg), $N_d$ = nitrogen in the diet (g/g), $N_{exc.}$ = nitrogen in the excreta (g/g).

MEI (metabolizable energy intake, kcal/ $kg^{0.70}$) was calculated to be = FI (kg) * AMEn (kcal/kg)/kg av. $BW^{0.70}$. RE (retained energy, kcal/ $kg^{0.70}$) was calculated to be = (Protein gain (g) * 5.66 + Fat gain (g) * 9.35)/ kg av. $BW^{0.70}$ (Okumura, 1979). HP (heat production, kcal/$kg^{0.70}$) was calculated to be = MEI – RE. The body composition (protein, fat and bone mineral density (BMC) will be reported dry matter (DM) g/kg of body weight.

**Statistical analysis**

For the determination of MEm (metabolizable energy for maintenance), linear regression analysis was performed. RE (retained energy) as the dependent variable was regressed on MEI

(metabolizable energy intake) according to Farrell (1974), the MEm was calculated by inverse prediction when RE = 0. The slope was used for determining $\kappa_g$ (efficiency of energy utilization for gain). A logarithmic curve was fitted between HP by MEI building parameters for $a*e^{b* MEI}$, where a = NEm (net energy of maintenance). The $\kappa_m$ (efficiency of energy utilization of maintenance) was calculated as NEm/ MEm (Sakomura, 2004). Body composition between NC and NC+Enz were analyzed by ANOVA within each feeding level, the means were separated by T-student. *P -value* was considered significant when ≤ 0.05 and a trend if <0.10. All analysis was determined with JMP12 (SAS institute, 2015)

## RESULTS

### Maintenance energy (MEm, NEm)

Maintenance energy could be MEm (metabolizable energy for maintenance), and NEm (net energy for maintenance. The first one is obtained with a linear equation and the second one with a logarithmic equations. The MEn was 151.8 ± 8.0 kcal/$kg^{0.70}$/d for experiment 1 when the birds were under restriction for 6 days (10 – 50 % feeding level), and 128.1 ± 5.9 kcal/$kg^{0.70}$/d for experiment 2 when the birds had 11 days (30 – 100% feeding level). In the first experiment, birds lost weight with all feeding levels because the feed intake was fixed with the feed intake of the previous day, and also the restrictions levels were too extreme. For the second experiment, the feed intake was different daily since it was upgraded with the *ad libitum* group, so the birds gained weight even with the 30%. In both trials the linear regression had a slightly prolongation to reach zero gain. Birds from the second experiment were analyzed only from the negative control group, no enzymes effect seeing at this point. The NEm was lower than MEm as

expected, 97.2 kcal± 7.9 kcal/kg$^{0.70}$/d, 97.9 kcal± 6.3 kcal/kg$^{0.70}$/d and for experiment 1 & 2 respectively (Table 5). The values for NEm are similar between experiments compared to the values for MEm which differ more. With NEm/MEm ratio, the efficiency for maintenance was 0.64 for experiment 1 and higher for experiment 2 (0.79). The efficiency for maintenance in the other hand is higher for experiment 1 (0.62), and lower for experiment 2 (0.55) (Table 5). Higher the intake, lower the efficiency. When a multi-enzyme blend (NC +Enz.) was added to NC in experiment 2 to have two diets and 8 feeding levels (16 treatments total). Retained energy (RE) was regressed on MEI (Figure 1), the NC+Enz decreased the MEm in 8.5 kcal (128.1 NC vs 119.6 kcal/kg$^{0.70}$/d NC+Enz.) (Table 6) which corresponds to 6.6% of the energy for maintenance, taking 8.5 kcal over kg feed intake, the energy savings from the enzymes varies from 75 kcal at *ad libitum* intake up to 265 kcal at 30% of feeding level, so the more feed restricted is the chick, the more the enzymes can spare energy for maintenance. Maintenance for NC represented 35% of the total energy intake and NC + Enz accounted for 32%. When NC was compared to NC+Enz within each feeding level, retained energy (RE) was always higher with the enzymes, however significant at 100% intake ($P<0.01$), and tendency at 80% of intake ($P<0.052$). On the other hand, HP was lower with enzymes at 70% ($P<0.050$), 80% ($P<0.041$), and tendency to be lower at 40% ($P<0.052$), and 100% ($P<0.096$) (Table 7).

**Body composition in fed restricted birds**

Body composition in feed restricted chicks can change protein and fat composition mainly. In experiment 1, the lowest feeding level (10%) had higher protein, 759 g/kg DM (Figure 2), and the highest (50%) had 728 g/kg DM while fat increased from 98 g/kg DM (10% intake) up to 163 g/kg DM (50% intake). Likewise, in experiment 2, protein decreased from 701 g/kg DM at

30% feeding level to 633 g/kg at 100% feeding level, while fat increased from 152 g/kg DM to 319 g/kg to 100% (Figure 3). These data shows that at low feeding levels, nutrients are directed to protein synthesis first and as feeding level increases, fat deposition increases. Comparing NC, and NC+Enz within each feeding level, fat g/kg tends to be higher with NC+Enz. ($P$<0.091) at 100% intake but no differences for other feeding levels ($P$>0.05). NC, and NC+Enz showed no significance for protein and mineral content g/kg were within each feeding level.

## DISCUSION

### Maintenance energy (MEm, NEm)

The partition of metabolizable energy is in retained energy as protein and fat primarily, and heat production. There is few research for updating the energy requirement for modern broilers for the past 10 years. Lopez and Lesson (2008) reported an elegant review in energy partition for broilers and research dated from 1970's up to 2005, so more studies are needed to study energy requirement and make it more efficient for protein deposition. Maintenance energy accounted for 34% of the ME (metabolizable energy) intake in broilers from 16-27 d of age. This value is smaller of the 42 – 44 % reported by Lopez and Leeson (2005) for 0 – 49 d, may be due to age difference in the study. Maintenance energy is higher in older animals because a bigger tissue is needed to maintain. The MEm (metabolizable energy for maintenance) found in two studies (152 – experiment 1, and 128 kcal/kg$^{0.70}$ – experiment 2) differ in 16%, while NEm (net energy for maintenance) was almost the same, (97, and 98 kcal/kg$^{0.70}$). The difference between MEm and NEm is the heat increment which is discounted in the last one. These results suggest that broilers in the first experiment spent more energy as heat increment probably for thermoregulation since

the feeding levels in experiment 1 were lower than experiment 2 (Table 1); however the temperature was kept the same for both experiments. Temperature was monitored according to age; however it should have monitored by BW. Since the BW of fed restricted chicks is low, the BW corresponds to smaller chicks, so the need of more temperature. The present MEm is in agreement with 158 kcal/$kg^{0.75}$ MEm for broilers in ground at 13 °C reported by Sakomura (2004) and 155 kcal/$kg^{0.60}$ reported by Lopez, and Leeson (2005); however birds were studied in cages in the present experiment, and the metabolic weight modifier was different between authors, $kg^{0.75}$ for Sakomura (2004), $kg^{0.60}$ for Lopez and Leeson (2005) and $kg^{0.70}$ in the present experiment supported by Noblet (2015). The 127 kcal/$kg^{0.75}$, MEm at 32 °C, and 112 kcal/$kg^{0.75}$, MEm at 23 °C reported by Sakomura (2005) is in close agreement to the 128 kcal/$kg^{0.70}$ found in experiment 2. The NEm is lower than MEm and it has been reported to be 119, 90, 96 kcal/$kg^{0.75}$ at 13 °C, 23°C, and 32 °C, respectively (Sakomura, 2004) which are in close agreement to the values found for experiment 1 (97 kcal/$kg^{0.70}$) and experiment 2 (98 kcal/$kg^{0.70}$) in the present study. MEm includes FHP (fasting heat production) and physical activity. According to Noblet, (2015) the FHP was 104 ± 6 kcal/$kg^{0.70}$, so if the MEm is 128 ± 6 kcal/$kg^{0.70}$ (experiment 2), activity would represent 24 kcal/$kg^{0.70}$ or 19% of the MEm. This activity value is higher than 8 -10% reported by Van Milgen et al., (2001). All maintenance components need to be studied by the same group to minimized variability due to methodologies and tools. The efficiency of energy utilization for maintenance is reported to be close to 80% (De Groote, 1974), and the present experiment 2 is in close agreement with 79%; however the efficiency for maintenance was 64% in experiment 2 probably for the high feed restriction (De Groote, 1974). On the other hand, the efficiency for gain was 62%, and 55% in experiment 2, it may be due to higher intake in experiment 2, decreases efficiency for gain. To the author's

knowledge there is no research that exogenous enzymes have been reported to decrease the maintenance energy, so no information is available for comparison. The energy savings from the enzymes depend on the feed intake. According to the present study, the more intake, the less the energy provided from the enzymes; however when for any reason feed intake is lower, the energy savings with the use of enzymes will represent more.

Protein represents a higher concentration of the body g/kg in dry matter (DM) at the highest restriction, and as feed intake is increased protein decreases and fat increases. Data reported in the present experiment follow the same trend as the data reported by Boekholt (1997) the nutrient utilization under feed restriction is directed to protein deposition first, then protein and fat are increased linearly as feed intake is increased.

**Table 1. MEI, RE, HP, and BW for experiments 1 & 2**

| Experiment 1 | | | | | Experiment 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Feeding level** | **[1]MEI** | **[2]RE** | **[3]HP** | **[4]BW (16-22 d), g** | **Feeding level** | **MEI** | **RE** | **HP** | **BW (16 - 27 d), g** |
| | **(kcal/kg$^{0.70}$ per day)** | | | | | **(kcal/kg$^{0.70}$ per day)** | | | |
| | | | | | Ad lib. | 373 | 135.7 | 237 | 865 |
| | | | | | 90% | 334 | 121.1 | 213 | 805 |
| | | | | | 80% | 314 | 105.6 | 209 | 741 |
| 50% | 142.0 | -6.1 | 148 | 436 | 70% | 292 | 91.7 | 200 | 681 |
| 40% | 116.2 | -24.2 | 140 | 407 | 60% | 261 | 74.0 | 187 | 639 |
| 30% | 87.9 | -35.0 | 123 | 390 | 50% | 228 | 56.9 | 171 | 600 |
| 20% | 60.1 | -57.5 | 118 | 356 | 40% | 193 | 36.9 | 156 | 554 |
| 10% | 31.6 | -76.1 | 108 | 317 | 30% | 155 | 18.7 | 137 | 500 |

Feeding level for experiment 1 doesn't correspond to experiment 2 because the feeding level was set up with the ad libitum feed intake of on d15. On experiment 2, the feeding level was adjusted daily during the trial based on the ad libitum intake. For instance, 50% of feeding level is close to 30% of the feeding level in experiment 2.

[1]RE = retained energy kcal/kg$^{0.70}$

[2]MEI = metabolizable energy intake, kcal/kg$^{0.70}$

[3]HP = heat production (MEI – RE), kcal/kg$^{0.70}$

[4]BW = body weight, g

**Table 2. Composition and nutrient calculations (g/100g as fed) of the diet**

| Ingredient, % | Grower 16-27 d |
|---|---|
| Yellow Corn (8.3% CP) | 59.62 |
| Soybean meal (47.5% CP) | 25.09 |
| Wheat middlings (16.7%CP) | 5.00 |
| Corn DDGS (29.4% CP) | 4.00 |
| Poultry Fat | 2.26 |
| DL-Methionine | 0.24 |
| L-Lysine HCl | 0.28 |
| L-Threonine | 0.11 |
| Calcium Carbonate | 0.93 |
| Dicalcium Phosphate | 1.17 |
| Sodium Chloride | 0.35 |
| Vitamin and mineral premix[1] | 0.54 |
| Propionic acid | 0.05 |
| Phytase[2] | |
| ***Calculated composition*** | |
| ME, kcal/kg | 3,008 |
| Crude Protein | 20.1 |
| Calcium[3] | 0.81 |
| Non-phytate phosphorus | 0.41 |
| Digestible lysine | 1.02 |
| Digestible methionine + cysteine | 0.77 |
| Digestible threonine | 0.67 |
| Digestible arginine | 1.06 |
| **Analyzed composition** | |
| AMEn, kcal/kg | 2966 |
| Crude protein | 22.1 |

[1]Supplied 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.

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

[3]Includes contribution from phytase of 0.10% Ca and 0.10% digestible P.

**Table 3. Dietary treatment**

| N° | Treatments | Abbrev. | Enzyme source | Principal enzyme | Minimum content (Units/kg feed) | Grower 16- 27 d |
|---|---|---|---|---|---|---|
| 1 | Negative control | NC | - | - | - | - |
| 2 | NC+ Multi-enzyme composite | NC + Enz | Carbohydrase from Aspergillus aculeatus | β-glucanase[1] | 2.5 FBG | 50 |
| | | | Carbohydrase from Trichoderma longibrachiatum | endo- 1,4 β xylanase[2] | 23.6 U | 100 |
| | | | | Cellulase | 70 – 87 U | |
| | | | Protease from Nocardiopsis prasina | Serine protease[3] | 5625 - 3750 PROT | 50 |
| | | | Phytase from Aspergillus oryzae | Phytase[4] | 1500 FTY | 150 |

[1]This carbohydrase is a multicomponent enzyme produced by submerged fermentation of *Aspergillus aculeatus*; however it has been standardized only for β-glucanase (*endo-1, 3 (4)-β-glucanase)*, so it has a guarantee value when analyzed in the final feed. The product also has hemicellulose and pectinase activities (Ravn, et al., 2015). Only the principal enzyme is cited because of the difficulty in the analysis of the other components. The analysis of the principal enzyme will be reported in results.

[2]The carbohydrase Xylanase is produced from *Trichoderma longibrachiatum*. It contains xylanase, cellulase, and *-1, 4-β-glucanase, and endo-1, 3 (4)-β-glucanase*. However, only 1, 4 endo-xylanase and cellulose are cited as principal enzymes because it has been standardized for this enzyme and guarantee values are provided.

[3]The serine protease has chymotrypsin specificity from Nocardiopsis prasina expressed in *Bacillus licheniformes*.

[4]The phytase is produced from *Aspergillus Oryzae.* The activity is 1500 FTY/kg of feed. The phytase is included on top the basal diet (NC) that has already 500 FTY/kg

**Table 4. Enzyme activity analysis in feed[1]**

| Enzyme | Treatment | Diet | Enzyme analyzed units/Kg | Target, U/kg | % of Guarantee |
|---|---|---|---|---|---|
| β-Glucanase U/kg[2] | NC + Enz | Starter | 112 | 80 | 140 |
| | | Grower | 83 | 80 | 104 |
| Xylanase, U/kg[3] | NC + Enz | Starter | 449 | 270 | 166 |
| | | Grower | 257 | 270 | 95 |
| Cellulase, U/kg[4] | NC + Enz | Starter | 133 | 70 | 190 |
| | | Grower | 97 | 70 | 139 |
| Protease PROT/Kg | NC + Enz | Starter | 5711 | 5625 | 102 |
| | | Grower | 3436 | 3750 | 92 |
| Phytase, FTY/Kg | NC | Starter | 487 | 500 | 97 |
| | | Grower | 374 | 500 | 75 |
| | NC + Enz | Starter | 1881 | 2000 | 94 |
| | | Grower | 2084 | 2000 | 104 |

[1]Samples from the diets were analyzed by the laboratory of Technical marketing Analytical Services – TMAS- Belvidere, New Jersey.

[2]When a multi-enzyme composite is added to one diet, the analytics can become cumbersome because β-Glucanase could be originated not only from the microorganism *Aspergillus aculeatus* but also from *Trichoderma longibrachiatum*. Each of these microorganisms produce multi-enzymes by themselves, so the analytical method measures in a different unit as the guarantee units but comparable to expected values U/kg.

[3, 4] the same scenario as with the β-Glucanase happens for the Xylanase measurements, so 2 different analytics have been performed to achieve the guarantee values.

**Table 5. Maintenance energy requirement (MEm, and NEm) for broilers 16 – 22d (experiment 1) and 16 -27d (experiment 2, only NC)**

| Experiment | Model expression | $R^2$ | [1]RMSE | Energy requirements kcal/kg BW0.70 per d | Efficiencies |
|---|---|---|---|---|---|
| Experiment 1 | [2]RE = -93.5 + 0.61 *[3]MEI | 0.91 | 7.99 | [4]MEm = 151.8 (145 - 160) | [5]kg = 0.62 |
| (10 - 50%) | [6]HP = 97.2 $e^{(0.0024*MEI)}$ | 0.87 | 7.87 | [7]NEm = 97.2 (92.3 - 102.1) | [8]km = 0.64 |
| Experiment 2 | RE = -68.54 + 0.55 *MEI | 0.98 | 5.86 | MEm = 128.1 (122 - 134) | kg = 0.55 |
| (30 - 100%) | HP = 97.9 $e^{(0.0023*MEI)}$ | 0.95 | 6.31 | NEm = 97.9 (93.7 - 102.3) | km = 0.79 |

[1]RMSE (root mean square error) means how far the data are from the model's predicted values.
[2]RE = retained energy kcal/$kg^{0.70}$
[3]MEI = metabolizable energy intake, kcal/$kg^{0.70}$
[4]MEm = metabolizable energy for maintenance, kcal/$kg^{0.70}$
[5]$k_g$ = efficiency of energy utilization for gain
[6]HP = heat production (MEI – RE), kcal/$kg^{0.70}$
[7]NEm = net energy for maintenance, kcal/$kg^{0.70}$
[8]$k_m$ = efficiency of energy maintenance

**Table 6. Metabolizable energy for maintenance with a multi-enzyme composite (experiment 2)**

| Diet | Retained energy, kcal/ $kg^{0.70}$/d | Predicted Mean (ME intake Kcal/$Kg^{0.70}$/ d) | Lower 95% | Upper 95% |
|---|---|---|---|---|
| NC | 0 | 128.1 | 123 | 133 |
| NC +Enz | 0 | 119.6 | 114 | 125 |
| Difference | | **8.5 kcal** | | |
| % savings with enzyme (8.5/128.1) x 100 | | **6.6 %** | | |

| **Feeding level** | **Feed intake (FI), g** | **Energy saving from the enzyme ME kcal/kg FI (8.5 / FI) x 1000** |
|---|---|---|
| 30% | 32 | 265 |
| 40% | 43 | 198 |
| 50% | 54 | 157 |
| 60% | 64 | 133 |
| 70% | 75 | 113 |
| 80% | 86 | 99 |
| 90% | 97 | 88 |
| 100% | 114 | 75 |

**Table 7. Metabolizable energy intake (MEI), retained energy (RE) and heat production (HP), kcal/kg$^{0.70}$ (exp. 2)**

| Feeding level | [1]MEI | | | [2]RE | | | | | [3]HP | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NC | NC + Enz | *Dif.* | NC | NC + Enz | *Dif.* | *[4]SE* | *P-value* | NC | NC + Enz | *Dif.* | *SE* | *P-value* |
| Ad lib. | 373 | 371 | *-1.9* | 135.7 | 147.1 | *11.4* | *2.2* | *<0.01* | 237 | 224 | *-13.3* | *2.2* | *0.096* |
| 90% | 334 | 333 | *-0.3* | 121.1 | 120.5 | *-0.5* | *2.9* | *0.897* | 213 | 213 | *0.3* | *3.4* | *0.952* |
| 80% | 314 | 310 | *-3.9* | 105.6 | 112.4 | *6.8* | *2.1* | *0.052* | 209 | 198 | *-10.7* | *3.1* | *0.041* |
| 70% | 292 | 289 | *-3.0* | 91.7 | 95.2 | *3.5* | *2.7* | *0.380* | 200 | 194 | *-6.5* | *3.1* | *0.050* |
| 60% | 261 | 261 | *-0.4* | 74.0 | 77.9 | *3.8* | *2.0* | *0.203* | 187 | 183 | *-4.3* | *2.3* | *0.214* |
| 50% | 228 | 227 | *-1.5* | 56.9 | 60.4 | *3.5* | *2.3* | *0.300* | 171 | 166 | *-5.0* | *3.0* | *0.257* |
| 40% | 193 | 192 | *-0.8* | 36.9 | 40.8 | *3.8* | *2.2* | *0.274* | 156 | 152 | *-4.6* | *1.4* | *0.052* |
| 30% | 155 | 155 | *-0.2* | 18.7 | 18.9 | *0.3* | *3.6* | *0.960* | 137 | 136 | *-0.4* | *2.4* | *0.905* |

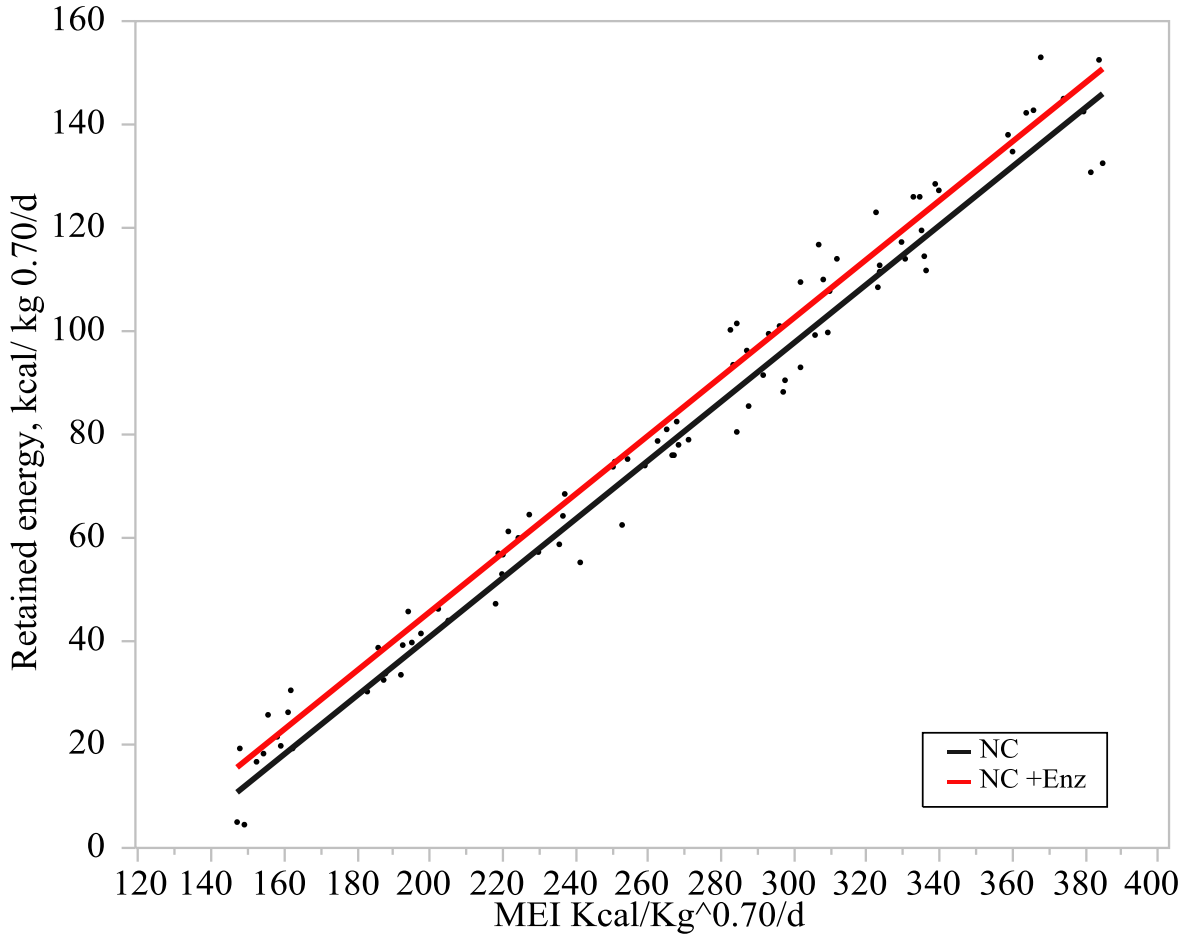
[1]MEI = metabolizable energy intake, kcal/kg$^{0.70}$
[2]RE = retained energy kcal/kg$^{0.70}$
[3]HP = heat production (MEI – RE), kcal/kg$^{0.70}$
[4]SE, standard error

**Table 8. Body composition in terms of protein, fat and BMC (g/kg) of broilers by feeding level (experiment 2)**

| Item | | 100% | 90% | 80% | 70% | 60% | 50% | 40% | 30% |
|---|---|---|---|---|---|---|---|---|---|
| Protein g/kg | NC | 639.8 | 647.8 | 654.0 | 661.3 | 668.9 | 675.3 | 687.7 | 701.0 |
| | NC +Enz | 638.2 | 647.5 | 653.5 | 659.3 | 667.3 | 675.5 | 686.3 | 700.4 |
| | *Dif.* | *-1.6* | *-0.3* | *-0.5* | *-2.0* | *-1.6* | *0.2* | *-1.5* | *-0.6* |
| | *SE* | *1.72* | *0.84* | *1.77* | *0.92* | *1.47* | *1.78* | *1.95* | *1.92* |
| | *P-value* | *0.530* | *0.820* | *0.845* | *0.160* | *0.457* | *0.944* | *0.616* | *0.830* |
| Fat g/kg | NC | 319.2 | 303.7 | 265.5 | 236.5 | 215.2 | 177.1 | 173.2 | 152.0 |
| | NC +Enz | 336.4 | 301.1 | 291.5 | 236.8 | 210.2 | 197.5 | 174.2 | 150.1 |
| | *Dif.* | *17.2* | *-2.6* | *26.1* | *0.2* | *-4.9* | *20.4* | *1.0* | *-1.9* |
| | *SE* | *9.50* | *8.06* | *13.70* | *11.12* | *6.40* | *6.57* | *7.80* | *10.10* |
| | *P-value* | *0.097* | *0.826* | *0.216* | *0.988* | *0.599* | *0.503* | *0.933* | *0.890* |
| [1]BMC g/kg | NC | 85.7 | 94.8 | 90.1 | 92.2 | 91.5 | 92.6 | 102.6 | 112.9 |
| | NC +Enz | 87.0 | 94.5 | 96.1 | 90.3 | 91.6 | 96.3 | 104.6 | 108.9 |
| | *Dif.* | *1.3* | *-0.3* | *5.9* | *-1.9* | *0.1* | *3.7* | *2.1* | *-3.9* |
| | *SE* | *3.00* | *1.47* | *3.29* | *2.80* | *0.98* | *1.85* | *1.68* | *2.40* |
| | *P-value* | *0.798* | *0.889* | *0.239* | *0.565* | *0.959* | *0.190* | *0.416* | *0.280* |

[1]BMC, bone mineral content
[2]SE, standard error mean

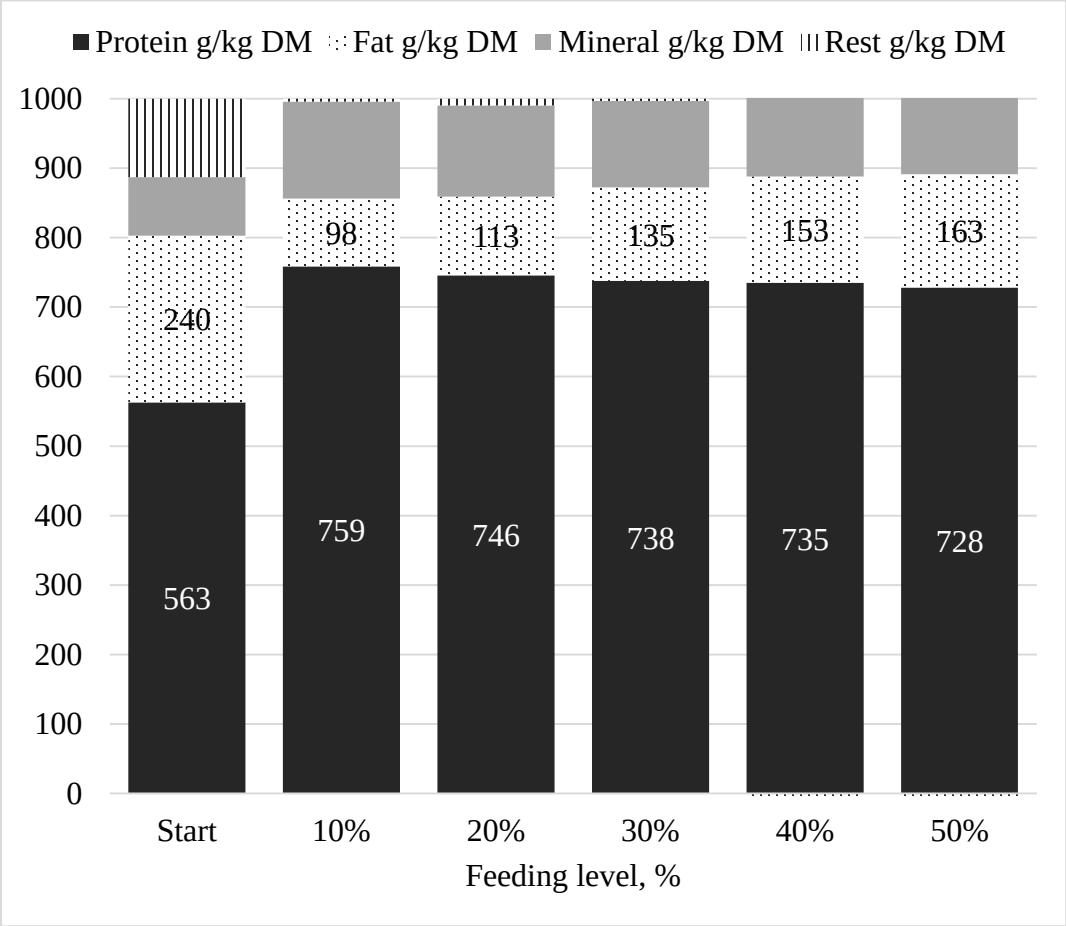
**Figure 1. Linear regression: Retained energy (RE) regressed on MEI (metabolizable energy intake) kcal/kg$^{0.70}$ (experiment 2)**




$R^2$ 0.98
RMSE 5.72
Lack of fit (P value 0.99)

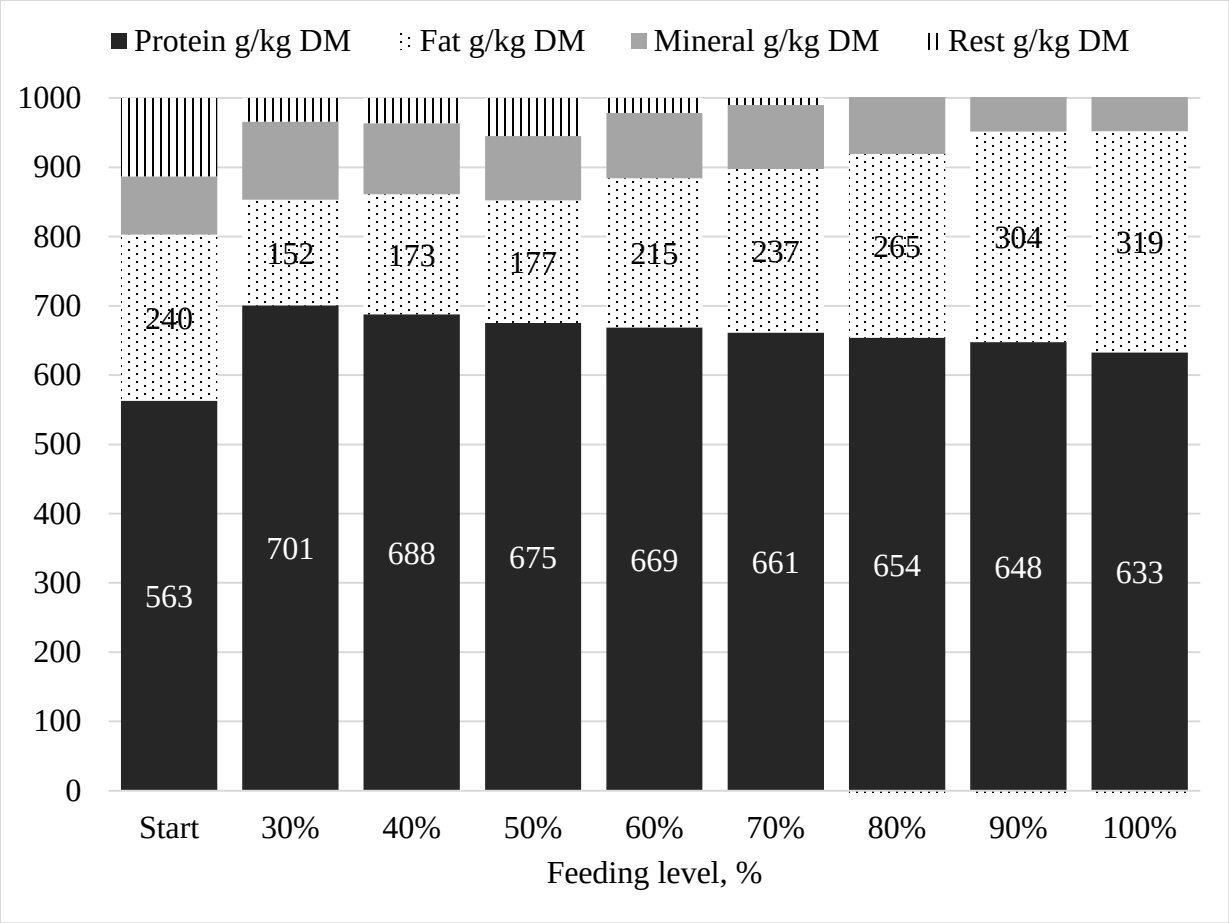
| **Term** | **P-value** |
|---|---|
| Intercept | <.0001* |
| MEI Kcal/Kg$^{0.70}$/d | <.0001* |
| Diet | 0.0002* |

**Figure 2. Body composition in broilers 10- 50% feeding level, 22d (experiment 1)**




DM, dry matter

**Figure 3. Body composition of broilers 30- 100% feeding level, 27d (experiment 2, only NC)**




NC, negative control
DM, dry matter

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# VII. DYNAMICS OF NUTRIENT UTILIZATION, HEAT PRODUCTION AND BODY COMPOSITION IN BROILER BREEDER HENS DURING EGG PRODUCTION

**ABSTRACT**

Changes in heat production and body composition in modern broiler breeders can provide means to understand nutrient utilization and an opportunity to improve feeding strategies. The aim of this study was to determine the dynamics of heat production and body composition in broiler breeders during production. Twelve Cobb 500 fast feather breeders wire caged were identified and evaluated every 3 weeks from 26 to 59 weeks of age, having 10 points of evaluation. At every point, the same breeders were moved to respiratory chambers connected to an open flow indirect calorimetry system, placing one breeder/chamber, one day before evaluation to allow them adaptation. Hens underwent 24 h of evaluation at every point to obtain volume of oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$) and with these values heat production (HP) kcal was estimated with the Brouwer equation (HP, kcal/d= 3.87 * $VO_2$ L/d + 1.23* $VCO_2$ L/d) and respiratory exchange ratio (RER) from $VCO_2/VO_2$. Data was separated by period of time during the day light (16 h) and dark (8 h). The same 12 breeders were scanned for body composition (lean and fat mass) using a dual X-ray absorptiometry (DEXA) one day before evaluation in chambers. The feed allocation was 123 g (352 kcal) at 26.3 wk and changed to 136 g (390 kcal) at 29.6 wk which was kept the same until the end of production. The statistical analysis was achieved using JMP 12 (SAS, 2015). A mixed model was used to evaluate calorimetry parameters HP kcal/d, $VO_2$, $VCO_2$ L/d and RER by age, time of day, and hen as random factor because it was repeatedly measured. A factorial design 2 x 10 (period of time x age) for calorimetry parameters kcal/$kg^{0.75}$ and, L/$kg^{0.75}$, and a CRD - one way ANOVA (age) with hen as random effect for body composition analysis, lean and fat gain g/d. Means were separated by Tukey-HSD test. HP was increased with age (d) in 0.28 kcal/d and the difference of light and dark period was 91 kcal/d ($P<0.01$). The amount of $VO_2$ consumed and $VCO_2$

produced was 0.058 and 0.046 L/d per each increase in age (d) respectively. During the light period, hens consumed more $VO_2$ (+17.5 L/d) and produced more $VCO_2$ (+19.2 L/d) (*P<0.01*). HP during the dark period was 83 kcal/$kg^{0.75}$ which could be considered the NEm (net energy for maintenance) and during the light period was 115 kcal/$kg^{0.70}$. RER decreased with age in -$0.1x10^{-3}$ per day suggesting more fat and/or protein being oxidized at later periods of production. Lean gain was negative after peak production (37 wk), and fat gain was the lowest at 40 wk, and after 54 wk. Lean body mass changed from 642 – 783 g/kg reaching the lowest at 37 and 50 wk and the highest at the beginning of production 26– 33 wk (*P*<0.001**). Fat body mass changed from 168 – 261 g/kg with the lowest at the beginning of production 26-33 wk and the highest at 50 wk of age (*P*<0.001**). Broiler breeder females may be using body energy reserves from 50 wk onward when the egg production has reduced below 50% because Heat Production kcal/$kg^{0.75}$ increased significantly at 54 and 59 wk compared to 50 wk (*P*<0.0002). Broiler breeder females change nutrient fuel use during egg production period. Indirect calorimetry and DEXA can be used to pursue further feed strategies to maximize egg production and maintain a healthy breeder before, and during egg production.

Key words

Breeders, calorimetry, heat production, body composition

## INTRODUCTION

The continuously increase in the broiler market requires increase in number and efficiency in the genetics of broiler breeders. Meat – type hens or broiler breeders have been intensively selected for growth rate, feed efficiency, and breast meat yield traits, but not necessarily for reproductive traits; in fact, these hens have less egg production than table-egg producing hens (Robinson et al., 2003). Therefore, management and nutrition of the broiler breeder is the most complex piece of the poultry production (Kleyn, 2013) because egg production in the parent stock, and meat production in the progeny are desired. Understanding the dynamics of heat production and body composition along egg production can provide insights of the nutrient utilization of broiler breeders; however few information of the modern broiler breeder has been published. Heat production (HP) can be measured by indirect calorimetry and by difference between MEI (metabolizable energy intake) and retained energy (Sakomura, 2004). Body composition can be different at the same body weight affecting the onset of sexual maturity, so the analysis of lean and fat mass are important (Wilson, et al., 1989). Body composition has changed over time resulting in leaner breeders being lean protein very important at the onset of sexual maturity (De Beer, and Coon, 2007). Salas (2012) evaluated the body composition of broiler breeders using DEXA (dual energy X-ray absorptiometry) and found a decrease in lean mass at 35 and 45 wk of age during production that couldn't be explained until Vignale (2014) who found the highest protein degradation during 30 -37 wk of age that explains the decrease in lean mass at 35 wk found by Salas. Indirect calorimetry measures $VO_2$ (volume of oxygen consumption), and $VCO_2$ (volumes carbon dioxide production) to estimate HP. It also provides the RER (respiratory exchange ratio $VCO_2/VO_2$) that explains nutrient oxidation. The values for RER are 1.0, 0.74, and 0.70 for carbohydrate, protein, and fat oxidation respectively in birds (McLean and Tobin, 1987).

Calorimetry can explain the nutrient oxidation, and DEXA body synthesis can provide means to understand the dynamics of nutrient utilization in broiler breeder hens, so the objective of the present study is to follow the same breeder during production from 26-59 wk of age and evaluate calorimetry parameters: $VO_2$, $VCO_2$, RER, HP, along with body composition: lean, fat mass and BMC (bone mineral content) and their interrelationships.

## MATERIALS AND METHODS

All management practices and procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC) # 13002.

### Birds and Housing

Twelve parent stock females from Cobb 500 fast feather (Cobb Vantress, Siloam Springs, AR) were selected from a population of 70 hens at 23 wk of age. Hens were selected to represent a normal population of hens at 23 wk (BW mean 2544 g ± 258 SD, 10% CV). Hens were transferred from floor pens to wire cages (47 cm high, 30.5 cm wide, 47 cm deep) equipped with an individual feeder and nipple drinker. Light stimulation was 16 h light and 8h dark from 26 – 59 wk. The same 12 hens were evaluated at 10 times during production (26, 30, 33, 37, 40, 43, 45, 50, 54, and 59 wk of age). Hens were moved to respiratory chambers for 24 h of evaluation after another 24 h of adaptation. Since the hens were maintained in wire cage with similar dimensions, wire floor, feeder and drinker type than the respiratory chambers, the adaptation period seemed to be enough. Temperature was kept at 21°C through production (Cobb 500, 2008) in cages and respiratory chambers. The light program was 18 hr. light: 6 hr. dark to

decrease the stress of feed restriction in the birds. Egg production was recorded daily and averaged for 12 hens at every week of evaluation.

***Respiratory chambers***

Respiratory chambers were made from polycarbonate plastic glass (61 cm long x 51 cm wide x 56 cm high) and equipped with 1 feeder and 1 nipple drinker according to the specifications of FASS, 2010 (Champaign, IL). The room for the respiratory chambers was the same as the rooms were the hens were kept in cages. It was equipped with two heating and air conditioning units. These units were controlled by a Honeywell programmable thermostat that automatically switches between cooling and heating within a 2°C range. Minimum ventilation was provided by two ventilation fans that exhaust to the outside and draw fresh air from the hall. Each ventilation fan is controlled by a timer. The on/off cycle can be adjusted as needed to maintain room air quality and desired $CO_2$ levels. To control humidity, the room was equipped with two de-humidifiers (GE, Madison, WI) running continuously. Relative humidity (RH) ranged from 50-80% depending upon on the RH of the environment. Temperatures (T) in the room were usually 4- 5 °C lower to ensure the temperature inside the chambers were 21 °C. The indirect calorimetry system was detailed in chapter 3, the air flow LPM (liter per minute) ranged from 12 -15 LPM depending on the size of the hen to have a range of $DCO_2$ ($CO_2$ out – $CO_2$ in) between 0.30 – 0.50. The gas evaluation in each chamber was measured every 12 minutes, so every chamber unit provided 5 readings during one hour, 120 readings in a day from which 67% is during the light time and 33% during dark time (7pm – 3am).

**Diet and feed program**

Hens were fed a commercial pelleted feed during evaluation (Cobb-Vantress, Siloam Springs, AR). Only 1 diet was used for the experiment (breeder 1) formulated to have 2860 kcal/kg of ME (metabolizable energy), and 15.5 % crude protein. Four batches of diet were received during the evaluation, and analyzed for proximal analysis upon arrival (Table 1). Feed and energy allowance was 123 g (352 kcal/d) at 26 wk and 136 g (390 kcal/d) at 30 wk and kept the same. Hens were fed every day at 7 AM in cages and chambers, the feed was finished after approximately an hour. Fresh water was provided *ad libitum* during evaluation.

**Body Composition Analysis**

Hens were scanned alive one day before every point of evaluation in the respiratory chambers using dual energy X-ray absorptiometry, DEXA scanner (GE, Madison, WI) with small animal body software module (Lunar Prodigy from GE encore version 12.2) for more details revise chapter 3 on this manuscript. Green lights were set up in the room to maintain the birds restful while scanning for about 3.5 – 4 minutes per hen. No chemicals or anesthesia were used and hens were scanned at the same time (around 1 PM) at every point of evaluation. Hens were returned to the respiratory chambers after scanning. Total tissue, lean, fat mass and BMC (bone mineral content) were adjusted to real body composition values analyzed by chemical analysis using equations previously developed (Salas, 2012).

**Calculation**

Data from indirect calorimetry were separated as time of day (light or dark) and averaged within a day. $VO_2$, $VCO_2$, RER ($VCO_2/VO_2$) were calculated as L/d (liters per day), and normalized to metabolic body weight $L/kg^{0.75}$ for comparative purposes. Heat production (HP) was obtained using the equation: HP kcal/d = 3.866 $VO_2$ L/d + 1.233 $VCO_2$ L/d (Brouwer, 1965). HP was also normalized $kcal/kg^{0.75}$. The body composition (lean, fat and bone mineral content (BMC) will be reported as absolute g and g/kg of body weight.

**Statistical analysis**

A mixed model was used to evaluate calorimetry parameters HP kcal/d, $VO_2$, $VCO_2$ L/d and RER by age (10 levels), time of day (2 levels: light and dark), and hen as random because of the repeated measurements. The normalization of calorimetry parameters by metabolic weight is meant to compare and parameters are reported to be the same over time, a mixed model with normalized data resulted in no differences, so a factorial design 2 x 10 (time of day x age) for calorimetry normalized parameters $kcal/kg^{0.75}$ and, $L/kg^{0.75}$ was analyzed. A complete randomized design, CRD - one way ANOVA (age) with hen as random effect for body composition, lean and fat gain g/d was performed. Means were separated by Tukey-HSD test. Multivariate correlation analysis between calorimetry parameters and body composition was also analyzed. A linear regression analysis was fit between fat tissue gain g/d and lean tissue gain g/d. *P* -value was considered significant when $\leq 0.05$ and a trend if $P<0.10$. All analysis were determined with JMP12 (SAS, 2015).

## RESULTS

### Calorimetry parameters

The calorific value of heat production (HP) is the result of gases evaluation, volume of oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$). Mixed models allowed to understand the dynamics of $VO_2$, $VCO_2$, and HP by means of age, and time of day (light, and dark) with repeated measurements. Gases and HP were increased by age being $VO_2$ 0.058 L higher per every increase of age (days), $VCO_2$ 0.046 L produced per day and heat 0.28 kcal per day ($P<0.001$). At the end of the trial (59 wk), HP was the highest because of a bigger body tissue, and lean mass formed during the production cycle. Hens consumed more oxygen during the light period (3 AM – 7PM) in +17.5 L because of higher activity and feeding. The parameter estimate of the mixed model provides half of this difference (Table 2) because two periods were evaluated (light and dark). The amount of $VCO_2$ produced in the light period was + 19.2 L more than in the dark period. $VO_2$ was always higher than $VCO_2$ in the light and dark periods (Figure 1). HP then was 91 kcal more in the light period than in the dark period, accounting for a more activity and heat increment of feed. Variation component due to hen was around 56, 46, and 54% for $VO_2$, $VCO_2$, and HP respectively, and the rest could be the environmental effect (Table 2). When gases and HP are normalized to metabolic body weight (BW $kg^{0.75}$) to provide comparison between ages of the hen, a factorial approach Time of day x age was performed (Table 3). The interaction effect was no significant ($P>0.05$), time of day was higher in 6.1 $L/kg^{0.75}$ (+ 27%) $VO_2$, 6.5 $L/kg^{0.75}$ (+ 30%) $VCO_2$, and HP 31 $Kcal/kg^{0.75}$ (+ 27%) ($P<0.01$). The respiratory exchange ratio (RER) was also higher during the light period (0.955 vs 0.907 in the dark period) meaning differences in nutrient utilization between light and dark periods (Figure 2). At the end

of the evaluation 59 wk, hens increased oxygen consumption L/kg$^{0.75}$ with respect to ages lower than 50 wk with exception of 30 wk which corresponds to peak production ($P$<0.01). Carbon dioxide production L/kg$^{0.75}$ shows a slightly different patter than $VO_2$, being the highest still at 59 wk compared to ages lower than 50 wk, except 26, 30, and 37 wk of age ($P$<0.01). Heat production kcal/kg$^{0.75}$ as consequence of higher $VO_2$ and $VCO_2$ at 59 wk, HP was higher at 59 wk compared to ages lower than 50 wk, except 30 wk. Respiratory exchange ratio (RER) was the lowest at 43 wk of age compared to other ages except 40, and 50 wk ($P$<0.01) meaning more fat and/or protein being oxidized at this ages compared to carbohydrates. The highest RER was found at 30 wk compared to other ages except 26 wk. ($P$<0.01).

**Body composition**

For body composition evaluation, a CRD design provides differences in tissue composition between ages (Table 4). Total mass which is equivalent to scale body weight (BW) was higher at 54 wk; however no differences was found between 54 wk, 59, 50, 45 and 43 wk of age ($P$<0.01). Lean mass, which corresponds to water + protein, was the highest at 59 wk (3031 g) compared to 26, 37, and 50 wk ($P$<0.01). The lowest lean was found at the beginning of production 26 wk compared to 33, 40, 43, 54 and 59 wk; there are some weeks (37, 45, and 50 wk) where the lean mass in absolute value was not different from the beginning of production ($P$<0.01). Fat mass was the highest at 50 wk compared to other ages except 54 wk. The smallest amount of fat was found to be at the beginning of production (26 wk) compared to ages higher than 37 wk ($P$<0.01). Body mineral content (BMC) reached the highest point at 50 wk (187 g) compared to other ages except 37, 45, 54, and 59 wk ($P$<0.01). The smallest amount of BMC was at 30 wk compared to 50 wk, only. Body composition expressed as in g/kg provides meaningful

information about the relative body composition between ages. Lean mass, g/kg was the highest at 26 wk compared to other ages except 30, 33, and 40 wk of age and the lowest at 50 wk compared to other ages except 45, and 54. Lean mass in absolute value as depicted in Figure 3, shows the lowest point at 26, 37, and 50 wk relative to 59 wk; and lean mass in g/kg shows the first low point at 37 wk compared to the initial body composition at 26 wk, and the second lowest point at 50 wk compared to 37 and 26 wk, and also 59 wk. At the end of the study 59 wk, lean mass (g) is higher than at the beginning 26 wk, 37, and 50 wk and when expressed as lean g/kg tends to increase at the end but still lower than at 26 wk but higher than 50 wk. Lean mass tend to decrease until 50 wk, but increase again after 50 wk. Fat mass as in g/kg was the lowest at the beginning of production compared to ages higher than 43 wk with the exception of 37 wk. The highest fat g/kg was at 50 wk compared to other ages except 45, and 54 wk ($P<0.01$). Fat composition tends to increase with age reaching the highest point at 50 wk compared to the beginning of production but, tends to drop at the end even though no significant (Figure 3, and 4). Bone mineral content (BMC) g/kg is the highest at 43 wk compared to 33, and 40 wk ($P<0.01$), and the lowest at 40 wk compared to 50 wk (Table 4). Lean gain g/d was variable during the egg production for the 12 hens evaluated ranged from -6.5 g/d at 37 wk to +10.4 g/d at 30 wk. Tissue gain g/d was calculated over the period between two proximate ages evaluated. For example, 10.5 g/d of lean tissue was BW 30 wk – BW 26 divided by the number of days between these two ages, and the same calculations for the next periods. Lean tissue gain at 37 wk was significant lower compared to 30, and 40 ($P<0.01$) suggesting protein tissue being oxidized during this period. Lean tissue was also negative at 50 wk compared to 30, and 40 wk ($P<0.01$). Fat gain g/d was the highest at 37, and 50 wk compared to 40, 54 and 59 wk ($P<0.01$). Figure 5 depicts the negative lean tissue gain at 37 wk, measured right after the highest times of egg

production, and Figure 6 shows the linear relationship between fat tissue gain and protein tissue gain. For every g/d of lean gain, fat gain decreases in 0.45 g/d.

**Relationship between egg production, heat production, RER, and body composition**

A pairwise correlation between egg production, and HP show a negative low correlation (-0.24) but significant ($P$<0.01), so when egg production decreases, heat production increases. Egg production does not correlate to lean mass but shows a negative small correlation (-0.18) with fat ($P$<0.05). RER shows not correlation with egg production and body composition. This may be due to RER shows instantaneous data while egg production and body composition are consequence of metabolism of previous ages. In effect, the ups and downs in the body composition along age, may be the cause of this low correlations with egg production and HP (Table 5). The dynamics of heat and body composition changes with age being HP the lowest at the beginning of egg production 26 wk and continuously increases in small amounts up to 45 wk, and after that a big jump of HP is seen (Figure 7). This HP behavior doesn't explain the lowest first point of lean mass g/kg seen at 37 wk and it slightly matches the 50 wk, second lowest point of lean tissue, before lean increases by 59 wk. Fat mass, g/kg increases at a higher rate than heat production but drops at 50 wk when HP keeps increasing (Figure 8). Heat and body composition change with age but the change along egg production (EP) is more important since the objective of meat-type breeders is produce chicks of high quality by producing good quality eggs. Both EP and HP star low at the beginning of the egg production (26 wk), peak production was reached at 30 wk and gradually decreased until the end of production (59 wk). When HP reached the highest point during the experiment (59 wk), EP was the lowest (Figure 9). HP in terms of egg production is an inefficient process because it's mostly used for maintenance energy requirement

(Chwalibog, and Thorbek, 1991). RER tends to decrease as with egg production until 43 wk of age; however it increases when EP drops (Figure 10). Lean mass and EP increase both up to peak production; at peak of production when lean tissue tends to drop, EP is gradually declining. When lean mass increases after 50 wk of age, EP keeps dropping (Figure 11). Fat mass, g/kg gradually increases while EP gradually decreases, that's why the negative correlation is positively significant (Figure 12).

## DISCUSION

### Calorimetry parameters and egg production

The amount of $VO_2$ and $VCO_2$ for modern broiler breeders is rarely reported. Past research with meat-type breeders reports 14.6 $L/kg^{0.75}$ for oxygen consumption (Waring, 1965) which is lower than the value reported in the present experiment, 20 $L/kg^{0.75}$. This may be due to modern breeders having more lean tissue than birds in 1965 in the same basis ($L/kg^{0.75}$). This increase represents 37% more oxygen in 2015 compared to 1965 accounting for an increase of 0.74 $L/kg^{0.75}$ of oxygen per year. It is well known that yield is higher in modern broilers and so in broiler breeders because of the genetic potential for more lean mass than before (Havenstein, 2003). When compared to broiler, broiler breeders have lower $VO_2$ and $VCO_2$ $L/kg^{0.75}$. Fedde (1998) reports $VO_2$ of 42 $L/kg^{0.75}$, and $VCO_2$ 40 $L/kg^{0.75}$ in broilers with BW 1.38 kg at 35d, which is almost twice the amount of $VO_2$ and $VCO_2$ found in breeders (20 $L/kg^{0.75}$). These amount of gases for broilers is also in agreement with data from chapter 4 and 5 of this manuscript. The reason of more gases consumption and production in broilers is consequence of high growth rate fed ad libitum while hens are fed restricted along egg production to avoid

increase in fatness and decrease in egg production. Maintenance energy is also higher in broilers (Sakomura, 2004). Regarding to heat production, past research shows that heat production in laying hens being around 200 kcal/d or 73 kcal/$kg^{0.75}$ fed 281 kcal (Waring and Brown, 1965). Daily HP averaged 287 kcal/d in this study, and 100 ± 3 kcal / $kg^{0.75}$ (range 96 – 106 kcal/ $kg^{0.75}$) being the highest at the of the experiment 59 wk. This high HP positively correlates with higher body lean mass, more heat is produced when body lean mass (g) is higher. This finding is in agreement with data from Teeter (1996) when oxygen required per unit protein synthesis was 380% greater than that for fat. Fasting HP over 3 days in meat-type hens was found to be 52 kcal/kg/d (Spratt, 1990) converting this value to metabolic $BW^{0.75}$, the HP would be 68 kcal/$kg^{0.75}$/d. This 68 kcal accounted for 75% of the maintenance energy (91 kcal/$kg^{0.75}$/d). Heat Production in the dark was 84 kcal/$kg^{0.75}$, which is similar to the maintenance energy reported by Spratt, 1990. However, the study was not design to follow maintenance requirements because the breeders were not fasted or underwent more stress conditions due to experimental procedure. RER provides means to differentiate nutrient utilization between carbohydrates, protein, and fat since these are the only nutrients assumed to release energy for maintenance of life in human and animals. The RER for the oxidation of carbohydrates, protein, and fat in chickens is 1, 0.72, and 0.70 respectively (MacLean and Tobin, 1987), since the diet is a balanced of carbohydrates, proteins and fats, one can compare only differences with respect to other age point. RER reached the lowest point at 40 – 43 wk of age which could mean more fat and/or protein oxidation compared to carbohydrates at the beginning of production, RER increases at 45 wk and remains low suggesting the hens using less carbohydrates at 45 wk compared to peak production, 30 wk. Salas (2001) reported hens using glucose for egg production at the beginning and fat utilization at the end of production. Salas used stable isotopes for this findings which is in partial agreement

with the results in the present experiment because RER other than peak production, 26, 33, 37 wk are not different than RER at 54, and 59 wk. High RER variability was seen during egg oviposition times and during day time, data were more clear at night when activity was decreased; however there are still differences between hens (50%), maybe more sophisticated models can explain hen's behavior during egg production. Data from RER is new and not information is being reported, so more research is needed to understand the significance of this value which can change with feeding strategies, individual bird variation, and genetics.

**Body composition, and egg production**

Lean tissue mass, g and g/kg reached the lowest point at 37 and 50 wk which is in full agreement with data found by Salas (2011) and Vignale (2014). Vignale, used $^{15}$N phenylalanine to calculate the rate of fractional protein synthesis and degradation and found the highest protein degradation at peak production (30 – 37 wk), suggesting the hen using protein breast for egg synthesis. This results is in agreement with the negative lean tissue gain found at 37 wk which was consequence of negative gain over 30 - 37 wk period, during peak production; however this negative tissue lean gain was different only compared to 30, and 43 wk and not to other ages because of high variability between hens. After 50 wk, egg production is low and the hen starts increasing lean tissue and protein synthesis increases as well, probably the hen is preparing to the next clutch or production cycle as it happens in nature. Lean mass was high at the beginning of production which matches with high protein synthesis shown by Vignale (2014). Van Emous (2015) reports breast muscle of 17.24% at 35 wk compared to 20.15% at 22 and 16.43% at 59 wk with high dietary protein. These findings are in partial agreement with the present study with respect to less breast muscle at 35 wk but not an increase in lean mass at 59 wk. Fat utilization is

also important in egg formation (Boonsinchai, 2015) and a balance of protein and fat utilization exists during the complex egg production process. In the present study, fat keeps increasing along with the age; however fat seems not be as important as lean tissue for egg production because lean tissue tends to decrease when the hen is at its highest egg production, contrary to fat which keeps increasing up to 50 wk where it reaches high positive fat gain. After 50 wk, fat component in the body tends to decrease suggesting body is using more fat as fuel than lean mass which increases at the end of production. Abdominal fat increases with age in broiler breeders 0.68 at 22 wk, 2.02 at 35 wk, and 2.27% at 59 wk (Van Emous, 2015) which is partial correlation to the present study. Even with only 12 hens, it is respectable how well the present experiment agrees with the results from other researchers who work with more hens. Bone mineral content (BMC) is the lowest at peak production (30 wk) compared to 50 wk. Since Ca and P account for 23, and 20% of the BMC (Caldas, 2015, chapter 2), data suggests high utilization of mineral utilization for egg shell formation during production. At 50 wk, hen decreases egg production <50%, so the mineral content seems to remain in the body in higher quantity than at peak production. Egg production in this experiment was close to the standard (Cobb, 2012) until peak production but lower than the standard at the end, probably because the handling of the same birds for 10 points of evaluation along production; however body composition was in agreement with other researchers that use different hens at each point of evaluation, so the data still valid. Finally, indirect calorimetry and DEXA can be used to pursue further feed strategies to maximize egg production and maintain a healthy breeder before, and during egg production.

**Table 1. Feed analysis from different batches of the same feed (breeder 1)**

| Batch | Dry Matter | Protein | Ash | Fat | Ca |
|---|---|---|---|---|---|
| | % | % | % | % | ppm |
| **#1** | 89.5 | 16.7 | 11.09 | 4.37 | 35598 |
| **#2** | 89.2 | 17.4 | 10.72 | 6.72 | 32787 |
| **#3** | 89.6 | 17.0 | 10.73 | 5.42 | 31663 |
| **#4** | 90.2 | 16.3 | 11.43 | 2.89 | 36159 |
| | Average | 16.9 | 11.0 | 4.9 | 34052 |
| | SD | 0.47 | 0.34 | 1.62 | 2171 |
| | CV, % | 2.8 | 3.1 | 33.4 | 6.4 |

**Table 2. Mixed model for $VO_2$, $VCO_2$, HP, and RER**

| | Y = [1]$VO_2$ L/d | Y = [2]$VCO_2$ L/d | Y = [3]HP kcal/d |
|---|---|---|---|
| Parameter estimates | Mean + SE | Mean + SE | Mean + SE |
| Intercept | 40.9 ± 1.5 | 40.3 ± 1.4 | 206 ± 7.3 |
| Age (d) | 0.058 ± 0.003 | 0.046 ± 0.003 | 0.28 ± 0.02 |
| Time of day (Light) | 8.78 ± 0.23 | 9.53 ± 0.24 | 45.3 ± 1.1 |
| | | | |
| Random effect | Variation component, % | | |
| Hen | 56 | 46 | 54 |
| | | | |
| Time of day | | | |
| Light | 66.4 | 63.40 | 333 |
| Dark | 48.9 | 44.20 | 242 |
| *Dif.* | *17.5* | *19.2* | *90.9* |
| $R^2$ | 0.91 | 0.90 | 0.91 |
| P-value | <.0001 | <.0001 | <.0001 |

[1]$VO_2$ = volume of oxygen consumption L/d
[2]$VCO_2$ = volume of carbon dioxide production L/d
[3]HP = Heat production kcal/d

**Table 3. Calorimetry parameters $VO_2$ L/kg$^{0.75}$, $VCO_2$ L/kg$^{0.75}$, HP kcal/kg$^{0.75}$ and RER by time of day and age**

| Time of day | [1]$VO_2$ L/kg$^{0.75}$/d | [2]$VCO_2$ L/kg$^{0.75}$/d | [3]HP kcal/kg$^{0.75}$/d | [4]RER ($VCO_2/VO_2$) |
|---|---|---|---|---|
| Light | 23.0 | 21.9 | 115 | 0.955 |
| Dark | 16.9 | 15.4 | 84 | 0.907 |
| *Dif. units* | *6.1* | *6.5* | *31* | *0.048* |
| *Dif. %* | *+27* | *+30* | *+27* | *+5* |
| *[5]SEM* | *0.37* | *0.37* | *1.82* | *0.006* |
| Age | | | | |
| 26 | 19.8$^{b}$ | 19.0$^{ab}$ | 100$^{bc}$ | 0.952$^{ab}$ |
| 30 | 20.4$^{ab}$ | 19.8$^{a}$ | 103$^{ab}$ | 0.966$^{a}$ |
| 33 | 19.8$^{b}$ | 18.5$^{bc}$ | 99$^{bc}$ | 0.931$^{bc}$ |
| 37 | 19.9$^{b}$ | 18.7$^{abc}$ | 99$^{bc}$ | 0.934$^{bc}$ |
| 40 | 19.8$^{b}$ | 18.2$^{bc}$ | 98$^{bc}$ | 0.919$^{cd}$ |
| 43 | 19.8$^{b}$ | 18.0$^{bc}$ | 98$^{bc}$ | 0.903$^{d}$ |
| 45 | 19.2$^{b}$ | 17.8$^{c}$ | 96$^{c}$ | 0.924$^{c}$ |
| 50 | 19.4$^{b}$ | 17.8$^{c}$ | 96$^{c}$ | 0.914$^{cd}$ |
| 54 | 20.1$^{ab}$ | 18.9$^{ab}$ | 101$^{abc}$ | 0.934$^{bc}$ |
| 59 | 21.2$^{a}$ | 19.8$^{a}$ | 106$^{a}$ | 0.933$^{bc}$ |
| *SEM* | *0.42* | *0.39* | *2.2* | *0.008* |
| *P-value* | | | | |
| Time of day | <0.01 | <0.01 | <0.01 | <0.01 |
| Age | <0.01 | <0.01 | <0.01 | <0.01 |
| Time of day x age | 0.912 | 0.850 | 0.927 | 0.673 |

Levels (a, b, c) not connected by same letter are significantly different
[1]$VO_2$ = volume of oxygen consumption L/kg$^{0.75}$/d
[2]$VCO_2$ = volume of carbon dioxide production L/kg$^{0.75}$/d
[3]HP = Heat production kcal/ kg$^{0.75}$/d
[4]RER = respiratory exchange ratio $VCO_2/VO_2$
[5]SEM = standard error mean

**Table 4. Body composition and tissue gain from 26 – 59 wk of age.**

| Age, wk | Total mass, g | Lean mass, g | Fat mass, g | [1]BMC, g | Lean mass, g/kg | Fat mass, g/kg | BMC, g/kg | Lean gain g/d | Fat gain g/d |
|---|---|---|---|---|---|---|---|---|---|
| 26 | 3335$^{e}$ | 2611$^{c}$ | 563$^{f}$ | 131$^{b}$ | 783$^{a}$ | 169$^{f}$ | 39$^{ab}$ | | |
| 30 | 3701$^{d}$ | 2857$^{abc}$ | 655$^{ef}$ | 130$^{b}$ | 771$^{ab}$ | 177$^{ef}$ | 35$^{ab}$ | 10.4$^{a}$ | 3.8$^{ab}$ |
| 33 | 3878$^{cd}$ | 2942$^{ab}$ | 721$^{def}$ | 132$^{b}$ | 758$^{abc}$ | 186$^{def}$ | 34$^{b}$ | -0.4$^{bc}$ | 3.6$^{abc}$ |
| 37 | 3844$^{cd}$ | 2719$^{bc}$ | 868$^{cd}$ | 160$^{ab}$ | 702$^{cde}$ | 224$^{bcd}$ | 41$^{ab}$ | -6.5$^{b}$ | 5.2$^{a}$ |
| 40 | 4015$^{bc}$ | 2933$^{ab}$ | 820$^{cde}$ | 132$^{b}$ | 732$^{abcd}$ | 203$^{cdef}$ | 33$^{b}$ | 10.3$^{a}$ | -2.2$^{c}$ |
| 43 | 4132$^{abc}$ | 2966$^{ab}$ | 880$^{bcd}$ | 150$^{b}$ | 719$^{bcde}$ | 212$^{bcde}$ | 36$^{ab}$ | 3.5$^{ab}$ | 0.7$^{abc}$ |
| 45 | 4161$^{ab}$ | 2831$^{abc}$ | 988$^{abc}$ | 162$^{ab}$ | 681$^{def}$ | 237$^{abc}$ | 39$^{ab}$ | 2.1$^{ab}$ | 2.8$^{abc}$ |
| 50 | 4336$^{a}$ | 2745$^{bc}$ | 1158$^{a}$ | 187$^{a}$ | 634$^{f}$ | 267$^{a}$ | 43$^{a}$ | -2.6$^{b}$ | 5.2$^{a}$ |
| 54 | 4386$^{a}$ | 2939$^{ab}$ | 1065$^{ab}$ | 165$^{ab}$ | 671$^{ef}$ | 242$^{ab}$ | 38$^{ab}$ | 2.7$^{ab}$ | -0.7$^{bc}$ |
| 59 | 4297$^{a}$ | 3031$^{a}$ | 945$^{bc}$ | 155$^{ab}$ | 708$^{cde}$ | 218$^{bcd}$ | 36$^{ab}$ | 1.2$^{ab}$ | -1.6$^{bc}$ |
| SEM | 70.4 | 70.8 | 50.7 | 9.7 | 15.4 | 10.2 | 1.7 | 2.3 | 1.3 |
| P-value | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

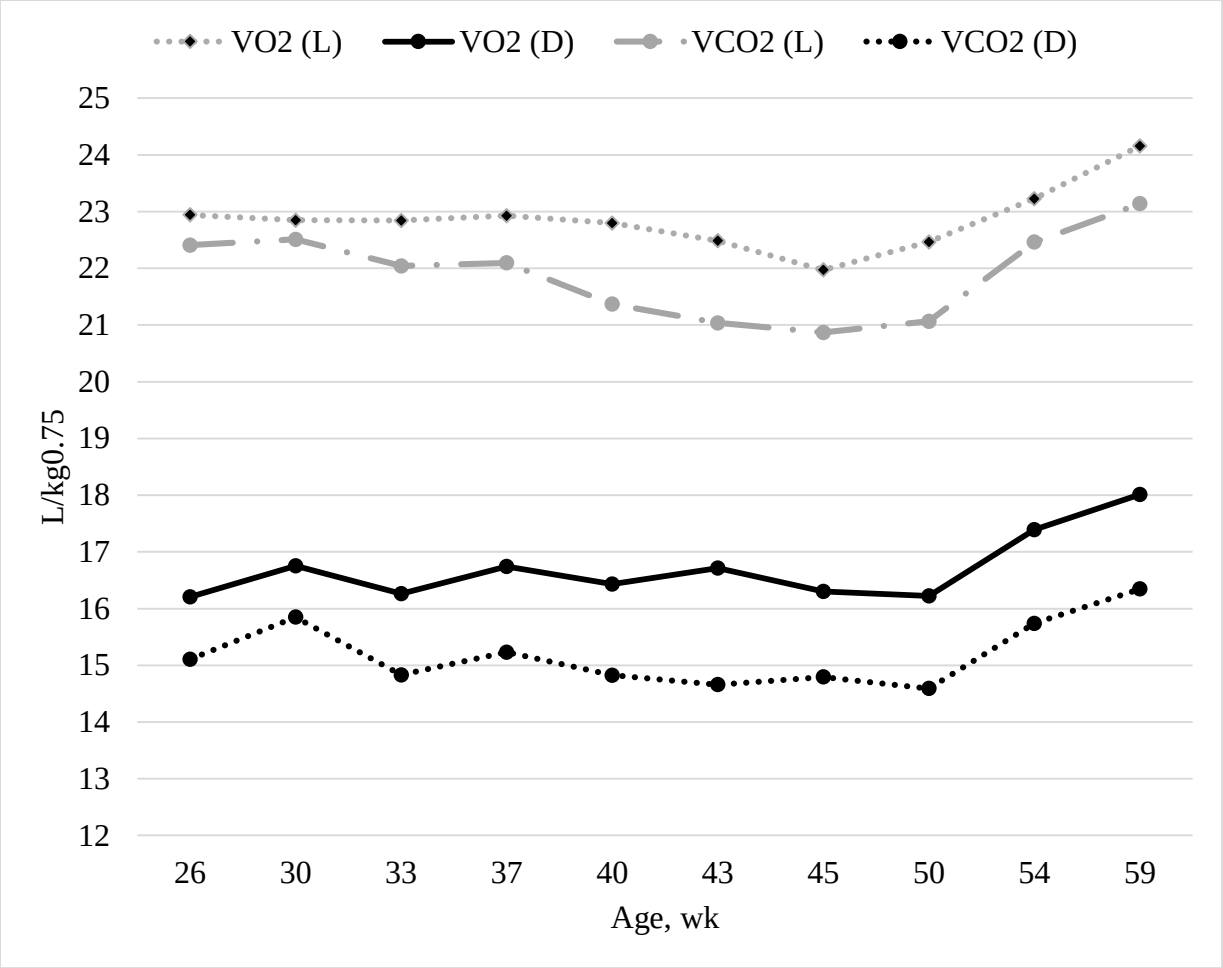
Levels (a, b, c, d, e, f) not connected by same letter are significantly different
[1]BMC = Bone mineral content
SEM standard error mean

**Table 5. Pairwise correlation between egg production, heat production and body composition**

| Variable 1 | Variable 2 | Correlation | Lower 95% | Upper 95% | *P*-value |
|---|---|---|---|---|---|
| [1]HP kcal/d | Body Lean mass, g | 0.36 | 0.193 | 0.508 | <.0001 |
| HP kcal/d | Body Fat mass, g | 0.43 | 0.275 | 0.569 | <.0001 |
| HP kcal/d | Egg Production, % | -0.24 | -0.403 | -0.056 | 0.011 |
| [2]RER | Body Lean mass, g | -0.077 | -0.253 | 0.105 | 0.406 |
| RER | Body Fat mass, g | -0.054 | -0.232 | 0.127 | 0.559 |
| RER | Egg Production, % | -0.069 | -0.115 | 0.249 | 0.463 |
| Body Lean mass, g | Body Fat mass, g | -0.18 | -0.349 | -0.002 | 0.048 |
| Body Lean mass, g | Egg Production, % | -0.01 | -0.197 | 0.168 | 0.875 |
| Body Fat mass, g | Egg Production, % | -0.18 | -0.349 | -0.002 | 0.048 |

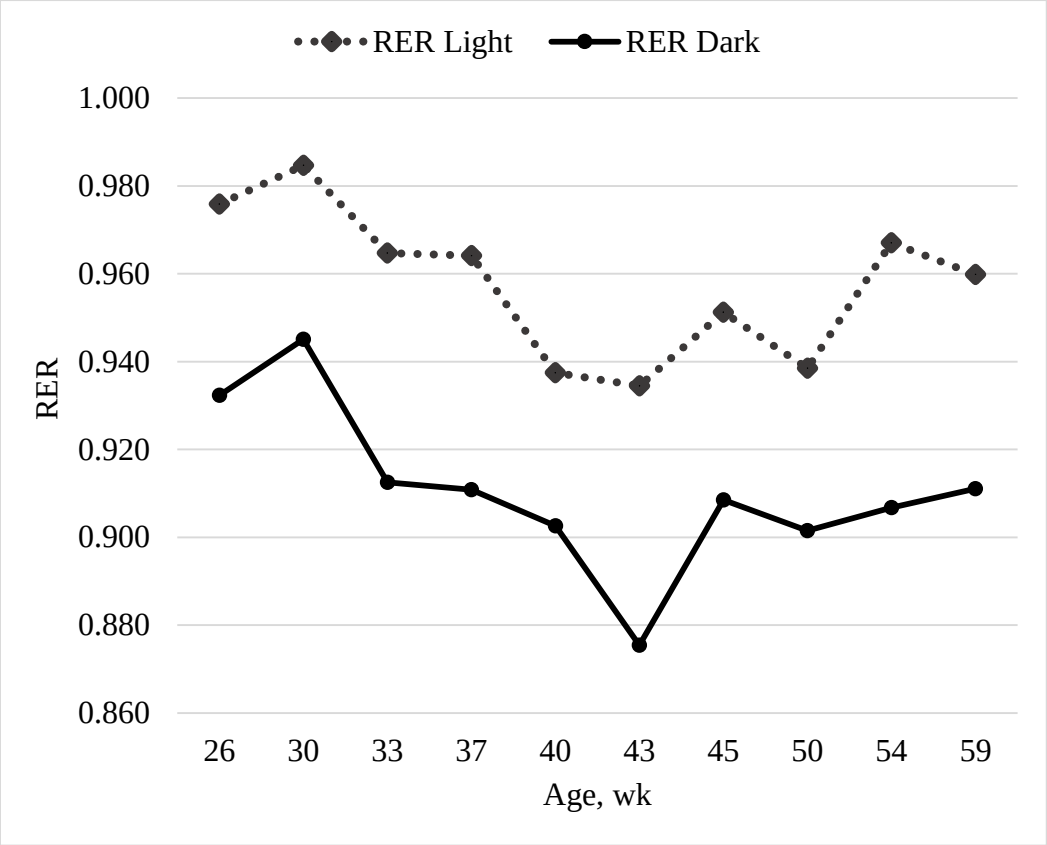
[1]HP = Heat production
[2]RER = Respiratory Exchange Ratio

**Figure 1. Oxygen consumption and carbon dioxide production during light and dark time**



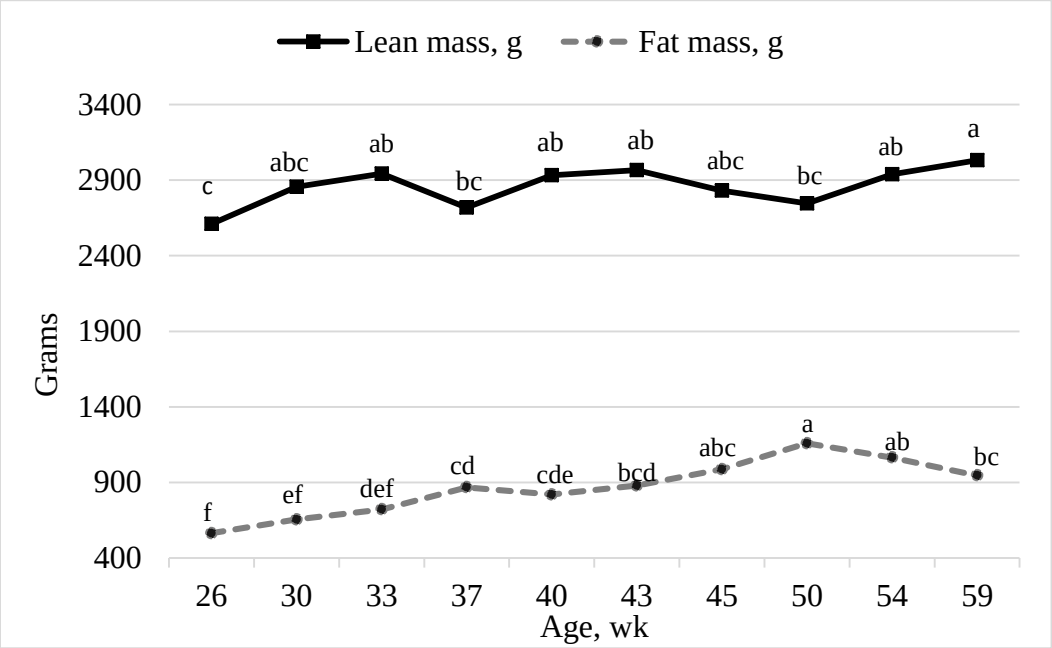

L Light
D dark
$VO_2$ = volume of oxygen consumption $L/kg^{0.75}/d$
$VCO_2$ = volume of carbon dioxide production $L/kg^{0.75}/d$

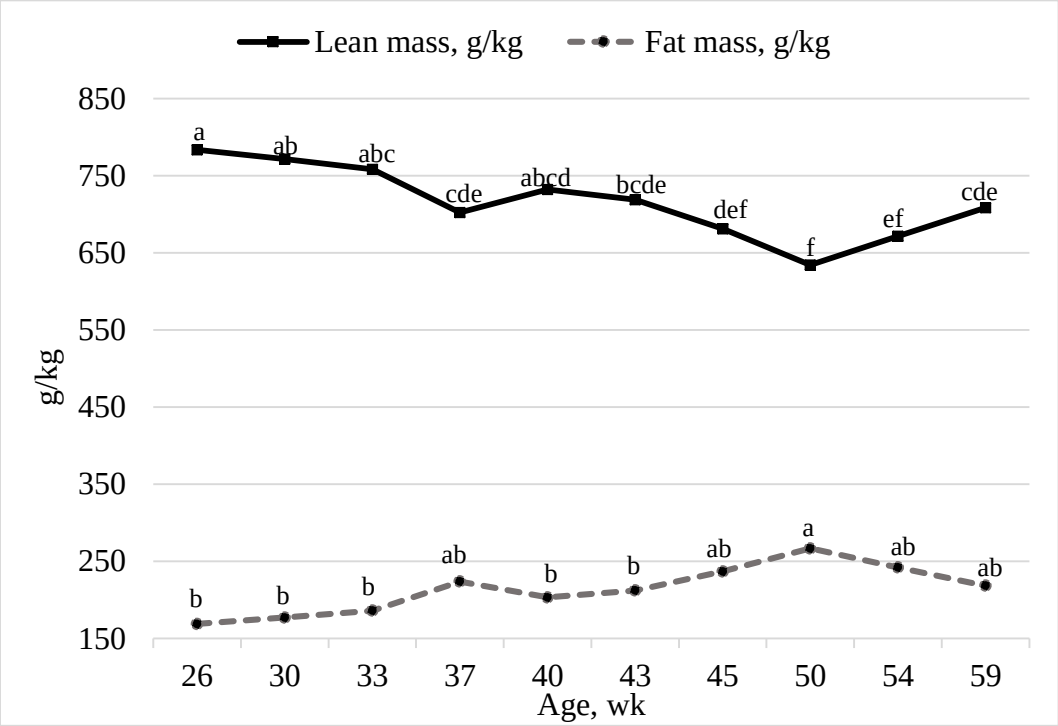
**Figure 2. Respiratory Exchange Ratio (RER) during light and dark time**




RER, respiratory exchange ratio

**Figure 3. Lean and body mass, g from 26 – 59 wk of age**




Levels (a, b, c, d, e, f) not connected by same letter are significantly different

**Figure 4. Body composition g/kg**

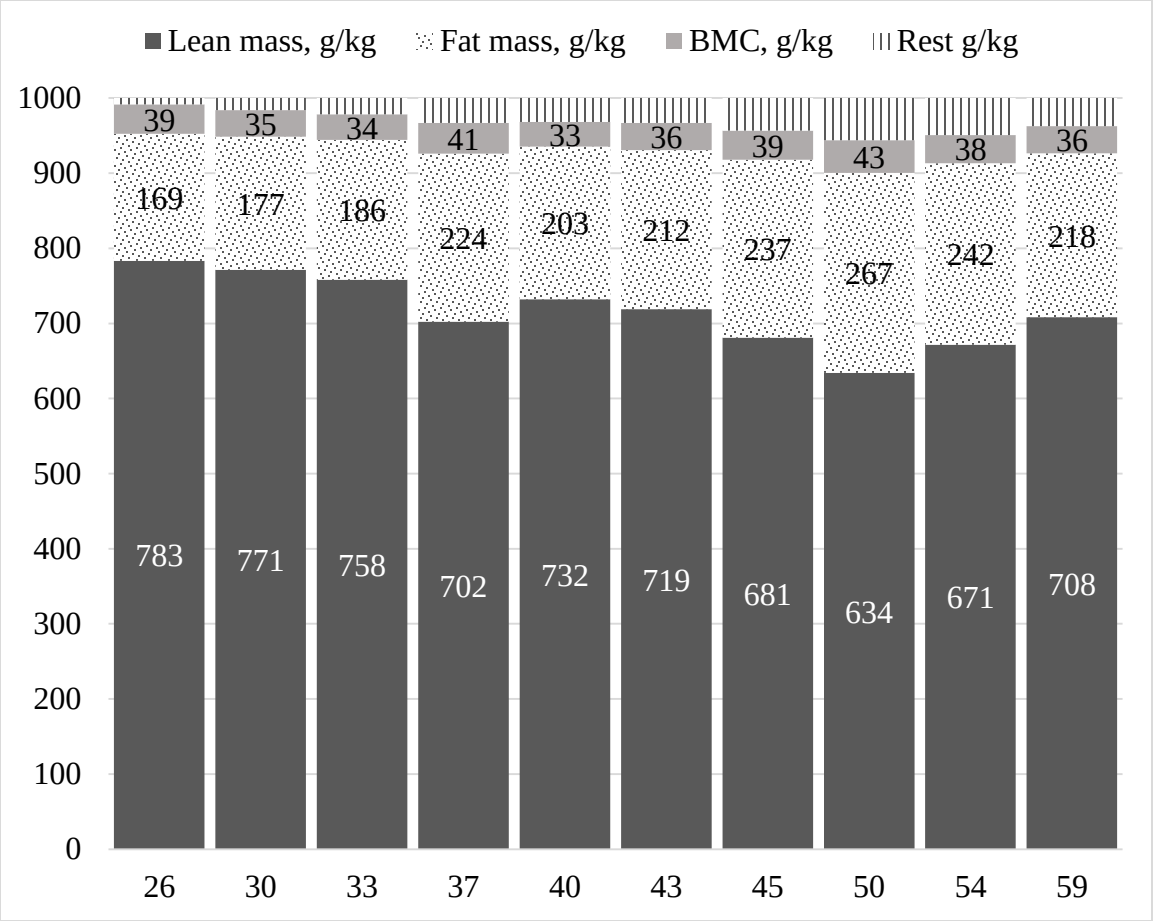


**Figure 5. Lean and fat gain, g/d from 26 – 59 wk of age**

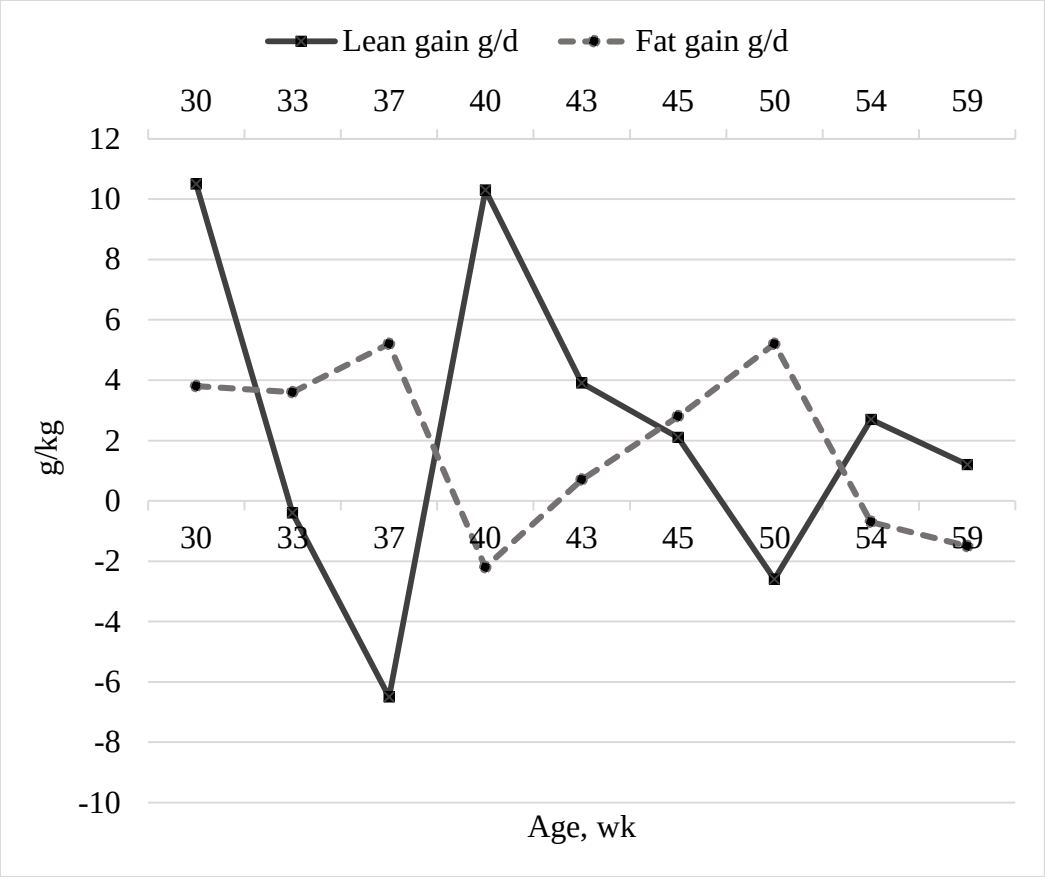
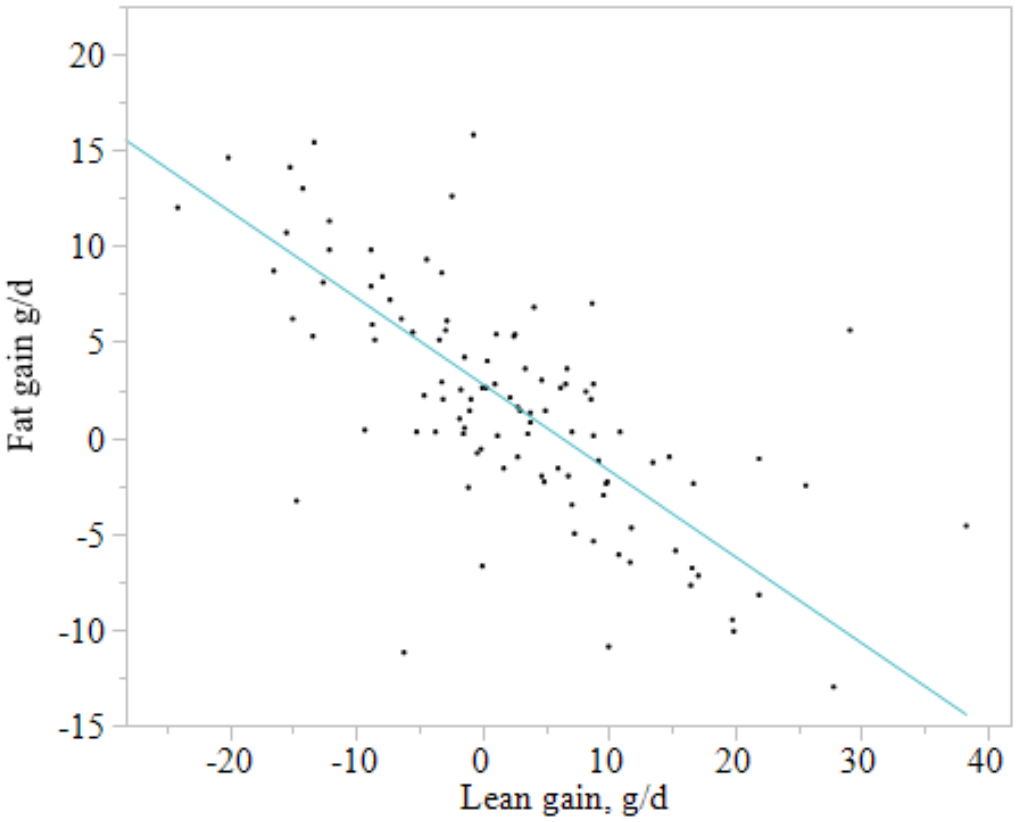


**Figure 6. Linear regression between fat tissue gain g/d vs lean tissue gain g/d**




Fat gain g/d = 2.811 – 0.45 x Lean gain g/d
Intercept $P<0.01$
Slope $P<0.01$
$R^2$ 0.68
RMSE 3.6
Lack of fit $P> 0.56$

**Figure 7. Relationship between body lean mass, and heat production**

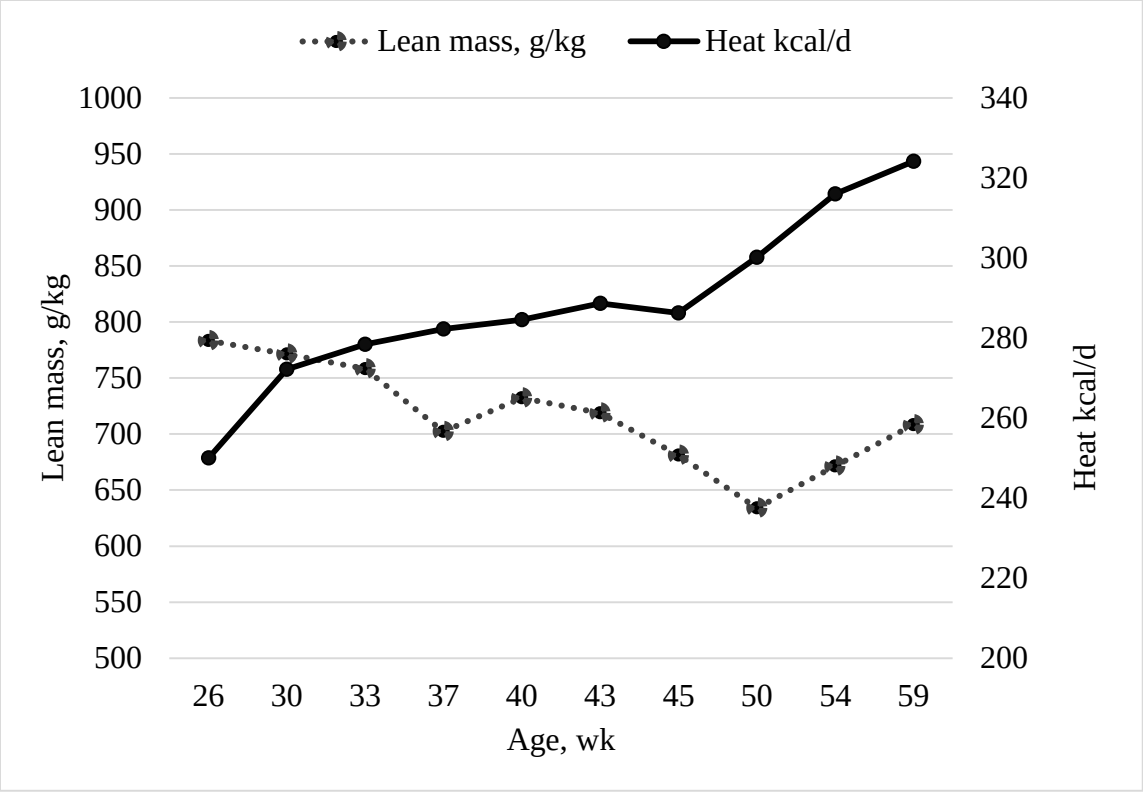


**Figure 8. Relationship between body fat mass, and heat production**

**Figure 9. Relationship between heat production, and egg production**

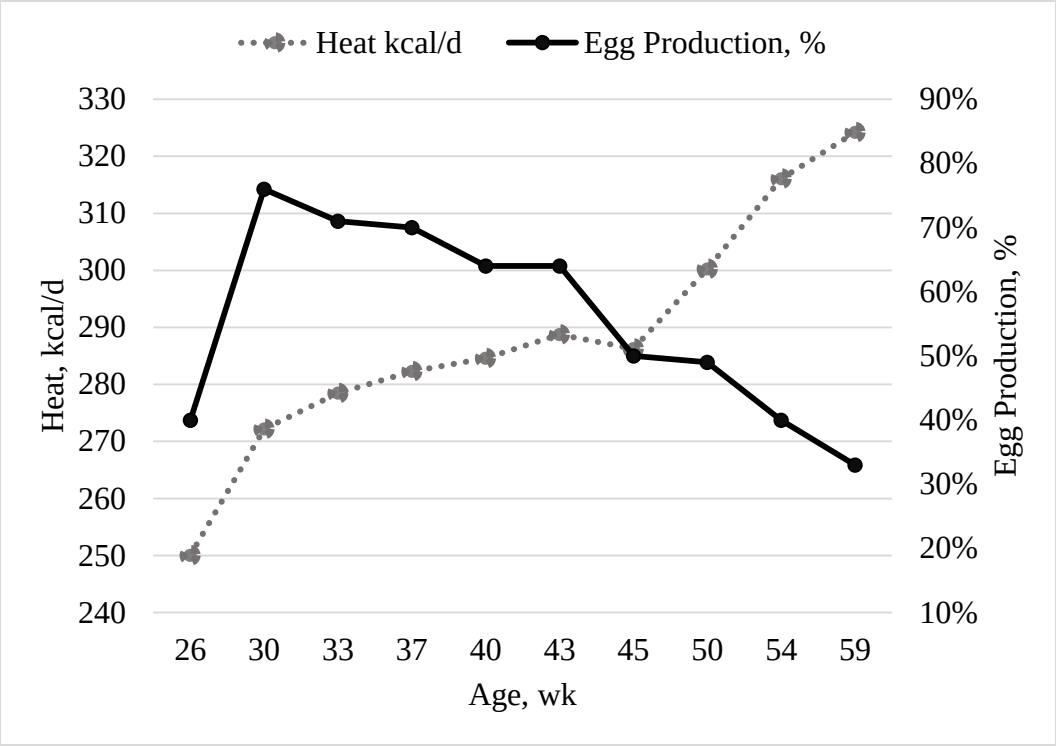


**Figure 10. Relationship between RER, and egg production**

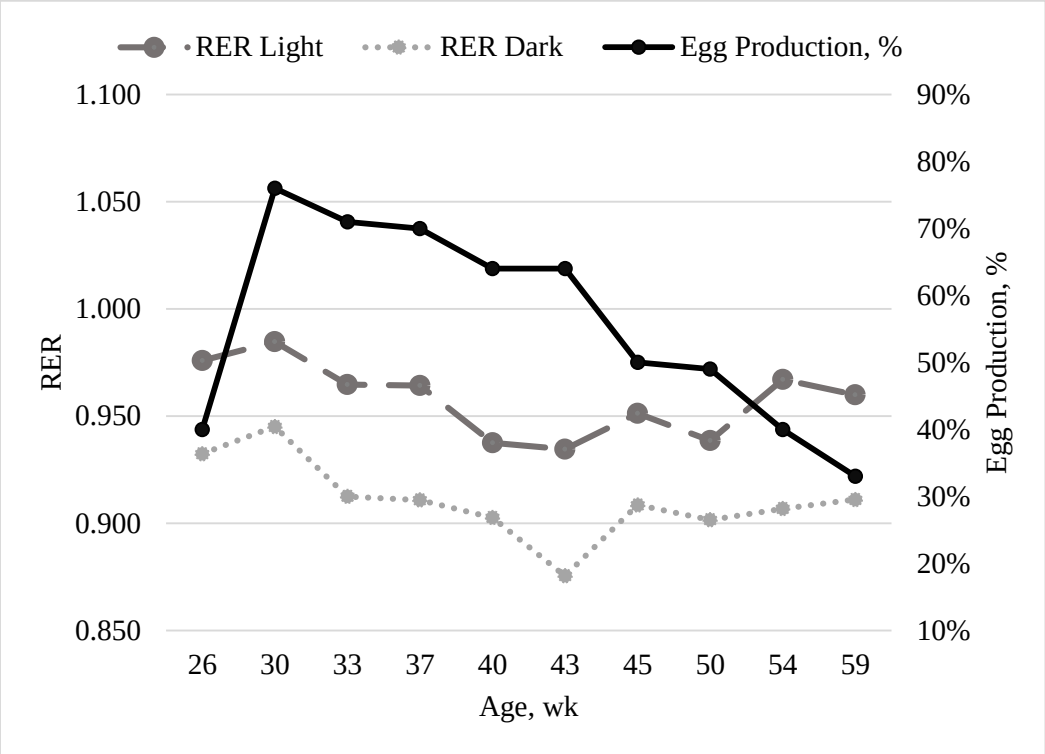


**Figure 11. Relationship between body lean mass, and egg production**

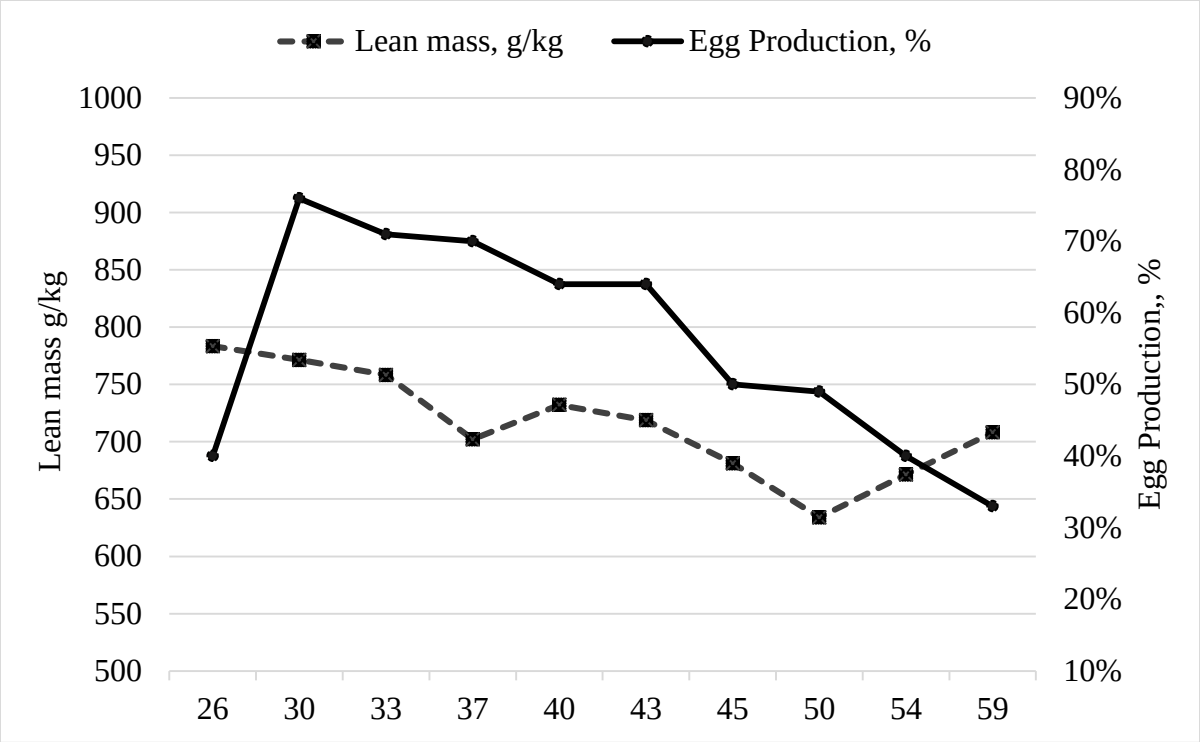


**Figure 12. Relationship between body fat mass, and egg production**

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# CONCLUSIONS

The overall results of these experiments conclude that body components (lean, protein, fat tissues and mineral content) have the same potential rate, said to be allometrically related, therefore can all be predicted from weight of one of these components. Dual energy X-ray absorptiometry (DEXA) after proper validation for precision, standardized for positioning, and equations built from chemical analysis to adjust body components proved to be precise, accurate and fast methodology to evaluated body components.

Exogenous enzymes, evaluated individually as protease, and carbohydrase – glucanase or as multi-enzyme composite (protease + glucanase + xylanase + phytase) decreased heat production by decreasing the maintenance energy, and improve protein utilization from the diets in broilers; however during grower (21-28d) a change from protein to fat accretion and back to protein gain is seen when enzymes are added. Further research is needed to confirm these findings and probably evaluation of individual enzymes with gradually combinations are needed to better use of enzymes in poultry feed.

Broiler breeders along egg production changed carbohydrate, protein and fat utilization. Calorimetry studies showed higher heat production at the end of production when the hen had highest body weight. Hens showed negative lean tissue and positive fat gain at 37 wk, and 50 wk compared to beginning of egg production (26 wk) and 40 wk. After 50 wk, lean tissue tends to increase and fat tissue to decrease. The 37 wk behavior has been explained before by Vignale, (2014) showing more protein degradation rate during peak egg production; however the 50 wk behavior needs to be studied further. It seems hens increasing lean tissue for the next clutch or production cycle as it happens in the wild, while fat tissue supports the maintenance energy.

**APENDIX A**

UNIVERSITY OF ARKANSAS

Office of Research Compliance

*MEMORANDUM*

TO: Craig N. Coon

FROM: Carol Rodlun, Program Manager
Institutional Animal Care
And Use Committee

DATE: February 15, 2013

SUBJECT: IACUC Modification Request **APPROVAL**
Expiration date: **May 14, 2015**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** the modification request (to add the use of metabolism chambers ) to Protocol **#12041- "THE EFFECT OF FEEDING COMMERCIAL EXOGENOUS ENZYMES TO BROILERS TO IMPROVE PERFORMANCE AND DIGESTION OF NUTRIENTS."** You may implement this modification immediately.

In granting its approval, the IACUC has approved only the modification request provided. Should there be any additional changes to the protocol during the research, please notify the IACUC in writing [via the Modification Request Form] **prior** to initiating the changes.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cc: Animal Welfare Veterinarian

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**APENDIX B**

UNIVERSITY OF ARKANSAS

Office of Research Compliance

*MEMORANDUM*

TO: Craig N. Coon

FROM: Carol Rodlun, Program Manager
Institutional Animal Care
And Use Commmittee

DATE: February 15, 2013

SUBJECT: IACUC Modification Request **APPROVAL**
Expiration date: **July 31, 2015**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** the modification request (to add the use of metabolism chambers ) to Protocol **#13002- "Evaluation of broiler breeder feeding regimes for pure-line and commercial type stock during rearing and production phases and calcium requirement during the production period."** You may implement this modification immediately.

In granting its approval, the IACUC has approved only the modification request provided. Should there be any additional changes to the protocol during the research, please notify the IACUC in writing [via the Modification Request Form] **prior** to initiating the changes.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cc: Animal Welfare Veterinarian

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