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Indirect calorimetry evaluations of energy utilization by laying hens: Nutrient and temperature effects

Rising, Russell Marshall, Ph.D.

The University of Arizona, 1988

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INDIRECT CALORIMETRY EVALUATIONS OF ENERGY UTILIZATION BY LAYING HENS: NUTRIENT AND TEMPERATURE EFFECTS

by

Russell Marshall Rising

A Dissertation Submitted to the Faculty of the

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In Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Russell Marshall Rising

entitled INDIRECT CALORIMETRY EVALUATIONS OF ENERGY UTILIZATION BY LAYING HENS: NUTRIENT AND TEMPERATURE EFFECTS

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

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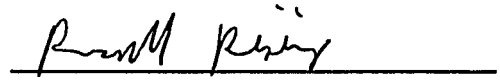
SIGNED: 

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ABSTRACT

A four-chamber indirect calorimeter was constructed to evaluate energy utilization by laying hens as affected by dietary energy, protein and amino acid levels and by housing temperature. Heat production was measured by indirect calorimetry and metabolizable energy was determined for each diet. Net energetic efficiency was calculated as the slope of the regression of energy balance (metabolizable energy intake - heat production) on metabolizable energy intake.

Hens fed diets formulated without protein specifications to provide amino acid levels equivalent to those provided by 14.5 or 16% protein, had higher ($P<.05$) net energetic efficiencies than hens fed diets formulated with specified protein levels. Addition of 3% animal fat to the higher (16% protein) amino acid diets produced the greatest increases ($P<.05$) in net efficiency, but decreased ($P<.05$) efficiency in the lower (14.5% protein) amino acid diet formulated without a protein specification. Metabolizable energy available for production was improved ($P<.05$) with animal fat additions to all but the diet formulated to the higher (16% protein) amino acid levels.

Animal fat, cottonseed oil, corn oil, cod liver oil and safflower oil were used to determine effects of fat source on energy utilization by laying hens at 21.1 C. Higher net efficiencies were obtained for the cottonseed and safflower oil diets (99.1 and 99.8%,

respectively) than for the basal diet (90.6%) or the other fat sources (cod liver oil = 86.0%, corn oil = 87.3%, animal fat = 91.1%).

Full vs. meal (two 1-hr periods daily) feeding and total sulfur amino acid deficiency effects on energy utilization were evaluated. Meal feeding reduced ($P<.05$) metabolizable energy available for production while increasing ($P<.05$) maintenance metabolizable energy requirements. Feeding regime did not reverse the decline ($P<.05$) in net efficiency (89.2-89.7 to 81.7-82.6%) observed with total sulfur amino acid deficiency.

Hens were fed either 7% animal fat or 7% corn oil and maintained at 10, 21.1 and 32.2 C to study effects of fat source and temperature on energy utilization. From 10 to 32.2 C, net energetic efficiency increased from 87.6 to 92.6% with animal fat. Corn oil showed the highest net efficiency (93.9%) at 10 C, while animal fat was highest (92.6%) at 32.2 C. Hens fed animal fat required the most ($P<.05$) maintenance metabolizable energy and had the highest ($P<.05$) fasting heat productions at each temperature.

CHAPTER 1

INTRODUCTION

Methods of reducing heat load on laying hens and maintaining egg production during periods of high temperature have been the objective of poultry nutrition research for many years. Poultry as well as all animals respond to heat stress by altering heat production in two ways: first, by lowering resting metabolic rates; and second, by reductions in feed consumption to reduce dietary induced thermogenesis (DIT) or heat increment. This second adaptive mechanism results in reduced energy available above maintenance for productive purposes (growth and egg output).

The amount of feed poultry consume is related to their energy requirements, assuming that all other nutrients (protein, vitamins and minerals) are in proper balance, and dietary energy represents the most expensive nutrient that must be provided. Improving energy utilization under adverse and non-adverse environments will improve productive efficiency in laying hens.

Previous work has demonstrated that added fat, with its lower percentage of DIT in comparison with protein and carbohydrate, is a very efficient energy source during heat stress. Added fat is effective in maintaining energy intakes and production during periods of chronic exposure to high environmental temperatures. Also, under

non-stress conditions fat supplementation increases energy intake resulting in more energy available for productive purposes.

Reduction in protein intake, achieved through improved dietary amino acid balance, provides another means by which diet alteration may improve energetic efficiency, and thus reduce heat loads. The effects of these changes have been investigated in some of our performance evaluation studies with laying hens, but are only now being studied in the calorimetry system developed during the past three years in our laboratory. The calorimetry system developed at the University of Arizona is the only one of the multi-chamber automated indirect type for experimental animals in the United States which allows 23-hr measurements.

Indirect calorimetry offers a rapid means of assessing the effects of varying proportions of protein, fat and carbohydrate on heat production without the problems associated with changes in resting metabolic rate resulting from alterations in body weight during the feeding period. Previous work in our laboratory has shown that dietary nutrient levels must be adjusted for changes in feed intake with increases in environmental temperature to prevent losses in energetic efficiency and laying hen performance.

Due to the ability to rapidly evaluate energy metabolism in laying hens with indirect calorimetry, the effects of formulating diets based on amino acid requirements, changing protein and fat proportions or nutrient deficiencies can be determined, and the appropriate adjustments made to maintain laying hen performance. Indirect calorimetry also allows the determination of energy metabolism when

various fat sources are used as energy supplements in laying hen diets. This rapid evaluation of dietary fat sources with indirect calorimetry allows the ability to evaluate a fat source, for possible use as a feed supplement, as price and availability of fat supplements change.

Indirect calorimetry was used in the following experiments to determine the usefulness of various dietary energy sources and the effects of environmental stresses on energy metabolism in laying hens.

CHAPTER 2

LITERATURE REVIEW

Indirect calorimetry is based on measurements of oxygen utilization and carbon dioxide evolution as a result of metabolic processes in a living organism.

Theory of indirect calorimetry

Brody's (1945) calculation of the ratio of carbon dioxide produced to oxygen consumed for the oxidation of pure carbohydrate, protein or fat gave respiratory quotients (RQ) of 1, .82 or .71, respectively. Since 1 mole of an ideal gas equals 22.4 liters, RQ is calculated by knowing either the volume or the weight of oxygen consumed and carbon dioxide released (Causey, 1971).

Heat of combustion values (caloric equivalents) associated with 1 liter of oxygen consumption and carbon dioxide production, for carbohydrate, fat and protein can be determined by oxidizing 1 g of pure carbohydrate, fat or protein and measuring the heat produced, amount of oxygen consumed and carbon dioxide released for each substance. Several workers have determined caloric equivalents of oxygen and carbon dioxide of pure carbohydrate, fat and protein and obtained values which are quite similar (Rubner, 1901; Loewy, 1911; Lusk, 1928; Carpenter, 1948).

Utilizing data provided by Rubner (1901), Lusk (1928) derived a table listing all the caloric equivalents at various RQ's and the corresponding percentages of fat and carbohydrate oxidized for each RQ value.

Since birds excrete excess nitrogen as uric acid, the caloric equivalent for protein in birds is lower due to the higher oxygen content and lower RQ obtained upon oxidation compared with urea. Barott et al. (1938), utilizing data provided by Coulson and Hughes (1931), recalculated the caloric equivalent of protein and utilized this value, along with the caloric equivalents for carbohydrate and fat provided by Carpenter (1948), to derive three linear simultaneous equations and coefficients for oxygen consumption, carbon dioxide production and protein oxidation. If oxygen consumption, carbon dioxide production and protein utilization are known, solving these equations enables the calculation of heat production, carbohydrate and fat utilization.

According to Romijn and Lokhorst (1961), total heat production can be calculated utilizing the formula derived by Barott et al. (1938) without accounting for protein oxidation. These workers measured respiratory exchange in a 3.5 kg North Holland cock for 4 days and calculated heat production with the formula derived by Barott et al. (1938) with and without accounting for protein oxidation. The largest differences they found in daily heat productions were 1.5%. These workers concluded that the formula derived by Barott et al. (1938) can be utilized to calculate heat production in poultry without the coefficient for protein oxidation.

History of calorimetry

Antoine L. Lavoisier, considered the founder of modern chemistry and the father of calorimetry, in the late 1700's discovered that animal heat was derived from the utilization of oxygen. In 1783, Lavoisier and de LaPlace constructed the first direct, open circuit calorimeter consisting of a chamber surrounded by an inner jacket filled with ice. An adiabatic outer jacket was filled with ice water to prevent heat exchange with the outside environment. Lavoisier and de LaPlace (1783) determined that it took 80 calories of latent heat to melt 1 g of ice and utilized this theory to measure heat loss by a guinea pig. These workers conducted the first measurement of respiratory exchange on animals. They utilized a closed circuit respiration apparatus consisting of large glass bell-jars sealed with mercury. After noting the initial volume of the system, the carbon dioxide exhaled by a guinea pig, previously housed in the system, was absorbed with concentrated sodium hydroxide thus decreasing the volume of the system. The change in volume equaled the volume of carbon dioxide liberated by the guinea pig. Oxygen consumption was determined by the amount of air necessary to return the system, after removal of the sodium hydroxide, to the initial volume. They found a positive relationship between heat loss and carbon dioxide production and suggested that the two processes were related (Lavoisier and de LaPlace, 1783).

Atwater and Rosa (1899) built the first calorimeter large enough for humans at Wesleyan College in Middletown, Connecticut. It operated as a closed circuit indirect calorimeter for measuring heat

production and also through the determination of fat and protein balances. It was a sealed system with air within the chamber passed through soda lime and sulfuric acid to absorb carbon dioxide and water, respectively. Oxygen was replaced by an external cylinder regulated by barometric pressure within the chamber. Their energy balance calculations were partly based on the assumption that protein contained 16% nitrogen and 53% carbon while fat contained 76% carbon. They determined protein oxidation by measuring nitrogen contents of the diet, urine and feces, and fat utilization was determined by accounting for all carbon losses through urine, feces and respiration. Assumed heats of combustion of protein and fat were 5.5 and 9.4 kcal/g, respectively, and from carbon and nitrogen balance values these workers were able to estimate heat production of the experimental subject. They determined latent heat loss as the weight of water absorbed by sulfuric acid, and that 585 kcal are required to evaporate 1 liter of water.

The Atwater-Rosa calorimeter also operated as a direct calorimeter by measuring heat transfer to a water filled heat exchanger. From the specific heat of water as 1.0 at 20 C and that 1 kcal is the amount of heat needed to raise the temperature of 1 liter of water from 21.5 to 22.5 C, they calculated heat production from the temperature differential of the known amount of water flowing through the heat exchanger.

Armsby (1908) built a respiration calorimeter similar to the Awater-Rosa apparatus for studying energy metabolism in cattle at the Pennsylvania Experiment Station in 1898. The operating theory was

similar to the Atwater-Rosa apparatus but contained a system for determining the amount of combustible gases (methane) excreted by cattle. This determination consisted of a platinized kaolin filled copper tube oven, maintained red hot, in order to oxidize the gases to carbon dioxide and water. The gases were trapped by an additional set of sulfuric acid and soda lime absorbers, and the final heat productions were corrected for heat loss as methane.

Haldane (1892) measured respiratory exchange in small animals with an indirect open circuit gravimetric calorimeter. Carbon dioxide and water were removed from air entering and exiting the system by sets of concentrated sodium hydroxide and sulfuric acid absorbers, assuming that chamber air only contained carbon dioxide and water liberated by the animal. It was assumed that the differences in weight between the accumulation of carbon dioxide and water and the change in the animal's weight over time equaled oxygen consumption. RQ's were calculated and heat production determined by utilizing the thermal equivalents for oxygen and carbon dioxide provided by Lusk (1928).

Energy utilization

Energy utilization in laying hens may be determined by measuring the efficiency of conversion of dietary metabolizable energy (ME) consumed into body weight change plus egg mass output (energy retention), with the difference between ME intake and energy retention being equal to heat production. Any two of the three parameters can be determined and used to calculate the third. There are two ways of evaluating energy retention; direct measurement of carcass and egg

energy by bomb calorimetry, or indirect calorimetry for measurements of heat production, which when subtracted from ME intake yields energy retention (Farrell, 1974).

Various investigators (Davis, Hassan and Sykes, 1972; Davis et al. 1973; Hoffman and Schiemann, 1973) have reported values for the energy content of laying hen carcasses and eggs as 5.0 and 1.6 kcal/g, respectively. Use of these values allows calculation of energy retention from determined body weight changes and egg mass output over time, thus eliminating the need for sacrificing the birds. Regression analysis (Farrell, 1974) is then used to calculate net energetic efficiency (NEE), energy retention, maintenance ME, maintenance feed and fasting heat production. Restricted feeding is utilized in combination with carcass analysis and requires from 7 to 28 days; while only a few days are required for indirect calorimetry studies to obtain the necessary data for the regression analyses. Restricted feeding of long duration leads to changes in basal metabolism and body composition thus changing the maintenance requirements during the test, and introducing uncontrolled errors for parameters derived from the slopes and intercepts calculated by regression analysis.

Utilizing indirect calorimetry, heat production is calculated from oxygen and carbon dioxide measurements and energy retention obtained by subtracting these values from ME intakes. The advantages of utilizing indirect calorimetry are rapid determination of heat productions and short restricted feeding times; thus basal metabolism is little affected. Indirect calorimetry reduces the errors associated

with carcass analysis, and shortens the total experimental period (Farrell, 1974).

There are reported differences in results obtained with carcass analysis and calorimetry (Farrell, 1974; Reid and Maiorino, 1984). Fuller, Dale and Smith (1983) have compared heat production measurements on 10-week old broiler cockerels determined by open-circuit respiration calorimetry with those obtained from the same group of birds utilizing carcass analysis data. Over two experimental periods of four days each, mean heat productions were 359 and 348 kcal/bird/d for the two methods in the first experiment, and 395 and 393 kcal/bird/d in the second experiment when measured by carcass analysis and calorimetry, respectively. These data represent differences of 3.0 and .6% for the first and second experiments, respectively, suggesting that the two methods are comparable for measuring energy metabolism in growing chickens. These results agree with the earlier work of Farrell (1972) who found average differences in heat production values of 1% between the two methods.

Temperature effects

Housing temperature and humidity have pronounced effects on laying hen performance. Lillie et al. (1976) fed 24-week old Leghorn pullets three diets containing 2220, 2648 and 3080 kcal ME/kg at dry bulb temperatures ranging from 13 to 29.5 C and relative humidities from 50 to 80% to evaluate the interaction of temperature, humidity and dietary energy on body weight change, hen-day egg production, egg weight, specific gravity, Haugh units (computed from egg weight and

albumen height) and feed intake (g hen-day) at 50% egg production. These workers found no significant effect of humidity on the above parameters with mean values of +1.8 g, 66.9%, 52.3 g, 3.81, 91.8 and 84.1 g for body weight change, egg production, egg weight, specific gravity, Haugh units and daily feed intake, respectively, during the six month trial. Considering temperature effects only, body weight change, egg production and egg weight decreased significantly from 3.0 to 2.0 g, 78.1 to 65.1%, and 58.1 to 52.2 g, respectively, with increasing temperature. Since the diets varied only in energy content, Lillie et al. (1976) suggest that reduced feed intake due to heat stress probably produced some nutrient deficiencies thus explaining the results obtained with increasing temperature.

Irrespective of temperature and relative humidity, Lillie et al. (1976) also observed the lowest egg production (70%) with the high energy diet and suggested lower feed consumption resulted in protein intakes below requirements. Protein intake for the low, intermediate and high energy diets were 17.8, 16 and 14 g/day, respectively, due to reduced feed intake with increasing dietary energy. Based on these results, Lillie et al. (1976) recommended the intermediate energy diet (2648 kcal ME/kg) for maximum laying hen performance in dry bulb temperatures from 13 to 21.5 C.

Utilizing carcass analysis to determine energy balance, Davis et al. (1972) subjected 31-week old Sterling White Links laying hens to constant temperatures of 10 or 35 C for three weeks. In comparison with hens at 10 C, those housed at 35 C showed significantly lower feed consumption (95 to 63 g dry matter/d), ME consumption (302 to 205

kcal/d), increased body weight loss (2.2 to 13.2 g/d) and reduced heat production (249 to 191 kcal/d) while no significant changes occurred in egg production or egg weight, thus significantly increasing the gross efficiency of egg production (egg calories/ME intake × 100) from 24 to 34%.

When 31-week old hens of the same strain were suddenly subjected to each temperature and allowed to acclimate for six weeks, Davis et al. (1972) found that feed consumption, body weight change and egg production returned to initial values after two weeks at 10 C. When suddenly exposed to 35 C, egg production, after an initial drop, returned to initial values after two weeks, but up to four weeks were needed for feed consumption and body weight change to return to "normal" values. These workers suggest that constant feed intake and heat production, along with stable body weight, are good indicators of adaptation to temperature regimes.

Fasting heat production is affected by diurnal rhythms, environmental temperature, body weight, feather loss, age and activity. Damme et al. (1987) studied the fasting heat production of Rhode Island Red and Light Sussex laying hens utilizing short term indirect calorimetry measurements of 24 to 36 hours. These workers found diurnal fasting heat productions ranging from 4.13 to 3.0 kcal/kg physiological body weight (PBW = $BW^{.75}$)/hr for day and night, respectively. They also found that nesting activity with and without oviposition increased fasting heat production an additional 1.8 kcal/kg PBW/hr and would last approximately two hours. When the hens were exposed to four different temperatures (13.5, 18.5, 24 and 29 C) for

two days, significant increases in fasting heat production were noted from an average of 2.82, for the three lower temperatures to 4.5 kcal/kg PBW/hr at 29 C. Ota and McNally, (1961) also found that White Leghorns produce 3.0 and 3.75 kcal/hr/kg body weight at 30 and 20 C ambient temperatures, respectively, showing that laying hens reduce heat production with increasing temperatures.

In a similar study, Olomu and Offiong (1983) evaluated the effect of the tropical climate of Nigeria, using 24-week old Warren Studler Sex-Sal-Link laying hens fed nine diets containing 16, 18 and 20% protein with each protein level fed at three different ME levels (2400, 2600 and 2800 kcal/kg), on egg production (hen-day %), egg weight, Haugh units, body weight change, feed and ME intakes. Maximum and minimum temperatures ranged from 26.8 to 35.2 C (summer) and 12.8 to 22 C (winter) with relative humidities ranging from 13.6 to 77.2% during the course of the study. Ignoring energy level, dietary protein produced no significant effects on the above parameters except that 20% protein significantly depressed feed consumption from 133 to 129 g/day, and ME intake from 345 to 335 kcal/day for the 18 and 20% protein diets, respectively. The highest energy level significantly depressed egg production and feed intake from 71.7 to 68.3% and 134.8 to 127.3 g/day, respectively, with a concomitant significant decrease in protein intake from about 24 to 22 g/day. In agreement with these authors is a report by Sugandi, Bird, and Atmadilaga (1975) who found that a diet containing 3050 kcal ME/kg depressed egg production in a tropical climate while a diet containing 2400 kcal/kg ME supported adequate egg production. Body weights increased significantly when hens were fed

the medium and high energy diets in comparison with the low energy diet. ME intake followed the same significant trends with increases from 324 for the low energy diet to 347 and 357 kcal/d, for the medium and high energy diets, respectively (Olomu and Offiong, 1983). According to these results, Studler Sex-Sal-Link laying hens were able to maintain performance in the tropics on a 16% protein, 2400 kcal/kg ME diet.

Unlike wild birds which reportedly do not adjust their basal metabolism with seasonal changes in temperature (King and Farner, 1961), a major adaptive mechanism for laying hens is the ability to alter metabolic rate. Changes in the lower critical temperature of laying hens in relation to seasonal acclimatization was evaluated by Arieli, Meltzer and Berman (1979). Over a period of three years, 1.5- to 2.5-year old White Leghorn × Rhode Island crossbred hens, acclimated to mean summer and winter temperatures of 25 and 15 C, respectively, were each periodically subjected to temperatures ranging from 2 to 32 C and oxygen consumption and body temperatures determined at each temperature. Older hens were chosen to eliminate the effect of growth on metabolism and their low egg production rates facilitated rectal temperature measurements. When ambient temperature (C) was regressed on mean oxygen consumption, Arieli et al. (1979) found differences in fasting oxygen usage, with no differences between slopes for the equations. This indicates a shift in basal metabolism during acclimation, but no difference in the rate of oxygen consumption per degree C. Taking the intersection of the above curves with the line derived for the thermoneutral oxygen consumption data as representing

the lower critical temperature, Arieli et al. (1979) found that hens acclimated to winter temperatures showed lower critical temperatures than summer acclimated hens. They calculated that, with seasonal temperature changes, lower critical temperatures changed .85 C per degree C change in mean ambient temperature, and that lower critical temperatures were related to mean ambient temperatures. These same workers found a similar relationship between summer and winter acclimatization of hens. Hens acclimated to winter temperatures showed greater body temperature variability when exposed to the high experimental temperatures than hens acclimated to summer temperatures. Since laying hens are bred for high productivity, lighter body weights inhibit their ability to modulate energy metabolism by changes in insulation, thus increasing or decreasing metabolic rate is their main adaptation mechanism during acclamation (Arieli et al., 1979).

According to Arieli et al. (1980) the thermoneutral zone is a temperature range where the energy requirement is minimal and constant. Utilizing the same experimental protocols described in a previous study (Arieli et al., 1979), Arieli et al. (1980) determined the upper critical temperature, evaporative water loss and the thermoneutral zone for White Leghorn × Rhode Island crossbred hens. These workers found that body temperature in both acclimated groups increased approximately .02 C per degree C change in test temperature, with the rate increasing to about .1 C at test temperatures above 26 C. The response curves for evaporative water loss and respiratory rate with housing temperature (C) were similar but changes in slopes occurred at lower temperatures in winter vs. summer acclimated hens. After determining upper critical

temperature from average changes in slopes obtained from the respiration rate and body weight response curves, Arieli et al. (1980) determined the thermoneutral zone by regressing acclimatization temperature (C) on upper and lower critical temperatures, respectively. These workers found that the thermoneutral zone decreases with increasing temperatures due to reduced changes in upper critical temperatures with increasing acclimatization temperatures. The intersection of both the lower and upper critical temperature regression curves represents the point (approximately 32 C) where there is no thermoneutral zone, maximum energetic efficiency and the maximum ability of the hen to increase heat dissipation and reduce heat production through evaporative water loss and reduced metabolic rate, respectively. Temperatures above this will reduce laying hen performance and cause physiological stress. According to Arieli et al. (1980), the small changes in upper critical temperatures (3 C) found in summer and winter acclimated hens, respectively, are due to the small contribution of insulative changes thus leaving basal metabolic rate change as the main adaptive mechanism. The ability to regulate heat loss to the air through insulation changes has a larger roll in adapting to lower temperatures thus lower critical temperatures show greater differences (11 C), respectively, with summer and winter acclimated hens than upper critical temperature.

In two experiments, de Andrade, Rogler and Featherston (1976) exposed 20-week old Single Comb White Leghorn laying hens to 21 and 32 C at 50% relative humidity and fed either a control or a high nutrient density (HND) diet to examine the effects of temperature and nutrient

interactions on egg production, feed consumption, feed efficiency, mortality, egg weight, specific gravity of the eggs, shell thickness, percent calcium and nitrogen in true shell (minus inner membranes), plasma calcium, percent hematocrit, percent ash in dry fat-free bone, partial pressure of blood carbon dioxide and rectal temperatures. These workers also determined the effect of temperature reversal on these same parameters. Elevated temperatures (32 C) significantly decreased egg production from 85.1 to 74.1% and egg weight from 60.4 to 54.8 g. In comparison with the control diet, the HND diet significantly improved egg production from 78 to 81.1% and egg weight from 56.2 to 59 g. At 32 C, the improved egg production seen with the HND diet (70.9 to 77.3%) suggests that the additional energy provided by soybean oil used in the HND diet maintained laying hen performance under heat stress. These workers suggest one reason for the hens' maintaining performance as reduced protein requirements resulting from reduced egg production normally seen with age. The HND diet decreased feed consumption from 99.8 to 92.1 g/bird/d at 21 C, but this decrease was not observed at 32 C.

Laying hens adjust feed consumption to abrupt changes in environmental temperature in approximately nine days (Powell et al., 1972). Jones and Barnett (1974) determined that turkey hens in production required 8 to 14 days to adjust feed consumption when exposed to 35 C. The effect of abruptly changing dietary energy levels and environmental temperatures on daily feed consumption, egg production and body weight change (g/21 days) were investigated by Jones, Hughes and Barnett (1976) who abruptly exposed 72-week old

Single Comb White Leghorn laying hens to 4.5 and 35 C after 7 days at 21 C. Diets containing 2671, 2853 and 2992 kcal ME/kg were used in their studies. In comparison with 21 C, no significant differences were found for feed consumption and egg production at 4.5 C with mean values of 111.2 and 71.9 g, respectively, while hens exposed to 35.0 C significantly decreased feed consumption and egg production (61.8 and 60.2 g) along with significant increases in body weight loss (-289 g). These workers found no significant differences in feed consumption and egg production with changes in dietary energy at each temperature. Compared with the intermediate energy diet, at each temperature, there was a trend for hens to eat less feed and produce less eggs when fed the higher energy diet, while the opposite occurred with the low energy diet. According to Jones et al. (1976) the hens adjusted feed intake when shifted to 4.5 or 35 C after one day, suggesting that hens adjust feed consumption immediately after abrupt temperature changes.

Total sulfur amino acid deficiency

The National Research Council (NRC, 1984) recommended methionine and cysteine requirements for laying hens are .32 and .23% of the diet, respectively, with 2900 kcal ME/kg.

Three methionine supplements (DL-methionine, calcium salt of methionine hydroxy analogue and an acid form of methionine hydroxy analogue) are currently used in poultry rations. Reid, Madrid and Maiorino (1982) determined biopotency of these three methionine sources, utilizing the slope-ratio technique, and the effects of different supplemental methionine levels on egg production and egg

output. Twenty-eight week old DeKalb Single Comb White Leghorn laying hens were fed various levels (0, .025, .05, .1 and .15%) of additional methionine, from all three sources, in a basal diet containing .229% methionine and .21% cysteine for a total sulfur amino acid (TSAA) content of .44%. Egg output when regressed on TSAA intake (mg/TSAA/bird/d) yielded slope values which were used to determine relative biopotency. After deriving the regression equations, Reid et al. (1982) found no significant differences among the three sources. This suggests that all three methionine sources are of the same relative biopotency and will equally support laying hen performance.

Reid and Maiorino (1984) determined the effect of dietary sulfur amino acid levels, (.47, .49 and .51%), including a toxic level (3.47%), on energy metabolism in 7-month old Single Comb White Leghorn laying hens at 15.6 and 32.2 C utilizing egg and carcass analysis to assess energy retention. The highest ME intake (kcal/bird/d), energy balance (kcal/bird/d) and energetic efficiency (%), and lowest heat production (kcal/bird/d), was 259, 98.7, 63.8 and 171.0, respectively, with the .51% TSAA diet at 32.2 C. For the same TSAA levels at 15.6 C, the values obtained were 359, 142, 62.2 and 228.8, respectively. There was approximately 45 kcal/bird/d more ME available above maintenance when hens were fed the higher non-toxic TSAA levels at 15.6 C than hens fed these diets at 32.2 C. No significant changes occurred in maintenance ME (kcal/bird/d) at either temperature with TSAA level. Reid and Maiorino (1984) suggested that improved protein quality lowered heat production and increased energetic efficiency and allowed laying hens to maintain egg production.

Fat type and lipogenesis

Waterman et al. (1975) determined the effect of feeding rats, pigs and chicks a basal diet supplemented with 20% tallow or safflower oil on adipose tissue and liver lipogenesis and on plasma triglycerides. Plasma triglycerides were depressed in rats, unchanged in chicks and increased in pigs with the feeding of safflower oil in comparison with tallow. These workers found that safflower oil significantly lowered hepatic fatty acid synthesis from 828 to 621 (expressed as nmoles radioactive acetate incorporated into fatty acids/g tissue/hr) in rats and hepatic fatty acid synthetase activities in chicks from 12.7 to 9.8 nmoles substrate metabolized/min/mg protein, respectively, in comparison with tallow supplementation. These workers suggested that polyunsaturated fatty acids depress hepatic lipogenesis to a greater extent than saturated fats.

Pearce (1968) determined the effect of feeding a basal diet supplemented isocalorically with 2% corn oil on liver acetyl-CoA carboxylase, citrate-cleavage enzyme and isocitrate dehydrogenase activities in two strains of laying hens (Hyline and Hybrid 4). Acetyl-CoA carboxylase activities decreased from 2.68 to .06 and from 1.59 to .20 μmole substrate metabolized/min/mg protein for the two strains studied, respectively. Corn oil feeding decreased citrate-cleavage enzyme activity from 18.31 to 5.97 and 15.39 to 8.05 μmole substrate metabolized/min/mg protein for the Hybrid 4 and Hyline hens, respectively.

Rationale

Laying hen performance is adversely affected by extremely high and low temperatures as a result of increased environmental stress leading to increased maintenance energy requirements. The amounts of energy above maintenance needed to support long-term egg production are lowered during environmental stress and become limiting.

This fact and the well established lower heat increment of fat in comparison with carbohydrate and protein (De Groote, Reyntens and Amich-Gali, 1971) led to the studies undertaken in this dissertation.

Using indirect calorimetry, the effects of protein quality, supplemental fat, type of fat, along with the interactions with temperature, were evaluated with laying hens.

CHAPTER 3

MATERIALS AND METHODS

Calorimetric studies have been conducted for many years, but until the advent of electronic analyzers and computer systems for data acquisition, it was difficult to obtain reliable data on large numbers of animals.

Development

Four environmental chambers were constructed from refrigerators according to plans provided by Darre (1985) and incorporated into a multi-chamber indirect calorimeter system (Figure 1) similar to the one described by Lundy, MacLeod and Jewitt (1978). Each chamber housed two hens in single cages (45.7 cm × 45.7 cm × 50.8 cm). Temperature (0 to 37 C), photoperiod (0 to 24 hr) and air flow (0 to 25 standard liters/min (SLPM)) were regulated in each chamber according to experimental needs. Except during sampling of chamber air, the system remained open to room air. Air flow was measured at the intake of each chamber by air mass flow transducers (model FMA-507V, Omega Engineering, Inc., Stamford, CT). Oxygen and carbon dioxide concentrations were measured every 15 minutes at the exhaust of each chamber by an electrochemical oxygen sensor (N-22, Ametek-Thermox Instruments Div., Pittsburgh, PA) and an infrared carbon dioxide sensor (model P-61, Ametek-Thermox instruments Div., Pittsburgh, PA),

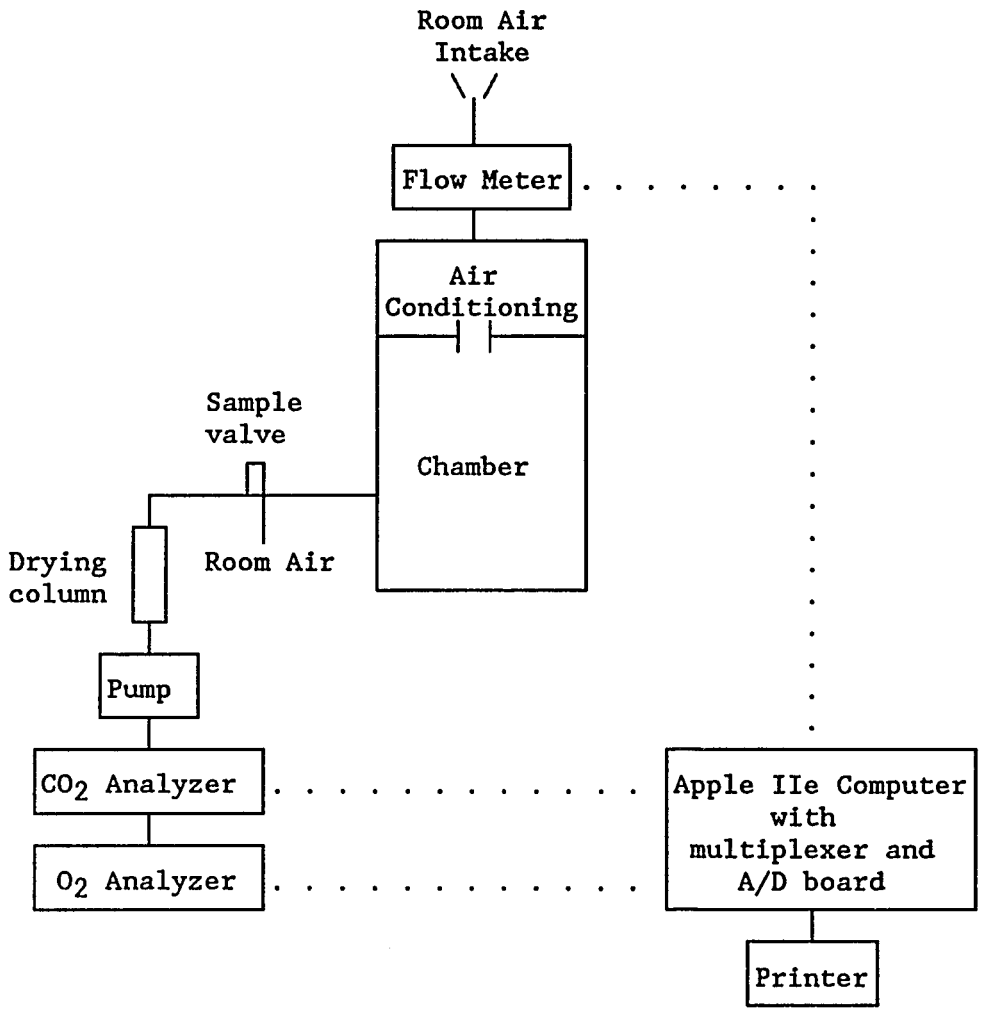

FIGURE 1. Schematic diagram of the open-circuit indirect calorimeter used for laying hen studies at the University of Arizona.

respectively, with sample flow (180 ml/min) controlled by an Ametek R-1 flow controller (Ametek-Thermox Instruments Div., Pittsburgh, PA). Respective analog signals from both sensors were processed by Ametek S-3A1 oxygen and Ametek CD-3A carbon dioxide analyzers (Ametek-Thermox Instruments Div., Pittsburgh, PA) which had been previously calibrated with an oxygen-carbon dioxide gas mixture of known concentration. Analog outputs from both analyzers and the flow transducers were converted to digital by an ADALAB analog/digital board (Interactive Microware, Inc., State College, PA) and all data processed by an Apple IIe computer.

Room air oxygen and carbon dioxide contents were recorded every 15 minutes and utilized as initial values for each sampling cycle. The exhaust air for each chamber was sampled every 15 minutes, for 2 minutes, with all four sampling cycles summarized hourly for 23 hours. Corrections to standard dry conditions (STPD) for room humidity, temperature and barometric pressure were applied to intake flow rates (McArdle, Katch and Katch, 1986) and an air volume correction factor applied to all exhaust oxygen and carbon dioxide volumes. Chamber sample air was assumed to be dry (passed through Drierite ($CaSO_4$)) and at room temperature and pressure during oxygen and carbon dioxide determinations.

Calorimetric procedures

Birds used in all experiments were Single Comb White Leghorn laying hens in full egg production (70-90%) weighing between 1500 and 1700 g. Hens were exposed to 14 hr of light per day and air flow

maintained in all chambers between 18 and 20 SLPM. Hens were maintained at constant temperatures, and water intake was unrestricted. Adaptation to experimental protocols was confirmed when hens showed constant feed intakes and heat productions for seven days (Davis et al., 1972). Up to four diets were evaluated simultaneously with measurements made for 23 hr per day with 1 hr used for cleaning, recording of feed intake and data acquisition.

Since dietary ME may change with temperature (Swain and Farrell, 1975) and possibly with age, diet and corresponding excreta samples were collected for each experiment from full-fed birds after adaptation to experimental diets and temperatures. Samples were analyzed for gross energy (GE) using a Parr adiabatic oxygen bomb calorimeter (model 1241, Parr Instrument Co., Moline, IL) and chromium oxide (Edwards and Gillis, 1959). ME values were calculated from GE and chromium oxide ratios.

Heat production was calculated from temperature and pressure corrected oxygen and carbon dioxide volumes according to the equation of Romijn and Lokhorst (1961). The correction for protein oxidation was ignored because the error in heat production is only .2% (Romijn and Lokhorst, 1966). Since adequate carbonate was included in all diets, no correction for carbon dioxide content of eggs laid during the experiments was necessary (Waring and Brown, 1965).

Data obtained for 3-5 days before restricted feeding, along with data obtained during restricted feeding, were expressed per kg physiological body weight ($PBW = BW^{.75}$) and subjected to regression analyses using energy balance, obtained as the difference between ME

intake and heat production, as the dependent variable and ME intake as the independent variable (Figure 2). Fasting heat production was estimated as the Y-intercept, and maintenance ME requirement as the X-intercept (Figure 2). The slope of the fitted regression line above the maintenance requirement represented the NEE of converting ME to retained energy. ME available above maintenance for productive purposes was calculated as the difference between maintenance ME requirements and total dietary ME intake.

Statistical comparisons of slopes and intercepts from the regressions were made according to Zar (1974). All regression and ad libitum data were subjected to analysis of variance across temperature and feeding regime and means were separated at the 5% level of probability ($P<.05$) using least significant difference (LSD) (Steel and Torrie, 1960).

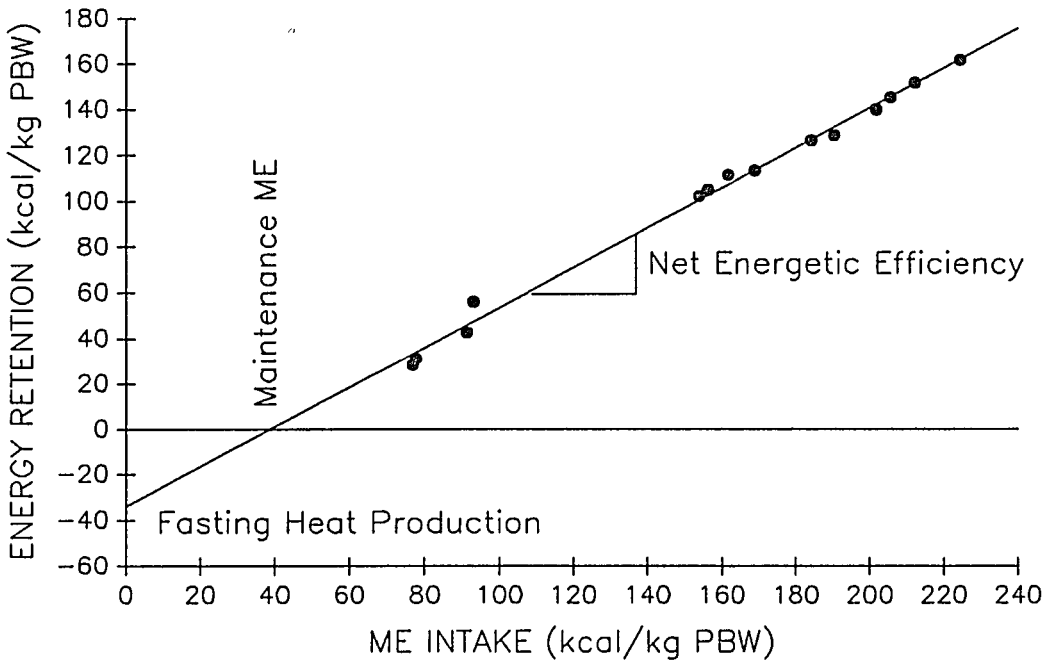

FIGURE 2. Regression of metabolizable energy (ME) consumption on energy retention.

CHAPTER 4

EFFECT OF DIETARY AMINO ACID AND PROTEIN LEVELS, AND ANIMAL FAT ON ENERGY UTILIZATION BY LAYING HENS

Evaluations of energy utilization by laying hens have been reported by a number of workers employing either energy balance trials or calorimetry (Waring and Brown, 1965; Grimbergen, 1970; van Es et al., 1970; Burlacu and Baltac, 1971; Davis et al., 1972, 1973). Studies using energy balance techniques based on carcass analyses have estimated energetic efficiencies of ME conversion to retained energy in the range of 60 to 70% depending on the diet employed, while higher efficiencies ranging from 80 to 90% have been obtained with calorimetry (Valencia, Maiorino and Reid, 1980a; Farrell, 1975). Davidson et al. (1968) compared the two techniques and found less heat production in calorimetry studies than with the carcass analysis technique; differences between the two methods were in the 3 to 12% range.

Valencia et al. (1980a) found lowered ($P<.05$) ME consumption and maintenance requirements by laying hens housed at 35 C in comparison with hens housed at 18.3 C. Dietary animal fat (AF) supplementation increased ME consumption at 35 C and allowed greater egg production during heat stress. Energetic efficiencies based on carcass energy analyses and egg energy output determinations, ranged from 78.2 to 80.1% and were not significantly ($P>.05$) affected by temperature or dietary AF treatments.

In another study, Valencia et al. (1980b) varied dietary protein from 12 to 20% in isocaloric diets at constant ambient temperatures of 21 and 32 C. Maintenance ME requirements were not affected by dietary protein level at 21 C; however, at 32 C maintenance ME was higher ($P<.05$) with 12 and 14% dietary protein in comparison with diets containing 16 to 20% protein. Energetic efficiencies were improved ($P<.05$) from 60.9 to 72.4% and from 50.6 to 70.6% with increased dietary protein at 21 and 32 C, respectively.

Before the availability of accurate oxygen and carbon dioxide analyzers, electronic flow meters and computer systems for data acquisition, indirect calorimeters (Blaxter and Graham, 1955; Hutchinson, 1955) suffered from low productivity, and high labor requirements. Automated calorimetry systems have now become more practical for research into dietary effects on energy utilization (Lundy et al., 1978), and the fasting metabolic rates of a large number (1120) of laying hens have been determined by Damme et al. (1987) using an indirect calorimetry system.

The objective of the present study was to evaluate energy utilization in laying hens fed diets formulated with different proportions of energy, protein and amino acids.

Materials and methods

Indirect calorimetry was utilized to determine energy utilization in laying hens fed different proportions of protein, amino acids and energy. Eight experimental diets were formulated using linear programming techniques to meet or exceed NRC (1984) recommendations for laying hens (Tables 1 and 2). The first four

TABLE 1. Percentage composition of experimental diets (Experiment 1)

Ingredient	NRC[1]	NRCAA[2]	HP[3]	HPAA[4]
	— Without Added Animal Fat —			
Ground milo	69.47	73.30	65.89	70.38
Soybean meal (48)	15.02	10.99	18.77	13.79
Dehydrated alfalfa meal	4.00	4.00	4.00	4.00
Ground limestone	8.58	8.58	8.33	8.58
Dicalcium phosphate	1.36	1.39	1.34	1.37
DL-Methionine	.12	.17	.17	.23
L-Lysine	----	.12	.05	.20
Salt	.35	.35	.35	.35
Vitamin mix[5]	1.00	1.00	1.00	1.00
Trace mineral mix[6]	.10	.10	.10	.10
	——— With 3% Animal Fat ———			
Ground milo	65.81	69.81	60.87	66.89
Soybean meal (48)	15.69	11.48	20.62	14.29
Dehydrated alfalfa meal	4.00	4.00	4.00	4.00
Ground limestone	8.57	8.57	8.56	8.57
Dicalcium phosphate	1.36	1.40	1.34	1.38
DL-Methionine	.12	.17	.16	.23
L-Lysine	----	.12	----	.19
Salt	.35	.35	.35	.35
Vitamin mix[5]	1.00	1.00	1.00	1.00
Trace mineral mix[6]	.10	.10	.10	.10
Animal fat	3.00	3.00	3.00	3.00

[1] NRC = 14.5% protein; formulated to meet National Research Council (NRC, 1984) amino acid and protein recommendations for laying hens.

[2] NRCAA = 12.9% protein; formulated without a minimum protein specification to meet NRC amino acid recommendations for laying hens.

[3] HP = 16% protein; formulated to exceed NRC amino acid recommendations for laying hens.

[4] HPAA = 14% protein; formulated without a minimum protein specification to have comparable amino acids to the HP diet.

[5] Supplied the following per kg of diet: 3965 IU vitamin A, 615 ICU vitamin D_3, 1.8 mg riboflavin, 11.2 mg niacin, 4.5 mg calcium pantothenate, 5.3 μg vitamin B_{12}, 2.2 IU d-α-tocopherol acetate, .9 mg menadione sodium bisulfite, 372 mg choline chloride, and 50 mg ethoxyquin.

[6] Supplied the following (ppm): 20 Fe, 60 Zn, 60 Mn, 4 Cu, and 1 Mo.

TABLE 2. Calculated nutrient composition of experimental diets (Experiment 1)

Nutrient	NRC[1]	NRCAA[2]	HP[3]	HPAA[4]
	— Without Added Animal Fat —			
Protein, %	14.50	12.91	16.00	14.03
ME[5], kcal/g	2.78	2.82	2.75	2.79
TSAA[6], %	.55	.55	.64	.64
Lysine, %	.68	.68	.84	.84
Calcium, %	3.75	3.75	3.66	3.75
Phosphorus, %	.55	.54	.56	.55
	——— With 3% Animal Fat ———			
Protein, %	14.50	12.84	16.45	13.95
ME[5], kcal/g	2.90	2.94	2.86	2.92
TSAA[6], %	.55	.55	.64	.64
Lysine, %	.69	.69	.84	.84
Calcium, %	3.75	3.75	3.75	3.75
Phosphorus, %	.55	.54	.56	.55

[1] NRC = formulated to meet National Research Council (NRC, 1984) amino acid and protein recommendations for laying hens.

[2] NRCAA = formulated without a minimum protein specification to meet NRC amino acid recommendations for laying hens.

[3] HP = formulated to exceed NRC amino acid recommendations for laying hens.

[4] HPAA = formulated without a minimum protein specification to have comparable amino acids to the HP diet.

[5] Metabolizable energy.

[6] Total sulfur amino acids.

experimental diets consisted of a 14.5% protein diet formulated to meet the laying hen requirements (designated NRC); a diet with similar amino acid levels (12.9% protein) without a minimum protein specification (designated NRCAA); a 16% protein diet with a higher level of amino acids (designated HP); and a diet with amino acids comparable to the HP diet (14% protein) without a minimum protein specification (designated HPAA). The remaining four diets were formulated to the same nutrient restrictions as the first four diets, but with 3% AF included in the formulation. These diets were designated NRC-F, NRCAA-F, HP- F and HPAA-F, respectively.

Six 12-month old Shaver 288 laying hens, in full production and weighing 1500 to 1600 g, were housed in the calorimeter chambers held at 21.1 ± .5 C. At the beginning of the experiment, diet and fecal samples were obtained for ME analysis. Following a 2-week adaptation period (Davis et al., 1972) during which the hens were fed ad libitum, the birds were full-fed for 3-5 days and then restricted to 60% of their ad libitum feed consumption for an additional 3-5 days to obtain data for regression analyses.

Results and discussion

Daily ad libitum feed intakes, and consequently ME intakes, showed considerable variation with dietary treatment whether expressed per bird or per kg PBW (Table 3). Birds fed the NRCAA diet consumed significantly less feed than those fed the NRC diet, suggesting that total nitrogen intake is important in maintaining feed consumption. Birds fed the NRC diet consumed 195.0 kcal/kg PBW/d when fed ad libitum

TABLE 3. Dietary protein and animal fat effects on nutrient intakes and energy utilization by laying hens (Experiment 1)

Criteria	NRC[1]	NRCAA[2]	HP[3]	HPAA[4]	NRC-F[1,5]	NRCAA-F[2,5]	HP-F[3,5]	HPAA-F[4,5]
Diet metabolizable energy (ME), kcal/g	2.66	2.79	2.65	2.74	3.11	3.00	2.97	2.84
Feed intake, g/b/d	112.3^{bc}	90.7^{e}	99.8^{de}	107.9^{cd}	100.4^{de}	97.4^{de}	125.8^{a}	120.2^{ab}
ME intake, kcal/b/d	298.8^{c}	253.2^{e}	264.9^{de}	295.8^{cd}	312.4^{bc}	291.9^{cd}	373.4^{a}	341.2^{b}
Protein intake, g/b/d	16.2	11.7	16.0	15.1	14.6	12.6	20.1	16.8
TSAA[6] intake, mg/b/d	651.3	517.0	668.7	733.7	562.2	535.7	830.3	817.4
Lysine intake, mg/b/d	763.6	625.8	848.3	927.9	702.8	672.1	1056.7	1045.7
Feed intake, g/PBW[7]/d	73.7^{bc}	59.9^{d}	68.2^{c}	75.6^{bc}	72.4^{bc}	72.1^{bc}	94.9^{a}	77.3^{b}
ME intake, kcal/PBW7/d	196.0^{cd}	167.1^{e}	180.9^{de}	207.1^{bc}	225.3^{b}	216.2^{bc}	281.7^{a}	219.6^{b}
Heat production, kcal/PBW[7]/d	144.0^{bc}	138.6^{cd}	130.7^{ef}	135.3^{de}	144.1^{bc}	156.5^{a}	147.6^{b}	125.2^{f}
Energy balance, kcal/PBW[7]/d	51.9^{e}	28.4^{f}	50.2^{e}	71.8^{cd}	81.2^{bc}	59.7^{de}	134.1^{a}	94.4^{b}
FHP8, kcal/PBW[7]/d	70.0^{ef}	81.7^{cd}	62.1^{f}	91.8^{b}	70.0^{ef}	73.3^{de}	101.8^{a}	89.1^{bc}
Maintenance ME, kcal/PBW[7]/d	112.8^{c}	123.9^{a}	102.4^{d}	116.0^{bc}	106.5^{d}	120.3^{ab}	122.5^{a}	107.2^{d}
Net energetic efficiency, %	62.2^{c}	66.1^{bc}	62.2^{c}	79.2^{a}	67.6^{b}	61.4^{c}	83.8^{a}	83.3^{a}
ME available for production, kcal/PBW[7]/d	83.2^{cd}	43.1^{e}	78.5^{d}	91.1^{bcd}	118.8^{b}	95.9^{bcd}	159.2^{a}	112.4^{bc}

$^{a-f}$ Means within a row not having common letter superscripts are significantly different ($P<.05$).

[1] NRC = 14.5% protein; formulated to meet National Research Council (NRC, 1984) amino acid and protein recommendations for laying hens.

[2] NRCAA = 12.9% protein; formulated without a minimum protein specification to meet NRC amino acid recommendations for laying hens.

[3] HP = 16% protein; formulated to exceed NRC amino acid recommendations for laying hens.

[4] HPAA = 14% protein; formulated without a minimum protein specification to have amino acids comparable to the HP diet.

[5] -F = 3% added animal fat.

[6] TSAA = total sulfur amino acids.

[7] PBW = physiological body weight = $BW^{.75}$.

and retained 51.9 kcal/kg PBW/d; lowering the total dietary protein to 12.9% decreased ME intake to 167.1 kcal/kg PBW/d and reduced energy balance to 28.4 kcal/kg PBW/d. As shown in Figure 3, NEE was the same (62.2 and 66.1%, respectively) for the NRC and NRCAA diets. Dietary total sulfur amino acid (TSAA) and lysine levels for these two diets (NRC and NRCAA) were the same, but the difference in feed consumption resulted in TSAA intakes which would be considered deficient (Guillaume and Summers, 1970; Reid and Weber, 1973; Reid et al., 1982). Valencia et al. (1980b) demonstrated increased heat production in laying hens having either low or high protein intakes with a minimal value in the range of 17 to 20 g protein per day.

Feeding a higher protein diet (HP) resulted in protein intakes which were similar to those obtained with the NRC diet. This 16% protein diet (HP) produced ME intakes which were significantly lower than those obtained with the NRC diet and significantly lower levels of heat production (Table 3). Birds fed the HP diet consumed 7.4% less feed per kg PBW and maintained the same energy balance. NEE was not significantly affected by the higher protein diet.

The most significant responses in the low fat diet series were obtained with the HPAA diet. Birds fed this diet maintained an energy balance of 71.8 kcal/kg PBW/d in comparison with 50.2 kcal/kg PBW/d for the HP diet. The improved energy balance resulted from a NEE of 79.2% for the birds fed the HPAA diet. The energy usage by these birds was significantly better than that of the NRC-fed birds, even with a slightly lower total protein intake. This appeared to be due to the higher intake of TSAA and is in agreement with previous work (Reid and

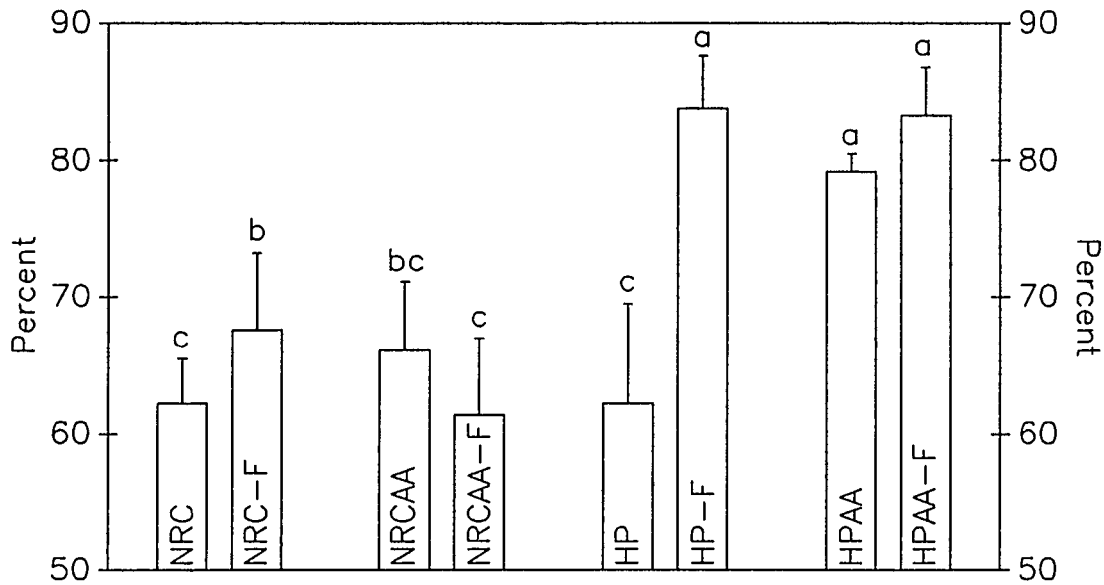

FIGURE 3. Effects of dietary protein (amino acid) level and animal fat supplementation on the net energetic efficiency of laying hens housed at 21.1 C (Experiment 1).

Maiorino, 1984) in which energy usage was evaluated by other methods. Latshaw (1981) reported that diets based on amino acid restrictions without a total protein specification, similar to the HPAA diet, were as effective for egg production as those which included a total protein restriction.

Addition of fat to poultry diets has been reported to improve performance and feed conversion, especially during periods of high temperature (Fuller and Rendon, 1979; Valencia et al., 1980a). Birds fed supplemental AF (3%) in the NRC diet (NRC-F) consumed 30.9% more ME per kg PBW than those fed the NRC diet alone, and had a 56.5% increase in energy balance. NEE was also improved ($P<.05$) from 66.2% for the NRC diet to 67.6%. Heat production levels which were not altered from the NRC-fed level by the addition of AF even though ME intake was increased (Table 3).

The NRCAA diet was also improved by the addition of animal fat. Birds fed the NRCAA-F diet showed a significant increase in ME intake over the NRCAA diet, but there was also a significant increase in heat production resulting in a greater than twofold increase in energy balance without a significant change in NEE.

Feeding supplemental fat in a higher protein diet (HP-F) increased ME intake by 55.7%, increased energy balance by 167.1% and improved energetic efficiency from 62.2% for the HP fed birds to 83.8% for those fed HP-F. The HPAA-F diet also produced improvements in heat production (7.5% decrease), energy balance (31.5% increase) and NEE (Table 3 and Figure 3).

These data indicate that feeding supplemental fat can significantly improve energy balance provided adequate amounts of essential amino acids and total protein are present in the diet. The energy balance of hens fed all four of the diet formulations in this study was significantly improved by the inclusion of 3% animal fat; however, the greatest improvement was noted with the HP-F diet which contained 16% protein and .65% TSAA.

Along with the greatest improvement ($P<.05$) in NEE, the HP-F diet also showed the greatest increase ($P<.05$) in maintenance energy requirements and fasting heat production (Figures 4 and 5) in comparison with the HP diet. This suggests that additional fat increases maintenance energy requirements and fasting heat production in laying hens.

Energy intakes above maintenance requirements represent the energy available for sustained productive purposes. This criterion was improved ($P<.05$) by the NRC-F, NRCAA-F and HP-F diets in this study (Figure 6). Using this criterion, the HP-F diet produced the highest ME intakes above maintenance. In the absence of added fat, the NRC diet provided ME above maintenance equivalent to the higher TSAA and lysine levels present in the HPAA diet.

The results of this study suggest that TSAA levels higher than those recommended by the NRC (1984) are needed for optimum energy utilization. The HPAA diet contained 14% protein with .65% TSAA, but supported energy utilization significantly better than the NRC diet which contained 14.5% protein with only .55% TSAA.

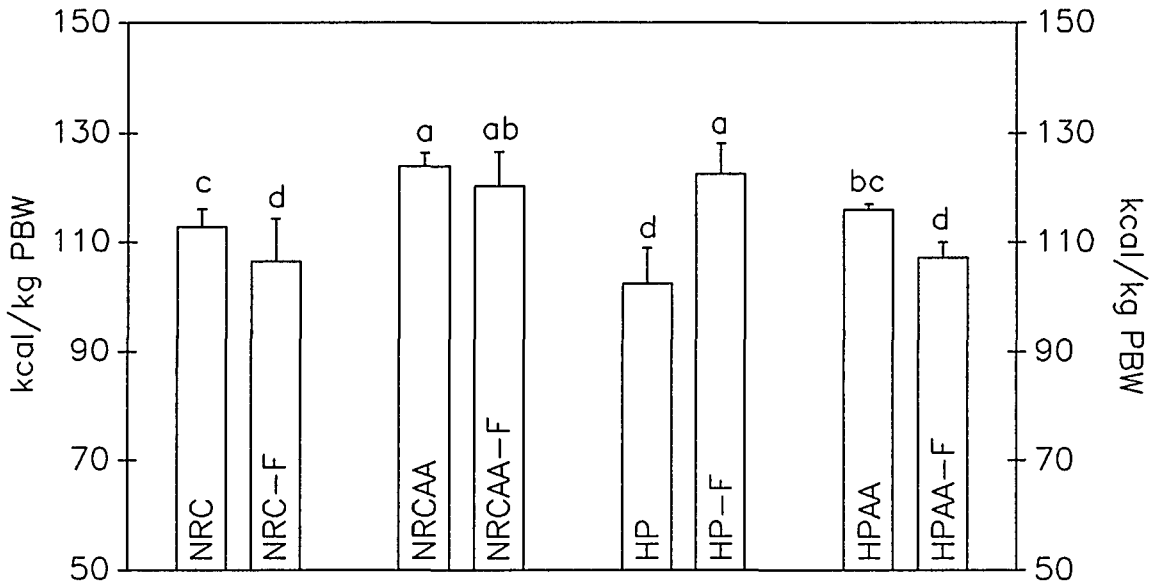

FIGURE 4. Effects of dietary protein (amino acid) level and animal fat supplementation on the maintenance metabolizable energy (ME) requirements of laying hens housed at 21.1 C (Experiment 1).

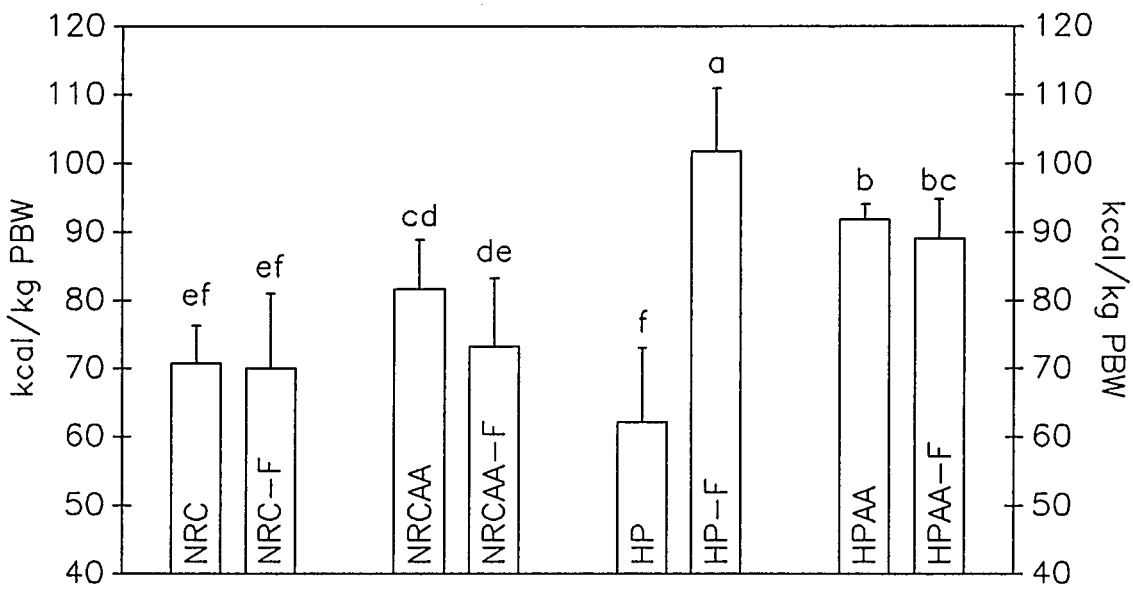

FIGURE 5. Effects of dietary protein (amino acid) level and animal fat supplementation on the fasting heat production of laying hens housed at 21.1 C (Experiment 1).

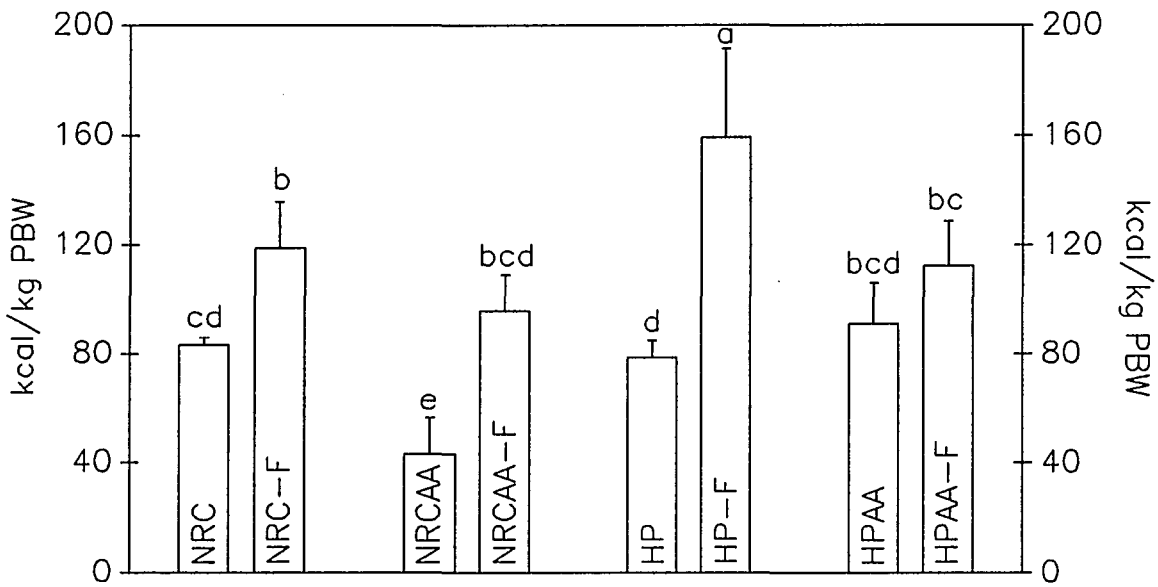

FIGURE 6. Effects of dietary protein (amino acid) level and animal fat supplementation on the metabolizable energy (ME) available for production by laying hens housed at 21.1 C (Experiment 1).

Effects of dietary protein on ad libitum feed intake were apparent when the diets were fed without supplemental fat. Increasing total protein from 14.5 to 16% (NRC vs. HP) lowered total feed intake by 13% (112.3 vs. 99.8 g/bird/d) indicating that laying hens tend to overconsume feed and energy when total protein is limiting in the diet.

Poultry feed formulation techniques, with the use of computers, have developed toward specifying individual amino acid levels and ignoring total protein intake. The results presented have suggested that total dietary protein is also important. The HP diet, with higher amino acid specifications than NRC, was improved by lowering total protein from 16 to 14.5% in the HPAA diet. This change improved ME intake, NEE and energy balance while such changes in formulations for the NRC and NRCAA diets were highly detrimental.

NRC recommended levels for TSAA are not adequate for laying hens and at least .65% methionine plus cysteine are required for maximum energetic efficiency.

The dramatic improvements noted for the NRCAA diet when fat was added (NRCAA-F) are not entirely explained. There were rather large improvements in energy balance and fasting heat productions attributable to animal fat. Some earlier studies (Reid et al., 1985; unpublished data) demonstrated increased availability of methionine with supplemental fat and this may explain the improvements noted here.

CHAPTER 5

EFFECT OF DIETARY FAT SOURCE ON ENERGY UTILIZATION

Fats and oils vary in the proportion of saturated and unsaturated fatty acids, and this may affect their utilization by laying hens. Assuming a NEE of glucose as 100%, De Groote et al. (1971) found no differences in NEE for lard (103.1), degummed soybean oil (102.9), fancy tallow (107.3), prime tallow (108.1) and brown grease (102.9). These workers did report improved greater tissue energy gains in growing chicks fed a prime tallow supplemented diet and suggested that tallow might be more efficiency utilized for growth relative to lard and soybean oil.

Fuller and Rendon (1977) determined body weight gain, feed consumption, caloric efficiency (body weight gain/feed consumed), heat production, dietary heat increment and fasting heat production of growing broiler chicks fed corn oil, palm oil, beef tallow, coconut oil, acidulated cottonseed soapstock, poultry fat and feed grade animal fat (FGAF) at 10 and 20% levels in a glucose monohydrate basal diet. Body weight gain was increased with all fat sources, except coconut oil and acidulated cottonseed soapstock. In comparison with the basal diet, these workers found decreased heat productions with all fat sources, except tallow and decreased heat increments with all the fat sources. Among all the fat sources, the highest heat productions were obtained with the more saturated fats. The highest caloric

efficiencies, along with the lowest heat increment values, were obtained with poultry fat, suggesting that similarities in composition between poultry fat and fat deposited in chickens reduces the amount of energy needed to metabolize supplemental dietary fat (Fuller and Rendon, 1977).

Hulan, Proudfoot and Nash (1984), using broiler chickens at 29 and 48 days of age, measured as body weight gain, feed conversion and carcass quality (commercial grading system) when fed either beef tallow, poultry grease, pork lard or rapeseed oil, singly and in various combinations, at 2-3% of the diet. Although not significant, combining all three fat sources resulted in the best feed conversion and carcass quality (Grade A), along with the highest body weight gains, compared with each fat source alone suggesting that a synergism between unsaturated and saturated fat sources improved broiler chicken performance. This was confirmed by mixing rapeseed oil (50:50) with each of the fats in a second experiment. Nash et al., (1984) also found that feeding of rapeseed oil with each fat lowered the saturated to unsaturated fatty acid ratio thus suggesting that fatty acid synergism improves performance in broiler chickens. Similar results were reported by Lall and Slinger (1973) who reported additive effects in dietary energy content when tallow and rapeseed oil were equally mixed suggesting that absorption is increased due to a balanced fatty acid make-up.

Supplementation of poorly utilized feedstuffs with unsaturated fats improves their utilization. Antoniou and Marquardt (1982) fed growing Leghorn cockerels rye and wheat based diets supplemented with

two levels of tallow or soybean oil (3 and 8%) to determine the effects on feed intake, body weight gain and feed-to-gain ratio (feed intake/body weight gain). These workers found that adding soybean oil to the poorly digested rye diet improved the above parameters in comparison with tallow additions. Similar improvements occurred with the addition of either fat to a wheat based diet but differences between fat types were not significant.

In an additional study, Antoniou and Marquardt (1982) fed either 8% tallow, coconut fat, soybean oil or sunflower oil in the same rye and wheat basal diets used previously, to determine the effects of these fats on the same parameters as defined in the previous study. Of the four fats used, greatest improvements in feed intake, body weight gain and feed-to-gain ratio occurred with unsaturated fat additions to the rye diets (soybean and sunflower oils) in comparison with saturated fat (tallow and coconut fats). The same trends occurred when these fats were added to the wheat basal but the differences between the saturated and unsaturated fats were not as pronounced. These workers suggested that either reduced bile salt excretions, nutrient interactions (pentosans in the rye tying up bile salts) or a proliferation of intestinal mucosa inhibiting enterohepatic circulation of bile salts contributed to the poor utilization of the rye - saturated fat diets.

Materials and methods

In this experiment, indirect calorimetry was utilized to determine the effect of AF and various unsaturated fat additions to a

basal diet on energy utilization in laying hens. Six diets were fed to 6-month old Hyline WS-36 laying hens maintained at 21.1 C in the calorimeter chambers. These diets consisted of a basal diet, and the basal diet supplemented with of 8% AF, cottonseed oil (CSO), cod liver oil (CLO), safflower oil (SFO), or corn oil (CO) (Table 4). The composition of each fat was determined by gas chromatographic analysis of fatty acid methyl esters prepared from chloroform:methanol (2:1, v/v) extracts of each fat by reaction with 12% boron trifluoride in methanol (American Oil Chemists' Society, 1972). A Shimadzu GC-8 gas chromatograph equipped with a Spectra-Physics SP4270 integrator was employed for these analyses. Analyzed compositions of the test fats are listed in Table 5.

At the beginning of the experiment, diet and fecal samples were obtained during ad libitum feeding for ME determinations. After adaptation to each diet (Davis et al., 1972), hens were restricted to 40% of their ad libitum intake for 3-5 days to obtain data for regression analysis.

Results and discussion

In comparison with the basal diet, total feed intake was reduced ($P<.05$) when hens were fed CSO, CLO and SFO; increased ($P<.05$) with CO; and unaffected by AF feeding (Table 6). Feed intakes normally decrease as dietary ME is increased (Fuller and Rendon, 1977). ME intake was increased ($P<.05$) with CO and AF, while no differences ($P>.05$) were obtained with the other fat treatments in comparison with the basal fed birds. Hens fed CLO, SFO, CO and AF had significantly

TABLE 4. Composition of experimental diets (Experiment 2)

Ingredient	
Ground milo, %	61.89
Soybean meal (48), %	22.38
Alfalfa (17), %	5.00
Ground limestone, %	7.59
Fat source[1], %	8.00
Dicalcium phosphate, %	1.31
DL-Methionine, %	.18
Salt, %	.35
Vitamin mix[2], %	1.00
Trace mineral mix[3], %	.10
Chromium oxide, %	.20
Total, %	108.00
Calculated nutrient composition[4]	
Protein, %	17.53
Metabolizable energy, kcal/g	2.67
Total sulfur amino acids, %	.65
Lysine, %	.75
Calcium, %	3.40
Phosphorus, %	.58

[1] Fat source = cottonseed oil, cod liver oil, safflower oil, corn oil or animal fat.

[2] Supplied the following per kg of diet: 3965 IU vitamin A, 615 ICU vitamin D_3, 1.8 mg riboflavin, 11.2 mg niacin, 4.5 mg calcium pantothenate, 5.3 μg vitamin B_{12}, 2.2 IU d-α-tocopherol acetate, .9 mg menadione sodium bisulfite, 372 mg choline chloride, and 50 mg ethoxyquin.

[3] Supplied the following (ppm): 20 Fe, 60 Zn, 60 Mn, 4 Cu, and 1 Mo.

[4] Nutrient composition of basal diet without added fat.

TABLE 5. Fatty acid composition of fats (Experiment 2)

Fatty Acid	Cottonseed oil	Cod liver oil	Safflower oil	Corn oil	Animal fat	Yolk fat
	%					
C16:0	11.3	10.2	7.2	11.8	26.5	25.3
C16:1	.3	11.1	.1	.3	4.1	6.2
C17	---	1.1	---	.1	1.5	.2
C18	1.8	2.4	2.4	1.8	16.1	8.6
C18:1	27.1	23.2	13.5	27.2	40.1	42.2
C18:2	58.0	1.5	75.5	57.4	3.5	12.1
C18:3ω6	.9	.6	---	.9	.2	.5
C20	.3	---	.3	.3	.4	.1
C22	---	9.2	.2	.2	---	---
Saturated	13.5	28.7	10.3	14.2	48.9	34.9
Monounsaturated	27.6	56.6	14.0	27.5	44.9	49.2
Polyunsaturated	58.9	14.7	75.7	58.3	6.2	15.9
Polyunsaturated ω3	---	11.1	.1	---	.2	1.1
Polyunsaturated ω6	.8	1.2	.1	.9	.2	2.5
P/S ratio	4.40	.49	7.35	5.66	.13	.46

TABLE 6. Effects of dietary fat source on energy utilization by laying hens (Experiment 2)

Criteria	Basal[1]	CSO[2]	CLO[3]	SFO[4]	CO[5]	AF[6]
Diet metabolizable energy (ME), kcal/g	2.81^{f}	3.35^{b}	3.28^{e}	3.31^{d}	3.34^{c}	3.37^{a}
Feed intake, g/b/d	99.2^{b}	84.7^{c}	87.0^{c}	88.0^{c}	110.8^{a}	97.5^{b}
ME intake, kcal/b/d	278.2^{c}	283.8^{c}	285.2^{c}	291.6^{c}	369.9^{a}	328.9^{b}
Heat production, kcal/b/d	107.5^{c}	104.6^{c}	132.9^{a}	122.6^{b}	120.1^{b}	131.1^{a}
Energy retention, kcal/b/d	170.8^{c}	179.2^{c}	152.4^{d}	169.1^{cd}	249.8^{a}	197.8^{b}
Feed intake, g/PBW[7]/d	70.1^{b}	58.8^{c}	58.4^{c}	59.9^{c}	80.6^{a}	67.9^{b}
ME intake, kcal/PBW[7]/d	196.6^{c}	197.2^{c}	191.3^{c}	198.3^{c}	269.2^{a}	229.1^{b}
Heat production, kcal/PBW[7]/d	75.9^{d}	72.7^{e}	89.1^{ab}	83.3^{c}	87.4^{b}	91.1^{a}
Energy retention, kcal/PBW[7]/d	120.6^{c}	124.5^{bc}	102.2^{d}	115.0^{cd}	181.8^{a}	138.0^{b}
Net energetic efficiency, (%)	90.6^{b}	99.1^{a}	86.0^{c}	99.8^{a}	87.3^{bc}	91.1^{b}
Maintenance ME, kcal/PBW[7]/d	63.3^{d}	72.9^{c}	74.0^{bc}	85.0^{a}	61.2^{d}	77.8^{b}
FHP[8], kcal/PBW[7]/d	57.3^{ab}	70.4^{c}	62.2^{b}	83.9^{d}	53.4^{a}	70.5^{c}
ME available for production, kcal/PBW[7]/d	133.3^{bc}	120.5^{c}	117.3^{c}	113.6^{c}	208.0^{a}	151.3^{b}

$^{a-e}$ Means within a row not having common letter superscripts are significantly different ($P<.05$).

[1] Basal = formulated to meet National Research Council (NRC, 1984) nutrient recommendations for laying hens.

[2] CSO = basal diet + 8% cottonseed oil.

[3] CLO = basal diet + 8% cod liver oil

[4] SFO = basal diet + 8% Safflower oil.

[5] CO = basal diet + 8% corn oil.

[6] AF = basal diet + 8% animal fat.

[7] PBW = physiological body weight = $BW^{.75}$.

higher ($P<.05$) heat production in comparison with the basal and CSO diets.

Among the fat sources fed to growing broiler chicks by Fuller and Rendon (1977), higher heat productions were associated with the saturated fats (tallow, poultry fat and FGAF) which is in partial agreement with the results of this study. Except for CLO, energy retention followed the same significant trends as ME intake with the higher values associated with CO and AF, respectively, while the lowest was associated with CLO (Table 6).

When expressed per kg PBW in Table 6 and in comparison with the basal diet, feed intakes for all fat sources, except for CO and AF, were reduced ($P<.05$). Hens fed diets with added CO and AF showed increased ($P<.05$) ME intake in comparison with the basal diet. In agreement with Fuller and Rendon (1977), the highest and lowest heat productions of 91.1 and 72.7 kcal/PBW/d occurred when hens were fed AF and CSO, respectively, suggesting that differences in fatty acid composition affects the metabolism of supplemental fats in laying hens; although heat production measurements are dependant on total feed intake. The amounts of energy retained by hens fed SFO and CO were similar to those fed the basal diet. In comparison with the basal diet in (Table 6), hens fed CO and CLO retained the most ($P<.05$) and the least ($P<.05$) amount of energy, respectively.

NEE for the SFO and CSO diets was quite high at 99.1 to 99.8%; while the CLO and CO diets showed the lowest ($P<.05$) NEE (Figure 7). The NEE data do not appear to be correlated with the degree of unsaturation. The higher efficiencies obtained with CSO and SFO in

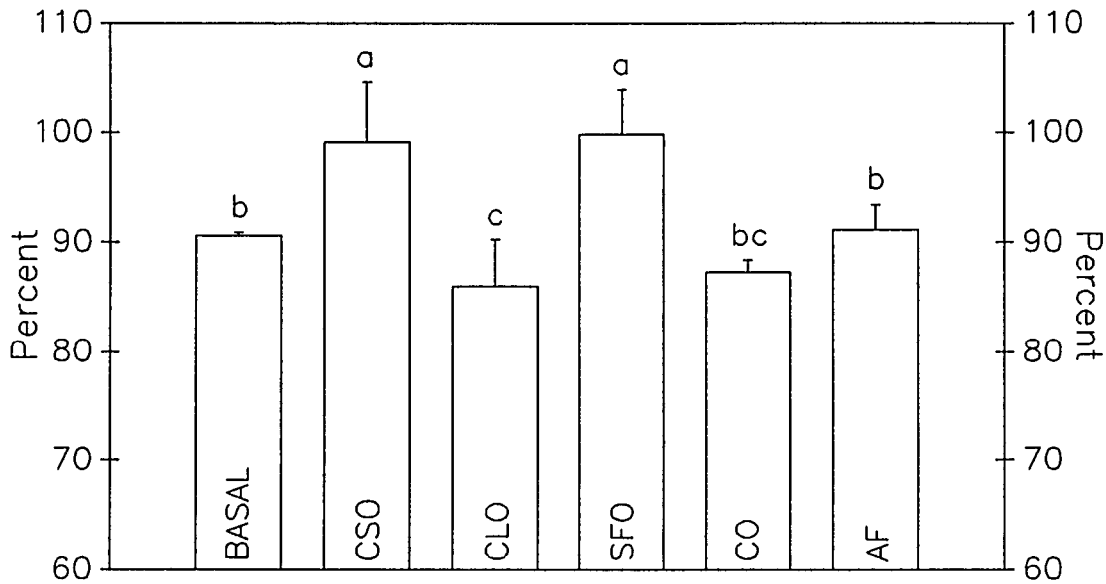

FIGURE 7. Effect of fat source on the net energetic efficiency of laying hens housed at 21.1 C (Experiment 2).

comparison with CLO and CO are not explained by the fatty acid composition data (Table 5).

Feeding CLO, CO or AF did not substantially change the NEE from that obtained for the basal fed birds, only CSO and SFO significantly improved energetic efficiency (Figure 7). With the exception of the CO treatment, supplemental fat significantly increased maintenance ME requirements of the laying hens in this study (Figure 8). Maintenance ME was 63.3 kcal/kg PBW for birds fed the basal diet (61.2 kcal for CO) and this was increased to 72.9 kcal with CSO, 74.0 with CLO, 85.0 with SFO and 77.8 with AF. The increased maintenance needs associated with supplemental fat, and the close similarity of ME intakes for the basal diet and the CSO, CLO, SFO treatments resulted in no improvements in ME available above maintenance for production with these sources (Figure 9). The CO and AF treatments each produced significant improvements in ME intake, and as a result of comparable maintenance ME needs for the basal and CO birds, ME above maintenance was significantly higher for the CO treatment. Animal fat produced increases in both ME intake and maintenance and an insignificant improvement in ME available above maintenance for production (Figure 9).

The differences obtained among the fat sources used in this study suggest differences in metabolism associated with fatty acid composition. De Groote et al. (1971) reported relative NEE of 102.9 to 108.1% for several fats using broiler chickens. In contrast, our study with laying hens indicates higher net efficiencies for two of the vegetable oils (CSO and SFO); but not for either CLO or CO.

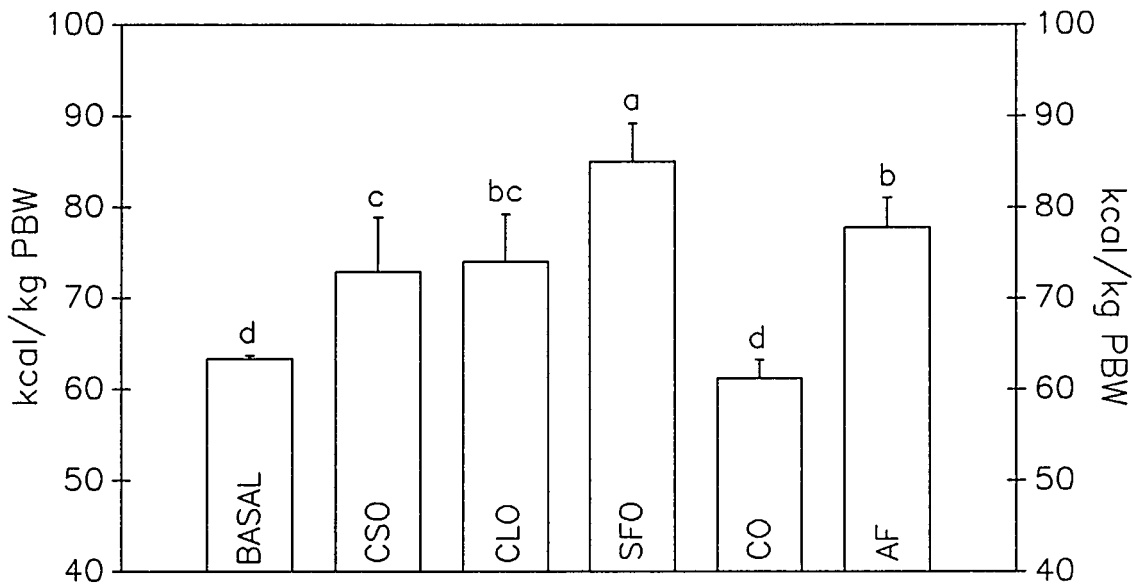

FIGURE 8. Effect of fat source on the maintenance metabolizable energy (ME) requirements of laying hens housed at 21.1 C (Experiment 2).

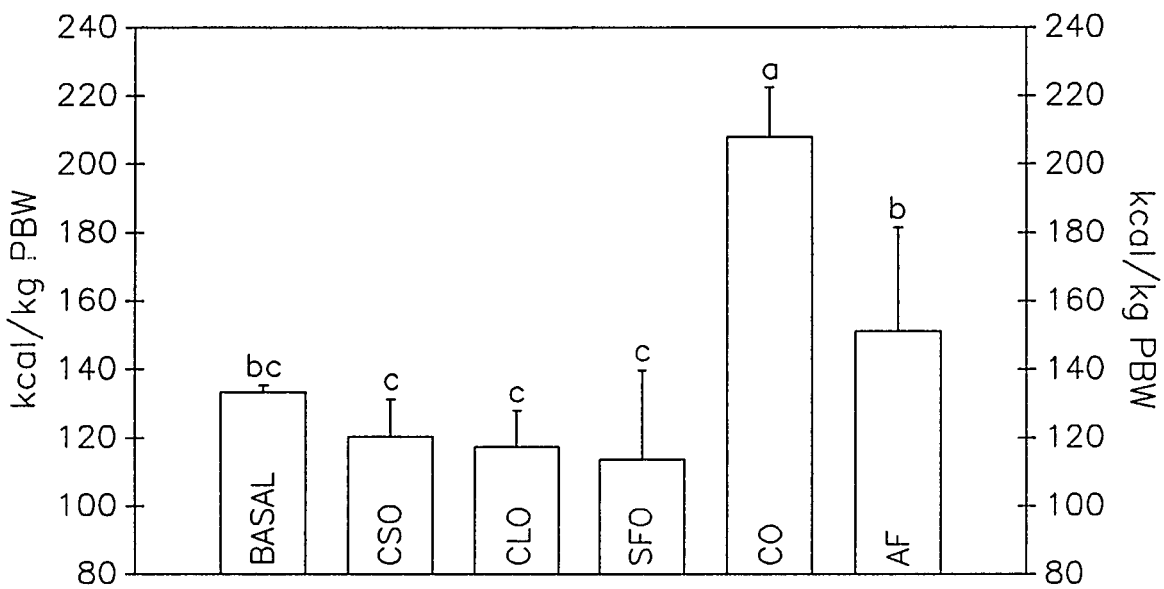

FIGURE 9. Effect of fat source on the metabolizable energy (ME) available for production by laying hens housed at 21.1 C (Experiment 2).

The study of Fuller and Rendon (1977) did show differences in energy utilization among the fats tested; the highest net efficiency was obtained with poultry oil for broilers. This might suggest that supplemental fat composition closely associated with that of the body depots results in improved efficiency. In our study, a comparison of egg yolk fatty acids with dietary fats failed to substantiate the findings of Fuller and Rendon (1977) in that the highest NEE was obtained with CSO and SFO, although not with CO which is quite similar to CSO, each of which has over five times the polyunsaturated fatty acid content of egg yolk (Table 7). CLO is probably the closest in fatty acid composition to egg yolk and produced a NEE significantly lower than the basal diet.

Recently, Rothwell and Stock (1987) compared lard, corn oil and a mixture of these using rats. They found that only the lard (saturated fat) increased energy gain while ME intakes were higher, expressed per kg PBW, for the mixed and saturated diets but not for corn oil. Resting heat production, measured as oxygen consumption, were increased by all fat supplements in the order of mixed greater than corn oil but unaffected by the lard. These results suggest that the laying hen metabolizes dietary fat in a different manner from the rat. Indeed the rat synthesizes fatty acids in both liver and adipose tissue while in poultry fatty acid synthesis occurs only in the liver.

The significant increases in maintenance ME requirements and heat productions which resulted from the supplemental fat, except for CO, are probably due to the requirements for saturation of the fatty acids prior to $\beta\epsilon\tau\alpha$-oxidation. Calculations of fasting fat metabolism

TABLE 7. Egg yolk fat composition, fasting fat use and net energetic efficiency (Experiment 2)

Criteria	Sat[1]	Mono[2]	Poly[3]	Fat Use[4]	NEE[5]
Basal	34.9	49.2	16.0	7.53[d]	90.6[b]
8% CSO	33.7	41.3	25.0	10.33[b]	99.1[a]
8% CLO	36.2	49.9	13.9	11.39[ab]	86.0[c]
8% SFO	34.3	28.7	37.0	11.70[a]	99.3[a]
8% CO	33.2	35.3	31.5	8.87[c]	87.3[bc]
8% AF	35.1	50.8	14.1	11.80[a]	91.1[b]

[a-d] Means within a column not having common letter superscripts are significantly different ($P<.05$).

[1] Saturated fatty acids, %

[2] Monounsaturated fatty acids, %

[3] Polyunsaturated fatty acids, %

[4] Under fasting conditions, g/b/d

[5] Net energetic efficiency, %

values by regression of fat use on ME intake suggest that the fats fall into two groups. The basal level of fat use (Table 7) was 7.52 ± .19 g/day (CSO, 10.33 ± 1.6; CLO, 11.39 ± 1.36; SFO, 11.70 ± 1.30; CO, 8.87 ± .54; and AF, 11.80 ± .99 g/day). These values roughly correlate with the maintenance ME values (Table 6).

In our study, a practical interpretation suggests CO or AF as the only fat tested which significantly increased ME intake, energy retention and ME available for production, and as the ones which would be expected to improve egg production in laying hens.

CHAPTER 6

EFFECT OF FAT SOURCE, ESSENTIAL AMINO ACID DEFICIENCY AND ENVIRONMENTAL TEMPERATURE ON ENERGY UTILIZATION

Dietary composition, environmental temperature and strain of bird have all been shown to affect energy utilization in laying hens. Farrell (1974) determined the energetic efficiency of 8 diets, containing from 2.17 to 3.65 kcal ME/g young crossbred cockerels fed above and below maintenance ME requirements. Combining all ME intake data above and below maintenance produced energetic efficiencies ranging from 79.3 (3.29 kcal/g diet) to 57.1% (2.75 kcal/g diet). Below maintenance data produced a combined energetic efficiency of 79.9%.

Farrell (1974) suggested that differences found in energetic efficiencies above and below maintenance were due to differences in chemical pathways for tissue synthesis, nutrient oxidation and activity. Birds fed below maintenance were less active thus reducing heat production and increasing energetic efficiency. Deighton and Hutchinson (1940) found that heat production of birds was increased 45% when standing rather then sitting.

Differences in energy metabolism of Black Australorp, Black Australorp * White Leghorn (crossbred) and White Leghorn laying hens were evaluated by Farrell (1975). Energetic efficiencies for the Black Australorp, crossbred, and White Leghorn hens were 79, 84 and 92%,

respectively. However, maintenance ME requirements were 83, 88 and 105 kcal/kg/d for the Australorp, crossbred, and White Leghorn, respectively. The lower maintenance ME values obtained for the Australorp and crossbred strains were probably due to low fasting heat productions (66 and 82 kcal/kg/d) and a low activity component due to the docile nature of the Australorp strains. The Australorp strains required 217 and 213 kcal ME/d as opposed to the Leghorn (183 kcal ME/d); both Australorp strains, due to their greater body weight, needed more ME for maintenance and production compared with the Leghorn.

Laying hens are frequently in negative energy balance during egg production and use body reserves to meet egg production requirements, (Farrell, 1975). Birds in positive or negative energy balance (Hoffman and Schiemann, 1973) had ME utilization efficiencies of 80 and 100%, respectively.

Temperature effects

Utilizing carcass analysis to determine energy retention, Davis et al. (1972) subjected 31-week old Sterling White-Links laying hens to a constant 10 or 35 C for three weeks to determine the effects of environmental stress on energy retention. In comparison with hens at 10 C, those at 35 C showed significant decreases in feed and ME consumptions, increased weight loss and reduced heat production, while no significant changes occurred in egg production or egg weight; and gross efficiency of egg production (egg calories/ME intake × 100) was increased from 24 to 34%.

Total sulfur amino acid deficiency

Reid and Maiorino (1984) determined the effect of various non-toxic levels of TSAA (.47-.51%) on energy utilization in laying hens at 15.6 and 32.2 C. Incremental increases in TSAA levels increased energy retention from 79.7 to 98.7 kcal/bird/d at 32.2 C, while added TSAA increased energy retention from 115.8 to 142 kcal/bird/d at 15.6 C. There was a trend for decreased heat production with increasing TSAA levels at 32.2 C. At 15.6 C, heat production averaged 225.4 kcal/bird/d for all non-toxic TSAA treatments with the lowest heat production of 222.1 kcal/bird/d occurring with the .49% TSAA level.

NEE at 32.2 C ranged from 52.1 to 63.8% with significant differences between the .49 and .51% TSAA levels. Energetic efficiency was increased (from 56.8 to 60.2%) with increasing TSAA levels at 15.6 C. No significant differences in energetic efficiency were obtained between temperatures at any TSAA level (Reid and Maiorino, 1984).

Reid and Maiorino (1984) also found no significant differences in ME intake among the treatments at 32.2 C (average intake of 270 kcal/bird/d). ME intakes were about 50 kcal/bird/d higher at 15.6 C and significant differences were noted between the .47 and the higher two TSAA diets. Since the highest energy retention and the greatest ME intake occurred when laying hens were fed the .51% TSAA diet at 15.6 C, Reid and Maiorino (1984) suggested that laying hens require 609 mg TSAA/bird/d and 17.3 g protein for maximum energy retention at this temperature.

Tallow and corn oil

Kussaibati, Guillaume and Leclercq (1981) reported that tallow contains approximately 26 and 62% saturated and unsaturated fatty acids, respectively, while corn oil contains 13 and 84%, with 67% of the unsaturated portion as linoleic acid. Utilizing a total collection procedure, Kussaibati et al. (1981) found no significant differences in dietary ME or true metabolizable energy (TME) between tallow and corn oil.

Farrell (1978), using broiler chicks (16-28 days old), fed 0, 4, 8 and 11% corn oil or 5 and 14% tallow and determined NEE with an indirect closed-circuit calorimeter. NEE for the basal diet was 77% and the data for the fat treatments were pooled to yield a NEE of 82%, suggesting no differences in efficiency between the diets supplemented with tallow or corn oil for growing broilers.

Utilizing carcass analysis, Fuller and Rendon (1979) have determined the energetic efficiency (energy gained/energy absorbed) of corn oil and poultry fat with 5-week old Cobb broilers. There were no significant differences in feed efficiency between the fats at any dietary level. In comparison with controls, caloric efficiency increased significantly with 10% corn oil and with both the 15 and 20% poultry fat additions. A greater fat deposition was found for poultry fat, in comparison with corn oil, due presumably to the similarity in composition of poultry fat to the fat deposited in chickens. This may explain the greater caloric efficiency and lower heat increment values found for diets supplemented with poultry fat. Assuming a gross energy for fat at 9 kcal/g, Fuller and Rendon (1979) found no significant

differences in calculated or determined dietary ME, with either fat source, suggesting that source and level of fat have no effect on dietary ME.

Fat source and lipogenic enzymes

Waterman et al. (1975) found that feeding male crossbred chicks 2% safflower oil significantly reduced hepatic fatty acid synthetase activity in comparison with feeding 2% tallow. These workers suggested that unsaturated fat sources reduce hepatic lipogenic enzyme activities to a greater extent than saturated fats. Similar results have been obtained by Pearce (1968) who found that two strains of laying hens (Hybrid 4 and Hyline) fed isocaloric diets with 2% corn oil showed reduced hepatic acetyl-CoA, citrate-cleavage enzyme and isocitrate dehydrogenase activities.

Iritani and Fukuda (1980) fed 5-week old male Wistar rats .5 to 10% corn oil in sucrose diets and determined triglyceride and phospholipid synthesis and fatty acid enzyme activities. When liver slices were incubated with radioactive glycerol and palmitic acid, substrate incorporation was significantly reduced in the 5 and 10% dietary corn oil groups. These results correlated with significantly decreased activities of hepatic α-glycerophosphate acyltransferase and diacylglycerol transferase with 5 and 2% corn oil, respectively. In comparison with .5% corn oil, fatty acid synthesis enzymes (glucose-6-phosphate dehydrogenase, malic enzyme, and acetyl-CoA carboxylase) were significantly reduced by as much as 66% by 10% corn oil.

The present studies were carried out to evaluate the effects of temperature, supplemental fat type and TSAA deficiency on energy utilization by laying hens.

Materials and methods

In three experiments, indirect calorimetry was utilized to determine the effect of fat source and TSAA deficiency (TSAAD) on energy utilization in laying hens exposed to constant temperatures of 10, 21.1 and 32.2 C. The first experiment consisted of four diets fed to eight 5-month old DeKalb XL laying hens housed in calorimeter chambers maintained at 21.1 ± .5 C. These diets consisted of a basal diet, two basal diets with 7% AF or 7% corn oil (CO) formulated in, and a basal diet formulated to be deficient in total sulfur amino acids (.52% TSAA) (Table 8).

After adaptation to experimental conditions (Davis et al., 1972), diet and fecal samples were obtained for ME analysis. Hens were then restricted consecutively to 20, 80, 30, 70, and 40% of their ad libitum intake over a 12-day period to obtain data for regression analysis above (70 and 80%) and below (20, 30 and 40%) maintenance ME requirements. In the second part of this study utilizing the same diets, hens were fed for 1 hr, twice a day (meal feeding), to determine if regulating feed intake would improve energy utilization.

The second study in this series employed the fat supplemented diets fed to the same hens, now seven months old, maintained at 32.2 ± .5 C. After hens returned to constant feed consumptions and heat productions, diet and fecal samples were again obtained for ME

TABLE 8. Composition of experimental diets (Experiment 3)

Ingredient	Diet type			
	Basal	7% AF	7% CO	TSAAD[1]
Ground milo, %	65.41	56.98	56.98	65.39
Soybean meal (48), %	20.54	21.97	21.97	20.69
Alfalfa (17), %	3.00	3.00	3.00	3.00
Ground limestone, %	7.95	7.93	7.93	7.95
Dicalcium phosphate, %	1.32	1.35	1.35	1.32
DL-Methionine, %	.13	.13	.13	----
Animal fat, %	----	7.00	----	----
Corn oil, %	----	----	7.00	----
Salt, %	.35	.35	.35	.35
Vitamin mix[2], %	1.00	1.00	1.00	1.00
Trace mineral mix[3], %	.10	.10	.10	.10
Chromium oxide, %	.20	.20	.20	.20
Calculated nutrient composition				
Protein, %	17.00	17.00	17.00	17.00
ME[4], kcal/g	2.87	3.17	3.25	2.88
TSAA[5], %	.65	.65	.65	.53
Lysine, %	.81	.85	.85	.82
Calcium, %	3.50	3.50	3.50	3.50
Phosphorus, %	.57	.56	.56	.58

[1] TSAAD = Total sulfur amino acid deficient diet.

[2] Supplied the following per kg of diet: 3965 IU vitamin A, 615 ICU vitamin D_3, 1.8 mg riboflavin, 11.2 mg niacin, 4.5 mg calcium pantothenate, 5.3 μg vitamin B_{12}, 2.2 IU d-α-tocopherol acetate, .9 mg menadione sodium bisulfite, 372 mg choline chloride, and 50 mg ethoxyquin.

[3] Supplied the following (ppm): 20 Fe, 60 Zn, 60 Mn, 4 Cu, and 1 Mo.

[4] Metabolizable energy.

[5] Total sulfur amino acids.

determinations. To reduce the probability of a change in fasting heat production and the onset of molt, hens were only restricted to 60% of the ad libitum intake for four days in this study.

The third study in this series again employed the diets fed to the same hens, now eight months old, maintained at 10 ± .5 C. Due to the large temperature decline required for this experiment, temperature was decreased 10 C per week until reaching 10 C. After hens attained constant feed consumptions and heat productions (Davis et al., 1972), diet and fecal samples were again obtained for ME determinations. Hens were then restricted as outlined in the second experiment of this series.

Results and discussion

In comparison with full-feeding the basal and TSAAD diets, meal feeding reduced ($P<.05$) feed intake approximately 12 g/PBW/d. Hens full fed the TSAAD diet produced more ($P<.05$) heat (61.3 vs. 70.9 kcal/PBW/d) than the basal diet while no changes occurred when both diets were meal-fed (70.9 vs. 72.3 kcal/PBW/d) (Table 9). These results suggest that an amino acid deficiency increases heat production, and that the effects were not reversed by meal feeding. In comparison with full feeding, meal feeding the basal diet reduced ($P<.05$) energy balance from 143.5 to 101.0 kcal/PBW/d; while meal feeding the TSAAD reduced ($P<.05$) energy balance from 119.4 to 84.4 kcal/PBW/d. Hens required more maintenance ME when full-fed the TSAAD diet vs. full feeding the basal diet. Meal feeding increased ($P<.05$) maintenance ME requirements from 42.6 to 59.4, and 49.9 and 54.9

TABLE 9. Effect of feeding regime on energy utilization by laying hens at 21.1 C (Experiment 3)

Criteria	BASAL	BASAL[1]	TSAAD[2]	TSAAD[1,2]
Feed intake, g/PBW/d[3]	74.7[a]	62.7[b]	72.4[a]	57.3[c]
Diet ME intake, kcal/PBW/d[3]	204.8[a]	171.9[b]	198.7[a]	157.1[c]
Heat production, kcal/PBW/d[3]	61.3[c]	70.9[b]	79.4[a]	72.3[b]
Energy retention, kcal/PBW/d[3]	143.5[a]	101.0[c]	119.4[b]	84.8[d]
FHP, kcal/PBW/d[3]	37.7[c]	53.1[a]	40.0[c]	45.0[b]
Net energetic efficiency, (%)	89.2[a]	89.7[a]	81.7[b]	82.6[b]
Maintenance ME, kcal/PBW/d[3]	42.6[d]	59.4[a]	49.9[c]	54.9[b]
ME for production, kcal/PBW/d[3]	162.2[a]	112.5[b]	148.8[a]	102.2[b]

[a-d] Means within a row not having common letter superscripts are significantly different ($P<.05$).

[1] Meal-fed = diet was available only during two 1-hr periods each day.

[2] Total sulfur amino acid deficient.

[3] Physiological body weight = $BW^{.75}$.

kcal/PBW/d for the two diets, respectively, suggesting an increase in the activity of enzymes associated with amino acid turnover. With meal feeding, hens fed the TSAAD diet showed significantly lower maintenance ME requirements (Table 9). The TSAAD diet reduced ($P<.05$) NEE from 89.2 and 89.7%, for the basal diets, to 81.7 and 82.6%. The increased maintenance ME requirements, along with low energy conversion efficiency for the hens fed the TSAAD diets under either feeding regime, resulted in less ($P<.05$) ME available for production. The amino acid deficiency increased heat production and maintenance ME requirements, limited ME available for production and may reduce laying hen performance (Table 9).

In Arizona, poultry in open housing are exposed to mean summer temperatures as high as 32 C while mean temperatures in winter can reach 8 C. We therefore decided to study hens under 10, 21.1 or 32.2 C constant temperature regimes.

Supplemental fat reduced ($P<.05$) feed intake at each of the three temperatures used (Table 10). This reduction was not sufficient to compensate for the increased energy density of the fat supplemented diets and, thus, daily ME intakes were increased at 10 and 21.1 C, but not at 32.2 C. These increased ME consumptions would be expected to translate into improved energy retentions if there were not a commensurate increase in heat production (Table 10). Animal fat did cause a increased ($P<.05$) in heat production over that obtained with the basal diet; while CO either reduced heat production or only slightly increased it at each of the three temperatures.

TABLE 10. Effects of fat source and temperature on energy utilization by laying hens (Experiment 3)

Criteria	10 C			21.1 C			32.2 C		
	BASAL[1]	AF[2]	CO[3]	BASAL[1]	AF[2]	CO[3]	BASAL[1]	AF[2]	CO[3]
Diet metabolizable energy (ME), kcal/g	2.74	3.19	3.13	2.74	3.22	3.25	2.74	3.22	3.26
Protein intake, g/b/d	21.0	18.1	19.6	17.9	18.2	14.1	15.7	14.4	14.2
TSAA[4] intake, mg/b/d	857.7	917.0	888.0	828.3	1025.4	812.5	727.1	807.8	821.9
Feed intake, g/PBW[5]/d	84.0^{a}	67.0^{c}	74.9^{b}	74.7^{b}	68.3^{c}	56.5^{de}	67.1^{c}	54.5^{e}	58.2^{d}
Diet ME intake, kcal/PBW[5]/d	230.0^{a}	213.8^{b}	234.8^{a}	204.8^{c}	219.9^{b}	183.7^{d}	184.0^{d}	175.4^{e}	189.6^{d}
Heat production, kcal/PBW[5]/d	90.8^{b}	97.1^{a}	84.6^{c}	61.3^{g}	86.7^{c}	67.4^{f}	78.4^{e}	81.1^{d}	67.4^{f}
Energy balance, kcal/PBW[5]/d	139.2^{bc}	116.6^{d}	150.2^{a}	143.5^{ab}	133.2^{c}	116.3^{d}	105.6^{e}	94.3^{f}	122.2^{d}
FHP6, kcal/PBW5/d	60.9^{b}	70.3^{a}	68.8^{a}	37.7^{e}	63.5^{b}	32.6^{f}	53.9^{c}	68.5^{a}	43.9^{d}
Maintenance ME, kcal/PBW[5]/d	70.6^{c}	82.8^{a}	73.8^{b}	42.6^{f}	70.5^{c}	40.7^{f}	62.7^{d}	74.5^{b}	50.8^{e}
Net energetic efficiency, %	87.1^{d}	87.6^{d}	93.9^{a}	89.2^{cd}	90.4^{bc}	82.9^{e}	86.7^{d}	92.6^{ab}	87.8^{d}
ME for production kcal/PBW[5]/d	159.5^{a}	131.0^{cd}	150.3^{ab}	153.7^{ab}	149.4^{abc}	143.0^{abc}	121.3^{de}	109.8^{e}	138.8^{bcd}

$^{a-g}$ Means within a row not having common letter superscripts are significantly different ($P<.05$).

[1] BASAL - Basal diet formulated to meet National Research Council (NRC, 1984) nutrient recommendations for laying hens.

[2] AF - 7% animal fat formulated into the basal diet.

[3] CO - 7% corn oil formulated into the basal diet.

[4] TSAA - total sulfur amino acids.

[5] PBW - physiological body weight - kg $BW^{.75}$.

[6] FHP - fasting heat production.

Hens maintained at 21.1 and 32.2 C and fed AF showed the highest ($P<.05$) heat productions in comparison with the basal and CO diets. The lowest ($P<.05$) heat productions occurred with CO at 10 and 32.2 C while the lowest heat production at 21.1 C occurred when hens were fed the basal diet.

Energy balance, which represents the net energy retained as tissue deposition and that appearing in eggs, reflected housing temperature and dietary treatments. At 10 and 32.2 C, the highest ($P<.05$) energy balances of 150.2 and 122.2 kcal/PBW/d, respectively, were obtained with CO; while the lowest (116.6 and 94.3 kcal/PBW/d) were obtained with AF. At 21.1 C, neither fat source improved energy balance in comparison with the basal diet (Table 10).

NEE data also suggest differences between the two fats. Corn oil improved NEE at the lower temperature; while AF was effective in improving NEE at 32.2 C and in each of these series the other fat caused no change ($P>.05$) in NEE. At 10 C, no differences in NEE were seen for the basal or AF treatments while an increase ($P<.05$) occurred with CO (Figure 10). Hens fed AF at 32.2 C showed the highest NEE (92.6%) while no change occurred with CO. Mean values across temperatures (Table 10) for hens fed AF show an increase in NEE with increasing temperature; which is in contrast to Reid and Maiorino (1984) who reported no effects of temperature on NEE for hens fed similar diets.

Maintenance ME requirements at 21.1 C ranged from 42.6, for the basal diet, to 70.5 kcal/PBW/d for the AF diet and were lower than those obtained for the other two temperatures suggesting that the hens

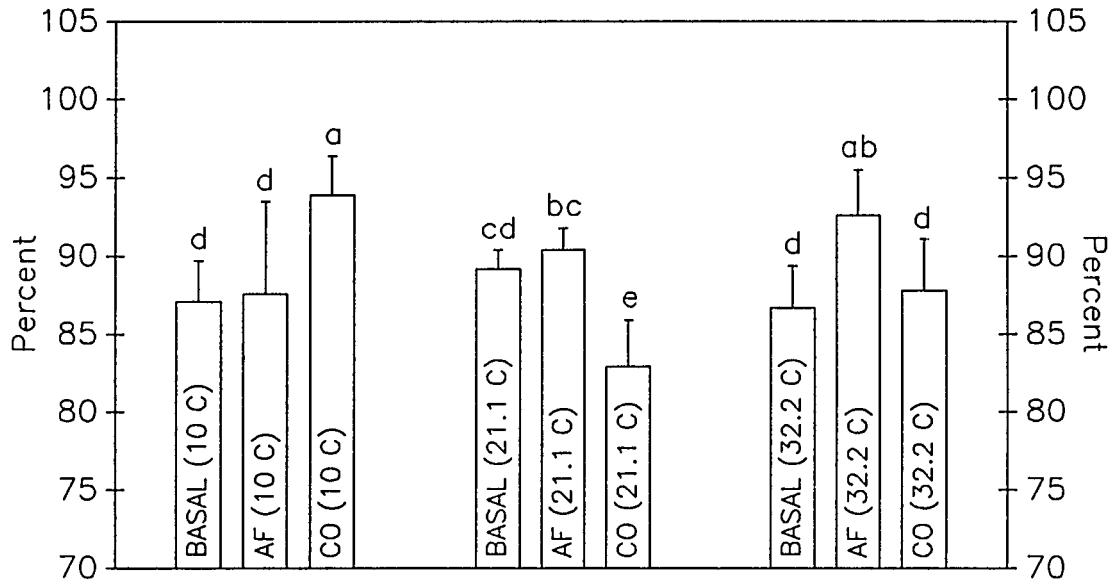

FIGURE 10. Effects of temperature and fat source on the net energetic efficiency of laying hens (Experiment 3).

are under the least amount of environmental stress at this temperature (Figure 11). Maintenance ME requirements for hens at 32.2 C ranged from 50.8 for the CO diet to 74.5 kcal/PBW/d for AF. The results in this study are in contrast with those of Farrell (1975) who reported maintenance ME requirements of White Leghorns as 133 kcal/PBW/d. However, maintenance ME is dependant on dietary formulation, temperature, age and strain of bird. Hens fed AF had the high ($P<.05$) fasting heat production and maintenance ME requirements at each of the three temperatures (Figures 11 and 12). Leeson, Summers and Bayley (1975) has also shown increased maintenance ME requirements for laying hens fed animal fat. Reduced fatty acid synthesis enzymatic activities (Pearce, 1968; Waterman et al., 1975) associated with unsaturated fats might partly explain the reduced maintenance ME requirements for hens fed supplemental CO, while higher activities of fatty acid synthesis enzymes are associated with more saturated fats. De novo synthesis of fatty acids from carbohydrates is energetically inefficient resulting in losses up to 20% in comparison with direct metabolism of carbohydrates for energy or deposition of fat tissues.

Since hens tend to overconsume energy when fed fat supplemented diets, the higher caloric density of the AF diet may also require more metabolic activity through increased enzyme activities, and may partly explain the higher heat productions and maintenance ME requirements seen in these treatments. Fasting heat production was significantly increased with AF at all three temperatures (Figure 12), in comparison with the basal diets, while CO feeding decreased fasting heat production when hens were maintained at 21.1 and 32.2 C. This further

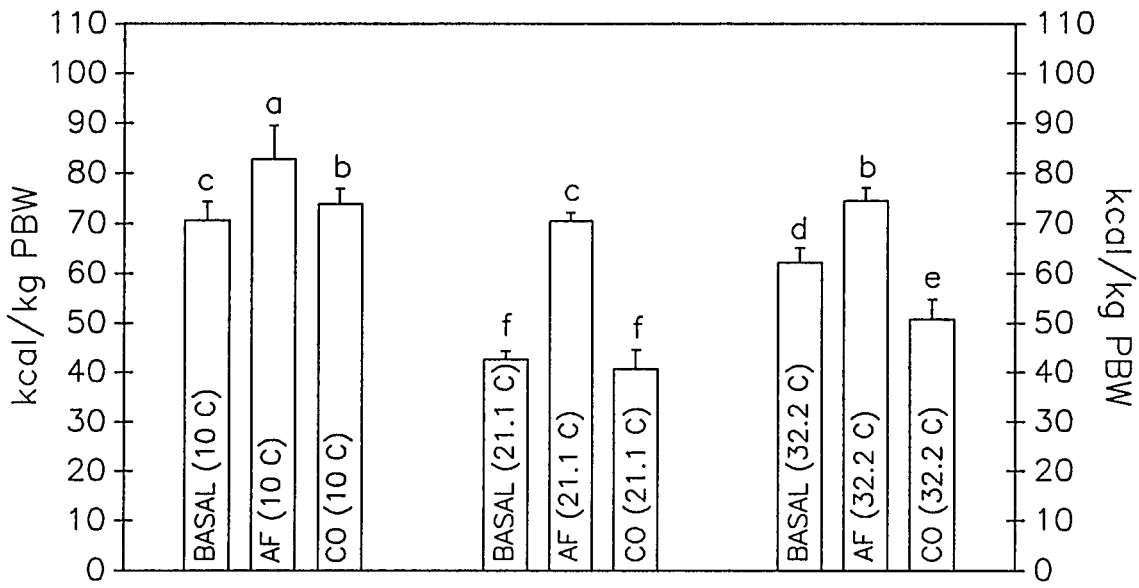

FIGURE 11. Effects of temperature and fat source on the maintenance metabolizable energy (ME) requirements of laying hens (Experiment 3).

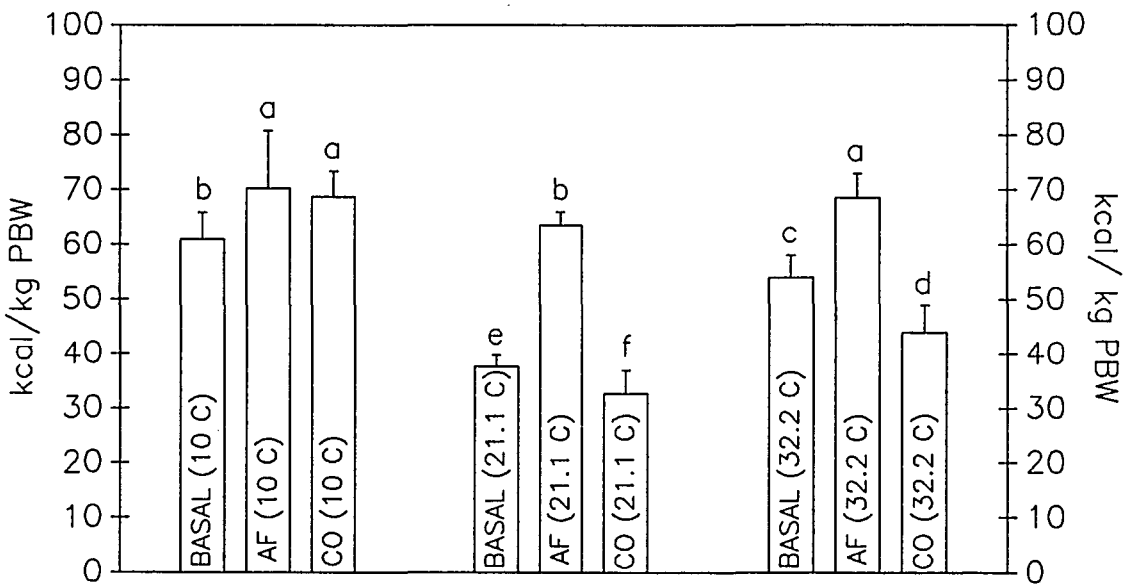

FIGURE 12. Effects of temperature and fat source on the fasting heat production of laying hens (Experiment 3).

indicates metabolic inefficiencies associated with maintenance functions.

There were no improvements in the amount of ME available for production with supplemental fat at either 10 or 21.1 C. At 21.1 C, values ranged from 153.7, for the basal diet to 143.0 kcal/PBW/d for the CO diet (Figure 13). Hens fed CO at 32.2 C had more ($P<.05$) ME available for production (138.8 kcal/PBW/d) in comparison with the basal and AF diets (Table 10 and Figure 13). CO fed birds at this temperature, consumed more feed (58.2 g/PBW/d) than AF (54.5 g/PBW/d) or basal treatments (67.1 g/PBW/d) and also had lower maintenance levels resulting in the increased ME above maintenance values.

Supplemental fat up to 7% of the diet, increased NEE for hens under cold and heat stress; but the two fats produced different responses. Corn oil was effective in improving NEE at 10 C, but not at either 21.1 or 32.2 C; while AF improved NEE at 21.1 and 32.2 C only.

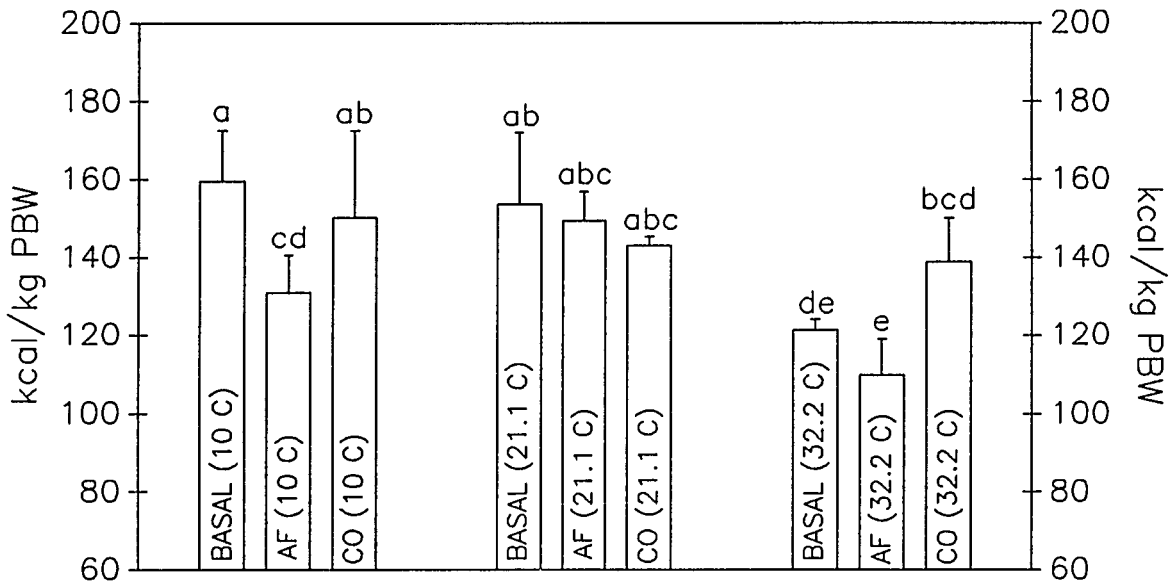

FIGURE 13. Effects of temperature and fat source on the metabolizable energy (ME) available for production by laying hens (Experiment 3).

CHAPTER 7

SUMMARY

It is possible to construct an indirect calorimeter for rapid determination of energy utilization by poultry and other small animals with limited resources. Energy utilization determined with indirect calorimetry eliminates some of the errors associated with other procedures due to the short duration of experiments.

Diets formulated by a least cost linear program to meet specific amino acid levels without a protein specification improved energy efficiency of laying hens provided that higher than National Research Council (1984) recommended levels of total sulfur amino acids (TSAA) were fed. Addition of animal fat to laying hen diets formulated without a protein specification improved the efficiency of dietary metabolizable energy conversion to net energy and increased the amount of ME available for production. Animal fat supplementation to diets containing adequate levels of amino acids but low in total protein content would be predicted to improve laying hen performance.

Total sulfur amino acid deficiency reduced net energetic efficiency, ME available for production and laying hen performance. These effects were not altered by feeding regime. Changing hens from an ad libitum to a meal feeding regime increased both maintenance ME requirements and fasting heat production.

Dietary fat, incorporated at levels up to 7% of the diet, increased ME consumption, energy balance, net energetic efficiency and ME available for production. Animal fat maintains ME intake and improves energetic efficiency in young laying hens under heat stress.

Feed intakes were reduced when diets containing 8% cottonseed oil, cod liver oil or safflower oil were fed to laying hens housed at 21.1 C and resulted in reduced ME available for production. Hens fed corn oil consumed more feed and had comparable energy conversion efficiencies, lower fasting heat productions and lower maintenance ME requirements than hens fed animal fat. This suggests that corn oil is a good fat supplement for laying hens under thermoneutral conditions.

Animal fat increased maintenance ME requirements and fasting heat productions in laying hens maintained at 10, 21.1 and 32.2 C in comparison with the other fat sources studied. Corn oil appeared to be a better fat supplement for maintaining laying hen performance under adverse conditions, while both animal fat and corn oil supplements improved laying hen performance under thermoneutral conditions.

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