MYCOTOXINS, FEEDING SYSTEM AND PROTEIN LEVEL EFFECTS ON BROILER PERFORMANCE

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CHAPTER I

INTRODUCTION

The new millenium comes to us with challenges for today's nutrition applicability. A bird has been manufactured over the years through genetic selection application, to produce higher live weight at lower feed consumption. This has been accomplished by the careful and detailed selection of fast growing broiler lines, leading to a modern broiler capable of reaching desirable slaughter characteristics in less than six weeks. It is debatable whether geneticists will further improve growth rate, and focus is beginning to be made on manipulating maintenance requirements and further how to adapt this bird to a leaner body type.

The broiler industry in the United States has reached an impressive status by producing more than 7 billion broilers in 1998 and a value of production of more than 15 billion dollars (USDA, 1999), thereby comprising the leading branch of agri-business. In an industry of such magnitude, changes that improve production are likely to lead to an increase in profitability. The market is demanding a leaner broiler and carcass composition, and continues to be a factor considered by all breeding companies.

Nutrition comes to play a key role controlling carcass composition. Today's bird has changed dramatically over the years, and new problems are faced. Not only are bird nutrient requirements changing annually, but also the adaptation period for stress has not been overcome. Ascites has been referred to as an incapacity of the bird to supply the necessary amounts of oxygen to the tissues, presumably due to elevated demand to fulfill

its metabolic-physiologic needs (Leeson et al., 1995). Genetic improvements in selection of fast growing birds have left the lungs with the task of oxygenating higher quantities of blood in the same amount of time in order to fuel metabolic processes.

Nutrition has the opportunity to face this problem by contributing a more balanced and appropriate ration to the bird. For this purpose we must understand substrate utilization, efficiencies, toxicities and imbalances so we can provide an efficient, balanced and adequate dietary nutrient density, thereby avoiding potential overloads that lead to higher oxygen demands. In addition, leaner poultry meat, more efficient substrate utilization, reduction in environmental pollution caused by phytate phosphorus (Nelson et al., 1971), nitrogen and ammonia excretion (Sims and Wolfe, 1994), is needed among others. Improved methods modeling nutrient use are needed.

Today's poultry industry continues to apply the metabolic energy system with success (NRC, 1994). This system supplies the bird with energy necessary to support the maintenance and growth processes. Unfortunately this system does not account for the different types of heat loss, one of them being associated with metabolic routes of the individual nutrients. Heat increment related to substrate utilization is different for the different nutrients regardless of the dietary level of MEn in the ration (Blaxter, 1989). Another type of heat loss is produced when the bird is submitted to environmental conditions that create a thermo-imbalance for which the bird is obligated to compensate for. This source of heat is necessary to maintain life processes, is separate from BMR (Van Kampen et al., 1979) and elevates the birds maintenance energy expenditure. It is important to understand that the MEn system does not account for substrate variability for either maintenance or growth energy demands.

Therefore, the studies described herein are directed towards providing additional insight into nutrient utilization efficiency and feeding systems to apply such insight. In order for efficiency of tissue accretion to be maximized, and energy expenditures associated with the metabolism of intermediate and waste products minimized, new methodologies are needed. This study is focussed primarily on the protein requirement and the need of the bird to consume the necessary amounts of the nitrogen compound in order to satisfy its tissue accretion capabilities.

REFERENCES

Blaxter, K. L., 1989. Energy Metabolism in Animals and Man. University Press, Cambridge, England.

Leeson, S., G. J. Diaz, and J. D. Summers, 1995. Poultry metabolic disorders and mycotoxins. University Books.

National Research Council, 1994. Nutrient requirements of domestic animals. Nutrient requirements of poultry. National Academy of Sciences, Washington, D. C.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski, and J. H. Ware, 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. J. Nutr. 101:1289-1294.

Sims, J. R., and D. C. Wolfe, 1994. Poultry waste management: Agricultural and enviromental issues. Adv. Agron., 52, 1.

United States Department of Agriculture, 1999. Poultry – Production and value 1998 summary. National Agricultural Statistics Service. Pou 3-1 (99).

Van Kampen, M., B. W. Mitchell, and H. Siegel, 1979. Thermoneutral zone of chickens as determined by measuring heat production, respiration rate, electromyographic and electroencephalographic activity in light and dark environments and changing ambient temperatures. J. Agri. Sci. 92:219-226.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The science of nutrition has been able to define poultry nutrient requirements to within small margins of error. Nutrition has also been able to establish that bird nutrient requirements decline with age. However, current standard ration formulation practice for broilers is divided into phases (Starter, grower, finisher, withdrawal) in which the bird has a constant percentage of each nutrient for a period of time (NRC, 1994). An abrupt change occurs when these phases are switched, producing either a lack or an excess of certain nutrients. Consequently, the bird will either "grow" into or out of as it matures and moves to the next phase. In order to create new alternatives in ration formulation and feeding it becomes necessary to define indispensable substrate metabolism as the bird moves from one requirement phase to the next. Energy is normally used as a ratio to bird nutrient needs and the effects of changing nutrient/ energy ratios as the bird matures is essential. The interaction of carbohydrate, lipid, and protein in an under or overfeeding condition potentially create a gap in maximizing substrate efficiency that may lead to fatter carcasses.

ENERGETICS

Energy may take various forms including chemical bond, kinetic, potential and nuclear, and therefore is expressed in a different variety of ways. Chemical bond energy,

critical to nutrition, is produced through the sun radiation and photosynthesis. Organisms utilize molecular energy from different substrates in order to satisfy energy needs for metabolic processes and indeed to sustain life. Metabolic processes used to convert chemical energy stored in the molecules of food into kinetic are ultimately what the animals use for work and heat (Scott et al., 1982).

METABOLIZABLE ENERGY: The feed energy evaluation system currently in use is metabolizable energy normally abbreviated as ME. When corrected to zero nitrogen balance, MEn is used (NRC, 1994). Chyme undergoes a variety of reactions that lead to the absorption of this energy and it is there plus urinary excreta losses that are accounted for by ME. Numerous internal factors such as energy, protein, lipid and carbohydrate content of the diet affect the degree of energy retention. The scheme of the different categories of energy derived from a feedstuff and its' relationship with the animal is illustrated in figure 1.

A number of discrepancies exist in the literature with regard to nomenclature used to classify energy. It has been somehow accepted that apparent metabolizable energy accounts for energy losses of excreta and urinary origin, but it does not for endogenous losses. In some cases, the results of an assay using full-fed laying hens has been labeled as apparent ME (AME) when a correction is done for the change in dietary ME due to supplemental fat (Horani and Sell, 1977). Mateos and Sell (1980), described the result of a similar assay as "AMEn" for nitrogen-corrected apparent ME without correcting for changes due to added fat.

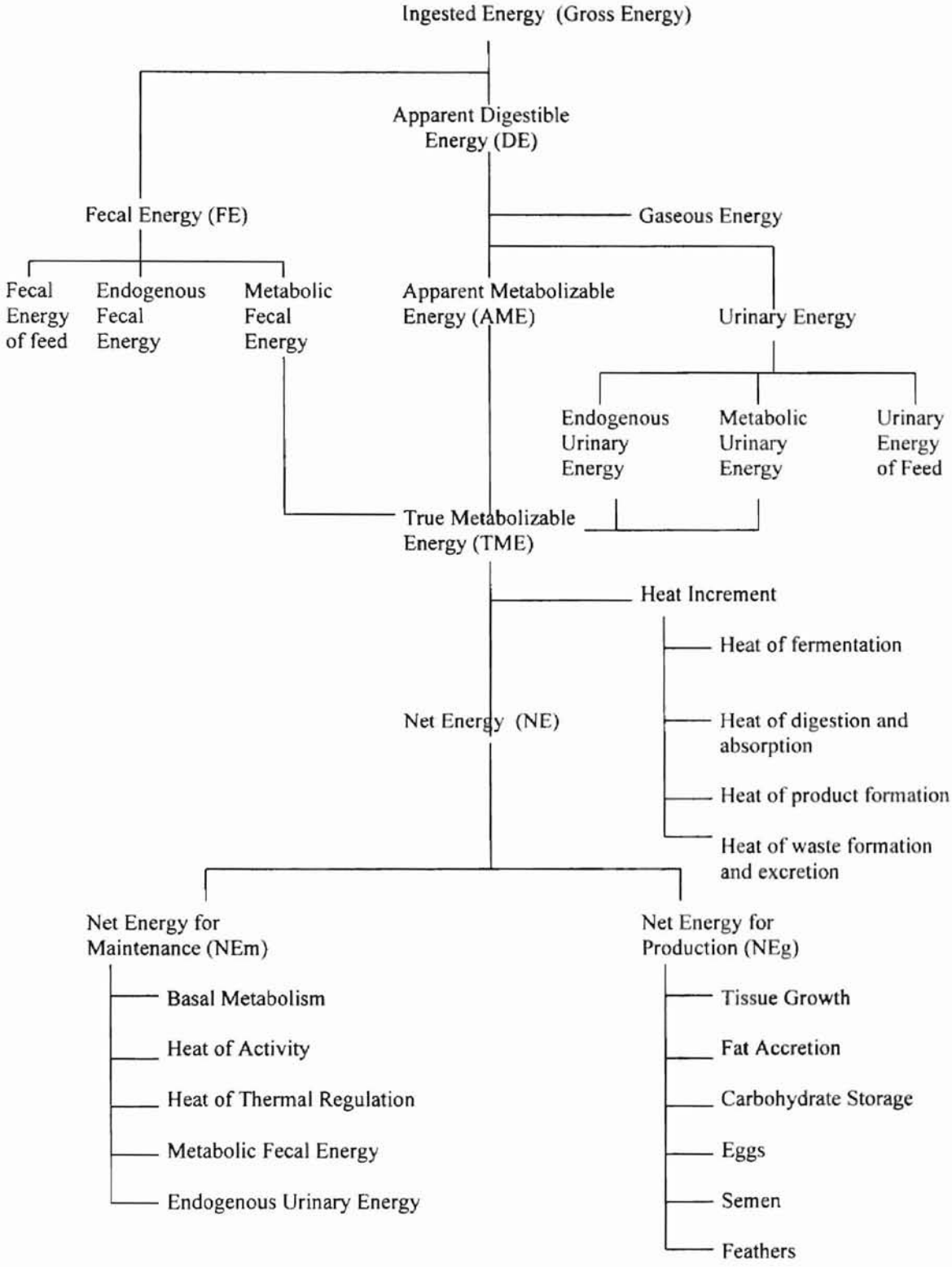

Figure 1. Partition of feed energy in the bird

Energy methodology, evaluation and classification of feedstuffs for poultry began with work conducted by Fraps at the Texas Agricultural Experiment Station (1912-1946). Most of the terminology applied today was derived from their work.

According to Fraps (1946), "metabolizable energy is the energy of the food eaten less the energy of the excrement derived from it, including both fecal and urinary as well as gases produced by fermentation. Gas production is minimal with poultry. The following expression may be used to define the various components as follows:

$$ME_f = GE_f - EE_f - UE_f - GPD_f$$

ME = metabolizable energy

GE = gross energy

EE = excreta energy

UE = urinary energy

GDP = gaseous products of digestion

$_f$ = feed origin

Fraps focussed primarily on the term "productive energy", but they actually determined the ME of various types of feedstuffs for poultry. They showed that when chicken excrement was combusted it resulted in a good indicator of the energy that was not digested by the chicken and was excreted in the feces. Titus (1961), later developed an alternate 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 the numerous environmental, genetic and management factors that can affect results of such studies. Hill and Anderson described detailed methodologies and procedures for the determination of metabolizable energy, 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

They determined fecal energy by using a ratio to chromic oxide. Chromic oxide is an indigestible marker and may be applied via following equation:

$EE_f = EE_t \times [\%\ Cr_2O_3$ per gram of diet / $\%\ Cr_2O_3$ per gram of excreta]

EE_t = total energy per gram of excreta

The metabolizable energy of individual feedstuffs were then calculated as follows:

$$ME_f = 3.64 - (ME_r - ME_t) / P$$

ME_f = metabolizable energy of the feedstuff

ME_r and ME_t = metabolizable energy of the reference and test diets

It may be noted that in the equations developed account for energy in both feed and excreta, and further that a correction for nitrogen retained by the bird is made. The value 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.

TRUE METABOLIZABLE ENERGY: As a result of Harris criticism (1966) of the conventional ME scheme because it included energy losses from the body as part of the fecal energy, a new model was proposed to take account of endogenous energy losses. Sibbald (1976, 1986) followed Harris' model and developed a system called "True Metabolizable Energy of Feedstuffs" in which he was trying to account for endogenous losses not derived from feed origin. Sibbalds' equations are as follows:

$$TME_n = IE - (FE_n + UE_n) + (F_m E_n + U_m E_n + U_e E_n)$$

IE = amount of energy as test material placed in the bird

FE_n and UE_n are then amount of energy excreted by the fed bird

$F_m E_n$, $U_m E_n$ and $U_e E_n$ are the amount of energy excreted by a fasted bird, considering these energy losses as a correction value for energy losses of endogenous origin.

In order to calculate $(FE_n + UE_n)$ it is necessary to apply the following correction formula:

$$(FE_n + UE_n) = (FE + UE) + k\,(IN - FN - UN)$$

k = constant estimating the gross energy content of the excretory products resulting from the catabolism of a unit weight of tissue nitrogen.

Hartel (1986) criticized Sibbalds' methodology and began a controversy that was known all around the scientific community debating whether differences between AME and TME values are real or largely a result of the methodologies applied. He proposed a different methodology and a different way of accounting for energy losses of endogenous origin.

After decades of modeling for ME_n determination, Hills' procedure is currently applied the most as demonstrated in NRC values (1994), and by the various types of feed formulation software programs available in the market. However, it must be kept in mind that none of these systems represent net energy, and the best that can be hoped for is that they are correlated with it.

NET ENERGY: The proportion of the feedstuffs' energy that the animal is going to utilize for maintenance and production purposes is referred to as "Net Energy (NE)". Net energy is the ME minus the energy lost as the heat increment. NE may be further subdivided into NE for maintenance (NEm) and production or gain (NEg) (NRC, 1994).

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 the metabolizable energy in the chick from 0-21days 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 error 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 its correlation with body composition. As described previously, NE is the difference between ME and energy loss as heat increment. This heat increment originates for several reasons. Heat increment include basal heat production (Bartels et al., 1973), heat production associated with environmental changes (Van Kampen et al., 1979; Meltzer, 1983), heat loss produced by the digestive process of feed (MacLeod and Shannon, 1978) heat produced by catabolic and anabolic reactions, and heat loss due to muscular activity (Boshouwers and Nicaise, 1985). Heat production can be affected by external factors like heat stress (Teeter et al., 1987) and also by the quantity and quality of the different substrates present in the feed (Sturkie, 1986). The amount of heat production subtracted from the 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)

This equation allows the estimation of NE values for maintenance and growth, by subtracting heat production from the metabolizable energy value.

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). An overview of protein and amino acid metabolism is illustrated in Figure 2. 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 acids 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 process involves the removal of the amino group and the resulting α-keto acid is then used for oxidation to CO_2, 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).

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

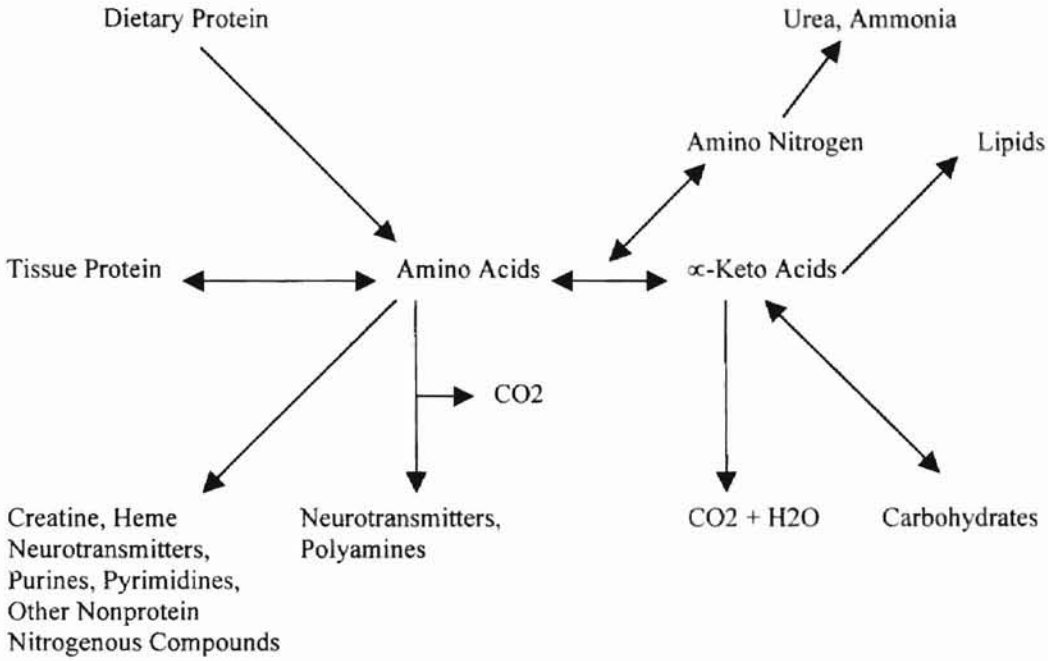

Figure 2. Overview of Protein and Amino Acid Metabolism

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 from 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 transamination, 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). We find these metabolic processes illustrated in Figure 3. 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).

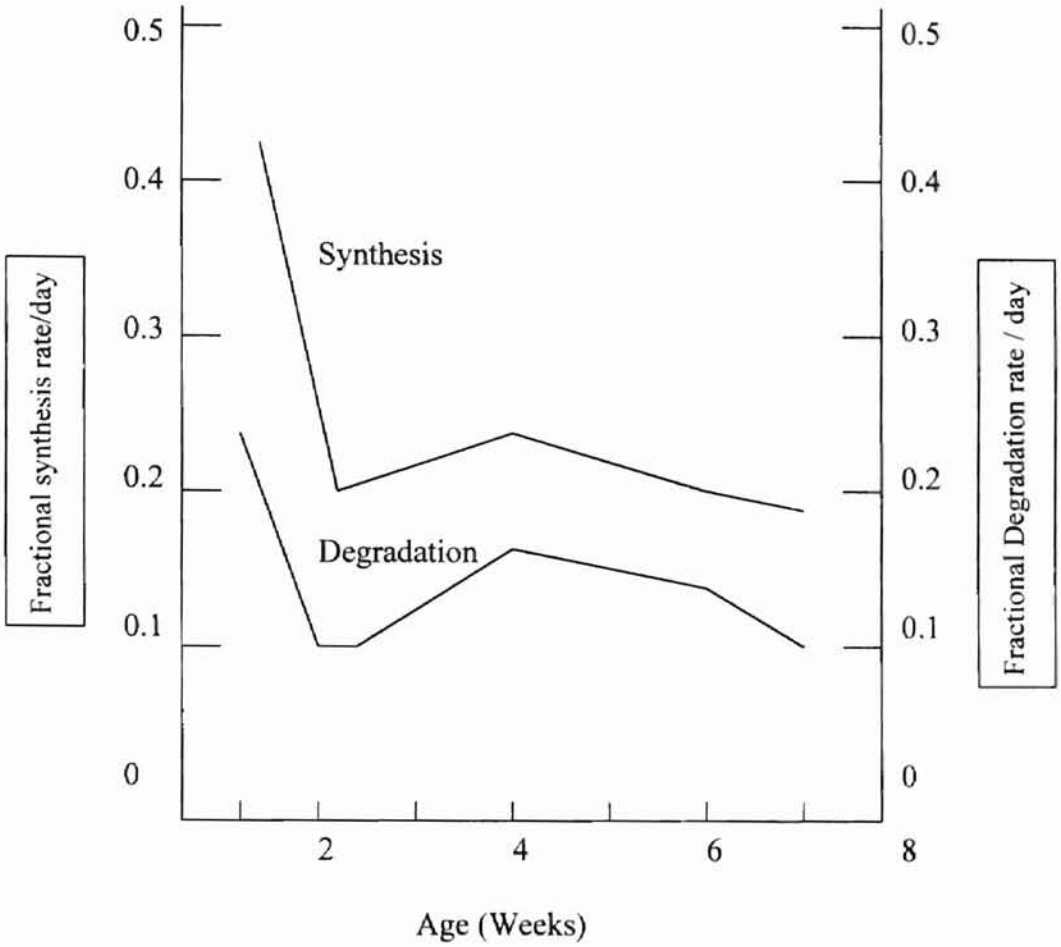

Figure 3. Rates of protein synthesis and degradation in chick skeletal muscle during growth (McDonald and Swick, 1981)

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 been known for a long time 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 occurring protein in eukaryotes, and it becomes covalently attached to amino groups on proteins, which are then selected for degradation. This process is illustrated in Figure 5.

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

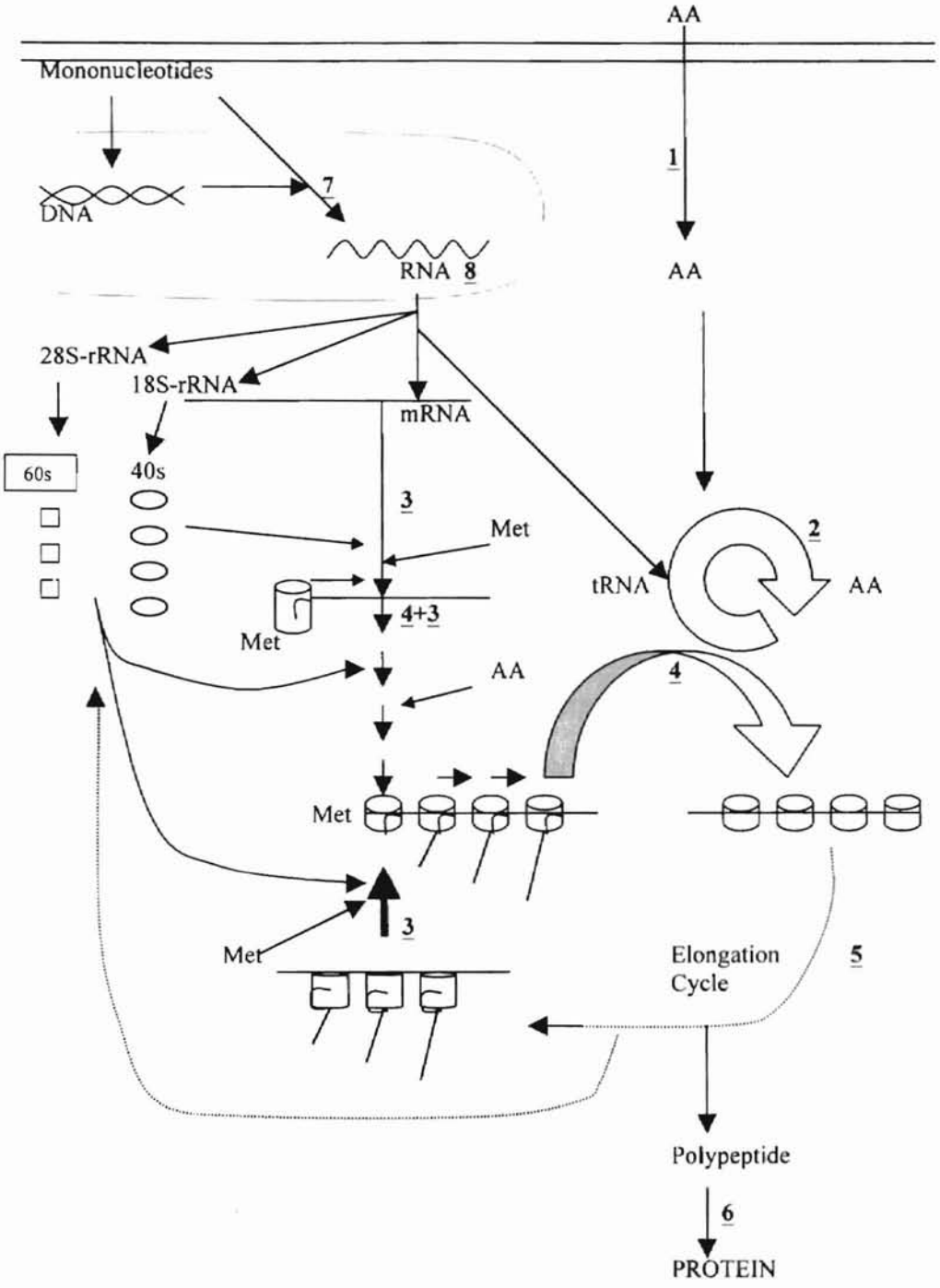

Figure 4. The major steps in protein synthesis and generation of the RNA fractions

Numbers in circles refer to: 1=transport of amino acids across the cell membrane, 2=activation of amino acids into aminoacyl-tRNA, 3=initiation, 4=elongation, 5=termination, 3+4+5=translation, 6=posttranslational processes, 7=transcription, 8=posttranscriptional processes.
AA=amino acids, DNA=deoxiribonucleic acids, RNA=ribonucleic acids, rRNA=ribosome RNA, mRNA=messenger RNA, tRNA=transfer RNA, MET=methionine, 40s, 60s=ribosomal subunits.

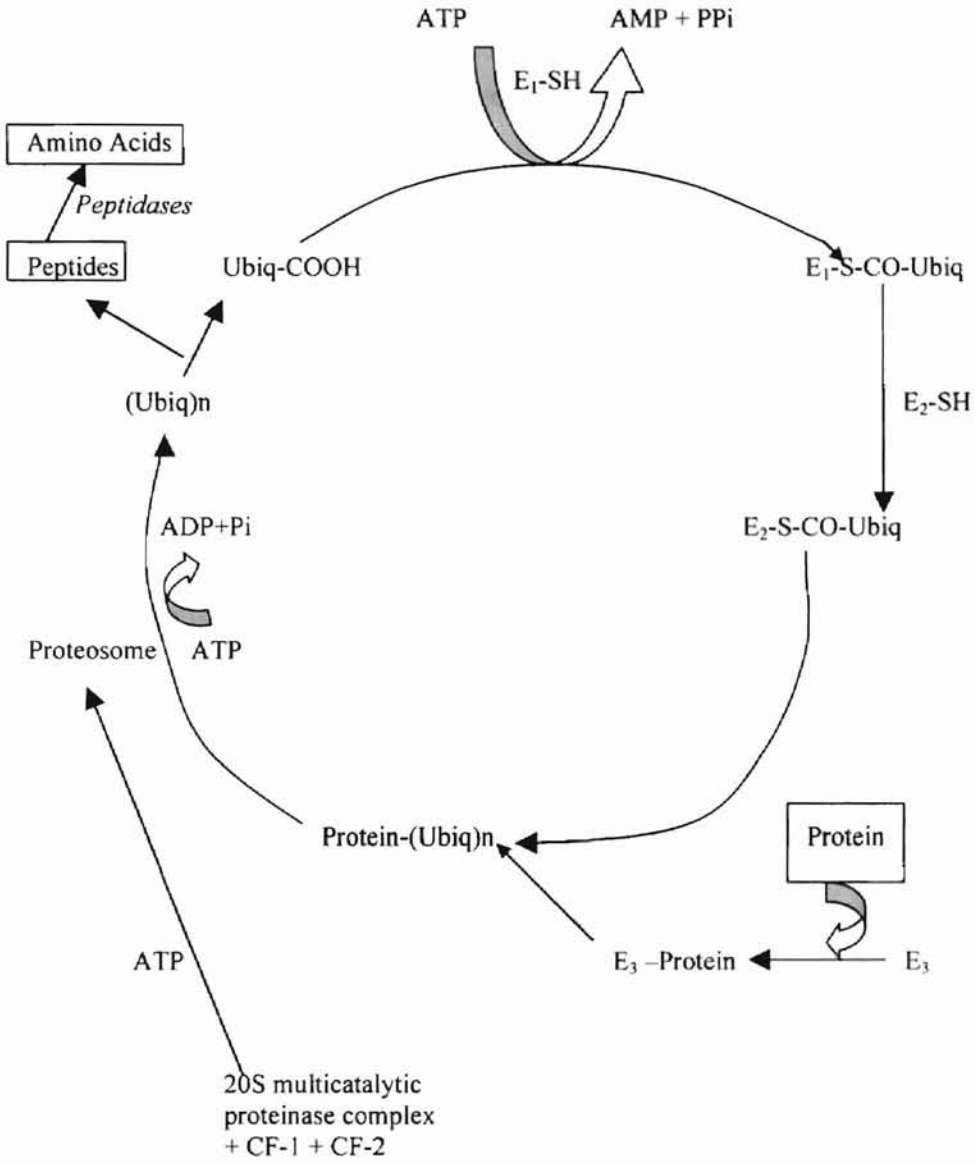

Figure 5. The ubiquitin pathway for protein degradation.

Ubiq=ubiquitin, E_1=ubiquitin-activating enzyme, E_2=ubiquitin-carrier protein, E_3=ubiquitin-protein ligase, CF-1and CF-2=conjugate-degrading factors.

are also used for synthesis of other purines such as adenine and guanine, and components of DNA (Scott et al., 1982). The incorporation of ammonia into uric acid requires both energy and building blocks. The synthesis of uric acid is costly in ATP 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 proventriculus and gizzard by hydrochloric acid and hydrolytic enzymes. The combined action of 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 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 prescence. 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 phenylalanine and phenyalanine 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 DEFFICIENCY: 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 it has be 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'Mello, 1994).

Antagonisms are characterized by a growth depression caused by a single amino acid, and may 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 that 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 concentartion. 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, phenyalanine 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 have 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. Such environmental concerns, make it necessary to reduce the dietary supply of protein. This study intends to evaluate a new approach for feeding poultry, particularly protein (Figure 6). The project seeks to evaluate the potential for daily diet alternate to more precisely satisfy bird nutrient needs.

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

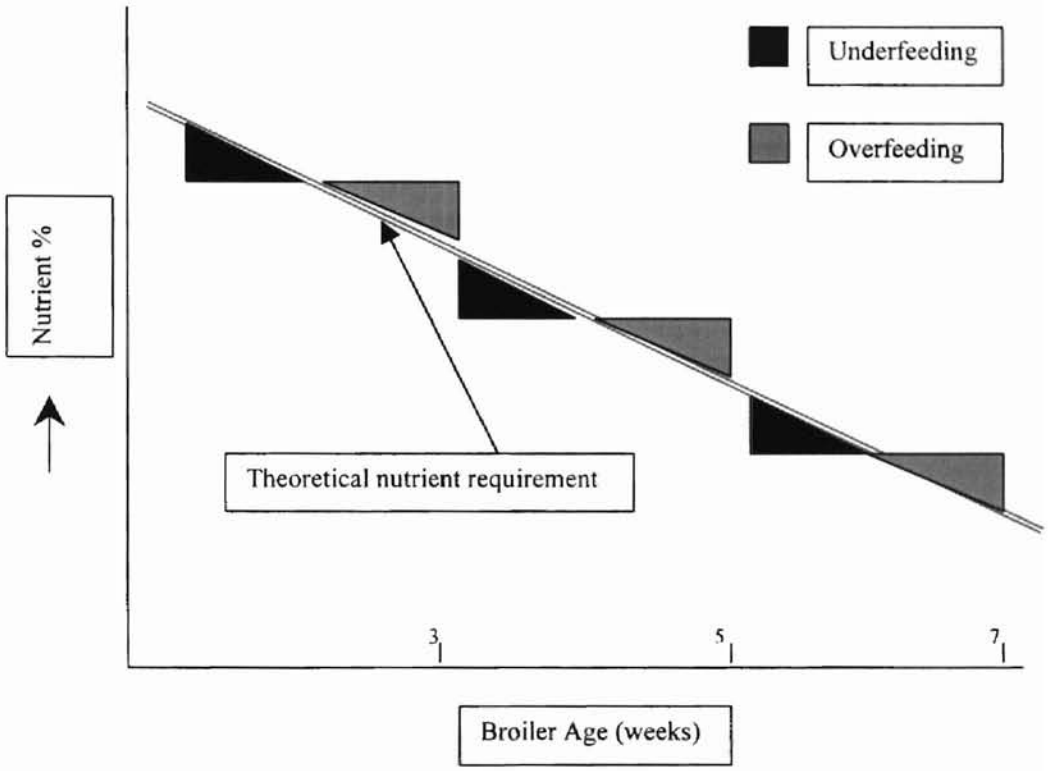

Figure 6. Nutrient Intake
(Feed Pattern vs. Requirement)

acids required by chicks. The most deficient amino acid will become the first limiting amino acid, being lysine in most cases. It usually does not make a difference if other amino acids are in moderate excess with 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).

DIETARY FACTORS AFFECTING CARCASS COMPOSITION

There are several factors that can affect the final composition of a carcass. They can be nutrition related or non-related issues that influence the contribution of carcass lean and fat proportions. Among the non-related factors we find environmental temperature (Swain and Farrell, 1975), sex (Summers et al., 1965; Thomas and Twining, 1971) and age (Combs, 1968; Edwards 1971; Kubena et al., 1972). Nutrition factors affecting composition are several, including water intake (Marks, 1980; 1981; Barbato et al., 1983), levels of salt in the feed (Marks and Washburn, 1983) or in the water (Lightsey et al., 1983), dietary protein and energy (Fraps, 1943; Pesti and Fletcher, 1983; Moran et al., 1968), physical characteristics of the diet (Reddy et al., 1962; and Pesti et al., 1983), amino acid supplementation (Summers and Leeson, 1985; Summers et al., 1988), among

others. How such factors interrelate with each other creates a complex array of possibilities with many variables.

The ratio between dietary calories and protein has been well studied and it has been determined that when this ratio increases, carcass fat also increases (Summers and Leeson, 1979; Jones and Wiseman, 1986), and when narrowed, a consistent enhancing in lean tissue accretion and reduced fat deposition has been observed (Fisher, 1984). Typically, a 139 Kcal of ME per 1% of dietary protein is recommended (NRC, 1994) and it is generally applied in most operations. However, during periods of humidity distress, reducing this ratio may also bring with it an increase in mortality if the bird heat load is adversely affected (Wiernusz and Teeter, 1993).

Dietary sources of starch, lipid and protein are submitted to a variety of physical and metabolic processes that will vary depending on the quantities of each and the needs of the bird at that specific point in time. In order to maximize protein accretion, amino acids must be available from dietary sources and body storage, but an excess of these always represent an unnecessary waste of nitrogen as mentioned previously (Klasing, 1998). However, the energetic cost for this protein gain is also more inefficient compared to those of other substrates. It has been reported that lipids have a theoretical efficiency of intake vs. synthesis of approximately 0.96 compared to 0.86 for protein accretion. When dietary protein is in excess it becomes even more inefficient, because it will be deaminated and either converted to glucose or fat with an efficiency of 0.66 (Blaxter, 1989).

Values for heat increment are also higher for protein metabolism (Forbes and Swift, 1944). There is general agreement that in simple stomached animals such as the

pig and the rat, the energy cost of fat deposition ranges from about 1.4 kJ ME/kJ fat deposited for feed consisting mostly of carbohydrate, to 1.15 kJ ME/kJ fat deposited for feed consisting mostly of triglycerdies (ARC/MRC Committee, 1974). However, the cost of protein deposition has been more difficult to assess. Due to the rapid chick growth, the amount of energy deposited as protein is small relative to that deposited as fat or dissipated as heat (Pullar and Webster, 1977). Estimates of protein deposition range close to 2.3 kJ ME/kJ protein deposited (Kielanowski, 1976; Pullar and Webster, 1977).

Simple stomached animals have apparent energetic efficiencies above maintenance of protein and fat accretion of 0.44 and 0.74, respectively, and 2.25 and 1.36 kJ ME/kJ for protein and fat respectively (Pullar and Webster, 1977). Thorbek (1970) estimated the energy cost of protein deposition to be 2.32 using an empirical estimate of maintenance requirement at the body weight of pigs. Unfortunately all these efficiency values do not take under consideration the total energy cost of synthesis, but only determine the difference between total protein accumulation and excretion. Only when quantification for total protein and fat synthesis is done, will it be possible to determine accurate energetic efficiencies of substrate utilization and thee contribution to growth.

Efficiency of MEn for tissue energy gain is 20.4% for protein in excess of that required to satisfy amino acid requirements, 47% for starch and 52% for fats (Mittelstaedt, 1990), and that net energy availability of amino acids is low due to inefficient carbon chain utilization and energy expenditure during uric acid synthesis and excretion.

Inefficiency itself is accompanied by carcass changes that will affect the final product. A calorie overload of protein or lipid origin forces the body to metabolize these

energy sources and increased fat deposition will occur (Marks and Pesti, 1984; Cabel and Waldroup, 1991; Summers et al., 1992).

FEED EFFICIENCY

Growth rate, mortality and feed efficiency have been considered the important variables for assessing flock success. Feed efficiency is calculated by dividing feed intake by live body weight and the result expresses the necessary amount of that feed capable of producing a kg of body weight.

Feed efficiency, body weight and feed consumption have been genetically selected over the years to be more profitable and indeed dramatic responses have been evidenced.

During the last 50 years, the age of slaughter has been substantially reduced, and the amount of feed required to produce a kg of live weight has been improved by almost 100% (Havenstein et al., 1994). Today's feed efficiency values range around 1.65-1.75. This improvement is due in part to the great demand of chicken meat in the United States, were per capita consumption has increased dramatically from 4.0 kg in 1950 (Tarver, 1986) to over 31.8 kg (Perez et al., 1991) in 1990. Last year (1999), chicken meat consumption per capita increased to 39.2 kg, showing an increase of almost 25% (USDA, 1999).

Sherwood (1977) reported a 225% increase in growth rate, from broilers raised in 1976 compared to ones raised in 1957 (Sherwood, 1977). Another study (Chambers et al., 1981) examined changes in carcass composition values, and observed that a 1978 broiler was capable of producing 230% more carcass weight than those in 1957. More

recent studies revealed that 1991 birds compared to 1957 for carcass composition, immune system characteristics and feed conversion, had dramatic improvements (Havenstein et al., 1994), even if compared with the studies in 1977 and 1981. These studies revealed that in a period of approximately 30 years, feed efficiency has been improved from 3.0 to 2.04. Today's bird continues to improve this variable to a maximum of 1.75 at 42 days of age.

The single largest factor affecting feed efficiency is the energy level of the feed. In the early 90's this was not a problem because broilers were usually fed diets that contain approximately 3000 kcal/kg for the starter period and a 100 to 200 increase for the finisher phase.

Because of variability and availability of high energy feed prices, and management problems discussed previously, we are seeing lower values than the ones used in previous days. Today it is more difficult to pinpoint a standard energy level for poultry ration. However, as the broiler gets older it does seem to adjust its intake in relation to dietary energy level (Table 1).

Bird sex is another source of variability that affects the feed efficiency. Male broilers are more feed efficient that females after about 30 days, presumably due to female birds depositing more carcass fat. Body fat takes 9 times as much feed energy to produce as muscle does, and the reasons for this is that lipids contain more energy than does protein per unit of weight. More important muscle is only about 20% protein by weight, the remaining being water (Leeson and Summers, 1997).

Age can also have a considerable effect. As birds get older feed efficiency deteriorates due to the fact that heavy birds use higher quantities of feed in order to maintain their body mass.

Temperature can also affect maintenance needs and feed consumption both of which affect feed conversion (Teeter et al., 1985; Teeter and Smith, 1986; Belay and Teeter, 1996).

Recently a factor called "feed-passage" has been observed in broilers, where undigested feed particles are seen in the excreta, and so consequently feed efficiency was affected. The exact cause of this problem is unknown, but is most likely the consequence of microbial challenges that create an inappropriate environment through the digestive tract (Leeson and Summers, 1997).

In summary, feed efficiency is a variable and moving target, and striving for optimal feed efficiency may not always be the most economical situation. A much more useful measure could be feed cost per kg of live or carcass weight. A useful starting point in re-evaluating efficiency of feed use is to consider conversion of feed energy into live-weight gain, or even the efficiency of substrate utilization. Table 2 illustrates the values for broilers up to 9 weeks of age (Leeson and Summers, 1997).

MYCOTOXINS

Mycotoxins are a group of compounds, of fungi origin, capable of producing negative health, production and economic effects. They can be found in crops before, during and after harvest, during storage and following processing (CAST, 1989). Mycotoxins have several modes of action, but repercussions over the birds' integrity is

Table 1. Effect of diet dilution from 35-49 days of age on broiler performance

Diet ME (kcal/kg)	Diet CP (%)	49 day body weight (grams)	Feed Intake 35-49 days (grams)	Feed:gain 35-49 days	Energetic efficiency (Mcal/kg gain)
3200	18	2950	2580	2.34	7.43
2900	16	2920	2760	2.49	7.19
2600	14	2880	2900	2.72	6.97
2300	13	2910	3270	2.99	6.70
1900	11	2910	3670	3.31	6.37
1600	9	2890	4300	4.01	6.41

Table 2. Energy conversion to live weight for broilers[1]

Age (weeks)	Male birds (/kg)	Female birds (/kg)	Mixed sex (/kg)
4	---	5.15	---
5	5.35	5.60	5.48
6	5.75	6.05	5.90
7	6.20	6.60	6.40
8	6.65	---	---
9	7.10	---	---

[1]Values expressed in Mcal of metabolizable energy/live weight gain.

the primary consequence of these compounds. Hundreds of mycotoxins are recognized (400), but the toxicity, occurrence and target organs varies among these naturally occurring toxins (Fredric, 1997).

AFLATOXINS: Aflatoxins are highly toxic and carcinogenic mycotoxins produced by fungal species of the genus *Aspergillus* in which the most common are *A. Flavus, A. Parasiticus* and other types of genus like the *Penicillium Puberulum*. Of these mycotoxins, Aflatoxin B1 is the most potent of all these toxins, binding to nuclear and mitochondrial DNA (Edds, 1979).

Aflatoxicosis effects include increased mortality from heat stress in broiler breeders (Cunningham, 1987), depression in egg production (Bryden *et al.*,1980), anemia, hemorrhages, and liver condemnations among other lesions. A bird can also suffer from paralysis and lameness (Lamont, 1979), as well as impaired performance (Okoye *et al.*, 1988). In broilers, clinical signs can be manifested by weight loss, ataxia, recumbency, decrease in feed and water consumption, nervous symptoms including leg weakness and dropping of wings (Rao and Joshi, 1993). Broilers that have been exposed to aflatoxins have a paler color than normal (Schaeffer *et al.*, 1988), due to a decreased absorption, transport, and deposition of dietary carotenoids (Tyczkowski *et al.*, 1987).

The adverse effects of aflatoxin on broiler performance depend on dose and time of exposure. Research indicates that levels of 0.5 ppm dietary Aflatoxin B1 can cause a significant decrease in body weight and feed intake when administered for 4 weeks whereas 1 ppm dietary aflatoxin for 1 or 2 weeks does not affect performance (Leeson *et al.*, 1995). According to Osborne *et al.*, (1982), the minimum dietary concentration of aflatoxin able to cause decreased growth in chickens appears to be 2.5 ppm.

Vacuolation of hepatocytes and lymphocytic depletion in the follicle medulla of the bursa of Fabricius are typically the first histologic lesions, followed by a reduction in weights of liver, bursa, spleen and thyroid, and petechial and ecchymotic hemorrhages on the medial surface of the thighs (Espada *et al.*, 1992). Liver tissue lesions are due to a congestion of the hepatic sinusoids, focal hemorrhages, centrolobular fatty cytoplasmic vacuolation or a possible necrosis, biliary hyperplasia, and nodular lymphoid infiltration. In the kidneys, the epithelial cells of convulated tubules are usually vacuolated (DaFalla *et al.*, 1987), and the tubular renal epithelium becomes cloudy swelled, and hydropic degenration is observed (Asuzu and Shetty, 1986).

OCHRATOXINS: Ochratoxin is another compound from fungal origin capable of producing harmful effects over livestock production. Manufacturers of this metabolite are *A. Ochraceus, A. Malleus, A. Ostianus, A. Petrakki,* among others (Leeson *et al.*, 1995). The most toxic of these compounds is Ochratoxin A, which is capable of inducing gross and microscopic lesions in the kidney and liver of livestock, and is a renal carcinogen in rats (Boorman *et al.*, 1992).

Performance is usually manifested by an increase in energy intake and heat production (Koh and Han, 1991). A reduction in weight gain is also characteristic (Huff *et al.*, 1984) and is typically accompanied by a poor feed conversion and an increase in mortality (Niemiec and Scholtyssek, 1989).

Ochratoxin, as well as aflatoxin, induces hypocarotenoidemia in a more sever manner and basically impairs the ability of the chicken to utilize dietary carotenoids for carcass pigmentation (Osborne *et al.*, 1982)

Methods of prevention or detoxification for aflatoxin contamination include grain quality control, fermentation, inactivation of microbial organisms, thermal inactivation and irradiation, among others (CAST, 1989). Adsorbent supplementation has also been suggested as an alternative for the prevention of alfatoxicosis (Masimanco *et al.*, 1973). Hydrated sodium calcium aluminosilicate recently demonstrated to prevent growth depression and organ affection of chicks when fed levels of Aflatoxin of up to 4 ppm (Ledoux *et al.*, 1999). As far as Ochratoxin A treatment is concerned, the addition of adsorbents such as 0.5% sodium calcium aluminosilicate have not had a significant effect at 2 ppm in broiler diets (Huff *et al.*, 1992). In contrast, Dale (1998) mentioned the need of testing the variety of commercial adsorbents that have for the most part only been tested *in vitro*. Another treatment that has not had substantial effect on Ochratoxin A toxicity is the addition of charcoal to diets containing 4 ppm of the toxin (Rotter *et al.*, 1989).

REFERENCES

ARC/MRC Committee, 1974. Food and Nutrition Research report. Stationary Office, London.

Asuzu, I. U. and S. N. Shetty, 1986. Acute aflatoxicosis in broiler chicken in Nsuka, Nigeria. Trop. Vet. 4:79-80.

Austic, R. E., 1986. Biochemical description of nutritional effects. In:Fisher, C. and Boorman, K. N. Nutrient Requirements of Poultry and Nutritional research. Butterworths, London.

Báker, D. H., 1989. Amino acid nutrition of pigs and poultry. In:Haresign, W. and D. J. A. Cole. Recent Advences in Animal Nutrition. Butterworths, London.

Baker, D. H., 1993. Digestible amino acids of broilers based upon ideal protein considerations, Arkansas Nutrition Conference Proceedings, 22.

Barbato, G. F., J. A. Cherry, and P. B. Siegel, 1983. Selection for body weight at eight weeks of age. 16. Restriction of feed and water. Poultry Sci. 62:1944-1948.

Bartels, H., P. Dejours, R. H. Kellogg, and J. Mead, 1973. Glossary of the respiration and gas exchange. J. Appl. Physio. 34:549-558.

Belay, T., and R. G. Teeter, 1996. Virginiamycin and caloric density effects on live performance, blood serum metabolite concentration, and carcass composition of broilers reared in thermoneutral and cycling ambient temperatures. Poultry Sci. 75:1383-1392.

Benevenga, N. J., and R. D. Steele, 1984. Adverse effects of excessive consumption of amino acids. Annual Review of Nutrition. 4:157-181.

Benzinger, T. H., and C. Kitzinger, 1949. Direct calorimetry by means of the gradient principle. Review of Scientific Instruments. 20:849-860.

Bertland, L. H., and N. O. Kaplan, 1970. Studies on the conformations oof multiple forms of chicken heart aspartate aminotransferase. Biochemistry, 9:2653-65.

Blaxter, K. L., 1989. Energy Metabolism in Animals and Man. University Press, Cambridge, England.

Boorman, G. A., M. R. McDonald, S. Imotot and R. Persing, 1992. Renal lesions induced by ochratoxin A in the F344 rat. Tox. Path. 20:236-245.

Boshouwers, F. M. G., and E. Nicaise, 1985. Automatic gravimetric calorimeter with simultaneous recording of physical activity for poultry. Br. Poult. Sci. 26:531-541.

Bryden, W. L., A. B. Lloyd and R. B. Cumming, 1980. Aflatoxin contamination of Australian animal feeds and suspected cases of mycotoxicosis. Aust. Vet. J. 56:176-180.

Brouwer, E., 1965. Report of sub-committee on constants and factors. In: Energy Metabolism, pp. 441-443. K. L. Blaxter ed. Academic Press, London.

Cabel, M. C., and P. W. Waldroup, 1991. Effect of dietary protein level and length of feeding on performance and abdominal fat content of broiler chickens. Poultry Sci. 70:1550-1558.

Chambers, J. R., J. S. Gavora, and A. Fortin, 1981. Genetic changes in meat-type chickens in the past twenty years. Can. J. Anim. Sci. 61:555-563.

Combs, G. F., 1968. Amino acid requirement of broilers and laying hens. Proc. Md. Nutr. Conf., 87-90

Committee on Medicine Aspects of Food Policy, 1984. Diet and cardiovascular disease. Report on Health and Social Subjects, No. 28. London, England.

Council for Agricultural Science and Technology, 1989. Pages 1-91 in: Mycotoxins: Economic and Health Risks. K. A. Nisi, ed. Council for Agricultural Science and Technology, Ames, IA.

Cromwell, G. L., 1994. Diet formulation to reduce the nitrogen and phosphorus in pig manure. Nutrient Management Symposium Proceedings. Chesapeake Bay Commission, Harrisburg, PA, December, 1994.

Cunningham, P., 1987. Mycotoxin problems appear to be growing worse. Poult. Times 34(24):19.

D'Mello, J. P. F., and D. Lewis, 1970. Amino acid interactions in chick nutrition. 3. Interdependence in amino acid requirements. Br. Poultry Sci. 11:367.

D'Mello, J. P. F., 1994. Responses of growing poultry to amino acids. In:D'Mello, J. P. F., Amino Acids in Farm Animal Nutrition. CAB International. Wallingford, UK.

DaFalla, R., A. I. Yagi and S. E. I. Adam, 1987. Experimental aflatoxicosis in Hybrotype chicks: sequential changes in growth and serum constituents and histopathological changes. Vet. Hum. Toxicol. 29:222-226.

Dale, N., 1998 Mycotoxin Binders: It's time for real science. Poult. Digest 57:38-39.

Deighton, T., 1939. A study of metabolism of fowls I. A calorimeter for the direct determination of the metabolism of fowls. J. Agricultural Science (Cambridge) 29:431-451.

Dukes, H. H., 1993. Duke's Physiology of Domestic Animals. Cornell University Press. Ithaca, New York.

Edds, G. T., 1979. Aflatoxins. N W. Shimmed(Ed) Vongrttrnvr on mycotoxins in animal feeds and grains related to animal health, food and drug administration. FDA/ BVM 79(139):80-164.

Edwards, H. M., 1971. Effect of type of supplementation on the body composition of broilers. Feedstuffs, Minneapolis. 43:66-70.

Espada, Y., Domingo, J. Gomez, and M. A. Calvo, 1992. Pathological lesions following an experimental intoxication with aflatoxin B1 in broiler chickens. Res. Vet. Sci. 53:275-279.

Fagan, J. M., E. F. Culbert, and L. Waxman, 1992. ATP depletion stimulates calium dependent protein breakdown in chick skeletal muscle. Am. J. Physiol., 262:E637-643.

Farrell, D. J., 1978. Rapid determination of metabolizable energy of foods using cockerels. Br. Poult. Sci. 19:303-308.

Filmer, D., 1991. A new system for livestock feeding. Feeds and Feed. Jul-Aug 1991.

Filmer, D., 1993. Applying nutrient allowances in practice – a new approach to growing poultry. The Eight International Poultry Breeders Conference Proceedings. Glasgow.

Fisher, C., 1980. Protein deposition in poultry. In Protein Deposition in Animals. P. J. Buttery & D. B. Linsday. Butterworths, London.

Fisher, C., 1984. Fat deposition in broilers. Pp. 437-470. In:Fats in Animal Nutrition. J. D. Wiseman, ed.Butterworths, London, England.

Forbes, E. B., and R. W. Swift, 1944. Associative dynamic effects of protein, carbohydrate, and fat. J. Nutr. 27:453.

Fraps, G. S., and E. C. Carlyle, 1942. Productive energy of some feeds and foods as measured by gains of energy by growing chickens. Texas Agric. Exp. Sta. Bull 625.

Fraps, G. S., 1943. Relation of the protein, fat and energy levels of the ration to the composition of chickens. Poultry sci. 22:421-424.

Fraps, G. S., 1946. Composition and productive energy of poultry feeds and rations. Texas Agric. Exp. Sta. Bull. 678.

Frederic. J. H., 1997. Diseases of Poultry. Poisons and Toxins. Iowa State University Press. 10:568-965.

Harper, A. E., N. J. Benevenga, and R. M. Wohlhueter, 1970. Effects of ingestion of disproportionate amounts of amino acids. Pysiol. Rev. 50:428.

Harris, L. E., 1966. Biological energy interrelationships and glossary of energy terms. Pub. 1411. National Academy of Sciences. National Research Council, Washington D. C.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and T. Larson, 1994. Growth, livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. Poultry Sci. 73:1785-1794.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and T. Larson, 1994. Carcass composition and yield of 1991 vs 1957 broilers when fed "typical" 1957 and 1991 broiler diets. Poultry Sci. 73:1795-1804.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and T. Larson, 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 randomized strain when fed "typical' 1957 and 1991 broiler diets. Poultry Sci. 73:1805-1812.

Hershko, A., and A. Ciechanover, 1982. Mechanisms of intracellular protein breakdown. Annu. Rev. Biochem., 51:335-364.

Hill, F. W., D. L. Anderson, R. Renner, and L. B. Carew, Jr., 1960. Studies of the metabolizable energy of grain and grain products for chickens. Poultry Sci. 39:573-579.

Horani, F., and J. L. Sell, 1977. Effect of feed grade animal fat on laying hen performance and on metabolizable energy of rations. Poultry Sci. 56:1972-1980.

Huff, W. E., J. A. Doerr, C. J. Wabeck, G. W. Chaloupka, J. D. May, and J. W. Merkley, 1984. The individual and combined effects on aflatoxin and ochratoxin A on various processing parameters of broiler chickens. Poult Sci. 63:2153-2161.

Huff, W. E., L. F. Kubena, R. B. Harvey and T. D. Phillips, 1992. Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poultry Sci. 71:64-69.

Jones, L. R., and J. Wiseman, 1985. Effect of nutrition on broiler carcass composition: Influence of dietary energy content in the starter and finisher phases. Br. Poult. Sci. 26:381-388.

Kielanowski, J., 1976. *Publs. Eur. Ass. Anim. Prod.* No. 15

Klasing, K. C., 1998. Comparative avian nutrition. CAB International. Wallingford, United Kingdom.

Koh, T. S. and S. J. Han, 1991. Effect of diet contaminated with subchronic levels of ochratoxin A on the lipid accumulation and heat production in chick. Korean J. Anim. Sci. 33:58-66.

Kubena, L. F., F. N. Reece, J. W. Deaton, and J. D. May, 1972. Heat prostration of broilers as influenced by dietary energy source. Poultry Sci. 51:1744-1747.

Kubena, L. F., R. B. Havery, W. E. Huff, M. H. Elissalde, A. G. Yersin, T. D. Phillips, and G. E. Rottinghaus, 1993. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and diacetoxyscirpenol. Poultry Sci. 72:51-59.

Kubena, L. F., R. B. Harvey, R. H. Bailey, S. A. Buckley, and G. E. Rottinghaus, 1998. Effects of a hydrated sodium calcium aluminosilicate on mycotoxicosis in young broiler chickens. Poultry Sci. 77:1502-1509.

Lamont, M. H., 1979. Cases of suspected mycotoxicosis as reported by veterinary investigation centers. Proc. Mycotoxins Anim. Dis., 3:38-39.

Lawes, J. B., and J. H. Gilbert, 1861. On the composition of oxen, sheep, pigs and of their increase whilst fattening. Journal of the Royal Agricultural Society of England. 21:1-92.

Ledoux, D. R., G. E. Rottinghaus, A. J. Bermudez, and M. Alonso-Debolt, 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poultry Sci. 78:204-210.

Leeson, S., G. Diaz, and J. D. Summers, 1995. Poultry metabolic disorders and mycotoxins. University Books. Guelph, Ontario, Canada.

Leeson, S., and J. D. Summers, 1997. Commercial poultry nutrition. University Books. Guelph, Ontario, Canada.

Lightsey, S. F., D. V. Maurice, and J. E. Jones, 1983. Dietary salt and abdominal fat in broilers. Poultry sci, 62:1352.

Mac Leod, M. G., and D. W. F. Shannon, 1978. Effects of food intake regulation on the energy metabolism of laying hens. Br. Poult. Sci. 19:349-369.

Marks, H. L., 1980. Water and feed intake of selected and nonselected broilers under *ad libitum* and restricted feeding regimens. Grwoth 44:205-219.

Marks, H. L., 1981. Role of water in regulating feed intake and feed efficiency of broilers. Poultry Sci. 60:698-707.

Marks, H. L., and K. W. Washburn, 1983. The relationship of altered water/feed intake ratios on growth and abdominal fat in commercial broilers. Poultry Sci. 62:263-272.

Marks, H. L., and G. M. Pesti, 1984. The roles of protein level and diet form in water consumption and abdominal fat pad deposition of broilers. Poultry Sci. 63:1617-1625.

Masimanco, N., J. Remacle, and J. Ramaut, 1973. Elimination of aflatoxin B1 by adsorbent clays in contamineated substrates. Ann. Nutr. Alimen. 23:137.

Mateos, G. G., and J. L. Sell, 1980. True and apparent metabolizable energy values of fat for laying hens: Influence of level of use. Poultry Sci. 59:369-373.

Matterson, L. D., L. M. Potter, M. W. Stutz, and E. P. Sengsen, 1965. The metabolizable energy of feed ingredients for chickens. Connecticut Agric Exp. Sta. Res. Rep. 7.

Maurice, D. V., 1981. Factors influencing carcass fat in broilers. Proceedings Georgia Nutrition Conference, p.32.

McDonald, M. L., and R. W. Swick, 1981. The effect of protein depletion and repletion on muscle-protein turnover in the chick. J. Biochem. 194:811-819.

Meltzer. A., 1983. Thermoneutral zone and resting metabolic rate of broilers. Br. Poult. Sci. 24:471-476.

Mittelstaedt, C. W., 1990. Feed Bioenergy Evaluation: Methodology as Applies to Growing Broiler.

Moran, E. T., Jr, J. D. Summers, and H. L. Orr, 1968. Back fat, qualitative measure of broiler carcass finish: technique, correlation with grade and effect of dietary caloric density. Food Technol. Champaign. 22:999-1002.

National Advisory Committee on Nutrition Education, 1983. A discussion paper on proposals for nutritional guidelines for health education in britain. London, Health Education Council.

National Research Council, 1994. Nutrient requirements of domestic animals: Nutrient requirements of poultry. National Academy of Sciences, Washington D.C.

Niemiec, J. and S. Scholtyssek, 1989. The response of broiler chickens to feed containing ochratoxin A. Annals of Warsaw Agricultural University SGGW-AR, Animal Science. 24:37-40.

Okoye, J. O., A. I. Asuzu, and J. C. Gugnani. 1988.Paralysis and lameness associated with aflatoxicosis in broilers. Avian Pathol 17:731-734.

Osborne, D. J., W. E. Huff, P. B. Hamilton, and H. R. Burtmeister, 1982. Comparison of ochratoxin, aflatoxin and T-2 toxin for their effects on selected parameters related to digestion and evidence for specific metabolism of carotenoids in chickens. Poult. Sci. 61:1646-1652.

Park, I. N., S. Shin, and R. R. Marquardt, 1991. Efects of niacin deficiency on the relative turnover rates of proteins in various tissues of Japanese quail. Int. J. Biochem., 23:1005-1012.

Perez, A. M., M. R. Weimar, and S. Cromer, 1991. pp: 110, in: U. S. Egg and Poultry Statistical Series, 1960-1990. United States Department of Agriculture, Economic Research Service, Station Bulletin 833, Washington D. C.

Pesti, G. M., and D. L. Fletcher, 1983. The response of male broiler chickens to diets with various protein and energy contents during the growing phase. Br. Poult. Sci. 24:91-99.

Pesti, G. M., T. S. Whiting, and L. S. Jensen, 1983. The effects of crumbling on the relationship between dietary density and chick growth, feed efficiency and abdominal fat pad weights. Poultry Sci. 62:490-494.

Pullar, J. D., and A. J. F. Webster, 1977. The energy cost of fat and protein digestion in the rat. Br. J. Nutr. 37:355.

Rao, V. N. and H. C. Joshi, 1993. Effect on certain drugs on acute induced aflatoxicosis in chicken. Indian Vet. J. 70:344-347.

Reddy, C. V., L. S. Jensen, L. H. Merrill, and J. McGinnis, 1962. Influence of mechanical alteration of dietary density on energy available for chick growth. J. Nutr. 77:428-432.

Rinehart, K. E., 1996. Environmental challenges as related to animal agriculture-poultry. In: Nutrient Management of Food Animals to Enhance and Protect the Environment. Kornegay, E. T., CRC Press, Inc.

Rotter, R. G., A. A. Frohlich, and R. R. Marquardt, 1989. Influence of dietary charcoal on ochratoxin A toxicity in Leghorn chickens. Can.J.Vet.Res. 53:449-453.

Schaeffer, J. L., J. K. Tyczowski, and P. B. Hamilton, 1988. Aflatoxin-impaired ability to accumulate oxycarotenoid pigments during restoration in young chickens. Poult. Sci. 67:619-625.

Scott, M. L., M. C. Nesheim, and R. J. Young, 1982. Nutrition of the chicken. M. L. Scott & Associates, Publishers.

Sherwood, D. H., 1977. Modern broiler feeds and strains; What two decades of improvement have done. Feedstuffs 49:70.

Sibbald, I. R., 1976. A bioassay for true metabolizable energy in feedingstuffs. Poultry Sci. 55:303-308.

Sibbald. I. R., 1976. The true metabolizable energy values of several feedingstuffs measured with roosters, laying hens, turkeys and broiler hens. Poultry Sci. 55:1459-1463.

Sibbald, I. R., 1976. The measurement of true metabolizable energy in poultry feedingstuffs. Proc. Guelph Nutr. Conf., Toronto, Canada. Pp. 102-114.

Sibbald, I. R., 1986. The T.M.E. system of feed evaluation:methodology, feed composition data and bibliography. Anim. Res. Ctr., Agricult. Canada. (85) 19.

Stevens, L., 1996. Avian biochemistry and molecular biology. Cambridge University Press. Stirling University, Scotland.

Sturkie, P. D., 1986. Avian Physiology. Springer-Verlag New York Inc. New York.

Summers, J. D., S. J. Slinger, and G. C. Ashton, 1965. The effect of dietary energy and protein on carcass composition with a note on a method for estimating carcass composition. Poultry Sci. 44:501-509.

Summers, J. D., and S. Leeson, 1979. Composition of poultry meat as affected by nutritional factors. Poultry Sci. 58:536-542.

Summers, J. D., and S. Leeson, 1985. Broiler carcass composition as affected by amino acid supplementation. Can J. Anim. Sci. 65:717-723.

Summers, J. D., S. Leeson, and D. Spratt, 1988. Yield and composition of edible meat from male broilers as influences by dietary protein level and amino acid supplementation. Can J. Anim. Sci. 64:241-248.

Summers, J. D., D. Spratt, and J. L. Atkinson, 1992. Broiler weight gain and carcass composition when fed diets varying in amino acid balance, dietary energy, and protein level. Poultry Sci; 72:263-273.

Swain, S., and D. J. Farrell, 1975. Effect of different temperature regimens on body composition and carry-over effects on energy metabolism of growing chickens. Poultry Sci. 54:513-520.

Swick, R. W., 1982. Growth and protein turnover in animals. Crit. Rev. Food. Sci. Nutr., 16:117-126.

Tarver, F. R., Jr., 1986. pp:20-22, in: Poultry and egg marketing National Food Review. United States Department of Agriculture, Economic Research Service, Washington, DC.

Teeter, R. G., M. O. Smith, F. N. Owens, S. C. Arp., S. Sangiah, and J. E. Breazile, 1985. Chronic heat stress and respiratory alkalosis: Occurrence and treatment in broiler chicks. Poultry Sci 64:1060-1064.

Teeter, R. G., and M. O. Smith, 1986. High chronic ambient temperature stress effects on broiler acid-base balance and their response to supplemental ammonium chloride and potassium chloride and potassium carbonate. Poultry Sci. 65:1777-1781.

Teeter, R. G., M. O. Smith, S. Sangiah, and F. B. Mather, 1987. Effect of feed intake and fasting duration upon body temperature and survival of thermostressed broilers. Nutri. Rep. Int. 35:531-537.

Thomas, O. P., and P. V. Twining, 1971. Broiler nutrition during the withdrawal period (7-8 $^{1}/_{2}$ weeks). Proc. Md. Nutr. Conf., 87-90.

Thorbek, G., 1970. Publs. Eur. Ass. Anim. Prod. 13:120.

Titus, H. W., 1961. The scientific feeding of chickens. 4th Ed., Interstate, Danville, Illinois.

Torchinsky, Y. M., 1937. Transamination: Its discovery, biological and clinical aspects. Trends Biochem. Sci.. 12:115

Tyczkowski, J. K. and P. B. Hamilton, 1987. Altered metabolism of carotenoids during aflatoxicosis in young chickens. Poult. Sci. 66:1184-1188.

USDA, 1999. Statistics and highlights of U. S. agriculture. National Agriculture Statistics Service. United States Department of Agriculture. Bulletin no. 964.

Van Kampen, M., B. W. Mitchell, and H. Siegel, 1979. Thermoneutral zone of chickens as determined by measuring heat production, respiration rate, electromyographic and electroencephalographic activity in light and dark environments and changing ambient temperatures. J. Agr. Sci. 92:219-226.

Wiernusz, C. J., and R. G. Teeter, 1993. Feeding effects on broiler thermobalance during thermoneutral and high ambient temperature exposure. Poultry Sci. 72:1917-1924.

Wiggins, D., P. Lund, and H. A. Krebs, 1982. Adaptation of ureate synthesis in chicken liver. Comp. Biochem. Physiol., 72B:565.

CHAPTER III

AFLATOXIN AND OCHRATOXIN EFFECTS ON PERFORMANCE OF MEAT TYPE STRAIN BIRDS[1]

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Abstract 1. Two experiments were conducted to study dietary mycotoxin effects.

2. The first experiment examined low concentrations of Aflatoxin B1 (1 or 0.5mg/kg) and Ochratoxin A (0.5 or 0.25mg/kg). Results indicated no effects on body weight gain, feed intake, feed efficiency, and organ weights and integrity. In the second experiment both mycotoxin values were increased to 2mg/kg and adsorbent supplementation was examined (Flobond®[1]; 2.5 kg, ton). A 2x2x2 factorial was applied in a randomized complete block design.

3. Final body weight was depressed by dietary ochratoxin at day 21 by 5.5% ($p<0.01$). The adsorbent increased body weight of birds by 5.1% ($p<0.01$). When birds were fed dietary aflatoxin, feed consumption increased 8% in birds supplemented with adsorbent ($p<0.01$). When birds were fed no adsorbent feed consumption was depressed by 5%($p<0.01$). Ochratoxin reduced total feed consumption by 7% ($p<0.05$). Body temperature was higher in birds consuming dietary aflatoxin ($p<0.05$). Total water consumption was improved by almost 7% when adsorbent was present ($p<0.05$). Dietary aflatoxin reduced hematocrit values by 4% ($p<0.05$). Lymphoid organ weights were reduced ($p<0.05$) when birds consumed dietary mycotoxins, and liver weights were affected by the presence of aflatoxin in the feed ($p<0.01$).

4. In conclusion, data quantitatively address mycotoxin effects and suggests a potential therapeutic alternative for the prevention of mycotoxicosis in broilers.

(Key words: broiler, mycotoxin, aflatoxin, ochratoxin, adsorbent)

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INTRODUCTION

Mycotoxins are a group of fungal derived compounds, capable of producing negative health consequences in all poultry classes. They can be found in crops at all stages of the production, harvest and storage processes (CAST, 1989). While hundreds of mycotoxins are recognized, the toxicity and target organ varies. Mycotoxins have several modes of action, but repercussions over the birds' integrity is the primary consequence of these compounds (Fredric, 1997).

Aflatoxins are highly toxic and carcinogenic mycotoxins produced by fungal species of the genus *Aspergillus*. The most common types of *Aspergillus* are *A. Flavus* and *A. Parasiticus*. Other fungi such as *Penicillium Puberulum* may produce aflatoxins. Of these mycotoxins, Aflatoxin B1 is the most potent, binding to nuclear and mitochondrial DNA (Edds, 1979). Consequences of aflatoxicosis include increased mortality from heat stress in broiler breeders (Cunningham, 1987), depression in egg production (Bryden *et al.*,1980), anemia, hemorrhages, and liver condemnations among other lesions. A bird can also suffer from paralysis and lameness (Lamont, 1979), as well as impaired performance (Okoye *et al.*, 1988). Effects on broiler performance include weight loss followed by depression, ataxia and recumbency (Leeson *et al.*, 1995).

Ochratoxin is another compound from fungal origin capable of producing harmful effects on livestock production. Microbial ochratoxin producers include *A. Ochraceus, A. Malleus, A. Ostianus, A. Petrakki,* among others (Leeson *et al.*, 1995). The most toxic of these compounds is ochratoxin A, which is capable of inducing gross and microscopic lesions in the kidney and liver of livestock, and is a renal carcinogen in

rats (Boorman *et al., 1992)*. The minimum dietary growth inhibitory concentration of ochratoxin A for the young broiler chick is 2 mg/kg, which is a lower concentration than that required by aflatoxin and T-2 Toxin which is 2.5 and 4mg/kg, respectively (Morehouse, 1985). This naturally occurring toxin induces a reduction in growth rate, and feed conversion at levels as low as 1.5mg/kg (Niemiec and Scholtyssek, 1989), as well as an increase in heat production in broilers (Koh and Han, 1991).

Methods of prevention or detoxification for aflatoxin contamination include grain quality control, fermentation, inactivation of microbial organisms, thermal inactivation and irradiation, among others (CAST, 1989). The addition of adsorbents has also been suggested as an alternative for the prevention of alfatoxicosis (Masimanco *et al.*, 1973). Hydrated sodium calcium aluminosilicate was recently demonstrated to prevent growth depression and organ damage in chicks fed up to 4mg/kg of aflatoxin (Ledoux *et al.*, 1999). In contrast, the addition of 0.5% sodium calcium aluminosilicate has not been observed to reduce ochratoxin A toxicity at 2mg/kg in broiler diets (Huff *et al.*, 1992).

Dale (1998) mentioned the need of testing the variety of commercial adsorbents that have for the most part only been tested *in vitro*. Similarly, charcoal supplementation to diets containing 4mg/kg of ochratoxin A has not been beneficial (Rotter *et al.*, 1989). Relatively little data regarding consequential effects of aflatoxin and ochratoxin combinations are in the literature. The potential for negative synergistic impact may enhance therapeutic effects of such compounds as hydrated sodium calcium aluminosilicate. Therefore, the objective of the study reported herein was to further

examine different mycotoxin levels as well as hydrated sodium calcium aluminosilicate therapeutic effect in poultry rations containing aflatoxin and ochratoxin combinations.

MATERIALS AND METHODS

Rations were formulated to contain 12.97 MJ/kg of metabolizable energy and 22.5% crude protein. Basal diet composition is given in Table 3. Dietary rations were analyzed for aflatoxin (AF) and ochratoxin (OC) content using HPLC[1] versions 96.3 and 97.4 respectively, and ELISA[2] procedure.

Birds and housing

Commercial Cobb x Cobb meat type strain chicks were obtained from a local Cobb hatchery. Birds were randomly allocated in calorimetry chambers. Feed and water was supplied *ad-libitum*. Light was provided continuously to all chambers, and temperature was adjusted to be at thermoneutral during the course of the study.

All calorimetry chambers were continuously monitored for oxygen consumption and carbon dioxide production. Heat production (HP) and net energy values (NE) were determined using indirect calorimetry methodologies by applying oxygen and carbon dioxide values to a heat production multiple regression equation (Brouwer, 1965).

Statistical Analysis

All data were analyzed statistically using the Mixed procedure of SAS® (SAS, 1996). Differences in treatment means were considered significant at $P \leq 0.05$. When multiple means differed, they were separated using a least squares means test.

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Experiment I

Three hundred meat type strain chicks were individually weighed and wing-banded. Chicks were randomly allocated in calorimetry chambers up to 2 weeks of age. The experimental design of the experiment was a completely randomized design with 6 replicates per treatment. Treatment 1 was the control group; treatment 2 contained 0.5 and 0.25mg/kg of AF and OC, respectively; treatment 3 contained 1 and 0.5mg/kg of AF and OC, respectively; treatment 4 contained 1mg/kg of AF, and treatment 5 contained 0.5mg/kg of OC. The chicks were fed for 2 weeks with individual body weight (BW), and feed consumption (FC) monitored on a weekly basis. Upon termination of the experiment 25 birds from each treatment were euthanized via carbon dioxide chamber, and the liver and bursae examined for macroscopic lesions and weight.

Experiment II

Three hundred meat type chicks was obtained, individually weighed, body temperature (BT) taken via rectum and wing-banded. The chicks were randomly allocated in calorimetric chambers up to week 3. The experimental design of the study was a 2x2x2 Factorial Arrangement of Treatments where AF, OC and adsorbent (AS) presence or absence determined the 8 dietary treatments, with 8 replications per treatment. Diets with AF, OC and AS presence contained 2mg/kg, 2mg/kg, and 2.5 kg/ton of these components, respectively. The chicks were fed up to 3 weeks with BW, FC, water consumption (WC) as well as BT being monitored on a weekly basis.

Upon termination of the experiment, 25 birds from each treatment were randomly selected for blood collection and necropsy. Blood was obtained via vena-puncture for hematocrit determination. The birds were euthanized via carbon dioxide chamber. At necropsy the thymus, spleen, liver and bursa of Fabricius of each bird was removed and organ weighed recorded. Tissue selections of thymus, spleen, liver, bursa of Fabricius and kidney were taken and fixed in 10% buffered neutral formalin for histopathologic examination. Lymphoid organs were evaluated for degree of pynkosis, karyorrhexis of lymphocytes and overall lymphoid depletion. Liver lesions were evaluated for bile duct proliferation, hepatocyte necrosis and hemorrhage. Kidneys were evaluated for tubular epithelium damage. Lesions of each organ were subjectively scored as follows: score 0-no lesion, score 1-mild severity, score 2-moderate severity, score 3-marked severity.

RESULTS

Experiment I

None of the variables monitored revealed any significant difference at any stage during the study. Body weights and feed intake values are shown in Table 4. Mycotoxin concentrations used in this study failed to produce an effect on either the integrity of the liver or its weight. Bursa weight and macroscopic integrity were not affected by the toxins and did not reflect significant differences between treatments. Liver and bursa weights are shown in Table 4. Oxygen consumption and carbon dioxide production values when applied to an indirect calorimetry equation to determine HP did not reveal significant differences nor for NE values.

Experiment II

No BW main or simple effect at day 7 was seen, but BW was depressed ($p<0.01$) by dietary OC at days 14 and 21 by 7.7% and 5.5% respectively. Adsorbent supplementation prevented depression in BW at days 14 and 21 by 4.6% and 5.1% respectively ($p<0.01$), when compared to non-supplemented treatments, as displayed in Table 5.

Feed consumption was reduced during the first week ($p<0.05$) for birds consuming dietary AF group by 5%. During the second week, a two-way interaction was observed, where treatments that consumed dietary AF had 10% higher FC with AS supplementation when compared to non-supplemented treatments ($p<0.05$). Also, in the absence of AS, the birds that consumed AF free diets had 7% higher FC. Adsorbent supplementation increased FC during the third week ($p<0.01$) by 5% when compared to non-supplemented treatments. During the third week, dietary OC reduced FC ($p<0.01$) by 4% when compared to control. Total feed consumption (TFC) in birds that consumed dietary AF with adsorbent supplementation was 8% higher when compared to non-supplemented AF treatments ($p<0.05$). As a main effect, when birds were fed no adsorbent, TFC was depressed by 5%. Dietary OC also reduced TFC by almost 7% ($p<0.01$).

No differences in WC were seen during the first week, but was 6% higher during the second week ($p<0.05$) in birds supplemented with AS, when compared to non-supplemented birds. During the third week, dietary AF depressed WC by 16% ($p<0.05$) when compared to AF-free treatments. Dietary AF fed without AS produced a 17% reduction ($p<0.01$) during the third week. Total water consumption (TWC) was

improved by 7% with AS supplementation ($p<0.05$) when compared to non-supplemented treatments, and AF also reduced WC values ($p<0.05$) when compared to AF-free treatments. WC is displayed in Table 6.

When WC and FC values are merged and analyzed as water:feed ratio, a main effect was seen for birds consuming dietary OC during the second week and for cumulative values ($p<0.05$). No differences were observed in feed efficiency throughout the study for any effect. However, AS supplementation analyzed as a main effect, improved by two points the feed efficiency of the birds.

Increase in BT was observed with dietary AF when compared to AF -free diets. Differences were seen at days 7, 14 and 21, in which birds consuming dietary AF had increased BT than birds consuming feed without it ($P<0.05$).

No differences in hematocrits were seen, with the exception of AF as a main effect ($P<0.05$), where dietary AF reduced hematocrit values of birds by 4% when compared to AF -free treatments. Organ weights were also affected with the presence of mycotoxins as shown in Table 6, with AF impacting liver and spleen weights. Liver weights were increased by 11% in birds consuming dietary AF ($p<0.01$). Spleen weights were increased 17% by dietary AF when compared to the control group. Ochratoxin depressed thymus weight due to the suppressive effect of this toxin on lymphoid organs of the bird. Adsorbent supplementation improved thymus and bursa weights maintaining weights similar to those of control.

Histopathologic scores revealed a three-way interaction of AF, OC and AS on the liver ($p<0.01$), where dietary AF produced mild lesions in hepatic tissue and AS supplementation prevented it. When OC interacted with AF, an increase in the

frequency and severity of the lesion was observed, but AS supplementation failed to prevent it. No other histopathologic observations were seen.

If we analyze Net energy on a "per kilogram of feed consumed" (NE), no difference was observed for any effect or period of the study. The same result was obtained for heat production (Table 7).

DISCUSSION

Experiment I

Reports by Kubena *et al.* (1998), Ledoux *et al.* (1999), Edds and Bortell (1983) suggest that BW is depressed when birds are exposed to higher levels of dietary AF and OC than the levels used in Experiment I. Feed intake did not reflect treatment differences, in contrast to data reported previously (Edds and Bortel, 1983), therefore it can be stated that the levels of dietary mycotoxin utilized in Experiment I may be below the minimum dietary growth inhibitory concentrations for these toxins.

The liver has been considered to be the target organ for AF in the broiler (Kubena *et al.*, 1993), but it is presumed that mycotoxin levels in this study were not sufficient enough to produce such effect. As described before by Ruff *et al* (1992), OC suppresses lymphoid organs, but as it was seen with AF effect in the liver, OC failed to produce such effect.

In conclusion, based upon the data reported in Experiment I, depressed growth rates and feed intake reduction caused by dietary mycotoxicosis of AF or OC origin might be evidenced when their concentrations in the feed are higher than the values used in this experiment.

Experiment II

In contrast with Experiment I, in Experiment II BW was depressed as seen by Kubena *et al.* (1998), Ledoux *et al.* (1999), Edds (1979). As reported previously (Edds and Bortel, 1983), feed consumption was affected in birds consuming dietary AF.

Kubena *et al* (1993; 1998) also reported liver and spleen lesions affected by dietary mycotoxin treatments. Adsorbent supplementation effectively prevented microscopic lesions in Experiment II. In agreement with Ruff *et al* (1992), the thymus was a target for OC. However, the adsorbent effectively prevented this OC toxicity. No histopathologic observations were seen in the study, except the AF effects on hepatic tissue. This may be due to the low mycotoxin concentrations used compared to previous studies. The duration of the study could have also been too short to allow lesions to develop.

In disagreement with Koh and Han (1991), dietary OC failed to increase heat production during all weeks of the study. The increase in BT seen in birds consuming dietary AF was observed at all phases of the study. Gale (1973) suggested that an increase in BT could be due to a positive heat balance due to skin vasoconstriction leading to an increase heat production. These cases of hyperthermia can be associated with a toxic effect of AF.

In conclusion, based upon the data reported in Experiment II, depressed growth rates and feed intake reduction caused by mycotoxicosis of AF or OC origin are reduced by supplementing with hydrated sodium calcium aluminosilicate. It can also be concluded that minimum dietary growth concentration for AF and OC are at least at levels of 2mg/kg for chicks raised up to 3 weeks of age.

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REFERENCES

BOORMAN, G. A., McDONALD, M. R., IMOTOT, S., & PERSING, R. (1992). Renal lesions induced by ochratoxin A in the F344 rat. *Toxicology and. Pathology*, **20**:236-245.

BROUWER, E., 1965. Report of sub-committee on constants and factors. In: *Energy metabolism*, pp. 441-443. K.L.

BRYDEN, W. L., LLOYD, A. B., & CUMMING, R. B. (1980) Aflatoxin contamination of Australian animal feeds and suspected cases of mycotoxicosis. *Australian Veterinary Medicine,* **56:**176-180.

CAST (1989) Mycotoxins: Economic and Health Risks. K. A. Nisi, ed. *Council for Agricultural Science and Technology*, Ames, IA. pp 1-91

CUNNUNGHAM, P. (1987) Mycotoxin problems appear to be growing worse. *Poultry Times*, No.**34**, 24:19.

DALE, N. (1998) Mycotoxin Binders: It's time for real science. *Poultry Digest,* **57**:38-39.

EDDS, G. T. (1979) Aflatoxins. N W. Shimmed, in: Vongrttrnvr on mycotoxins in *Animal feeds and grains related to animal health, food and drug administration. FDA/ BVM* (Eds), Vol. 79, No.139, pp.80-164.

EDDS, G. T., & BORTELL, R.R. (1983) Biological effects of aflatoxin in poultry. in: Aflatoxin and *Aspergillus flavus*. DIENER, R.L. & J.W. DICKENS, J. W. (Eds). *Southern Cooperative Services Bulletin.* 279. Auburn University, Alabama. pp.55-61.

FREDERIC. J. H. (1997) Diseases of Poultry, In: *Poisons and Toxins.* Iowa State University Press.

GALE, C. C. (1973) Neuroendocrine aspects of thermoregulation. *Annual. Revision of. Physiology*, **35**:391-430.

HUFF, W. E., KUBENA, L. F., HARVEY, R. B., & PHILLIPS, T. D. (1992) Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. *Poultry Science*, **71**:64-69.

KOH, T. S. & HAN, S. J. (1991) Effect of diet contaminated with subchronic levels of ochratoxin A on the lipid accumulation and heat production in chick. *Korean Journal of Animal Science*, **33**:58-66.

KUBENA, L. F., HARVEY, R. B., HUFF, W. E., ELISSALDE, M. H., YERSIN A. G., PHILLIPS, T. D. & ROTTINGHAUS, G. E. (1993) Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and diacetoxyscirpenol. *Poultry Science*, **72**:51-59.

KUBENA, L. F., HARVEY, R. B., BAILEY, R. H., BUCKLEY, S. A., & ROTTINGHAUS, G. E. (1998) Effects of a hydrated sodium calcium aluminosilicate on mycotoxicosis in young broiler chickens. *Poultry Science*, **77**:1502-1509.

LAMONT, M. H. (1979) Cases of suspected mycotoxicosis as reported by veterinary investigation centers. *Proceedings: Mycotoxins Animal. Diseases*, No. 3, pp. 38-39.

LEDOUX, D. R., ROTTINGHAUS, G. E., BERMUDEZ, A. J., & ALONSO-DEBOLT, M. (1999). Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. *Poultry Science*, **78**:204-210.

LEESON, S., DIAZ, G., & SUMMERS, J. D. (1995*). Poultry metabolic disorders and mycotoxins*. University Books. Guelph, Ontario, Canada.

MASIMANCO, N., REMACLE, J., & RAMAUT, J. (1973) Elimination of aflatoxin B1 by adsorbent clays in contamineated substrates. *Annual Nutrition Alimen* **23**:137.

MOREHOUSE, L. G. (1985). Mycotoxins of veterinary importance in the United States, in: LACEY, J. (Eds) *Trichothecene and Other Mycotoxins*. John Wiley and Sons Ltd, New York, pp. 383-410.

NIEMIEC, J. & SCHOLTYSSEK, S. (1989). The response of broiler chickens to feed containing ochratoxin A. *Annuals of Warsaw Agricultural University SGGW-AR, Animal Science*, **24**:37-40.

OKOYE, J. O., ASUZU, A. I., & GUGNANI, J. C. (1988) Paralysis and lameness associated with aflatoxicosis in broilers. *Avian Pathology*, **17**:731-734.

ROTTER, R. G., FROHLICH, A. A., & MARQUARDT, R. R. (1989) Influence of dietary charcoal on ochratoxin A toxicity in Leghorn chickens. *Canadian.Journal of Veterinary Research*, **53**:449-453.

RUFF, M. D., HUFF, W. E., & WILKINS, G. C. (1992) Characterization of the toxicity of the mycotoxins, aflatoxin, ochratoxin and T-2 toxin in game birds. *Avian Diseases*, **63**:34-39.

SAS Institute, SAS/STAT 1996. Changes and enhancements guide. Version 6.12., SAS Institute Inc., Cary, NC.

Table 3

Basal ration composition[1]

Ingredients	%
Yellow grain corn	58.3
Soybean meal dehulled	29.2
Pro-Pak	5.0
Vegetable fat	3.6
Calcium carbonate	1.24
Dicalcium phosphate	0.65
Salt	0.34
DL-Methionine	0.15
Vitamin premix[2]	0.1
Mineral premix[3]	0.1
Copper sulfate	0.03
Selenium	0.002

[1] Contained (by calculation) 22.64% CP, 1%calcium, 0.62% total phosphorus and 12.97 MJ of ME/kg of diet.
[2] Premix contained: vitamin A 720 *m*g, vitamin D_3 8 *m*g, vitamin E 10.0mg/g, vitamin B_{12} 3.5 mg/g, riboflavin, 2.2 mg/g, niacin 6.6 mg/g, d-pantothenic acid 7.055 mg/g; choline 176.36 mg/g; menadione 0.52 mg/g, and d-biotin, 44 mg/g.
[3] Premix contained manganese, 12%; zinc, 8%; iron, 6%; copper, 10%, iodine, 0.1% and calcium, 18%.
Basal ration is based on minimum values, binder level of inclusion was 2.5kg/ton or 0.25%.

Table 4

Body weight, feed intake, and organ weight for birds reared in experiment 1.[1]

Treatment[2]	Final body weight (g)	Total feed intake (g)	Liver weight (g)	Bursa Weight (g)
Control	347.0	375.9	11.48	0.801
AF 0.5 / OC 0.25	329.2	364.1	12.09	0.755
AF 1.0 / OC 0.5	324.6	354.2	11.85	0.813
AF 1.0	342.8	380.7	12.26	0.785
OC 0.5	331.5	367.2	12.42	0.824
P value >	NS	NS	NS	NS

[1]Each value represents the mean of 6 replicate measurements for feed intake and all birds submitted to a dietary treatment.
[2] AF = dietary aflatoxin (mg/kg), OC = dietary ochratoxin (mg/kg).

Table 5

Weekly body weight, and feed intake for birds reared in experiment II [1] *(g).*

Treatment [3]	BW day7[2]	FI week 1	BW day14	FI week 2	BW day21	FI week 3	Total FI
Control	123.0	92.3	321.1^{a}	259.9^{b}	587.5^{b}	400.9^{ab}	752.9^{b}
AF	123.5	96.0	314.3^{ab}	249.0^{c}	575.8^{bc}	381.1^{bc}	726.6^{bc}
OC	121.1	91.2	299.1^{bc}	237.9^{c}	564.1^{bc}	384.9^{bc}	714.1^{c}
AF OC	125.6	93.0	282.5^{c}	216.5^{d}	537.8^{c}	360.3^{c}	699.4^{d}
AS	120.3	90.5	330.3^{a}	261.9^{b}	606.4^{a}	402.1^{ab}	754.7^{b}
AF AS	123.0	97.5	329.6^{a}	275.4^{a}	618.5^{a}	417.4^{a}	789.9^{a}
OC AS	116.6	86.3	308.6^{b}	250.3^{bc}	583.1^{b}	401.4^{ab}	738.2^{bc}
AF OC AS	122.3	92.5	304.5^{b}	235.3^{c}	572.0^{bc}	386.2^{bc}	713.6^{cd}
AS	NS	NS	0.01	0.01	0.01	0.05	0.01
AF	NS	0.05	NS	0.05	NS	NS	NS
OC	NS	NS	0.01	0.01	0.01	0.05	0.01
AF x OC	NS	NS	NS	0.01	NS	NS	NS
AF x AS	NS	NS	NS	0.05	NS	NS	0.05
OC x AS	NS	NS	NS	NS	NS	NS	NS

[a,b,c,d] Means within a column without common superscript differ ($P<0.05$).

[1] Each value represents the mean of 7 replicate measurements.

[2] BW = body weight, FI = feed intake.

[3] AF = dietary aflatoxin; OC = dietary ochratoxin; AS = adsorbent supplementation.

Table 6

Water consumption/bird (ml), and organ weight (g) for birds reared in experiment II [1].

Treatment [3]	WC[2] week2	WC week3	Total WC	Liver	Thymus	Spleen	Bursa
Control	472.7^{abc}	832.9^{ab}	1602^{ab}	17.02^{c}	1.68^{b}	0.67^{c}	1.62^{c}
AF	443.0^{c}	771.4^{bc}	1523^{bc}	18.71^{ab}	1.50^{bc}	1.00^{a}	1.63^{bc}
OC	491.0^{a}	902.6^{a}	1697^{a}	18.57^{ab}	1.52^{bc}	0.79^{bc}	1.81^{abc}
AF OC	463.9^{bc}	701.0^{c}	1433^{c}	20.55^{a}	1.45^{c}	0.92^{ab}	1.55^{c}
AS	499.5^{a}	884.6^{ab}	1692^{a}	18.22^{bc}	2.11^{a}	0.87^{abc}	2.05^{a}
AF AS	501.6^{a}	922.6^{a}	1750^{a}	19.49^{ab}	1.80^{ab}	0.81^{abc}	1.91^{ab}
OC AS	492.8^{a}	865.0^{ab}	1674^{ab}	17.47^{c}	1.60^{b}	0.75^{bc}	1.96^{ab}
AF OC AS	483.2^{ab}	866.3^{ab}	1644^{ab}	20.94^{a}	1.71^{b}	0.89^{ab}	1.66^{bc}
AS	0.05	0.05	0.05	NS	0.01	NS	0.05
AF	0.05	NS	0.05	0.01	NS	0.05	NS
OC	NS	NS	NS	NS	0.01	NS	NS
AF x OC	NS	NS	NS	NS	NS	NS	NS
AF x AS	NS	NS	NS	NS	NS	NS	NS
OC x AS	NS	NS	NS	NS	NS	NS	NS

[a,b,c,d] Means within a column without common superscript differ ($P<0.05$).

[1] Each value represents the mean of 7 replicate measurements.

[2] WC = water consumption.

[3] AF = dietary aflatoxin; OC = dietary ochratoxin; AS = adsorbent supplementation.

Table 7

Heat production and net energy efficiency for birds reared in experiment II[1]

Treatment[2]	Heat production 0-7 days MJ / bird	Heat production 7-14 days MJ / bird	Heat production 14-21 days MJ / bird	Net energy Efficiency
Control	0.509	1.558	2.561	52.7
AF	0.550	1.699	2.637	48.8
OC	0.520	1.701	2.659	48.3
AF OC	0.539	1.702	2.339	49.4
AS	0.481	1.541	2.868	48.7
AF AS	0.506	1.473	2.791	51.0
OC AS	0.512	1.681	2.627	49.4
AF OC AS	0.532	1.654	2.664	48.2
AS	NS	NS	NS	NS
AF	NS	NS	NS	NS
OC	NS	NS	NS	NS
AF x OC	NS	NS	NS	NS
AF x AS	NS	NS	NS	NS
OC x AS	NS	NS	NS	NS

[1]Each mean represents 8 replication measurements.
[1]AF = dietary aflatoxin; OC = dietary ochratoxin; AS = adsorbent supplementation

CHAPTER IV

A TIME DEPENDANT EVALUATION OF THE BROILERS' 0 TO 42 DAY DIETARY PROTEIN REQUIREMENT[1]

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ABSTRACT An experiment was conducted whereby classical (CFS), constant dietary protein fed as starter, grower and finisher was contrasted with a dynamic adjustment (DFS). For DFS, dietary protein was changed daily to meet a projected daily protein need. The experiment comprised a 2x3 factorial composed of three different protein levels, high (NRC +2.2% CP), medium (NRC CP requirement), and low (NRC – 2.2% CP), and two feeding systems (classical, dynamic). Feed ingredients used in the study were tested for MEn, amino acid and protein content, and such values used to assure ration content per formulation. Diets were formulated to be isocaloric during the various feeding periods and to exceed indispensable amino acid requirements by at least 3% (NRC, 1994). This study utilized male Cobb 500 birds, randomly allocated in 54 floor pens up to 6 weeks of age. Upon completion of the starter (21d), grower (35d) and finisher (42d) phases, 3 birds from each pen were processed for carcass composition. Body weight (BW) and feed intake (FI) was higher ($P<0.01$) for birds consuming low protein diets ($P<0.01$). Birds in DFS treatments exhibited higher ($P<0.01$) FI at all the phases of the study while BW did not differ and feed conversion (FC) was elevated ($P<0.01$). When protein intake was quantified, dynamic treatments had significantly lower intakes and a more efficient protein utilization with regard to BW, carcass weight, breast weight and breast as a percentage of carcass ($P<0.01$). However, birds on DFS as well as low protein diets also had a higher abdominal fat ($P<0.01$) and total carcass fat ($P<0.01$), as well as higher leg quarter weights and dressing percentages ($P<0.01$). Mortality caused by ascites was more frequent in CFS ($P<0.05$) when compared to DFS, particularly for birds that consumed high protein diets. In conclusion, dynamic protein feeding has the potential to elevate protein utilization efficiency, but fails to optimize

overall feed conversion. It also resembles the importance of substrate impact on oxygen requirement as evidenced by the increase in ascites incidence in the high protein treatments.

(Key words: protein, broiler, carcass, energy, ascites)

INTRODUCTION

Broiler protein and amino acid requirements have classically been determined in timed growth phases or feeding periods (Almquist, 1947), and up to today the same criteria is being applied when feeding broilers (NRC, 1994). Within such periods nutrients are provided in a constant quantity. When periods are switched to the next interval, an immediate reduction in protein and amino acid content occurs. For example, the protein requirement (NRC, 1994) established for today's broiler is divided into 3 phases. A starter protein of 23% CP fed to 3 weeks of age, a grower phase with 20% CP fed from 3 to 6 weeks, and a finisher phase with 18% CP fed to 8 weeks of age. Energy is set at 3200 Kcal/kg of MEn during all the phases (NRC, 1994). Often times this feeding strategy fills the physical contrasts of feedmills as they can only efficiently provide and deliver 3 to 4 feed types without significant manufacturing problems.

The NRC (1994) and numerous publications (Almquist, 1947; Hurwitz *et al.*, 1978; Baker *et al.*, 1978; Klain *et al.*, 1965; D'Mello, 1974; Edwards *et al.*, 1956; Moran, 1981; Nelson *et al.*, 1960) make it evident that broiler protein and amino acid requirements decline with bird age. Such changes presumably occur in continuous fashion and not abruptly on specific days. If so, then NRC requirements satisfy bird nutrient needs only for an instant and during the rest of this phase the bird is either

underfed or overfed dietary protein. Underfeeding results in the bird not having nutrients to maximize production, while and overfeeding can represent a lower economic efficiency for protein and in addition, overfeeding would contribute to pollution concerns (de Lange, 1993).

An alternative requirement approach, herein termed dynamic feeding, offers ideology of expressing requirements as decay functions. The goal is to provide a closer nutrient fulfillment according to bird daily needs. Not only nutritional advantages will become apparent. but also other non-nutrition benefits.

Calculations reveal that a male broiler, with average feed intake per NRC's recommendations, will have a 70 gram higher protein intake than a bird fed on the dynamic system. Reducing protein consumption would increase net energy values of the feed due to carbohydrates' higher efficiency for lipogenesis compared to protein (Pullar and Webster, 1974; Blaxter, 1989; Mittelstaedt , 1990), but also decrease feed cost because high protein diets are typically more expensive.

For that purpose, an experiment was conducted whereby classical (NRC type) versus a dynamic adjustment was compared in order to evaluate broiler performance, protein efficiency, and evaluate broiler composition with diets varying in protein supply.

MATERIALS AND METHODS

An experiment utilizing 1080 Cobb x Cobb male broilers was conducted to examine effects of 3 dietary crude protein levels (NRC, NRC+ 2.2%, NRC- 2.2%) and 2 feeding systems (classical, dynamic) arranged in a factorial arrangement of treatments. Classical feeding entailed providing dietary protein as a constant proportion of the diet,

while dynamic declined daily as displayed in Figure 7. The declining dietary crude protein was achieved by gradually blending grower ration into starter, finisher into grower, and a withdrawal ration into finisher. At the beginning of the starter, grower and finisher periods chicks received 100% of their respective ration.

Chicks were placed in 54 floor pens with 9 pens per treatment, wing-banded and randomly allocated 20 chicks per pen. All pens were blocked by position forming a randomized complete block experimental design.

To assure ration composition, feed ingredients were analyzed for amino acid content using a Beckman 6300 AA analyzer following AOAC (1990) procedure[3], ether extract and starch analysis (AOAC, 1994) and MEn utilizing adult cockerels (Hill *et al.*, 1958). Experimental diets were thus formulated to be isocaloric at 3100 Kcal MEn/Kg (Table 8). Feed and water were supplied *ad-libitum* and temperature was adjusted as specified in the Cobb 500 broiler management guide (1994).

Since feed for the DFS was changed daily, FI was determined for all pens on a daily basis. Individual BW was determined on days 0, 21, 35 and 42. Carcass specific gravity and dry matter were used to estimate carcass lean and fat content according to Wiernusz *et al.* (1999).

On day 42 a bird selected from each pen was weighed, fasted for 36 hours and randomly allocated to calorimetry chambers (Belay and Teeter, 1993; Wiernusz and Teeter, 1993) for quantification of fasted heat production. Heat production was quantified using indirect calorimetry (Brouwer, 1965). Upon completion of the

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calorimetry phase all birds were euthanized via CO_2 chamber and scanned for body composition via whole body bone densitometer (Hologic QDR™-1000/W)[4].

All data were statistically analyzed using the "mixed" procedure of SAS® (SAS Institute, 1996). Interactions and main effets were considered significant at $P \leq 0.05$. When multiple means differed, they were separated using a least squares means test.

RESULTS

With few exceptions, no significant ($P>0.1$) interactions were detected for all the variables monitored throughout the study. Consequently, only main treatment effects were analyzed and discussed. The study was successfully completed with birds achieving weights comparable to the anticipated growth curve. Live weight increased linearly with age, reaching 760, 1755 and 2289 grams at 21, 35 and 42 days, respectively. Day 21 revealed a 2-way interaction for BW were higher BW ($P<0.01$) were observed in birds consuming HP diets in the CFS when compared to the DFS. Main effects for protein level averaged over feeding system were evident for each of the feeding phases monitored, as LP diets showed higher values ($P<0.01$) when compared to MP and HP diets as displayed on Table 9. Body weight values for DFS and CFS were similar ($P>0.1$) study.

Feed consumption was elevated ($p<0.05$) for the DFS treatments when compared to the CFS for the grower phase as well as for total FI values ($p<0.05$). The LP treatments had a marked elevation ($p<0.01$) in consumption throughout the study, with birds exhibiting 7% and 9% higher FI than the MP and HP treatments, respectively.

[4] Hologic, Waltham, MA 02154.

Birds receiving LP diets in the DFS treatments consumed over 400 grams more than chicks fed the HP diets under the CFS ($p<0.01$).

Due to FI changes for the DFS and LP treatments, these birds were less efficient ($P<0.05$) in FC throughout the study as displayed in Table 9. Similar to feed consumption, FC differences occurred in all the phases of the study, when DFS values were compared to CFS ($p<0.05$), being CFS 4 points higher than DFS. However, protein level did not show a main effect on feed conversion.

Carcass processing for the 3 different feeding phases expressed a similar pattern for most variables analyzed (Table 10). Hot carcass weights (HC) were higher for birds receiving LP diets by at least 5% ($p<0.01$), and the end of the three feeding phases. Dressing percentage was not impacted ($P>0.1$) by treatment at day 21, but was increased during grower and finisher phases for birds consuming LP diets, particularly in the DFS ($p<0.01$). Dressing was also higher for birds in the DFS at the end of each phase, but it was significant only during the grower phase ($p<0.05$) when compared to CFS treatments.

Breast weight was elevated ($P<0.01$) for birds consuming CFS diets for the starter and grower phases but at day 42 weights were similar for all treatments. Breast as a percentage of HC was increased for CFS and HP diets ($p<0.01$), with the exception of day 42 where DFS and CFS values were similar. Abdominal fat mass and as a percentage of HC increased with period and was higher ($P<0.01$) in DFS and LP diets.

Leg quarters as a percentage of HC were similar for all treatments throughout the study. However, their weight was higher for those birds in the LP treatments at the end of grower and finisher phases ($p<0.01$), when compared to MP and HP treatments.

Results for carcass composition are displayed in Table 11. Leaner birds as a percentage of BW were seen on birds in the CFS and HP diets ($p<0.01$) throughout the different phases of the study. However, total protein mass was similar for all treatments. Carcass fat percentage was higher ($P<0.01$) for DFS and LP diet treatments for all phases of the study, but fat carcass mass was similar at days 35 and 42 across treatments. Higher gross energy values were observed in carcass on birds that consumed the DFS and LP treatments ($p<0.01$).

Fasting heat production, correlated ($r = 0.66$; $P<0.001$) with lean mass (Figure 8). Birds in CFS-HP diets had a basal heat production 16% higher than birds that received CFS-LP diets during their productive life, even though no effect was detected presumably due to low replication obtained within treatments for this specific variable.

Scanning results (Table 12) are in agreement with carcass composition analysis. Birds receiving CFS and LP treatments exhibited higher ($p<0.01$) lean tissue percentage and lower ($p<0.01$) lipid tissue percentage. Lipid mass in the whole bird was higher ($p<0.01$) in DFS and LP birds, particularly those in the DFS-LP treatment having 33% higher lipid mass compared to HP treatments. As displayed in carcass composition results, grams of lean tissue for the scanned birds were similar across treatments. However, bone mineral content and density results were increased ($p<0.01$) for birds consuming LP diets when compared to MP and HP.

Substrate (protein, lipid, carbohydrate) was determined by multiplying ration content by feed consumption. Birds fed the LP and DFS diets had 15% and 3.5% less ($p<0.01$) protein intake than those in the HP and CFS diets, respectively. This was consistent during all phases of the study (Table 13). Protein efficiency was determined,

LP diets had improved efficiency ($p<0.01$), and DFS when compared to CFS were similar for most stages of the study, but there was trend seen for the DFS to be more efficient, particularly at day 42, where DFS had 2.3% better efficiency ($p<0.1$).

Energy content of the carcass was higher in all instances of the study for birds in the LP, particularly under the DFS treatments. Efficiency of metabolizable energy retention was also higher ($p<0.01$) in birds consuming LP diets when compared to MP and HP ($p<0.01$), and 4% higher in DFS diets. Net energy efficiency of a live bird expressed the same results, where birds receiving LP were more efficient when compared to MP and HP.

Mortality birds manifested a higher ascites syndrome incidence for birds in the CFS as well as the HP and MP treatments[4], as displayed in Table 9. Birds in the CFS-HP diets had a total incidence of 7.4%, were birds in the DFS-LP treatments had no cases of ascitic syndrome reported ($p<0.05$), as displayed in Figure 9. Dynamic feeding system birds reported almost half the cases that the CFS did ($p<0.05$).

DISCUSSION

Overall growth extent and feed conversion were within anticipated results for Cobb 500 broiler (1994). Dietary protein effects on body weight gain results are in agreement with previous studies, where LP diets were able to support growth when a proper balance of indispensable amino acids is maintained (Lipstein *et al.*, 1975; Spencer, 1984; Summers *et al.*, 1985; Schutte, 1987; Sell *et al.*, 1989; Parr *et al.*, 1991; Sell, 1993).

[4] Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, OK 74078.

However, feed intake increased ($P<0.05$) in birds consuming LP diets, a result similar to other workers (Lipstein *et al.*, 1975; Waldroup *et al.*, 1976; Moran, 1979; Moran *et al.*, 1992). This effect has been noted previously (Maddy *et al.*, 1960; Waldroup *et al.*, 1976; Parr *et al.*, 1991) and has been hypothesized to be the result of "self-selection" of amino acids. The low protein diets have an increased ratio of indispensable (EAA) to dispensable amino acids (NEAA), and this elevation might increase the requirement for NEAA. Consequently, limiting dietary protein, as in the case of the LP diets may have resulted in the elevated feed intake, due to either amino acid imbalance increasing NEAA requirements or a NEAA deficiency. If NEAA is the explanation for this increase in FI, then supplementation with NEAA at its optimal concentration in the diet should reduce the catabolism of EAA for NEAA synthesis.

Addition of NEAA, in some cases, has failed to overcome increased appetite (Fancher *et al.*, 1986; Deschepper *et al.*, 1994; Pichasov *et al.*, 1990). Protein synthesis requires both EAA and NEAA during mRNA translation, thus, manifesting that cellular requirements do not distinguish between essential and nonessential amino acids.

Feed efficiency for most of the study reported herein, and in accordance with other studies (Twining *et al.*, 1974; Uzu, 1982; Bedford *et al.*, 1984; Jensen, 1991; Moran *et al.*, 1992; Holsheimer *et al.*, 1991), was reduced when the LP diets were fed. The elevated feed consumption was not, wit LP diets, associated with increased growth, and consequently feed conversion declined. Fate of the added energy consumption will be discussed below.

The carcass parts yield data follow a same pattern of results for the three different feeding phases. Carcass lipid and abdominal fat decreased when protein in the diet was

higher. This effect has been observed previously and can be explained due to fact that HP diets involve a metabolic route that will increase heat production, in consequence of the degradation of excess amino nitrogen to uric acid (Bartov, 1979). It can also be associated with a decrease in net efficiency of energy utilization for growth (MacLeod, 1991). This effect was seen during the BMR phase of this study, when birds during a typical fast and rest period, making body composition the primary inducing agent of metabolic heat production. A positive correlation (r=0.67; P<0.001) was observed between grams of lean and heat production, strongly suggesting that body composition had a marked impact on maintenance of chickens. This correlation would be expected to increase, and the heat production range to be wider on full fed growing chickens, when protein synthesis known to have a higher energy cost than fat deposition (Millward, *et al.*, 1976) would create a bigger gap between units of body protein units when plotted against heat production. In contrast to this study, previous studies have suggested that variation in heat production did not have an important regulatory role in body composition (Mac Leod, 1990). However, it has also been suggested that maintenance energy costs per unit of weight may also have been greater with an increased proportion of fat-free body mass (Mac Leod, 1991).

In agreement with previous studies (Summers *et al.*, 1992; Deschepper *et al.*, 1995), total carcass protein data in terms of grams is conclusive, and expresses no differences among treatments. Is when a percentage of carcass is determined, that the lean in LP and DFS treatments is reduced, this is due to an increase in lipid content as seen in abdominal fat results and fat determination by multiple regression and scanning results. However, since protein intake was lower as the protein in the diet was lower, and

carcass and body protein was the same among dietary treatments, it was seen that the efficiency in utilization of this substarte was higher among LP and DFS treatments during all stages of this study. The LP-DFS diet was the most efficient with 44.4% of protein retention, improving this retention by 20% when compared to the CFS-HP diets and 15% when compared to the CFS-MP NRC phase and nutrient recommendations. These results are in agreement with results observed by Parr and Summers (1991), and Deschepper and De Groote (1995). A positive correlation (r=0.987; $p<0.0001$) between protein intake and carcass protein suggests that protein retention was not much influenced by the amino acid balance of the diet. However, it was also seen that avoiding excess amino acid and protein intake led to a higher efficiency.

It can be assumed that body composition has a great influence over maintenance requirements, thus oxygen demand, and so the propensity to ascites. It has been established that growth rate is the major factor contributing to oxygen demand, but when body composition is higher in protein and protein metabolism rather than lipid, this demand can increase some more. It has been suggested by Leeson *et al* (1995) that when dietary protein is excess by 4%, it will not only be catabolized and deaminated to uric acid and fat synthesis, but also increase the oxygen demand to 2 and 1 liters, respectively. This was a dietary effect seen in this study, where ascites was dramatically increased as the protein intake increased. A strong positive correlation was observed (r=0.467; $p<0.0001$), for ascites and protein intake, confirming the dietary effect of protein over oxygen demand and subsequent ascitic incidence.

In conclusion, protein accretion is strictly dependant on EAA and NEAA supply and ratio, having these a marked influence on performance as witnessed by protein

efficiency, protein and fat composition. Protein supply can also influence maintenance costs as witnessed by BMR heat production results as well as ascites incidence. Implications of what the adequate protein supply should reside on environmental and marketing demand conditions.

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REFERENCES

Almquist, H. J., 1947. Evaluation of amino acid requirements by observations on the chick. J. Nutr. 34:543.

Association of Official Analytical Chemists, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC.

Association of Official Analytical Chemists, 1994. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Washington DC.

Baker, D. H., M. Sugahara, and H. M. Scott, 1968. The glycine-serine interrelationship in chick nutrition. Poult. Sci. 47:1376.

Bartov, I., 1979. Nutritional factors affecting quantity and quality of carcass fat in chickens. Federation Proceedings. 38:2927-2639.

Bedford, M. R., and J. D. Summers, 1985. Influence of the ratio of essential to non essential amino acids on performance and carcase composition of the broiler chick. Br. Poult. Sci. 26:483-491.

Belay, T. and R. G. Teeter, 1993. Broiler water balance and thermobalance during thermoneutral and high ambient temperature expossure. Poult. Sci. 72:116-124.

Blaxter, K. L., 1989. Energy Metabolism in Animals and Man. University Press, Cambridge, England.

Brouwer, E., 1965. Report of sub-committee on constants and factors. In: Energy Metabolism, pp. 441-443. K. L. Blaxter ed. Academic Press, London.

Cobb Vantress, Inc., 1994. Cobb 500 Broiler Management Guide. Siloam Springs, Arkansas.

De Lange, C. F. M., 1993. Diet formulation to minimize contribution of livestock to environmental pollution, Arkansas Nutrition Conference Proceedings. (9).

Deschepper, K., and G. De Groote, 1995. Effect of dietary protein, essential and non essential amino acids on the performance and carcase composition of male broiler chickens. Br. Poult. Sci. 36:229-245.

D'Mello, J. P. F., 1974. Plasma concentrations and dietary requirements of leucine, isoleucine and valine: Studies with the young chick. J. Sci. Food Agric. 25:187.

Edwards, H. M., Jr., L. C. Norris, and G. F. Heuser, 1956. Studies on the lysine requirement of chicks. Poult. Sci. 35:385;

Hill, F. W., and D. L. Anderson, 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587-604.

Hurwitz, S., D. Sklan, and I. Bartov, 1978. New formal approaches to the determination of energy and amino acid requirements of chicks. Poult. Sci. 57:197.

Fancher, B. L., and L. Jensen, 1986. Effects of feeding reduced dietary protein upon broilers from 3 to 6 weeks of age while maintaining essential amino acid requirements. Poultry Sci. 65 (Suppl. 1):40.(Abstr.)

Jensen, L. S., 1991. Broiler performance as affected by intact proteins versus synthetic amino acids. Proceedings of the Georgia Nutrition Conference, pp. 83-89.

Holsheimer, J. P, and W. M. M. A. Janssen, 1991. Limiting amino acids in low protein maize-soybean meal diets fed to broiler chicks from 3 to 7 weeks of age. Br. Poult. Sci. 32:151-158.

Klain, G. J., H. M. Scott, and B. C. Johnson, 1960. The amino acid requirement of the growing chick fed a crystalline amino acid diet. Poult. Sci. 39:39.

Leeson, S., G. Diaz, and J. D. Summers, 1995. Poultry metabolic disorders and mycotoxins. University Books. Guelph, Ontario, Canada.

Lipstein, B., S. Bornstein, and I. Bartov, 1975. The replacement of some of the soybean meal by the first limiting amino acid in practical broiler diets 3. Effects of protein concentration and amino acid supplementation in broiler finisher diets on fat deposition on the carcass. Br. Poult. Sci. 16:627-635

Mac Leod, M. G., 1990. Energy and nitrogen intake, expenditure and retention at 20° in growing fowl given diets with a wide range of energy and protein contents. Br. J. of Nutr. 64:625-637

MacLeod, M. G., 1991. Fat deposition and heat production as responses to surplus dietary energy in fowls given a wide range of metabolisable energy:protein ratios. Br. Poult. Sci. 32:1097-1108

Maddy, K., L. Machlin, and R. Gordon, 1960. The effect of excess amino acids on the requirements for glycine. Poultry Sci. 39:1271. (Abstr.)

Millward, D. J., P. J. Garlick, and P. J. Reeds, 1976. The energy cost of growth. Proceedings of the Nutrition Society. 35:339-349.

Mittelstaedt, C. W., 1990. Feed Bioenergy Evaluation: Methodology as Applies to Growing Broiler. Thesis Dissertation.

Moran, E. T., Jr., 1979. Carcass quality changes with broiler chicken after dietary protein restriction during the growing phase and finishing period compensatory growth. Poultry Sci. 58:1257-1270.

Moran, E. T., Jr., 1981. Cystine requirement of feather-sexed chickens with sex and age. Poult. Sci. 60:1056.

Moran, E. T., Jr., R. D. Bushong, and S. F. Bilgili, 1992. Reducing dietary crude protein for broilers while satisfying amino acid requirements by least-cost formulation: live performance, litter composition, and yield of fast-food carcass cuts at six weeks. Poultry Sci. 71:1687-1694.

National Research Council, 1994. Nutrient requirements of domestic animals: Nutrient requirements of poultry. National Academy of Sciences, Washington D.C.

Nelson, T. S., R. J. Young, R. B. Bradfield, J. B. Anderson, L. C. Norris, F. W. Hill, and M. L. Scott, 1960. Studies on the sulfur amino acid requirement of the chick. Poult. Sci. 39:308.

Parr, J. F., and J. D. Summers, 1991. The effect of minimizing amino acid excesses in broiler diets. Poultry Sci. 70:1540-1549.

Pinchasov, Y., C. X. Mendonca, and L. S. Jensen, 1990. Broiler chick response to low protein diets supplemented with synthetic amino acids. Poultry Sci. 69:1950-1955.

Pullar, J. D., and A. J. F. Webster, 1974. The energy cost of fat and protein deposition in the rat. Br. J. Nutr. 37:355-363.

SAS Institute, SAS/STAT 1996. Changes and enhancements guide. Version 6.12., SAS Institute Inc., Cary, NC.

Sell, J. L., P. R. Ferket, C. R. Angel, S. E. Scheideler, F. Escribano, and I. Zatari, 1989. Performance and carcass characteristics of turkey toms as influenced by dietary protein and metabolizable energy. Nutr. Rep. Int. 40:979-992.

Sell, J. L., 1993. Influence of metabolizable feeding sequence and dietary protein on performance and selected carcass traits of tom turkeys. Poultry. Sci. 72:521-534.

Spencer, G. K., 1984. Minimum Protein Requirements of Turkeys Fed Adequate Levels of Lysine and Methionine. M.S. thesis, University of Arkansas, Fayetteville, AR.

Summers, J. D., D. Spratt, and J. L. Atkinson, 1992. Broiler weight gain and carcass composition when fed diets varying in amino acid balance, dietary energy, and protein level. Poultry Sci. 71:263-273.

Summers, J. D., and S. Leeson, 1985. Broiler carcass composition as affected by amino acid supplementation. Canad. J. Ani. Sci. 65:717-723.

Twining, P. V., Jr., O. P. Thomas, E. H. Bossard, and J. L. Nicholson, 1974. The effect of amino acid and protein level on body composition of $8^{1}/_{2}$ week broilers. Proceedings of the Maryland Nutrition Conference, pp. 89-95.

Uzu, G., 1982. Limit of reduction of the protein level in broiler feeds. Poultry Sci. 61:1557-1558 (Abstr.)

Waldroup, P. W., R. J. Mitchell, J. R. Payne, and K. R. Hazen, 1976. Performance of chicks fed diets formulated to minimize excess levels of essential amino acids. Poultry Sci. 55:243-253.

Wiernusz, C. J., and R. G. Teeter, 1993. Feeding effects on broiler thermobalance during thermoneutral and high ambient temperature expossure. Poult. Sci. 72:1917-1924.

Wiernusz, C. J., B. C. Park, and R. G. Teeter, 1999. Prediction of carcass fat, protein, and energy content from carcass dry matter and specific gravity of broilers. Asian J. of An. Sci. 12:42-48.

Table 8. Experimental diet composition (%)

	Starter			Grower			Finisher			Withdrawl		
	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low
Ingredient												
Gr. yellow corn	55.62	49.15	59.78	62.17	56.36	66.05	67.32	62.32	71.65	68.25	62.86	66.05
Soybean meal dh.	28.61	34.19	25.85	24.84	30.11	21.33	22.72	26.26	20.27	22.85	26.16	14.10
Pro-Pak	7.0	7.0	5.02	4.5	4.55	2.866	2.4	3.7	------	0.6	2.0	2.90
Vegetable fat	6.8	7.8	6.7	6.2	7.0	6.318	5.5	6.6	5.6	5.8	6.5	5.044
Limestone	1.31	1.25	1.5	1.199	1.293	1.4	1.5	1.324	1.809	1.776	1.8	1.5
Dicalcium phosp.	------	0.013	0.4	0.5	0.248	1.2	0.109	------	0.488	0.332	0.206	1.5
Salt	0.34	0.327	0.33	0.34	0.198	0.3	0.22	0.18	0.18	0.18	0.18	0.18
Vitamin premix[1]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Copper sulfate	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ethoxiquin	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Mineral premix[2]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Selenium	0.0018	0.0018	0.0018	0.0018	0.0017	0.0016	0.0017	0.0018	0.0017	0.0018	0.0018	0.0020
L-Isouleucine	------	------	0.044	------	------	0.09	------	------	0.04	------	------	0.036
DL-Methionine	0.1	0.02	0.1	------	0.02	0.042	0.007	------	0.05	------	0.2	------
L-Cystine	------	------	0.063	0.04	------	0.058	------	------	------	------	------	------
L-Threonine	------	------	------	------	------	0.066	-------	------	0.085	------	0.06	0.09
L-Lysine	------	------	------	------	------	0.058	------	------	0.06	------	------	0.058
L-Arginine	------	------	------	------	------	------	------	------	0.01	------	------	0.055
Analysis												
MEn Kcal/kg	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100
Crude protein (%)	22.64	24.79	20.49	19.69	21.83	17.54	17.72	19.86	15.57	16.73	18.88	14.59
Calcium (%)	1.0	1.0	1.03	0.901	0.90	1.01	0.80	0.90	0.836	0.835	0.914	1.094
Phosphorus av(%)	0.551	0.574	0.554	0.555	0.53	0.618	0.414	0.53	0.40	0.40	0.43	0.649
Methionine (%)	0.575	0.58	0.575	0.42	0.44	0.40	0.349	0.438	0.334	0.31	0.371	0.302
Lysine (%)	1.30	1.47	1.16	1.109	1.26	1.0	0.972	1.263	0.867	0.907	1.054	0.793

[1]Premix contained vitamin A, 3,527 IU; vitamin D_3, 1,322 IU; vitamin E, 11,9 IU; vitamin B_{12}, 3,5 *mg*; riboflavin, 2,2 mg; niacin 6,6 mg; pantothenic acid, 7,05 mg; choline, 176,3 mg; menadione, 291 mg; folic acid, 441 mg; thiamin, 882 mg; and biotin, 44 mg/kg.

[2]Premix contained manganese, 12%; zinc, 8%; iron, 6%; copper, 10%; iodine, 0.1%; and calcium, 18%.

Table 9. Body weight gain, feed efficiency, ascites incidence, by feeding phase

	Classical			Dynamic			
	Low	Medium	High	Low	Medium	High	
							P<
Body weight[4] (g)							
21d[1, 2, 3]	763[a]	749[b]	767[a]	766[a]	750[b]	746[b]	0.001
35d[1]	1762[ab]	1711[bc]	1735[bc]	1796[a]	1715[bc]	1687[c]	0.001
42d[1]	2342[ab]	2267[bc]	2285[bc]	2375[a]	2276[bc]	2236[c]	0.011
Feed efficiency							
21d[1, 2]	1.32[ab]	1.30[bc]	1.27[c]	1.35[a]	1.32[ab]	1.31[abc]	0.021
35d[1, 2]	1.53[ab]	1.50[b]	1.47[c]	1.55[a]	1.55[a]	1.54[ab]	0.001
42d[2]	1.64[a]	1.62[ab]	1.58[b]	1.66[a]	1.65[a]	1.65[a]	0.049
Ascites incidence (%)							
21d	0.0	1.23	1.23	0.0	0.0	0.61	NS
35d	0.0	1.23	2.47	0.0	1.23	1.85	0.049
42d[1]	2.47[ab]	4.32[a]	3.70[a]	0.0[b]	2.47[ab]	3.08[a]	0.040
Total[1, 2]	2.47[c]	6.79[ab]	7.4[a]	0.0[d]	3.7[c]	5.55[b]	0.002

[a-d] Means within a row with unlike superscripts differ.
[1]Protein level main effect ($P<0.05$).
[2]Feeding system main effect ($P<0.05$).
[3]Protein level x Feeding system ($P<0.05$).
[4]Each value represents the mean of individual body weights in 9 replicate measurements.

Table 10. Carcass processing, by feeding phase

	Classical			Dynamic			
	Low	Medium	High	Low	Medium	High	
							P<
Hot carcass (g)							
21d [1,]	559 [a]	538 [c]	549 [abc]	553 [ab]	545 [bc]	541 [bc]	0.044
35d [1, 3]	1343 [b]	1288 [c]	1292 [c]	1376 [a]	1297 [c]	1269 [c]	0.001
42d [1]	1725 [a]	1646 [b]	1668 [b]	1786 [a]	1653 [b]	1636 [b]	0.001
Dressing (%)							
21d	73.0	71.4	71.2	71.9	72.2	72.1	NS
35d [1, 2]	71.7 [ab]	70.7 [cd]	70.0 [d]	72.1 [a]	71.2 [bc]	70.7 [cd]	0.001
42d [1]	73.2 [ab]	72.1 [b]	72.2 [b]	73.9 [a]	72.1 [b]	72.4 [b]	0.022
Breast (g)							
21d [2]	103.8 [bc]	108.7 [a]	106.1 [ab]	100.2 [c]	102.3 [bc]	103.3 [bc]	0.005
35d [2]	312.1 [a]	297.0 [c]	310.1 [ab]	298.5 [bc]	296.5 [c]	294.8 [c]	0.022
42d	396.6 [b]	388.6	384.1	390.4	376.4	385.3	NS
Breast (%)							
21d [1, 2, 3,]	18.56 [cd]	20.14 [a]	19.28 [b]	18.04 [d]	18.73 [bc]	19.05 [bc]	0.001
35d [1, 2, 3]	23.21 [ab]	23.01 [b]	23.92 [a]	21.62 [c]	22.83 [b]	23.26 [ab]	0.001
42d [1, 3]	22.95 [ab]	23.66 [a]	23.01 [ab]	22.01 [c]	22.76 [bc]	23.43 [ab]	0.005
Abdom. fat (g)							
21d [1, 2]	10.81 [bc]	8.65 [d]	8.52 [d]	13.46 [a]	11.36 [b]	9.61 [cd]	0.001
35d [1, 2]	32.49 [b]	24.68 [c]	21.30 [d]	38.39 [a]	29.87 [b]	25.54 [c]	0.001
42d [1, 2]	49.20 [b]	36.35 [c]	34.73 [c]	58.38 [a]	39.40 [c]	35.74 [c]	0.001
Abdom. fat (%)							
21d [1, 2]	1.93 [bc]	1.59 [de]	1.54 [e]	2.43 [a]	2.08 [b]	1.77 [cd]	0.001
35d [1, 2]	2.40 [b]	1.89 [cd]	1.65 [d]	2.77 [a]	2.28 [b]	1.99 [c]	0.001
42d [1, 2]	2.83 [b]	2.15 [cd]	2.06 [d]	3.24 [a]	2.36 [c]	2.15 [cd]	0.001
Leg quarters (g)							
21d	173.9	166.7	174.5	174.6	171.4	171.4	NS
35d [1]	430.0 [b]	417.3 [c]	416.6 [c]	448.1 [a]	421.0 [bc]	414.9 [c]	0.001
42d [1]	548.4 [ab]	521.2 [c]	541.2 [bc]	564.5 [a]	526.6 [c]	524.2 [c]	0.001

[a-e] Means within a row with unlike superscripts differ.
Each value represents the sample mean of 27 individual birds per treatment.
[1]Protein level main effect ($P<0.05$).
[2]Feeding system main effect ($P<0.05$).
[3]Protein level x Feeding system ($P<0.05$).

Table 11. Carcass composition, by feeding phase

	Classical			Dynamic			
	Low	Medium	High	Low	Medium	High	P<
Protein (% hot carcass)							
21d [1,2]	18.25 [c]	18.80 [a]	18.91 [a]	17.65 [d]	18.21 [c]	18.51 [b]	0.001
35d [1,2]	18.08 [a]	18.11 [a]	18.30 [a]	17.72 [c]	17.78 [bc]	18.06 [ab]	0.001
42d [1,2]	17.78 [bc]	18.02 [a]	18.03 [a]	17.62 [c]	17.76 [bc]	17.94 [ab]	0.002
Protein (g hot carcass)							
21d [2]	102.3 [a]	104.6 [a]	104.5 [a]	94.7 [b]	98.0 [ab]	100.7 [ab]	0.044
35d	243.1	228.7	246.8	237.4	230.0	231.0	NS
42d	308.3	290.7	307.1	309.0	297.2	291.0	NS
Fat (% hot carcass)							
21d [1,2]	12.17 [b]	9.58 [d]	9.06 [d]	15.01 [a]	12.37 [b]	10.97 [c]	0.001
35d [2]	12.93 [c]	12.79 [c]	11.96 [c]	14.49 [a]	14.26 [ab]	13.03 [bc]	0.001
42d [1]	14.24 [ab]	13.17 [c]	13.15 [c]	14.94 [a]	14.30 [ab]	13.53 [bc]	0.002
Fat (g hot carcass)							
21d [2]	68.4 [b]	54.5 [de]	50.2 [e]	80.1 [a]	66.4 [bc]	59.9 [cd]	0.001
35d	174.3 [abc]	160.3 [c]	163.6 [bc]	193.3 [a]	184.4 [ab]	166.9 [bc]	0.05
42d	247.4 [a]	211.0 [c]	225.8 [bc]	261.3 [a]	239.7 [ab]	219.7 [bc]	0.002
Energy (kcal/g of hot carcass)							
21d [1,2]	6.09 [b]	5.95 [d]	5.92 [d]	6.25 [a]	6.11 [b]	6.03 [c]	0.001
35d [2]	6.16 [bc]	6.14 [c]	6.10 [c]	6.24 [a]	6.23 [ab]	6.16 [bc]	0.001
42d [1]	6.23 [ab]	6.17 [c]	6.17 [c]	6.27 [a]	6.24 [ab]	6.19 [bc]	0.002

[a-e] Means within a row with unlike superscripts differ.
Each value represents the sample mean of 27 individual birds per treatment.
[1]Protein level main effect ($P<0.05$).
[2]Feeding system main effect ($P<0.05$).
[3]Protein level x Feeding system ($P<0.05$).

Table 12. Whole body scanning and basal metabolic rate heat production

	Classical			Dynamic			
	Low	Medium	High	Low	Medium	High	
							P<
Lean [1, 2] (% body weight)	81.2 [b]	84.3 [a]	85.4 [a]	79.8 [b]	80.8 [b]	84.0 [a]	0.001
Lean (g)	1849	1883	2002	1841	1864	1870	NS
Fat [1, 2] (% body weight)	17.0 [a]	14.1 [b]	13.0 [b]	18.3 [a]	17.5 [a]	14.5 [b]	0.001
Fat [1, 2] (g)	385 [a]	315 [b]	308 [b]	422 [a]	404 [a]	325 [b]	0.001
Bone Mineral Density[1] (g/cm^2)	0.169 [a]	0.157 [bc]	0.157 [bc]	0.173 [a]	0.162 [ab]	0.149 [c]	0.002
Bone Mineral Content[1, 3] (g)	38.7 [a]	34.1 [bc]	35.2 [bc]	40.5 [a]	37.3 [ab]	31.9 [c]	0.001
Heat production (KJ/L/h)	36.57	38.23	42.61	39.19	40.23	38.14	NS

[a-d] Means within a row with unlike superscripts differ.
[1]Protein level main effect ($P<0.05$).
[2]Feeding system main effect ($P<0.05$).
[3]Protein level x Feeding system ($P<0.05$).
[4]Each value represents the mean of 9 individual birds per treatment.

Table 13. Protein intake, protein efficiency, efficiency of metabolizable energy use, and net energy efficiency of a live bird.

	Classical			Dynamic			
	Low	Medium	High	Low	Medium	High	P<
Protein intake (g/bird)							
21d [1, 2]	206.8 [c]	219.5 [b]	242.1 [a]	193.1 [d]	204.3 [c]	223.4 [b]	0.001
35d [1]	324.0 [c]	340.0 [b]	371.6 [a]	322.0 [c]	337.2 [b]	360.0 [a]	0.001
42d [1]	182.9 [bc]	193.9 [b]	213.7 [a]	179.5 [c]	189.2 [bc]	209.0 [a]	0.001
Total [1, 2]	713.8 [de]	753.5 [c]	827.4 [a]	694.6 [e]	730.8 [cd]	792.5 [b]	0.001
Protein efficiency*							
21d [1]	49.5 [a]	47.7 [ab]	43.2 [c]	49.0 [a]	48.0 [ab]	45.0 [bc]	0.001
35d [1]	45.8 [a]	40.8 [bc]	40.3 [bc]	46.1 [a]	42.5 [b]	39.5 [c]	0.001
42d [1]	43.2 [a]	38.6 [bc]	37.1 [c]	44.4 [a]	40.7 [b]	36.6 [c]	0.001
Efficiency of ME use**							
21d	36.8	35.2	33.8	37.4	36.4	35.5	NS
35d [1, 2]	41.8 [a]	35.0 [c]	36.4 [bc]	41.9 [a]	40.3 [ab]	37.9 [abc]	0.003
42d [1]	42.4 [a]	35.5 [b]	37.2 [b]	43.2 [a]	40.0 [ab]	36.2 [b]	0.005
Net energy efficiency of a live bird***							
21d	50.5	49.1	47.4	51.9	50.3	49.1	NS
35d [1,2]	58.1 [a]	49.5 [c]	51.8 [bc]	58.1 [a]	56.5 [ab]	53.4 [abc]	0.006
42d [1]	57.8 [a]	49.1 [b]	51.2 [bc]	58.3 [a]	55.2 [ab]	49.9 [c]	0.005

[a-e] Means within a row with unlike superscripts differ.
Each value represents the sample mean of 27 individual birds per treatment.
[1]Protein level main effect ($P<0.05$).
[2]Feeding system main effect ($P<0.05$).
[3]Protein level x Feeding system ($P<0.05$).

*Protein intake (g) / carcass protein (g).
**Gross carcass energy (Kcal) / ME intake (Kcal).
***Efficiency of ME use (Kcal) / dressing %.

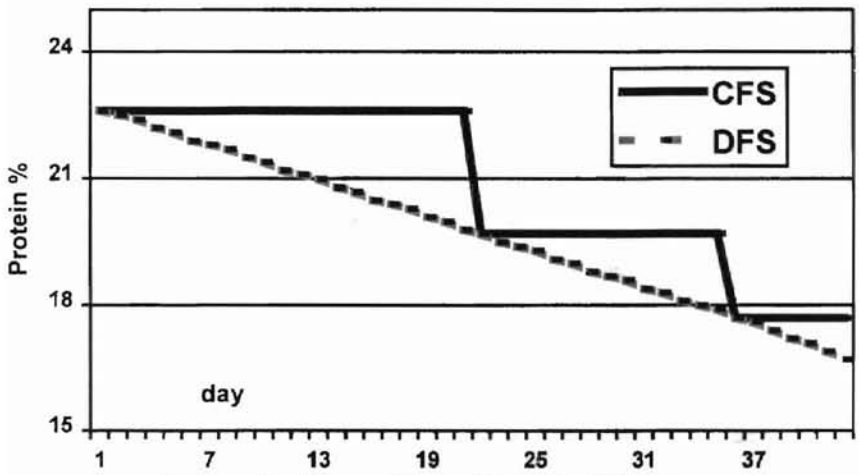

Figure 7. Dynamic vs Classical Feeding System

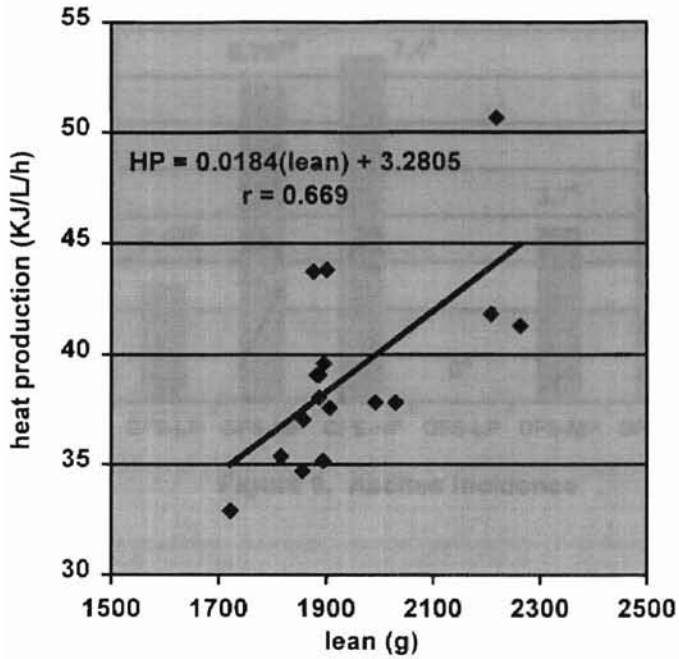

Figure 8. BMR heat production vs lean body content

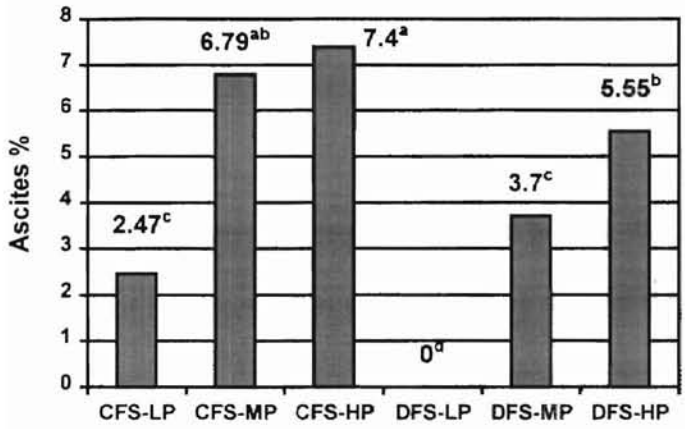

Figure 9. Ascites incidence

CHAPTER V

SUMMARY AND CONCLUSION

It can be concluded by the series of studies reported herein, that in order to maximize broiler efficiency it is necessary to understand the relationship between nutrition, genetics and environment. It was observed that environment played a key role in performance and it can impact productivity in a profound way. Pathogen agents such as mycotoxins not only jeopardize the health and integrity of a flock, but can also reversibly affect growth and production. It is because of this reason that measures must be taken to ensure a proper collection, processing, and manufacturing of feedstuffs in order to provide the animal with nutrients that can be efficiently utilized. However, microorganism such as fungi can very easily be taken for granted and usually is not within the reach of commercial broiler farms to ensure feed quality. Therefore therapeutic solutions must be available in the market in order to prevent this type of adversity.

Genetics has produced a high growth rate broiler, capable of doubling its body weight in a short period of time. Therefore, it is also indispensable that nutritionists posses the knowledge and capability to adapt and adjust nutrient supply in order to ensure adequate substrate quantity and availability. It was also seen that protein supply and

balance can impact performance. It was concluded in that particular study that protein can adversely affect broiler performance. It not only had an effect over body and carcass composition, but it also impacted specific nutrient utilization. Regardless of the number of investigations done concerning dietary protein supply, emphasis in the balance of dispensable and indispensable amino acids is still necessary, particularly their ratio, where so much is still unclear.

Ascites keeps on bringing serious consequences to profitability of broiler flocks. However, little or no data has been reported to the date expressing the impact of protein supply on this variable. It is because of this, that this study provided a series of ideals and opportunities for research that could potentially provide nutritionists with a therapeutic tool in order to minimize ascites incidence in broiler flocks. It was also concluded that dynamic feeding systems by eliminating the abrupt change in protein supply, as well as all the other nutrients minimized ascites incidence in a significant manner.

Today, a considerable interest has raised concerning environmental issues, particularly pollution. It has been concluded that a more appropriate management of excretion products derived from livestock production is needed, since it will negatively impact the world in a long-term basis. The better protein utilization and efficiency evidenced in the low protein diets can lead to a lower nitrogen excretion, thus reducing ammonia and nitrogen pollution.

It has been hypothesized that today's commercial broiler has almost reached its maximum genetic potential, and it has also been stated that very little is left for

manipulation and selection of broilers for the increase in growth and feed conversion rates. Understanding energy expenditure can help explain what can be adapted in the bird's maintenance and production energy requirements, thus potentially decreasing unnecessary energy expenditures therefore increasing energy available for gain. This will only be accomplished with energy studies that analyze energy partitioning from the feed and their effect on energy metabolism of the animal for all the various types of activities. It was found that heat production has a linear increase with lean content that lead to a reduction in energy retained and an increase in energy lost as heat and energy spent to dissipate that heat. Since the bird depends on non-evaporative cooling in order to thermo-regulate itself, this can significantly affect energy requirement for maintenance.

It has been well established that today's market demands a leaner bird. and it is also evident that protein feeds are typically more costly. In the study it was found that with less amount of dietary protein supply, the broiler could reach maximum lean accretion, therefore reducing energy expenditure for deamination and excretion processes, increasing protein and energetic efficiency, and potentially enhancing profitability.

In conclusion, studies reported in this dissertation manifest the impact that nutrition can have on broiler and carcass performance and characteristics. It is encouraged that future investigations of dietary protein and its relationship with the animal's metabolism be made. It is essential to posses a profound understanding of protein metabolism and its interaction with maintenance and production costs, if we are to maximize substrate utilization and enhance productivity. It can be finally stated that the need for an energetic system that involves net energy availability from the various

types of feedstuffs is needed. This area has the potential to decrease calorie overload at its maximum, thus, reducing body fat percentage and mass, therefore increasing lean tissue.

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