ENERGETIC EFFICIENCY OF SUBSTRATE SOURCE AS RELATED TO BROILER PROTEIN AND LIPID ACCRETION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Chapter II

Chapter IV

Chapter VI

CHAPTER I

INTRODUCTION

Today's commercial broiler is the fastest growing and most efficient bird ever produced; it represents the combined efforts of genetics and management. However, with this tremendous potential also comes greater susceptibility to different types of stress. Because growth taxes numerous physiological systems and because stress consequences typically are additive, we should not be surprised that modern birds frequently exhibit unwanted stress. Knowledge related to bird energetics, stress management and waste production are evolving and new management approaches are being employed. More thorough understanding of energy metabolism is fundamental to improving profitability of production enterprises.

Consumer demand for lean poultry products necessitates that product leanness and uniformity be improved. "Ice pack" will decrease and "value added" products will increase. As a result, technologies resulting in greater protein production, not overall bird mass, will be emphasized. The shift of focus to the profit center of proteinaceous tissue mass necessitates that nutritional advances occur which enable muscle growth at optimal rates while minimizing fat accretion. Though this direction may slow bird growth rate, which has been artificially enhanced via lipid accretion, research directed at reducing the growth-depressing consequences of stress may help offset this dilemma. Nonetheless,

technological developments must occur within the bounds of increasing environmental restrictions, which are becoming more intense.

To date, the metabolizable energy (ME_n) system has been accepted as the standard for ration formulation (NRC, 1994). However, the ME_n system by definition, does not quantitatively predict bird feed energy deposition. Any heat increment change alters ME_n utilization and thereby can affect the cellular energy/nutrient ratios. Alterations in the cellular energy/nutrient ratio may enhance fat deposition. For example, recent and ongoing studies directed at evaluating the ME_n system indicate that cellular energy supply does not necessarily reflect ME_n consumption. The greater heat increment from protein ME_n calories vs those from starch and fat make low protein diets lipogenic. Oxygen required per unit protein synthesis is 380% greater than that for fat (Teeter and Wiernusz, 1994). Deeper understanding and application of cellular energy-nutrient relationships will be required to produce leaner birds.

Energetic efficiency of ME_n use for tissue gain depends upon numerous variables. Efficiency varies with substrate source, for lipogenesis being approximately 75, 84, and 61% for carbohydrates, fats and proteins, respectively (De Groote, 1969; Chudy and Schiemann, 1971; Hoffmann and Schiemann, 1971). The high availability of fat ME_n for tissue gain, however, requires that fat is used for lipogenesis (Bossard and Combs, 1961). Utilization of protein for tissue energy gain depends upon the biological value of the protein source and should not be constant (De Groote, 1973). Indeed, one could summarize that the bird's energetic efficiency for use of protein or any substrate is the net

result of partitioning consumed substrate energy into maintenance needs verses accretion of protein and fat.

Recommendations for dietary protein concentration for optimum rates of lean tissue accretion range from high (Kubena et al., 1972) to low levels complemented with specific amino acids (Waldroup et al., 1976). Whether the carcass leanness associated with feeding high protein diets is attributable to substrate limitations (amino acids), or due to greater heat production per kcal ME_n for dietary amino acids carbohydrate and fat is subject to debate. Research conducted in our laboratory by Mittelstaedt (1990) examined the true metabolizable energy (TME) utilization of carbohydrate, protein and fat sources for energy, protein and fat gain. Despite similar TME consumption among the energy supplemented groups, carcass energy was impacted significantly. Total carcass energy gain was 17, 27, and 30% greater for the gelatin, starch, and corn oil groups, than for birds fed the basal diet. Estimated energy gain from the basal ration was similar among the energy supplemented groups due to nearly identical feed consumptions. However, total calories gained differed ($P<.05$) across experimental groups with the highest value of 436 kcal/bird observed for the corn oil group verses only 167 kcal/bird for the gelatin. As a result, energetic efficiency varied among the energy supplemented groups. Efficiency of ingredient TME usage for carcass energy deposition averaged 50.0, 39.1, and 19.9%, respectively for supplemental corn oil, starch and gelatin, respectively.

An additional consequence of low protein ME_n utilization efficiency is that the birds heat load is increased. Elevated heat load has little consequence when birds are housed at or below thermoneutral temperatures. However, if the bird's heat load is

elevated by high ambient temperature distress, without a concomitant increase in heat dissipation, elevated heat load can be devastating (Wiernusz and Teeter, 1993). Belay and Teeter (1992) fed birds fed various protein levels and calorie/protein ratios. Increasing dietary energy and (or) narrowing calorie-protein ratios by relaxing restrictions on amino acid balance (which necessitated increased dietary protein) significantly impacted bird performance. Improving amino acid balance and lowering dietary crude protein concentration increased survival both in the thermoneutral environment (4.4%) and within the heat distressed environment (10.8%; $\underline{P} < .05$). Lowering crude protein (at adequate amino acid balance) for heat distressed broilers certainly can prove beneficial. Research is needed to identify which amino acid excess cause the greatest risk.

Diets formulated, based on the ME_n system do not necessarily correlate with bird energy retention; the calorie-nutrient ratios of depot tissue can vary independent of metabolizable energy. In order for the broiler to achieve maximum protein deposition with minimal fat accretion, an energy-requirement scheme must account for the variation in substrate-mediated heat production.

The objective of the studies described herein was to investigate the energetic efficiencies of carbohydrates, fats, and proteins for tissue accretion using an indirect calorimetry system developed at the Oklahoma State University Avian Climatological Research Center. Additionally, other objectives included the development of a model for the prediction of carcass fat content and the effects of two methionine sources on broiler thermobalance, carcass and production variables. Data collected should increase the understanding of energy metabolism for all poultry classes. By restricting those substrates with high obligatory heat production, utilizable ME_n (net energy) of the diet will be

enhanced which in turn should reduce the need for fat supplementation. Quantitative data related to substrate energy values will enable diets to be formulated with more realistic energy/nutrient ratios which should impact carcass composition favorably. And finally, the ability to reduce dietary heat increment should increase growth rate and feed efficiency of broilers.

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CHAPTER II

REVIEW OF LITERATURE

Introduction

A clear understanding of energy and protein metabolism for all poultry classes is fundamental for diet formulation and profitable production enterprises. The metabolizable energy (ME_n) system currently is accepted as the standard for ration formulation. (NRC, 1994). However, by definition ME_n does not quantitatively predict energy deposition by birds. Any difference in heat increment alters energy retention and thereby may affect cellular energy/nutrient ratios. Other factors affecting ME_n utilization include distress factors such as disease agents (bacteria, viruses and protozoa), social stress created by other animals and man, malnutrition, toxicities, and the thermal environment. Diets based on ME_n do not necessarily correlate with bird energy retention and may have calorie-nutrient ratios varying independently of metabolizable energy. For maximum protein deposition with minimal fat accretion, an energy scheme is needed to account for variations in heat production. This study will be devoted to enhancing ration utilizable ME_n (net energy) by reducing the need for protein supplementation, increasing bird heat distress tolerance, and optimizing lean tissue growth without excessive fat accretion through feeding diets to produce optimal cellular energy/nutrient ratios.

BIOENERGETICS AND THERMODYNAMICS

The bioenergetic field deals with metabolic energy transformations in living things according to the physical laws governing energy transformation. The first law of thermodynamics states that the total energy of a system, including its surroundings, remains constant (energy can not be created or destroyed). However, within that total system energy may be transformed into another form of energy. For example, chemical energy may be transformed into heat, electrical energy, radiant energy, or mechanical energy in living systems. The second law of thermodynamics states that the total entropy (disorder) of a system must increase if a process is to occur spontaneously. Entropy becomes maximum in a system as it approaches true equilibrium. These laws dictate that the heat produced from varies metabolic processes is the same as it would be if feed were allowed to combust to the same end-products.

The first law of thermodynamics asserts that the total amount of energy in an isolated system remains constant. When the energy content of a system changes, the sum of all forms of energy given off by the system must be equal to the magnitude of the change. The first law is only concerned with initial and final energetic states of the system. The principle of conservation of matter also is taken into account in the first law because matter and energy are inseparable according to the theory of relativity. Matter and energy are different expressions of the same thing. In animal systems, the energy equivalent of work, plus the maintenance energy of the animal, plus the heat increment of feed equals the energy generated from the oxidation of nutrients of the feed.

Hess's law of Constant Heat Summation states that all forms of energy are quantitatively convertible to heat. This law states that in going from a particular set of reactions to a particular set of products, the change in enthalpy is the same whether the reaction takes place in one step or in a series of steps. Oxidation of substrates within an animal body is quite different from oxidation in a bomb calorimeter, however from a thermodynamic point of view these facts are incidental. According to Hess's law the physical and physiological heat values of all nutrients, with the exception of protein, are the same.

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

THERMOBALANCE

HEAT RETENTION: Birds and mammals are homeotherms and consequently maintain a relatively constant core body temperature. However, diurnal body temperature cycles have been detected and are influenced by age, sex, season, time of day, work, digestion, drinking of water and environmental temperature (Dawson, 1975; Dukes, 1977). Overall animals have a temperature gradient declining from the body core to the peripheral tissues. The animals total heat load is dependent upon metabolism, the reactions by which

chemical energy is transformed into heat, and the environmental temperature which the animal is subjected too. In order to maintain a constant body temperature the animal must balance its rate of heat dissipation against its rate of heat gain. Heat is added to the body by metabolism; in hot environments the body may gain heat by radiation, convection and/or conduction. However, with poultry the principle problem relates to reduced nonevaporative heat dissipation at high ambient temperatures.

The body temperature is determined by the balance between the amount of metabolic heat produced and the quantity of heat lost from the body to the environment (Figure 1). For the body temperature to remain constant the heat production must equal the heat dissipated. Sturkie (1986) proposed the following equation to describe the process:

Energy balance requires that HP = NHD + EHD

where

HP = metabolic heat production

NHD = nonevaporative heat dissipation

EHD = evaporative heat dissipation

HEAT PRODUCTION: Total heat production is the sum of basal heat production (Bartels et al., 1973), heat production to maintain a constant body temperature when ambient temperature is low (van Kampen et al., 1979) or heat loss is high (Meltzer, 1983, 1987), heat production due to intake and digestion of food (MacLeod and Shannon, 1978; MacLeod et al., 1979), heat production due to synthesis and production, and finally heat production due to muscular activity (Boshouwers and Niciase, 1985). Heat stressed birds

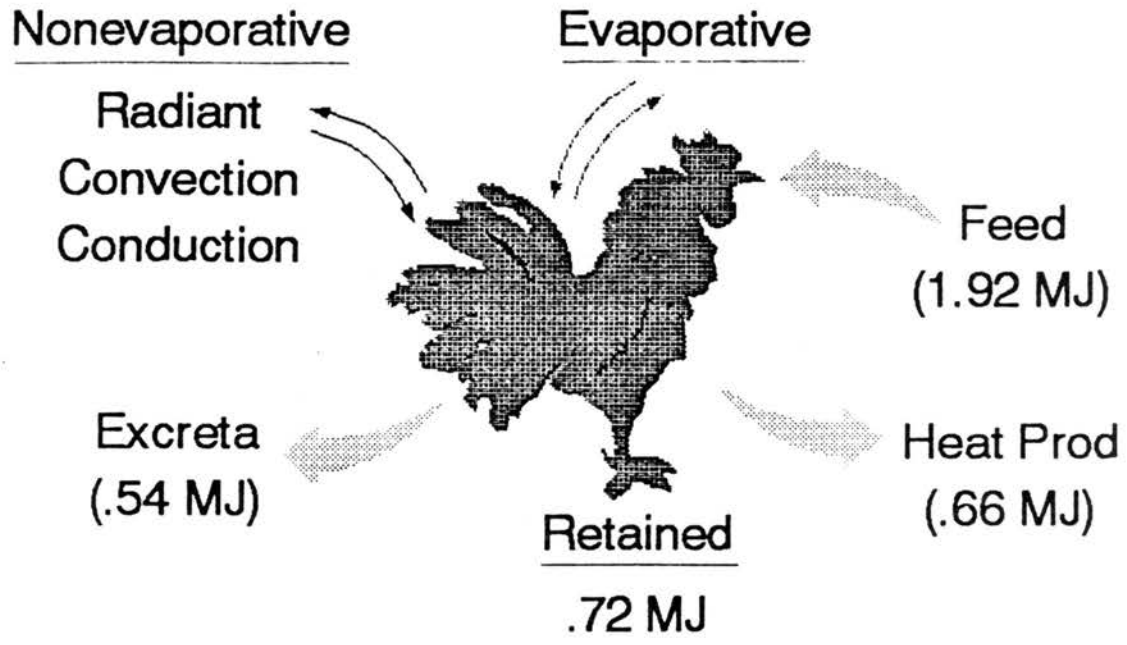

Figure 1. Principle energy exchanges between bird and environment

reduce their heat production via consuming less feed which in turn reduces substrate availability (Teeter and Smith, 1987).

As discussed, heat is produced by animals for a variety of purposes and its quantification is critical to many disciplines. Heat production may be estimated directly by measuring the heat lost from the body through radiation, convection, conduction, (Deighton, 1939; Benzinger and Kitzinger, 1949) and evaporation (direct calorimetry), by analysis of comparable carcasses at the start and end of a trial (comparative slaughter technique) and from the exchange of respiratory gases (indirect calorimetry). Both direct and indirect methods were originated by Lavoisier in 1777. Each method supplies essentially the same information however the difficulty and labor involved for each are quite different.

Indirect calorimetry provides a quantitative estimate of heat production using the gaseous reactants and end products of metabolism. Indirect calorimetry estimates the heat generated by respiratory gas exchange. Most methods depend on the measurement of oxygen consumption, carbon dioxide production and possibly methane production and/or urinary nitrogen excretion. Methane is a by-product of microbial fermentation in the rumen (Kleiber, 1961) and is usually not considered when estimating poultry heat production. No correction is usually applied for nitrogen excretion since Romijn and Lokhorst (1961, 1964, 1966) indicated that the error resulting from this omission is about .2% and should not exceed 1.5% even at a high rate of protein catabolism in poultry. The preferred equation for determining heat production (HP) was developed by Brouwer, 1965:

HP = 16.18 (L oxygen consumed) + 5.02 (L carbon dioxide produced)

Gas volumes are in liters at standard temperature and pressure.

The measurement of heat production by indirect calorimetry in poultry can be used to determine the energy of a feed that is available to the bird for maintenance and growth (Shannon and Brown, 1969; Burlacu et al., 1970a, 1970b) or it can be used to estimate the energy required by the bird exposed to a specific set of conditions. Factors affecting energy requirements include energy required for maintenance and production (Waring and Brown, 1965, 1967; Burlacu and Baltac, 1971), tissue synthesis (Shannon and Brown, 1970), effects of temperature (Romijn and Lokhorst, 1966; van Kampen, 1974; Farrell and Swain, 1977), deficiencies (Klieber, 1945), nutrient imbalances (Baldini, 1961), and diseases (Sykes, 1970, 1986).

The ratio of volume or moles of CO_2 produced to the volume or moles of O_2 consumed is known as the respiratory quotient (RQ). RQ's are only applicable when the biological substrates are completely oxidized to CO_2 and H_2O and the nitrogenous excretion products (Zuntz and Schumburg, 1901). Respiratory quotient is dependent on the form of nitrogen is excreted, in birds, uric acid is the major excretory product and in mammals, it is urea which predominates among the nitrogen end products. Typical RQ's (Table 1) for carbohydrate are 1.0; for mixed fats .7; and for mixed protein .81. Each specific fatty acid and protein may have distinctive RQ. The RQ's of fats with short-chain fatty acids is about .8 and long-chained fatty acids are typically around .7 (Church and Pond, 1988).

Table 1. The heat equivalent of oxygen and of carbon dioxide under different circumstances (Blaxter, 1989)

Substrate alteration	RQ	O_2 consumed (kJ/L)	CO_2 produced (kJ/L)	Heat increment J/J
Lipid oxidation	.71	19.7	27.8	.04
Protein catabolism	.81	19.2	23.8	.33
Carbohydrate	1.00	21.2	21.2	.05
Lipid synthesis	1.10	21.7	19.7	-

When indirect calorimetry is used to estimate RQ, CO_2 output, and O_2 uptake of the lungs are measured, but in actuality the gases should be measured at the cellular level. The measurements only define correct energy data in a steady state condition when the gas exchange from the tissue is equal to that of the lungs. In short term experiments (experiments run for just a few hours), with low total O_2 consumption and CO_2 production the possible changes in O_2 and CO_2 content of the body fluids may cause very large errors in the energy production data (Boshouwers and Nicaise, 1983).

Several investigators have reported RQ values lower than .7 in fasting fowl (Romijn and Lokhorst, 1961; Farrell, 1974; Morrison and Leeson, 1978). Boshouwers and Nicaise (1983) stated that RQ values lower than .7, discounting experimental error, must originate from a nonsteady state or from metabolic interconversions in which oxygen is used for the partial oxidation of the biological substrates. Bleibtreu (1901) reported RQ values greater than 1.0, he explained this high RQ by the synthesis of fat to carbohydrate which results in a partial liberation of O_2.

HEAT DISSIPATION: To preserve homeothermy or homeostasis animals must conserve heat in cold environments and dissipate it in hot environments (Kleiber, 1961). For dissipation, heat produced within the body must be transported to the surface of the body for conductive and vascular convection dissipation or for evaporative loss to the mucosa lining of the upper respiratory tract. Transfer of heat from the animal's core by nonevaporative heat dissipation to the environment depends on the temperature differential between its surface area and deep core temperature as well as its surface area

where peripheral blood flow may be regulated (Nolan et al., 1978). Heat from the body surface will flow to the environment when the surroundings are cooler than the birds body temperature. Compared with large birds small birds have a large surface area to volume ratio. As the ratio of surface area to volume increases efficiency of nonevaporative heat dissipation increases (Sturkie, 1986).

Thermophysiology involves the physiologic measurements concerning the mechanisms of heat exchange between the organism and its environment (Slonim, 1974). Heat transferred through the tissues to the surface of the skin can be lost from the body by both nonevaporative and evaporative heat dissipation. Nonevaporative or sensible heat dissipation as it is sometimes called occurs in three main processes: radiation, conduction, and convection (Ota and McNally, 1961; Roller and Dale, 1963; Hoch, 1971; Olson and Mather, 1974).

Radiation heat is in the form of electromagnetic waves and the rate of heat exchange is proportional to the ambient temperature surface-temperature differential. Radiation is supported by the internal steps comprising heat flow through circulatory convection and conduction from the body core, through the shell, to the skin surface and heat flow by conduction from the skin through the cover layer to the outer edge of cover (Walsberg et al., 1978).

Conduction is the heat transfer through a medium without material movement or transfer. A warm molecule collides with a cool one and transfers some of its kinetic energy too that molecule. Thermal conductivity depends on the medium to which the heat

is being lost be it air, water, or the ground. The knowledge of conductive heat flow is inadequate to formulate accurate whole bird conductive heat dissipation.

Convection is the heat transported by streams of molecules from a warm place to a cool one. For convective heat flow the internal step includes heat flow by circulatory convection and conduction from the body core through the shell to the skin surface, heat flow by conduction from the skin to the outer edge of the cover and heat flow by conduction through the boundary layer to the outer edge.

In poultry, evaporation of moisture occurs from the skin surface and from the respiratory tract (Sturkie, 1986). When water is evaporated, energy is lost at a rate of 586 calorie/gram water evaporated at 20 C. Evaporative water loss occurs at the skin due to passive diffusion of water vapor through the skin. Neither sweat glands nor sebaceous glands are present in the skin of birds. The amount of moisture that may be lost through the skin under heat distress is therefore limited.

Evaporative heat dissipation (EHL) occurs mainly from the upper respiratory tract. A significant proportion of the total heat loss is lost through respiratory evaporative heat dissipation when birds are panting. As the air passes over the wet surfaces of the respiratory tract, the air becomes saturated near the body temperature. Some of the heat is lost back to the upper respiratory tract and some of the water vapor is condensed as the bird exhales, however as long as the inspired air is not saturated and equal to the temperature of the body the expired air contains more heat than the inspired air.

THERMOBALANCE: ENVIRONMENTAL INTERACTIONS

The thermal environment is determined by the combination of ambient temperature, relative humidity, wind, precipitation, photoperiod, solar radiation intensity, and cloud cover. The ideal combination of thermal environment determinants, the combination that minimizes bird heat production at maintenance, is defined as the thermoneutral environment. Since birds are principally raised in confinement, critical factors are reduced to ambient temperature, relative humidity and wind velocity. Divergence of these factors from the ideal pattern results in animal distress accompanied by decreased growth rate, feed consumption and feed efficiency as well as increased mortality. A greater understanding of basic nutritional/physiological-thermal environment interactions must be understood such that managerial practices may be developed to reduce animal distress and thereby increase productivity.

A thermoneutral environment or comfort zone is defined as one which the animal does not need to increase energy expenditure to either warm or cool the body. The critical temperature is defined as the point at which an animal must increase its heat production to prevent body temperature from falling or increase the rate of heat dissipation to prevent body temperature from rising (Figure 2).

Total productivity and production efficiency of all animals declines as the ambient temperature diverges from the thermoneutral zone. In broilers substantial declines in productivity occur as a result of alterations in feed consumption and feed utilization. At an environment below the thermoneutral zone, broiler maintenance requirement for energy is enhanced thereby forcing greater feed consumption to maintain normal levels of

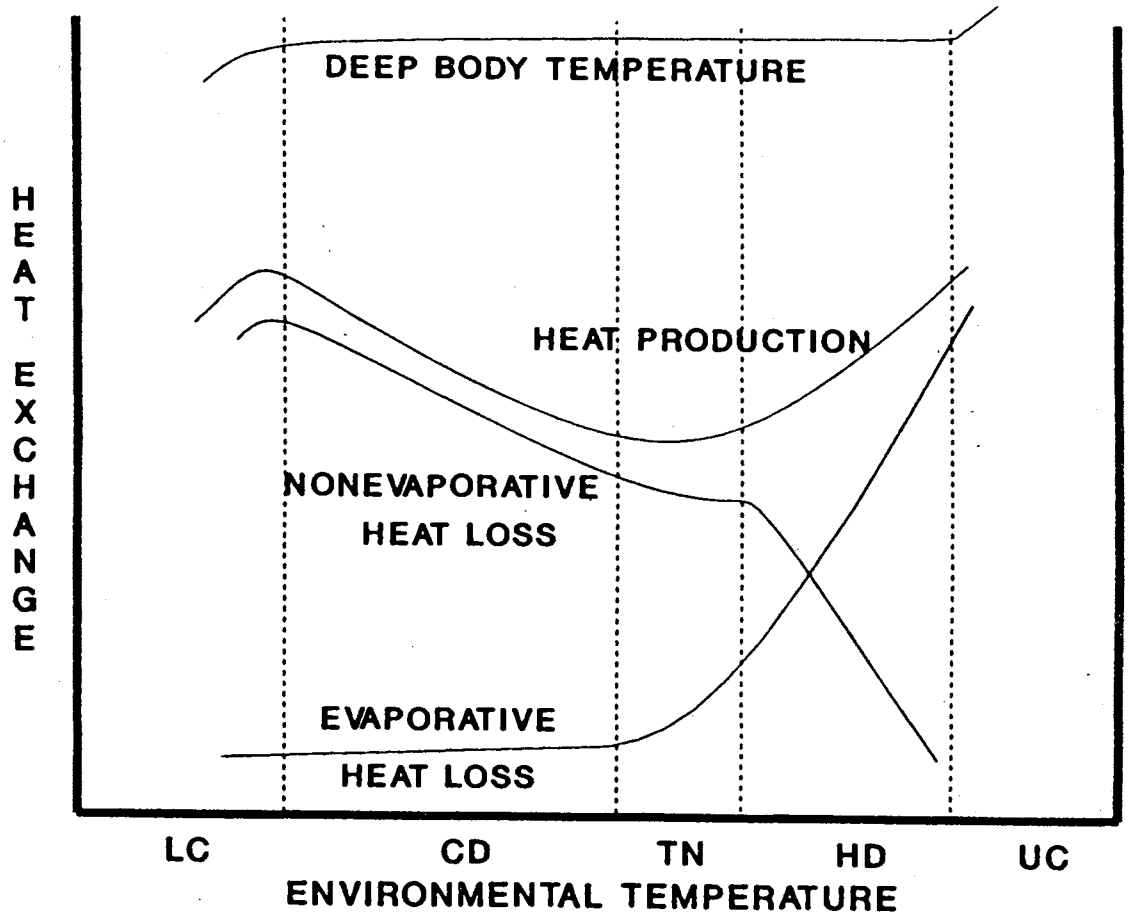

Figure 2. Ambient temperature effects on core body temperature, heat production, and nonevaporative and evaporative heat loss (LC= lower critical; CD=cold distress; TN=thermoneutral; HD=heat distress; UC=upper critical

production. Feed efficiency declines in these cases due to the added energy requirement to maintain body temperature. At environmental temperatures above the thermoneutral zone maintenance requirements for energy are also increased, but feed intake is reduced thereby lowering productivity and production efficiency.

Heat production and heat exchange vary with environmental temperature with the least amount of energy being produced and exchanged within the thermoneutral zone and referred to as the basal metabolic rate. Animals lose heat by conduction, convection and radiation from the body surface, and by evaporation of water from the body surface, lungs and oral surfaces. The rate of heat exchange depends on the difference between the body surface temperature and the environmental temperature. Surface temperature may be altered by constriction or dilation of blood vessels in the skin or extremities, thus increasing or decreasing surface temperature in relation to the environment. During ambient temperature distress, birds will increase respiration rate, cardiac output and decrease blood pressure to enhance blood flow to the extremities and tissues of the respiratory system (Michael and Harrison, 1987). Other factors affecting heat loss include posture, sweating, panting, and insulation of subcutaneous fat.

Environment plays a key role in heat production, but defining the thermoneutral zone in which the heat production is minimal has been shown to be variable (Stanier et al., 1984). Barott and Pringle (1941) reported the thermoneutral zone for the hen to be between 22.8 and 27.7 C, while Randall (1943) suggests a range from 19 to 29 C. van Kampen and Romijn (1970) provided a line of best fit to the results of heat production and environmental temperature from -5 to 40 C:

$$HP = 0.2453\ Ta^2 - 18.90\ Ta + 803.3$$

where

HP = heat production $kJ \cdot Kg^{-.75}$

Ta = ambient temperature (C)

This equation may be used to estimate heat production in widely differing temperatures.

The range of air temperatures over which birds are in thermoneutral zone varies from a few degrees C in younger birds to as much as 30 C in penguins (Le Maho, 1983). The heat production within the thermoneutral zone also depends on the level of feed intake prior to the measurement. The higher level of feed intake, the higher the heat production (Sturkie, 1986; Wiernusz and Teeter, 1993) and the heat increment will persist for a period of time before the basal metabolic rate is reached again.

In a thermoneutral environment, Brody (1964) indicated about 25% of the body heat is dissipated by moisture vaporization and about 75% of the heat is lost through sensible heat. Sensible heat may be measured by either direct or indirect calorimetry. Insensible heat must be estimated by determining the amount of water vapor lost by the animal. Farrell and Swain (1977) reported that evaporative heat loss was 85% of HP at 35 C and declined to 30% at 2 C and Wiernusz and Teeter (1993) observed similar trends.

COLD DISTRESS: Homeotherms exposed to low temperatures increase their metabolic rates to maintain deep core temperature. When an animal is subjected to its lower critical temperature, the limit of heat production capacity is reached. If the environmental

temperature becomes colder, the deep core body temperature begins to fall, metabolic heat production decreases and may lead to death by hypothermia.

Birds in cold environments must increase heat production in order to maintain homeostasis. Increased heat production may include biochemical calorigensis, heat increment due to feeding and muscular contraction from shivering or activity. Biochemical calorigensis refers to the basal metabolic rate and is a form of respiratory metabolism known as obligatory nonshivering thermogenesis. In birds the possible role of regulatory nonshivering thermogenesis like that of mammals, where enhancement of chemical calorigensis occurs in brown adipose tissue under sympathetic control, remains controversial. Heat production due to feeding may increase the basal metabolic rate as much as 20% and is referred to the heat increment of feed (Calder and King, 1974; Berman and Snapir, 1965). Shivering produces the largest sources of added heat production.

When the ambient temperature falls below the lower critical temperature, heat production increases due to shivering. The heat produced by shivering is derived largely from the oxidation of fatty acids (Marsh and Dawson, 1982). The rate at which the heat production increases in response to exposure of low ambient temperatures depends on the insulation of the tissues and feathering. The greater the insulation the lower the rate of heat production presumably due to greater retention. Smaller birds have less insulation and more surface area then larger birds and as a result the rate of increase in heat production is greater.

Shivering occurs in a variety of muscle masses such as muscles of the leg, neck and in the pectoral muscle (Hillman et al., 1977). Shivering is evident at an early age and it coincides with the development of homeothermy (Odum, 1942). Randell (1943) and Freeman (1966) reported shivering was not pronounced or was absent in chicks 2 to 24 hours old, however in a more recent study electromyographic activity was found in day old chicks (Saarela, 1976). Birds acclimated to cold temperatures have an altered shivering response. Arieli et al. (1979) reported that during cold exposure and with birds shivering, oxygen consumption is higher in cold acclimated birds than summer acclimated birds. No differences in basal metabolic rate were seen between the two groups of birds and it appears that, when exposed to cold distress, the difference comes from an increased capacity for oxidative catabolism. Cold exposure increases heart size resulting in an increase supply of blood to the muscles (Aulie, 1977). Davison (1973) suggested that gluconeogensis is activated during cold exposure in neonatal fowl since a rapid increase in plasma free fatty acids and a drop in plasma triglycerides was observed shortly after cold exposure.

HEAT DISTRESS: As the environmental temperature rises within the thermoneutral zone temperature regulation is maintained by increased heat dissipation. At temperatures above the thermoneutral temperature the capacity of nonevaporative heat dissipation may be exceeded resulting in an increased body temperature and metabolic rate which may result to death in hyperthermia.

At ambient temperatures above the thermoneutral zone, the heat production increases as a result of an increase in body temperature (Wiernusz and Teeter, 1993). The increased temperature of tissues results in an acceleration of chemical reactions and consequently in an increased oxygen requirement and heat production, known as the van't Hoff-Arrhenius effect.

High ambient temperature-relative humidity distress has been shown to reduce broiler body weight gain, feed efficiency, and survival. The birds total heat load is due to a combination of environmental heat and metabolic heat production. Birds produce and therefore must dissipate considerable amounts of heat daily as their net energetic efficiency is less than 25%. Heat distressed broilers must remove generated body heat in order to maintain body temperature at near normal levels. As the ambient temperature rises heat loss associated with nonevaporative cooling declines markedly as the temperature differential between the bird and its environment is reduced.

During increased high ambient temperature exposure, the bird's heat load is increased due to the environmental heat gain and the energy cost associated with activation of metabolic processes required for heat dissipation (Balnave, 1974; Meltzer, 1987). Heat dissipation is enhanced by postural adjustments to increase surface area (Baldwin, 1974), vasodilation of unfeathered extremities (Nolan et. al, 1978), by increasing water intake (Farrell and Swain, 1977; Belay and Teeter, 1993), by increasing urine volume (van Kampen, 1981; Belay and Teeter, 1993) and elevating respiration rate from the basal 25 breathes per minute to as much as 250 (Frankel et al., 1962; Simon, 1981; Wiernusz and Teeter, 1993). Arieli et al. (1980) estimated 4 $mg \cdot Kg^{-1} \cdot min^{-1} \cdot C^{-1}$ of

evaporative water loss when broilers were subjected to ambient temperatures above 26 C. Respiration rate is of particular importance as a considerable amount of water is evaporated from the mucous membranes of the respiratory tract. During heat distress, blood flow to this region is doubled to provide water for evaporation (Freeman, 1984).

Barott and Pringle (1946) measured a 17% increase in bird heat production at ambient temperatures above the thermoneutral zone. They concluded that this increase resulted from the energetic cost of panting. However Romijn and Vreugdenhil (1969) did not observe an increase in heat production due to panting in fowl between 35 and 40 C even though the respiration rate increased from 30 to 150 breaths per minute. The true cost of panting requires further study because an increase in the metabolic demand of muscles involved in panting may be offset by a decreased metabolic demand of other tissues (Weathers and Schoenbaechler, 1976).

Increased respiration rate during heat distress is critical to maintain constant body temperature. However, the increased respiration rate lowers the partial pressure of carbon dioxide in the blood resulting in a lower bicarbonate concentration and elevated blood pH. When chickens are exposed to heat distress, both partial pressure of carbon dioxide and bicarbonate in blood decreases thereby increasing blood pH (Bottje et al., 1985; Teeter et al., 1985).

Birds under heat distress must deal with the conflict between the hyperthermic response to increase tidal volume for greater evaporative heat loss and the need for minimizing hypercapnic alkalosis by reducing tidal volume. When the body temperature reaches about 44 C respiration rate reaches a peak of 150 to 260 breathes per minute

(Frankel et al., 1962; Kassim and Sykes, 1982), tidal volume decreases as respiration rate increases, but this is not enough to prevent minute volume from increasing. This pattern of respiratory events is termed phase I panting which is followed by phase II if hyperthermia proceeds further. Phase II panting is characterized by a slow deep breathes which increases the tidal volume. Phase II panting results in a acute respiratory alkalosis (Yousef, 1985)

FEED ENERGY-METABOLISM

BASAL METABOLISM: The basal metabolic rate or standard metabolic rate is defined as the heat production occurring by an animal at rest, awake, fasted and housed within its thermoneutral zone. Under these conditions the rate of energy metabolism is a function of surface area since heat loss is closely tied to this factor (Brody, 1964). Surface area per unit body weight declines with increasing body weight and basal metabolism per unit weight declines with increasing body weight. However, surface area is a difficult parameter to estimate and numerous attempts have been made to relate it to body weight (Brody, 1964). Typically body weight will be raised to a power, most commonly .75, which is now regarded as the universal metabolic weight. Brody (1964) suggested weight$^{.73}$ be used as a reference base for basal-energy metabolism in mature animals of different species including a weight range from mice to elephants.

Brody reported that for mature birds of different species the exponent varies from .62 to .70. Metabolic weight for poultry is commonly reported as W$^{.66}$, since this value gives a better estimate when comparing poultry within species. If the correct power is

chosen and body temperature and animal composition are constant, then heat production per unit metabolic weight is relatively constant. Under basal conditions, heat energy is produced from various energy sources to offset heat loss and maintain constant body temperature.

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

ENERGY METABOLISM SCHEMES: The total energy contained in feed is termed gross energy (GE). However only a portion of gross energy actually appears in animal products such as meat, eggs or milk. Blaxter (1989) estimated the efficiency of ME_n utilization to be 45, 68, and 60% for meat, egg, and milk production. Considerable energy losses occur in the feces and heat through oxidative processes (Figure 3).

Feed energy absorbed in the gastrointestinal tract is termed digestible energy. The apparent digestible energy of the ration or individual nutrients such as carbohydrates, proteins and fats may be estimated by subtracting fecal energy from gross energy. This value is termed apparent because the endogenous losses have not been accounted for.

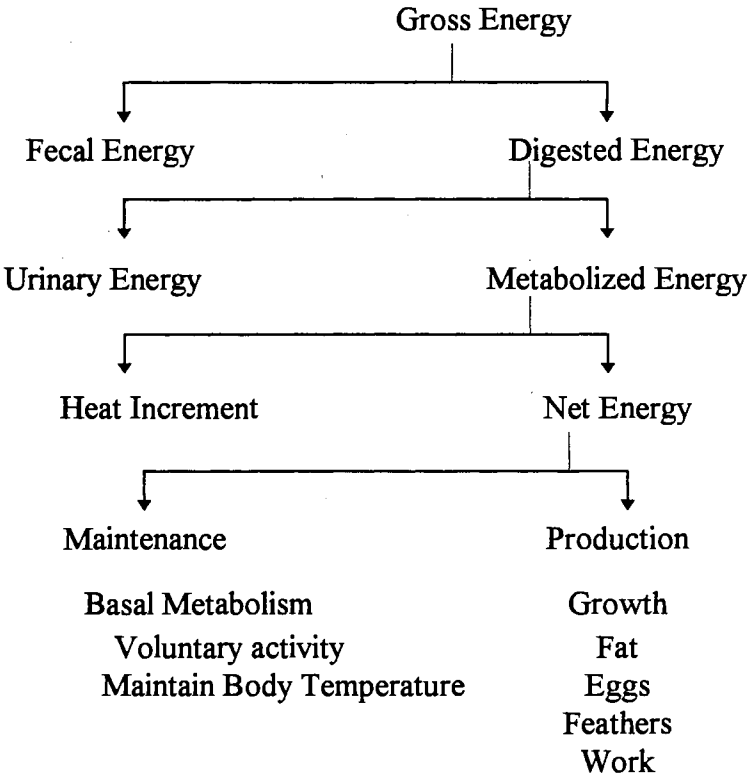

Figure 3. Energy Distribution and Utilization Scheme

Metabolizable energy losses may be estimated by subtracting gas and urinary energy loss from apparent digestible energy yielding apparent metabolizable energy.

Metabolizable energy (ME) is the energy provided for metabolism including tissue assimilation and oxidation. However, during ME utilization wasted heat is given off. Shortly after these molecules are absorbed into the gastrointestinal tract there is an increase in heat production referred to as the heat increment of the feed. Subtracting the wasted heat from ME provides an estimate of net energy, or the energy actually contained in tissues or utilized for work. The net energy is available to maintain body temperature, to provide energy for work and other activities, to be stored as adipose tissue and for growth and production.

NET ENERGY: The net energy efficiency of a feedstuff may be expressed as:

$$NE_m = RE/IE$$

NE_m = net energy for maintenance (kcal/g)

RE = retained energy

IE = gross energy consumed

Net energy is estimated by determining retained energy (energy balance) and may be a positive or negative quantity. Two basic techniques have been developed for acquiring this information. The balance trial, first used by Lawes and Gilbert (1861), attempts to measure the difference between energy input and output. An alternative technique is the comparative slaughter technique, which seeks to relate energy input to changes in body composition.

The comparative slaughter technique can be quite accurate, but it is laborious, time consuming and forces one to assume initial animal composition. It estimates the actual amount of energy retained by the animal or conversely the measurement of all forms of energy loss. The variability that exists at 3 or 4 weeks of age, when the majority of comparative slaughter trials start, is great when you consider the differences in body composition.

In growing and fattening animals the measurement of energy retention has been proven useful in broilers, cattle and swine. In work done with broilers, the energy value of whole carcasses can be determined by bomb calorimeter after the initial and final slaughter groups have been homogenized. Heat production is calculated as the difference between the energy intake and the energy of body weight gain plus that of the excreta.

The comparative slaughter technique has several potential sources of error because it is difficult to obtain a truly representative sample of birds. Davidson and Mathieson (1965) suggested that the composition of birds killed at the beginning of a trial is probably representative of the remainder when very young birds are used. However, Fraps and Carlyle (1939) and Hanlen (1939) reported, variability exists within older birds especially due to their varying fat content. Errors may also be produced in the measurement of ME using either a total collection or marker and relating it to the entire experiment since Sibbald et al. (1960) and Burlacu et al. (1970b) indicated that the ME especially in young chicks may change with age and level of intake (Deighton and Hutchinson, 1940; Tasaki, 1969).

A method for calculating net energy values for feedstuffs from determined ME values has been described by DeGroote (1974). The studies of Hoffman and Schiemann (1971) and Chudy and Schiemann (1971) has shown that the utilization of ME for carbohydrates, fats, and proteins for lipogenesis is 75, 84, and 61%, respectively. These values are in agreement with Burlacu et al. (1970a, 1970b) and Mittelstaedt and Teeter (submitted). Work conducted by Mittelstaedt and Teeter using precision fed birds, to evaluate gelatin, starch and corn oil has established that the ME_n utilization for energy gain varies by over 150% with each nutrient class (Table 2). The three ME_n sources evaluated were added on top of the basal ration (fixed intake) supporting maintenance and growth and supplying 125% of NRC amino acid recommendations. Protein gain was not impacted by energy supplementation class while fat gain increased with elevated ME_n utilization efficiency. Energetic efficiencies for the various substrates would be higher if the authors did not factor out bird maintenance. Therefore, one might speculate, that the increased carcass leanness obtained by feeding high protein rations could be a result of reduced cellular energy supply.

Despite these discrepancies in nutrient utilization, the ME system is still the main energy utilization scheme used in the poultry industry. The use of protein as an energy source is inefficient because economically it is more expensive then that of fat or carbohydrate, much energy is required for the formation of glucose from amino acids, and the excess nitrogen produced must be removed by the formation of uric acid. In order for the broiler to achieve maximum protein accretion efficiency a net energy system is needed if maintenance and production can be specified.

Table 2. ME_n energy retention utilizing nutritionally complete limit fed rations.

Variable	Basal	Gelatin	Starch	Corn oil
Ingredient ME_n intake	--	897.6	782.6	887.4
Energy gain due Ingredient	--	183.1	367.9	462.2
Efficiency (%)	--	20.4[b]	47.0[a]	52.0[a]

[ab]Means within a row with unlike superscripts differ ($P<.05$)

DIET COMPOSITION

The degree of carcass fatness is well documented to be impacted by both non-nutritional and nutritional factors. The effects of age (Combs, 1968; Edwards, 1971; Kubena et al., 1972b), sex (Summers et al., 1965; Thomas and Twining, 1971) and ambient temperature (Swain and Farrell, 1975; Howlider and Rose, 1987) are well known among the non-nutritional factors. Fraps (1943) first described the nutritional effects of varying dietary ingredients on broiler carcass fat, since that time numerous studies have investigated the effects of calorie/protein ratio on broiler carcass composition during high ambient temperature heat distress.

CALORIE/PROTEIN RATIO: The decline in feed intake, growth rate and survivability of broilers exposed to high ambient temperature-relative humidity distress (HD) has long been documented (Squibb et al., 1959). The HD broiler retains unexpressed potential for growth, as force feeding such birds to higher feed consumption levels was reported to increase growth rate (Smith and Teeter, 1987a, 1987b; Teeter et al., 1987; Teeter and Smith, 1985, 1987). The added growth, however, was accompanied by increased mortality suggesting that the added heat of metabolism exacerbated the HD state. Indeed, Wiernusz and Teeter (1993) reported that bird heat production (HP) increased by 44% as feed consumption rose from 0 to 9% of bird body weight. As a result, rations producing less heat per Kcal ME_n consumed would be expected to induce better performance during HD.

A number of dietary manipulations have been proposed to enhance growth rate of broilers. Kubena et al. (1972a) recommended increased ration protein and amino acid fortification levels to counter the reduced feed intake. In sharp contrast, Waldroup et al. (1976) recommended reducing dietary crude protein level with crystalline amino acid fortification to maintain indispensable amino acid concentration. The basic premise here was to reduce ration heat increment.

Adams et al. (1962) reported that increasing ration caloric density (CD) by substituting fat for corn at a constant calorie : protein ratio (CPR) was beneficial in overcoming high ambient temperature effects on gain. The authors indicated that the CD effect occurred independent of environmental temperature which was confirmed by Dale and Fuller (1979, 1980). However, specific environmental effects can not be ruled out as studies (Fisher and Wilson, 1974; Valencia et al., 1980; Abdelkarim et al., 1985; Sinurat and Balnave, 1974, 1986) have suggested that the gain response is less pronounced at higher ambient temperatures. Though fat calories have less heat increment per kcal ME_n than carbohydrate or protein, the fact that rations supplemented with fat frequently result in higher energy consumption (Kubena et al., 1972b) could affect the reduced heat increment per Kcal ME_n and actually increase HP.

Carcass fat content is well documented to be increased by high ambient temperature (Swain and Farrell, 1975; Howlider and Rose, 1987; Sonaiya et al., 1990), by low dietary crude protein and higher CPR (Summers and Leeson, 1979; Jones and Wiseman, 1985; Jones, 1986; Bartov, 1987). The increased carcass fat during HD may reflect metabolic adjustments to reduce HP as Pullar and Webster (1974) reported 2.22

kcal heat produced per kcal protein retained in contrast to just 1.38 kcal heat per kcal fat. Rations formulated to contain low CPR are advantageous for reducing carcass fat, but their use during HD may elevate mortality if the bird heat load is adversely affected.

PROTEIN INTAKE, DIGESTION AND ABSORPTION

The ingestion of feed increases both heat production and the retention of energy in the body The relationship between daily energy retention and the enthalpy of combustion of feed intake is curvilinear with the retention per unit feed ingested falling as intake increases (Blaxter, 1989). Part of this is due to the decline in the metabolizable energy of unit feed intake increases, however, even when retention and metabolizable energy intake are plotted the relationship is still not linear. In most species the slope is higher below maintenance than it is above and there is evidence that the slope becomes lower at very high intakes.

The elevated heat production associated with feed intake is commonly referred to as heat increment or specific dynamic action of the feed. The resulting heat is produced by oxidative reactions that are not coupled with energy transfer mechanisms or the result of incomplete transfer of energy and is partly due to heat production resulting from work of excretion by the kidney and increased muscular activity of the gastrointestinal tract, respiration, and circulatory systems resulting from nutrient metabolism. This phenomenon is most pronounced with proteins or amino acids and relatively small effects are observed with fat or carbohydrate (Blaxter, 1989).

Immediately upon the digestion of feed, there is a stimulation of the vagus nerve in the gastric mucosa which starts the secretion of gastric juice into the proventriculus. This juice contains hydrochloric acid, mucins, and proteinases. Dietary protein entering the gastrointestinal tract is partially hydrolyzed by the action of the endopeptidases (pepsin, trypsin, chymotrypsin, elastase, and enterokinase) and the exopeptases (carboxypeptidase A and B). Pepsinogen is secreted by the peptic cells of the proventriculus and is converted to pepsin upon exposure to the acid. The polypetides resulting from pepsin digestion are broken down further in the small intestine by trypsin, chymotrypsin, and by elastase. The enthalpy of hydrolysis of proteins in the lumen of the gut has been estimated to be about .1 to .2% of the energy of the substrates hydrolyzed and the standard free energy of peptide bond is about 3 kcal/mol (Blaxter, 1989). The resulting action of these enzymes form terminal peptide bonds which are attacked by aminopeptidases, carboxypeptidases and other specific peptidases present in the lumen or mucosa of the small intestine.

Endopeptidases secreted in the proventriculus and by the pancreas degrade 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. Peptides that are absorbed into the mucosal cell, are hydrolyzed into free amino acids by intracellular peptidases located in the cytoplasm of the intestinal

mucosal. The amino acids enter the portal blood stream from the mucosal cells as free amino acids and only trace amounts of peptides can be detected in plasma. Most of this work has been done in mammals, but there is evidence that similar events occur in the chicken.

PROTEIN AND AMINO ACID METABOLISM

PROTEIN SYNTHESIS: The concentration of amino acids is higher intracellularly than extracellularly. Transport of amino acids across the cell membrane is by an active processes requiring the hydrolysis of high energy bonds. Specific groups of amino acids are transported by selective systems. The neutral amino acids (alanine, glycine, proline, serine) are transported by a sodium-dependent A-system, whereas leucine and other amino acids with hydrocarbon side chains (serine, threonine, tyrosine) are transported by a sodium-independent L-system. Other groups of amino acids are transported by other systems. The A-system is stimulated by insulin, whereas the L-system is not. Waterlow et al. (1978) calculated the entry rates of individual amino acids into rat skeletal muscles and concluded that the entry rates of arginine, methionine, and histidine were only slightly higher than their requirements. These rates of transport of these amino acids across the cell membranes may be of importance for the rate of muscle protein synthesis in growing animals.

Protein synthesis requires the activation of intracellular amino acids into aminoacyl-transfer RNA (aminoacyl-tRNA) and their coupling into the sequence characteristic of muscle proteins. The last step, involving the ribosomes and messenger

RNA, is referred as translation; later, the polypeptide chains undergo posttranslational processing in order to achieve characteristic structures. Several forms of RNA are generated in the cell nucleus from deoxyribonucleic acid (DNA) by transcription and modified by posttranscriptional pathways in the nucleus and cytoplasm. The energy for protein synthesis (about 4 mol ATP/mol peptide bond; Blaxter, 1989) is supplied from the hydrolysis of ATP and guanosine 5'-triphosphate (GTP); these high energy phosphate bonds are regenerated in the mitochondria. The reader is referred to Lehninger (1975) for a more detailed treatise of protein synthesis.

PROTEIN CATABOLISM: Proteolysis is catalyzed by proteinases. Like the proteinases of the digestive tract, the hepatic proteinases are divided into endozymes and exozymes. The endozymes cleave native proteins into smaller polypeptides and the exozymes hydrolyze the smaller polypeptides into amino acids. The major hepatic endopeptidases are cathepsins B and D, both are present in liver lysosomes. Cathepsin B cleaves peptide bonds at the carboxyl side of basic amino acids and its major substrates are albumin, ribonuclease and cytochrome C, but enzymes like arginase and alanine transferase are also broken down. Catepsin D cleaves hemoglobin but also attacks albumin and intracellular proteins. The major exopeptides are located in the cytosol, but some exopeptidases are contained in the lysosomes where the major part of hepatic proteolysis takes place.

Tissue proteins are degraded at different rates and the breakdown of proteins in skeletal muscles cells are accomplished by proteinases. The rate of proteolysis in muscle cells is low compared with the liver and corresponds to the lower content of proteinases

found in muscle cells. Most proteinases activity has been found in the myofibril cell fraction, while hepatic proteolysis seems to be associated with and regulated by lysosomes. Lysosomes have not been identified in muscle cells, however evidence for the existence of lysosome-like particles in muscle fibers has been observed by Pennington (1977).

PROTEIN AND FAT ENERGETIC EFFICIENCY: The principal aim of animal production is the production of high quality protein in the form of meat, milk, and eggs for human consumption. To maximize accretion of protein in muscle, a considerable amount of fat also is deposited. This is inefficient and no longer wanted by the consumer. Protein synthesis (protein deposition, protein in milk, eggs) requires a large amount of energy. A through understanding of the relationships between the energy requirements for protein production is prerequisite for improving efficiency.

The energy cost for fat and protein accretion is simply the increment of feed energy required to obtain a defined increment in body protein or fat (Table 3). The amount of energy required for fat deposition can be estimated in adult animals because the protein gain is minimal and the amount of energy needed to maintain energy balance is constant. In species such as the pig and rat, the energy cost of fat deposition ranges from about 1.4 kJ ME/kJ fat deposited for feed consisting predominantly of carbohydrate to 1.15 kJ ME/kJ fat deposited for feed rich in triglycerides (ARC/MRC Committee, 1974). The energy cost of protein deposition is more difficult to assess. Even during rapid growth the amount of energy deposited as protein is small relative to that deposited as fat or dissipated as heat. Confounding this rapid growth rate, the division of feed

Table 3. The theoretical efficiency (J/J) with which the energy of nutrients is employed in synthesis, calculated from the stoichiometry of transport and synthesis (Blaxter , 1989)

Dietary substrate	Product	Estimated efficiency	Heat increment
Carbohydrate	Glycogen	0.95	0.05
	Body fat	0.80	0.20
Lipid	Body fat	0.96	0.04
Protein	Body fat	0.66	0.33
	Body protein	0.86	0.14

energy between the maintenance requirement and that for protein and fat accretion changes continuously.

EFFICIENCY OF PROTEIN AND FAT DEPOSITION: Efficiencies of utilization of metabolic energy have been measured by determining energy retention when two different amounts of metabolizable energy are given. Lean protein growth is less efficient energetically than fattening. Pullar and Webster (1977) concluded that in simple stomached animals, such as the rat and the pig, the apparent energetic efficiencies above maintenance of protein and fat accretion were 0.44 and 0.74, respectively. Table 4 lists estimates of the energy costs of protein and fat deposition in pigs and rats. All the values listed with the exception of Pullar and Webster (1977) depend on certain assumptions as to the relationship between body weight and metabolizable energy requirement for maintenance. Thorbeck (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. Kielanowski and Kotarbinska (1970), Close et al. (1973) and McCracken and Weatherup (1973) assumed that the maintenance energy requirement of the growing pigs was proportional to body weight$^{.75}$. In experiments of Gadeken et al. (1973), Schiemann (1963) and Thorbek (1970), the maintenance energy requirement was assessed from measurement of energy balance. However, body weight still had to be included as a major factor in the regression analysis. In the Pullar and Webster (1977) experiment, body weight was not included as a variable for regression analysis because weight differences between the fatty and lean rats were small. Webster and Pullar's values of 2.25 and 1.36

Table 4. Estimates of the metabolizable energy requirement for protein and fat deposition in pigs and rats

Species	Diet ME (kJ/g dm)	Protein deposition[1]	Fat deposition[1]	Assumed maintenance requirement (kJ/kg W/24 h)	Source
Pigs	-	2.80	1.36	424 $W^{.75}$	Kielanowski and Kotarbinska, 1970
	16.2	1.85	1.42	375-460 $W^{.75}$	Gadekan et al., 1973
	14.1	1.75	1.45	418 $W^{.75}$	Close et al., 1973
	13.4	2.05	1.33	7029 + 33.4 W	Thorbek, 1970
	14.6	2.19	1.20	7029 + 33.4 W	Thorbek, 1970
	14.4	1.98	1.37	7029 + 33.4 W	Thorbek, 1970
	-	2.06	1.40	548 $W^{.75}$	Schiemann, 1963
Rats	-	1.32	1.32	468 $W^{.75}$	McCracken and Weatherup, 1973
	13.4	2.32	1.53	Lean, 63.9 + 310 W Fat, 19.6 + 380 W	Pullar and Webster, 1974
	18.3	2.25	1.36	-	Pullar and Webster, 1977

[1]Energy requirement (kJ ME/kJ tissue)

kJ ME/kJ for protein and fat, respectively, are in close agreement with the other values presented in Table 4 suggesting that the assumptions made by the other authors may be correct.

The values for the energy costs of protein and fat deposition are arbitrary since they do not describe the total energy costs of synthesis, but simply relate the difference between total protein synthesis and degradation to increments of metabolizable energy. The energy cost of synthesizing protein and fat additional to that which is deposited in the growing body is included within the maintenance requirement. Until reliable measurements can be made of total protein and fat synthesis in animals, it will not be possible to asses the true contribution of the energy costs of protein and fat synthesis to the energy requirements for growth.

EXCESS PROTEIN AND AMINO ACID: Any surplus amino acids are absorbed by the intestine and conserved by the kidney. Because amino acids are not stored and excessive amounts of both amino acids and ammonia, a major end product of amino acid metabolism, are toxic, excess of amino acids must be catabolized and eliminated from the body. Amino acid catabolism provides carbon and energy for glucose synthesis via gluconeogensis.

Typical feed formulations based on natural feed ingredients provide excess of both dispensable and indispensable amino acids. Not only does an oversupply of amino acids result in waste, because the animal does not convert the excess amino acids into body proteins, but excess amino acids also may depress animal performance leading to

inefficient and uneconomical animal production. By using crystalline amino acids in diets, amino acid excesses can be reduced. Although crystalline amino acids have been used to lower dietary protein levels for over 30 years, concern remains as to whether low protein-amino acid supplemented diets can maximize animal performance and carcass lean accretion.

AMINO ACID AVAILABILITY AND UTILIZATION: Crystalline amino acids are more digestible than protein-bound amino acids (Izquierdo et al., 1988). Protein-bound amino acids can differ in digestibility. Therefore, an additional factor that may account for enhanced animal performance and/or carcass quality, when animals are fed crystalline amino acid supplemented diets, is increased in amino acid utilization due to greater absorption of free than protein-bound amino acids. However, absorption may be too rapid Rolls et al. (1972) and Batterham and Bayley (1989) suggested crystalline amino acids may arrive at the cell at a time prior to the other indispensable amino acids supplied by intact protein. Being in excess at this time, it is deaminated and its nitrogen is lost in urine. This apparent imbalance could enhance oxidation of limiting essential amino acids. Following a meal, dietary proteins serve as the principle source of amino acids entering plasma. The postprandial increase in amino acid concentrations may not mirror the relative amounts of the same amino acids in the feed protein, because protein and amino acids may affect gastric function, rates of amino acid release from protein will differ, rates and extents of digestion of protein sources differ, absorption rates and sites for individual amino acids and peptides differ, and certain amino acids compete for absorption when

presented to the intestinal mucosa (Adibi and Mercer, 1973; Savoie et al., 1988; Matthews, 1990).

In pigs (Batterham and Bayley, 1989), chicks (Baker and Izquierdo, 1985), and rats (Rafalski et al., 1975) diets containing both crystalline amino acids and intact proteins must be consumed frequently for maximum amino acid utilization. Efficiency of nitrogen utilization by growing boars was lower when fed once daily than when fed twice or four times daily; no difference was noted between feeding two or four times daily (Partridge et al., 1985). Otto et al. (1990) also reported that lysine utilization by chicks fed a diet containing either free or protein bound lysine was equivalent when fed either four times daily or *ad libitum*. Thus, when animals are fed *ad libitum*, crystalline amino acids should be fully utilized.

The balance of amino acids in a feedstuff or a combination of feeds can influence animal performance. Improperly balanced diets potentially reduce feed intake, nutrient metabolism, growth rate, and impact carcass composition. The ideal amino acid balance concept suggests that nutritionists must not lose sight of the practical implications of utilizing multiple ingredients to achieve diets with better balance. Although many feedstuffs have excellent amino acid profiles, high levels in mixed diets may have detrimental effects on feed intake (Wahlstrom et al., 1983).

EFFECT OF PROTEIN LEVEL ON AMINO ACID REQUIREMENTS: The level of indispensable amino acids required for maximum performance is affected by the level of dietary protein. Under cases where dietary protein levels vary from deficiency to

adequate, the requirement has been suggested to vary directly with protein level (Boomgaardt and Baker, 1973). In cases where dietary protein intakes are adequate for maximum growth, amino acid requirements, as a percent of the diet, appear to increase at a decreasing rate as dietary protein levels increase (Baker et al., 1975). The requirements for amino acids depend upon the variables being measured (Robbins et al., 1979) as it is often assumed that more amino acids are required to maximize lean tissue deposition followed by feed efficiency and growth rate. In most studies, requirements have been based on total body weight gain or feed efficiency and not on lean carcass deposition. Thus, if amino acids are added to low protein diets to meet animal requirements, deficiency in certain other amino acids may limit lean growth.

THEORETICAL EFFICIENCY OF AMINO ACID USE: Recent research related to broiler amino acid and energy nutrition has established the following: 1.) efficiency of ME_n utilization 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 and Teeter, submitted), 2.) that broiler consumption of excess amino acids during acute heat distress increases bird mortality (Belay and Teeter, 1991) and 3.) free energy liberation upon catabolism of different amino acids to Kreb cycle or glycolytic pathway intermediate varies by over 400%. Low net energy availability of amino acids is due to a combination of inefficient carbon chain utilization and uric acid synthesis-excretion. Least cost diet formulation normally necessitates that excess amino acids be fed. Assumptions made regarding excess dietary amino acids frequently include equal ME_n utilization efficiency

compared to other nutrient classes and that excess amino acid consumption are inconsequential. Research is needed to identify those amino acids that produce the greatest heat upon catabolism such that appropriate restrictions may be placed on amino acid excesses for heat distressed birds and further such that calorie/protein ratios may be adequately adjusted to reflect the true cellular energy and amino acid supply.

Whether the reduced ME_n utilization efficiency for gain should be attributed to protein per se or to specific amino acids has not been addressed. The problem is compounded by the apparent lack of thermodynamic data regarding energy changes accompanying amino acid metabolism to various intermediates. Questions also exist regarding how far metabolism must progress before heat production is no longer obligatory. Conceptually, amino acid metabolism to Kreb cycle or glycolytic intermediate would appear to most adequately describe obligatory heat production as carbon skeleton fate thereafter would be determined by animal need. Utilizing Gibb's free energy values to predict free energy (entropy+enthalpy) change upon such amino acid metabolism reveals that energy liberation is indeed strongly influenced by the specific amino acid (Table 5). As such, the energetic efficiency for individual amino acids is needed so that ration ME_n may be adequately adjusted.

The Gibbs free energy values represent a combined enthalpy and entropy value. From the vantage of heat production enthalpy would presumably be of greatest interest. However, the importance of entropy can not be eliminated as a factor potentially influencing bird heat production as life itself is a state of highly controlled entropy. Isoenthalpic rations differing in entropy liberation upon catabolism, could theoretically

Table 5. Obligatory Gibbs free energy liberation[1] for glucose, palmitic acid and selected amino acids utilizing available thermodynamic data

Substrate	Product(s)	Gibb's Free Energy[2]
Glucose	Lactate	-.32
Palmitic	Acid Triglyceride	-.04
Alanine	Pyruvate	-.02
Valine	Succinyl COA	-1.64
Leucine	Acetoacetic acid	
	+ Acetyl COA	-2.87
Isoleucine	Succinyl COA	-4.35
Glycine	Pyruvate	-1.34
Threonine	Acetyl COA + Pyruvate	-2.80
Cystine	Pyruvate	-2.81
Tyrosine	Fumaric acid	-3.60
	+ Acetoacetic acid	

[1] NADH produced was oxidized to H_2O

[2] A more negative number reflects greater energy liberation

increase animal heat production. Unfortunately, literature values for the necessary metabolites are not readily available.

An additional consequence of low amino acid ME_n utilization efficiency is that the birds heat load is increased. Elevated heat load is of little consequence when birds are housed at thermoneutral temperatures and below. However, if the birds heat load is elevated during high ambient temperature distress, without a concomitant increase in its ability to dissipate heat, the consequences of elevated heat load can become significant. Indeed, studies with birds fed varying protein and calorie/protein ratios, indicate that increasing dietary energy and/or narrowing calorie-protein ratios by relaxing restrictions on amino acid balance (necessitating increased dietary protein) significantly impacts bird performance. Improving amino acid balance and lowering dietary crude protein numerically increased survivability in the thermoneutral environment (4.4%) and significantly so by 10.8% within the heat distressed environment (Belay and Teeter, 1993). Broiler producers will most certainly benefit by lowering crude protein (at adequate amino acid balance) of rations fed to heat distressed broilers. Research is needed to identify the high risk amino acid categories.

APPLICATION AND BENEFIT

A thorough understanding of energy metabolism for all poultry classes is fundamental to profitable production enterprises. Though the specific mechanisms of dietary protein and fat utilization, as discussed, are well defined relatively little research has been conducted to quantitatively estimate energetic efficiencies. Research is needed to

identify nutrients producing the greatest heat upon catabolism such that appropriate restrictions may be placed on nutrients supplied in excess and further that nutritionists may adequately adjust diets for all birds to better reflect cellular energy and amino acid supply.

The purpose of this study is to evaluate metabolizable energy utilization for tissue energy gain for carbohydrates, fats, and proteins. Additionally, the effects of amino acid analogues on broiler thermobalance and predictive equations for carcass fat and protein determination will be examined. Data collected will be used to determine if the metabolizable energy system reflects the cellular energy supply of the broiler. Increased understanding and application of cellular energy-nutrient relationships will be required to produce leaner birds. The trend towards increased consumer demand for leaner poultry products at nominal cost will necessitate that product leanness, uniformity and be supplied by poultry producers worldwide.

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CHAPTER III

An Evaluation of DL-Methionine and DL-2-Hydroxy-4-Methylthio Butanoic Acid on Broiler Thermobalance, Feeding Pattern and Performance during Thermoneutral and Cycling High Ambient Temperature Exposure[1].

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ABSTRACT Two experiments were conducted to evaluate effects of DL-methionine (DLM) and DL-2-hydroxy-4-methylthio butanoic acid (DL-HMB) on broiler growth rate, feed consumption and pattern, survivability, thermobalance and carcass composition variables during exposure to either heat distress (HD) cycling temperature (24 to 35 C) or to a thermoneutral (TN) environment. Birds in Experiment 1 were fed a 23% crude protein corn-soybean based ration containing either 0.22% DLM or isomolar DL-HMB through 21-d posthatching. In Experiment 1, birds were fed a 20% crude protein diet containing either 0, .05, .25% DLM or isomolar amounts of DL-HMB through 49-d. Methionine source had no impact ($\underline{P} > .1$) on weight gain, feed consumption, survivability, carcass weight, chill weight, dressing percentage, breast weight or total fat content. Heat distress increased ($\underline{P} < .05$) heat production (H; 4.84 vs. 4.39 kcal/Kg$\cdot^{.66}$h), respiration rate (RR; 169 vs. 34 breaths/minute) and evaporative heat dissipation (E; 3.19 vs. 1.95 kcal/Kg$\cdot^{.66}$h), while reducing ($\underline{P} < .05$) sensible heat dissipation (S; 1.30 vs. 2.82 kcal/Kg $\cdot^{.66}$h). At 24 C, H, E, S, and RR were similar for DLM and DL-HMB supplemented birds. Broilers supplemented with DL-HMB and exposed to HD had 11 % greater ($\underline{P} <$ .05) H compared to those provided DLM. The added heat produced by the DL-HMB supplementation was dissipated by elevated ($\underline{P} < .05$) E with no effect on mortality. Methionine source had no impact on S, RR or mortality. In Experiment 2, diets of two caloric densities (CD; 2945 and 3200 ME_n kcal/Kg diet) were supplemented with either DLM or DL-HMB and fed to birds housed at 24 C or cycling HD. Responses were similar to Experiment 1, with the exception that methionine source had no effect on H or E at HD. Birds supplemented with DL-HMB supplemented birds consumed more ($\underline{P} <$

.05) feed during the wk 4 five h period prior to peak ambient temperature and thus had an increased heat load during HD. Birds consuming the higher CD diet had an increased ($P <$ .05) live weight gain, carcass weight, dressing percentage, fat pad weight, and carcass dry matter within the HD group and an elevated carcass weight and dressing percentage within the TN group.

(*Key words:* heat distress, broilers, DL-methionine, DL-HMB, thermobalance)

INTRODUCTION

Corn and soybean based diets require methionine supplementation to elicit optimal broiler performance. DL-2-hydroxy-4-methylthio butanoic acid (DL-HMB) and DL-methionine (DLM) are used routinely, as sources of supplemented methionine. Within thermoneutral (TN) environments, Garlich (1985), Twining and Hochstetler (1982) and Kreig (1962) have reported that broilers supplemented with DLM or DL-HMB had similar weight gains or feed efficiencies. In contrast, Picard (1979), Schutte and van Weerden (1981) and van Weerden et al. (1983) have observed superior growth rates and feed efficiencies from DLM than from DL-HMB supplements. Swick et al. (1989) reported that feed efficiency was improved with DL-HMB compared to DLM during heat distress (HD), but they were unable to successfully repeat their results in a following study (1990) although they still detected a numerical advantages for DL-HMB. Swick et al. (1992) using 6, 6 week old cockerels indicated a tendency ($P < .11$) for DL-HMB supplemented birds to have lower body temperature than DLM supplemented birds during HD. Because the diets employed by Swick et al. exceeded the methionine needs of the bird, perhaps methionine source impacts bird thermobalance. This has led to confusion by industry personnel seeking enhanced productivity of HD broilers. If DL-HMB does ameliorate HD consequences, then it must be impacting bird thermobalance via heat production,

evaporative heat dissipation, sensible heat dissipation, respiration rate or respiration efficiency.

The objectives of the experiments described herein were to assess the metabolic effects of DLM and DL-HMB on broiler thermobalance (heat production, evaporative heat dissipation, sensible heat dissipation, respiration rate, respiration efficiency, change in body heat content) during weeks 4 to 7 under cycling high ambient temperature conditions.

MATERIALS AND METHODS

Experiment 1: The first study was conducted to evaluate the metabolic effects of DLM and DL-HMB on thermobalance and performance of broilers from 4 to 7 wk of age exposed to cycling HD. Eight hundred Ross x Ross mixed sex broilers were fed a 23% crude protein corn-soybean starter diet containing either 0.22% DLM or 0.25% DL-HMB (Table 1). Birds were assigned randomly to 4 floor pens (2/methionine source) and reared through 28-d posthatching under optimal brooding conditions. On Day 28, 576 birds were allotted randomly to 32 x 30.5 x 38.1-cm wire compartments within a thermostatically and humidistatically controlled environmental chamber under continuous tungsten filament lighting and switched to the grower ration. The 16 replications of 6 chicks each were blocked such that chamber position effects were accounted for. Dietary treatments included: 1.) grower ration (Table 1) not fortified with methionine (birds previously fed DLM); 2.) As 1 (birds previously fed DL-HMB); 3.) As 1 + 0.05% DLM 4.) As 2 + DL-HMB to equal 0.05% DLM; 5.) As 1 + 0.25% DL-methionine; 6.) As 2 + DL-HMB to equal 0.25% DLM. Maximum daily ambient temperature was increased by 3.3 C/d, during the first 3-d of this study, such that ambient temperature cycled between 24 and 35 C for the remaining 24-d. This temperature cycle produced 6 h each day a temperature exceeding 32 C and 12 h of constant 24 C; the remaining 6 h consisted of increasing or decreasing at 1.3 C/h to complete the cycle. In all cases, methionine source

comparisons were consistent throughout the experiment. Birds classified as either DL-HMB or DLM consuming chicks consumed that methionine source both during the starter and grower periods; birds fed the methionine-free grower had been fed either DLM or DL-HMB in the starter. Relative humidity was maintained at 55%. Both feed and water were available for *ad libitum* consumption

Experiment 2: The second study was conducted to evaluate the metabolic effects of DLM and DL-HMB on broiler thermobalance and performance during 4 to 7 wk thermoneutral and cycling HD ambient temperatures. A total of 1536 male Cobb x Cobb broilers were fed one of two caloric density (CD) diets at a calorie/crude protein of 140 supplemented with either .18% DLM or DL-HMB to equal the DLM supplemented birds (Table 1). The treatments were arranged in a factorial arrangement consisting of 12 reps per treatment and reared through 21-d posthatching. On Day 21, birds were allotted randomly to individual 32 x 30.5 x 38.1-cm wire floor compartments housed within two thermostatically and humidistatically controlled environmental chambers, one mimicking the HD conditions described in Experiment 1 with the second held constant at 24 C. The 24 replications of 6 chicks were blocked such that chamber position effects were balanced. Dietary treatments included grower ration (Table 1) containing 2945 or 3200 ME_n Kcal/Kg supplemented with either .11 or .08% DL-methionine or isomolar amounts of DL-HMB. In all cases birds received the same caloric density diet, and methionine source throughout the experiment. As in Experiment 1, maximum daily ambient temperature was increased by 3.3 C/d, during the first 3 study days; however, the peak ambient temperature exceeded the projected 35 C by 3 C due to equipment limitation and the prevailing Oklahoma environment. Consequently birds were exposed to 38 C during weeks 4, 5, and 7 and nightly ambient temperature averaging 28 C. The temperature cycle provided 8 h daily excess of 32 C and 12 h of 29 C or below. As in Experiment 1, both feed and water were available for *ad libitum* consumption.

Data Collection, Calculations and Measurements:

Production Variables. Body weight was measured on Days 1, 21 (Experiment 2), 28 (Experiment 1), and 49 posthatching. Live weight gain was calculated by subtracting the starting bird weight from the ending weight. Feed consumption, feed efficiency, percent survival, and feed efficiency adjusted for mortality were measured from Days 1-28, 28-49 in Experiment 1 and from Days 1-21 and 21-49 in Experiment 2. Adjusted feed efficiency was calculated as: live weight gain + weight of dead birds divided by feed consumed. Feeding pattern was estimated by weighing feeders 5 h prior to peak temperature, at peak temperature, and 5 h post peak temperature during the first 3-d of wk 4, 5, and 6 of Experiment 2.

Carcass Measurements. On Day 49, surviving birds were weighed, hung on a rail, stunned, exsanquinated, by severing the jugular and carotid veins, passed through a scalding vat, plucked by machine and hand eviscerated. Carcasses were weighed and chilled in ice water for 4 h. Following chilling, carcasses again were weighed, with specific gravity, breast weight, and fat pad weight (Experiment 2) recorded, and whole carcass dry matter determined in a forced air (60 C) oven. Carcass fat was estimated as: 74.19-(87.33 x specific gravity) + (.88 x dry matter percentage).

Thermobalance Measurements. On Day 29, 72 male broilers (Experiment 1) and on Day 22, 60 male broilers (Experiment 2) were selected at random from the large scale environmental chamber, aseptically prepared and abdominally implanted with a radiotelemetry temperature transmitter (Mini-Mitter Telemetry System, Sunriver, Oregon 97707) and returned for recovery. Prior to surgery, anesthetic induction and maintenance was achieved by a intramuscular ketamine HCl injection (40 mg/kg of body weight) and halothane oxygen mixture using Bain none rebreathing system, respectively. Birds used for the thermobalance data were rotated between the large scale environmental chamber and the 24 metabolic chambers (51 x 34 x 41 cm) to allow the birds to become adapted to the metabolic chambers and handling. Rotation began 7-d prior to thermobalance

recording, providing the birds opportunity to become acquainted with chamber surroundings and handling. Ambient temperatures in the large scale chamber and the metabolic chambers were similar. Bird thermobalance response variables including heat production (H), evaporative heat dissipation (E), body temperature (BT), change in body heat content (HC), sensible heat dissipation (S), respiration rate (RR), respiration efficiency (RESEFF; cal/breath) were recorded on Days 35, 42 and 48 for Experiment 1 and on Days 28, 35, 42, and 48 for Experiment 2, as described by Wiernusz and Teeter (1993).

Serum Blood chemistries. On Day 49, 300 birds were selected randomly for blood samples which was collected from the ulnaris vein using a 3 mL syringe. Serum variables including glucose, triglycerides, total protein, creatinine, aspartate aminotransferase, albumin, uric acid, unsaturated iron-binding capacity, lactate dehydrogenase, alanine transaminase, creatine pyruvate kinase, Na, Cl Mg, K, Ca, P, and Fe were measured as described by Cason and Teeter (1994).

Statistical analysis

Oxygen, CO_2 and H_2O differential concentration between incoming and outgoing respiratory chamber gases along with BT and RR were regressed against time, time^2 and time^3 to establish polynomial equations describing the data. Quantitative estimates for each variable, attributed to bird metabolism, were made by integrating variable functions over specified time intervals. All integrated values as well as live weight gain, feed consumption, feed efficiency, adjusted feed efficiency, percent survival, and carcass and serum variables were analyzed by ANOVA using the General Linear Models procedure of the SAS Institute (1982). When a significant F statistic was noted, treatment means were separated by the Duncan multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Experiment 1: Results of the first study are shown in Tables 2 to 4. No significant differences ($P > .1$) between DLM and DL-HMB were detected for live weight gain, feed efficiency or mortality at 28-d posthatching (Table 2). Likewise, no differences ($P > .1$) were noted for production variables associated with methionine level or source at 49-d of age (Table 2). This suggests that the basal ration was adequate for sulfur amino acids. The basal ration was .07% below NRC (1994), at Day 28 and .05% above NRC on Day 49 of the heat exposure period. Balnave and Oliva (1990) suggested that HD reduced the birds need for methionine and may explain the lack of methionine effect. (FIELD OBSERVATION REFERENCE) Nonetheless, no advantage was noted for DL-HMB over DLM as suggested by Swick (1989; 1990). Carcass variables including carcass weight, chill weight, dressing percentage, breast weight and breast percentage of carcass as well as carcass fat were not influenced by methionine source or level (Table 2). Consequently, the data were pooled over methionine level, but not source or consumption history for thermobalance analysis.

The main effects of TN and HD thermobalance measurements yielded classical physiological responses (Table 3) to increased ambient temperature (van Kampen, 1974; 1981; Wiernusz and Teeter, 1993). Birds exposed to elevated ambient temperature had decreased ($P < .05$) S presumably due to the reduced differential between body temperature and ambient temperature. The reduced S forced the birds to elevate ($P < .05$) E (1.95 vs 3.19 Kcal/weight$^{.66}$/h) in an effort to maintain body temperature homeostasis via increased respiration rate. In this study, H increased ($P < .05$) from 4.39 to 4.84 Kcal/weight$^{.66}$/h presumably due to the increased energy expenditure associated with the elevated ($P < .05$) respiration rate (169 vs. 34 breaths/minute). In other experiments using fasted birds (Wiernusz and Teeter, 1993), H decreased from 5.02 to 4.54 Kcal/weight$^{.66}$/h during HD. Apparently fasted birds have the capacity to moderate H

when exposed to HD. Feed consumption prior to thermobalance was not measured in this study, but it would be expected to impact thermobalance profoundly.

Within the TN environment, H, S, RESEFF, and RR were similar (Table 4) between birds supplemented with either DL-HMB or DLM. Within the HD environment, birds supplemented with DL-HMB had 11 % greater ($P < .05$) H on Days 35, 42, and 49 compared to birds receiving DLM supplementation. The added H by the DL-HMB supplemented broilers was reflected in increased ($P < .05$) E. Consequently, no differences ($P > .1$) were observed for S, RR or RESEFF. Possible explanations include an altered feed consumption pattern and unidentified metabolic effects. Though total feed consumption for the DL-HMB and DLM sources was similar ($P > .1$) through 21 (1,378 vs. 1,389 g/bird) and 49-d (2,325 vs. 2,315 g/bird), any effect on the daily feed consumption pattern would impact TB. The DL-HMB effect, whatever its mode of action, was established by 21-d of age, as it was not impacted thereafter by subsequent methionine level (0, .05 .25%). The second experiment was conducted to judge the repeatability of this intriguing physiological response and to gain information related to mode of action.

Experiment 2: Results for Experiment 2 are presented in Tables 5 to 13. No two or three way interactions between caloric density, methionine source, and environment were detected, therefore, only the main effects of each factor will be discussed. Results for CD are displayed in Tables 5 to 7. Birds consuming the lower CD diet had reduced ($P < .05$) 21-d live weight gain and gain/feed compared to birds fed the 3200 Kcal/Kg diet, similar to previous reports (Belay and Teeter, 1992). Though feed consumption did not differ ($P > .1$) for the two energy sources total energy consumed was 8% greater ($P < .05$) for birds consuming the higher CD as reported by Kubena et al. (1972). Elevated live body weight gain for birds consuming the 3200 kcal/Kg diet during wk 3 to 7 was the result of increased ($P < .05$) feed and total ME_n consumption. Survivability was not impacted by

CD to Day 21, but tended ($P = .09$) to be reduced with the high CD diet similar to other works (Belay and Teeter, 1992). Carcass weight, chill weight, dressing percentage, specific gravity, fat pad weight (g), carcass fat (%), and dry matter (%) were greater ($P < .05$) for birds consuming the 3200 kcal/Kg diet in agreement with previous reports (Jones and Wiseman, 1985; Bartov, 1987; Belay and Teeter, 1992). The short term (2h) thermobalance measurements, estimated 4 times weekly during wk 4 to 7 indicated no differences between the two caloric densities. However, considering the difference between diets in ME_n consumption (Table 7) and estimated energy gain as protein and fat to estimate heat production yielded a 49-d value of 8632 vs 8367 kcal heat production for the high vs low caloric density ration. Any caloric effect on mortality would depend on feed consumption pattern suggesting that feed consumption pattern may have masked the overall impact of the elevated energy consumption. The trend for increased mortality coupled with previous work indicates that elevated CD is associated with elevated HD mortality and that longer thermobalance periods are needed.

Increasing ambient temperature (Table 8) reduced ($P < .05$) Day 21 to 49-d live weight gain by 17% in agreement with others (Squibb et al., 1959; Cowan and Michie, 1978; Belay and Teeter, 1992). Feed consumption, feed efficiency, survivability, carcass weight, chill weight, dressing percentage were adversely impacted ($P < .05$) by HD, while percent carcass fat was increased ($P < .05$). Fat pad as a percent of carcass weight during HD averaged 2% compared to 1.8% for birds housed in 24 C. The increased carcass fat during HD reported in this study and by other investigators (Howlider and Rose, 1987; Sonaiya et al, 1990; Belay and Teeter, 1992) may reflect metabolic adjustments to reduce H, as H is positively correlated with lean tissue accretion (Teeter and Wiernusz, 1994). Thermobalance comparisons between TN and HD responses (Table 9) were similar to Experiment 1, with the exception that H was 3 % higher in HD compared to 9% in Experiment 1. Wiernusz and Teeter (1993) observed that feed consumption during HD increases bird H by .14 kcal per unit feed consumed.

Methionine source effects for live performance from hatching to 49-d of age are displayed in Table 10. No differences (P >.1) between DLM and DL-HMB were observed in broiler performance or carcass variables, similar to Experiment 1. Heat production, S, E, HC, BT, RR, RESEFF, and RQ (Table 11) were similar between birds supplemented with the two methionine sources. In contrast to Experiment 1, where H was elevated 15% during HD, birds supplemented with DL-HMB had only a 3.2 % greater (P >.1) H compared to birds consuming DLM. Whether a lower ambient temperature exposure would have enabled a H response, similar to Experiment 1, is not known. However, despite the similar overall H, bird feeding pattern measurements indicated that during wk 4 (1st heat stress exposure) birds supplemented with DL-HMB had an elevated (P < .05) feed consumption (g/bird/h) during a 5 h period prior to peak high temperature (Table 12) while no differences (P > .1) were observed between the methionine sources after peak temperature or during the cool evening hours. The fact that the feed consumption pattern effect was not reflected in H may be the result of thermobalance measurements occurring after HD acclimation and possibly the birds ability to shunt nutrient metabolism to cooler time periods (Teeter et al., 1992). Nonetheless, the data may reflect a problem with DL-HMB supplemented birds to adjust feed intake as mediated by HD acclimation. As such, the data are in partial agreement with Experiment 1 in this regard.

Serum blood chemistries for ambient temperature, methionine source, and caloric density are shown in Table 13. No differences (P > .1) were noted for serum blood chemistries associated with methionine source or caloric density. Consequently, the data were pooled over methionine source and caloric density for analysis. Birds housed in the 24 C ambient temperature had higher (P < .05) Sodium, Na, Ca, Mg, Fe, TP, LD, and CPK than birds exposed to HD. Birds consuming the 3200 kcal/Kg diet (Table 2 of Appendix) had higher (P < .05) uric acid and P and reduced (P < .05) CPK concentration when compared with birds consuming the lower CD diet. Methionine source had no

impact on the serum analytes measured. Additional tables are found in the appendix of Chapter 3.

Conclusion: The combined data of Experiments 1 and 2 suggest that methionine source may impact the chicks ability to acclimate to heat stress. Additional studies are needed focusing on methionine source effects during the early acclimation process.

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TABLE 1. Composition of basal diets in Experiment 1 and caloric density (CD) diets used in experiment 2

	Experiment 1		Experiment 2			
	Starter	Grower	Starter		Grower	
Ingredients			CD 1	CD 2	CD 1	CD 2
	(%)					
Ground corn	55.38	61.00	64.93	53.43	68.49	61.21
Soybean meal (48.5%)	37.03	30.29	31.23	37.03	--	30.00
Soybean meal (44.5%	--	--	--	--	27.56	--
Animal fat	4.00	4.63	--	5.65	--	4.70
Deflourinated phosphate	1.98	1.82	1.87	1.94	1.83	2.00
Calcium carbonate	.61	1.23	1.00	1.00	1.20	1.20
Sodium chloride	.35	.40	.35	.35	.40	.40
Vitamin mix[1]	.25	.28	.30	.30	.30	.30
Trace mineral mix[2]	.10	.10	.10	.10	.10	.10
Variables[3]	.25	.25	.22	.20	.12	.09
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis						
ME_n Kcal/Kg	3100	3200	2945	3200	2945	3200
CP (%)	23.00	20.00	21.01	22.81	18.20	19.96
Calorie:CP ratio	135	160	140	140	160	160
Calcium (%)	1.00	1.02	.93	.96	1.00	1.03
Phosphorus (% av)	.50	.49	.48	.49	.48	.49

[1]Mix supplied per kilogram of diet: vitamin A, 14,109 I.U.; cholicalciferol, 5291 I.U.; vitamin E, 47.62 I.U.; vitamin B_{12}, .014 mg; riboflavin, 8.82 mg; niacin, 26.5 mg; d-pantothenic acid, 28.2 mg; choline, 705.5 mg; menadione, 1.16 mg; folic acid, 1.176 mg; pyridoxine, 3.52 mg; thiamine, 3.52 mg; d-biotin, .176 mg.

[2]Mix supplied per kilogram of diet: Ca, 160 mg; Zn, 100 mg; Mn, 120 mg; Fe, 75 mg; Cu, 10 mg; Iodine, 2.5 mg.

[3]Variables consisted of DL-methione, DL-HMB and washed builders sand in proportions specified in the materials and methods.

TABLE 2. DL-methionine and DL-HMB effects on body weight,, live weight gain, feed consumption, feed efficiency, feed efficiency adjusted for mortality, survivability, and carcass measurements of straight run Ross x Ross in Experiment 1

Variable	DL-methionine	DL-HMB
Live performance (1 to 28-d)		
Live body weight (g)	929	930
Gain (g)	881	875
Feed cons. (g)	1389	1378
Gain / feed	.63	.63
Adj. gain / feed	.65	.65
Survivability (%)	97.1	97.0
Live performance (28 to 49-d)		
Live body weight (g)	2008	2009
Gain (g)	1079	1077
Feed cons. (g)	2315	2325
Gain / feed	.40	.40
Adj. gain / feed	.50	.50
Survivability (%)	92.7	92.9
Carcass measurements		
Carcass weight (g)	1439	1440
Chill weight (g)	1504	1510
Dressing %	72	72
Carcass fat (%)	13.5	13.5
Breast weight (g)	263	266
Breast percentage	18.3	18.6
Specific gravity	1.043	1.043
Carcass dry matter (%)	38.1	37.8

TABLE 3. Heat distress (4-7 week) effects on heat production (H), sensible heat loss(S), evaporative heat loss (E), change in heat content (HC), body temperature (BT), respiration rate(RR), respiration efficiency (RESEFF), and respiratory quotient (RQ) of straight run Ross x Ross broilers in Experiment 1

Variable	24 C	35 C
H, Kcal/MWT per h	4.39[b]	4.84[a]
S, Kcal/MWT per h	2.82[a]	1.30[b]
E, Kcal/MWT per h	1.95[b]	3.19[a]
HC, Kcal/MWT per h	-0.06[b]	0.29[a]
BT, C	41.2[b]	42.8[a]
RR, breaths/min	34[b]	169[a]
RESEFF, cal/breath	2.20[a]	1.08[b]
RQ	.90	.91

[a,b] Means within a row with no common superscripts differ significantly (P<.05)

TABLE 4. Heat distress (4-7 week), DL-methionine and DL-HMB effects on heat production (H), sensible heat loss(S), evaporative heat loss (E), change in heat content (HC), body temperature (BT), respiration rate(RR), respiration efficiency (RESEFF), and respiratory quotient (RQ) of straight run Ross x Ross broilers in Experiment 1

	24 C		35 C	
Variable	DL-methionine	DL-HMB	DL-methionine	DL-HMB
H, Kcal/MWT per h	4.46[b]	4.33[b]	4.59[b]	5.10[a]
S, Kcal/MWT per h	2.95[a]	2.69[a]	1.10[b]	1.49[b]
E, Kcal/MWT per h	1.97[c]	1.93[c]	3.01[b]	3.38[a]
HC, Kcal/MWT per h	-.04[b]	-.08[b]	.26[a]	.31[a]
BT, C	41.2[b]	41.1[b]	42.6[a]	42.9a
RR, breaths/min	33[b]	35[b]	177[a]	162[a]
RESEFF, cal/breath	1.89[ab]	2.50[a]	.92[b]	1.23[b]
RQ	.89	.90	.91	.91

[a,b,c] Means within a row with no common superscripts differ significantly ($P < .05$).

TABLE 5. Caloric density effects on body weight, live body weight gain, feed consumption, survivability, feed efficiency and feed efficiency adjusted for mortality, and carcass measurements of Cobb x Cobb broilers in Experiment 2

Variable	2945 kcal/Kg	3200 kcal/Kg
Live performance (1 to 21-d)		
Live body weight (g)	624.9[b]	664.9[a]
Gain (g)	581.9[b]	622.2[a]
Feed cons. (g)	894.6	890.4
Gain / feed	.63[b]	.67[a]
Adj. gain / feed	.64[b]	.69[a]
Survivability (%)	98.5	97.8
Live performance (21 to 49-d)		
Live body weight (g)	1919.0[b]	1993.0[a]
Gain (g)	1275.5[b]	1318.1[a]
Feed cons. (g)	2960.4[b]	3116.2[a]
Gain / feed	.43	.43
Adj. gain / feed	.45	.45
Survivability (%)	94.4	91.5
Carcass measurements		
Carcass weight (g)	1419.5[b]	1501.4[a]
Chill weight (g)	1517.1[b]	1588.9[a]
Dressing %	72.0[b]	73.1[a]
Fat pad (g)	24.8[b]	30.2[a]
Breast weight (g)	243.4	248.9
Specific gravity	1.048[b]	1.045[a]
Carcass fat (%)	11.7[b]	12.7[a]
Carcass DM (%)	33.1[b]	34.4[a]

[a,b]Means within a row with no common superscripts differ significantly ($P < .05$).

Table 6. Caloric density effects on heat production (H), sensible heat loss (S), evaporative heat loss(E), change in heat content (HC), body temperature (BT), respiration rate (RR), respiration efficiency (RESEFF), and respiratory quotient (RQ) of 4-7 week old male Cobb x Cobb broilers in Experiment 2

Variable	2945 kcal/Kg	3200 kcal/Kg
H, Kcal/MWT per h	4.09	4.08
S, Kcal/MWT per h	2.91	2.58
E, Kcal/MWT per h	1.7	1.9
HC, Kcal/MWT per h	.30	.32
BT, C	41.6	41.6
RR, breaths/min	99	99
RESEFF, cal/breath	.68	.74
RQ	.82	.81

Table 7. Caloric density effects on ME_n consumption, protein and fat gain, and heat production of male Cobb x Cobb broilers in Experiment 2

Variable	2945 kcal/Kg	3200 kcal/Kg
ME_n consumption, kcal	11352	11961
Protein, g	258.2	268.2
Protein, kcal	1441	1499
Fat, g	166.1	190.6
Fat, kcal	1544	1830
Total, kcal	2985	3329
ME_n efficiency, %	26.3	27.8
Heat production, kcal	8367	8632

TABLE 8. Ambient temperature effects on body weight, live body weight gain, feed consumption, survivability, feed efficiency and feed efficiency adjusted for mortality, carcass measurements of 21-49-d old male Cobb x Cobb broilers in Experiment 2

Variable	24 C	35 C
Live performance		
7 week weight (g)	2450.6[a]	1761.3[b]
Gain (g)	1418.7[a]	1174.8[b]
Feed cons. (g)	3022.4	2596.2
Gain / feed	.47[a]	.44[b]
Adj. gain / feed	.47[a]	.45[b]
Survivability (%)	98.7[a]	87.3[b]
Carcass measurements		
Carcass weight (g)	1636.2[a]	1284.8[b]
Chill weight (g)	1729.0[a]	1377.0[b]
Dressing %	71.7[a]	73.1[b]
Fat pad (g)	29.7[a]	25.3[b]
Breast weight (g)	285.0[a]	207.2[b]
Specific gravity	1.049[a]	1.045[b]
Carcass fat (%)	11.9[a]	12.7[b]
Carcass DM (%)	33.1[a]	34.0[b]

[a,b]Means within a row with no common superscripts differ significantly ($P < .05$).

TABLE 9. Ambient temperature effects on heat production (H), sensible heat loss (S), evaporative heat loss(E), change in heat content (HC), body temperature (BT), respiration rate (RR), respiration efficiency (RESEFF), and respiratory quotient (RQ) of 4-7 week old male Cobb x Cobb broilers in Experiment 2

Variable	24 C	35 C
H, Kcal/MWT per h	4.72	4.86
S, Kcal/MWT per h	3.68[a]	2.16[b]
E, Kcal/MWT per h	1.29[b]	2.69[a]
HC, Kcal/MWT per h	-.07[b]	.66[a]
BT, C	40.9[b]	42.4[a]
RR, breaths/min	41[b]	161[a]
RESEFF, cal/breath	1.04[a]	.54[b]
RQ	.80	.82

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

Table 10. DL-methionine and DL-HMB effects on body weight, live body weight gain, feed consumption, survivability, feed efficiency and feed efficiency adjusted for mortality, and carcass measurements of male Cobb x Cobb broilers in Experiment 2

Variable	DL-methionine	DL-HMB
Live performance (1 to 21-d)		
Live body weight (g)	644.3	645.6
Gain (g)	601.4	602.8
Feed cons. (g)	907.8	910.8
Gain / feed	.65	.65
Adj. gain / feed	.67	.67
Survivability (%)	98.0	98.4
Live performance (21 to 49-d)		
Live body weight (g)	1953.3	1958.6
Gain (g)	1297.6	1296.0
Feed cons. (g)	3034.0	3042.6
Gain / feed	.43	.43
Adj. gain / feed	.45	.45
Survivability (%)	92.8	93.1
Carcass measurements		
Carcass weight (g)	1456.8	1464.1
Chill weight (g)	1545.0	1561.0
Dressing %	72.4	72.4
Fat pad (g)	27.4	27.6
Breast weight (g)	245.7	246.6
Specific gravity	1.047	1.047
Carcass fat (%)	12.3	12.3
Carcass DM (%)	33.8	33.7

Table 11. DL-methionine and DL-HMB effects on heat production (H), sensible heat loss (S), evaporative heat loss(E), change in heat content (HC), body temperature (BT), respiration rate (RR), respiration efficiency (RESEFF), and respiratory quotient (RQ) of 4-7 week old male Cobb x Cobb broilers in Experiment 2

Variable	DL-methionine	DL-HMB
H, Kcal/MWT per h	4.74	4.83
S, Kcal/MWT per h	2.98	2.86
E, Kcal/MWT per h	1.95	2.03
HC, Kcal/MWT per h	.12	.37
BT, C	41.65	41.67
RR, breaths/min	103	99
RESEFF, cal/breath	.76	.81
RQ	.81	.81

TABLE 12. DL-methionine, and DL-HMB effects on broiler feed consumption pattern of Cobb x Cobb broilers in Experiment 2

Variable	DL-methionine	DL-HMB
Week 4		
Preheat	7.5 [b]	7.9 [a]
Postheat	7.1 [c]	7.2 [bc]
TN	7.2 [bc]	7.3 [bc]

[a,b,c]Means with no common superscripts differ significantly ($P < .05$).

TABLE 13. Ambient temperature effects on serum blood chemistries of 49-d old male Cobb x Cobb broilers in Experiment 2

Variable	24 C	35 C
Na, mEq/L	149.02[a]	146.68[b]
K, mEq/L	4.31	3.74
Ca, mg/dL	7.99[a]	7.65[b]
Mg, mEq/L	1.87[a]	1.81[b]
Cl, mEq/L	114.16	113.19
P, mg/dL	5.78	5.70
Fe, μg/dL	152.5[a]	142.46[b]
Gluc, mg/dL	220.18	224.05
Trig, mg/dL	63.13	61.91
TP, g/dL	3.22[a]	2.99[b]
Crea, mg/dL	0.31	0.31
LD, U/L	2767.42[a]	2229.13[b]
AST, U/L	298.41	308.20
Alb, mg/dL	0.97	0.96
Uric, mg/dL	7.42	6.97
UIBC, μg/dL	76.52	85.59
ALT, U/L	4.78	4.19
CPK, U/L	7189.47[a]	6744.35[b]

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

Gluc = glucose, Trig = triglycerides, TP = total protein, Crea = creatinine,
AST = aspartate aminotransferase, Alb = albumin, Uric = uric acid,
UIBC = unsaturated iron-binding capacity, LD = lactate dehydrogenase,
ALT = alanine transaminase, CPK = creatine pyruvate kinase.

APPENDIX A

CHAPTER III

TABLE 1. DL-methionine and DL-HMB effects on serum blood chemistries of 49-d old male Cobb x Cobb broilers in Experiment 2

Variable	DL-methionine	DL-HMB
Na, mEq/L	148.51	147.19
K, mEq/L	4.01	4.05
Ca, mg/dL	7.76	7.87
Mg, mEq/L	1.84	1.84
Cl, mEq/L	113.31	114.04
P, mg/dL	5.64	5.84
Fe, μg/dL	145.85	149.10
Gluc, mg/dL	224.04	220.19
Trig, mg/dL	65.48	59.55
TP, g/dL	3.13	3.08
Crea, mg/dL	0.32	0.30
LD, U/L	2491.95	2504.59
AST, U/L	306.33	300.28
Alb, mg/dL	0.99	0.94
Uric, mg/dL	7.11	7.27
UIBC, μg/dL	78.97	83.14
ALT, U/L	4.68	4.28
CPK, U/L	6999.24	6934.58

Gluc = glucose, Trig = triglycerides, TP = total protein, Crea = creatinine,
AST = aspartate aminotransferase, Alb = albumin, Uric = uric acid,
UIBC = unsaturated iron-binding capacity, LD = lactate dehydrogenase,
ALT = alanine transaminase, CPK = creatine pyruvate kinase.

TABLE 2. Caloric density effects on serum blood chemistries of 49-d old male Cobb x Cobb broilers in Experiment 2

Variable	2945 kcal/Kg	3200 kcal/Kg
Na, mEq/L	148.06	147.64
K, mEq/L	3.98	4.08
Ca, mg/dL	7.78	7.86
Mg, mEq/L	1.83	1.84
Cl, mEq/L	113.22	114.13
P, mg/dL	5.60 [a]	5.88 [b]
Fe, μg/dL	148.58	146.38
Gluc, mg/dL	223.74	220.50
Trig, mg/dL	66.44	58.59
TP, g/dL	3.10	3.11
Crea, mg/dL	0.30	0.32
LD, U/L	2539.07	2457.48
AST, U/L	293.17	313.45
Alb, mg/dL	0.97	0.95
Uric, mg/dL	6.78 [a]	7.61 [b]
UIBC, μg/dL	75.91	86.20
ALT, U/L	4.81	4.15
CPK, U/L	7200.30 [a]	6733.51 [b]

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

Gluc = glucose, Trig = triglycerides, TP = total protein, Crea = creatinine,
AST = aspartate aminotransferase, Alb = albumin, Uric = uric acid,
UIBC = unsaturated iron-binding capacity, LD = lactate dehydrogenase,
ALT = alanine transaminase, CPK = creatine pyruvate kinase.

TABLE 3. Caloric density, DL-methionine and DL-HMB effects on body weight, live body weight gain, feed consumption, survivability, feed efficiency and feed efficiency adjusted for mortality on 1-21-day old male Cobb x Cobb broilers

	2945 kcal/Kg		3200 kcal/Kg	
Variable	DL-methionine	DL-HMB	DL-methionine	DL-HMB
3 week weight (g)	621.9[b]	628.0[b]	666.7[a]	663.2[a]
Gain (g)	578.5[b]	585.3[b]	624.2[a]	620.2[a]
Feed cons. (g)	908.2	909.4	907.4	912.3
Gain / feed	.62[b]	.64[b]	.67[a]	.67[a]
Adj. gain / feed	.64[b]	.64[b]	.69[a]	.69[a]
Survivability (%)	98.1	98.8	97.5	98.0

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

TABLE 4. Caloric density, DL-methionine and DL-HMB effects on body weight, live body weight gain, feed consumption, survivability, feed efficiency and feed efficiency adjusted for mortality on 21-49-d old male Cobb x Cobb broilers housed in 24 C environment

	2945 kcal/Kg		3200 kcal/Kg	
Variable	DL-methionine	DL-HMB	DL-methionine	DL-HMB
7 week weight (g)	2131.2	2145.8	2169.5	2155.9
Gain (g)	1406.4	1416.1	1433.6	1418.7
Feed cons. (g)	3068.9	3091.1	3038.1	3067.1
Gain / feed	.46	.46	.47	.46
Adj. gain / feed	.46	.47	.48	.48
Survivability (%)	100.0	98.6	97.9	98.3

TABLE 5. Caloric density, DL-methionine and DL-HMB effects on body weight, live body weight gain, feed consumption, survivability, feed efficiency and feed efficiency adjusted for mortality on 21-49-day old male Cobb x Cobb broilers housed in 24-35 C cycling temperature

	2945 kcal/Kg		3200 kcal/Kg	
Variable	DL-methionine	DL-HMB	DL-methionine	DL-HMB
7 week weight (g)	1699.3[b]	1699.7[b]	1813.2[a]	1832.8[a]
Gain (g)	1143.4[b]	1135.9[b]	1206.9[a]	1213.1[a]
Feed cons. (g)	3068.9	3091.1	3038.1	3067.1
Gain / feed	.40	.40	.38	.39
Adj. gain / feed	.43	.43	.43	.43
Survivability (%)	88.7	90.5	84.7	85.2

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

TABLE 6. Thermoneutral (3-7 week) and overall (0-7 week) DL-methionine and DL-HMB effects on carcass weight, chill weight, dressing percentage, fat pad, breast weight, specific gravity, percent carcass fat and carcass dry matter

	2945 kcal/Kg		3200 kcal/Kg	
Variable	DL-methionine	DL-HMB	DL-methionine	DL-HMB
Carcass weight (g)	1612.2[b]	1602.1[b]	1667.3[a]	1663.1[a]
Chill weight (g)	1708.9	1702.1	1738.4	1766.6
Dressing %	71.1[b]	71.4[b]	72.3[a]	72.9[a]
Fat pad (g)	29.7	27.3	30.3	31.5
Breast weight (g)	282.0	283.7	287.5	287.0
Specific gravity	1.050	1.049	1.049	1.047
Carcass fat (%)	11.5	11.5	12.3	12.4
Carcass DM (%)	33.0	33.0	33.9	33.9

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

TABLE 7. Heat distress (3-7 week) and overall (0-7 week) DL-methionine and DL-HMB effects on carcass weight, chill weight, dressing percentage, fat pad, breast weight, specific gravity, percent carcass fat and carcass dry matter

	2945 kcal/Kg		3200 kcal/Kg	
Variable	DL-methionine	DL-HMB	DL-methionine	DL-HMB
Carcass weight (g)	1233.4[b]	1250.5[b]	1334.5[a]	1340.8[a]
Chill weight (g)	1327.2[b]	1340.2[b]	1415.5[a]	1435.2[a]
Dressing %	72.0[b]	71.4[b]	73.8[a]	73.1[a]
Fat pad (g)	20.6[b]	21.6[b]	29.0[a]	30.0[a]
Breast weight (g)	201.9	205.9	211.3	209.8
Specific gravity	1.047	1.047	1.044	1.044
Carcass fat (%)	12.0[b]	11.7[b]	13.5[a]	13.4[a]
Carcass DM (%)	33.3[b]	33.1[b]	34.8[a]	34.7[a]

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

CHAPTER IV

Prediction of Carcass Fat, Protein, and Energy Content from Carcass Dry Matter and Specific Gravity of Broilers[1]

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ABSTRACT Three experiments were conducted to develop and test equations predicting carcass fat and protein content. In the first study, 24 broiler carcasses were selected based upon visual abdominal fat appraisal (low, medium, high) and assayed for specific gravity (SG), dry matter (DM), fat, protein, and ash. In Experiment 2, birds were fed rations containing 2 caloric densities (2880, 3200 kcal ME/Kg diet) and assayed as described above on weeks 2, 3, 4, 5, and 6. Carcass fat was elevated ($\underline{P} < .05$) with elevated caloric density. In both studies predictive variables were significantly correlated with determined carcass fat, protein, and ash. Pooled across the 2 studies, data were used to form SG, DM, and/or age based equations for predicting carcass composition. Results were tested in Experiment 3, where birds reared to 49-d consumed either 2880, 3200, or 3574 kcal ME/Kg diet while exposed to constant 24 or cycling 24 to 35 C ambient temperatures. Both dietary and environmental effects impacted ($\underline{P} < .05$) carcass composition with AOAC analyzed fat increasing from 12.4 to 15.7%, predicted fat ranged from 13.4 to 16.8% with caloric density. Heat distress reduced ($\underline{P} < .05$) analyzed carcass protein (18.9 vs 18.3%) and predicted protein (18.2 vs 17.5%). Predicted equation values for carcass fat and protein were correlated with the analyzed values at R = .96 and R = .77, respectively. Results suggest that prediction equations based on DM and SG may be used to estimate carcass fat and protein of broilers consuming diets that differ in caloric density and for broilers exposed to either normal or elevated ambient temperatures.
(*Key words:* broiler, predictive equation, fat, protein, dry matter, specific gravity)

INTRODUCTION

Increasing the lean yield of poultry products is one goal of the poultry industry as it strives to meet consumer interests. Efforts have been underway for years to reduce carcass fat through genetic, nutritional and pharmacological techniques. However, meaningful progress for these areas requires rapid and economical methods for determining carcass composition. Current methods are both laborious and costly.

Specific gravity and dry matter content of carcasses have been used to estimate carcass and body composition of both mammalian species and poultry (Garrett, 1968; Jones *et al.*, 1978; Chambers and Fortin, 1984; Lewis and Perry, 1991). These methods are based upon the premise that fat and nonfat (ash, protein) components have unique densities (Pearson *et al.*, 1968). Specific gravity has been criticized as being impractical for poultry due to the potential for air entrapment in the internal carcass cavity and air sacs in many of the limb bones Feduccia (1975). Indeed, air entrapment is an important source of error (Garrett, 1968; Miles, 1976). Fortin and Chambers (1981) attempted to circumvent this problem by measuring specific gravity of individual parts to predict carcass fat, but they found no improvement suggesting that other errors may be involved. One additional source of error could be cellular hydration, increased cellular hydration accompanies glycogen storage and would increase specific gravity.

The objectives of the studies reported herein were to evaluate the relationship between chemically determined whole carcass fat, protein, energy, and ash composition with whole carcass specific gravity, dry matter and age combinations, and further to

propose and test resulting predictive equations under a variety of dietary and ambient temperature treatment regimes.

MATERIALS AND METHODS

Experiment 1

To assess the potential of carcass specific gravity and dry matter to predict carcass composition, 24 Cobb x Cobb, 52-d old birds were selected from 325 processed broiler carcasses based on visual appraisal of abdominal fat. Chicks were raised on rice hull litter and allowed to consume a 23% crude protein diet composed of corn and soybean meal through 21 days posthatching. Birds were selected to fall within three categories (low, medium, and high amounts) of abdominal fat to ensure that the range of carcass composition was large. Bird processing was accomplished by hanging the weighed birds on a rail, stunning, exsanquinating by severing the jugular and carotid veins, passing the carcass through a scalding vat, plucking by machine and hand eviscerating. Carcasses were weighed and chilled in ice water for 4 h. Following chilling, carcasses again were weighed and specific gravity was measured. Immediately following specific gravity measurement, all broiler carcasses were placed in polypropylene freezer bags and frozen until analyzed. Carcasses were homogenized by the method of McDonald (1993) and analyzed for nitrogen, dry matter percentage, fat (ether extract), energy, and ash (AOAC, 1990).

Statistical Analysis

Standard statistical procedures (Steel and Torrie, 1960) were used to obtain the simple and multiple correlation coefficients as well as linear regression equations for predicting carcass fat, protein, ash, and energy. In Experiment 2 and 3, an ANOVA was performed using the General Linear Model of SAS (1982). When a significant F statistic was detected, means were separated using Duncan's multiple range test.

Experiment 2

The second study was conducted to expand the data base of carcass specific gravity, dry matter and AOAC composition values such that predictive equations might be developed. Birds consumed rations containing two different caloric densities (Table 1; 2880, 3200 kcal ME_n/Kg diet) and were processed on week 2, 3, 4, 5, and 6. so that age effects also could be appraised. With the exception of the chicks fed the two different diets and processed at five different ages, all procedures were as described for Experiment 1.

Experiment 3

The third experiment was conducted to evaluate prediction equations formed from Experiments 1 and 2 in birds fed two caloric densities (2880, 3200 kcal ME_n/Kg diet; Table 1) and exposed to two ambient temperatures (24 , 24 to 35 C). Birds were raised in rice hull-covered floor pens and fed a 23% corn and soybean meal-based diet through 21-d posthatching. On day 22, 240 birds were allotted randomly to wire-floored grower battery compartments (82 x 61 x 38 cm) housed within a thermostatically (24, 24 to 35 C)

controlled environmental chambers. The 4 treatment groups contained 16 replicates of six birds arranged in blocks such that chamber positions could be included in the analysis of variance. All birds were allowed to consume feed and water *ad libitum*. Maximum daily ambient temperature was increased 3.3 C/day during the first 3-d of the study for chicks housed within the 24 to 35 C temperature. The ambient temperature cycled providing 6 h daily in excess of 32 C, 12 h of constant 24 C, and 6 h increasing or decreasing at a rate of 1.3 C/h. On Day 49, posthatching birds were slaughtered and similar measurements were obtained as in Experiment 1.

RESULTS AND DISCUSSION

Experiment 1

Linear regression equations for predicting carcass fat, protein, ash, and energy along with R^2 and mean square errors are presented in Table 2. Correlations between analyzed and predicted carcass composition (Table 3) produced R of .98, .89, .71, and .92 for carcass fat, protein, ash, and energy, respectively.

Experiment 2

Results of live weight, carcass weight and laboratory assayed carcass fat, protein, ash, specific gravity, and energy for the five age groups are shown in Table 4. Mean live weight increased ($\underline{P} < .05$) from 343 to 2387 g at 2 and 6 weeks of age, respectively. Carcass weight, percent dry matter, and fat had similar trends. Percent dry matter increased ($\underline{P} < .05$) and specific gravity decreased through 4 weeks posthatching, presumably due to an increased ($\underline{P} < .05$) fat content as reported by others (Kubena et al.,

1974; Tzeng and Becker, 1981; Summers et al., 1992). Percent ash was low at week 2 compared to the older aged birds, while protein as a percent of wet carcass weight remained unchanged ($\underline{P} > .1$) throughout the 6 week study.

An increased caloric density of the diet increased ($\underline{P} < .05$) dry matter and specific gravity of bird carcasses at 4, 5, and 6 weeks of age, and is reflected by the higher ($\underline{P} < .05$) fat content at each of these ages. Birds fed the higher caloric density had 5, 4, and 8% increase ($\underline{P} < .05$) in carcass energy at 4, 5, and 6 weeks of age, respectively. Caloric density had no significant effect ($\underline{P} > .1$) on live body weight, carcass weight, or protein; in contrast other studies have observed increases in these components with weight (Jones and Wiseman, 1985; Bartov, 1987; Belay and Teeter, 1992).

Correlation coefficients between 2 component predicative equations (specific gravity, dry matter percentage) and analyzed carcass composition produced R of .98, .87, .67, and .85 for carcass fat, protein, ash, and energy, respectively, similar to Experiment 1. Regression equations for predicting carcass composition were pooled between Experiments 1 and 2 and displayed in Table 5.

Experiment 3

This study was conducted to evaluate accuracy of prediction equation for broilers fed diets containing 3 caloric densities (2800, 3200, 3574 kcal ME_n/Kg diet; Table 1) while exposed to two ambient temperatures (24, 24 to 35 C). No interaction between caloric density and ambient temperature was detected for prediction or AOAC analyzed carcass fat, protein, dry matter, ash, and energy, therefore, only the main effects of each will be discussed. Both diet and ambient temperature significantly altered carcass

composition (Tables 6 and 7). Carcass fat and energy, averaged over ambient temperature, increased ($P < .05$) from 12.4 to 15.7% and 6.35 to 6.62 kcal/g, respectively with increasing caloric density, while carcass protein and ash remained constant ($P > .1$). Heat distress reduced ($P < .05$) carcass protein but had no effect ($P > .1$) on carcass fat, energy, and ash.

Correlation coefficients between predicted and analyzed carcass compositions were .96, .77, .86, and .79 for carcass fat, protein, ash, and energy, respectively (Figures 1-4). The correlations observed for carcass fat were high reflecting a strong capability to predict carcass fat and energy differences among the caloric densities. No differences ($P > .1$) were observed between analyzed or predictive carcass protein and ash. However, heat distress reduced ($P < .05$) analyzed carcass protein (18.9 vs 18.3%) and predicted carcass protein (18.2 vs 17.5%) compared to birds housed within 24 C. In conclusion, the regression equations, obtained in Experiments 1 and 2, for predicting carcass fat, energy, protein, and ash showed strong correlations with AOAC determinations. The ability of prediction equations to separate treatment means, among birds housed in different thermal environments and fed diets ranging from 2880 to 3574 kcal ME/Kg diet is encouraging.

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TABLE 1. Diets used in Experiments 1, 2, and 3

Ingredients and analysis	2880 kcal ME/Kg	3200 kcal ME/Kg	3574 kcal ME/Kg
		(%)	
Ground corn (8.8% CP)	60.49	52.10	50.53
Soybean meal (48.5% CP)	34.74	36.40	31.73
Animal fat	.90	7.65	13.16
Dicalcium phosphate (22% Ca; 18.5% P)	1.60	1.58	2.13
Limestone (38% Ca)	1.25	1.25	1.34
NaCl	.41	.41	.41
Vitamin mix[1]	.30	.30	.30
DL-methionine, 99%	.21	.21	.21
Trace mineral mix[2]	.10	.10	.10
Total	100.00	100.00	100.00
Calculated analysis			
ME, kcal ME/Kg	2880	3200	3574
CP, g/kg	20.57	20.64	19.85
Ca	1.00	1.00	1.10
P, available	.44	.42	.48
Na	.16	.16	.17
K	1.00	1.01	1.02
Cl	.25	.25	.25

[1]The vitamin mix contained the following per kilogram of diet: vitamin A, 14,109 IU (retinyl acetate); cholicalciferol, 5,291 IU; vitamin E, 47.6 IU (dl-α-tocopheryl acetate); vitamin B_{12}, .014 mg; riboflavin, 8.82 mg; niacin, 26.5 mg; d-pantothenic acid, 28.2 mg; choline, 705.5 mg; menadione, 1.16 mg; folic acid, 1.176 mg; pyridoxine, 3.52 mg; thiamin, 3.52 mg; d-biotin, .176 mg.

[2]The mineral mix contained the following per kilogram of diet: Ca, 160 mg; Zn, 100 mg; Mn, 120 mg; Fe, 75 mg; Cu, 10 mg; I, 2.5 mg, Se, .15 mg.

Table 2. Specific gravity, % dry matter, coefficient of determination (R), regression equations, and root mean square error for the estimation of carcass fat, protein, ash, and energy in Experiment 1

Variable	Intercept	Specific gravity	% Dry matter	R	RMSE
Carcass fat	429.294	-398.566		.93	1.558
	-30.887		1.28	.97	1.077
	126.370	-137.904	.894	.98	.910
Carcass protein	-76.346	90.389		.79	.717
	27.406		-.272	.77	.748
	-40.194	59.281	-.107	.81	.718
Carcass ash	5.764	-4.513		.22	.194
	.246		.023	.39	.184
	-14.043	12.530	.058	.48	.180
Carcass energy	30.210	-23.025		.87	.134
	3.677		.072	.88	.127
	15.494	-10.362	.043	.90	.121

Table 3. Analyzed and predicted carcass fat, protein, energy, and ash and correlation coefficients of analyzed and predicted variables in Experiment 1

Variable	Analyzed	Predicted	Correlation coefficients
Carcass fat, %	12.53±4.40	12.33±1.07	.97
Carcass protein, %	18.26±1.30	18.20±1.01	.80
Carcass ash, %	1.00±0.24	1.02±0.10	.65
Carcass energy, kcal/g	6.15±0.26	6.12±0.26	.85

Table 4. Live weight, carcass weight, and carcass composition of the 2 to 6 week old broiler consuming two caloric density rations in Experiment 2

Bird age	Diet	Live weight	Carcass weight	Carcass Composition					
				DM	Fat	Protein	Ash	Specific gravity	Energy
(wk)	(kcal ME/Kg)	(g)	(g)	(%)	(% of AS IS)				kcal/g
2	2880	352^{e}	193^{e}	29.5^{d}	8.78^{cd}	17.8^{b}	.90^{c}	1.056^{ab}	5.96^{ed}
	3200	340^{e}	170^{e}	28.4^{d}	7.34^{d}	17.9^{b}	.91^{c}	1.060^{a}	5.80^{e}
3	2880	804^{d}	498^{d}	30.4^{cd}	8.90^{cd}	18.1^{ab}	1.10^{abc}	1.051^{bc}	5.97^{de}
	3200	930^{d}	591^{d}	32.9^{bc}	11.30^{b}	18.4^{ab}	1.40^{a}	1.051^{bc}	6.10^{bcd}
4	2880	1474^{c}	978^{c}	32.0^{bc}	9.61^{bcd}	18.4^{ab}	1.09^{abc}	1.053^{b}	5.90^{de}
	3200	1461^{c}	975^{c}	36.0^{a}	15.36^{a}	17.8^{ab}	1.32^{ab}	1.047^{c}	6.27^{abc}
5	2880	1998^{b}	1356^{b}	33.6^{b}	11.82^{b}	18.6^{ab}	.86^{c}	1.046^{c}	6.03^{cde}
	3200	2115^{b}	1419^{b}	36.2^{a}	14.91^{a}	17.8^{b}	1.40^{a}	1.040^{d}	6.29^{ab}
6	2880	2385^{a}	1691^{a}	32.2^{bc}	9.83^{bcd}	18.9^{a}	.98^{bc}	1.050^{bc}	5.98^{de}
	3200	2387^{a}	1692^{a}	37.3^{a}	15.87^{a}	18.3^{b}	1.42^{a}	1.038^{d}	6.49^{a}

$^{a-e}$Means within a column with no common superscripts differ significantly ($P < .05$).

Table 5. Specific gravity, % dry matter, age, coefficient of determination (R), regression equations, and root mean square error for the estimation of carcass fat, protein, ash, and energy in Experiments 1 and 2

Variable	Intercept	Specific gravity	% Dry matter	Age	R	RMSE
Carcass fat	407.636	-377.735			.87	1.989
	-23.610		1.06		.96	1.096
	7.471			.086	.41	3.693
	74.192	-87.328	.877		.97	1.010
	83.977	-97.166	.931	-.028	.97	.905
Carcass protein	-39.862	55.291			.50	.903
	22.377		-.129		.45	.927
	17.784			.008	.14	1.028
	-26.996	44.086	-.034		.50	.904
	-37.733	54.881	-.093	.030	.69	.757
Carcass ash	10.951	-9.386			.22	.381
	-.233		.041		.37	.362
	1.009			.002	.10	.389
	-16.325	14.369	.072		.42	.356
	-15.568	13.608	.076	-.002	.44	.356
Carcass energy	30.907	-23.666			.74	.200
	4.05		.062		.76	.194
	5.842			.005	.35	.280
	16.514	-11.131	.038		.78	.186
	17.029	-11.648	.041	-.001	.79	.186

115

Table 6. Caloric density effects on analyzed and predicted carcass fat, protein, and ash percentage in Experiment 3

Variable	2800 kcal/Kg	3200 kcal/Kg	3574 kcal/Kg
Analyzed fat (%)	12.41^{c}±3.34	14.41^{b}±2.83	15.74^{a}±3.24
Predicted fat (%)	13.41^{c}±4.07	14.62^{b}±3.09	16.79^{a}±3.57
Analyzed Protein (%)	18.59±1.82	18.45±1.91	18.73±2.06
Predicted Protein (%)	17.89±1.35	17.76±1.31	17.62±2.29
Analyzed Ash(%)	1.02±0.16	1.06±0.17	1.06±0.18
Predicted Ash(%)	1.18±0.31	1.28±0.33	1.36±0.34
Analyzed Energy, kcal/g	6.35^{b}±.22	6.53^{b}±.19	6.62^{a}±.20
Predicted Energy, kcal/g	6.20^{b}±.23	6.28^{b}±.20	6.38^{a}±.20

^{a,b,c} Means within a row with no common superscripts differ significantly ($P < .05$).

Table 7. Ambient temperature effects on analyzed and predicted carcass fat, protein, and ash percentage in Experiment 3

Variable	24 C	24-35 C
Analyzed fat (%)	14.20±1.42	14.33±1.38
Predicted fat (%)	15.15±2.19	15.07±2.02
Analyzed protein (%)	18.90[a]±1.90	18.29[b]±1.94
Predicted protein (%)	18.18[a]±2.01	17.49[b]±2.05
Analyzed ash(%)	1.06±.17	1.02±.16
Predicted ash(%)	1.30±.32	1.25±.34
Analyzed Energy, kcal/g	6.48±.21	6.52±.21
Predicted Energy, kcal/g	6.28±.22	6.30±.22

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

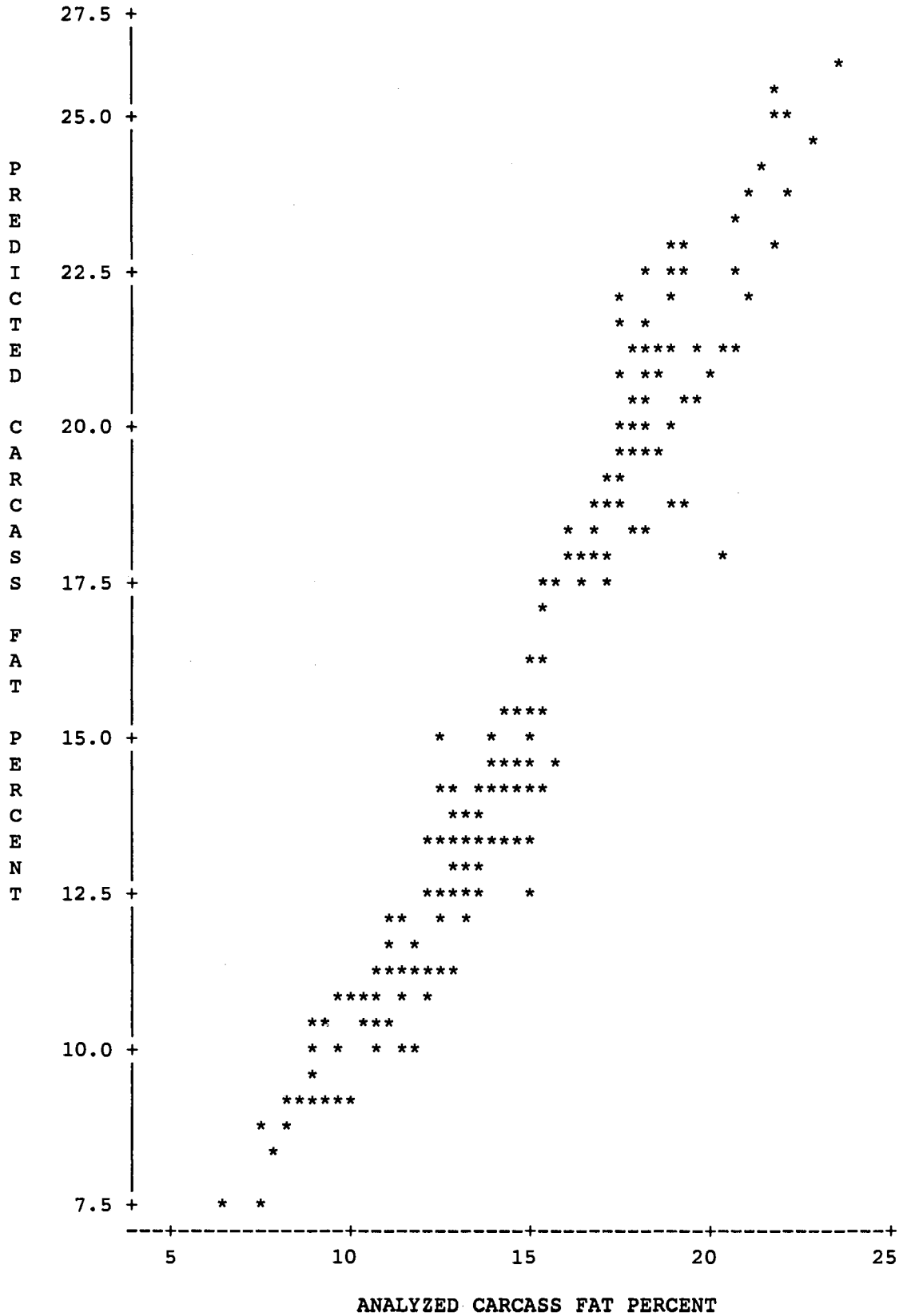

Figure 1. Analyzed and predicted carcass fat percentage in Experiment 3.

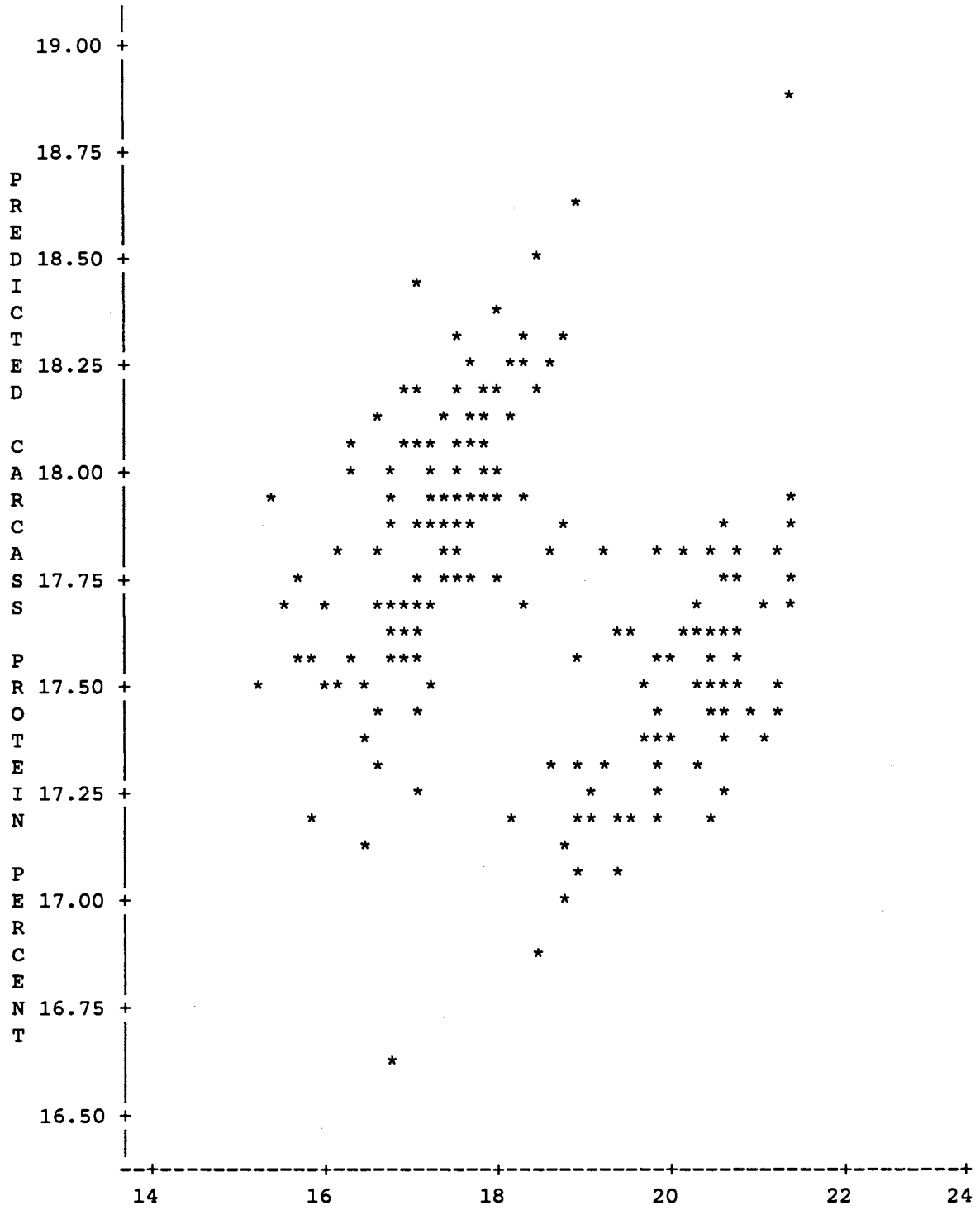

Figure 2. Analyzed and predicted protein percentage in Experiment 3.

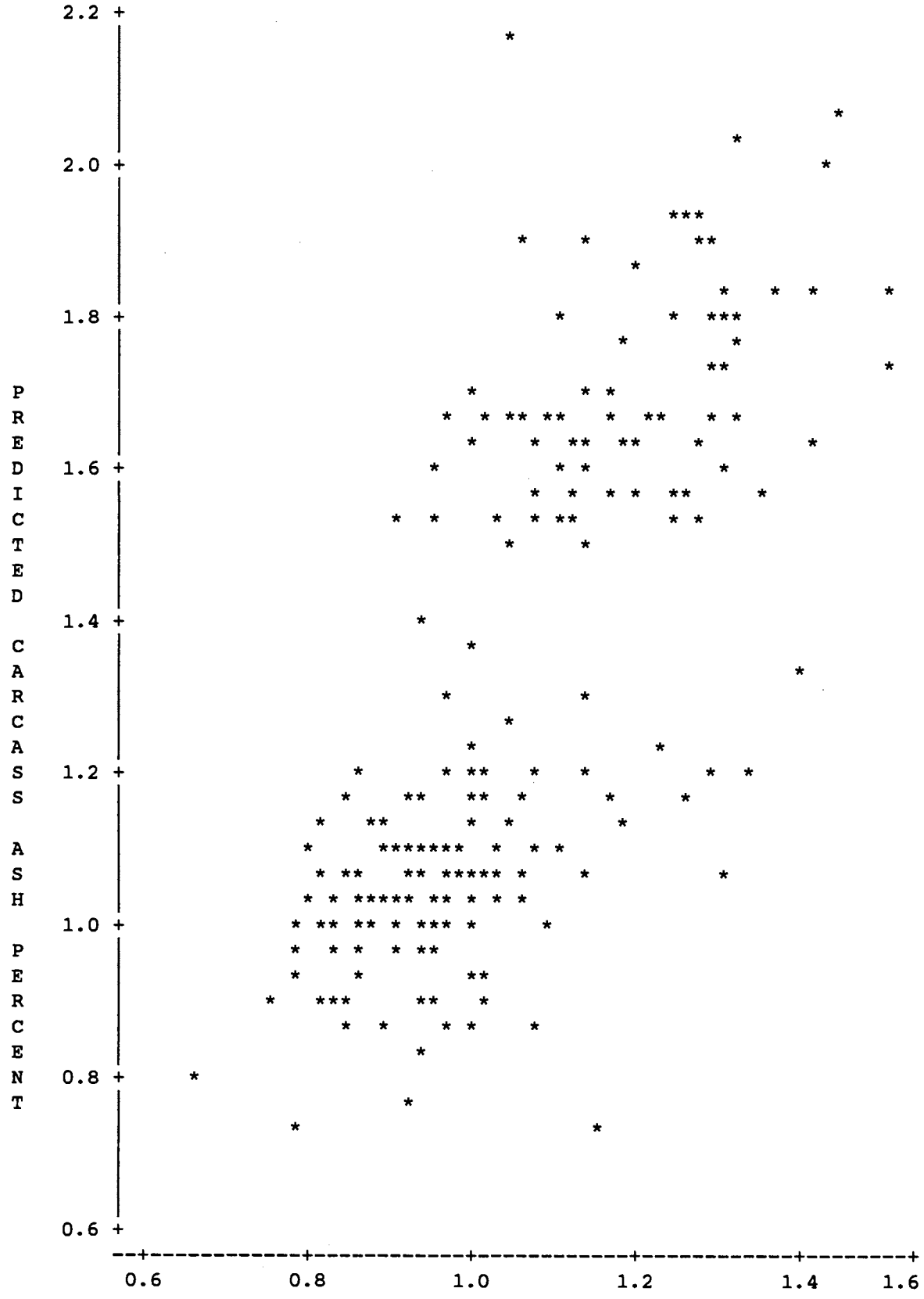

Figure 3. Analyzed and predicted ash percentage in Experiment 3.

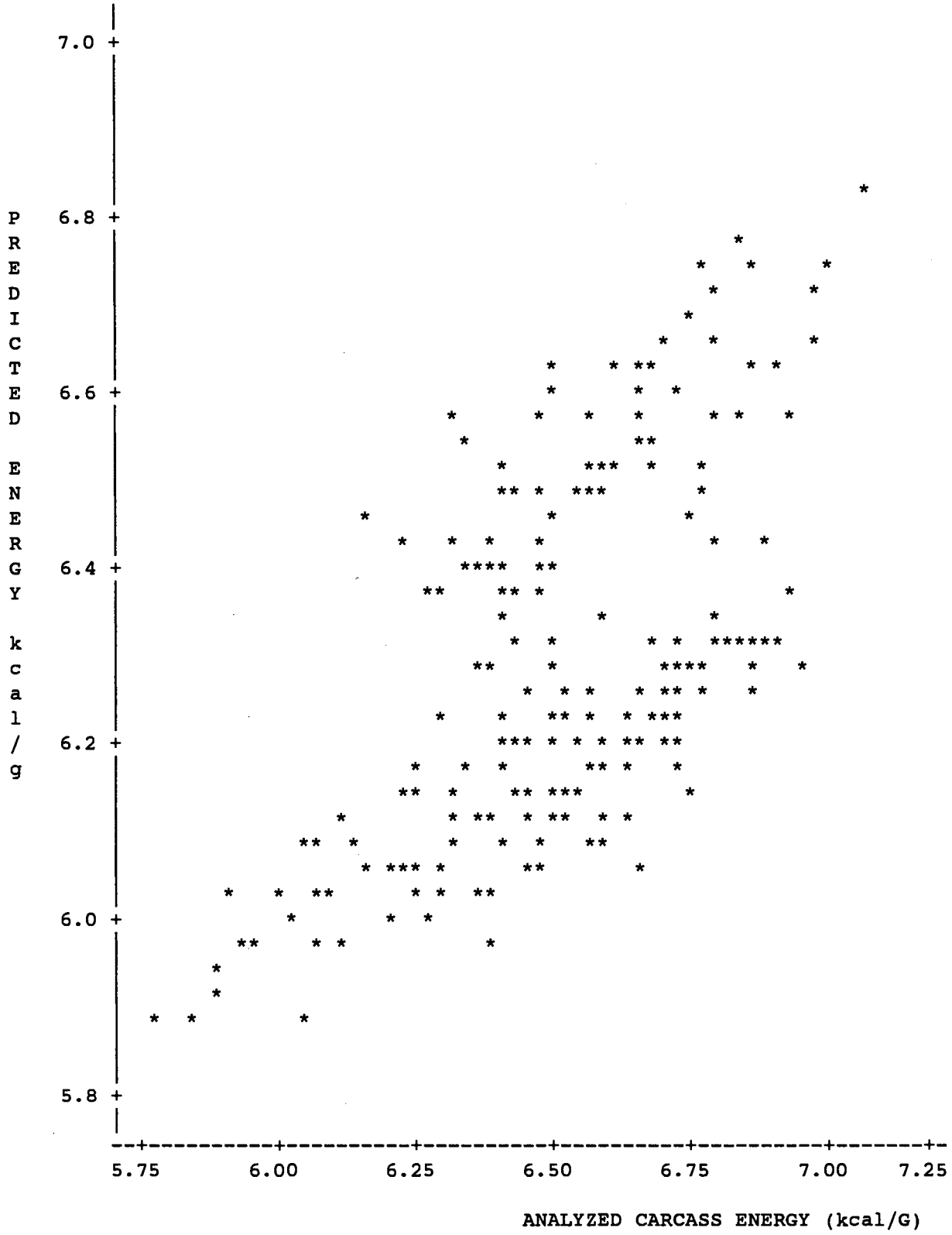

Figure 4. Analyzed and predicted energy in Experiment 3.

Chapter V

An Evaluation of A Metabolizable Energy System for Carbohydrates, Proteins and Fats as Determined by Broiler Carcass Composition[1]

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ABSTRACT Three experiments were conducted to determine AME_n efficiency for gain of a corn-soy basal ration alone or supplemented with isocaloric quantities of corn starch, isolated soy-protein, and corn oil. In Experiment 1 we determined the AME_n to be 3.24, 3.95, 4.25, and 8.75, kcal/g dry matter for the basal ration, corn starch, isolated soy-protein, and corn oil, respectively. In the second and third experiment, birds were fed the two isocaloric diets; basal plus corn starch, basal plus isolated soy-protein, and basal plus corn starch and urea during a 28 day growing period. Despite similar ($\underline{P} > .1$) AME_n consumption and carcass protein gain, birds supplemented with corn starch had greater ($\underline{P} < .05$) carcass fat gain than birds supplemented with isolated soy-protein (142.9 vs 125.6 g/bird). Experiment 3 yielded similar responses. Although no differences ($\underline{P} > .1$) were observed in AME_n intake, carcass fat gain was less ($\underline{P} < .05$) with supplemental soy-protein than starch (134.8 vs 121 g); fat pad weight and dry matter followed similar trends. Birds consuming basal plus corn starch and urea gained 128 g of fat had fat pad weight similar ($\underline{P} > .1$) to the basal plus corn starch supplement. Results suggest that rations formulated using AMEn as an index of energy content do not adequately predict energy retention of various diet components. Consequently, calorie-nutrient ratios of tissue depots can vary independently of metabolizable energy.

(*Key words:* broiler, metabolizable energy, net energy, carcass)

INTRODUCTION

The metabolizable energy (ME) system is utilized by the vast majority of the world's poultry industry for formulating diets. It is trusted to provide an accurate ranking of comparative energy values for various feeds. However, ME yields both heat (heat

increment) and net energy, i.e., energy for maintenance and production. One method for calculating net energy values for feedstuffs from determined ME values was described by DeGroote (1973). Studies by Hoffman and Schiemann (1971) and Chudy and Schiemann (1971) suggest that for lipogenesis, the ME utilization of carbohydrates, fats, and proteins was 75, 84, and 61%, respectively. These values are in general agreement with values from Burlacu and Baltic (1971), Burlacu et al. (1970) and Mittelstaedt and Teeter (submitted). Lipid gain of precision fed birds varied by over 150% between dietary protein and dietary lipid. Therefore, one might speculate that increased carcass leanness, often seen with birds fed high protein rations, could be a result of a reduction in the cellular energy supply.

Adams et al. (1962) reported that increasing the caloric density of the diet by substituting fat for corn at a constant calorie/protein ratio alleviated the effects of high ambient temperature on gain. The authors indicated that this caloric density effect occurred independent of environmental temperature, which was confirmed by Dale and Fuller (1980). However, specific environmental effects can't be ruled out as other studies (Fisher and Wilson, 1974; Abdelkarim et al., 1985) have suggested that the gain response to added fat is reduced by higher ambient temperatures. Although fat calories have a lower heat increment per kcal ME_n than carbohydrate or protein, feed intakes of diets supplemented with fat frequently are higher (Kubena et al., 1972); this could affect the reduced diet heat increment per kcal ME_n and actually increase heat production due to the increased energy intake.

Despite these discrepancies in nutrient utilization, the ME system still is the main energy utilization scheme used in the poultry industry. The use of protein as an energy source usually is inappropriate because economically protein is typically more expensive per unit ME then fat or carbohydrate is; energy is required to form glucose from amino acids, and the excess nitrogen produced must be removed by uric acid synthesis. In order to formulate diets for maximum protein accretion without excess, a net energy system is needed so that production rate can be predicted accurately.

The objective of the study reported herein was to determine the efficiency of fat accretion from ME from carbohydrate and soy-protein.

MATERIALS AND METHODS

Experiment 1 and 2

Two experiments, repeated in time, were conducted to estimate the AME_n of corn oil, corn starch and isolated soy-protein as described by (Fisher and McNab, 1987). Apparent metabolizable energy values were converted to AME_n using 8.73 Kcal/g N excreted as proposed by Titus (1956). To quantify AME and AME_n of these ingredients, the metabolizable energy content of the basal diet was used to estimate basal energy contribution of the basal diet to the diets supplemented with specific ingredients. Contribution of each test ingredient metabolizable energy to total bird energy balance during the 3-d feeding period was computed by difference (Dale and Fuller, 1982). Test ingredient AME and AME_n content were estimated as the slope of the regression line

relating ingredient AME contribution to ingredient consumption (Fisher and McNab, 1987).

Cobb x Cobb male broilers were fed a 23% crude protein corn-soybean based diet and reared through 42 days posthatching. Seven days prior to the experimental period, 35 birds were selected at random and placed within individual plexiglas metabolic chambers (51 x 34 x 41 cm). Seven experimental diets were formulated by adding either nothing or corn starch (10 & 20%), corn oil (5 & 10%) and isolated soy protein (12 & 24%) to the basal diet mash (Table 1). Following a 7-d adaptation period to the experimental rations and respiratory chambers, the birds were fasted for 24 h and the experiment was initiated. Test rations were provided for *ad libitum* consumption during next 3-d. Upon completion of the feeding period, birds again were fasted (24 h) to complete the excreta collection period. Feed consumption was monitored and total excreta voided during the feeding and final fasting period were collected daily and dried at 60 C in a forced air oven. Feed and fecal moisture, nitrogen and energy were determined as described by the AOAC (1990). Gross energy was determined with an adiabatic bomb calorimeter and crude protein was calculated as 6.25 times the nitrogen content.

Experiment 3

This experiment was conducted to estimate composition of carcass gain by birds supplemented with isocaloric (ME) amounts of corn starch and isolated soy-protein. Male Cobb x Cobb chicks were fed a 23% crude protein corn-soybean based ration and reared on rice-hull covered floor pens through 21 d of age. At 22-d of age, 160 birds were

transferred to individual 82 x 61 x 38 cm wire-floored cages housed within an environmentally controlled room (24 ± 1 C). Feed, representing the respective treatments, and water was offered for *ad libitum* consumption. On Day 49, birds were weighed, hung on a rail, stunned, exsanquinated by severing the jugular and carotid veins, passed through a scalding vat, plucked by machine and hand eviscerated. Carcasses were weighed and chilled in ice water for 4 h. Following chilling, carcasses were weighed, specific gravity was recorded, and carcass were dried at 60 C in a forced air drying oven for dry matter determination. Carcass fat was estimated (Wiernusz et al., 1995) as: 74.19-(87.33 x specific gravity) + (.88 x dry matter percentage) and carcass protein (McDonald, 1993) as: (carcass weight - carcass fat - (carcass weight x .035)) x .30.

Experiment 4

This experiment was conducted to estimate the composition of carcass gain of birds fed: 1.) basal ration (Table 1); 2.) basal plus glycolic acid; 3.) basal plus corn starch; 4.) basal plus isolated soy-protein; 5.) basal plus corn starch plus urea. Treatments 2, 3, and 4 were isocaloric (ME) and Treatments 2 and 4 were isonitrogenous. With the exception of utilizing 240 birds (48 replications/treatment) and measuring breast weight, all procedures were as described for Experiment 2.

Statistical Analysis

Treatments were arranged in a randomized complete block design (Steel and Torrie, 1960). A multiple regression procedure was applied to determine the AME_n value of the various energy supplements. The two component regression equation (ingredient intake and ingredient AME_n consumption) yielded a slope that equaled the AME_n value of the test ingredient. In Experiments 2 and 3, an ANOVA was performed using the General

Linear Models procedure of the SAS Institute (1982). When an F statistic was significant, treatment means were separated using the SAS® probability of difference procedure.

RESULTS AND DISCUSSION

Experiments 1 and 2

Results of the first and second experiments are shown in Tables 2 and 3. No run treatment interactions ($P > .1$) were detected, consequently, the data were pooled over the two studies for each test ingredient. Feed intake remained acceptable and independent of the contribution of test ingredient; intake ranged from 392 to 439 g for the 3-d feeding period (Table 2). Regression analysis (Appendix C) estimated the AME and AME_n for corn starch, corn oil and the isolated soy protein to be 4.19, 8.83 and 4.58 while the AME_n was estimated to be 3.95, 8.75 and 4.25, respectively (Table 3). As expected, the AME_n values consistently were lower than AME, similar to observations reported by Wolynetz and Sibbald (1984). In all cases, the values were within the range of other published work. National Research Council (1994) reported AME_n values ranging from 8.6 to 10.8 kcal/g for corn oil, 3.7 kcal/g dry matter for isolated soy-protein, and 3.92 kcal/g dry matter for corn starch (Mittelstaedt and Teeter, submitted).

Experiment 3

Experiment 3 results are presented in Table 4. Although feed and AME_n consumption, live weight gain, and carcass weight were similar ($P > .1$), fat gain and carcass dry matter percentage were significantly affected by energy source. Fat pad weight was 16% greater ($P < .05$) for birds supplemented with corn starch than for birds supplemented with isolated soy-protein. While both treatments exhibited similar ($P > .1$)

protein gain, birds supplemented with corn starch had greater ($\underline{P} < .05$) fat gain (143 vs 126 g/bird). No differences ($\underline{P} > .1$) were observed for dressing percentage, carcass chill weight, or carcass specific gravity between the energy supplements. Observations of this experiment are in agreement with others (DeGroote, 1969; Mittelstaedt and Teeter, submitted) and demonstrate that rations formulated to provide equal amounts of metabolizable energy do not necessarily produce equal energy retention.

Experiment 4

We expected that energy intake of birds fed the glycolic acid supplement to be depressed (Pinchasov and Jensen, 1989). Had this occurred, we could have established how intake of the basal diet would impact carcass fat and protein gain in the same range as the supplemented test diets. Caloric value of the energy supplement then could be quantified by difference (Dale and Fuller, 1982). Unfortunately, no differences ($\underline{P} > .1$) were detected between intakes of the basal diet and the basal plus glycolic acid diet. Consequently, the data were pooled over glycolic acid levels as shown in Table 5. Basal and ingredient AME_n intake and live weight gain were similar ($\underline{P} > .1$) for the energy supplemented groups, while the unsupplemented group consumed 5 percent more ($\underline{P} < .05$) basal AME_n energy than the energy supplemented groups. This made it infeasible to determine the contribution of the basal diet to carcass fat and protein accretion for birds consuming isolated soy-protein, corn starch, and corn starch plus urea. Birds consuming the corn starch + urea supplement had a lower ($\underline{P} < .05$) feed efficiency compared to the other groups, possibly due to energy expended to excrete nitrogen in the form of uric acid.

Carcass weight, dressing percentage, chill weight, specific gravity, breast weight, and protein gain were similar ($\underline{P} > .1$) between the energy supplemented groups. Overall, protein gain averaged 465.9, 472.3, and 463.2 g for the isolated soy-protein, corn starch, and corn starch + urea, respectively. Despite similar AME_n intakes, carcass fat gain was again greater ($\underline{P} < .05$) with added starch than addition of isolated soy-protein (134.8 vs 121 g). Fat pad weight and dry matter content of the carcass followed similar trends. As a result energetic efficiency estimated as AME_n varied among the energy supplemented groups. Birds supplemented with isolated soy-protein had lower carcass energy retention than corn starch as judged by the quantity of carcass fat with the birds supplemented with corn starch plus urea being intermediate.

In conclusion, rations formulated to provide similar amounts of ME_n did not produce equal energy retention. Carcass calorie-nutrient ratios changed independently of metabolizable energy intake. In order to formulate balanced diets for maximum protein accretion and minimal fat accretion, an energy-requirement scheme needs to be devised to account for differences in substrate-mediated heat production.

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TABLE 1. Composition of basal diets used in Experiments

Ingredients and analysis	Percentage
Ground corn (8.8% CP)	56.05
Soybean meal (48.5% CP)	37.10
Animal fat	2.48
Dicalcium phosphate (22% Ca; 18.5% P)	2.42
Limestone (38% Ca)	.93
NaCl	.40
Vitamin mix[2]	.30
DL-methionine, 99%	.22
Trace mineral mix[3]	.10
Total	100.00
Calculated analysis	
ME, kcal/kg	3,004.40
CP	23.00
Ca	1.00
P, available	.44
Na	.16
K	1.00
Cl	.25

[1]The vitamin mix contained the following per kilogram of diet: vitamin A, 14,109 IU (retinyl acetate); cholicalciferol, 5,291 IU; vitamin E, 47.6 IU (dl-α-tocopheryl acetate); vitamin B_{12}, .014 mg; riboflavin, 8.82 mg; niacin, 26.5 mg; d-pantothenic acid, 28.2 mg; choline, 705.5 mg; menadione, 1.16 mg; folic acid, 1.176 mg; pyridoxine, 3.52 mg; thiamin, 3.52 mg; d-biotin, .176 mg.

[2]The mineral mix contained the following per kilogram of diet: Ca, 160 mg; Zn, 100 mg; Mn, 120 mg; Fe, 75 mg; Cu, 10 mg; I, 2.5 mg, Se, .15 mg.

Table 2. Apparent metabolizable energy (AME_n) measurements for corn starch (CS), corn oil (CO), and isolated soy-protein (SOY) during the 3-d experimental period in Experiments 1 and 2

	Ration						
Variables	basal	10%CS	20%CS	5%CO	10%CO	12%SOY	24%SOY
Feed intake, g	399	398	426	439	415	392	399
Basal intake, g	399^{ab}	356^{abcd}	343^{bcd}	415^{a}	374^{abc}	335^{dc}	298^{d}
Ingredient intake, g	-	42^{d}	83^{b}	24^{e}	41^{d}	57^{c}	101^{a}
Fecal output, g	126	106	126	133	111	125	126
Energy intake, kcal	1800^{b}	1802^{b}	1952^{ab}	2124^{a}	2033^{ab}	1840^{ab}	1903^{ab}
Fecal energy, kcal	464^{ab}	391^{b}	463^{ab}	499^{a}	413^{ab}	454^{ab}	443^{ab}
Energy balance, kcal	1336^{b}	1411^{ab}	1488^{ab}	1625^{a}	1620^{ab}	1386^{ab}	1460^{ab}
Nitrogen intake, g	16^{d}	15^{d}	21^{bc}	18^{d}	15^{cd}	22^{b}	26^{a}
Fecal nitrogen, g	11^{cd}	10^{d}	14^{b}	14^{bc}	9^{d}	15^{b}	18^{a}
Nitrogen balance, g	5^{ab}	5^{ab}	7^{a}	4^{b}	6^{ab}	7^{a}	8^{a}

$^{a-d}$Means within a row with no common superscript differ significantly ($P < .05$)

Table 3. AME and AME_n analysis for the basal, corn starch, corn oil and isolated soy protein

Ingredient	AME (kcal/g)	AME_n (kcal/g)
Basal Ration	3.35±.11	3.24±.10
corn starch	4.19±.28	3.95±.27
corn oil	8.83±.40	8.75±.42
isolated soy	4.58±.12	4.25±.10

Table 4. Isolated soy-protein and corn starch effects on broiler live performance and carcass composition in Experiment 3

Variable	Ration	
	Isolated soy-protein	Corn starch
Live bird measures		
Final weight (g)	2417.2	2438.0
Live weight gain (g)	1773.7	1783.9
Feed Intake (g, DM)	3172.8	3224.9
Feed efficiency (gain/feed)	.51	.51
Basal AME_n intake (kcal)	9520.2	9520.4
Ingredient AME_n intake (kcal)	1028.0	1025.4
Carcass measures		
Carcass weight (g)	1778.7	1790.6
Dressing percentage	73.6	73.4
Chill weight (g)	1846.5	1862.7
Specific gravity	1.052	1.052
Fat pad (g)	17.2^b	20.4^a
Fat gain (g, DM)	125.6^b	142.9^a
Protein gain (g, DM)	471.1	472.6
Dry matter (%)	31.0^b	32.1^a

[a,b] Means within a row with no common superscripts differ significantly ($P < .05$).

Table 5. Basal isolated soy-protein, corn starch, and corn starch plus urea effects on broiler live performance and carcass composition in Experiment 4

Variable	Ration			
	Basal	Isolated soy-protein	Corn starch	Corn starch+urea
Live bird measures				
Final live weight (g)	2314.4	2331.7	2381.1	2343.3
Live weight gain (g)	1680.5^{b}	1729.3^{ab}	1773.3^{a}	1737.0^{ab}
Feed Intake (g)	2933.1^{c}	3069.9^{b}	3114.6^{b}	3189.4^{a}
Feed efficiency (gain/feed)	.53^{a}	.52^{a}	.52^{a}	.50^{b}
Basal AME_n intake (kcal)	9586.4^{a}	9275.3^{b}	9275.3^{b}	9275.3^{b}
Ingredient AME_n intake (kcal)	-	1160.6	1177.1	1170.9
Carcass measures				
Carcass weight (g)	1695.0^{b}	1710.5^{ab}	1754.9^{a}	1724.9^{ab}
Dressing percentage	73.0^{b}	73.3^{ab}	73.7^{a}	73.3^{ab}
Chill weight (g)	1765.2^{b}	1777.6^{ab}	1827.2^{a}	1796.3ab
Specific gravity	1.053	1.054	1.053	1.053
Breast weight (g)	336.0	337.2	344.8	347.1
Fat pad (g)	17.6^{b}	19.8^{b}	26.4^{a}	24.3^{a}
Fat gain (g, DM)	111.4^{c}	121.0^{b}	134.8^{a}	128.3^{ab}
Protein gain (g, DM)	448.8^{b}	465.9^{a}	472.3^{a}	463.2^{ab}
Dry matter (%)	30.7^{c}	31.0^{bc}	31.7^{a}	31.4^{ab}

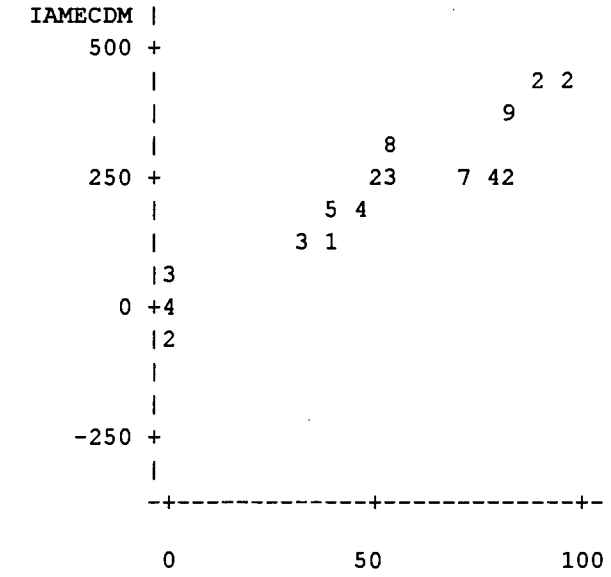
^{a,b} Means within a row with no common superscripts differ significantly ($P < .05$).

APPENDIX B
CHAPTER V

corn starch

IAMECDM=ingredient AME consumption (kcal/g DM)
INCONDM=ingredient consumption (g DM)

Plot of IAMECDM*INCONDM. Symbol is value of CHAMB.



NOTE: 9 obs hidden.

corn starch

Model: M1
Dependent Variable: IAMECDM

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	434687.23109	434687.23109	214.686	0.0001
Error	23	40495.19460	2024.75973		
C Total	24	475182.42569			

Root MSE	44.99733	R-square	0.9148
Dep Mean	169.97714	Adj R-sq	0.9105
C.V.	26.47258		

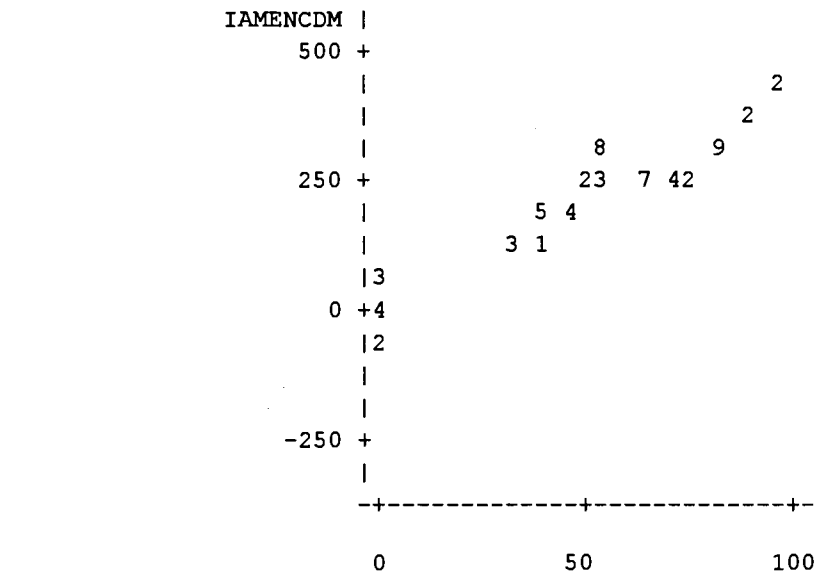
Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	11.215995	14.47201135	0.775	0.4474
INCONDM	1	4.187850	0.28581787	14.652	0.0001

corn starch

IAMECDM=ingredient AMEN consumption (kcal/g DM)
INCONDM=ingredient consumption (g DM)

Plot of IAMENCDM*INCONDM. Symbol is value of CHAMB.



NOTE: 9 obs hidden.

corn starch

Model: M2
Dependent Variable: IAMENCDM

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	386774.76232	386774.76232	211.607	0.0001
Error	23	36555.90438	1827.79522		
C Total	24	423330.66669			

Root MSE	42.75272	R-square	0.9136
Dep Mean	162.29064	Adj R-sq	0.9093
C.V.	26.34331		

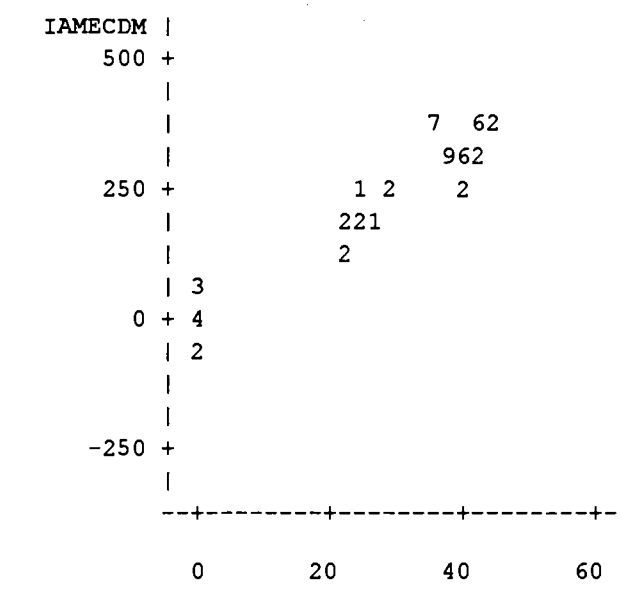
Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	12.534428	13.75010187	0.912	0.3728
INCONDM	1	3.950315	0.27156038	14.547	0.0001

corn oil

IAMECDM=ingredient AME consumption (kcal/g DM)
INCONDM=ingredient consumption (g DM)

Plot of IAMECDM*INCONDM. Symbol is value of CHAMB.



NOTE: 11 obs hidden.

corn oil

Model: M1
Dependent Variable: IAMECDM

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	540548.29748	540548.29748	484.843	0.0001
Error	25	21182.98440	1114.89392		
C Total	26	561731.28188			

Root MSE	33.39003	R-square	0.9623
Dep Mean	196.90498	Adj R-sq	0.9603
C.V.	16.95743		

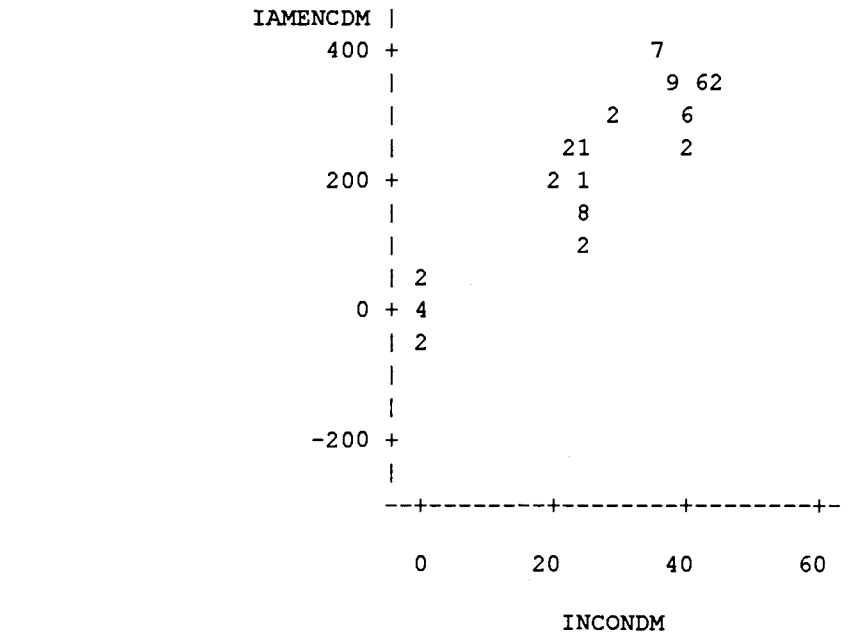
Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	8.896817	11.22472127	0.793	0.4378
INCONDM	1	8.379577	0.40092454	22.019	0.0001

corn oil

IAMECDM=ingredient AMEN consumption (kcal/g DM)
INCONDM=ingredient consumption (g DM)

Plot of IAMENCDM*INCONDM. Symbol is value of CHAMB.



NOTE: 11 obs hidden.

corn oil

Model: M2
Dependent Variable: IAMENCDM

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	531126.22684	531126.22684	416.009	0.0001
Error	25	24257.64616	1276.71822		
C Total	26	555383.87300			

Root MSE	35.73119	R-square	0.9563
Dep Mean	198.09140	Adj R-sq	0.9540
C.V.	18.03773		

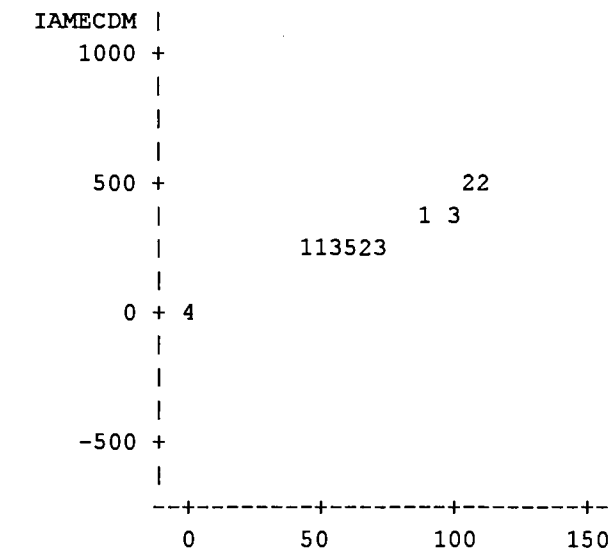
Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	11.728993	12.01175100	0.976	0.3411
INCONDM	1	8.750739	0.42903566	20.396	0.0001

isolated soy-protein

IAMECDM=ingredient AME consumption (kcal/g DM)
INCONDM=ingredient consumption (g DM)

Plot of IAMECDM*INCONDM. Symbol is value of CHAMB.



NOTE: 12 obs hidden.

isolated soy-protein

Model: M1
Dependent Variable: IAMECDM

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	781670.53633	781670.53633	1286.921	0.0001
Error	21	12147.91763	607.39588		
C Total	22	793818.45395			

Root MSE	24.64540	R-square	0.9847
Dep Mean	223.89719	Adj R-sq	0.9839
C.V.	11.00746		

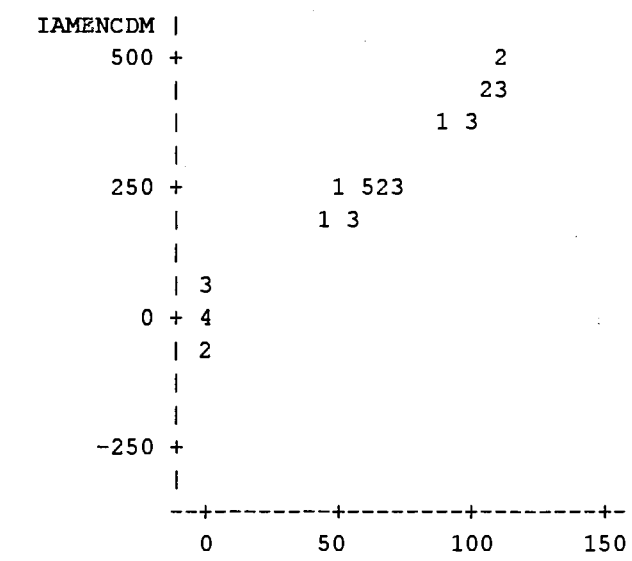
Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	1.894720	8.11823941	0.233	0.8178
INCONDM	1	4.581862	0.12772211	35.874	0.0001

isolated soy-protein

IAMECDM=ingredient AMEN consumption (kcal/g DM)
INCONDM=ingredient consumption (g DM)

Plot of IAMENCDM*INCONDM. Symbol is value of CHAMB.



NOTE: 9 obs hidden.

isolated soy-protein

Model: M2
Dependent Variable: IAMENCDM

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	671776.72728	671776.72728	1639.183	0.0001
Error	21	8196.48281	409.82414		
C Total	22	679973.21008			

Root MSE	20.24411	R-square	0.9879
Dep Mean	206.47137	Adj R-sq	0.9873
C.V.	9.80480		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.665193	6.66844697	0.100	0.9215
INCONDM	1	4.247590	0.10491291	40.487	0.0001

CHAPTER VI

Effect of Dietary Crude Protein on Energetic Efficiency of Broilers[1]

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Running Head: CRUDE PROTEIN AND ENERGETIC EFFICIENCY

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ABSTRACT Three experiments, replicated in time, were conducted to quantify effects of equalized AME_n consumption from isolated soy protein, corn oil and starch on bird heat production, carcass protein and fat accretion, and serum chemistries. In addition, the studies sought to quantify these response variables for rations containing 23, 20 and 17% CP. A fasted bird group provided data to assess the birds maintenance needs, where regressing energy loss of fasted birds on initial protein mass yielded the following equation: Energy Loss = 740.9 - 4.32 x protein mass ($P=.16$; $R^2 = .94$), which was used to estimate maintenance energy needs. Birds were fed the basal ration at 6% of body weight$^{.66}$ (MWT) per day. Even though this was approximately 80% of the *ad libitum* consumption exhibited by reference birds from the same flock, growth rate was much less than anticipated. As a result, maintenance energy needs, estimated to be 957 kcal, utilized the bulk of the energy consumed and provided insufficient dietary energy for protein accretion. Utilization of the carbon-nitrogen balance equations enabled carcass lipid and protein monitoring, such that the use of 10.8 g of endogenous lipid stores provided 98.5 kcal of energy for protein accretion. Protein accretion was elevated by supplemental carbohydrate, corn oil and isolated soy-protein and averaged 63.5 g vs 57.4 g for the basal alone. Similar to the birds consuming the basal diet, energy supplemented groups lost an average of 14.8 g carcass fat which supplied 134 kcal for protein accretion. Lowering the dietary crude protein from 23 to 17% increased protein efficiency ratio from .77 to .91 and reduced the quantity of excess amino acid that were catabolized. The increased energy gain at elevated crude protein could negate a higher heat increment for the amino acids, however, this is confounded by greater dietary fat intake and therefore a lower heat

increment with the high protein ration. Though the limit feeding technique theoretically enables more direct comparison of substrate energetic efficiency, our results were compromised by the creation of an energy deficiency.

(*Key words:* broiler, energetic efficiency, utilization of metabolizable energy, net energy, carcass)

INTRODUCTION

Deeper understanding of energy metabolism should improve precision of diet formulation and enhance profit of production enterprises. The ME_n system is the current standard for ration formulation (NRC, 1994). However, by definition, the ME_n system does not account quantitatively for heat increment. Any heat increment change alters ME_n utilization and may alter the cellular energy/nutrient ratios; an increase in this ratio should increase fat accretion.

Carcass fat is influenced by several factors. The factor investigated most widely is the dietary calorie to protein ratio (Summers and Leeson, 1979; Jones and Wiseman, 1985; Bartov, 1987; Belay and Teeter, 1992). Decreasing the dietary calorie/protein ratio increases broiler fat pad weight and carcass fat. Sonaiya et al. (1990) suggested that carcass fat is reduced by reducing the caloric density of the diet and by increasing its protein content. Belay and Teeter (1991) also observed that increasing dietary energy and/or narrowing the calorie/protein ratio impacts bird performance. Lowering dietary crude protein numerically increased survivability in a thermoneutral environment (4.4%) and significantly enhanced survival (10.8%) in a cycling heat distressed environment. If

these responses are related to heat production than energetic efficiency would vary with substrate.

Mittelstaedt and Teeter (submitted), using precision fed birds, evaluated the ME_n retention from gelatin, starch, and corn oil. Metabolizable energy utilization for energy (fat) gain varied by over 150% across these nutrient classes. Efficiency of ingredient TME for carcass energy deposition averaged 50, 39 and 20%, respectively for corn oil, starch and gelatin. Therefore, one might speculate, that the increased carcass leanness obtained by feeding high protein rations could be due to reduction in the cellular energy supply. Whether the reduced ME_n utilization of protein for fat gain should be attributed to protein per se or to specific amino acids has not been addressed. This problem is perpetuated by the apparent lack of thermodynamic data regarding energy changes accompanying substrate metabolism to various tissues. Inefficient use of ME_n with its increased heat production also has implication for heat stress as Wiernusz and Teeter (1993) reported; regressing heat production on feed intake (0, 3, 6, 9% body weight$^{.66}$; MWT per day) yielded .48 kJ/g feed per unit MWT for birds housed at 24 C and .58 kJ/g feed per unit MWT for birds exposed to heat distress (35 C). Although some of the added heat production is related to the cost of heat dissipation, efficiency of various substrates for tissue accretion would enable one to define heat stress risk and heat load more precisely.

The objective of this study reported herein was to quantify and contrast equalized AME_n consumption of isolated soy protein, corn oil and starch on heat production, carcass protein and fat accretion, and serum chemistries. In addition, the study sought to quantify these response variables for rations containing 23, 20 and 17% CP.

MATERIALS AND METHODS

Cobb x Cobb male broilers were fed a 23% crude protein corn soybean-based diet and reared through 35-d posthatching. Seven days prior to the experimental period, 35 birds were selected at random and placed in individual plexiglas metabolic chambers (51 x 34 x 41 cm). Nine experimental diets were formed by adding on top of a nutritionally complete 23% CP basal ration (Table 1) either: 1.) nothing; 2.) 15% corn starch; 3.) isolated soy protein AME_n to equal AME_n provided by 15% cornstarch; 4.) corn oil AME_n to equal AME_n provided by 15% corn starch; 5.) 50-50 AME_n contribution of starch and isolated soy protein to equal the AME_n provided by 15% corn starch; 6.) 50-50 AME_n contribution of corn oil and isolated soy protein to equal the AME_n provided by 15% corn starch; 7.) 50-50 AME_n contribution of starch and corn oil to equal the AME_n provided by 15% corn starch. Diets 8 and 9 (Table 1) consisted of 17% and 20% CP plus the addition of limiting amino acids (where warranted) caloric densities were equal to that of the 23% CP basal ration. The experiment was replicated over time for a total of 100 birds (10 birds/treatment). Following a 7-d adaptation period to the experimental rations and respiratory chambers, the birds were fasted 24 h and the experiment was initiated. Test rations were provided at 6% of bird MWT/day offered in 4 equal feedings during the next 3-d. The AME_n for corn starch, corn oil, and isolated soy-protein had been estimated previously (Wiernusz and Teeter, submitted) to be 3.95, 8.75, and 4.25, respectively. In addition to these 9 diets, a fasted negative control formed the 10th treatment and was used

to estimate basal metabolic rate. This fasted group was fed the 23% CP ration during the adaptation period, but remained fasted during the 3-d experiment. Upon completion of the test period, birds again were fasted (24 h) to complete the excreta collection period. Water was supplied for *ad libitum* consumption throughout the experiment.

Throughout the 4-d experimental period, birds were limit fed treatment diets. Production of O_2 (L) and CO_2 (L) by individual birds was monitored as described by Wiernusz and Teeter (1993). Total excreta were collected, dried in a 60 C oven, air equilibrated and weighed (Dale and Fuller, 1982). Excreta and feed samples were ground to pass through a screen with 1 mm holes, and representative sub-samples were retained and assayed for water, nitrogen and ash contents (AOAC, 1990). Feed and excreta were assayed for gross energy (calorie/g) and carbon (%) by combustion in a Model 1261 Parr adiabatic bomb calorimeter as described by Harjo and Teeter (1994). The AME and AME_n value of each diet was calculated (Fisher and Wilson, 1974) and balances for nitrogen, carbon and energy were calculated by difference between consumption and loss. The calculation for carbon balance accounts for respiratory carbon loss by converting the CO_2 (L) production to g of carbon using: [(CO_2 (L) x molecular weight of CO_2 (44)]/(22.4L/mole CO_2 gas at STP x percent carbon in CO_2 (27.27). Protein and fat accretion (g) were estimated using predictive equations: protein gain = (CO_2 production x .146) + AME intake x .822) - (AME_n intake x .840) and fat gain = carbon gain - (protein gain x .53)/.77. Tissue energy accretion as protein and fat were calculated by using 5.72 and 9.31 kcal per g, respectively (McDonald and Teeter, 1994). Efficiency of ME_n and

net energy utilization were determined by dividing the total tissue energy gain by total ME_n and gross energy consumed, respectively.

Serum Blood chemistries. Blood samples were collected from the ulnaris vein using a 3 mL syringe. Serum variables including glucose, triglycerides, total protein, creatinine, albumin, uric acid, triiodothyronine (T_3), thyroxine (T_4), and lactate were measured as described by Cason and Teeter (1994).

Statistical Analysis

Chamber oxygen, CO_2, were regressed against time, time squared and time cubed such that polynomial equations describing the data were formed. Quantitative estimates for each variable were made by integrating variable functions over specific time intervals and adjusting for the control chamber as appropriate. Data were analyzed by analysis of variance using the General Linear Models procedure of SAS (SAS Institute, 1985) to determine the effects of diet and the effects on body weight gain, nitrogen balance, carbon balance, O_2 consumption, CO_2 production, respiratory carbon loss, protein and fat accretion, heat production, energetic efficiency, and serum blood chemistries. When a significant F statistic was noted, treatment means were separated using the SAS® probability of difference procedure. Standard statistical procedures (Steel and Torrie, 1960) were used to obtain correlation coefficients as well as linear regression equations.

RESULTS AND DISCUSSION

Results are presented in Tables 2 to 7. No significant differences ($P > .1$) between the 3 runs were detected for the variables monitored during the 4-d experimental periods,

consequently, the data were pooled across runs for analysis. Data were divided into 4 parts for statistical analysis and presentation. Data partitioning included the fasted birds, birds consuming the basal ration only, birds consuming the basal ration plus energy supplements and birds consuming the various protein diets (23, 20 and 17% crude protein).

Fasted Birds

The fasted bird group provided data (Tables 2 and 3) to assess the bird's maintenance needs for the trial. All the birds deprived of feed lost ($P < .01$) body weight (-341.7 g), carcass energy (-913.1 kcal), protein (-19.5 g),and fat (-87.7 g) during the 4-d experimental period. Both protein and body mass losses were variable ranging from -21 to -18 g and -387 to -287 g, respectively. Heat production gradually decreased throughout the experiment and averaged 4.1 kcal/MWT/h. Energy loss also varied among birds, however, energy loss was well correlated ($R^2 = .94$) with bird initial protein mass (computed as final protein mass adjusted for protein loss) suggesting that protein mass may be used to assess maintenance energy need. Regressing energy loss on initial protein mass yielded the following equation (Figure 1): Energy Loss = 740.9 - 4.32 x g protein mass ($R^2 = .94$), which was used to estimate bird maintenance energy needs in the discussion below. Estimates derived from this predictive equation are approximately 80% of the maintenance energy needs estimated by Hurwitz et al. (1978) using fed birds where maintenance energy requirement was considered to equal 8.0 x g body weight$^{.66}$. The

20% difference may be random error, reflect the decline in heat production associated with feed deprivation or may be an overestimation by Hurwitz et al (1978).

Birds Fed the Basal Diet

Birds consuming the basal ration at 6% of MWT per day, which was approximately 80% of the *ad libitum* consumption exhibited by reference birds from the same flock, did not grow as much as expected. Whether consumption by this reference group was depressed for some reason is unknown. Weight gain averaged only 25 g throughout the experiment; this is about 55% of the growth rate observed in other trials. As a result, maintenance energy needs, estimated to be 957 kcal, utilized the bulk of the energy consumed and gave the birds insufficient dietary energy to support protein accretion. Utilization of the carbon-nitrogen balance equations enabled carcass lipid and protein monitoring such that the use of endogenous lipid stores for protein accretion could be detected. In this study, birds fed the basal ration lost 10.8 g of depot fat which provided 100.5 kcal of energy for protein accretion. These birds gained 57.4 g of protein in contrast to 19.5g protein lost by the fasted birds. An inverse relationship was observed between fat and protein accretion ($R^2 = -.4$; $\underline{P} < .05$) indicating that the energy insufficiency was large. The original objective for this treatment was to use it as an aid in partitioning the energy gain associated by the energy supplemented groups into the independent effects of basal and supplemental energy source. However, because the birds

were repartitioning their energy stores to support protein growth, our modeling approach was inadequate.

Birds Consuming Supplements

It was not possible to separate the basal and energy supplement groups for the reasons discussed above (basal consuming birds). Protein accretion was elevated by the carbohydrate, corn oil and isolated soybean protein additions averaging 63.5 g vs the 57.4 g for the basal alone. Similar to the birds fed the basal diet, those fed the energy supplements lost carcass fat, averaging -14.8 g which supplied 134 kcal for protein accretion. Fat loss tended to be greater than observed for the basal group (-10.8 g) when either starch or protein was added to the basal ration. This suggests that when indispensable amino acids are present, energy needed for protein accretion can be supplied by fat depots. Presumably, the starch provided energy not only for protein accretion but also carbon skeletons for dispensable amino acid synthesis. The trend for the elevated fat loss is consistent with a high energy need occurring with an elevated protein accretion rate. Once again, an inverse relationship was detected between fat accretion and protein gain.

Birds Fed Various Protein Levels

Interpretation of results of isocaloric diets with different calorie/protein ratios (Table 4), also was complicated by the restricted feeding technique. Even at *ad libitum* intakes one would need to differentiate between the energetic efficiencies of corn starch,

corn oil and dietary protein to interpret the data. Under the restricted feeding regime one also must contend with a variable contribution of depot lipids. The addition of corn oil to the basal ration incrementally reduced lipid loss and increased energy gain at constant protein accretion. However, heat production was not increased by increasing the protein level. Lowering the dietary crude protein from 23 to 17% increased protein efficiency ratio from .77 to .91 indicating that the quantity of excess amino acid catabolized also declined. The increased energy gain at elevated crude protein could negate a higher heat increment for the amino acids, however, this is confounded with greater dietary fat (lower heat increment) consumption with the high protein ration. Fat addition to the high protein ration was necessary to maintain caloric density as the lower energy soybean meal was substituted for corn grain. Other factors responsible for the failure of added protein to increase heat production include the extra caloric effect of fat, error in the assumed AME content of the corn and soy sources and the fact that birds consuming the 17% crude protein ration wasted slightly more feed.

Differences ($\underline{P} < .05$) were noted for serum blood chemistries associated with diet and feeding and are presented in Table 6. Fasting birds for 24 h increased ($\underline{P} < .05$) glucose, triglycerides, total protein, and uric acid, while creatinine, T4, and lactate were increased by fasting 24 h prior to blood sampling (Table 7).

In summary, the potential for crystalline amino acid supplementation to enhance broiler production efficiency by improving cellular energy supply was explored through reduced protein consumption. This hypothesis is based on the fact that the metabolizable energy system does not account for bird heat production and the premise that heat increment is higher for amino acids than for other substrates. Though our feeding

technique theoretically enables more direct comparison of substrate energetic efficiency, the results were compromised by the creation of a simultaneous energy deficiency. The birds attempted to compensate for their energy deficit by mobilizing depot lipid to support protein synthesis. Both energy and protein supplementation impacted protein accretion and lipid mobilization. Precise evaluation of energetic efficiency was made impossible because energy sources included mobilized depot lipids, the basal ration and the energy supplement (corn starch, corn oil, isolated soy-protein). Protein accretion was impacted by both energy and protein supplementation. Bird maintenance energy needs were closely correlated with body protein mass and as such played a varying role. This type of information would not be detected in trials not utilizing carbon and nitrogen balance and can explain some of the variability in the literature. Data are presented in the appendix to this chapter, providing equations that may be used to estimate maintenance energy needs based on protein mass, an illustration of bird depot lipid-protein accretion relationships under restricted feeding conditions and accretion-energy balance relationships for isocaloric diets with different calorie/protein ratio. Though the study was not successful in terms of separating treatments according to energetic efficiency (the stated objective) it provided a basis for meaningful future research. Research to establish critical energetic relationships should have profound implications.

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TABLE 1. Composition of basal diets

Ingredients and analysis	23%CP	20%CP	17%CP
Ground corn	56.05	64.45	71.93
Soybean meal	37.10	29.50	22.00
Dicalcium Phosphate	2.42	2.45	2.50
Corn oil	2.50	1.50	0.70
Limestone	0.93	0.95	0.96
NaCl	0.50	0.50	0.50
Vitamin mix[1]	0.25	0.25	0.25
Trace mineral mix[2]	0.10	0.10	0.10
DL-methionine	0.15	0.18	0.27
L-lysine-HCl	-	0.08	0.36
L-threonine	-	0.04	0.16
L-arginine-HCl	-	-	0.26
L-tryptophan	-	-	0.01
Total	100.00	100.00	100.00
Calculated analysis			
ME, kcal/kg	2999.61	3002.55	3004.71
CP	23.01	20.19	17.05
Ca	1.10	1.10	1.10
P, available	.58	.58	.58
Na	.22	.22	.22
K	.87	.87	.87
Cl	.25	.25	.25

[1]The vitamin mix contained the following per kilogram of diet: vitamin A, 14,109 IU (retinyl acetate); cholicalciferol, 5,291 IU; vitamin E, 47.6 IU (dl-α-tocopheryl acetate); vitamin B_{12}, .014 mg; riboflavin, 8.82 mg; niacin, 26.5 mg; d-pantothenic acid, 28.2 mg; choline, 705.5 mg; menadione, 1.16 mg; folic acid, 1.176 mg; pyridoxine, 3.52 mg; thiamin, 3.52 mg; d-biotin, .176 mg.
[2]The mineral mix contained the following per kilogram of diet: Ca, 160 mg; Zn, 100 mg; Mn, 120 mg; Fe, 75 mg; Cu, 10 mg; I, 2.5 mg, Se, .15 mg.

Table 2. Feed, AME, and AME_n consumption along with nitrogen, carbon, and energy estimated by a summation of intake and excretion of birds under various feeding regimes

Variable	Fasted	Basal	Basal+ingredient
Initial weight, g	3006.3	3049.2	2946.3
Final weight, g	2664.7^{b}	3074.2^{a}	3034.0^{a}
Live weight gain, g	-341.7^{c}	25.0^{b}	87.8^{a}
Feed consumption			
Basal, g	0.0^{b}	336.8^{a}	326.0^{a}
Ingredient, g	0.0^{b}	0.0^{b}	42.6^{a}
AME intake, kcal[1]	0.0^{c}	1190.8^{b}	1355.6^{a}
AME_n intake, kcal[2]	0.0^{c}	1131.2^{b}	1284.2^{a}
Nitrogen[3]			
Intake, g	0.0^{c}	11.8^{b}	14.8^{a}
Excreta, g	3.1^{b}	5.0^{ab}	6.7^{a}
Gain, g	-3.1^{b}	6.8^{a}	8.0^{a}
Carbon[4]			
Intake, g	0.0^{c}	153.7^{b}	165.2^{a}
Excreta, g	4.8^{b}	33.5^{a}	35.3^{a}
Respiratory, g	73.7^{b}	100.5^{a}	108.2^{a}
Gain, g	-78.5^{b}	19.7^{a}	21.6^{a}
Energy[5]			
Intake, kcal	0.0^{c}	1532.6^{b}	1713.3^{a}
Excreta, kcal	47.2^{b}	341.8^{a}	357.9^{a}
Respiratory, kcal	865.9^{b}	1049.6^{a}	1169.7^{a}
Gain, kcal	-913.1^{b}	229.6^{a}	228.1^{a}

^{abc}Means within a row with no common superscript differ significantly ($P < .05$)
[1]AME (dm) = (energy intake - fecal energy) / dry matter intake
[2]AME_n (dm) = (energy intake - fecal energy) - (8.22 x nitrogen gain)/ dry matter intake
[3]Nitrogen gain = g nitrogen consumed - g nitrogen excreted
[4]Carbon gain = g carbon consumed - g carbon excreted - carbon lost as CO_2
[5]Energy gain = kcal energy consumed - kcal energy excreted - kcal energy lost as heat

TABLE 3. Carcass composition measurements live measures, and growth efficiencies of birds under various feeding regimes

Variable	Fasted	Basal	Basal+ingr.
Carcass measurements			
Carcass weight, g	2060.3[a]	2323.0[a]	2279.9[a]
Dressing percentage	75.3	75.5	75.1
Specific gravity	1.047	1.051	1.052
Carcass dry matter, g	31.8	31.3	31.9
Live bird measures and efficiencies			
Heat production, kcal/MWT/h	4.1[b]	5.4[a]	5.9[b]
Body weight gain, g	-341.7[b]	41.0[a]	77.3[a]
Protein gain, g	-19.5[b]	57.4[a]	63.5[a]
Fat gain, g	-87.7	-10.8	-14.8
Protein efficiency ratio	-	.77	.71
Dmegrowth, kcal	-	313.4	531.6
Tmegrowth, kcal	-	411.9	666.7
ME maintenance, kcal	-	957.0	932.7

[ab]Means within a row with no common superscript differ significantly ($P < .05$)
[1]Protein efficiency ratio = protein gain/protein intake
[2]Dmegrowth = dietary ME consumed-energy required for maintenance
[3]Tmegrowth = Dmegrowth + ME from depot fat
[4]ME maintenance = energy required for maintenance

Table 4. Feed, AME, and AME_n consumption along with nitrogen, carbon, and energy estimated by a summation of intake and excretion of birds fed 23, 20, and 17% CP diets

Variable	23%	20%	17%
Initial weight, g	3049.2	2970.3	2931.0
Final weight, g	3074.2a	3022.7	2955.5
Live weight gain, g	25.0	52.3	24.5
Feed consumption			
Intake, g	336.8	333.9	316.9
AME intake, kcal[1]	1190.8	1203.7	1119.7
AME_n intake, kcal[2]	1131.2	1143.2	1068.3
Nitrogen[3]			
Intake, g	11.8[a]	11.3[a]	9.3[b]
Excreta, g	5.0[a]	4.4[a]	3.4[b]
Gain, g	6.8	6.9	5.9
Carbon[4]			
Intake, g	153.7[a]	149.5[ab]	136.6[b]
Excreta, g	33.5[a]	29.2[b]	26.4[b]
Respiratory, g	100.5	106.2	105.5
Gain, g	19.7	14.1	4.6
Energy[5]			
Intake, kcal	1532.6[a]	1498.5[ab]	1387.0[b]
Excreta, kcal	341.8[a]	294.8[b]	267.3[b]
Respiratory, kcal	1099.6[a]	1166.9	1091.7
Gain, kcal	229.6[a]	136.8[a]	-6.2[b]

[abc]Means within a row with no common superscript differ significantly ($P < .05$)

[1]AME (dm) = (energy intake - fecal energy) / dry matter intake

[2]AME_n (dm) = (energy intake - fecal energy) - (8.22 x nitrogen gain)/ dry matter intake

[3]Nitrogen gain = g nitrogen consumed - g nitrogen excreted

[4]Carbon gain = g carbon consumed - g carbon excreted - carbon lost as CO_2

[5]Energy gain = kcal energy consumed - kcal energy excreted - kcal energy lost as heat

TABLE 5. Carcass composition measurements live measures, and growth efficiencies of birds fed 23, 20, and 17% CP diets

Variable	23%	20%	17%
Carcass measurements			
Carcass weight, g	2323.0	2292.0	2256.8
Dressing percentage	75.5	76.3	75.8
Specific gravity	1.051	1.059	1.058
Carcass dry matter, g	31.3	30.7	30.8
Live bird measures and efficiencies			
Heat production, kcal/MWT/h	5.4	5.6	6.4
Body weight gain, g	41.0	41.5	40.7
Protein gain, g	57.4	58.2	55.6
Fat gain, g	-10.8	-21.5	-35.5
Protein efficiency ratio[1]	.77	.82	.91
Dmegrowth, kcal[2]	313.4	230.1	221.4
Tmegrowth, kcal[3]	411.9	426.2	545.9
ME maintenance, kcal[4]	957.0	973.7	955.9

[1]Protein efficiency ratio = protein gain/protein intake
[2]Dmegrowth = dietary ME consumed-energy required for maintenance
[3]Tmegrowth = Dmegrowth + ME from depot fat
[4]ME maintenance = energy required for maintenance

TABLE 6. Feeding and diet effects on serum blood chemistries of male Cobb x Cobb broilers

		Variable								
Trt[1]	Day[2]	Gluc, mg/dL	Trig, mg/dL	TP, g/dL	Crea, mg/dL	Alb, mg/dL	Uric, mg/dL	T_3, ng/ml	T_4, µg/dl	Lactate, mg/dL
1	1	214.2^{ab}	29.4^{e}	4.27^{ab}	0.16^{def}	1.55^{a}	5.16^{cd}	0.11	0.84^{e}	31.4
	2	214.0^{ab}	23.1^{e}	3.94^{abc}	0.22^{ab}	1.44^{ab}	5.16^{cd}		1.56^{abcde}	37.7
2	1	209.2^{ab}	50.8^{d}	4.01^{abc}	0.17^{cdef}	1.43^{ab}	6.08^{bc}	0.55	1.10^{cde}	39.8
	2	201.1^{ab}	23.2^{e}	3.82^{abc}	0.22^{abc}	1.33^{bc}	3.86^{defg}		1.58^{abcde}	40.7
3	1	213.2^{ab}	63.6^{dc}	3.89^{abc}	0.14^{ef}	1.36^{abc}	4.20^{def}	1.10	1.17^{cde}	33.9
	2	209.1^{ab}	19.8^{e}	3.51^{c}	0.20^{abcd}	1.19^{c}	2.42^{g}		1.60^{abcde}	40.0
4	1	212.8^{ab}	86.5	3.95^{abc}	0.16^{def}	1.44^{ab}	4.54^{cde}	0.76	1.26^{bcde}	41.0
	2	198.2^{b}	24.5^{e}	3.95^{abc}	0.20^{bcde}	1.36^{abc}	3.14^{efg}		1.92^{abc}	39.8
5	1	210.8^{ab}	52.5^{d}	4.26^{ab}	0.15^{ef}	1.43^{ab}	9.22^{a}	0.67	1.05^{cde}	35.8
	2	200.1^{ab}	21.0^{e}	3.59^{c}	0.20^{abcd}	1.28^{bc}	3.12^{efg}		2.18^{a}	36.4
6	1	199.2^{ab}	71.5^{abc}	4.26^{ab}	0.14^{f}	1.41^{ab}	4.68^{cde}		.80^{e}	30.8
	2	201.0^{ab}	22.0^{e}	3.80^{bc}	0.22^{ab}	1.18^{c}	2.49^{g}		2.08^{ab}	42.5
7	1	219.8^{ab}	47.8^{d}	4.46^{a}	0.16^{ef}	1.46^{ab}	7.26^{b}		1.04^{cde}	30.8
	2	216.8^{ab}	13.8^{e}	3.82^{abc}	0.25^{a}	1.31^{bc}	3.61^{defg}		1.93^{abc}	41.2
8	1	217.8^{ab}	57.8^{cd}	4.01^{abc}	0.14^{f}	1.38^{abc}	6.75^{b}		1.05^{cde}	34.8
	2	202.4^{ab}	24.7^{e}	4.01^{abc}	0.20^{bcde}	1.38^{abc}	3.28^{efg}		1.88^{abcd}	36.2
9	1	226.1^{a}	78.5^{ab}	3.84^{abc}	0.16^{def}	1.34^{abc}	3.38^{efg}	0.84	1.08^{cde}	39.6
	2	199.6^{ab}	23.8^{e}	3.68^{bc}	0.20^{bcde}	1.28^{bc}	2.83^{efg}		1.38^{abcde}	42.1
10	1	213.6^{ab}	69.9^{bc}	3.95^{abc}	0.16^{def}	1.41^{ab}	4.69^{cde}	0.67	1.00^{de}	31.5
	2	203.4^{ab}	26.6^{e}	4.01^{abc}	0.21^{abc}	1.40^{ab}	3.54^{efg}		1.24^{bcde}	40.1

$^{a-g}$Means within a column with no common superscript differ significantly ($P < .05$)
Gluc = glucose, Trig = triglycerides, TP = total protein, Crea = creatinine, Alb = albumin, Uric = uric acid, BUN = blood urea nitrogen T_3 = triiodothyronine, T_4 = thyroxine

[1]Treatment: 1. fasted throughout experiment, 2. basal (23%CP), 3. basal+corn oil, 4. basal+corn starch, 5. basal+isolated soy-protein, 6. basal+corn oil+corn starch, 7. basal+isolated soy-protein+corn oil, 8. basal+isolated soy-protein+corn starch, 9. 17%CP, 10. 20%CP
[2]Day: 1. fed experimental treatments, 2. fasted 24 h

TABLE 7. Feeding and fasting effects on serum blood chemistries of male Cobb x Cobb broilers

Variable	Fed	Fasted
Glucose, mg/dL	214.3[a]	202.4[b]
Triglycerides, mg/dL	64.8[a]	22.7[b]
Total Protein, g/dL	4.00[a]	3.81[b]
Creatinine, mg/dL	0.15[b]	0.21[a]
Albumin, mg/dL	1.41[a]	1.32[b]
Uric acid, mg/dL	5.70[a]	3.20[b]
Thiiodothyronine, T_3, ng/ml	0.75	-
Thyroxine, T_4, μg/dl	1.07[b]	1.69[a]
Lactate, mg/dL	36.05[b]	39.80[a]

[ab]Means within a row with no common superscript differ significantly ($P < .05$)

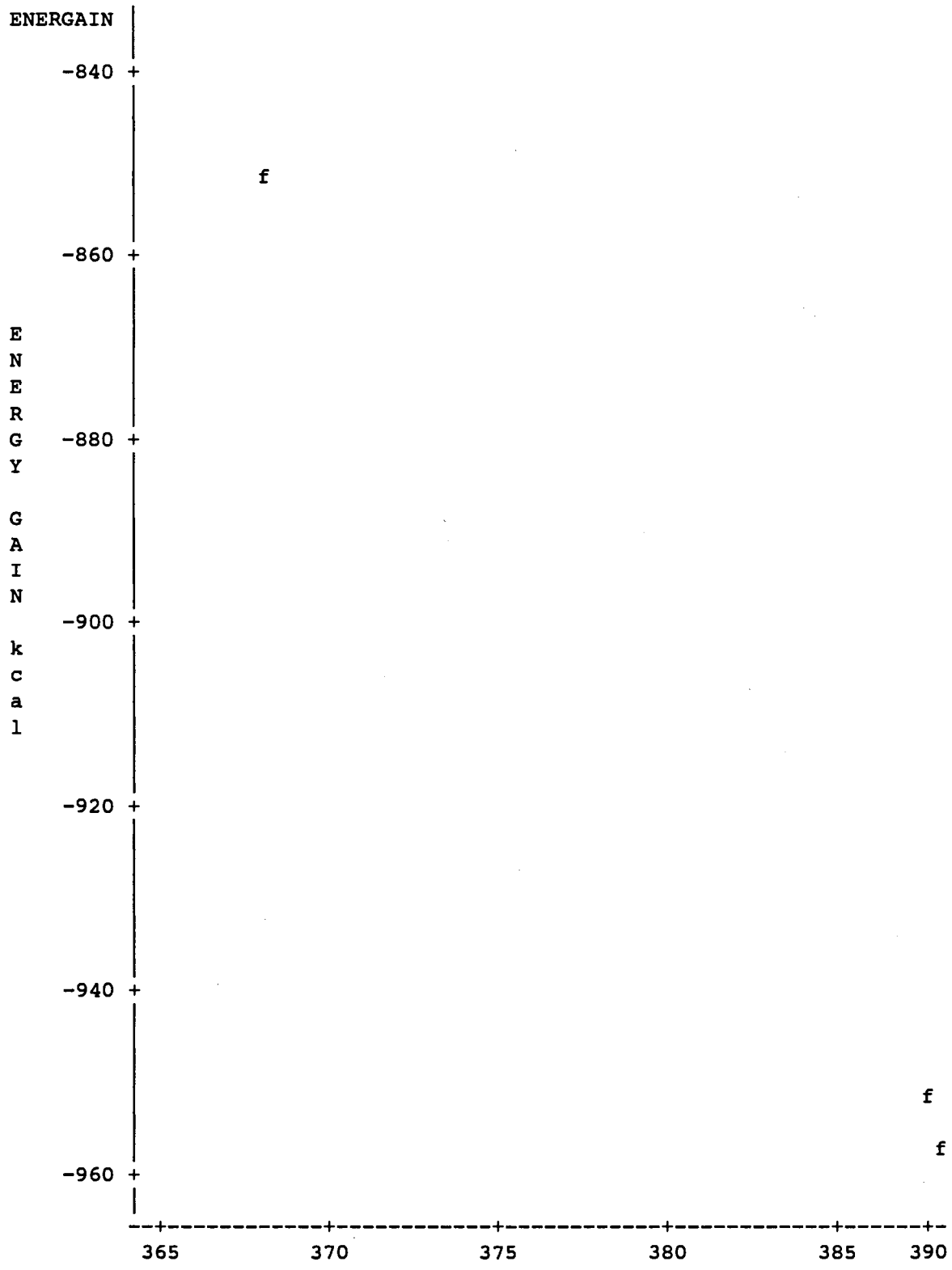


FIGURE 1. ESTIMATE OF BIRD MAINTENANCE NEEDS

Model: M1
Dependent Variable: ENERGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	5609.89641	5609.89641	15.499	0.1584
Error	1	361.94526	361.94526		
C Total	2	5971.84167			

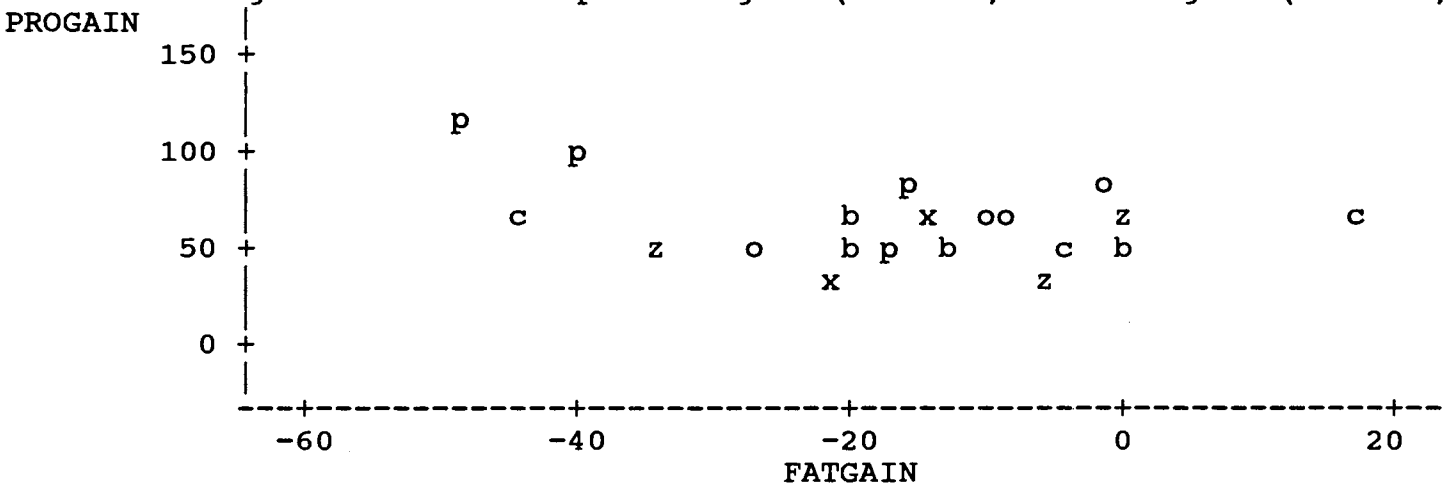
Root MSE	19.02486	R-square	0.9394
Dep Mean	-913.10755	Adj R-sq	0.8788
C.V.	-2.08353		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	740.895338	420.27026706	1.763	0.3285
PROINT	1	-4.326249	1.09889322	-3.937	0.1584

APPENDIX C
CHAPTER VI

Figure 1. Plot of protein gain (PROGAIN) and fat gain (FATGAIN)



Dependent Variable: PROGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	1298.94381	1298.94381	3.843	0.0634
Error	21	7097.96401	337.99829		
C Total	22	8396.90782			

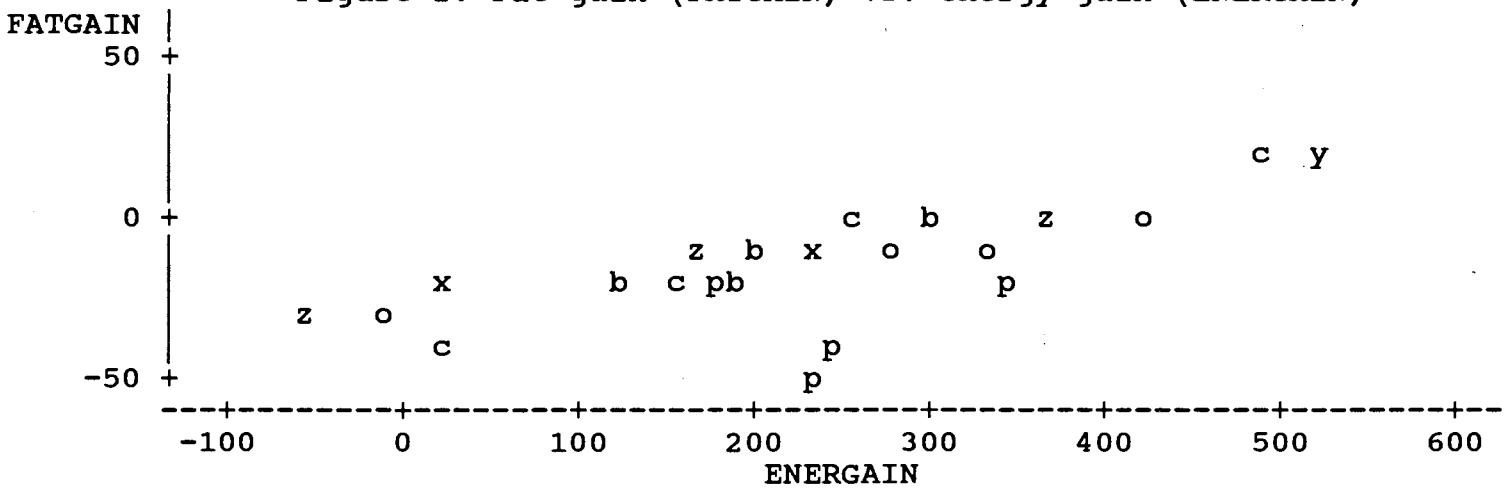
Root MSE	18.38473	R-square	0.1547
Dep Mean	63.49580	Adj R-sq	0.1144
C.V.	28.95425		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	56.699402	5.16864960	10.970	0.0001
FATGAIN	1	-0.459969	0.23463378	-1.960	0.0634

Statement: Protein gain is negatively correlated with fat gain

Figure 2. Fat gain (FATGAIN) vs. energy gain (ENERGAIN)



Dependent Variable: FATGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	3243.56583	3243.56583	23.521	0.0001
Error	21	2895.94002	137.90191		
C Total	22	6139.50585			

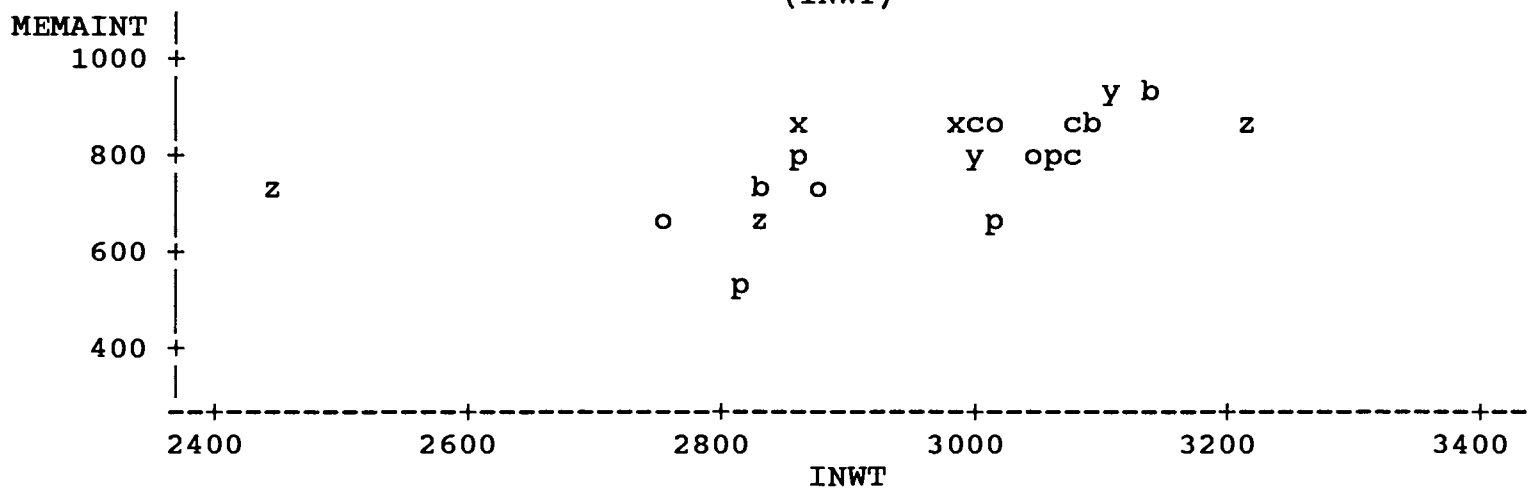
Root MSE	11.74316	R-square	0.5283
Dep Mean	-14.77577	Adj R-sq	0.5058
C.V.	-79.47581		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	-33.293522	4.53592447	-7.340	0.0001
ENERGAIN	1	0.081166	0.01673595	4.850	0.0001

Statement: As depot fat loss is lowered bird energy gain is increased.

Figure 3. Metabolizable energy required for maintenance (MEMAINT) vs. initial weight (INWT)



Dependent Variable: MEMAINT

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	104929.55592	104929.55592	17.560	0.0004
Error	21	125486.94267	5975.56870		
C Total	22	230416.49859			

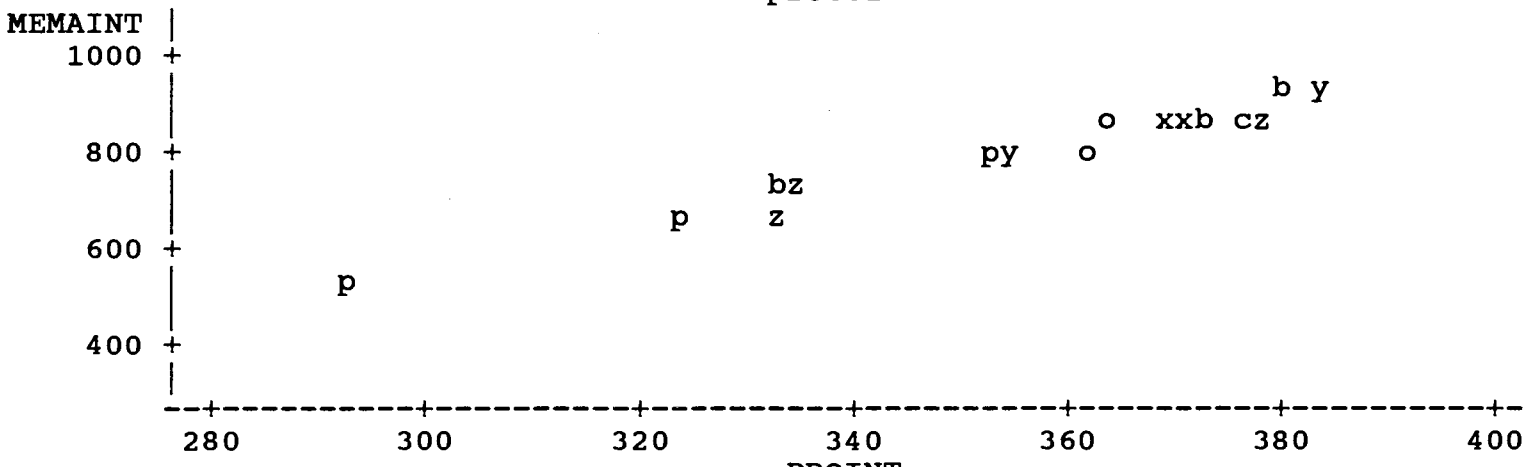
Root MSE	77.30180	R-square	0.4554
Dep Mean	795.28149	Adj R-sq	0.4295
C.V.	9.72006		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	-421.126309	290.72902730	-1.449	0.1622
INWT	1	0.410665	0.09800054	4.190	0.0004

Statement: As weight is increased metabolizable energy required for maintenance is also increased.

Figure 4. Metabolizable energy required for maintenance (memaint) vs. initial bird protein



Dependent Variable: MEMAINT

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	230416.49859	230416.49859	.	.
Error	21	0.00000	0.00000		
C Total	22	230416.49859			

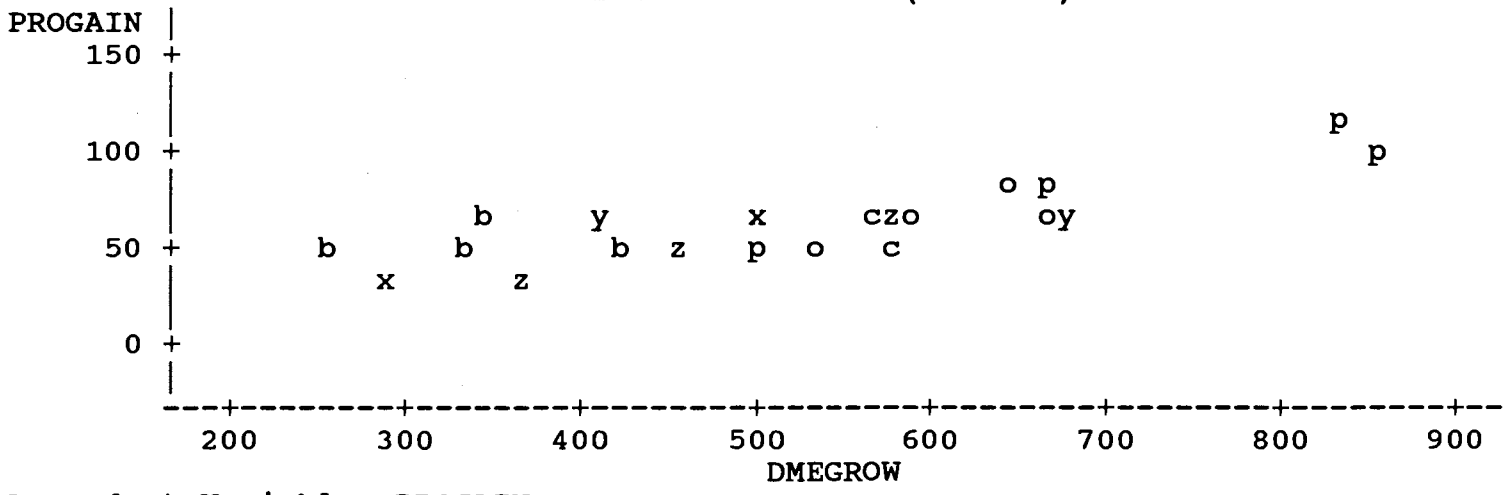
Root MSE	0.00000	R-square	1.0000
Dep Mean	795.28149	Adj R-sq	1.0000
C.V.	0.00000		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	-740.900000	0.00000000	.	.
PROINT	1	4.326200	0.00000000	.	.

Statement: Predictive equation relating metabolizable energy required for maintenance and initial bird protein.

Figure 5. Protein gain (PROGAIN vs dietary energy consumption minus energy required for maintenance (DMEGROW)



Dependent Variable: PROGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	5288.79106	5288.79106	35.734	0.0001
Error	21	3108.11676	148.00556		
C Total	22	8396.90782			

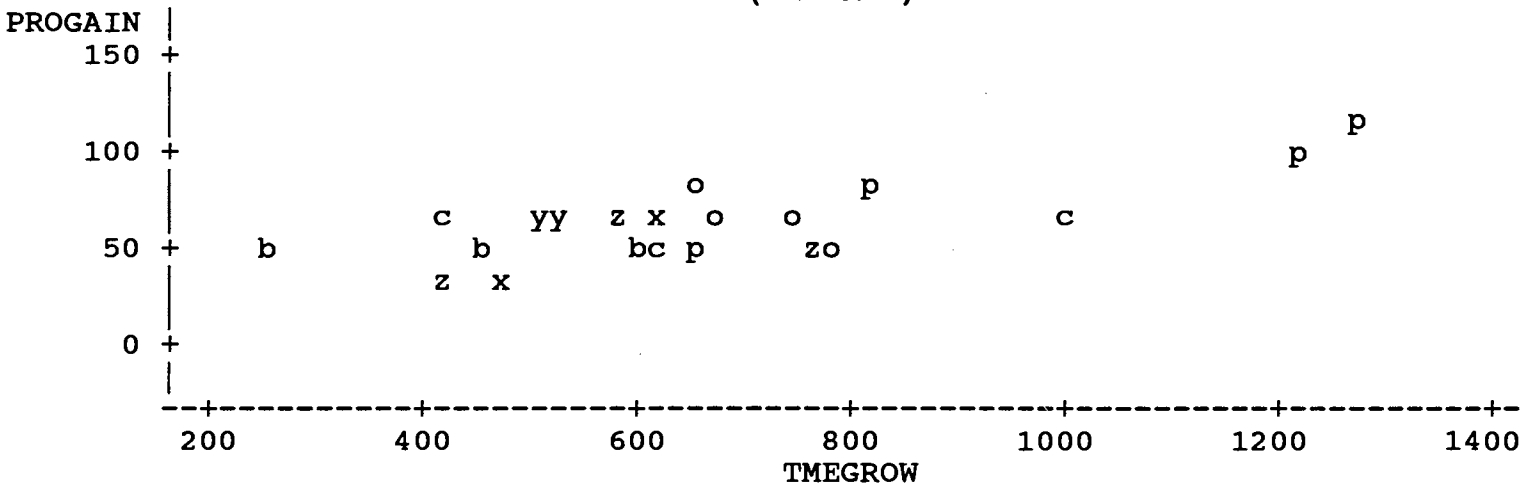
Root MSE	12.16575	R-square	0.6298
Dep Mean	63.49580	Adj R-sq	0.6122
C.V.	19.15994		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	11.280730	9.09577097	1.240	0.2286
DMEGROW	1	0.098217	0.01643039	5.978	0.0001

Statement: There does not appear to be a relationship between protein gain and DMEGROW. This likely occurs because the birds are utilizing body lipids as an energy source.

Figure 6. Protein gain (PROGAIN) vs DMEGROW plus energy from depot fat loss (TMEGROW)



Dependent Variable: PROGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	4890.83873	4890.83873	29.294	0.0001
Error	21	3506.06910	166.95567		
C Total	22	8396.90782			

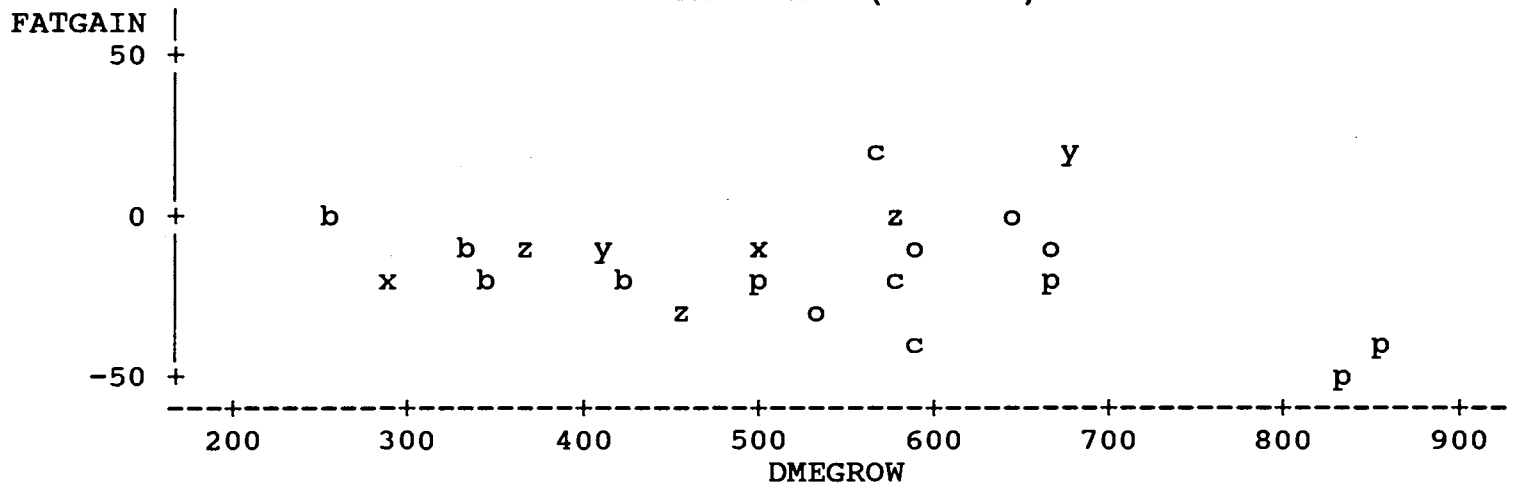
Root MSE	12.92113	R-square	0.5825
Dep Mean	63.49580	Adj R-sq	0.5626
C.V.	20.34959		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	22.563623	8.02823417	2.811	0.0105
TMEGROW	1	0.061397	0.01134375	5.412	0.0001

Statement: The TMEGROW term is somewhat more responsive to protein accretion than merely using metabolizable energy consumption, since it includes a correction for maintanence and lipid mobilization.

Figure 7. Fat gain (FATGAIN) vs dietary energy consumption minus energy required for maintenance (DMEGROW)



Dependent Variable: FATGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	304.77435	304.77435	1.097	0.3068
Error	21	5834.73150	277.84436		
C Total	22	6139.50585			

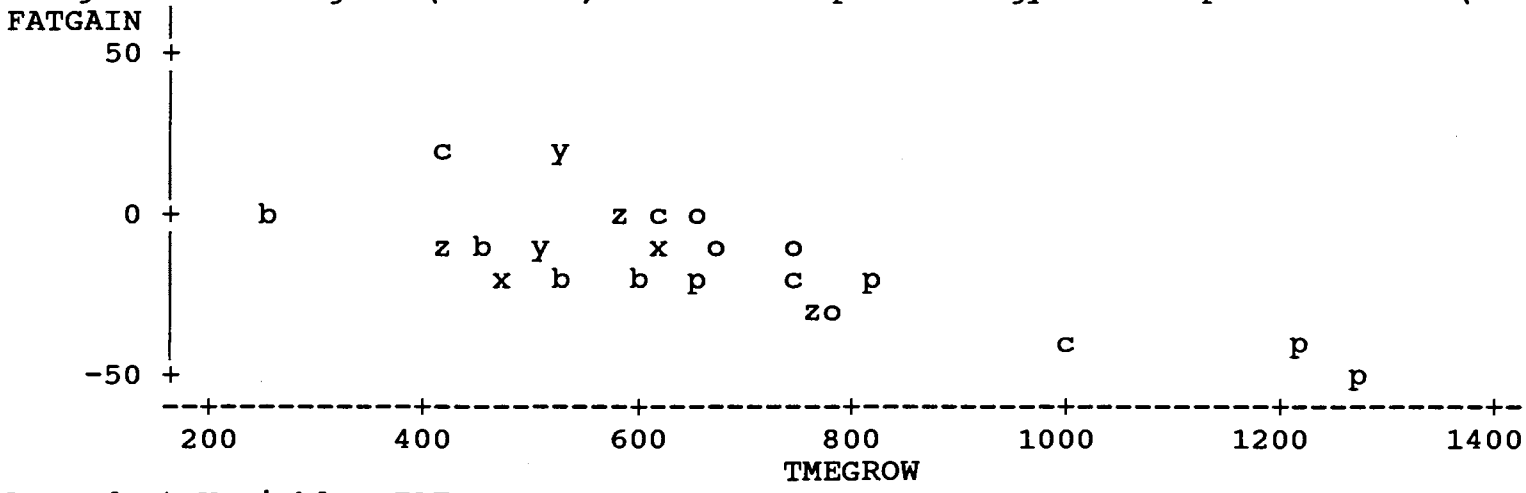
Root MSE	16.66866	R-square	0.0496
Dep Mean	-14.77577	Adj R-sq	0.0044
C.V.	-112.81079		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	-2.241276	12.46238866	-0.180	0.8590
DMEGROW	1	-0.023578	0.02251177	-1.047	0.3068

Statement: Similar to the protein gain vs DMEGROW relationship, the fat gain and DMEGROW do not appear to be related.

Figure 8. Fat gain (FATGAIN) vs DMEGROW plus energy from depot fat loss (TMEGROW)



Dependent Variable: FATGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	3673.94931	3673.94931	31.292	0.0001
Error	21	2465.55654	117.40745		
C Total	22	6139.50585			

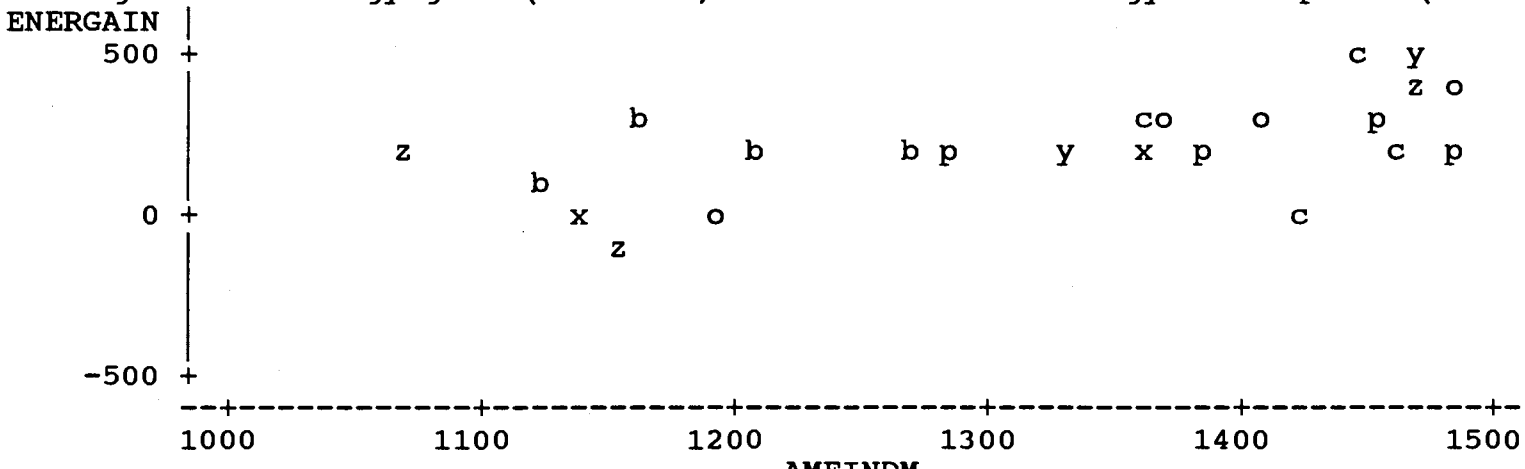
Root MSE	10.83547	R-square	0.5984
Dep Mean	-14.77577	Adj R-sq	0.5793
C.V.	-73.33270		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	20.700643	6.73235920	3.075	0.0057
TMEGROW	1	-0.053214	0.00951270	-5.594	0.0001

Statement: Despite fat gain not related to DMEGROW, fat gain appears to be negatively correlated with TMEGROW.

Figure 9. Energy gain (ENERGAIN) vs metabolizable enrgy consumption (AMEINDM)



Dependent Variable: ENERGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	194313.17292	194313.17292	13.692	0.0013
Error	21	298032.02465	14192.00117		
C Total	22	492345.19758			

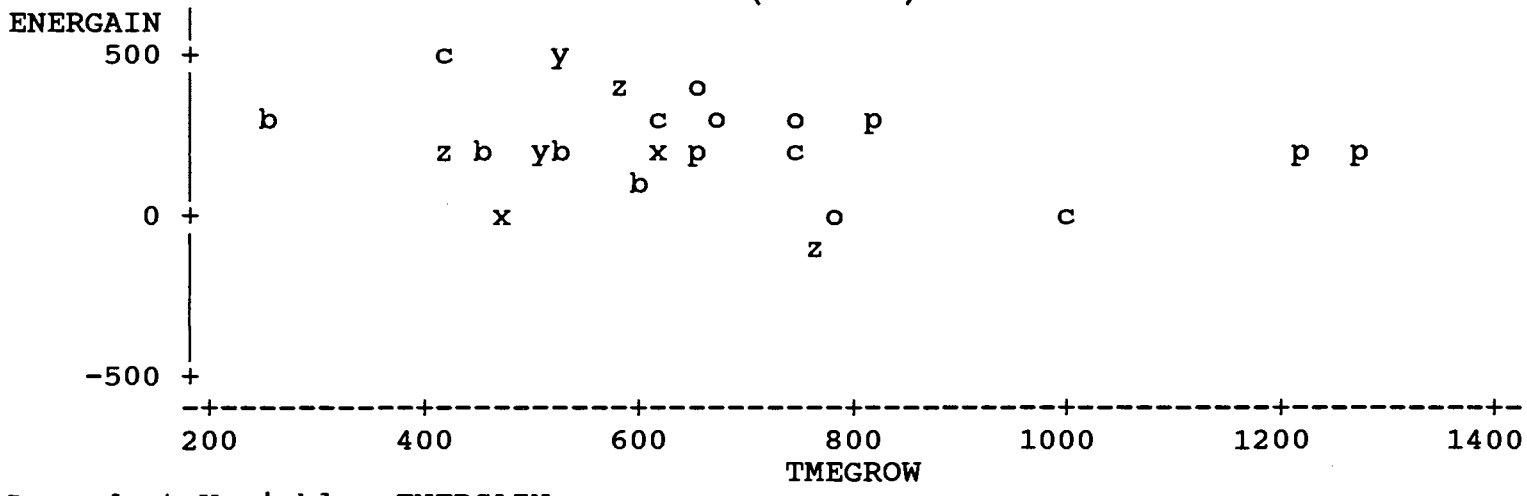
Root MSE	119.13019	R-square	0.3947
Dep Mean	228.14541	Adj R-sq	0.3658
C.V.	52.21678		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	-695.971374	250.97773821	-2.773	0.0114
AMEINDM	1	0.696442	0.18821573	3.700	0.0013

Statement: Due to maintentance variability and varying utilization of depot lipids for protein synthesis energy gain was poorly related with metabolizable energy intake.

Figure 10. Energy gain (ENERGAIN) vs DMEGROW plus energy from depot fat loss (TMEGROW)



Dependent Variable: ENERGAIN

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	23709.32288	23709.32288	1.062	0.3144
Error	21	468635.87469	22315.99403		
C Total	22	492345.19758			

Root MSE	149.38539	R-square	0.0482
Dep Mean	228.14541	Adj R-sq	0.0028
C.V.	65.47815		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	318.267803	92.81700741	3.429	0.0025
TMEGROW	1	-0.135181	0.13114873	-1.031	0.3144

Statement: Adjusting metabolizable energy consumption for maintenance and fat mobilization yields reponse to energy gain

CHAPTER VII

Energetic Efficiency of Isolated-Soy Protein and Amino Acids, Starch, and Starch and Urea for Tissue Accretion in Broilers fed Isocaloric Rations[1]

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ABSTRACT An experiment was conducted to quantify efficiency of ME use from isolated soy protein, corn starch, and crystalline amino acids for tissue accretion. Birds were limit fed the basal and basal plus energy supplement during day 24 to 45. No differences ($\underline{P} > .1$) were observed in live weight, protein gain, and feed efficiency between the birds consuming basal diet alone and those receiving the energy supplements. Oxygen consumption and heat production averaged 1013 L and 5000 kcal, respectively, for the 21-d trial. Birds not supplemented with energy had lower ($\underline{P} < .05$) AME_n consumption, CO_2 production, fat and carbon gain, and final body dry matter percentage than those receiving the energy supplements. Total body energy ranged from 4 to 15% higher for the isolated soy-protein, corn starch energy supplements. Despite similar ($\underline{P} > .1$) AME_n consumption among the energy supplemented groups, energy and fat gain were impacted significantly. Energy gain was 10% lower ($\underline{P} < .05$) for the isolated soy-protein compared to corn starch energy supplement. Fat gain per MWT ranged from an average of 8.2 to 10.2 g for the isolated soy-protein and corn starch-supplemented groups, respectively. Estimated energy and fat gain due to supplement per MWT was greater ($\underline{P} < .05$) for the starch supplement compared to other energy supplements. Total calorie gained from supplemented diets was highest (28 kcal/bird) for the corn starch group compared to -.5 kcal/bird for those supplemented with isolated soy-protein. Data presented suggest that high protein diets promote leaner broiler carcasses primarily because net energy efficiency for lipogenesis is lower for protein than for starch.

(*Key words:* broiler, energetic efficiency, net energy, thermobalance, carcass

INTRODUCTION

Deeper understanding of energy metabolism should improve precision of diet formulation and enhance profit of production enterprises. The ME_n system is the current standard for ration formulation (NRC, 1994). However, by definition, the ME_n system does not account quantitatively for heat increment. Any heat increment change alters ME_n utilization and may alter the cellular energy/nutrient ratios; an increase in this ratio should increase fat accretion.

Carcass fat is influenced by several factors. The factor investigated most widely is the dietary calorie to protein ratio (Summers and Leeson, 1979; Jones and Wiseman, 1985; Bartov, 1987; Belay and Teeter, 1992). Decreasing the dietary calorie/protein ratio increases broiler fat pad weight and carcass fat. Sonaiya et al. (1990) suggested that carcass fat is reduced by reducing the caloric density of the diet and by increasing its protein content. Belay and Teeter (1991) also observed that increasing dietary energy and/or narrowing the calorie/protein ratio impacts bird performance. Lowering dietary crude protein numerically increased survivability in a thermoneutral environment (4.4%) and significantly enhanced survival (10.8%) in a cycling heat distressed environment.

Mittelstaedt and Teeter (submitted), using precision fed birds, evaluated the ME_n retention from gelatin, starch, and corn oil. Metabolizable energy utilization for energy (fat) gain varied by over 150% across these nutrient classes. Efficiency of ingredient TME for carcass energy deposition averaged 50, 39 and 20%, respectively for corn oil, starch and gelatin. Therefore, one might speculate, that the increased carcass leanness obtained by feeding high protein rations could be due to reduction in the cellular energy supply.

Whether the reduced ME_n utilization of protein for fat gain should be attributed to protein per se or to specific amino acids has not been addressed. This problem is perpetuated by the apparent lack of thermodynamic data regarding energy changes accompanying substrate metabolism to various tissues. Inefficient use of ME_n with its increased heat production also has implication for heat stress as Wiernusz and Teeter (1993) reported; regressing heat production on feed intake (0, 3, 6, 9% body weight$^{.66}$; MWT per day) yielded .48 kJ/g feed per unit MWT for birds housed at 24 C and .58 kJ/g feed per unit MWT for birds exposed to heat distress (35 C). Although some of the added heat production is related to the cost of heat dissipation, efficiency of various substrates for tissue accretion would enable one to define heat stress risk and heat load more precisely.

The objective of this study reported herein was to quantify and contrast equalized AME_n consumption of isolated soy-protein, corn starch, and crystalline amino acids on bird utilization of ME_n, serum metabolites, and carcass protein and fat accretion.

MATERIALS AND METHODS

The study was conducted to evaluate the energetic efficiency of isolated soy-protein, amino acids, starch, and starch plus urea. Ingredient AME_n was previously estimated to be 3.95, 4.25, and 3.62 kcal/g DM for starch, isolated soy-protein, and amino acids, respectively. Prior to, initiating this experiment, the Cobb x Cobb male broilers used were fed a 23% crude protein corn-soybean based ration and reared through 21-d posthatching. Following a 7-d adaptation period to the respiratory chambers (51 x 34 x 41 cm) as described by Wiernusz and Teeter (1993), the 60 birds were fasted for 24 h and the experiment was initiated. Twenty-four birds, selected at random from the fasted

population, were sacrificed by cervical dislocation, placed in freezer bags, and frozen for later analyses. The remaining 36 birds were fed restricted amounts of the test diets; hence, each bird received the same quantity of basal/MWT/d during the 21-d experiment. The AME_n for corn starch and isolated soy-protein was estimated previously (Wiernusz and Teeter, submitted) to be 3.95 and 4.25, respectively. The estimated ME_n of the amino acid mixture was calculated from NRC (1994) ME_n estimates. Upon completion of the feeding period, birds again were fasted (24 h) to complete the excreta collection period. Water was supplied for *ad libitum* consumption throughout the experiment.

Six experimental diets were formed by adding to a 26% CP basal ration (Table 1), which supplied 150% of the birds essential nutrient needs, either 1.) basal alone; 2.) basal plus 15% corn starch; 3.) basal plus isolated soy protein to equal AME_n provided by 15% corn starch; 4.) basal plus crystalline amino acids (Table 2) to equal analyzed amino acids in isolated-soy protein; 5.) basal plus corn starch to equal the calculated AME_n provided by amino acids (assumed 100% digestibility; 6.) basal plus corn starch to equal the calculated AME_n provided by amino acids plus urea to equal the nitrogen in the amino acids.

Throughout the 21-d experimental period, birds were fed treatment diets. Production of O_2 (L) and CO_2 (L) by individual birds was monitored as described by Wiernusz and Teeter (1993). On Day 42, birds were individually weighted, sacrificed by cervical dislocation and placed in freezer bags at -20C until analyzed. Total excreta were collected biweekly, dried in a 60 C oven, air equilibrated and weighed (Dale and Fuller, 1982). McDonald and Teeter (submitted) estimated the NH_3 loss from excreta to the

atmosphere to be negligible and was therefore not estimated here. Excreta and feed samples were ground to pass through a screen with 1 mm holes, and representative sub-samples were retained and assayed for water, nitrogen and ash contents (AOAC, 1990). Feed and excreta were assayed for gross energy (calorie/g) and carbon (%) by combustion in a Model 1261 Parr adiabatic bomb calorimeter as described by Harjo and Teeter (1994). The AME and AME_n value of each diet was calculated (Fisher and Wilson, 1974) and balances for nitrogen, carbon and energy were calculated by difference between consumption and loss. The calculation for carbon balance accounts for respiratory carbon loss by converting the CO_2 (L) production to g of carbon using: [(CO_2 (L) x molecular weight of CO_2 (44)]/(22.4 L/mole CO_2 gas x percent carbon in CO_2 (27.27). Protein and fat accretion (g) were estimated using prediction equations: protein gain = (CO_2 production x .146) + AME intake x .822) - (AME_n intake x .840) and fat gain = carbon gain - (protein gain x .53)/.77. Tissue energy accretion as protein and fat were calculated by using 5.72 and 9.31 kcal per g, respectively (McDonald and Teeter, 1994). Efficiency of ME_n and net energy utilization were determined by dividing the total tissue energy gain by total ME_n and gross energy consumed, respectively.

Serum Blood chemistries. Blood samples were collected from the ulnaris vein using a 3 mL syringe. Serum variables including glucose, triglycerides, total protein, creatinine, albumin, Mg, blood urea nitrogen, uric acid, and lactate were measured as described by Cason and Teeter (1994).

Statistical Analysis

Chamber oxygen, CO_2, were regressed against time, time squared and time cubed such that polynomial equations describing the data were formed. Quantitative estimates for each variable were made by integrating variable functions over specific time intervals and adjusting for the control chamber as appropriate. Data were analyzed by analysis of variance using the General Linear Models procedure of SAS (SAS Institute, 1985) to determine the effects of diet and the effects on body weight gain, nitrogen balance, carbon balance, O_2 consumption, CO_2 production, respiratory carbon loss, protein and fat accretion, heat production, energetic efficiency, and serum blood chemistries. When a significant F statistic was noted, treatment means were separated using the SAS® probability of difference procedure. Standard statistical procedures (Steel and Torrie, 1960) were used to obtain correlation coefficients as well as linear regression equations.

RESULTS AND DISCUSSION

Results of the initial slaughter group are presented in Table 3. The determined percent dry matter, protein, fat, ash, nitrogen, carbon and gross energy were 29.45, 62.83, 28.39, 9.09, 9.39, 55.98, and 6.14 kcal/g dm, respectively. The AME_n determined during the experiment value (kcal/kg dm) for the isolated soy-protein supplement was slightly lower ($P < .05$) than for other test diets (Table 4; 3.45 vs 3.51).

Bird growth rate and feed efficiency (Table 4) were comparable to NRC (1994); 21 to 45-d growth estimates were 1396 g gain and .6 gain/feed. No differences ($P > .1$) were observed for live weight, protein gain, and feed efficiency between the birds

consuming basal alone and the energy supplements. Oxygen consumption and heat production averaged 1013 L and 5000 kcal, respectively, for the 21-d trial as shown in Tables 4, 5, and 6. Birds consuming the basal diet alone had lower ($\underline{P} < .05$) AME_n consumption (7797 vs 8171 kcal/kg diet), CO_2 production (872 vs 907 L), fat (121 vs 148 g dm) and carbon (243 vs 260 g) gain, and final body dry matter percentage (30.7 vs 31.7) compared to the energy-supplemented groups. Total body energy ranged from 4 to 15% higher for the isolated soy-protein, corn starch energy supplements compared to birds fed the basal diet alone.

No differences ($\underline{P} > .1$) were detected for dry body weight, protein, and ash gain which averaged 454, 292, and 34 g, respectively, (Table 5). Correlation coefficients between bird weight gain with protein, fat, ash and energy were .83, .81, .74, and .69, respectively. The efficiency of AME_n utilization (NE/AME_n) averaged 38% which falls within the range of other reported values of DeGroote (1974; 35.5%), Jackson et el., (1982; 29%), Macleod (1990; 34-55%), and McDonald and Teeter (submitted; 36%). Differences in efficiencies may be due to breed, gender, and amounts of feed consumed.

The AME_n consumption was lower ($P < .05$) for birds supplemented the isolated soy-protein supplement compared to other energy supplements. No differences ($\underline{P} > .1$) were observed between dietary treatments for O_2 use and heat production which averaged 1013 L and 5000 kcal, respectively, for the 21-d trial. Carbon dioxide production increased ($\underline{P} < .05$) in birds supplemented the corn starch compared to isolated soy-protein. Respiratory quotient increased ($\underline{P} < .05$) with all supplements except isolated soy-protein and amino acids simulated soy diet.

Despite equal AME_n consumption between the isolated soy-protein and corn starch energy supplements (Table 8), body fat gain estimated by comparative slaughter was greater ($P < .05$) with starch (153 vs 132 g). Final bird dry matter was positively correlated ($R^2 = .84$) with fat gain and also was greater ($P < .05$) in birds supplemented with corn starch vs isolated soy-protein. Similar to fat gain, birds consuming the corn starch had increased ($P < .05$) energy and carbon gain compared to the other dietary treatments at 3133 kcal/g dm and 278.2 g, respectively (Table 6). No differences ($P > .1$) were observed in carbon, nitrogen, and energy gain between the other dietary treatments, except that birds fed corn starch to equal AME_n of crystalline amino acids had a greater ($P < .05$) energy gain then birds fed basal alone.

Dietary treatment effects on nitrogen, carbon and energy balance estimated by intake, gain, and excretion are presented in Table 7. Basal, AME_n, and ingredient intake along with gain per MWT are shown in Table 8. Despite similar ($P > .1$) AME_n consumption among the energy supplemented groups, bird energy and fat gain were significantly different. Total body energy ranged from 4 to 15% higher for the isolated soy-protein, corn starch energy supplements compared to basal diet. Energy gain was 10% lower ($P < .05$) for the isolated soy-protein compared to corn starch supplement. Fat gain was impacted ($P < .05$) by substrate and averaged 8.2 to 10.2 g dm for the isolated soy-protein and corn starch supplemental groups, respectively.

Estimated gain due to basal ration was similar among the energy supplement groups due to nearly identical AME_n consumption. Energy and fat gain due to supplement per MWT was greater ($P < .05$) for the starch supplement compared to other energy

supplements (Table 9). Total calorie gain was highest at 28 kcal/bird for the corn starch group compared to -4 kcal/bird for the isolated soy-protein groups. Protein and dry matter gain were similar ($P > .1$) among energy supplements.

Differences ($P < .05$) were noted for serum blood chemistries associated with diet and feeding (Table 10). Birds fasted for 24 h had lower ($P < .05$) glucose, triglycerides, total protein, creatinine, albumin, Mg, blood urea nitrogen, uric acid, and lactate compared to fed birds as summarized in Table 11.

In summary, the potential for starch and crystalline amino acid supplementation to enhance broiler production efficiency by improving cellular energy supply was examined. The hypothesis is based on the fact that the metabolizable energy system does not account for heat production and the premise that heat increment is higher for amino acids than for other substrates. High protein diets promoted leaner broiler carcasses primarily as a result of a lower net energy efficiency for lipogenesis. Rations formulated using the ME_n system do not necessarily correlate with bird energy retention and may produce different calorie/nutrient ratios. A greater heat increment for protein ME_n calories vs. those from starch and fat make low protein rations lipogenic. Increased understanding and application of cellular energy-nutrient relationships will be required to produce leaner birds. For maximum protein deposition with minimal fat accretion, an energy requirement scheme accounting for the variation in substrate-mediated heat production is needed.

In conclusion, an overestimation of nitrogen retention was observed using the difference technique (nitrogen intake-nitrogen excretion) compared to comparative slaughter method (final carcass nitrogen-initial carcass nitrogen). It appears that excreta

nitrogen is lost when dried in a 60 C oven (Dale and Fuller, 1982) which would impact AME_n values. Dale and Fuller (1982) measured nitrogen loss during freeze and oven drying and indicated no differences between the two techniques, but did not compare fresh or raw samples to samples that were dried. In retrospect, birds in this study were fed unequal AME_n due to differences in nitrogen concentrations between the supplements. This would indicate birds consuming the isolated soy-protein supplement received less AME_n thence calculated. Future studies need to address nitrogen loss when drying, comparing fresh verses dried samples may be an option, and adjustments of ingredient AME_n values may need to addressed.

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TABLE 1. Composition of basal diet

Ingredients and analysis	Percentage of diet
Ground corn (8.8% CP)	42.44
Soybean meal (48.5% CP)	45.85
Animal fat	6.50
Dicalcium phosphate (22% Ca; 18.5% P)	2.00
Limestone (38% Ca)	1.55
$NaHCO_3$	.50
NaCl	.36
Vitamin mix[2]	.45
DL-methionine, 99%	.25
Trace mineral mix[3]	.10
Total	100.00
Calculated analysis	
ME, kcal/kg	3139.00
CP	26.17
Ca	1.20
P, available	.51
Na	.25
K	1.00
Cl	.26

[1]The vitamin mix contained the following per kilogram of diet: vitamin A, 14,109 IU (retinyl acetate); cholicalciferol, 5,291 IU; vitamin E, 47.6 IU (dl-α-tocopheryl acetate); vitamin B_{12}, .014 mg; riboflavin, 8.82 mg; niacin, 26.5 mg; d-pantothenic acid, 28.2 mg; choline, 705.5 mg; menadione, 1.16 mg; folic acid, 1.176 mg; pyridoxine, 3.52 mg; thiamin, 3.52 mg; d-biotin, .176 mg.

[2]The mineral mix contained the following per kilogram of diet: Ca, 160 mg; Zn, 100 mg; Mn, 120 mg; Fe, 75 mg; Cu, 10 mg; I, 2.5 mg, Se, .15 mg.

Table 2. Amino acid composition, crude protein and dry matter percentage of corn, soybean meal, and isolated soy-protein

Amino Acid	Corn (W/W%)	Soybean meal (W/W%)	Isolated soy-protein (W/W%)
Taurine	0.14	0.06	0.00
Hydroxyproline	0.01	0.00	0.00
Aspartic Acid	0.54	5.33	9.23
Threonine	0.29	1.82	2.99
Serine	0.35	2.18	3.57
Glutamic Acid	1.49	8.19	14.52
Proline	0.72	2.40	4.25
Lantionine	0.00	0.00	0.00
Glycine	0.32	1.96	3.36
Alanine	0.59	2.05	3.65
Cystine	0.21	0.76	0.96
Valine	0.40	2.19	3.97
Methionine	0.17	0.69	2.03
Isoleucine	0.30	2.12	3.92
Leucine	1.01	3.65	6.59
Tyrosine	0.25	1.68	3.11
Phenylalanine	0.41	2.39	4.37
Hydroxylysine	0.00	0.02	0.04
Histidine	0.25	1.24	2.09
Ornithine	0.00	0.02	0.03
Lysine	0.26	2.91	5.07
Arginine	0.40	3.39	6.15
Tryptophan	0.07	0.72	1.12
Total	8.18	45.77	81.02
Crude Protein	8.54	47.68	84.49
Dry Matter	86.84	89.55	92.06

Table 3. Initial body wieght and composition of 24-d old male broilers

Initial body weight, g	762.70
Initial body composition, dm	
Dry weight, g	224.49
Protein, g	140.98
Fat, g	63.81
Ash, g	20.41
Carbon, g	125.69
Nitrogen, g	21.07
Energy, kcal	1379.7

Table 4. Dietary treatment effects on broiler feed, AME, and AME_n consumption along with live performance and oxygen, carbon dioxide, and heat production

	Diet					
Variable	Basal	Isolated-soy	Starch=AME_n of isolated soy	Starch=AME_n of amino acids	Amino acid=aa of isolated soy	Starch+urea=AME_n and N of AA
AME, kcal/g, dm	3.70^{b}	3.70^{b}	3.71^{ab}	3.69^{ab}	3.77^{a}	3.73^{ab}
AME_n, kcal/g, dm	3.53	3.45	3.54	3.52	3.53	3.47
Gain/feed	$.63^{a}$	$.62^{ab}$	$.58^{b}$	$.60^{ab}$	$.59^{ab}$	$.58^{b}$
Live performance						
Initial weight, g	756.83	754.60	770.33	748.33	763.20	759.00
Final weight, g	2144.17	2177.80	2180.83	2124.83	2146.20	2157.33
Live weight gain, g	1387.33	1423.20	1410.5	1376.5	1383.00	1398.33
Feed consumption						
Intake, g	2210.08^{c}	2304.90^{abc}	2405.23^{a}	2289.53^{bc}	2317.04^{abc}	2372.21^{ab}
AME intake, kcal[1]	8174.0^{c}	8529.3^{abc}	8896.0^{a}	8454.0^{bc}	8751.6^{ab}	8840.4^{ab}
AME_n intake, kcal[2]	7797.2^{c}	7946.6^{bc}	8487.8^{a}	8070.4^{abc}	8178.5^{abc}	8232.1^{ab}
Gas variables						
O2 consumption, L	995.94	1005.08	1028.63	1009.22	1024.67	1013.76
CO2 production, L	872.54^{b}	871.59^{b}	935.49^{a}	908.40^{ab}	911.52^{ab}	913.87^{ab}
Heat production, kcal	4898.31	4932.50	5100.24	4992.68	5056.16	5016.79
Respiratory Quotient	$.88^{bc}$	$.87^{c}$	$.91^{a}$	$.90^{a}$	$.89^{ab}$	$.90^{a}$

^{abc}Means within a row with no common superscript differ significantly ($P < .05$)

[1]AME (dm) = (energy intake - fecal energy) / dry matter intake

[2]AME_n (dm) = (energy intake - fecal energy) - (8.22 x nitrogen gain)/ dry matter intake

Table 5. Dietary treatment effects on broiler comparative slaughter estimates of body weight, protein, fat, and ash gains during the 24 to 45-d experiment as well as bird dry matter

	Diet					
Variable	Basal	Isolated-soy	Starch=AME_n of isolated soy	Starch=AME_n of amino acids	Amino acid=aa of isolated soy	Starch+urea=AME_n and N of AA
Body weight, g dm[1]						
Initial	222.91	222.25	226.88	220.40	224.78	223.54
Final	658.25	676.11	706.00	672.73	677.70	678.10
Gain[5]	435.34	453.86	479.11	452.32	452.92	454.56
Body protein, g dm[2]						
Initial	140.05	139.63	142.54	138.47	141.22	140.44
Final	436.62	440.99	424.12	431.35	435.87	432.56
Gain	296.58	301.36	281.58	292.88	294.64	292.12
Body fat, g dm[3]						
Initial	63.29	63.10	64.42	62.58	63.82	63.47
Final	184.53	195.68	230.93	209.72	216.67	208.72
Gain	121.24[b]	132.58[b]	166.51[a]	147.14[b]	152.84[ab]	145.25[b]
Body ash, g dm[4]						
Initial	20.26	20.20	20.62	20.04	20.43	20.32
Final	55.88	54.36	55.10	54.30	56.02	54.78
Gain	35.62	34.15	34.47	34.26	30.86	34.45
Initial body, dm	29.45	29.45	29.45	29.45	29.45	29.45
Final body, dm	30.74[b]	31.08[ab]	32.41[a]	31.72[ab]	31.68[ab]	31.43[ab]

[abc]Means within a row with no common superscript differ significantly ($P < .05$)

[1]Initial weight = initial bird live weight x .2945

[2]Initial protein = initial bird weight (g, dm) x .6283
Final protein = final bird weight (g, dm) x nitrogen in bird (g) x 6.69

[3]Initial fat = initial bird weight (g, dm) x .2839
Final fat = final bird weight (g, dm) x percent fat

[4]Initial ash = initial bird weight (g, dm) x .0909
Final ash = final bird weight (g, dm) x percent ash

[5]Gain = final-initial

Table 6. Dietary treatment effects on broiler comparative slaughter estimates of body energy, nitrogen, and carbon gains during the 24 to 45-d experiment

	Diet					
Variable	Basal	Isolated-soy	Starch=AME_n of isolated soy	Starch=AME_n of amino acids	Amino acid=aa of isolated soy	Starch+urea=AME_n and N of AA
Body energy, kcal dm[1]						
Initial	1369.78	1365.74	1394.22	1354.40	1381.31	1373.70
Final	4062.58[b]	4183.21[b]	4527.58[a]	4262.70[b]	4282.40[b]	4270.60[b]
Gain[4]	2692.79[c]	2817.47[bc]	3133.34[a]	2908.29[b]	2901.09[bc]	2896.89[bc]
Nitrogen, g dm[2]						
Initial	20.93	20.87	21.31	20.70	21.11	20.99
Final	65.26	65.92	63.40	64.48	65.15	64.66
Gain	44.33	45.05	42.09	43.78	42.14	43.66
Carbon, g dm[3]						
Initial	124.78	124.42	127.01	123.38	125.83	125.14
Final	368.55[b]	376.89[b]	405.22[a]	383.02[b]	381.74[b]	383.45[b]
Gain	243.76[b]	252.47[b]	278.21[a]	259.64[b]	255.90[b]	258.31[b]

[ab]Means within a row with no common superscript differ significantly ($P < .05$)
[1]Initial energy = initial bird weight(g, dm) x 6.14 kcal/g
Final energy = final bird weight (g, dm) x gross energy (kcal/g)
[2]Initial nitrogen = initial bird weight (g, dm) x .0939
Final nitrogen = final bird weight (g, dm) x percent nitrogen
[3]Initial carbon = initial bird weight (g,dm) x .5598
Final carbon = final bird weight (g, dm) x percent carbon
[4]Gain = final-initial

Table 7. Dietary treatment effects on broiler nitrogen, carbon, and energy balance estimated by a summation of intake and excretion

	Diet					
Variable	Basal	Isolated-soy	Starch=AME_n of isolated soy	Starch=AME_n of amino acids	Amino acid=aa of isolated soy	Starch+urea=AME_n and N of AA
Nitrogen, g dm						
Intake, g	90.17^{b}	115.94^{a}	91.40^{b}	90.44^{b}	113.77^{a}	117.66^{a}
Excreta, g	45.84^{d}	70.89^{ab}	49.31^{c}	46.66^{cd}	69.68^{b}	74.00^{a}
Gain, g	44.33	45.05	42.09	43.78	42.14	43.66
Carbon, g dm						
Intake, g	1052.49^{c}	1084.76^{abc}	1135.74^{a}	1072.24^{bc}	1113.54^{ab}	1118.01^{ab}
Excreta, g	341.29^{bc}	365.36^{ab}	356.37^{ab}	325.95^{c}	369.32^{a}	370.12^{a}
Respiratory, g	467.44^{b}	466.93^{b}	501.16^{a}	486.65^{ab}	488.32^{ab}	489.58^{ab}
Gain, g	243.76^{b}	252.47^{b}	278.21^{a}	259.64^{b}	255.90^{b}	258.31^{b}
Energy						
Intake, kcal	10631.9^{c}	11089.6^{abc}	11456.2^{a}	10820.00^{bc}	11214.5^{ab}	11183.3^{ab}
Excreta, kcal	3040.83^{bc}	3339.65^{a}	3222.67^{ab}	2919.01^{c}	3257.22^{ab}	3269.60^{ab}
Respiratory, kcal	4898.31	4932.50	5100.24	4992.68	5056.16	5016.79
Gain, kcal	2692.79^{c}	2817.47^{bc}	3133.34^{a}	2908.29^{b}	2901.09^{bc}	2896.89^{bc}

^{abc}Means within a row with no common superscript differ significantly ($P < .05$)

Table 8. Dietary treatment effects on broiler basal, AME_n, and ingredient consumption, energy, fat, protein, and dry matter gains estimated per unit body weight[66] (MWT)

Variable	Diet					
	Basal	Isolated-soy	Starch=AME_n of isolated soy	Starch=AME_n of amino acids	Amino acid=aa of isolated soy	Starch+urea=AME_n and N of AA
Intake/MWT						
Basal intake,g dm	136.6	135.84	137.98	135.68	137.23	136.80
AMEn Intake, kcal/g dm	481.85	472.47	488.10	478.23	484.52	474.68
Ingredient intake, g dm	0.0	11.16	12.49	9.39	10.31	12.07
Gain/MWT						
Energy, kcal	166.39^{c}	174.41^{bc}	192.15^{a}	180.48^{ab}	178.51^{bc}	178.66^{bc}
Fat, g dm	7.50^{c}	8.21^{bc}	10.20^{a}	9.13^{abc}	9.41^{ab}	8.95^{abc}
Protein, g dm	18.31	18.64	17.80	18.18	18.10	18.02
Dry matter, g dm	26.89	28.09	29.39	28.07	27.86	28.04

^{abc}Means within a row with no common superscript differ significantly ($P < .05$)

Table 9. Dietary treatment effects on broiler energy, fat, protein, and dry matter gains estimated per unit body weight[66] (MWT) partitioned into energy supplement components

Variable	Diet					
	Basal	Isolated-soy	Starch=AME_n of isolated soy	Starch=AME_n of amino acids	Amino acid=aa of isolated soy	Starch+urea=AME_n and N of AA
Gain due to supplement per MWT						
Energy, kcal	0.0^{b}	-4.19^{b}	28.34^{a}	13.03^{b}	12.84^{b}	10.20^{b}
Fat, g dm	0.0^{bc}	$-.50^{c}$	3.51^{a}	1.30^{bc}	2.15^{ab}	$.81^{bc}$
Protein, g dm	0.0	1.55	-.93	.20	-.45	.36
Dry matter, g dm	0.0	1.43	2.35	1.24	.91	1.27

^{abc}Means within a row with no common superscript differ significantly ($P < .05$)

TABLE 10. Feeding and diet effects on serum blood chemistries of male Cobb x Cobb broilers

		Diet					
Variable	Day	Basal	Isolated-soy	Starch=AME_n of isolated soy	Starch=AME_n of amino acids	Amino acid=aa of isolated soy	Starch+urea=AME_n and N of AA
Glucose, mg/dL	1	244.0^{a}	249.8^{a}	249.0^{a}	242.0^{a}	247.7^{a}	239.8^{a}
	2	211.8^{b}	208.8^{b}	209.7^{b}	208.0^{b}	207.3^{b}	204.2^{b}
Triglycerides, mg/dL	1	83.5^{b}	89.6^{ab}	104.5^{a}	94.0^{ab}	83.2^{b}	90.3^{ab}
	2	30.0^{c}	25.8^{c}	27.5^{c}	25.8^{c}	26.7^{c}	24.8^{c}
Total Protein, g/dL	1	3.60^{abc}	3.74^{a}	3.42^{abcd}	3.43^{abcd}	3.65^{ab}	3.38^{abcd}
	2	3.45^{abcd}	3.36^{abcd}	3.14^{d}	3.30^{bcd}	3.53^{abcd}	3.19^{cd}
Creatinine, mg/dL	1	$.23^{ab}$	$.26^{a}$	$.22^{ab}$	$.24^{ab}$	$.23^{ab}$	$.23^{ab}$
	2	$.22^{ab}$	$.20^{b}$	$.21^{ab}$	$.22^{ab}$	$.20^{b}$	$.24^{ab}$
Albumin, mg/dL	1	1.21^{ab}	1.30^{a}	1.21^{ab}	1.21^{ab}	1.30^{a}	1.16^{abc}
	2	1.13^{bc}	1.14^{bc}	1.13^{bc}	1.08^{bc}	1.18^{abc}	1.05^{c}
Mg, μeq/L	1	1.91^{ab}	2.11^{a}	1.94^{ab}	1.91^{ab}	1.99^{ab}	1.98^{ab}
	2	1.93^{ab}	1.81^{b}	1.86^{b}	1.88^{b}	1.86^{b}	1.78^{b}
BUN, mg/dL	1	1.01^{c}	1.36^{c}	1.07^{c}	$.93^{c}$	1.13^{c}	20.61^{a}
	2	$.85^{c}$	$.80^{c}$	$.91^{c}$	$.87^{c}$	$.94^{c}$	5.0^{b}
Uric acid, mg/dL	1	8.82^{bc}	12.70^{a}	6.76^{d}	8.25^{cd}	10.4^{b}	10.21^{b}
	2	3.70^{e}	3.16^{e}	2.89^{e}	3.98^{e}	4.24^{e}	3.68^{e}
Lactate, mg/dL	1	52.17^{ab}	49.00^{ab}	49.17^{ab}	53.50^{a}	46.67^{ab}	48.84^{ab}
	2	47.0^{ab}	38.40^{ab}	43.83^{ab}	46.17^{ab}	37.00^{b}	43.33^{ab}

[a-g]Means within a column with no common superscript differ significantly ($P < .05$)

[2]Day: 1. fed experimental treatments, 2. fasted 24 h

TABLE 11. Feeding and fasting effects on serum blood chemistries of male Cobb x Cobb broilers

Variable	Fed	Fasted
Glucose, mg/dL	245.3[a]	207.8[b]
Triglycerides, mg/dL	90.9[a]	26.8[b]
Total Protein, g/dL	3.53[a]	3.33[b]
Creatinine, mg/dL	0.23[a]	0.21[b]
Albumin, mg/dL	1.23[a]	1.12[b]
Mg, μeq/L	1.97[a]	1.85[b]
Blood urea nitrogen, mg/dL	4.44[a]	1.58[b]
Uric acid, mg/dL	9.41[a]	3.62[b]
Lactate, mg/dL	49.91[b]	42.74[a]

[ab]Means within a row with no common superscript differ significantly ($P < .05$)

CHAPTER VIII

SUMMARY AND CONCLUSION

The trend towards increased consumer demand for leaner poultry products will necessitate that product leanness and uniformity be provided. Fewer birds will be produced for "ice pack" with a corresponding increase in "value added" products. As a result, technologies resulting in greater protein production, in lieu of overall bird mass, will be emphasized. The shift of profit center focus to proteinaceous tissue mass necessitates that nutrition advances occur which enable optimal muscle growth with minimal fat accretion. Though this direction will slow bird growth rate, which has been artificially enhanced via lipid accretion, research directed at reducing the growth depressing consequences of stress may help offset this dilemma. Nonetheless, technology development must occur within the ever increasing environmental restrictions, which are by necessity becoming more severe.

To date the ME_n system has been accepted as the standard for ration formulation. However, it is recognized that, by definition, the ME_n system does not quantitatively predict bird feed energy deposition. Any heat increment change will also alter ME_n utilization and thereby affect cellular energy/nutrient ratios. Any unplanned alteration in cellular energy/nutrient ratio would make fat deposition extent uncertain. For example, recent and ongoing studies directed at evaluating the metabolizable energy system

indicate that cellular energy supply is not necessarily reflective of ME_n consumption. A greater heat increment for protein ME_n calories vs. those from starch and fat make low protein rations lipogenic. Increased understanding and application of cellular energy-nutrient relationships will be required to produce leaner birds. Oxygen required per unit protein synthesis is 380% greater than that for fat.

Research was conducted to (1) assess the metabolic effects of DL-methionine (DLM) and DL-2-hydroxy-4-methylthio butanoic acid (DL-HMB) on broiler thermobalance (heat production, evaporative heat dissipation, sensible heat dissipation, respiration rate, respiration efficiency, change in body heat content) during weeks 4 to 7 under cycling high ambient temperature conditions, (2) evaluate relationships between chemically determined whole carcass fat, protein energy, and ash composition with whole carcass specific gravity and dry matter and age combinations, and to both propose and test resulting predictive equations under a variety of dietary and ambient temperature treatment regimes, (3) determine the efficiency of carbohydrate, and soy-protein AME_n, utilization for fat accretion in birds fed corn starch and isolated soy-protein, (4) quantify and contrast equalized AME_n consumption of isolated soy protein, corn oil and starch on bird heat production, carcass protein and fat accretion, and serum chemistries and to quantify these response variables for rations containing 23, 20 and 17% CP, and (5) quantify and contrast equalized AME_n consumption of isolated soy-protein, corn starch, and crystalline amino acids on bird utilization of ME_n, serum metabolites, and carcass protein and fat accretion.

In the first study, two experiments were conducted to evaluate effects of DLM and DL-HMB on broiler growth rate, feed consumption and pattern, survivability, thermobalance and carcass composition variables during exposure to cycling temperature (24-35 C) heat distressed and thermoneutral (24 C) environments. Methionine source had no impact on weight gain, feed consumption, survivability, carcass weight, chill weight, dressing percentage, breast weight or total fat content. Heat distress increased heat production, respiration rate, and evaporative heat dissipation, while reducing sensible heat dissipation. At 24 C, heat production, evaporative and sensible heat dissipation, and respiration rate were similar for DLM and DL-HMB supplemented birds. However, broilers supplemented with DL-HMB and exposed to heat distress had greater heat production compared to DLM birds. The added heat produced by the DL-HMB supplementation was dissipated by elevated evaporative heat dissipation with no mortality consequence. These methionine sources did not differ in their impact on sensible heat dissipation, respiration rate, or mortality. In Experiment 2, diets having a different caloric density diets (2945, 3200 ME_n kcal/Kg diet) supplemented with either DLM or DL-HMB were fed to birds housed at 24 C or cycling heat distress. Responses were similar to Experiment 1, with the exception that methionine source had no effect on heat production or evaporative heat dissipation under heat distress conditions. However, the DL-HMB supplemented birds consumed more feed during the five h period prior to peak ambient temperature; this increased their heat load during heat distress. Birds consuming the higher caloric density diet had an increased live weight gain, carcass weight, dressing percentage, fat pad, and carcass dry matter within heat distress and an elevated carcass

weight and dressing percentage in a thermoneutral environment. The combined data of Experiments 1 and 2 suggest that methionine source may impact the chicks ability to acclimate to heat stress. Additional studies are needed focusing on effects of methionine source on the early acclimation process.

In the second study, three experiments were conducted to develop and test equations predicting carcass fat and protein content. Carcass fat was elevated when caloric density was increased. In Experiments 1 and 2, predicted variables were significantly correlated with determined carcass fat, protein, and ash. Data were pooled across the 2 studies and used to form specific gravity, dry matter, and/or age based equations for predicting carcass composition. Results were tested in Experiment 3, where birds reared for 49-d consumed either 2880, 3200, or 3574 kcal ME/Kg diet while exposed to constant 24 or cycling 24 to 35 C ambient temperatures. Both dietary and environmental effects impacted carcass composition with fat increasing with caloric density, while heat distress reduced carcass protein. Predicted values for carcass fat and protein were highly correlated with analyzed values. Results suggest that prediction equations based on dry matter and specific gravity based may be used to estimate carcass fat and protein of broilers consuming diets differing in caloric density and exposed to normal or elevated ambient temperatures.

The three experiments in the third study were conducted to determine the apparent metabolizable energy content corrected for zero nitrogen balance (AME_n) for gain of a corn-soy basal ration alone and when supplemented with isocaloric quantities of corn starch, isolated soy-protein, and corn oil. Experiment 1 determined the AME_n to be 3.24,

3.95, 8.75, and 4.25 kcal/g dry matter for the basal ration, corn starch, isolated soy-protein, and corn oil, respectively. In the second and third experiment, despite similar AME_n consumption and carcass protein gain, birds supplemented with corn starch had elevated carcass fat gain compared to birds supplemented with isolated soy-protein. Experiment 3 yielded similar responses. Although no differences were observed in AME_n intake, carcass fat gain was lowest with the isolated soy-protein supplement, while bird fat pad weight and dry matter percentage followed similar trends. Results suggest that diets formulated by the ME_n system, do not necessarily correlate with bird energy retention and that dietary calorie/nutrient ratios are not proportional to metabolizable energy. In order for the broiler to achieve maximum protein deposition with minimal fat accretion, an energy metabolism scheme is needed to account for the variation in substrate mediated heat production.

The fourth study was conducted to quantify effects of equal AME_n consumption from isolated soy protein, corn oil and starch on heat production, carcass protein and fat accretion, and serum chemistries. In addition, effects of 23, 20 and 17% CP were examined. To estimate maintenance need, some birds were fasted. Energy loss during 4-d by fasted birds was proportional to initial protein mass increasing by 4.3 kcal/g initial protein mass with an intercept of 741 kcal.. In this study, maintenance utilized the bulk of the diet consumed so that insufficient dietary energy was available for protein accretion. Utilization of the carbon-nitrogen balance equations enabled both whole body carcass lipid and protein to be monitored. Endogenous lipid stores provided energy for protein accretion. Protein accretion was elevated by the carbohydrate, corn oil and isolated

soybean protein additions. Similar to the birds consuming the basal diet, birds given energy supplement lost carcass fat which supplied energy for protein accretion. Lowering the dietary crude protein from 23 to 17% increased the protein efficiency ratio indicating that the quantity of excess amino acid being catabolized was reduced. The increased energy gain at elevated crude protein conflicts with the concept of a higher heat increment for amino acids, however, the higher protein diet also contained more dietary fat which should have a lower heat increment. Although the controlled feeding technique enables substrate energetic efficiencies to be directly compared, the results were compromised by the creation of an energy deficiency.

In the final study, we estimated efficiency of isolated soy protein, corn starch, and crystalline amino acid ME use for tissue accretion. No differences were observed for live weight gain, protein gain, or feed efficiency between the birds consuming basal diet and birds given energy supplements. Oxygen consumption and heat production averaged 1013 L and 5000 kcal, respectively, for the 21-d trial. Birds not receiving supplemental energy had lower AME_n consumption, CO_2 production, fat and carbon gain, and carcass dry matter percentage as compared to the energy supplemented groups. Total body energy ranged from 4 to 15% higher for the birds receiving energy supplements. Despite similar AME_n consumption among the energy supplemented groups, bird energy and fat gain were impacted significantly. Energy gain was 10% lower ($\underline{P} < .05$) from the supplemental energy from isolated soy-protein than the supplemental energy from corn starch. Estimated energy and fat gain due to supplement per MWT was greater for the starch supplement than the other energy supplements. Total calorie gained from the energy

supplement was greatest for the corn starch supplemented group and lowest for isolated soy-protein supplemented groups. High protein diets promote leaner broiler carcasses primarily as a result of a lower net energy being available for lipogenesis.

In summary, research was conducted to examine the potential for crystalline amino acid supplementation to enhance broiler production efficiency by improving cellular energy supply. This hypothesis is based on the fact that the metabolizable energy system does not account for heat production and the premise that heat increment is higher for protein than other energy sources. For maximum protein deposition, with minimal fat accretion, an energy metabolism scheme is needed that accounts for differences among substrates in heat production.

In conclusion, an overestimation of nitrogen retention was observed using the difference technique (nitrogen intake-nitrogen excretion) compared to comparative slaughter method (final carcass nitrogen-initial carcass nitrogen). It appears that excreta nitrogen is lost when dried in a 60 C. In retrospect, birds in this study were fed unequal AME_n due to differences in nitrogen concentrations between the supplements. This would indicate birds consuming the isolated soy-protein supplement received less AME_n thence calculated. Future studies need to address nitrogen loss when drying, comparing fresh verses dried samples may be an option, and adjustments of ingredient AME_n values may need to addressed.

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