MODELING BROILER ENERGY AND PROTEIN METABOLISM

By

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FORMAT OF THESIS

This thesis presented in the Journal of Applied Poultry Science and Poultry Science style and format allowing for independent chapters to be suitable for submission to scientific journals. Four papers have been prepared from research data collected at Oklahoma State University Oklahoma Agricultural Experiment Station to partially fulfill the requirements for the degree of Doctor of Philosophy. Each paper is complete in itself containing an abstract, introduction, materials and methods, results, discussion, and literature cited section.

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CHAPTER I

INTRODUCTION

Today's poultry industry continually strives to enhance both efficiency of applied production and yield of edible parts per unit feed input. Improvements in efficiency have largely come from genetic progress, though nutrition has also contributed. Nonetheless, impetus for nutritional evolution has frequently been the fact that nutritionists are formulating rations for birds that differ from the past. Such appears to be the case today as, though ration ME values appear static, the value of ME to poultry has increased significantly. An examination of Agristats data indicates that the ME content of rations has been lowered over time, a fact that well correlates with improved efficiency. However, the consequence of this is that the industry is continuously chasing the evolving genetics. A case study in our laboratory (Table 1) indicates that 1999 Cobb broilers had a ME savings of 880 Kcal over NRC (1994) data at equalized body weight.

Table 1. A comparison of the 1994 NRC and 1999 Cobb broiler partitioning of consumed energy (E) into maintenance and gain.

	Cobb-94	Cobb-99	Saving
Live Weight (g)	2588	2588	--
Bird Age (Days)	49	45	4 days
ME Cons (Kcal/b)	15994	15114	880 Kcal
Maint. E (Kcal/b)	5672	5244	428 Kcal
E. Gain (Kcal/b)	10321	9870	451 Kcal
Carc. E/E. gain	0.414	0.444	296 Kcal

Calculation indicates that the bulk of energy savings (49%) is in the form of maintenance (428 Kcal), with 296 Kcal saved for gain and about 156 kcal remaining unaccounted. The latter, we believe, represents reduced activity by the bird.

Nutrition comes to play a key role controlling carcass composition. Today's bird has changed dramatically over the years, but new problems are also faced. Not only are the nutrient requirements changing annually, but also the adaptation period for stress has not been overcome. It is well known that poultry, like many other animals, are insufficient in converting feed to protein. To achieve maximum broiler performance, the dietary CP content must provide sufficient levels of EAA and NEAA to allow maximum protein synthesis and meet the demands of metabolic processes. Reduction in nitrogen excretion and improvement in the efficiency of nitrogen deposition can be obtained by matching amino acid composition of the diet with the amino acid requirement of the broiler for maintenance and meat production. The availability of commercial synthetic amino acids allows this to be done with low protein diets by avoiding an excess of each amino acid above the requirement. Keshaverz (1991) postulated that low protein diets increase the tolerance of birds to elevated temperatures because the heat production associated with the utilization of protein is greater when compared to carbohydrates and fats. However, there is conflicting evidence as to whether low protein diets supplemented with amino acids can support maximum growth rate. Twining et al. (1974) reported that

broiler chicks receiving a low protein starter diet exhibited inferior body weights and feed conversions at 4 weeks of age when compared with the control diet.

In practical corn-soybean meal based broiler diets, methionine is considered first limiting followed by lysine, arginine, valine, and threonine (Han et al., 1992). However, lysine is the amino acid to which all others are proportionally related (i.e. ideal protein concept; Baker and Han, 1994; Baker, 1997). Additionally, lysine is generally expressed in ratio to energy, as dietary caloric density largely regulates voluntary feed intake (Leeson et al., 1996; McKinney and Teeter, 2004). Lysine is largely viewed as a pivotal nutrient because lysine has no major precursor role, and there has been extensive work to quantify digestible lysine need in broilers reared under a wide range of dietary and environmental circumstances (Han and Baker, 1993; Emmert and Baker, 1997).

Intrinsic factors determine a broiler's overall capacity to synthesize and accumulate muscle (Lawrence and Fowler, 1997). However, whether or not the inherent upper limit is realized largely depends on the dietary supply of essential amino acids, as well as energy, as protein accretion is energetically costly (4 to 7 moles of ATP per peptide bond formed; Bequette, 2003). As maximum meat yield at optimal efficiency is a principle goal, nutritionist's routinely tweak nutrient to calorie ratios in an attempt to provide an ideally balanced ration.

The energy expenditure or heat production of an animal is essentially a function of two processes. Firstly, the biochemical transformation of a nutrient to an end product is often associated with a loss of energy for the animal. For example, Baldwin (1995)

calculated that only 0.84 of glucose energy can be conserved as tripalmitin and ATP; reminder is lost as heat. Second, energy expenditure is due to biophysical processes requiring ATP (Miligan and Summers, 1986). Both, the conversion of nutrients to ATP and the actual ATP utilization contribute to the heat production. Different authors have quantified the energy efficiencies of the main biochemical processes. (Birkett and de Lange, 2001).

The work of Schulz (1978) is very advanced and includes the fate of each nutrient into ATP equivalents. It includes the catabolic and anabolic processes for carbohydrates, amino acids and lipids. The basic premise of Schulz's work is that excess amino acids are converted to either glucose or ATP, which can then be used for other purposes.

The yield of ATP from an oxidizable substrate is highly dependent on the cytoplasmic equivalents. The ATP yields of proteins fats and carbohydrates are calculated the same way as the heats of combustion of proteins and fats with substitution of appropriate farmula of - ΔHc values with the potential net cytoplasmic ATP yield for the individual amino acids, fatty acids and glycerol.

Studies were conducted to evaluate bird need for indispensable amino acids, lysine and the theoretical yields of ATP's by amino acids consumed in excess of accretion.

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CHAPTER II

REVIEW OF LITERATURE

Introduction

Bird Age

The growth rate of broiler increases from day 0 to 10, reaching a maximum of about 20% per day (Nitsan et al., 1991). The weekly proportionate increase in body weight declines as the chicken ages (Marks and Pesti, 1984). Therefore, it would be expected that the relative amount of energy required for growth per unit metabolic size also declines. As the relative amount of energy required for growth declines, any excess energy consumed will result in increased rate of daily fat accretion. At early ages, birds must rapidly adapt to digestion of an exogenous diet in which nutrients are absorbed from the intestine. During this early growth stage, energy is supplied predominantly by carbohydrates in order to achieve their genetic potential. Some of the factors that may influence early growth rate include amount of yolk sack residue, quality and intake of feed and water, pancreatic and intestinal enzyme levels, gastrointestinal tract surface area, nutrient transporters, and overall nutrient digestibility. Also, the change in energy metabolism from lipids to carbohydrates must be considered.

BIOENERGETICS AND THERMODYNAMICS

The bioenergetics field deals with metabolic energy transformations in living things according to the physical laws governing energy transformation. The first law of thermodynamics (Klotz, 1986 in Harper's Biochemistry) states that the total energy of the system, including its surroundings, remains constant (energy cannot be created or destroyed). However, within that total system energy may be transformed into another form of energy. For example, chemical energy may be transformed into heat, electrical energy, radiant energy, or mechanical energy in living systems. The second law of thermodynamics states that the total entropy (disorder) of a system must increase if a process is to occur spontaneously. Entropy becomes maximum in a system as it approaches true equilibrium. These laws dictate that the heat produced from various metabolic processes is the same as it would be if feed were allowed to combust to the same end-products. The first law of thermodynamics asserts that the total amount of energy in an isolated system remains constant. When the energy content of a system changes, the sum of all forms of energy given off by the system must be equal to the magnitude of the change. The first law is only concerned with initial and final energetic states of the system. The principle of conservation of matter also is taken into account in the first law because matter and energy are inseparable according to the theory of relativity. Matter and energy are different expressions of the same thing. In animal systems, the energy equivalent of work, plus the ME of the animal, plus the heat increment of feed equals the energy generated from the oxidation of nutrients of the feed.

Hess's law of Constant Heat Summation (1850, Atkins and Beran in General Chemistry) states that all forms of energy are quantitatively convertible to heat. This law states that in going from a particular set of reactions to a particular set of products, the

change in enthalpy is the same whether the reaction takes place in one step or in a series of steps. Oxidation of substrates within an animal body is quite different from oxidation in a bomb calorimeter, however from a thermodynamic point of view these facts are incidental. According to Hess's law the physical and physiological heat values of all nutrients, with the exception of protein, are the same.

The end products of protein oxidation within the body and the bomb calorimeter are different because waste products of protein metabolism within the body are capable of further oxidation to produce carbon dioxide and water. In poultry, the end product is mainly uric acid, which accounts for 80% of metabolized nitrogen and secondly ammonia which accounts for 10% total nitrogen (Sturkie, 1986) and in mammals the major nitrogenous end product in urea.

FEED ENERGY METABOLISM

BASAL METABOLIC RATE: The basal metabolic rate of standard metabolic rate is defined as the heat production occurring by an animal at rest, awake, fasted and housed within its thermo neutral zone. Under these conditions the rate of energy metabolism is a function of surface area since heat loss is closely tied to this factor (Brody, 1964). Surface per unit body weight declines with increasing body weight. However surface area is a difficult parameter to estimate and numerous attempts have been made to relate it to body weight. However, surface area is a difficult parameter to estimate and numerous attempts have been made to relate it to body weight (Brody, 1964). Typical body weight will be raised to a power, most commonly 0.75, which is now regarded as the universal metabolic weight. Brody (1964) suggested weight $^{0.75}$ be used as a reference base for

basal-energy metabolism in mature animals of different species including a weight range from mice to elephants.

Brody reported that for mature birds of different species the exponent varies from 0.62 to 0.70. Metabolic weight for poultry is commonly reported as $W^{0.66,}$ since this value gives a better estimate when comparing poultry within species. If the correct power is chosen and body temperature and animal composition are constant, then heat production per unit metabolic weight is relatively constant. Under basal conditions, heat energy is produced from various energy sources to offset heat loss and maintain constant body temperature.

The basal state is seldom achieved with assurance in animals because of the varying time period required to achieve the post absorptive state and the physical, mental and emotional distress created by the experimental conditions. Misson (1974) found that laying hens required a 3 day exposure to the experimental situation before basal values could be achieved and that the time required to reach the post-absorptive state was influenced by body weight requiring 24 hours for birds below 2.5 Kg and 48 hours for those above.

THERMOBALANCE: Birds and mammals are homeotherms and consequently maintain a relatively constant core body temperature. However, diurnal body temperature cycles have been detected and are influenced by several factors like age, sex, work and digestion (Dawson, 1975; Dukes, 1977). The animal's total heat is determined by metabolism, the reaction by which chemical energy is transformed into heat, and the environmental temperature which the animal is subjected to. For the animal to maintain constant heat in

the body the rate of heat dissipation should balance heat gain. Heat is added to the body by metabolism. In poultry the problem is related to nonevaporative heat dissipation at high ambient temperatures. Sturkie (1986) proposed the following equation

Energy balance requires that HP = NHD + EHD

HP = metabolic heat production

NHD = nonevaporative heat dissipation

EHD = evaporative heat dissipation

HEAT PRODUCTION: Birds, like mammals, are homoeothermic. They produce heat to maintain a relatively constant body temperature. Although birds and mammals are homoeothermic, birds have a number of thermoregulation characteristics different from mammals. The most obvious is feathers. Feathers are great insulation which is good for cold weather but bad for hot weather. Feathers tend to hold heat in and not let it escape easily from the chicken's body. Another difference is that birds have no sweat glands. Most mammals perspire when they are hot, and evaporation of this perspiration from their skin is extremely effective in reducing body temperature. Nevertheless, birds have a couple of special features that do help them during hot weather. Their relatively high body temperature makes it easier for them to lose heat to the air around them. Also the bird's respiratory system is very effective at cooling. The air sacs of the bird allow inhaled air (which is usually cooler than body temperature) to reach deep into the abdominal cavity and of course when the bird exhales, heat is removed from its body. The bird also has a panting mechanism (gular flutter) that it uses during hot weather to evaporate water from its throat and reduce its body temperature. Thus panting in birds is analogous to perspiring in mammals and is extremely effective at cooling the bird.

ENERGY METABOLISM SCHEMES: The total energy contained in feed is termed as gross energy (GE). However, only a portion of gross energy actually appears in animal products such as meat, eggs or milk. Blaxter (1989) estimated the efficiency of ME. ME is the energy available to the bird that is not subsequently lost in feces and urine. Gaseous energy losses from the digestive tract have been considered too small to affect the estimate of ME significantly. NE (net energy) is the ME of the food corrected for the energy losses that result from the assimilation of the dietary nutrients. This energy loss is frequently termed the heat increment of digestion. The remaining NE is available for maintenance and production. Growth and egg production are the only products of NE that do not result entirely in heat emission.

Titus (1961) developed an alternative way to determine ME of individual feed ingredients based upon digestibility coefficients, and subsequently incorporated factors such as moisture, protein, ether extract, nitrogen free extract, and crude fiber contents into the formula. Later, Hill et al. (1960), and Matterson et al. (1965) suggested that determined values are more accurate than calculated values due to numerous environmental, genetic and management factors that can affect results of such studies. Hill and Anderson described detailed methodologies and procedure for the determination of ME as follows:

$$ME = GE - EE - 8.22 \times N$$

ME = metabolizable energy per gram of dry diet consumed

EE = excreta energy per gram of dry diet consumed

N = grams of nitrogen retained

The value of 8.22 Kcal/g is the residual energy values in the excreta as uric acid originating from the catabolism of nitrogen compounds retained as protein tissue. However, these equations do not subdivide endogenous energy losses from urinary and excreta origin.

Net energy is the ME – energy lost as heat increment. NE may be further divided into NE for maintenance (NEm) and production or gain (NEg). It has been estimated that NE is 84 % of ME in chick from 0 – 21 days of age (Sturkie, 1986).

The proportion of NE can change considerably due to factors of animal and/ or feedstuff origin, but it has been estimated to be around 84% of ME in the chick from 0 – 21 days of age (Sturkie, 1986). Unfortunately in practice the concept of NE is not estimated and it is used infrequently. Ecologists even consider the proportion of energy related from the feed that is used for maintenance and growth as a part of ME.

In order to estimate NE for growth we must quantify the amount of energy that is completely retained. Originally there were a couple of methodologies used to determine NE. The first one was developed by Lawes and Gilbert (1861) in which they attempted to measure the difference between energy input and energy output. The second methodology is a comparative slaughter technique that correlates energy input to changes in body composition. However, this second methodology is laborious as well as time consuming and has the potential for errors due to difficulty in obtaining a representative sample of birds. There have been other attempts trying to establish a system to estimate NE but controversy has occurred when values are contradicting.

Net energy deals directly with the profitable portion of the feed, therefore a NE quantification system may have value to understand substrate efficiency and it correlation with body composition. As described previously, NE is the difference ME and energy loss as heat increment. The amount of heat produced subtracted from ME value of a feed results in the NE value. For this purpose calorimetry can play a key role in quantifying this variable, and that is why a direct or an indirect technique can be applied. Direct calorimetry deals the amount of heat lost due to thermoregulation mechanisms such as radiation, convection, conduction and evaporation (Deighton, 1939, Benzinger and Kitzinger, 1949). Indirect calorimetry methods originally estimated the amount of heat lost by incorporating respiratory gases such as oxygen, carbon dioxide and methane in the case of ruminants, into an equation. Brouwer (1965) proposed the following equation:

$HP = 16.18\ (Kj/L) \times O_2 + 5.02\ (Kj/L) \times CO_2$

HP = heat production (Kj/L/hour)

O_2 = oxygen consumption (L/hour)

CO_2 = carbon dioxide production (L/hour)

PROTEIN METABOLISM

An increased consumer demand for leaner products for today's consumption pattern is evident and is presumably due to high lipid foods negative effects on human health (NACNE, 1983; CMAFP, 1984). In order to produce leaner poultry products it is helpful to understand the metabolism of proteins and its relation with lipid, carbohydrate and energy metabolism.

PROTEIN CHARACTERISTICS: Proteins are constituents of all cells and are essential

to sustain life under any condition. These compounds are formed by chains of amino acids linked together by peptide bonds attached to the carboxyl side of one and the amino group of the next amino acid. It is the order of these amino acids that determines the chemical, biological and physical characteristics of a specific protein. Proteins' molecular weight ranges from 5000 to 1 million depending on the structure of the protein. Proteins can serve as regulators of metabolism (enzymes and hormones), structural components of membranes, muscles and connective tissues, transport molecules, osmoregulators, and body defenders via immunoglobulins (Dukes, 1993).

Proteins consumed in the feed are hydrolyzed in the intestinal lumen and in the mucosal cells of the gastrointestinal tract by proteases and peptidases, resulting in free amino acids that are mostly transported to hepatocytes via the portal blood. The liver then controls the distribution of amino acids across the body and receives a constant supply of amino acids as a result of the catabolism of tissue proteins.

In most animal species the total amino acid concentration ranges between 35 and 65 mg/dl of blood plasma (Dukes, 1993). The prevalent amino aids are glutamine, alanine and glycine. Free amino acids are submitted to catabolism in almost all tissues but especially in the intestinal mucosa, liver, brain, kidney and liver. The catabolic procecss involves the removal of the amino group and the resulting ∝-keto acid is then used for oxidation to CO2 and with a portion of its energy conserved as adenosine triphosphate (ATP), glucose and lipids.

Nitrogenous waste products also originate from catabolism of proteins. In some terrestrial species that waste product is ammonia, which is then converted and released as

urea. In poultry and most reptiles, the waste products are metabolized principally into uric acid for excretion. Other species such as aquatic animals excrete excess nitrogen as ammonium ions (Dukes, 1993). Excretion by uric acid method works costlier for the animal than by uric acid.

To synthesize proteins, all the amino acids that make up the various proteins must be present in adequate amounts. Some types of amino acids cannot be synthesized by the body and must be supplied by proteins or amino acids present in the feed, and they are called essential or indispensable amino acids. Others can be synthesized if nitrogen is available in the body. In poultry, the carbon skeletons of amino acids come from intermediates of carbohydrate metabolism For example, amino acids such as serine and glycine come for 3-phosphoglyceric acid, alanine and pyruvic acid. Aspartic and glutamic acid proceed from oxalacetate and ∝-ketoglutarate in the citric acid cycle (Scott et al., 1982). A metabolic process known as transmutation plays a major role in the efficiency of dietary nitrogen use, and it is thought that this process with the excess of one amino acid utilizes it to synthesize another in short supply. In this way amino groups will be used to synthesize nonessential amino acids and the nitrogen source will avoid excretion and the energy expenditure associated with it (Scott et al., 1982).

PROTEIN SYNTHESIS, TURNOVER AND DEGRADATION: Intracellular proteins are being constantly synthesized and degraded throughout the life of a cell. The rate at which this process occurs is termed turnover and has been studied for several types of proteins in a variety of tissues (Stevens, 1996). By 1980, most protein synthesis and degradation studies have taken place on skeletal muscle tissue, showing the accumulation

of body protein happening at a rate of 0.6% of the body weight per day in the commercial broiler, and about 0.3% of the body weight per day in the laying hen (Fischer, 1980). Today this rate is higher and quicker. The rate of protein synthesis is always higher than the rate of protein accumulation because of turnover. Protein turnover may be about 5 times higher than the dietary nitrogen intake considering that approximately 80% of the amino acids that come from turnover are used again (Swick, 1982).

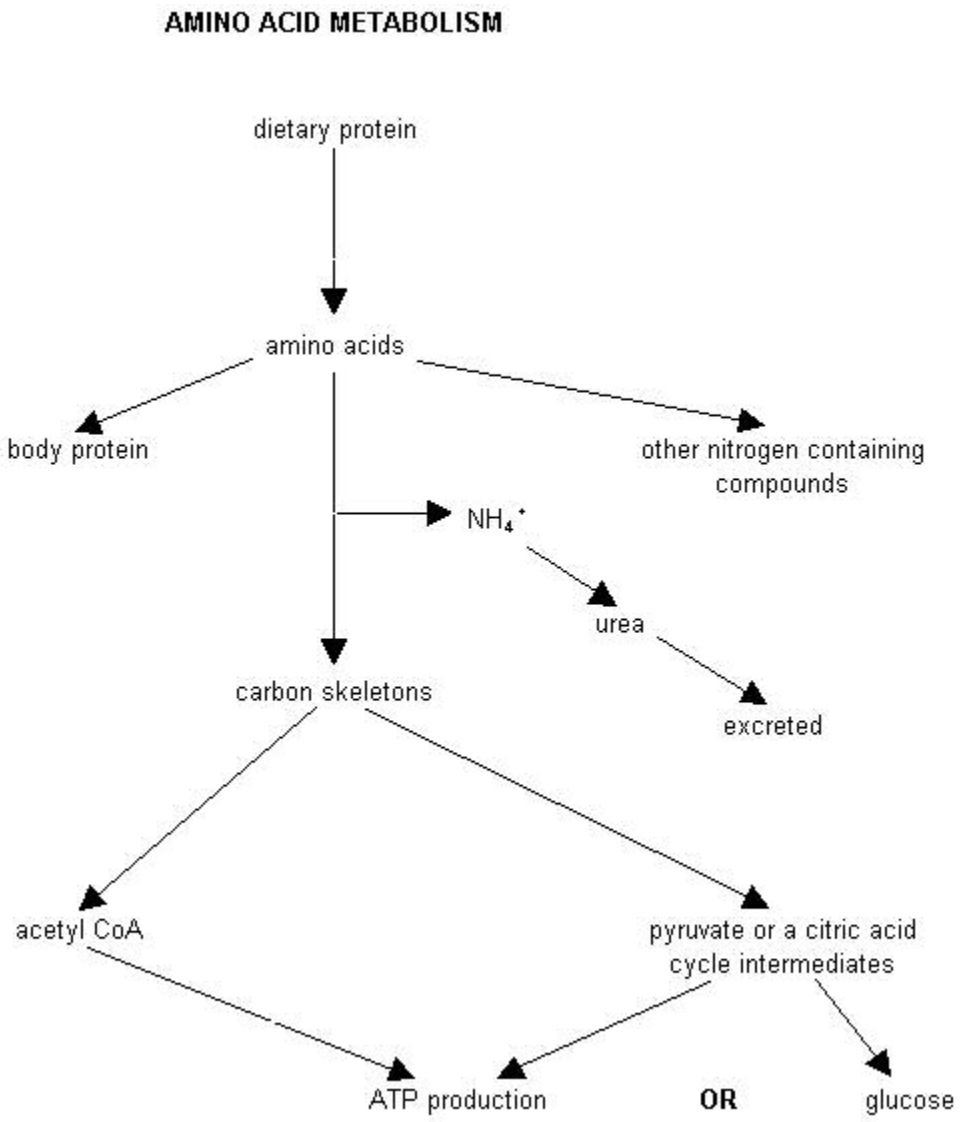

Figure **1. Overview of Protein and Amino Acid Metabolism**

The rates of protein turnover measured in the different tissues of Japanese quail are higher in the liver followed by the heart and the brain and lowest in pectoral muscle (Park et al., 1991). The free amino acid pool in tissues is about 0.5% of the total protein tissue (Stevens, 1996). Protein degradation is often more important in regulating protein turnover that protein synthesis. The mechanism of protein synthesis and catabolism is well understood, but the mechanism of protein turnover is less well defined. The biochemical details of protein synthesis, including the role of messenger ribonucleic acid (mRNA), ribosomes and transcription factors, have long been known and are similar for birds and other vertebrates (Torchinsky, 1937). The major steps involved in protein synthesis and the factors required are illustrated in Figure 4.

Proteins can be divided into short and long-lived (Hershko and Ciechanover, 1982). Long-lived proteins are taken up into lysosomes and degraded by a group of proteolytic enzymes called cathepsins. Short-lived proteins are generally degraded by an energy dependent pathway, i.e. ATP is required for proteolysis. Substantial effort has been made to unravel the mechanism by which short-lived proteins are selected for degradation via ATP-dependent mechanisms. This process often involves modification by the protein ubiquitin. In the skeletal muscle of the broiler there are a number of different pathways of proteolysis, which include lysosomal and non-lysosomal routes, some of which require ATP and ubiquitin (Fagan et al., 1992). Ubiquitin is a widely occuring protein in eukaryotes, and it becomes covalently attached to amino groups on proteins, which are then selected for degradation.

NITROGEN EXCRETION: Poultry secrete waste or excess nitrogen mostly as uric acid

rather than urea. Uric acid is a purine, synthesized by a series of reactions that are also used for synthesis of other purines such as adenine and guanine, and components of DNA (Scott et al., 1982). This incorporation of ammonia into uric acid requires both energy and building blocks. The synthesis or uric acid is costly in ATO and organic carbon. Immediate precursors of uric acid biosynthesis are glycine, glutamine, aspartate, bicarbonate and formyltetrahydrofolate. The three amino acids may arise directly from proteolysis or of dietary origin. Glutamine may arise from glutamate via glutamine synthetase. Glutamate itself and aspartate may arise from transamination of other amino acids, in this way the nitrogen from other several amino acids can be transferred to aspartate, glutamate or glutamine (Bertland and Kaplan, 1970). The rate-limiting step in uric acid formation is the enzyme amidophosphoribosyltransferase (Wiggins et al., 1982).

PROTEIN INGESTION, DIGESTION AND ABSORPTION: Proteins are consumed as a component of the dietary ration and are attacked in the proventriculous and gizzard by hydrochloric acid and hydrolytic enzymes. The combined action hydrochloric acid and hydrolytic enzymes denature the proteins' structure into single strands so that peptide linkages are exposed. Some native proteins create resistance to this catalytic process because they contain bonds that the birds' proteinases do not have access to, but the acid state of the proventriculus and gizzard aid to break down the protein so that most of the

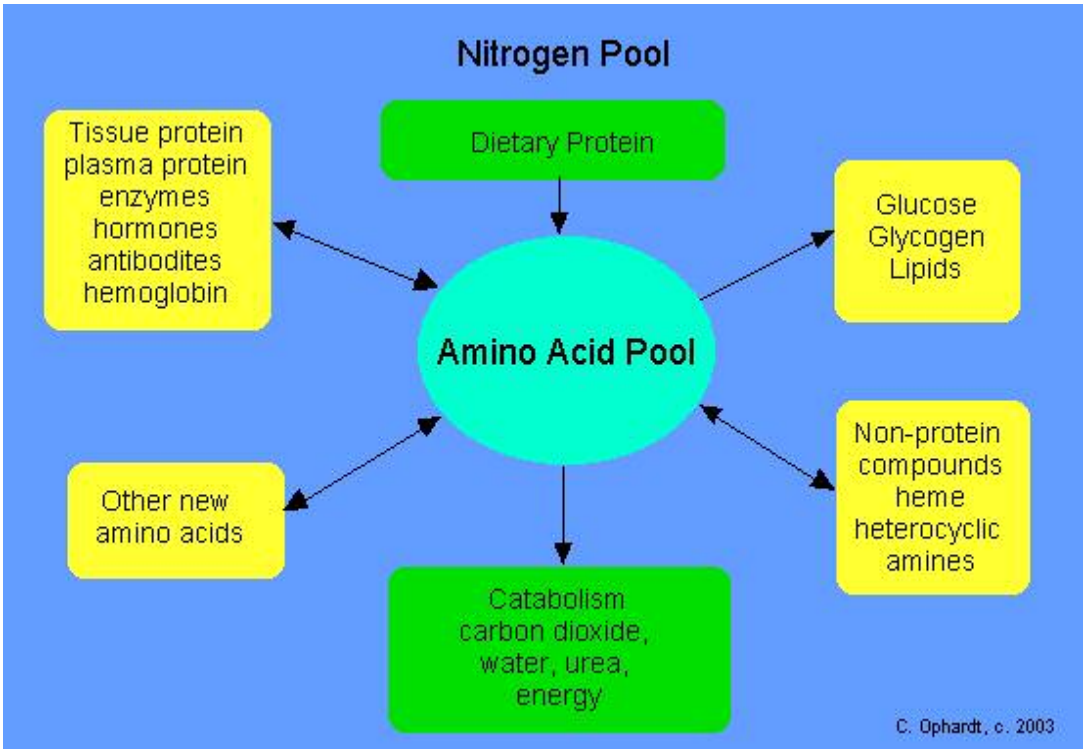

Figure 2. Nitrogen and Amino Acid pool

pepsin-sensitive peptide bonds are exposed. Pepsin is responsible for initiating the proteolytic process, and results in an increased accessibility of peptide bonds to hydrolysis by proteolytic enzymes of the small intestine. In the small intestine, trypsin, chymotrypsin and elastase further hydrolyze the peptides even more, exposing numerous terminal peptide bonds that are attacked by a new set of enzymes: aminopeptidases, caroxypeptidases and other specific peptidases present in the lumen or mucosa of the small intestine. Each enzyme plays a sequential role in degradation, therefore if one is inhibited or not present in sufficient concentration a decrease in digestion may occur.

Food in the gastrointestinal tract stimulates vagal nerve, which in turn initiates

secretion of gastric juice into the proventriculus by the gastric mucosa. This juice is rich in mucin, hydrochloric acid and proteinases. Pepsinogen is then secreted by the peptic cells of the proventriculus, and autocatalytically activated by hydrochloric acid presence. A highly acid condition predominates in the proventriculus (pH 1.5-2); followed by a buffer effect of feed that increases pH to 3.5-5. Pepsin is known to hydrolyze several different peptide linkages having a more pronounced effect between leucine and valine, tyrosine and leucine, phenylalanine and tyrosine bonds.

The endopeptidases secreted in the proventriculus and by the pancreas are capable of degrading proteins to small peptides containing from 2 to 6 amino acids (oligopeptides) and some free amino acids. Some hydrolysis of these small peptides takes place by the action of peptidases that are present in desquamated mucosal cells but most of oligopeptide breakdown does not occur within the intestinal lumen. Most amino acids and small peptides are absorbed into enterocytes via active carrier-mediated processes, but passive absorption also occurs. Those small peptides that are absorbed into the mucosal cell are hydrolyzed into free amino acids by intracellular peptidases located in the cytoplasm of the intestinal mucosa. These amino acids go the liver via portal blood stream as free amino acids.

AMINO ACID TOXICITY AND DEFICIENCY: There is evidence to indicate that amino acids themselves may precipitate negative effects in diverse classes of farm livestock. These effects may emerge due to the intake of indispensable and dispensable amino acids absorbed in quantities and patterns which are disproportionate to the required for optimum tissue utilization. These manifestations of adverse effects can be due to what

has been referred to as “imbalance”.

The term amino acid imbalance has been defined as a change in the pattern of amino acids in the diet that precipitate food intake and growth depressions that may be completely alleviated by supplementation of the first limiting amino acid (Harper et al., 1970). The primary manifestation of adverse effect is a reduction in food intake, which also reduces intake of limiting amino acids that lead to a reduced growth rate. This particular problem can be overcome by simply adding a higher amount of the most limiting amino acid. When diets contain marginal levels of threonine, excess of serine result in a growth depression that can be overcome by higher levels of dietary threonine. Excess serine increases the activity of threonine dehydrogenase and threonine aldolase (Scott et al., 1982). Threonine imbalance is also produced when chicks fed low threonine diets are then fed additional tryptophan or branched chain amino acids (D’ Amello, 1994).

Antagonisms are characterized by a growth depression caused by a single amino acid, and may be caused by structurally related amino acids. The most common antagonism seen in poultry is that of excess lysine impairing the utilization of arginine increasing the requirement markedly. The ratio of dietary lysine to arginine cannot be much greater than 1.2:1 before growth retardation occurs with small additional amounts of lysine. It was also seen that excess arginine depressed growth of chicks fed a lysine deficient diet, which was reversed by the addition of supplementary lysine (D’Mello and Lewis, 1970). Due to their uricotelism, poultry are unable to synthesize arginine and are particularly sensitive to this interaction. The most significant contributory factor to the

antagonism is the enhanced activity of kidney arginase in chicks fed excess lysine, resulting in increased catabolism of arginine (Austic, 1986). If arginase activity is suppressed by the use of a specific inhibitor, then the susceptibility of chicks to the lysine-arginine antagonism is attenuated. In this case the depression of food intake presumably arising from lysine-induced disruption of brain uptake and metabolism of other amino acids and their biogenic amines.

Another type of antagonism can be seen in the case of branch chain amino acids. Excesses of leucine may be severely growth depressing unless additional isoleucine and valine are added to the diet, inducing a rapid fall in plasma valine concentration. Similarly, an excess of isoleucine and valine can cause growth depression that may be alleviated by leucine supplementation (D'Mello and Lewis, 1970). An excess in branch chain amino acids may, additionally, induce a depletion of brain pools of other amino acids, particularly those which are the precursors of the neurotransmitters. Dietary excesses of three branch chain amino acids reduced brain concentrations of noradrenaline, dopamine and 5-hydroxytryptamine in the chick. However, this effect may be overcome with supplementation of the neurotransmitters' precursors, phenylanine and tryptophan (D'Mello., 1994). Unique toxic effects may be precipitated on feeding excess quantities of individual amino acids by virtue of their particular structural or metabolic features. In some cases an acute growth depression caused by excesses of some individual amino acids has had significant lesions in tissues and organs (Benevenga and Steele, 1984). It has also been observed that methionine is probably the most toxic amino acid in livestock (Baker, 1989). Methionine toxicity is usually characterized by growth depression, but this pattern has also been observed when excess threonine is fed to

chicks. Other amino acids that may have toxic effects are tyrosine, phenylalanine, tryptophan and histidine, but only when they are present at levels of 2 to 4 % of the diet. Glycine can be toxic to chicks if the diet is deficient in niacin or folic acid (Scott et al., 1982).

PROTEIN FEEDING: Before the 1940's, most poultry acquired a good proportion of their needs, particularly protein needs, from foraging at free range. Generally their diets were supplemented by both mash and whole grain feeding. Broilers are now fed on a 3 to 5 diet system: starter, grower and finisher, containing typically 23-24%, 20-22% and 18-20% crude protein respectively. This commercial feed program shows the decline in percentage protein in the diet with age of the birds. The ideal percentage protein as well as most other nutrients declines progressively with age, where-as the diets obviously have to decline in steps. This leads to inefficiency in the use of protein, as alternate periods of under and over-feeding of protein are inevitable (Filmer, 1993).

During the periods of under-feeding, birds are clearly short of the ideal levels of protein, and so their performance falls short of their genetic potential. During periods of overfeeding, the unwanted protein has to be deaminated and excreted as uric acid through the kidneys. This involves unnecessary energy expenditure and the ingestion of extra water resulting in litter with high nitrogen, sulfur and water content. This may also result in high ammonia levels, sticky and wet litter, hock burns and breast blisters. This ammonia and other noxious and smelling nitrogenous pollutants in litter are now seen as environmentally unacceptable and is a source of criticism of current farming practice from the public (Filmer, 1991). Baker (1993) reported the ideal protein concept for

poultry as well as swine, indicating that the use of the ideal protein concept in feed formulation would minimize nitrogen excretion in waste products.

The availability and use of synthetic amino acids has allowed nutritionists to lower the dietary crude protein content, thus reducing nitrogen excess and environmental impact. This trend will continue as more economically useful amino acids are made available for animal feeding.

The major environmental concerns as they relate to groundwater protection are nitrogen and phosphorus, while other environmental concerns include odors and pathogens (Rinehart, 1996). Cromwell (1994) noted that livestock and poultry excrete approximately 158 million tons of dry matter manure in the United States which translates to 800,000 tons of nitrogen. Consequently, law regulations makers have started to regulate phosphorus (Maryland), while nitrogen excretion and ammonia pollution are issue of concern in The Netherlands (Cromwell, 1994). Such environmental concerns make it necessary to reduce the dietary supply of protein.

PROTEIN QUALITY: Quality of proteins present in the feed is due to a combination of factors including quantity, digestibility, and amino acid balance. This last factor represents the most important variable because a feedstuff rarely contains all the amino acids required by chicks. The most deficient amino acid will become the first limitating amino acid, being lysine in most cases. It usually does not make a difference if other amino acids are in moderate excess in the exceptions of antagonisms or toxicity. Excessive amino acids are generally used for energy, but at a high energetic cost.

Currently, corn and soybean-meal are the two most common feed ingredients used to manufacture commercial broiler rations. In the case of corn, lysine is the first limiting amino acid, even though efforts have been made to genetically engineer corn varieties with higher lysine content. Soybeans' limiting amino acid is methionine and is rich in lysine therefore these two ingredients complement each It is because of this that it must be clear that a diet deficient in protein is due to amino acid limitations and not because there is a lack of nitrogenous compounds (Klasing, 1998).

DIET COMPOSITION

CALORIE/PROTEIN RATIO: The decline in feed intake, growth rate and survivability of broilers exposed to high ambient temperature-relative humidity distress (HD) has long been documented (Squibb et al., 1959). Amino acid requirements may be identified as those for maintenance, carcass growth and feather growth, on the basis of their respective amino acid profiles. This compartmentalization provided the basis for formulation of a model that was tested in both chickens and turkeys. The model calculates the requirements of each amino acid to satisfy a definite target function in terms of growth but makes no allowance for any possible interaction among dietary amino acids or for amino acid balance. Interactions have been demonstrated in chickens between lysine and arginine. The important of such interaction appears minor in normal diets in which requirements are satisfied for all essential amino acids and large excesses of amino acids are avoided, but they may become significant under conditions of protein underfeeding or overfeeding.

When growth or feed efficiency are plotted as functions of the limiting dietary amino acid level, the values of the response variables increase until a plateau is reached. The level of dietary amino acid that marks the beginning of the plateau depends on the elevation of the plateau of the variable (growth or feed efficiency). Improvement in the amino acid balance would be expected to modulate the requirement level by changing the dependence of growth or feed efficiency on the dietary amino acid concentration.

TABLE 1 Nutrient Requirements of Broilers as Percentages or Units per Kilogram of Diet (90 percent dry matter)

Nutrient	Unit	0 to 3 Weeks	3 to 6 Weeks	6 to 8 Weeks
Arginine	%	1.25	1.10	1.00
Glycine + Serine	%	1.25	1.14	0.97
Histidine	%	0.35	0.32	0.27
Isoleucine	%	0.80	0.73	0.62
Leucine	%	1.20	1.09	0.93
Lysine	%	1.10	1.00	0.85
Methionine	%	0.50	038	0.32
TSAA	%	0.90	072	0.60
Phenylalanine	%	0.72	0.65	0.56
Proline	%	0.60	0.55	0.46
Threonine	%	0.80	0.74	0.68
Tryptophan	%	0.20	0.18	0.16
Valine	%	0.90	0.82	0.70

The 0 – 3, 3 – 6 and 6 – 8 week interval for nutrients requirements are based on chronology for which research data were available; however, these nutrients requirements are often implemented at earlier age intervals or on a weight of feed consumed basis

Many factors may influence the requirement, the foremost being the genotype and age of the bird. Other factors include the level of other nutrients (which may affect growth rates), disease states and environmental conditions such as temperature, humidity, water quality and air quality.

Although the absolute values for amino acid requirements vary greatly, the relative levels required are much more stable. This observation has led to the concept of an ideal amino acid balance or ideal protein. The ideal protein ratio for broiler chickens is shown in Table1. By convention the values are expressed relative to lysine. Values for the ideal protein ratio are still being evaluated. Recently, for example, Baker and Han (1994)

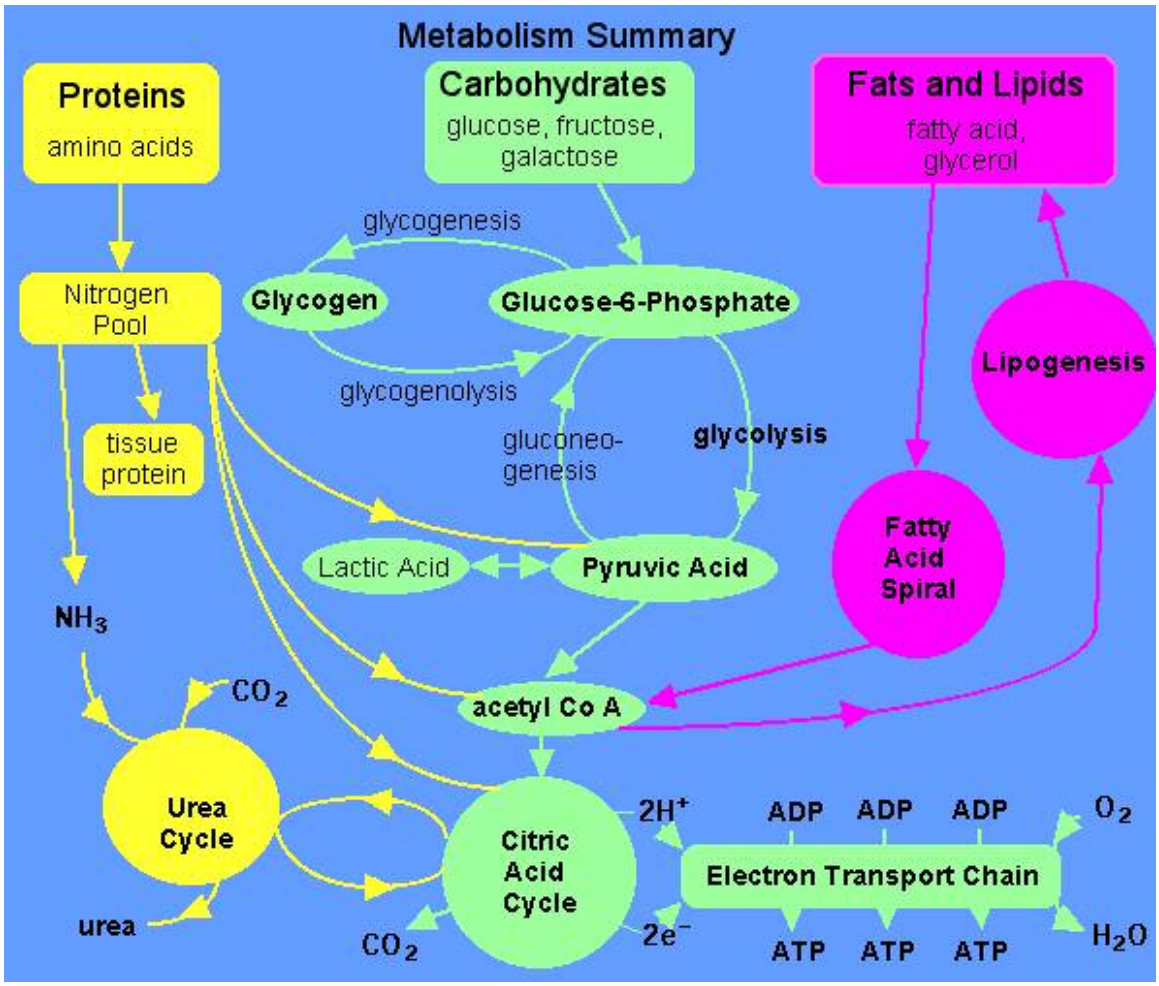

Figure 3. Summary of Metabolism. Fate of Carbohydrates, Fats and Proteins

suggested that in starter chicks the threonine levels should be increased to 85% of the value for lysine.

LIMITING AMINO ACIDS: Assuming all other nutrient requirements are met, production of meat or eggs will be determined by the level of the amino acid that is most limiting. If that amino acid is supplemented in the diet to requirement, production will increase to a level which is determined by the next limiting amino acid. Dietary supplementation with the second amino acid will result in further increases in production to a level determined by the third limiting amino acid.

Limiting amino acids in diets can also be established by reducing incrementally the protein levels in a balanced diet until performance is depressed. Supplementation with the first limiting amino acid will return performance to former levels and further decreases in protein levels can be made until the level of the second limiting amino acid drops below requirement.

In poultry diets consisting of corn and soybean meal as the major ingredients the order of limiting amino acids is usually methionine, lysine, threonine, arginine, and valine (Baker et al., 1993). Amino acid requirements have been established for poultry and this data is available from the NRC 1994. The challenge would be to formulate diets which provide as closely as possible the amino acid requirements of the bird and at the same time are economic. This can present some difficulties because the amino acid profiles of raw feed materials do not match the requirements of the birds. Amino acid imbalances may occur when using too much protein. Antagonism can exist when the excess of an amino acid is associated with a deficiency of another whose requirements will therefore be increased. For example, if the lysine to arginine ratio exceeds 1.2, reduced growth rates of young birds may occur. Also, excess leucine, which may occur when using high levels of gluten meal or blood meal causes, reduced growth. Feed intake is depressed through a metabolic effect. Catabolism of valine and isoleucine is also stimulated.

COMPOSITION OF GAIN: Increased carcass fat that has accompanied rapid growth of the modern broiler chickens continues to be a health concern of consumers. In addition, abdominal and visceral fat are waste products to the poultry processor and add to waste

management problems. Various nutritional and management techniques have been employed in attempts to control fat accumulation by reducing growth rate of poultry during the early growth period.

Pollution with nitrogen originating from animal wastes has become a concern in developed as well as developing nations (Deschepper and De Groote, 1994). Hence, reduction in the nitrogen excretion and the efficiency of nitrogen accumulation in the tissue can be met with matching amino acid composition of the diet with the amino acid requirement of the broiler. Availability of various amino acids in the market has made it possible to supplement synthetic amino acids to the diets there by meeting the protein requirement of the broiler. Consequently, birds fed low protein diets have shown to dissipate less heat (Cobb Vantress, 2003). Moreover, reduced nitrogen excretion lowers ammonia build up in the poultry house. Another consequence of waste nitrogen is that its conversion into uric acid lowers the energetic efficiency of protein use as an energy source. This has created fundamental misinterpretation of protein effects when fed to poultry. For example, feeding elevated protein to broilers improves "leanness" but not by elevating lean mass, but by increasing heat production and reducing lipid content. Hence dietary proteins are fed to broiler in an effort to satisfy indispensable amino acids and dispensable amino acids consumption needs.

A review of the literature shows that there is a broad debate on the topic of synthetic amino acids being supplied to low protein diets and its subsequent impact on the performance of broilers. Twining *et al*. (1974) has shown that birds fed low protein diets never gained the bodyweights and feed conversions equal to the control diet. However, when they consumed the finisher diets according to the NRC requirements,

birds gained weight through compensatory growth this led to the final performance almost equal to that of the controls. However, even though compensatory growth occurs, it should be noted that, the composition of the chicken would change only in terms of fat accumulation (Moran, 1979, Lipstein et al, 1975). These authors concluded that broilers overeat and this results in fat accumulation in the carcass. Uzu (1982), Jensen (1991), Moran *et al* (1992) and Holsheimer and Janssen (1991) all claim that maximum performance cannot be reached by fortifying low protein diets with synthetic amino acids. On the other hand, Schutte (1987) and Parr and summers (1991) have shown that optimal performance can be reached by supplementing diets with synthetic amino acids. There is also substantial work showing the influence of dietary protein and energy levels on the composition of the carcass (Bartov et al., 1974; Lipsteion and Bornstein, 1975; Summers et al., 1988). Although the effect of dietary protein on carcass composition is basically an amino acid effect, as demonstrated by several workers (Carew and Hill, 1971; Bornstein and Lipstein, 1975a, b; Lipstein and Bornstein, 1975; Lipstein *et al*., 1975) there are considerably limited amount of work done to learn the influence of amino acid supplementation on increased protein deposition in the carcass.

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CHAPTER III

Effect of Low-Protein Diets on Growth Performance and Body Composition of Broiler Chicks

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RUNNING TITLE: Low Protein Diets

Abbreviation Key: EAA = essential amino acids, NEAA = non essential amino acids CP = Crude Protein

INTRODUCTION

Pollution with nitrogen originating from animal wastes has become a concern in developed as well as developing nations (Deschepper and De Groote, 1994). Hence, reduction in the nitrogen excretion and the efficiency of nitrogen can be met by matching the amino acid composition of the diet with strict amino acid requirement of the broiler. Availability of various amino acids in the market make it possible to supplement synthetic amino acids to the diets thereby meeting the amino acid requirement of the broiler at reduced dietary protein.

Advantages potentially exist for birds fed low protein diets. Birds fed low protein diets have been shown to dissipate less heat (Cobb Vantress, 2003). Energy retention is the difference between consumption and excretion plus heat dissipation. Ration metabolisable energy (ME) value is predictable while fed heat production (HP) can be more variable. Heat production is influenced by feeding level, dietary heat increment, environment, and body size (Stanier *et al*, 1984; Blaxter, 1989).

The relation of heat production with body weight is curvilinear adding yet another variability source. Consequently, accounting for such variability may help to make management programs more repeatable. Heat production, measured by indirect calorimetry, may be utilized to estimate the energy expended for basal metabolic rate (BMR), maintenance, activity, tissue gain, environmental perturbations, and reproductive activities (Waring and Brown, 1965; Bornstein *et al*, 1979; Pinchasov and Galili, 1990; Spratt *et al*, 1990a&b). Better understanding of such relationships may enable producers to feed not only to BWT but also specific BC, thereby assisting in fine-tuning ration formulation.

Basal Metabolic Rate (BMR) relates to mature animals (Brody, 1964) though such relationship likely exist for growing animals as well, the application of such principle in young growing broilers, this is necessary to assure interface of protein calorie need. Skinner et al., (1996) observed that fasting young birds created a body temperature homeostasis after 15h of feed removal in contrast to the 36h needed for adults. Research is needed in young birds to create the counterpart of BMR, such might be termed MBR (Metabolic Basal Rate).

Moreover, reduced nitrogen excretion lowers ammonia build up in the poultry house. Another consequence of waste nitrogen is that its conversion into uric acid lowers the energetic efficiency of protein use as an energy source. This has created fundamental misinterpretation of protein effects when fed to poultry. For example, feeding elevated protein to broilers improves "leanness" but not by elevating lean mass, but by increasing heat production and reducing lipid content. Hence dietary proteins are fed to broiler in an effort to satisfy indispensable amino acids and dispensable amino acids consumption needs. Consequently, accounting for such variability may help to make management programs more repeatable. Better understanding of such relationships may enable producers to feed not only to BWT, but also specific BC, thereby assisting in fine-tuning ration formulation for optimal performance.

A review of the literature shows that there is a broad debate on the topic of synthetic amino acids being supplied to low protein diets and its subsequent impact on the performance of broilers. Twining *et al*. (1974) has shown that birds fed low protein diets never exhibit the bodyweight and feed conversion equal to the control diet. However, when they consumed the finisher diets according to the NRC requirements,

birds gained weight through compensatory growth and this led to final performance being almost equal to that of the controls. However, even though compensatory growth occurs, it should be noted that, the composition of the chicken would change only in terms of fat accumulation (Moran, 1979, Lipstein et al, 1975). These authors concluded that broilers overeat and this results in fat accumulation in the carcass. Uzu (1982), Jensen (1991), Moran *et al* (1992) and Holsheimer and Janssen (1991) all claim that maximum performance cannot be reached by fortifying low protein diets with synthetic amino acids. On the other hand, Schutte (1987) and Parr and Summers (1991) have shown that optimal performance can be reached by supplementing diets with synthetic amino acids. There is also substantial work showing the influence of dietary protein and energy levels on the composition of the carcass (Moran et al., 1968; Bartov et al., 1974; Lipsteion and Bornstein, 1975; Summers et al., 1988). Although the effect of dietary protein on carcass composition is basically an amino acid effect, as demonstrated by several workers (Carew and Hill, 1971; Bornstein and Lipstein, 1975a,b; Lipstein and Bornstein, 1975; Lipstein *et al*., 1975) there are limited amounts of work done to learn the influence of amino acid supplementation on protein deposition in diets varying in crude protein content. Therefore, the purpose of the present experiment was to study the effects of feeding low protein diets supplemented with synthetic amino acids on performance and carcass composition (tissue accretion, energetic efficiency, metabolic rate)

MATERIALS AND METHODS

Floor Pen Study

This study was conducted to evaluate the effects of various levels of protein on performance and carcass parameters. Seven hundred and twenty day-old Cobb x Cobb birds were received from a commercial hatchery and were randomly distributed into 72 pens (10 birds / pen). Chicks were pooled randomly and were placed on concrete floor covered with wood shavings as litter. Birds were reared under optimum growth conditions recommended by breeders. Starter feed was provided in open tray feeders for fist seven days and then after, hanging feeder were used simultaneously for feeding. Corn-soy based starter mash diet (Table 1) was offered ad libitum . Automatic nipple drinkers were placed prior to arrival of chicks and water was available ad libitum. Birds were wing banded immediately after its arrival from the hatchery. Feed consumption and daily mortality was recorded on prescribed data capture farm. Treatment format contained starter, grower, and finisher components and during these three stages, treatments were examined in a factorial arrangement with two sexes and two enzyme levels (Table 1). This provided a total of 8 dietary treatment profiles. Birds were regularly taken out during the days, 9, 18, 27, 36, 47 and 55 days from the floor pens for Basal Metabolic Rate.

During the floor pen part of the trial, starter (0-18 days), grower (18-47), and finisher (47-55) feed consumption, individual body weights at 0, 10, 18, 27, 36, 47, and 55 day of ages, and mortalities were recorded. Individual body weights of birds were recorded whenever birds were removed from pens for chamber studies for feed conversion correction. Because this part of the study also served as a pool for metabolic phase of the study and six birds from each pen were processed for GIT size and weight at the end of each growth phase, birds got fewer as the study progressed. At the end of the

study, there were two birds remaining. After fasting the birds for 24 hours for the heat production data, birds were then scanned

Metabolic Study

The metabolic chamber periods were conducted on days 10, 18, 27, 36, 47, and 55. During these 6 periods, treatments were examined in a factorial arrangement with two sexes and four protein levels for all the three phases (starter grower and finisher). 72 birds (9 birds per treatment) were selected and randomly assigned to the metabolic chambers. There were nine replicates for each treatment. Birds were kept in the chambers and were fasted 24 hours for heat production data. The chambers were checked twice daily for mortality, general conditions, temperature, lighting, water and feed conditions and any unanticipated events were documented. After completion of the chamber trials, all birds were sacrificed for scanning and body composition evaluation. Both metabolic chambers and general operational procedures have been described elsewhere (Wierusz and Teeter, 1993; Belay and Teeter, 1993).

Data Analysis

The data were analyzed using ordinary least squares (SAS, 1991). The model included treatments sexes and ages as the main effects. Interaction between main effects was included in the model. Mean separation was accomplished using Least Significant Difference (Steel and Torrie, 1960). Regression technique was used to estimate feed consumption for body weight homeostasis. Carbon dioxide production and O_2 consumption were regressed for each bird as the differential concentration between incoming and outgoing gas concentration multiplied by the air flow rate. Subsequently,

heat production was estimated from liters of O_2 consumed and CO_2 produced (Brouwer, 1965).

Results and Discussion

The study was successfully completed with results displayed in Table 2 , 3, and 4. During the starter phase, there were no significant differences between the treatments for the body weights (Table 2). Body weights of the birds ranged from 360 g for the NRC – 4.5 % deficient diet to 404 g for the NRC treatment birds. On an average the females weighed more than the males during the starter period (400 g vs 385 g). During the grower period, the NRC – 4.5% birds were significantly lower than the other treatment birds. These results continued to the finisher phase suggesting that low protein diets fortified with EAA can only offset the substitution of CP to some extent.

In order to achieve optimal performance of broiler chickens, the necessity of providing EAA from intact protein rather than from a large quantity of synthetic amino acids was observed in the present investigations and confirms observations from the literature (Pinchasov et al., 1990, Edmonds et a., 1985). The reason for the failure of low-CP diets fortified with EAA to support maximum performance is not clear.

Birds were removed from the cages at regular intervals and were randomly assigned to the metabolic chambers for heat production data and scan analysis data. Birds were removed from the metabolic chambers at the end of 24 hours and were euthanized for body protein and body fat data (Table 3). Regression equations were developed to yield equations predicting the protein and fat (grams) for the birds in the cages. Bird protein and fat in grams were regressed over the body weight to the third power to come up equations.

Birds fed NRC – 4.5% deposited the least amount of protein for all the phases (Table 3). There were no differences between the other treatments. During the grower phase, the protein deposition ranged from 206.3 g for the NRC treatment to 180.9 g for the NRC – 4.5% treatment. Similar trend was followed for the finisher phase. Birds fed the control diet were able to deposit 536.8 g of protein vs 486.6 g for the reduced dispensable N treatment. Similar trend was followed for the fat deposition. Birds fed the control diet were able to deposit more fat when compared to the deficiency treatment.

Results of present experiment together with previous research with low protein diets indicate that reducing dietary CP with broilers by decreasing the levels of both NEAA and EAA in excess of the requirement adversely affects production at all levels. The same results occur when the decrease in CP disregards EAA requirements (Salmon et al., 1983, Summers and Leeson, 1984; Marus et al., 1988; Summers et al., 1988; Roth et al., 1989). Further additions of EAA and potassium to low-CP feeds thought to be marginal to need have failed to correct these problems (Fancher and Jensen, 1989a; Pinchasov et al., 1990; Holsheimer and Jensen, 1991). The implications of these findings are that the spectrum of NEAA must be fully represented as such if EAA values corresponding the minimum requirements are to optimize performance.

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TABLE 1. Composition of experimental broiler feeds formulated to satisfy essential amino acid needs at and 4.5% below the National Research Council (NRC, 1994) recommended crude protein levels, percentage "as is" basis

	Starter				Grower				Finisher			
Ingredients	1	2	3	4	5	6	7	8	9	10	11	12
Corn	58.48	65.06	67.71	72.54	60.67	60.71	70.17	76.43	51.41	55.89	61.1	61.1
Soybean meal	29.42	19.43	18.4	16.19	28.12	27.95	19.28	12.6	21.02	16.8	11.92	11.92
Fat, Soybean	3.59	2	2.03	1.68	4.75	4.77	3.32	2.25	4.31	3.68	2.87	2.87
Corn glutenen meal	2.31	4.25	1.44	-	-	-	-	-	-	-	-	-
Propak	2	5	6.1	-	-	-	-	-	-	-	-	-
Wheat midds	-	-	-	-	-	1.6	1.5	1.62	20	20	20	20
Fish Meal	-	-	-	-	1.03	1	1	1.5	-	-	-	-
Dicalcium phosphate	1.46	1.85	1.87	1.89	1.43	1.43	1.5	1.55	1.01	1.04	1.08	1.08
Calcium carbonate	1.24	1.07	0.96	0.77	1.55	1.56	1.58	1.53	1.42	1.43	1.44	1.44
Choline chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.07	-	-	-	-
Salt	0.49	0.49	0.49	0.49	0.44	0.44	0.44	0.43	0.44	0.36	0.36	0.36
Vitamin premix[1]	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Mineral premix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
selenium[3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Lysine	-	0.29	0.32	0.33	0.01	-	0.29	0.49	-	0.14	0.32	0.32
DL Methionine	0.03	0.06	0.12	0.17	0.08	0.07	0.16	0.22	0.05	0.1	0.12	0.12
Threonine	-	0.05	0.11	0.19		0.04	0.11	0.2	-	-	0.05	0.13
Tryptophan	-	-	0.02	0.05		0.01	0.04	0.06	-	-	0.01	0.04
Arginine			0.08	0.28		-	0.16	0.37	-	-	-	0.17
Valine			0.02	0.13		-	0.05	0.15	-	-	-	0.1
Calculated analysis												
ME, kcal/kg	3150	3150	3150	3150	3200	3200	3200	3200	3200	3200	3200	3200
Crude protein, %	22.64	21.16	19.68	18.21	20	18.5	17	15.5	18	16.5	15	15
ME/CP ratio												
Methionine	0.45	0.45	0.47	0.52	0.43	0.43	0.47	0.5	0.35	0.38	0.38	0.38
Lysine	1.18	1.17	1.18	1.17	1.08	1.07	1.05	1.04	0.86	0.85	0.85	0.85
Sulphur amino acids	1.01	0.99	0.88	0.86	0.83	0.82	0.81	0.8	0.73	0.73	0.70	0.70
Calcium	1	1	1	1	1	1	1	1	0.8	0.8	0.8	0.8
Available phosphorus	0.45	0.44	0.44	0.44	0.38	0.38	.038	0.38	0.3	0.3	0.3	0.3

[1]Supplied per kilogram of diet: vitamin A, 10,141 IU (retinyl acetate); cholecalciferol, 3,086 IU; vitamin E, 23.92 IU (dl-α-tocopheryl acetate); menadione, 2.87 mg; thiamine, 2.20 mg; riboflavin, 7.72 mg; niacin, 60.30 mg; d-pantothenic acid, 12.46 mg; pyridoxine, 3.75 mg; vitamin B_{12}, 0.017 mg; folic acid, 1.066 mg; d-biotin, 0.127 mg.

Table 2. Body weight, Feed Conversion Ratio as influenced by protein levels for Starter, Grower and Finisher periods

Dietary treatment		Starter		Grower		Finisher	
Sex	Trt	BW	FCR	BW	FCR	BW	FCR
Interactive effect means							
M	NRC	404.3	1.42[a]	1602.4	1.45	3417.8	1.74
M	NRC – 1.5	386.4	1.49[a]	1452.5	1.47	3341.8	1.74
M	NRC – 3.0	388.3	1.45[a]	1534.8	1.46	3184.2	1.70
M	NRC – 4.5	360.3	1.60[b]	1421.1	1.53	3091.3	1.67
F	NRC	400.0	1.47[a]	1454.2	1.50	2941.0	1.81
F	NRC – 1.5	399.3	1.47[a]	1477.3	1.46	2992.0	1.88
F	NRC – 3.0	413.4	1.44[a]	1468.7	1.51	2846.4	1.89
F	NRC – 4.5	387.3	1.45[a]	1316.2	1.60	2648.3	1.94
Main effect means							
M		384.8	1.49	1502.7[a]	1.47[a]	3258.9[a]	1.71[a]
F		400.0	1.45	1429.1[b]	1.52[b]	2857.0[b]	1.87[b]
	NRC	402.1	1.45 [a]	1528.3[a]	1.47[a]	3179.4[a]	1.78
	NRC – 1.5	393.0	1.48 [a]	1465.0[a]	1.46[a]	3166.9[a]	1.81
	NRC – 3.0	400.9	1.44 [a]	1501.7[a]	1.47[a]	3015.3[ab]	1.76
	NRC – 4.5	373.8	1.52 [b]	1368.7[b]	1.57[b]	2869.9[b]	1.80

Source of Variation	Starter		Grower		Finisher	
	BW	FCR	BW	FCR	BW	FCR
Sex	0.1392	0.1242	0.0181	0.0141	< 0.0001	0.0019
Trt	0.2159	0.0385	0.0023	0.0002	0.0104	0.8559
Sex x Trt	0.6914	0.0221	0.2099	0.2661	0.8793	0.5117

[a- e]Means within a column with different superscripts differ ($P < 0.05$).

[2]Feed conversion ratio (FCR) = feed consumption / body weight gain.

Table 3. Protein (grams) and Fat (grams) deposition for the Starter Grower and Finisher Phases for the Experimental period

Dietary treatment		Starter		Grower		Finisher	
Sex	Trt	Protein	Fat	Protein	Fat	Protein	Fat
Interactive effect means							
M	NRC	67.2	37.8	218.7	218.7	567.3	512.5
M	NRC – 1.5	63.3	35.4	188.6	188.6	561.8	509.0
M	NRC – 3.0	62.9	34.5	208.7	208.7	532.3	484.0
M	NRC – 4.5	57.6	31.2	189.1	189.0	509.7	460.1
F	NRC	65.9	36.9	194.0	193.4	506.2	454.7
F	NRC – 1.5	64.3	35.7	194.7	194.6	514.1	467.0
F	NRC – 3.0	67.1	37.3	197.2	197.1	494.8	449.4
F	NRC – 4.5	62.8	34.0	172.6	172.6	463.4	424.1
Main effect means							
M		62.8	34.7	201.3[a]	201.2[a]	542.8[a]	491.8[a]
F		65.0	36.0	189.6[b]	189.7[b]	494.7[b]	491.2[b]
	NRC	66.6	37.3[a]	206.3[a]	206.3[a]	536.8[a]	483.6[a]
	NRC – 1.5	63.9	36.0[a]	191.6[a]	191.6[ab]	538.0[a]	488.0[a]
	NRC – 3.0	65.0	36.0[a]	202.9[a]	203.0[a]	513.6[a]	467.0[a]
	NRC – 4.5	60.1	32.7[b]	180.9[b]	181.0[a]	486.6[b]	442.1[b]

Source of Variation	Starter		Grower		Finisher	
	BW	FCR	BW	FCR	BW	FCR
Sex	0.1606	0.2334	0.0398	0.0398	< 0.0001	< 0.0001
Trt	0.0554	0.0168	0.0069	0.0069	0.0002	0.0032
Sex x Trt	0.4553	0.4617	0.2374	0.2374	0.8104	0.7891

[a- e]Means within a column with different superscripts differ ($P < 0.05$).

[1]Initial body composition determined by whole bird chemical analysis; final body compositions were based on dual energy x-ray absorptiometry measurements adjusted as described by Mckinney et al. (2005).

Table 4. Fat to protein ratio (%) and Energy retained (Kcal/g) for the Starter Grower and Finisher Phases for the Experimental period

Dietary treatment		Starter		Grower		Finisher	
Sex	Trt	FatProRat	Energy	FatProRat	Energy	FatProRat	Energy
Interactive effect means							
M	NRC	0.56	1.85[a]	0.52[b]	2.23[a]	0.28[b]	2.32[c]
M	NRC – 1.5	0.56	1.80[e]	0.74[a]	1.81[e]	0.90[a]	1.54[d]
M	NRC – 3.0	0.56	1.82[bc]	0.76[a]	2.22[ab]	0.90[a]	2.36[bc]
M	NRC – 4.5	0.56	1.82[bc]	0.75[a]	2.18[c]	0.90[a]	2.33[bc]
F	NRC	0.56	1.82[b]	0.76[a]	2.20[ab]	0.89[a]	2.41[a]
F	NRC – 1.5	0.56	1.79[d]	0.75[a]	2.19[bc]	0.90[a]	2.41[ab]
F	NRC – 3.0	0.57	1.82[bc]	0.75[a]	2.20[b]	0.90[a]	2.45[a]
F	NRC – 4.5	0.56	1.81[c]	0.75[a]	2.15[c]	0.9[a]	2.49[a]
Main effect means							
M		0.56	1.80	0.69	2.11	0.75	2.14
F		0.56	1.81	0.75	2.18	0.90	2.44
	NRC	0.56	1.84	1.00	2.22	0.90	2.37
	NRC – 1.5	0.56	1.75	1.00	2.00	0.90	1.97
	NRC – 3.0	0.55	1.82	1.00	2.21	0.90	2.41
	NRC – 4.5	0.54	1.82	1.00	2.17	0.90	2.41

Source of Variation	Starter		Grower		Finisher	
	FatProRat	Energy	FatProRat	Energy	FatProRat	Energy
Sex	0.7646	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Trt	0.3822	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Sex x Trt	0.7304	< 0.0039	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a- e]Means within a column with different superscripts differ ($P < 0.05$).

[1]Initial body composition determined by whole bird chemical analysis; final body compositions were based on dual energy x-ray absorptiometry measurements adjusted as described by Mckinney et al. (2005).

FatProRat = Fat to Protein Ratio

CHAPTER IV

Refinement of Novel Estimations of Poultry Body Composition and Evaluation Dual Energy X-Ray Absorptiometry as a Method for Rapid Broiler Body Composition Assessment

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Section Preference: Education and Production
Research Note: _____ Full-Length Paper:____X__
RUNNING TITLE: Body composition of poultry by DEXA
Abbreviation Key: DEXA = dual energy x-ray absorptiometry, BMC = bone mineral content, CNB = carbon and nitrogen balance

ABSTRACT

Two experiments were conducted to validate and or refine methodologies for quantifying body composition in poultry. In the first experiment, constants classically

used to derive body composition in C and N balance studies were evaluated for application in poultry. In Experiment 2, the efficacy of using dual energy x-ray absorptiometry (DEXA) to rapidly assess body composition in poultry was examined. In Experiment 1, broilers ranging in body weight from 1,660 to 2,240 g were sacrificed, and used for either measuring whole bird composition or determining the composition of the protein and lipid fractions. In Experiment 2, broilers ranging in body weight from 280 to 3,075 g were sacrificed, and DEXA measurements of lean, fat, and bone mineral content were obtained. The birds were then chemically assayed to determine protein, lipid, and ash for comparison. Results from Experiment 1 demonstrate that though poultry protein and lipid tissue do not greatly differ in composition compared to other species, the differences may significantly impact the assessment of body composition, and should therefore be considered as a source of error in C and N balance studies using poultry. Results from Experiment 2 demonstrate that DEXA measurements failed to accurately quantify the body composition of poultry when direct comparisons are made. Instead, DEXA measurements must be applied to regression equations that inter-relate DEXA measurements with compositions obtained by chemical analysis.

(*Key words:* carbon nitrogen balance, dual energy x-ray absorptometry, body composition, poultry)

INTRODUCTION

The principle goal of poultry producers is to consistently meet consumer demand for product taste and nutritional acceptability in a profitable manner. Accomplishing this requires that the end product be defined and that the criteria for success be centered on obtaining that defined product, and not entirely on live performance characteristics. As demonstrated by McKinney and Teeter (2004), body weight and FCR improvements obtained by increasing dietary caloric density did not in all cases equate into increased lean mass but rather greater amounts of carcass fat. Assuming fat to be at some level a waste product undesirable to the consumer, production decisions based solely on body weight and FCR are potentially misguided. Therefore, nutritional, environmental, and managerial decision consequences on body composition must be quantified.

Though numerous methods exist for estimating the body composition of animals used in nutritional studies (Hendrick, 1983; Topel and Kauffman, 1988), comparative slaughter has historically been the method applied in experiments with poultry. This methodology, however, is time consuming, difficult to apply to an entire growth curve, requires bird destruction, and the assumption that the composition of birds initially examined is the same as those incorporated into an experiment (Blaxter, 1967). However, according to work presented by Wolynetz and Sibbald (1987), the initial slaughter group may not be necessary for comparison purposes, which would result in a considerable reduction in the required resources.

Measures of C, N, and energy content of the feed and excreta, and CO_2 production have also been used for assessing body composition in poultry (Farrel, 1974). Advantages of using C and N balance (CNB) as compared to comparative slaughter are

measurements of the same animal can be repeated over time, as animal sacrifice is not required, and that the importance of initial body composition uniformity is negated (Blaxter, 1967). As outlined by Farrel (1974), there are assumptions associated with CNB: 1) that energy is retained only in the form of fat and protein tissue; 2) the composition of fat and protein are constant; and 3) poultry protein and lipid tissue are not significantly different in composition compared to other species.

Regarding the latter assumption, one would not expect sizable tissue compositional differences to exist between species. However, as CNB is already susceptible to analytical errors (Blaxter, 1967), examination of this assumption is warranted. Additionally, estimates for fat and protein tissue constituents are dated (Armsby, 1903; Blaxter and Rook, 1953; and Brouwer, 1965).

Advancements in dual energy x-ray absorptiometry (DEXA) have resulted in the availability of fan beam technology, which enables faster scan acquisition (Koo et al., 2004). This has sparked interest in the use of DEXA technology as a non-invasive method for assessing body composition in experiments with animals reared for consumption. A large body of data exists validating DEXA for accurately measuring soft tissues (lean and fat tissues) and bone mineral content in swine (Lukashi et al., 1999; Chauhan et al., 2003; and Koo et al., 2004) as piglets are used extensively as models for human infant studies (Fiorotto et al., 1986). However, little evidence is available verifying DEXA use for poultry.

An experiment conducted by Mitchell et al. (1997) is the only known evaluation of DEXA for quantifying lean and lipid tissues in poultry. They found the technology to fall short of accurately assessing bird lean and lipid content, but did suggest that the

technique may be applicable with software and or hardware modifications. However, this conclusion was based on results of simple linear regression analysis. Perhaps more sophisticated statistical models are needed.

Therefore, two experiments were conducted with the first directed at validating and/or refining estimations of protein and fat constituents with specificity to poultry. In the second experiment, DEXA was evaluated for accuracy and precision in quantifying soft tissue (lean and fat tissue) and bone mineral content (BMC) in poultry.

MATERIALS AND METHODS

Experiment 1 – Validation and or refinement of constants for poultry tissue constituents

Twenty-four broilers ranging in body weight from 1,660 to 2,240 g were obtained commercially, fasted (24 h), and euthanized by carbon dioxide asphyxiation. After autoclaving (20 h; 11 psi; 116 °C), the birds were equilibrated to ambient temperature. Each bird was then homogenized (including feathers) with a commercial grade blender and samples of each homogenate were obtained and frozen (20°C) until analysis. Twelve of the samples were randomly selected and partitioned by ether extraction into protein and lipid factions for analysis of DM, N, ash, (AOAC, 1990) and C (Harjo, 1994). The remaining homogenates of the whole bird were analyzed for DM, ash, ether extract C and N (AOAC, 1990). These samples were used to evaluate whether whole bird ether extract could be accurately estimated using the compositions of the protein and lipid fractions determined from the first 12 samples analyzed. Equations used were as follows:

(Eq. 1) TP = N x (1 / % N in P)

(Eq. 2) PC = TP x % C in P

(Eq. 3) LC = TC – PC

(Eq. 4) EE = LC / % C in L

where: TP = total protein (g), P = pure protein (g), PC = carbon as protein (g), TC = total carbon (g), L = pure lipid (g), LC = carbon as lipid (g), and EE = estimated whole bird ether extract (%).

Experiment 2 – Evaluation of DEXA for measuring body composition in poultry

All scans were obtained using a fan beam dual energy x-ray absorptiometer operated in the infant whole body mode. Rat-scan software was used for scan analysis. A total of 35 broilers ranging in body weight from 280 to 3,075 g were obtained commercially, and fasted and euthanized as described in Experiment 1. Previous work in this laboratory (unpublished) and that of Lukaski et al. (1999) demonstrated that animal positioning on the scanning surface does not impact scan results. However, for consistency all birds were scanned individually (5 times) in a prostrate position with the long axis of the bird perpendicular to the length of the table. After scanning the birds were immediately autoclaved and sampled for chemical analysis as previously described.

Data Analysis

In both experiments, bird served as the experimental unit. Regression analysis was used initially to compare DEXA measurements of body composition with those obtained by proximate analysis. Subsequently the effectiveness of these developed regression models in relating DEXA results with measures obtained through proximate analysis were evaluated using General Linear Models of SAS (2000).

RESULTS AND DISCUSSION

Experiment 1 – Validation and or refinement of constants for poultry tissue constituents

The C and N content of protein and lipid determined herein (Tables 1) were in close agreement with those values traditionally accepted (Armsby, 1903, Blaxter and Rook, 1953 and Brouwer, 1965) and utilized in assessing body composition through CNB techniques. For example, nitrogen as a percent of protein averaged 15.9 ± 0.06%, essentially matching that which is generally applied (16.0%) across numerous protein sources. Carbon as a proportion of protein and lipid was determined as 52.96 ± 0.14 and 74.0 ± 1.4%, respectively. The latter of which exhibited the most variability across samples measured and averaged slightly lower than the other constants evaluated (Table 2). Nonetheless, values determined herein for the C and N contents of protein and lipid resulted in the best overall estimation of whole bird ether extract when applied to equations 1 through 4 (Table 2). In comparison, estimates obtained with protein and lipid C and protein N estimates of Armsby (1903) and Blaxter and Rook (1953) resulted in roughly an 8% overestimation of whole bird ether extract (Table 2; Figure 1). Using constants proposed by Brower (1965), whole bird ether extract estimates were still inflated, but only slightly (approximately 2%). As it was successfully demonstrated that whole bird ether extract could be accurately computed from the composition of protein and lipid determined from independent samples, this approach was accepted as a means for estimating whole bird ether extract. Therefore, procedures were modified for Experiment 2 in that ether extract was estimated by determined C and N constants rather than AOAC (1990) methods. However, for simplification, ether extract estimated in this manner may be referred to as fat determined by proximate analysis.

Experiment 2 – Evaluation of DEXA for measuring body composition in poultry

An example of a DEXA scan and the information that appears in the scan report is shown in Figure 2. Note that lean tissue is not further subdivided in the report into its protein and water constituents. Thus, in order to directly compare DEXA measurements from the report with values obtained from proximate analysis, actual bird water content must be determined. To estimate this, the difference between the body weight of the bird and the sum of its protein, lipid, and ash (dry matter basis) were determined. Bird protein and fat were then regressed on the estimated bird water content. As indicated by the lack of a significance coefficient (Table 3), zero bird water content was associated with the lipid parameter. This was expected as the water in adipose tissue is predominantly associated with its vasculature and connective tissues (Pitts et al., 1971; Digirolamo and Owens, 1976). As a result, direct comparisons could be made between DEXA measures of lean, and the protein determined by chemical analysis plus the estimated bird water content (Figure 3). However, this was done only to illustrate direct relationships. Bird water content and protein were not coupled when developing regression models.

On the basis of simple linear regression, DEXA failed to accurately measure lipid and ash as determined by proximate analysis (Table 4), which agrees with conclusions reached by Mitchell et al. (1997). Error associated with BMC as it relates to ash are most likely a consequence of the hollow bone structure of poultry, as programming software was developed for mammals (Kelly, 2004). Additionally, BMC was not determined per se, rather the ash content of the whole body was measured. This potentially explains the consistent under-estimation of DEXA measure of ash.

In an attempt to correct for these errors, forward stepwise regression procedures (Neter, 1990) were used to develop more complex predictive models (Table 4), incorporating more parameters and their cross-products. Indeed, with these equations, the accuracy in which DEXA measurements could be inter-related with chemically determined values increased markedly. This was demonstrated through comparisons between DEXA measurements adjusted using these regression equations (αDEXA) and those obtained through chemical analysis (Table 5). No significant ($P > 0.05$) differences were detected between predicted (αDEXA) and determined values for any of the variables monitored. Additionally, the sum of αDEXA estimates of protein, lipid, water, and ash closely matched the body weights of the birds when they were initially scanned.

An inherent limitation of the proposed predictive models is the fact that the body weights of birds used in the experiment did to completely encompass the entire growth curve, depending on the end product desired (i.e., Cornish hen verses birds reared for breast meat). As such, using these equations to estimate the body composition of birds weighing more than 3,000 grams requires extrapolating beyond the models inference base, which with polynomial equations particularly, leads to erroneous estimations of the dependent variable.

This limitation of the models was clearly observed in an effort to quantify broiler body compositions using DEXA in a study designed to compare broiler rearing conditions typically found in different parts of the world. As part of this study, broilers were selected to represent body weights of approximately 500, 1,500, 2,500, and 3,500 grams. Application of the predictive equations to DEXA measurements appeared to work well (based on body weight accountability: the sum of the predicted body components vs.

gravimetric weight) in every case except for birds outside the inference base of the models. For birds weighing more the 3,000 grams, the predicted protein and lipid (as a % of body weight) was greatly underestimated. Concomitantly, predicted water content was largely exaggerated. As drastic changes of this manner were viewed as physiologically infeasible, the only logical explanation was that the models were failing in this zone of body weights.

In pending data to expand the inference base of the models, equations were modified by first fitting, for each body composition measure, trend lines to data that fell within the scope of the original equations (Figure 5). In assuming that these trend lines represent mid-points or bird population means, αDEXA estimates for protein, lipid, water, and ash were assigned to each trend line. Subsequently, DEXA measures were regressed on these mid-points to modify the equations so as to encompass the entire growth curve (Table 6). Note, however, that variability among birds is attenuated with these modified equations and thus would only be of use when describing a population as was the case here (Figure 6).

In conclusion, research reported herein has demonstrated that though poultry protein and lipid tissue may not appear to differ significantlyin composition compared to other species, these differences significantly impact the assessment of body composition and should therefore be considered as a source of error in C and N balance studies with poultry. Furthermore, DEXA technology can be used to rapidly assess body composition in poultry, however, not directly. For this technology to be of value, regression equations inter-relating DEXA measurements of BMC, and lean and lipid tissue, with PA determined protein, ether extract, ash, and water content are required.

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Table 1. Carbon and nitrogen concentrations in the whole carcass and protein and lipid factions of broilers

	Sample number													
Faction[1], %	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SEM
Total														
Carbon	61.0	58.8	61.1	60.6	61.2	58.5	58.5	59.2	60.1	59.3	57.0	57.5	59.4	0.41
Nitrogen	10.9	12.4	11.0	10.8	9.6	10.2	10.3	10.0	9.6	9.9	10.0	11.3	10.5	0.24
Protein														
Carbon	52.9	53.4	52.9	52.5	53.6	52.6	52.2	53.6	52.5	53.5	52.7	53.3	52.9	0.14
Nitrogen	15.5	15.9	15.9	15.7	16.0	15.7	15.6	16.3	16.0	15.9	15.9	15.8	15.9	0.06
Ether Extract[2]														
Carbon	80.0	79.9	79.5	78.9	74.3	70.6	71.6	69.9	72.9	71.0	65.3	68.9	74.0	1.4

[1]Dried, ash-free basis.

[2]Nitrogen averaged 0.2 percent.

Table 2. Ether extract predicted from protein and lipid tissue carbon and nitrogen concentrations

		%			
Reference	Tissue	Carbon	Nitrogen	Predicted ether extract[1]	Predicted ether extract error[2]
Armbsy, 1903	Protein	52.5	16.7	24.7	8.62[a]
	Lipid	76.5	–		
Blaxter and Rook, 1953	Protein	51.2	16.0	24.5	7.94[a]
	Lipid	74.8	–		
Brouwer, 1965	Protein	52.0	16.0	23.3	2.19[b]
	Lipid	76.7	–		
Present experiment	Protein	52.9	15.9	22.8	0.01[b]
	Lipid	74.0	–		

[a,b]Means within a column with different superscripts differ ($P < 0.05$).

[1]Calculated as: total carbon – (protein x protein carbon) / lipid carbon).

[1]Ether extract (EE) determined by proximate analysis (AOAC, 1990) was 22.8 percent.

[2]Calculated as: ((EE determined by proximate analysis – EE predicted) / EE determined by proximate analysis) x 100.

Table 3. Regression equation of water content (W) on protein (P) and lipid (L) in whole bird carcasses (W = a + b P + c L)

Parameter	Coefficient	Standard error	Probability
a	78.23313	18.45308	0.0002
b	3.41462	0.20961	< 0.0001
c	-0.00639	0.21166	0.9761

Table 4. Regression equation coefficients relating dual energy x-ray absorptiometry (DEXA) measurements with proximate analysis values

	DEXA variables							
Dependent variable	Intercept	Lean	Lipid	Lean^2	Lipid^2	Lean x Lipid	Lean^2 x Lipid^2	R^2, %
Protein[1], g	-8.90481^{+}	0.21571**						96.91
	-11.13536**	0.18779**	0.15961**					99.42
	-15.14152**	0.19686**	0.15655**	-2.99e^{-6}				99.43
	-15.15759**	0.2024**	0.11615**	-4.0e^{-6*}	4.494e^{-5*}			99.44
	-15.14395**	0.20176**	0.12014**	3.5e^{-6}	4.909e^{-5+}	4.07e^{-6}		99.44
	-10.9351^{**4}	0.2019**	-7.42e^{-3}	7.0e^{-6*}	1.5831e^{-4**}	9.223e^{-5**}	-4.4712e^{-11**}	99.49
Lipid[2], g	Intercept	Lipid	Lean	Lipid^2	Lean^2	Lean x Lipid	Lean^2 x Lipid^2	
	102.47233**	0.60302**						58.54
	-44.29583**	0.21388**	0.17341**					96.27
	-39.60899**	0.07553	0.18212**	1.5789e^{-4}				96.42
	-12.03683	0.14717**	0.11281**	1.0157e^{-4+}	2.184e^{-5**}			96.75
	-11.97534	0.16515**	0.10993**	1.2025e^{-4+}	2.409e^{-5**}	1.835e^{-5}		96.76
	-4.94566[4]	-0.04790	0.11017**	3.0268e^{-4**}	1.825e^{-5**}	1.4249e^{-4*}	-7.4679e^{-11**}	96.88
Ash[3], g	Intercept	BMC	Lean	Lipid	Lean x BMC	Lipid x BMC	Lean x Lipid	
	7.2132**	1.29866**						82.64
	-0.46329	0.42645**	0.02246*					99.02
	-0.24389	0.30121**	0.02344**	7.24^{-3*}				99.05
	-1.59568**	0.383**	0.02479**	7.61e^{-3**}	-5.672e^{-5**}			99.10
	-2.17225**	0.43985**	0.02713**	8.57e^{-3}	-1.2359e^{-4**}	3.0354e^{-4**}		99.15
	-1.64751^{**4}	0.16682^{+}	0.02813**	0.01159	-5.8e^{-6}	5.1384e^{-4**}	1.634e^{-5**}	99.20
Water[3], g	Intercept	Lean	Lipid	Lean^2	Lipid^2	Lean x Lipid	Lean^2 x Lipid^2	
	41.99924**	0.73998**						97.06
	34.0781**	0.64084**	0.5668**					99.76
	18.63859*	0.6758**	0.55503**	-1.153e^{-5**}				99.76
	18.65469*	0.67025**	0.5955**	-1.052e^{-5*}	-4.501e^{-5}			99.76
	18.66013*	0.66999**	0.5971**	-1.032e^{-5+}	-4.3366e^{-5}	-1.62e^{-6}		99.76
	8.33059[4]	0.66965**	0.91016**	-1.76e^{-6}	-3.1142e^{-4**}	-2.3796e^{-4**}	1.09734e^{-11**}	99.79

[1]Calculated as: nitrogen × 6.29.
[2]Calculated as: ((EE determined by proximate analysis – EE predicted) / EE determined by proximate analysis) x 100.
[3]Determined using AOAC (1990) procedures.
[2]Equation used to adjust DEXA measurements to proximate analysis data.
$^{+}$Significant ($P < 0.1$).

Table 5. Comparison of adjusted dual energy x-ray absorptiometry (αDEXA) and proximate analysis measurements of total broiler protein, fat, ash, water, and body weight[1,2,3]

	Total body constituents, g									
	Protein		Fat		Ash		Water		Body Weight	
Weight class	PA[4]	αDEXA	PA[5]	αDEXA	PA[6]	αDEXA	PA[6]	αDEXA	Scale	αDEXA
A	56	59	30	33	9	9	245	241	341	343
B	191	195	134	135	29	30	769	767	1,231	1,227
C	414	411	334	337	60	59	1,468	1,476	2,289	2,283
D	517	515	453	458	74	74	1,841	1,840	2,902	2,886

	Probability				
Source of variation	Protein	Fat	Ash	Water	Body Weight
Weight class	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Method	NS	NS	NS	NS	NS
Weight class x method	NS	NS	NS	NS	NS
Pooled SEM[7]	0.14	0.17	0.14	0.13	0.14

[1] Adjusted using regression equations relating DEXA measurements with proximate analysis results.
[2]Log transformations of the data were performed for statistical analysis.
[3]Reported values are the anti-log of the resultant least square means.
[4]Calculated as: nitrogen × 6.29 based on Experiment 1 results.
[5]Calculated as: ((EE determined by proximate analysis – EE predicted) / EE determined by proximate analysis) x 100.
[6]Determined using AOAC (1990) procedures.
[7]Based on analysis of log transformed data.

Table 6. Proposed equation coefficients relating dual energy x-ray absorptiometry (DEXA) measurements with proximate analysis values for broilers weighing more than 3000 grams

		DEXA variables								
Dependent variable[1]	Intercept	BMC	Lipid	Lean	Lipid^2	Lean^2	Lean x Lipid	Fat x BMC	Lean x BMC	Lean^2 x Lipid^2
Protein	-6.13349*	–	0.1119*	0.18308*	3.567e-5*	3.7e^{-6*}	4.728e^{-5*}	–	–	-1.252e^{-11*}
Lipid	-5.6813*	–	0.03129*	0.10041*	6.536e-5*	2.336e-5*	9.6e^{-5*}	–	–	-1.2042e^{-11*}
Water	5.79504*	–	0.76994*	0.68501*	-3.797e-5*	-1.373e-5*	-1.5077e-4*	–	–	2.43437e-11*
Ash	-1.6675*	0.01579	0.02434*	0.02658*	–	–	1.44e^{-6}	-2.54e^{-6}	-3.95e^{-6*}	–

[1]Determined by trend-line analysis of adjusted dual energy x-ray absorptiometry measurements (αDEXA).
*Significant ($P < 0.05$).

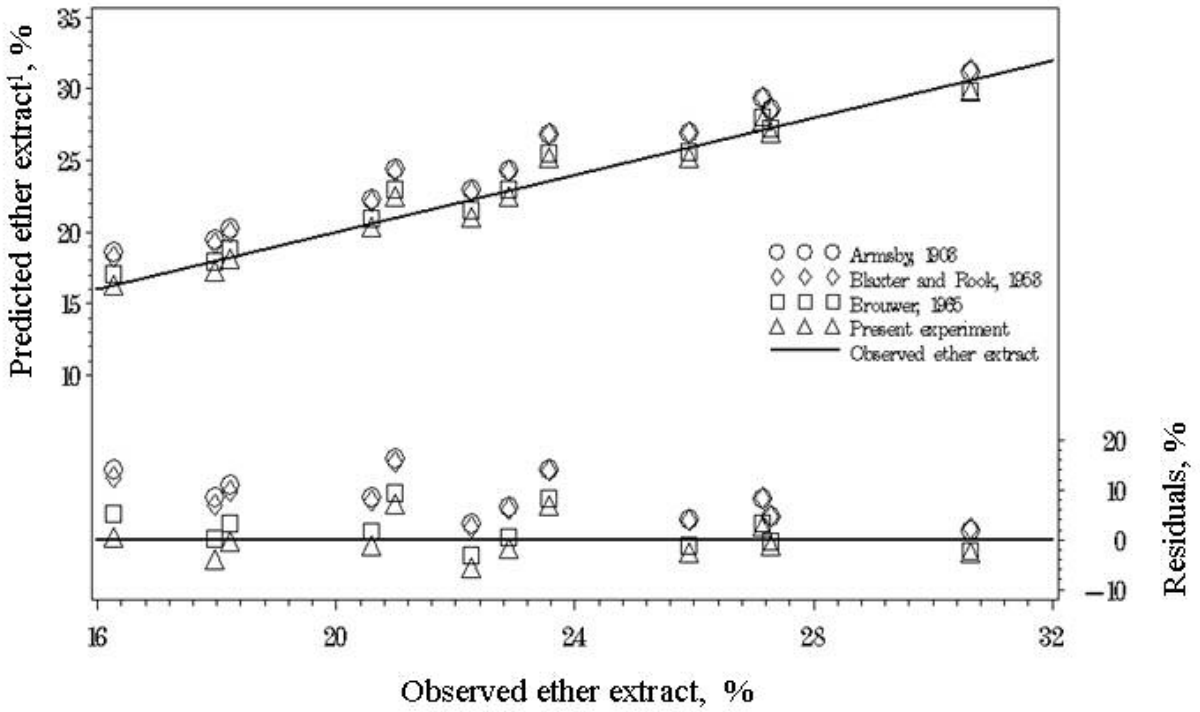
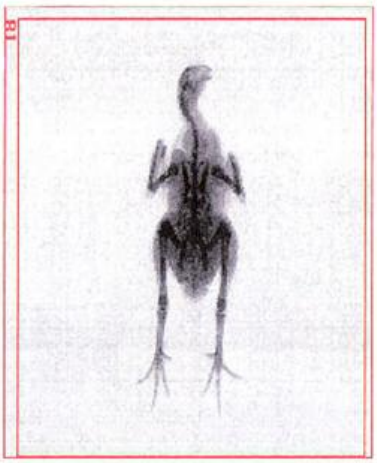


Figure 1. Ether extract estimated using proposed carbon and nitrogen contents of protein and lipid and the corresponding residual errors

[1]Calculated as: TC – (TP – C in P) / C in F, where: TC = total whole bird carbon, TP = total whole bird protein, P = protein, F = fat, and C = carbon.



DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	
GLOBAL	334.56	56.20	0.168	
RI	334.56	56.20	0.168	
NETAVG	334.56	56.20	0.168	
	Fat (g)	**Lean+BMC (g)**	**Total Mass (g)**	**% Fat**
GLOBAL	325.3	2614.8	2940.1	11.1
RI	325.0	2615.1	2940.1	11.1
NETAVG	325.3	2614.8	2940.1	11.1

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Figure 2. Dual energy x-ray absorptiometry scan image and results of a chicken.

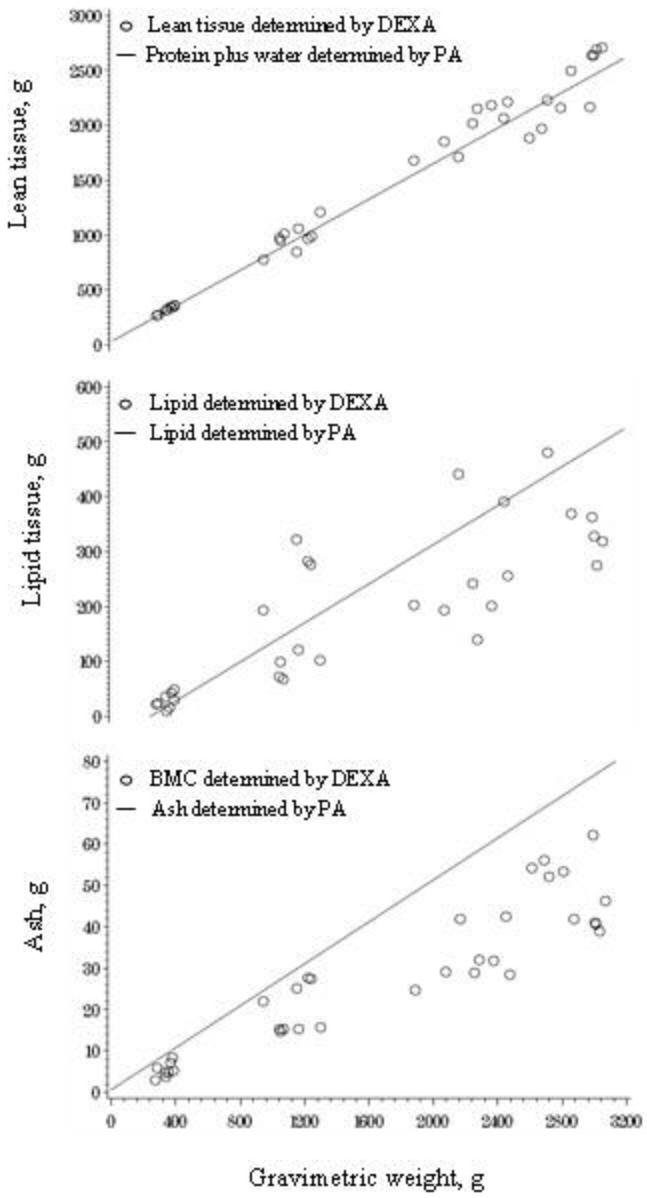

Figure 4. Relationship between body composition measured by dual energy x-ray absorptiometry (DEXA) and proximate analysis (PA)

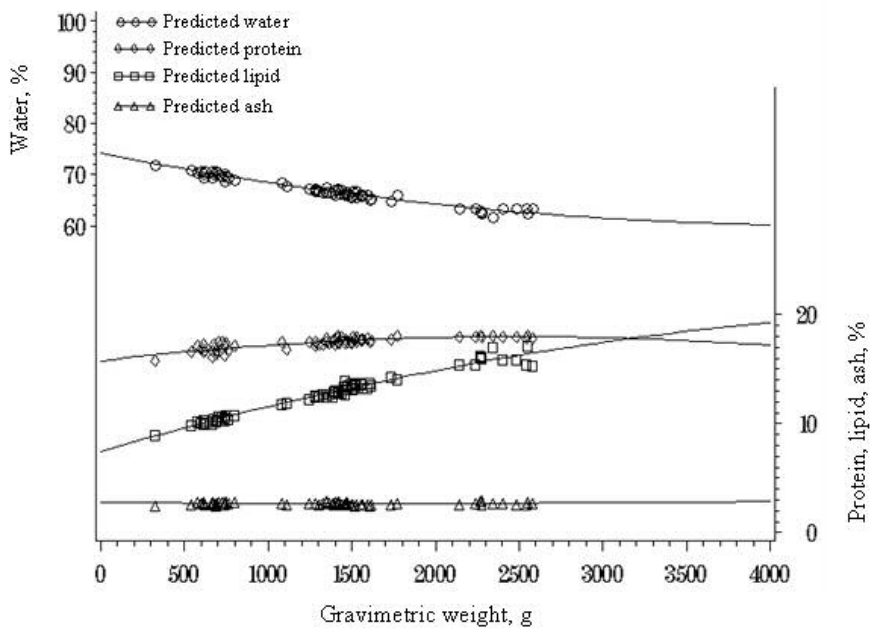

Figure 5. Independent application of regression equations that relate dual energy x-ray absorptiometry measurements to proximate analysis results and trend-line analysis to expand the applicable body weight range

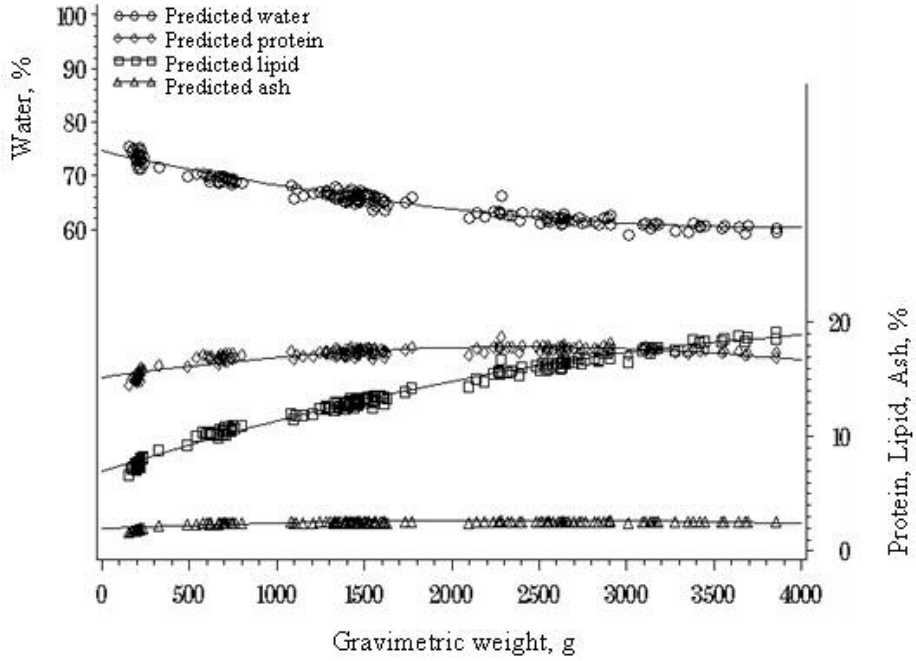

Figure 6. Broiler water, protein, lipid, and ash predicted from regression equations that relate DEXA measurements with proximate analysis results

CHAPTER V

Predicting Effective Caloric Value of Nonnutritive Factors: IV. Nutrient to calorie ratios as influenced by pelleting

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RUNNING TITLE: Pelleting influences on nutrient-calorie ratios

Abbreviation Key: ECV = effective caloric value; BWG = body weight gain; FCR = feed conversion ratio; M = mash; C = mash steam pelleted and crumbled; P = mash steam pelleted and sifted; PD = protein deposition; LD = lipid deposition; $kLys_{PD}$ = efficiency of dietary lysine for protein deposition; kRE = apparent energetic efficiency

ABSTRACT

Three experiments of similar design were conducted to first, evaluate dietary lysine in ratio with effective caloric value (ECV) altered either through the addition of soybean oil or by feed form, and second, to develop mathematical models that describe lysine requirement based on body composition. Studies utilized male and female broilers over age intervals ranging from 1 to 10 (Experiment 1), 19 to 29 (Experiment 2), and 45 to 55 (Experiment 3) days. Treatments evaluated were structured as 4 suboptimal dietary lysine levels by 2 (Experiment 1) or 3 (Experiments 2 and 3) ECV treatments. ECV treatments examined were: 1) unprocessed mash (M), 2) M plus 187 kcal ME_n/kg of soybean oil (M187), and 3) M steam pelleted (P). No significant sex × treatment interactions were detected therefore sex effects were combined. In all experiments, increasing dietary lysine level resulted in greater feed intake, weight gain, protein and lipid tissue gain, and feed efficiency. No significant dietary ECV effects were detected in Experiments 1 and 2. However, overall in Experiment 2 results suggest that calories provided in the diet can be replaced on a one-to-one basis by calories spared through reduced activity. In Experiment 3, P fed birds had higher feed intake, weight gain, protein and lipid gain, and feed efficiency compared to M187. Responses to P and M187 were equal when lysine intake was used as a covariate in the model. Regression models were successful in inter-relating body composition with lysine need. Models indicated that current recommendations for dietary lysine fail to sufficiently meet lysine

requirement for the fist 10 d of age. Afterwards, recommended dietary lysine levels exceed requirements particularly towards the end of the growth curve.

(Key words: broilers, pelleting, lysine, effective caloric value)

INTRODUCTION

Intrinsic factors determine a broiler's overall capacity to synthesize and accumulate muscle (Lawrence and Fowler, 1997). However, whether or not the inherent upper limit is realized largely depends on the dietary supply of essential amino acids, as well as energy, as protein accretion is energetically costly (4 to 7 moles of ATP per peptide bond formed; Bequette, 2003). As maximum meat yield at optimal efficiency is a principle goal, nutritionist's routinely tweak nutrient to calorie ratios in an attempt to provide an ideally balanced ration.

In practical corn-soybean meal based broiler diets, methionine is considered first limiting followed by lysine, arginine, valine, and threonine (Han et al., 1992). However, lysine is the amino acid to which all others are proportionally related (i.e. ideal protein concept; Baker and Han, 1994; Baker, 1997). Additionally, lysine is generally expressed in ratio to energy, as dietary caloric density largely regulates voluntary feed intake (Leeson et al., 1996; McKinney and Teeter, 2004). Lysine is largely viewed as a pivotal nutrient because lysine has no major precursor role, and there has been extensive work to quantify digestible lysine need in broilers reared under a wide range of dietary and environmental circumstances (Han and Baker, 1993; Emmert and Baker, 1997).

In addition to pelleting, numerous other nonnutritive factors encountered in broiler production such as stocking density (Cravener et al., 1992; Puron et al., 1997), lighting

program (Buyse et al., 1996; Ingram et al., 2000), ventilation (Lott et al., 1998), and feed processing techniques (i.e., pelleting; Acar et al., 1991; Schiedeler, 1995; Moritz et al., 2001) are well documented to impact body weight (BW) and feed conversion ratio (FCR). If the paradigm is accepted that these responses are consequences of managerial-husbandry decisions that either "take away" or "add to" energy provided by the diet, then nonnutritive entities of broiler production need to be considered as variables directly influencing the ration formula.

Therefore, the following experiments were conducted to evaluate the efficacy of expressing lysine in ratio with calories added to the diet in two ways. 1) by increasing ME through substrate addition, or 2) through reduced activity energy expenditure by differing broiler management circumstances. Secondly, data will be utilized to develop a model that enables lysine requirement to be inter-related with body composition.

MATERIALS AND METHODS

General Information

In a related experiment that was directed at evaluating the ECV of pelleting under conditions mimicking those found commercially, male and female broilers (Cobb 500) were obtained from a commercial hatchery following sexing and vaccination for Marek's disease. The chicks were wing-banded and allotted by sex to floor pens (3.5 × 2.0 m) with used litter top-dressed with fresh wood shavings. The lighting program followed was 23L:1D and the stocking density was 45 birds per pen. Birds were reared with ad libitum access to feed and water on starter (0-18 d), grower (18-35 d), and finisher (35-60 d) diets (Table 1) formulated to meet or exceed nutrient recommendations of the Cobb

Broiler Nutrition Guide (2003). Treatments during the starter phase were: 1) unprocessed mash (M) and 2) M steam pelleted and crumbled (C). Treatments for the grower and finisher phases were: 1) M; 2) M plus soybean oil (187 kcals ME_n / kg diet; M187); and 3) M steam pelleted and sifted (P). Pens assigned to C in the starter phase were randomly re-assigned to either M187 or P in the grower and finisher phases. At the outset of this experiment, 25 wing-bands from each pen were randomly selected. Spray paint was applied to the wing bows of the birds with pre-selected wing-bands so that those birds could be easily identified for use in the experiments reported herein.

The aim of the experiments described herein was also to evaluate dietary ECV, namely the efficacy of expressing dietary nutrients in ratio to ECV. Experiments were of similar design and utilized male and female broilers (obtained from the aforementioned bird population) over age intervals ranging from 1 to 10 (Experiment 1; EXP1), 19 to 29 (Experiment 2; EXP2), and 45 to 55 (Experiment 3; EXP3) days. During the test periods, the birds were housed individually in floor pens (46 x 60 x 60 cm) equipped with a stainless steel feeder, a nipple drinker, and fresh wood-shavings. Feed and water were provided for ad libitum consumption and the same lighting program stated previously was followed. The general treatment structure for the three experiments was four dietary lysine levels and 2 (M and M187; Experiment1) or 3 (M, M187, and P; Experiments 2 and 3) ECV treatments in a factorial arrangement. Note, dietary ECV treatments formerly assigned were maintained in these experiments. Body weight gain (BWG), feed intake (FI), and whole-body protein (PD) and lipid (LD) deposition were quantified in each assay. Further, digestible lysine (LI) and metabolizable energy (MEI) intake, feed conversion ratio (FCR), apparent efficiency of energy retention (retained energy/energy

intake; kRE), and the efficiency of lysine for whole-body protein deposition (protein gain/lysine intake; $kLys_{PD}$) were calculated.

To obtain whole-body PD and LD, initial and final body composition was determined using dual-energy x-ray absorptiometry (DXA) as described by McKinney et al. (2005). In brief, birds were fasted (8 h), anesthetized (Skinner-Noble et al., 2005), and scanned 4 consecutive times in the prone position. Equations developed by McKinney et al. (2005) were used to adjust DXA measurements to match what would otherwise have been obtained by proximate analysis (AOAC, 1990). As a check of DXA results, the summation of the adjusted bird protein, water, lipid, and ash were compared with the gravimetric weight. Body weight calculated from adjust DXA measurements not within ± 5% of the respective gravimetric weight were excluded and the accepted scans for each bird were combined for analysis.

Diets

The preparation of the experimental diets involved several steps. First, for each experiment, a basal diet (Table 2) was formulated to 105% of recommended nutrient concentrations (Cobb Broiler Nutrition Guide, 2003), with the exception of lysine. Second, four premixes with graded levels of lysine were formulated to be iso-caloric, iso-nitrogenous and equal in Na^+ and Cl^- ions, utilizing L-lysine-HCL, NaCL, $NaHCO_3$, glutamic acid, corn starch, and Solka-Floc®. Four additional premixes were formulated in the same manner with the exception that the energy level was increased by adding soybean oil at the expense of Solka-Floc®. The experimental diets were then prepared by mixing proportions of the basal diet (95%) and the premixes (5%). In Experiments 2 and 3, half of the diets of the base energy level were steam conditioned and pelleted.

Experimental design

All dietary lysine levels examined were deficient relative to that recommended (Cobb Broiler Nutrition Guide, 2003). Not focusing replication around the zone where responses to lysine plateau allowed a wider range of lysine levels to be evaluated. In addition, this approach ensured response linearity, which enables the lysine requirement to be projected based on slope analysis. Experiment 1 had a 2 × 4 factorial arrangement of dietary treatments. Diets of four lysine levels (3.9, 5.2, 6.5, and 7.8 g/kg) were fed as: 1) M (3,053 kcal ME_n / kg diet) and 2) M187 (3240 kcal ME_n / kg diet). Experiments 2 and 3 had a 3 × 4 factorial arrangement of dietary treatments. Dietary lysine levels in Experiments 2 and 3 were: 3.5, 4.8, 6.1, and 7.4, and 3.0, 4.3, 5.6, and 6.9 g/kg, respectively. In Experiment 2, each of the lysine levels were fed as: 1) M (3,131 kcal ME_n / kg diet); 2) M187 (3,318 kcal ME_n / kg diet); and 3) P (3,131 kcal ME_n / kg diet). In Experiment 3, each of the lysine levels were fed as: 1) M (3,174 kcal ME_n / kg diet); 2) M187 (3,361 kcal ME_n / kg diet); and 3) P (3,174 kcal ME_n / kg diet).

Data Analysis

Bird served as the experimental unit and the experiments were analyzed as a completely randomized design. Data were analyzed using General Linear Models of SAS (2000), with probability values of $P < 0.05$ considered significant. When a significant F-statistic was detected, least square means were used for treatment comparisons. Orthogonal

polynomial contrasts were used to test for linear and curvilinear responses with respect to lysine level.

Modeling procedures were based on forward stepwise regression (Neter et al., 1990). Factors were added to the regression model until three conditions were met: 1) adding factors to the model did not result in a substantial (R^2 improvement < 2 %) increase in the model R^2; 2) all factors in the model were significant at $P < 0.10$; and 3) the resulting model matched known properties of the independent variables.

RESULTS AND DISCUSSION

No significant sex × lysine treatment interactions were detected in any of the experiments. This was expected as prior research with both swine (Susenbeth, 1995) and poultry (Han and Baker, 1993) have shown no sex differences when diets of sub-optimal lysine content were fed. Limited data is available as to differences between male and female responses to pelleting. However, it has been suggested (Nir and Hillel, 1995) that females are slightly less responsive to pellets. However, similar observations were obtained from two independent studies, one utilizing males (McKinney and Teeter, 2004), the other females (Skinner-Noble et al., 2005), as to the activity calories spared due to pelleting. In the present study no sex × ECV treatment interactions were detected indicating that males and females response similarly to pelleting. As no significant sex × dietary treatment interactions were detected sex effects were combined (Table 3, 4, and 5).

No significant ECV × lysine level interactions were detected for FI, LI, or MEI. Overall, there was a tendency for feed consumption to increase in a linear manner (P <

0.09) as dietary lysine level was increased. Observations that a bird's appetite increases to a point as dietary lysine is increased are well documented throughout the literature (Tesseraud et al. 1992; Edwards et al., 1999; Fatufe et al., 2004). Lysine fed in excess of need has the opposite effect. Dietary ECV did not influence feed consumption in EXP1 or EX2. However in EXP3, birds provided pellets consumed more ($P < 0.05$) feed, lysine, and energy compared to those fed M187, which appeared to have a depressed appetite. It is generally accepted that voluntary intake is largely controlled by energy consumption (Leeson et al. 1996; McKinney and Teeter, 2004). However, birds fed P consumed more energy overall compared to the M187 fed group. This would suggest that calories spared by reduced activity are not perceived in a manner that would regulate consumption.

No ECV × dietary lysine level interactions were detected for BWG, PD, or LD in EXP 2 and EXP 3. A significant interaction was observed in EXP1. Birds fed the lowest lysine level and M had significantly higher ($P < 0.05$) BWG, PD, and LD compared to birds fed the same lysine level and M187. Then as the dietary lysine levels increased, M187 fed birds surpassed those receiving M. It should be noted that birds on the low lysine M187 treatment did not consume feed well. Either birds on this treatment did not adapt to the experimental cages or as Jensen (1965) reported, the supplemental dietary energy resulted in an energy-lysine imbalance Unfortunately, birds of this age are unable to consume whole pellets so Jensen's (1965) hypothesis that pelleting exacerbates a deficiency could not be evaluated.

In EXP2 and EXP3, BWG, PD and LD increased (EXP2; linearly $P < 0.05$) as more dietary lysine was provided. Based on the work of Urdaneta-Rincon and Leeson (2004)

this is a result of increased tissue synthesis rather than a reduction in tissue degradation. Similarly, Tesseraud et al. (1992) demonstrated that both fractional synthesis and degradation of protein increases with lysine consumption, only fractional synthesis occurs at a higher level. In EXP3, P resulted in significantly more PD and LD compared to M187 and M birds. This may be partially attributed to the greater lysine consumption of birds fed P. However, when lysine consumption was included in the model as a covariate, a significant F-statistic for ECV was obtained. Means separation revealed the P still yielded higher PD. Additionally, though not statistically different, it should be noted that in EXP2, treatment P and M187 were virtually the same with respect to BWG, PD, and LD, and had similar lysine intakes.

In general FCR and kRE were improved and $kLys_{PD}$ reduced as dietary lysine was increased. Regarding the latter, several reports have suggested that the efficiency of lysine utilization declines with increased LI (Batterham et al., 1990; Gahl et al., 1991; Fatufe et al., 2004). In contrast, Möhn et al. (2000) reported that lysine utilization did not decline with increasing LI. It ultimately may depend on the manner in which lysine efficiency is quantified (Lys/Lys verses Lys/Prot). There is still discussion as to the composition of protein under conditions of sub-optimal lysine. For example, Skan and Noy (2004, 2005) contend that the lysine content of chicken tissue remains constant when dietary lysine is deficient. Conversely, Edwards et al. (1999) and Fatufe et al. (2004) contend that during lysine deficiency the lysine content of the tissue proportionally decreases. Nonetheless, lysine content of tissues was not evaluated in this study and $kLys_{PD}$ is expressed as lysine consumed per unit protein gain. This is an apparent

estimation and subject to error if the lysine content of the animal actually changes (i.e., increase collagen vs. breast tissue).

Indeed, the results of this experiment were somewhat variable. This may have resulted from 1) birds individually housed; 2) lack of appropriate replication; 3) birds in EXP2 not having an adaptation period to pellets; or 4) an inadequate experimental period. However, overall the data suggests that calories provided in the diet can be replaced by calories spared through reduced activity. Additionally, there was a general lack of interaction between energy (provided in either form) and lysine. Moreover, results give support to the importance of considering broiler management in establishing nutrient to energy ratios.

An objective of this study was to develop a mathematical model that inter-relates broiler lysine requirement with the body composition for the entire growth curve. As P was not included as a treatment in EXP1, data for the entire growth curve was unavailable. Therefore, P was excluded from the data set for modeling purposes.

Numerous variables expressed both as measured and per unit metabolic body size ($BW^{0.67}$) evaluated in the development of this model. The parameters which yielded the best model for predicting daily total (TL, g) and digestible lysine (DL, g) consumption were 1) dietary :ME_n:Lysine ratio (EL; g/kcal); 2) mean PD (g) and 3) daily PD (g). The resulting equations were:

(Eq. 1) TL = 0.46335 – (0.00007321 × EL) + (0.00121× mean PD) + (0.04227× daily PD); (R^2 = 91.6%)

(Eq. 2) DL = 0.41308 – (0.00005846 × EL) + (0.00110 × mean PD) + (0.03878× daily PD);(R^2 = 91.0%)

To evaluate the estimated daily total and digestible lysine requirement, as it compares to current recommendations (Cobb Vantress, 2003), data provided from the field (Wiernusz, 2005) were applied to the equations.

Results from these equations are illustrated in Figure 1. Based on these estimations, current recommendations do not sufficiently supply the broiler with the required amount of lysine for the first 10 days of age. After that, lysine is fed in excess particularly towards the end of the growth curve. Indeed, nutritionist's build buffers into the ration formula to protect against, for example, feed mixing mistakes, but is possible with these equations to quantify that allowance.

Potentially, information obtained from these equations could be used to modify the existing ideal protein model. Furthermore, it may be possible to integrate these equations into a more mechanistic approach whereby producers could specify, for example, desired bird composition, total days available for production, and rearing conditions, and these models would provide dietary provisions necessary to achieve that target.

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Table1. Composition of diets used to rear broilers to the ages evaluated in Experiments 1, 2, and 3

	Age interval, d and Treatments[1,2]				
	0 to 18	18 to 35		35 to 60	
Ingredient, %	M and P	M and P	M187	M and P	M187
Corn	58.12	64.89	60.73	68.72	64.31
Soybean meal (48% CP)	32.66	24.59	25.61	22.76	23.63
Soybean oil	2.90	2.93	6.4	3.32	6.89
Poultry by-product meal	1.50	3.00	3.00	0.50	0.50
Monocalcium phosphate	1.36	1.05	0.94	1.20	1.21
Limestone	1.32	1.04	0.94	1.08	1.07
NaCl	0.34	0.29	0.32	0.34	0.35
$NaHCO_3$	0.25	0.32	0.27	0.30	0.28
Vitamin premix[3]	0.28	0.24	0.24	0.25	0.25
Trace mineral premix[4]	0.09	0.09	0.09	0.09	0.09
Selenium premix	0.04	0.04	0.04	0.04	0.04
$CuSO_4$	0.002	0.002	0.002	0.002	0.002
Choline chloride	0.001	–	–	–	–
DL-Methionine	0.22	0.22	0.19	0.12	0.12
Lysine	0.076	0.157	0.10	0.10	0.08
Arginine	0.03	0.05	0.03	0.05	0.03
Threonine	0.03	0.05	0.02	0.05	0.04
AmeriBond 2x	0.75	1.00	1.00	1.00	1.00
Coccidiostat	0.05	0.05	0.05	0.08	0.08
Ethoxyquin	0.012	0.012	0.012	0.012	0.012
Calculated Analysis					
ME_n (kcal / kg)	3,053	3,131	3,318	3,174	3,361
CP, %	22.1	19.8	19.8	17.5	17.5
Arg	1.49	1.30	1.30	1.16	1.16
Lys	1.26	1.14	1.14	0.96	0.96
Met	0.55	0.52	0.52	0.42	0.42
TSAA	0.94	0.88	0.88	0.76	0.76
Ca	0.90	0.80	0.80	0.72	0.72
P, available	0.44	0.40	0.40	0.37	0.37

[1]Treatments: M = mash; M187 = M plus soybean oil (187 kcal ME_n/kg); P = M steam pelleted and sifted.

[2]From 0 to 18 days, P was crumbled.

[3]Supplied per kilogram of diet: vitamin A, 10,141 IU (retinyl acetate); cholecalciferol, 3,086 IU; vitamin E, 23.92 IU (dl-α-tocopheryl acetate); menadione, 2.87 mg; thiamine, 2.20 mg; riboflavin, 7.72 mg; niacin, 60.30 mg; d-pantothenic acid, 12.46 mg; pyridoxine, 3.75 mg; vitamin B_{12}, 0.017 mg; folic acid, 1.066 mg; d-biotin, 0.127 mg.

[4]Supplied per kilogram of diet: Ca,160 mg; Zn, 100 mg; Mn, 120 mg; Fe,75 mg; Cu, 10 mg; I, 2.5 mg.

Table 2. Basal diets used in Experiments 1, 2, and 3

	Experiment		
Ingredient	1	2	3

Corn	62.0	70.2	76.2
Corn gluten meal	15.0	13.9	11.4
Wheat bran	5.6	2.1	–
Soybean oil	3.2	3.0	3.0
Poultry by-product meal	1.58	1.28	1.22
Soybean meal	1.58	1.26	0.53
Mono-calcium phosphate	1.56	1.46	1.34
Limestone	1.54	1.39	1.22
Corn Starch	1.26	–	–
Potassium sulfate	1.26	0.87	0.95
Arginine	0.99	0.81	0.71
Pellet binder	0.79	1.05	1.05
$NaHCO_3$	0.72	0.62	0.54
Vitamin premix[1]	0.68	0.27	0.28
Isoleucine	0.33	0.23	0.18
Serine	0.33	–	–
Threonine	0.29	0.23	0.23
Glycine	0.29	0.13	0.29
Valine	0.28	0.18	0.18
Histidine	0.22	0.15	0.07
Methionine	0.21	0.18	0.09
Tryptophan	0.13	0.11	0.09
Trace mineral premix[2]	0.095	0.095	0.100
Coccidiostat	0.053	0.079	0.079
Selenium premix	0.011	0.021	0.028
Ethoxyquin	0.013	0.013	0.013
$CuSO_4$	0.002	0.002	0.002
Choline chloride	–	0.116	0.026
NaCl	–	0.105	0.175
Calculated Analysis			
ME_n (kcal / kg)	3,214	3,296	3,341
CP, %	22.01	19.93	17.68
Arg[3]	1.44	1.25	1.11
Lys[3]	0.39	0.35	0.30
Met[3]	0.55	0.50	0.41
TSAA[3]	0.84	0.78	0.69
Ca	0.95	0.84	0.76
P, available	0.46	0.42	0.39

[1]Supplied per kilogram of diet: vitamin A, 10,141 IU (retinyl acetate); cholecalciferol, 3,086 IU; vitamin E, 23.92 IU (dl-α-tocopheryl acetate); menadione, 2.87 mg; thiamine, 2.20 mg; riboflavin, 7.72 mg; niacin, 60.30 mg; d-pantothenic acid, 12.46 mg; pyridoxine, 3.75 mg; vitamin B_{12}, 0.017 mg; folic acid, 1.066 mg; d-biotin, 0.127 mg.
[2]Supplied per kilogram of diet: Ca,160 mg; Zn, 100 mg; Mn, 120 mg; Fe,75 mg; Cu, 10 mg; I, 2.5 mg. [3]True digestible basis according to the listing of Ajinomoto Heartland, Incorporated (2001).

Table 3. Broiler growth performance and whole body protein and lipid deposition and retention efficiencies as influenced by dietary treatment,

Experiment 1

Dietary treatment[1]		Intake			Deposition[3]			Efficiency		
ECV	Lys[2], %	Diet	Lys[2]	ME_n	BWG	Protein	Lipid	FCR[4]	$kLys_{PD}$[5]	kER[6]
Interactive effect means		(g)		(kcal)	(g)			(g/g)		(%)
M	0.39	146	0.57	446	50^{c}	4.7^{c}	0.1^{d}	3.00	9.7	7.1
M	0.52	144	0.75	439	57^{c}	6.0^{c}	0.7^{cd}	2.60	12.7	14.9
M	0.65	162	1.05	494	83^{b}	9.7^{bc}	2.7^{bc}	1.88	9.7	15.8
M	0.78	187	1.46	571	88^{b}	10.3^{b}	3.3^{b}	2.17	7.4	15.9
M187	0.39	86	0.33	278	36^{d}	1.5^{d}	-1.8^{e}	2.42	4.4	-4.4
M187	0.52	155	0.81	503	61^{c}	5.8^{c}	0.7^{cd}	2.58	8.2	8.5
M187	0.65	211	1.45	685	95^{b}	12.4^{b}	4.4^{b}	2.32	8.2	13.6
M187	0.78	270	2.11	875	127^{a}	18.4^{a}	7.9^{a}	2.15	9.0	20.4
Main effect means										
M		160	0.95	487	70	7.7	1.7	2.41	9.9	13.4
M187		181	1.17	585	80	9.5	2.8	2.37	7.4	9.5
	0.39	116^{c}	0.45^{d}	362^{b}	43^{d}	3.1^{c}	-0.8^{d}	2.71	7.1	1.4^{b}
	0.52	150^{bc}	0.78^{c}	471^{b}	59^{c}	5.9^{b}	0.7^{c}	2.59	10.4	11.7^{a}
	0.65	186^{abc}	1.24^{b}	589^{ab}	89^{c}	11.0^{a}	3.6^{b}	2.10	8.9	14.7^{a}
	0.78	229^{a}	1.78^{a}	723^{a}	108^{a}	14.3^{a}	5.6^{a}	2.16	8.2	18.2^{a}
Source of variation						Probability				
ECV		0.9702	0.4464	0.6470	0.3624	0.5660	0.5766	0.6577	0.2092	0.1273
Lys		0.0074	< 0.0001	0.0054	< 0.0001	< 0.0001	< 0.0001	0.6762	0.7577	0.0127
Linear		0.0809	0.0009	0.0701	< 0.0001	< 0.0001	< 0.0001	0.4186	0.6832	0.0512
Quadratic		0.9534	0.6284	0.9599	0.2036	0.3029	0.3040	0.7327	0.4593	0.4328
ECV × Lys		0.1373	0.0708	0.1057	0.0027	0.0018	0.002	0.8854	0.7913	0.4929
Pooled SEM		10.87	0.55	33.60	2.12	0.43	0.25	0.21	1.28	1.82

[a- e]Means within a column with different superscripts differ ($P < 0.05$).

[1]M = unprocessed mash; M187 = M plus soybean oil (187 kcal ME_n/kg diet).

[2]Expressed as true digestible lysine based on the listing of Ajinomoto Heartland, Incorporated (2001).

[3]Initial body composition determined by whole bird chemical analysis; final body compositions were based on dual energy x-ray absorptiometry measurements adjusted as described by Mckinney et al. (2005).

[4]Feed conversion ratio (FCR) = feed consumption / body weight gain.

[5]Efficiency of dietary lysine for protein deposition ($kLys_{PD}$) = protein deposition/lysine consumption.

[6]Efficiency of energy retention (kER) = ((protein deposition × 5.65 + lipid deposition × 9.31)/Energy (ME_n basis) consumption) × 100.

Table 4. Broiler growth performance and whole body protein and lipid deposition and retention efficiencies as influenced by dietary treatment, Experiment 2

Dietary treatment[1]	Intake	Deposition[3]	Retention

ECV	Lys[2], %	Diet	Lys[2]	ME_n	BWG	Protein	Lipid	FCR[4]	$kLys_{PD}$[5]	kER[6]
Interactive effect means		(g)		(kcal)		(g)		(g/g)		(%)
M	0.35	490	1.71	1,533	135	22	16	3.64	12.9	17.7
M	0.48	668	3.21	2,091	244	41	31	2.84	12.7	24.7
M	0.61	718	4.38	2,248	318	56	43	2.30	12.8	31.8
M	0.74	819	6.06	2,565	376	65	51	2.21	10.6	32.4
M187	0.35	648	2.27	2,149	209	34	26	3.23	14.4	19.3
M187	0.48	574	2.75	1,904	229	38	29	2.53	14.6	26.8
M187	0.61	735	4.48	2,439	342	59	47	2.17	13.0	31.1
M187	0.74	815	6.03	2,704	396	70	57	2.07	11.5	34.1
P	0.35	700	2.45	2,191	273	46	36	2.83	18.7	27.2
P	0.48	594	2.85	1,860	226	38	30	2.65	13.4	26.2
P	0.61	712	4.34	2,228	285	49	38	2.53	11.4	28.2
P	0.74	863	6.13	2,703	398	68	54	2.20	10.9	32.0
Main effect means										
M		674	3.84	2,109	268	46	35	2.75	12.2	26.7
M187		693	3.88	2,299	294	50	40	2.50	13.4	27.8
P		717	3.94	2,246	295	50	39	2.55	13.6	28.4
	0.35	612[c]	2.14[d]	1,958[c]	205[c]	34[c]	26[c]	3.23[a]	15.3[a]	21.4[c]
	0.48	612[c]	2.94[c]	1,952[c]	233[c]	39[c]	30[c]	2.67[b]	13.6[ab]	25.9[b]
	0.61	721[b]	4.40[b]	2,305[b]	315[b]	55[b]	42[b]	2.33[c]	12.4[bc]	30.4[a]
	0.74	832[a]	6.07[a]	2,657[a]	390[a]	68[a]	54[a]	2.16[c]	11.0[c]	32.8[a]
Source of variation						Probability				
ECV		0.8513	0.9775	0.7756	0.8037	0.8685	0.767	0.3575	0.5164	0.8149
Lys		0.0006	< 0.0001	0.0006	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0005	< 0.0001
Linear		0.0856	< 0.0001	0.0875	0.0017	0.001	0.0029	< 0.0001	0.0182	0.0002
Quadratic		0.2670	0.2748	0.2577	0.2988	0.2787	0.3096	0.4576	0.7612	0.9861
ECV × Lys		0.655	0.9431	0.6659	0.4284	0.4726	0.4835	0.3927	0.1839	0.2115
Pooled SEM		19.29	0.12	61.72	10.24	1.87	1.64	0.06	0.37	0.69

[a- d]Means within a column with different superscripts differ ($P < 0.05$).
[1]M = unprocessed mash; M187 = M plus soybean oil (187 kcal ME_n/kg diet); P = M steam pelleted and sifted.
[2]Expressed as true digestible lysine based on the listing of Ajinomoto Heartland, Incorporated (2001).
[3]Based on dual energy x-ray absorptiometry measurements adjusted as described by Mckinney et al. (2005).
[4]Feed conversion ratio (FCR) = feed consumption / body weight gain.
[5]Efficiency of dietary lysine for protein deposition ($kLys_{PD}$) = protein deposition/lysine consumption.
[6]Efficiency of energy retention (kER) = ((protein deposition × 5.65 + lipid deposition × 9.31)/Energy (ME_n basis) consumption) × 100.

Table 5. Broiler growth performance and whole body protein and lipid deposition and retention efficiencies as influenced by dietary treatment, Experiment 3

Dietary treatment[1]		Intake			Deposition[3]			Retention		
ECV	Lys[2], %	Diet	Lys[2]	ME_n	BWG	Protein	Lipid	FCR[4]	$kLys_{PD}$[5]	kER[6]
Interactive effect means		(g)		(kcal)		(g)		(g/g)		(%)

M	0.30	1,412	4.24	4,482	245	40.5	50.5	5.86	9.6^{bc}	15.6
M	0.43	1,515	6.51	4,809	306	55.5	75.7	5.21	8.8^{bc}	21.8
M	0.56	1,520	8.51	4,824	351	60.3	80.0	4.54	7.1^{bc}	22.5
M	0.69	1,663	11.48	5,279	395	59.5	81.0	4.51	5.1^{c}	20.2
M187	0.30	1,233	3.70	4,144	222	39.6	52.0	5.55	10.7^{bc}	17.1
M187	0.43	1,137	4.89	3,820	321	49.2	69.0	4.13	10.3^{bc}	24.5
M187	0.56	1,445	8.09	4,857	353	58.6	76.3	4.69	7.0^{bc}	20.6
M187	0.69	1,505	10.39	5,059	524	79.0	111.0	2.96	7.8^{bc}	29.7
P	0.30	1,079	3.24	3,426	322	69.1	91.4	3.34	22.9^{a}	39.1
P	0.43	1,557	6.69	4,940	423	74.4	102.7	3.75	11.0^{b}	27.6
P	0.56	2,230	12.49	7,078	570	102.9	142.7	4.09	8.2^{bc}	26.7
P	0.69	2,086	14.40	6,622	680	104.6	161.9	3.38	7.1^{bc}	30.8
Main effect means										
M		1,528^{b}	7.68^{ab}	4,849^{ab}	324^{b}	54.0^{b}	71.8^{b}	5.03^{a}	7.6^{b}	20.0^{b}
M187		1,330^{b}	6.77^{b}	4,470^{b}	355^{ab}	56.6^{b}	77.1^{b}	4.34^{ab}	8.9^{b}	23.0^{b}
P		1,738^{a}	9.20^{a}	5,517^{a}	499^{a}	87.8^{a}	124.7^{a}	3.64^{b}	12.3^{a}	31.0^{a}
	0.30	1,241^{c}	3.72^{d}	4,017^{c}	263^{b}	49.7^{b}	64.7^{b}	4.92	14.4^{a}	23.9
	0.43	1,403^{bc}	6.03^{c}	4,523^{bc}	350^{b}	59.7^{ab}	82.5^{ab}	4.37	10.0^{b}	24.6
	0.56	1,732^{ab}	9.70^{b}	5,587^{ab}	425^{ab}	73.9^{ab}	99.6^{ab}	4.44	7.4^{bc}	23.3
	0.69	1,752^{a}	12.09^{a}	5,653^{a}	533^{a}	81.0^{a}	118.0^{a}	3.62	6.7^{c}	26.9
Source of variation						Probability				
ECV		0.0072	0.0043	0.0295	0.0226	0.0046	0.0029	0.0358	0.0036	0.0076
Lys		0.0027	< 0.0001	0.0027	0.0071	0.0691	0.0320	0.2847	< 0.0001	0.8227
Linear		0.0111	< 0.0001	0.0112	0.0868	0.0992	0.1228	0.4948	0.0002	0.8793
Quadratic		0.5779	0.4541	0.5636	0.9370	0.8593	0.9862	0.5843	0.5276	0.7763
ECV × Lys		0.1938	0.3146	0.2039	0.9201	0.9398	0.9004	0.5828	0.0094	0.2783
Pooled SEM		52.07	0.31	166.73	26.34	4.10	6.36	0.20	0.48	1.28

[a- d]Means within a column with different superscripts differ ($P < 0.05$).

[1]M = unprocessed mash; M187 = M plus soybean oil (187 kcal ME_n/kg diet); P = M steam pelleted and sifted.

[2]Expressed as true digestible lysine based on the listing of Ajinomoto Heartland, Incorporated (2001).

[3]Based on dual energy x-ray absorptiometry measurements adjusted as described by Mckinney et al. (2005).

[4]Feed conversion ratio (FCR) = feed consumption / body weight gain.

[5]Efficiency of dietary lysine for protein deposition ($kLys_{PD}$) = protein deposition/lysine consumption.

[6]Efficiency of energy retention (kER) = ((protein deposition × 5.65 + lipid deposition × 9.31)/Energy (ME_n basis) consumption) × 100.

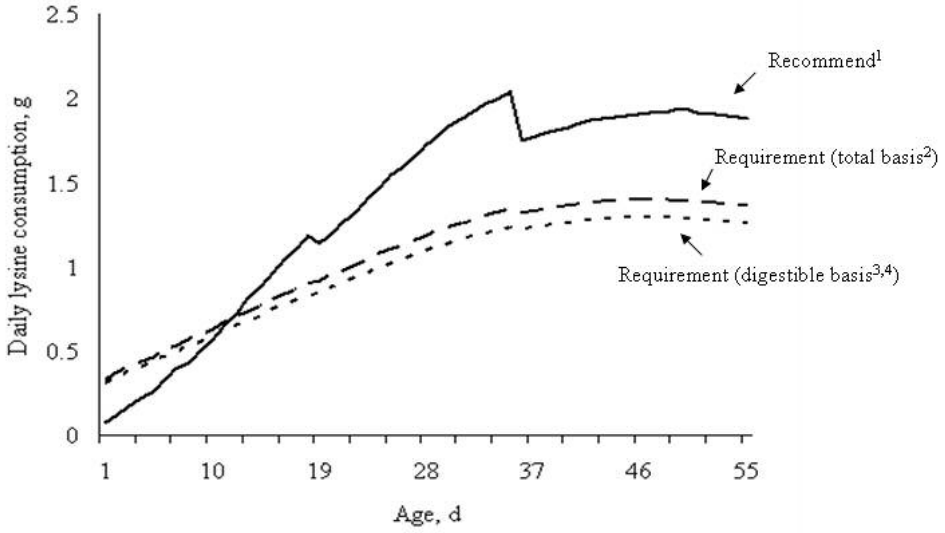

Figure 1. Recommended and predicted total and digestible daily lysine requirement of male and female broilers

[1]Cobb Broiler Nutrition Guide (2003).

[2]Total daily lysine requirement = $0.46335 - 7.321e^{-5} \times$ ME (kcal kg^{-1}) lysine (g) ratio + $1.21e^{-3} \times$ mean daily whole body protein + $4.227e^{-2} \times$ daily whole body gain.

[3]Digestible daily lysine requirement = $0.41308 - 5.846e^{-5} \times$ ME (kcal kg^{-1}) lysine (g) ratio + $1.1e^{-3} \times$ mean daily whole body protein + $3.387e^{-2} \times$ daily whole body gain.

[4]Digestibility coefficients reported by Ajinomoto Heartland, Incorporated (2001).

CHAPTER VI

Modeling Broiler Energetics at the Cellular Level

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Section Preference: Education and Production
Research Note: _____ Full-Length Paper: ____X__
RUNNING TITLE: Amino Acids vs ATP yield
Abbreviation Key: EEA = Essential amino Acids, NEAA = non essential amino acids, CNB = carbon and nitrogen balance

Abstract: Data originating from a commercial broiler growth curve and were coupled with published chick metabolic data in order that the effects of varying dietary protein on chick energy metabolism might be model through 2.5 Kg live weight. The metabolic data included various estimations of fractional protein synthesis (FSR) and fractional degradation rates (FDR). Both FSR and FDR declined curvilinearly with increasing bird age. Fractional protein synthesis declined from 35 % for the young chick to just 15 % for the 2.5 Kg - 42 day old broiler. The FDR declined from 28 % for the young chick to 10 % for the 2.5 KG bird. Coupling these values with the bird protein accretion model developed in this laboratory yielded overall estimates of synthesis and degradation as grams per day. For the 2.5 Kg bird total synthesis averaged 645.6, degradation averaged 190.1 yielding an accretion 455 grams. Previous work (chapter 3 of this thesis) established that dietary protein reduction of 3% was without consequence (P>0.10) for feed intake, live weight gain, and protein and lipid accretion. Application of these diets to lower dietary protein by 0, 1.5 and 3.0% subsequently lowered crude protein consumption from 758.6 g to 691.6 and 643.9, respectively. Simultaneously; overall AA catabolism declined from 272.5 to 205.5 and 157.7 grams thereby reducing the catabolizable amino acid energy from 1384.2 to 1070.5 and 862.8 Kcal. Catabolizable energy per gram of amino acid catabolized, however; increased from 5.08, for the 0% crude protein reduction, to 5.21 and 5.47 Kcal per gram for the two protein reductions, while ATP synthesis per gram catabolizable AA rose from 0.212 moles/g AA for the 0% protein reduction to 0.221 and 0.232 for the 1.5 and 3.0% reduction. As a result heat production (assuming glucose is substituted weight per weight for catabolizable AA)

declined linearly from 640 Kcal (NRC treatment) to 513 Kcal for the 3% crude protein reduction. In conclusion bird energy need for FSR is ~ 26.3% of MEn consumption. While varying dietary CP has the potential to alter HP by 127 Kcal, this only amounts to 0.90% of consumption. More fruitful advances in overall metabolic efficiency for broiler energy need are found in other avenues of energy expenditure such as activity, thermoregulation and hygiene management.

INTRODUCTION

Maximization of broiler production efficiency is one of the primary objectives of any of the poultry industry. Feed costs approximately 60 – 70 % of the poultry production industry making efficiency of substrate utilization critical. Substrates are oxidized for maintenance and accretion. Maintenance may be defined as energy for all non accretion functions such as thermoregulation, general homeostasis and activity to support various needs such as feed and water acquisition. If less substrate catabolism occurs than anabolism then growth is achieved.

Consumed substrates can be categorized as indispensable (cannot be synthesized by the bird) versus dispensable substrates that can be synthesized on an as-needed basis. Indispensable amino acids are thereby defined as those that cannot be synthesized by the animal, are needed metabolically and therefore must be supplied in the diet. These amounts are not the same for all production phases (Morris et al., 1999).

Amino acid requirements are typically set by feeding graded levels of amino acids to the bird and monitor the result. Several factors change simultaneously like feed intake (Denbow, 1989; Kuenzel, 1994), tissue accretion and accretion composition (Kang et al., 1985; Klasing et al., 1987). Changing a single AA has been used to determine AA

requirements. For example, requirements are typically estimated by increasing dietary concentrations of an AA, coupled with the monitoring of the growth plateau. These effects may however be influenced by the total dietary protein (Morris et al., 1999; Sklan and Noy, 2003) suggesting complex relationships between AA's.

Growth consists only in part, of protein synthesis, whereas efficiency of utilization is dependent not only upon the protein synthesis but also the amount of AA being catabolized. Previous studies have estimated protein synthesis and catabolism using flooding doses of a labeled AA (Kang et al., 1985; Klasing et al., 1987). As such the fractional synthesis rate (FSR) and fractional degradation rate (FDR) may be determined. The effect of varying dietary AA combinations on FI, growth and accretion may be estimated from carcass accretion. The impact of dietary AA combinations on the quantity of AA available for catabolism may be calculated as the difference between intake and carcass deposition. However, this approach would not account for overall protein turnover which would elevate synthesis making the isotope approach necessary.

Bioenergetics: Broiler maintenance energy averages 32% of the MEn consumption through 3Kg body weight (Beker, personal communication). Protein turnover is considered to be an important component of maintenance but the amount is not readily determined. Indeed protein turnover of birds fed at maintenance would likely be exacerbated due to reduced feed intake. Therefore, such estimates must come from isotope studies with FSR and FDR estimates attained with full-fed birds. Such data, when applied to the birds growth and composition curve, enables placing quantitative value on energy need associated with protein metabolism. For such measures bird mass must be viewed in moles instead of crude protein or mass in Kg as ATP requirements are

determined by molar and not weight relationships.

A mole of glucose would yield 36 ATP's, consequently, this represents 39 % of efficiency (Harpar,1939). Other energy is lost as heat. Metabolic efficiency for glucose is based upon a mole of ATP containing 7.3 KCals, thereby the 36 ATP's would account for only 270 Kcals of the 686 KCals present in Glucose. Energy expenditure is due to biochemical and biophysical processes requiring ATP (Summers and Leeson, 1985). Schulz (1978) has modeled catabolism to represent the yields of ATP for each AA, concluding that excess AA is converted into ATP or glucose depending upon animal need.

Any evaluation of energy metabolism must be based upon the nutrient composition and subsequent metabolic path. The identification of nutrients and cofactors involved in intermediary metabolism are described in Table 1 for Gross Energy, Carbon, Hydrogen, Oxygen, Nitrogen, Sulfur contents of the AA found in animal tissue. Calculation of ATP yield has been done through Stoicheometry equations for the fate of these amino acids according to the equations taken from Schulz (1978; Table 2).

Amino Acid Catabolism: Muscle protein turnover could be considered as an important component of protein metabolism during growth (Garlick et al., 1976). The rate of growth of muscle tissue accretion is a function of the relative rates of protein synthesis degradation and accretion. The chick is a good model because it grows at a rapid rate (MacDonald and Swick., 1981). A contrasting of diet and chick amino acid contents found in Table 3, 4 and 5 for standard feeding periods. Note, diet composition changes with time to reflect chick AA needs and economic limitations. Understanding of the Fractional Synthesis and Fractional Degradation Rates, when coupled with growth

composition, provide an insight into the phenomena of growth and maturation. In many studies (Maruyama et al., 1978) it was reported that protein turnover was correlated with slightly higher rates of protein synthesis. MacDonald and Swick, (1981) showed a

Table 1: Composition of indispensable and dispensable amino acids[1]

	Mwt[1]	C	H	O	N	S	Delta Gc
Indispensable AA							
Histidine	155	6	6	2	3		846.5
Isoleucine	131	6	13	2	1		855.8
Leucine	131	6	13	2	1		856
Lysine	146	6	14	2	2		771.6
Methionine	149	5	11	2	1	1	664.8
Phenylalanine	165	5	12	2	2		1110.6
Threonine	119	4	9	3	1		490.7
Tryptophan	204	11	12	2	2		1345.2
Tyrosine	181	9	11	3	1		1061.7
Valine	117	5	11	2	1		698.3
Dispensable AA							
Alanine	89	3	7	2	1		387.1
Arginine	174	6	14	2	4		893.5
Aspartic Acid	116	4	6	3	1		382.6
Glutamic Acid	130	5	8	3	1		536.4
Glycine	75	2	5	2	1		230.5
Cysteine	121	6	11	3	2	2	394.6
Proline	115	5	9	2	1		513.5
Serine	105	3	7	3	1		347.7

[1]Mwt = Molecular weight
[2]C= Carbon, H = Hydrogen, N=Nitrogen, S=Sulfur, O=Oxygen
[3]ΔGc estimates the energy release by complete combustion extracted from
Data collected from Schulz (1978) and Handbook of Biochemistry selected data for Molecular Biology

reduction in fractional synthesis rate of chick breast muscle from 1 to 2 week of age. However, this area warrants further research. Maruyama et al (1978) estimated the whole protein turnover rate in chickens to be 64g/Kg body weight per day. The purpose of this writing is to model energy metabolism using commercial growth date and published metabolism information.

Material and Methods: Data presented in chapter 3 of this dissertation and tables here in were applied to a commercial growth curve for the cobb bird (Table 7) and feed

consumption tables such that the amino acid consumption for birds fed NRC, NRC-1.5 and NRC – 3% dietary crude protein with indispensable amino acid fortified to maintain amino acid need might be computed. Crude protein effect on amino acid combustion to gross energy (ΔGc) and ATP yield were obtained from values presented in Table 2. Estimates for FSR, FDR and protein turnover (TO) are presented in Table 8. Total protein synthesis and degradation was computed as the product of the fractional values with bird protein whole body content. Moles of chick AA contained in the chick whole body protein were determined as the summation of (whole body protein x chick AA composition) / AA molecular weights for 20 amino acids. Energy as ATP need for protein accretion was estimated as moles AA accrued x 8 ATP per mole. Total ATP per mole chick protein accrued was estimate as described in table 9.

Statistical Analysis: Values presented represent discrete estimation and generally accepted composition data. As a result the combustion of terms has no error estimation other than that associated with FSR, FDR, bird protein content and portioning of whole body protein into AA. Though these each have error it is not possible to estimate error associated with the model presented. Summation of protein synthesis was accomplished by regressing daily values on bird age to generate a 3^{rd} power polynomial and integrate there of to probably a total value for the 41 day old bird with a mean live weight of 2.554 Kg.

Table 2: Amino Acid catabolism to CO_2 water and uric acid with accompanying moles of ATP produced[1]

	Moles O_2 used	Moles CO_2 Produced	Moles H_2O Produced	Moles Uric Acid Produced	Moles ATP Produced	Moles H_2O Produced
Indispensable AA						
Histidine	5	4.5	1.5	1.5	22.5	1.5
Isoleucine	7.5	5.5	5.5	0.5	40.5	5.5
Leucine	7.5	5.5	5.5	0.5	39.5	5.5
Lysine	7	5	5	0.5	36	5
Methionine	7	4.5	1 $(SO_4)^{2-}$	0.5	21.5	1 $(SO_4)^{2-}$
Phenylalanine	10	8.5	4.5	0.5	37.5	4.5
Threonine	4	3.5	3.5	0.5	20.5	3.5
Tryptophan	11.5	10	4	1	42	4
Tyrosine	9.5	8.5	4.5	0.5	41.5	4.5
Valine	6	4.5	4.5	0.5	31.5	4.5
Dispensable AA						
Alanine	3	2.5	2.5	0.5	15.5	2.5
Arginine	5.5	4	3	2	28	3
Aspartic Acid	3	3.5	2.5	0.5	15.5	2.5
Glutamine	4.5	4	3	1	22	3
Glycine	1.5	1.5	1.5	0.5	6.5	1.5
Cysteine	4	2.5	1 $(SO_4)^{2-}$	0.5	12.5	1 $(SO_4)^{2-}$
Proline	5.5	4.5	3.5	0.5	29.5	3.5
Serine	2.5	2.5	2.5	0.5	12.5	2.5
Glutamic Acid	4.5	5.5	3.5	0.5	24.5	3.5

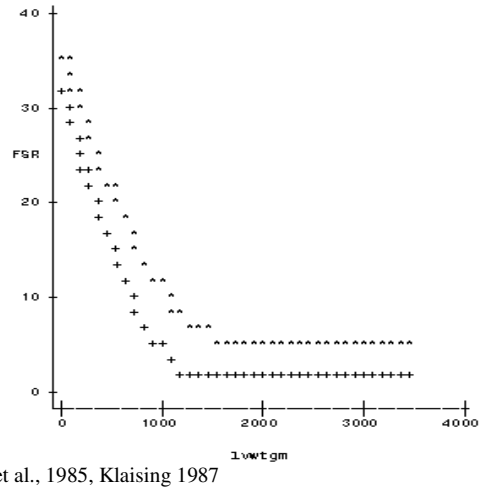
[1] Data extracted from Schulz(1978)

Table 3. Amino Acid Content of Chick[1]

Amino Acid	Chick Amino Acid
Indispensable AA	
Histidine	2.91
Isoleucine	4.07
Leucine	7.59
Lysine	7.5
Methionine	1.76
Phenylalanine	4.05
Threonine	4.5
Tryptophan	0.77
Tyrosine	3.03
Valine	5.33
Dispensable AA	
Alanine	6.81
Arginine	7.02
Aspartic Acid	9.3
Glutamic Acid	14.7
Glycine	8.5
Cysteine	1.73
Proline	6.78
Serine	4.53

[1] =Data extracted from Harold et al., 1953, Sklan and Noy., 2005 data also modified using regression equations and personal communication

Figure 1. Plot of FSR and FDR values collected from literature[1]



[1] = Klang et al., 1985, Klaising 1987

Table4. Commercial weight guide for males, females and As-Hatched chick live weight, daily gain and as hatched feed conversion rates

Age	Male weight (g)	Daily live weight gain	Female weight (g)	Daily live weight gain	As-Hatched weight (g)	Daily live weight gain	Daily feed per Bird(as-hat)	Cum Cons Per Bird(as-hat)	F.C.R As-hatched
0	42	0	42	0	42	0			
1	49	7	48	6	48	6			
2	61	12	58	10	59	11			
3	76	15	73	15	75	15			
4	97	21	91	18	94	19			
5	121	24	113	22	117	23			
6	149	28	139	26	144	27			
7	182	33	169	30	175	31	150.0	150	0.857
8	218	36	202	33	210	35	34.1	184	0.877
9	258	40	238	36	248	38	39.7	224	0.903
10	301	43	278	40	289	41	45.5	269	0.931
11	348	47	321	43	334	45	51.5	321	0.960
12	398	50	366	45	382	48	57.6	378	0.991
13	451	53	414	48	433	51	63.7	442	1.022
14	508	57	465	51	486	54	69.9	512	1.052
15	567	59	519	54	543	56	75.9	588	1.083
16	629	62	575	56	602	59	81.9	670	1.113
17	694	65	633	58	663	62	87.7	757	1.142
18	761	67	693	60	727	64	93.7	851	1.170
19	831	70	756	63	793	66	99.6	951	1.198
20	904	73	820	64	862	68	105.3	1056	1.226
21	978	74	885	65	932	70	110.8	1167	1.252
22	1055	77	953	68	1004	72	116.6	1283	1.279
23	1134	79	1021	68	1077	74	122.5	1406	1.305
24	1214	80	1092	71	1153	75	128.5	1534	1.331
25	1296	82	1163	71	1230	77	134.4	1669	1.357
26	1380	84	1235	72	1308	78	140.0	1809	1.383
27	1466	86	1308	73	1387	79	145.6	1954	1.409
28	1553	87	1382	74	1467	80	150.8	2105	1.435
29	1641	88	1457	75	1549	81	155.8	2261	1.460
30	1730	89	1532	75	1631	82	160.7	2422	1.485
31	1820	90	1607	75	1714	83	165.2	2587	1.510
32	1911	91	1682	75	1797	83	169.1	2756	1.534
33	2003	92	1758	76	1881	84	172.5	2929	1.557
34	2096	93	1834	76	1965	84	175.8	3104	1.580
35	2189	93	1909	75	2049	84	179.0	3283	1.602
36	2283	94	1984	75	2134	84	181.7	3465	1.624
37	2377	94	2059	75	2218	84	184.3	3649	1.645

38	2471	94	2133	74	2302	84	186.8	3836	1.666
39	2566	95	2206	73	2386	84	189.1	4025	1.687
40	2660	94	2278	72	2469	83	191.1	4216	1.708
41	2754	94	2350	72	2552	83	193.0	4409	1.728

1) Personal communication with Cobb Vantress (2003)
2) FCR (feed conversion ratio) estimated as Feed consumption/live weight

Results and Discussion: Data were successfully compiled from multiple studies so that the influence of dietary crude protein level on bird energy need might be examined. As the FSR and FCR values were collected from 2 laboratories (klang et al., 1985, klaising**,** 1987), it was necessary to model the values so that the growth entire curve might be represented. Figure 1 displays the composite FSR and the FDR. Note the FSR values are modeled at a higher confidence level than FDR as the R^2 for FSR = 0.9944 for FSR and While the R^2 = 0.9600. Consequently FDR for purposes of this writing was estimated as FSR – daily protein accretion. The FSR rate exceeded FDR in every case. Using Figure 1 equation and the methodology described yielded the following balance sheet

Table 5. Protein Synthesis, Protein Degradation in grams

Proein Synthesis (grams)	646.6
Protein Degradation (grams)	190.1
Protein Synthesis (Molar)	5.42
Protein Synthesis (ATP need)	43.4
CHO to supply ATP	217

Protein Synthesis expressed in FSR and degradation expressed in FDR

```
PredDailyProSyn2 = BirdProt * PredFSR2 /100;
PredDailyProdeg2 = PredDailyProSyn2 - Dailyprot;
```

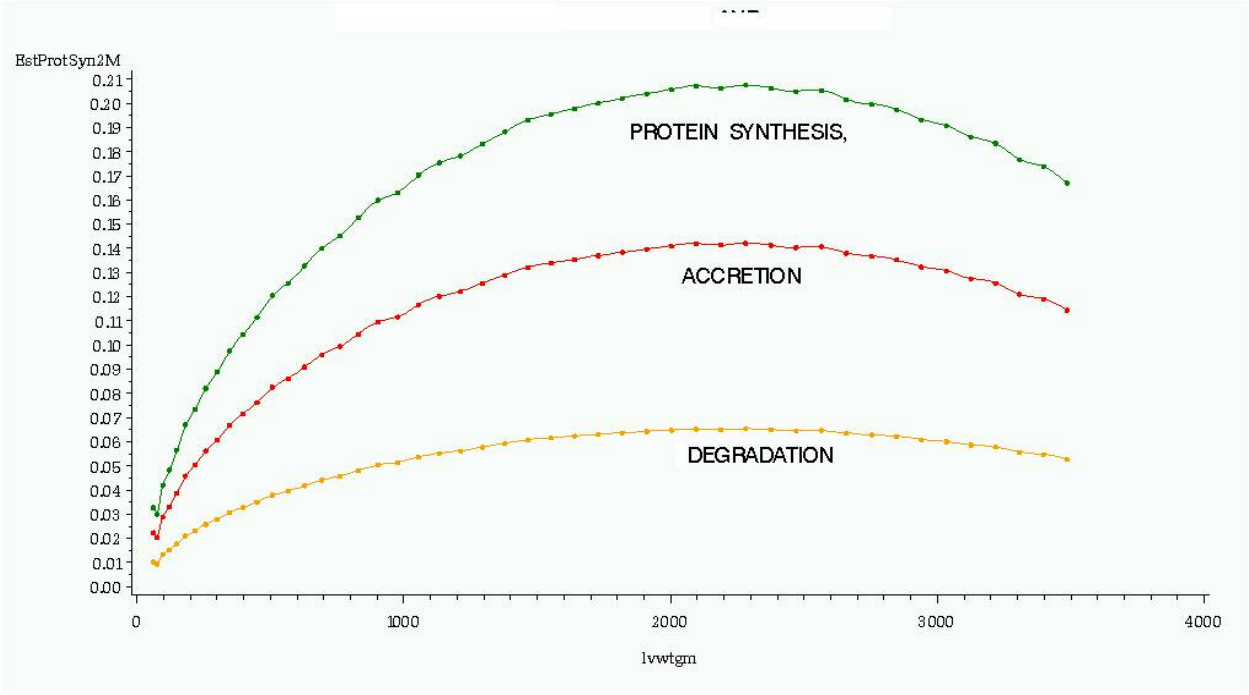
Table 6. FSR[1] and FDR[2] values collected from the literature

Tissue Source	Age	FSR	FDR	TO
Pectoralis Muscle	35	11.34	6.03	1.88
Sorotoris Muscle	35	10.54	5.7	1.85
Gastrocnemois Muscle	35	10.8	5.08	2.13
Breast Muscle	17	538	394	1.37
Breast Muscle	42	546	434	1.26
Breast Muscle	49	704	512	1.38
Liver	14	100	63.3	1.58
Liver	21	114.4	73.5	1.56
Liver	28	108.1	70.6	1.53
Whole Body	7	34	22.0	1.55
Whole Body	14	31.5	23.0	1.37
Whole Body	21	29.7	22.9	1.30
Whole Body	28	25.8	20.9	1.23

FSR = Fractional Synthesis rate
FDR = Fractional Degradation rate

In summary, modeling data estimates that 3526 Kcal are utilized for protein metabolism including synthesis, degradation and obligatory catabolism of consumed excess amino aicds. Dietary crude protein modification is possible to 3 % reduction. Reducing dietary protein to 3 % lowers HP from 640 to 513 Kcals.

Figure 2. Overall Protein synthesis (grams) accretion (grams) and degradation (grams) for broilers fed diets containing NRC specifications.



[1] Protein Synthesis = DailyProt * 1.46;
[2] Protein Degradation = EstProtSyn2g - Dailyprot;

As the bird consumes 13,386 Kcal to MEn to reach the desire weight. The values above correspond to a change in energy expenditure of just 127 Kcal of MEn. Such changes are nominal and other avenues directed at bird energy expenditure will be needed to enhance production efficiency. Efforts directed towards energy expenditure for activity and/or immunological response offer better opportunity for enhancing energetic efficiency of broiler production. Alternatively, perhaps improving genetics will alter the sum need energy expended for FSR and FDR.

TABLE 7. Composition of experimental broiler feeds formulated to satisfy essential amino acid needs at and 4.5% below the National Research Council (NRC, 1994) recommended crude protein levels, percentage "as is" basis

	Starter					Grower				Finisher		
Ingredients	1	2	3	4	5	6	7	8	9	10	11	12
Corn	53.74	56.27	58.99	72.5 4	59.57	62.08	65.03	76.43	73.28	74.68	78.12	78.12
Soybean meal	29.47	25.68	21.41	16.1 9	22.51	18.76	14.02	12.6	16.77	18.43	16.55	16.55
Fat, Soybean	5	5	5	1.68	5	5	5	2.25	1.65	2.16	1.92	1.92
Corn glutenen meal	5	5	5	-	5	5	5	-	5	1.45	-	-
Dicalcium phosphate	2.08	2.12	2.16	1.89	1.43	1.35	1.39	1.55	1.07	1.07	1.09	1.09
Calcium carbonate	1.07	0.98	0.89	0.77	1.55	1.56	1.58	1.53	1.44	1.42	1.44	1.44
Choline chloride	0.13	0.01	0.01	0.01	0.01	0.01	0.05	0.07	-	-	-	-
Salt	0.38	0.49	0.49	0.49	0.44	0.44	0.44	0.43	0.29	0.29	0.36	0.36
Vitamin premix[1]	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Mineral premix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
selenium[3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Lysine	0.11	0.24	0.38	0.33	0.11	-	0.38	0.49	0.12	0.10	0.18	0.18
DL Methionine	0.02	0.06	0.12	0.17	-	0.07	0.16	0.22		0.01	0.04	0.04
Calculated analysis												
ME, kcal/kg	3150	3150	3150	3150	3200	3200	3200	3200	3200	3200	3200	3200
Crude protein, %	22.64	21.16	19.68	18.2 1	19.68	18.21	16.73	15.5	18	16.5	15	15
ME/CP ratio												
Methionine	0.45	0.45	0.47	0.52	0.43	0.43	0.47	0.5	0.35	0.38	0.38	0.38
Lysine	1.18	1.17	1.18	1.17	0.89	0.89	0.89	0.89	0.76	0.76	0.76	0.76
Sulphur amino acids	0.88	0.88	0.88	0.86	0.83	0.82	0.81	0.8	0.73	0.73	0.73	0.73
Calcium	1	1	1	1	1	1	1	1	0.8	0.8	0.8	0.8
Available phosphorus	0.45	0.44	0.44	0.44	0.38	0.38	.038	0.38	0.3	0.3	0.3	0.3

[1]Supplied per kilogram of diet: vitamin A, 10,141 IU (retinyl acetate); cholecalciferol, 3,086 IU; vitamin E, 23.92 IU (dl-α-tocopheryl acetate); menadione, 2.87 mg; thiamine, 2.20 mg; riboflavin, 7.72 mg; niacin, 60.30 mg; d-pantothenic acid, 12.46 mg; pyridoxine, 3.75 mg; vitamin B_{12}, 0.017 mg; folic acid, 1.066 mg; d-biotin, 0.127 mg.
[2]Supplied per kilogram of diet: Ca,160 mg; Zn, 100 mg; Mn, 120 mg; Fe,75 mg; Cu, 10 mg; I, 2.5 mg.

Table8. Energy balance associated with the production of a 2.5 Kg broiler including energy for Protein synthesis, amino acid energy in Protein and the obligatory heat production for amino acid reduction replaced by glucose

Protein Synthesis (2.5 Kg bird) in Kcals	HP for Protein level relative to 3 % reduction	Total Protein HP estimate (Kcals)
829 + 2570	NRC = 127	3526
829 + 2570	NRC - 1.5 = 52.7	3451.7
829 + 2570	NRC – 3.0 = 0	3399

HP = Heat Production

```
HP = (totcataakcals - (TotalATPY * 7.3) - totcataaNkcalloss) + (( (choincrease / 180 ) *
688) - (36*7.3*choincrease / 180));
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VITA

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Scope and Method of Study: In today's economic climate efficient feed conversion into broiler tissue is essential for successful poultry production. Sophisticated knowledge of broiler nutrient requirements and nutrient content of feed ingredients are needed. Though genetic improvement has produced the most efficient broiler to date, questions related to its nutrients need are expected to continue. Refinements in dietary and environmental factors influencing energetic efficiency need to be developed. The aim of these studies was to evaluate and model broiler protein and energy metabolism in order that better feeding regimen be developed to enhance the quality of production.

Findings and Conclusions: Nutrition comes to play a key role controlling carcass composition. Today's bird has changed dramatically over the years, but new problems are also faced. Not only are the nutrient requirements changing annually, but also the adaptation period for stress has not been overcome. It is well known that poultry, like many other animals, are insufficient in converting feed to protein. To achieve maximum broiler performance, the dietary CP content must provide sufficient levels of EAA and NEAA to allow maximum protein synthesis and meet the demands of metabolic processes. Reduction in nitrogen excretion and improvement in the efficiency of nitrogen deposition can be obtained by matching amino acid composition of the diet with the amino acid requirement of the broiler for maintenance and meat production. Regression equations were developed in order to interpret ATP yield from substrates along with evaluation of Heat Production from various Protein levels.

ADVISER'S APPROVAL: Dr. Robert Teeter