INFLUENCE OF DIETARY AMINO ACID ADEQUACY ON PERFORMANCE AND MUSCLE PROTEIN TURNOVER IN POULTS

by

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INTRODUCTION

The high cost of a unit of dietary protein relative to energy in conjunction with declining costs of supplemental methionine and other amino acids has spurred considerable interest in the implementation of low protein diets for commercial poultry production. Widespread acceptance of reduced protein levels necessitates a better understanding of essential amino acid requirements for maximum performance. Providing amino acids at levels beyond requirements, as is currently practiced with dietary protein at recommended levels, represents an inefficient utilization of high cost protein ingredients. The advent of crystalline amino acids provides a feasible solution to the enigma of reducing dietary protein levels while maintaining adequate concentrations of the most limiting amino acids.

Skeletal muscle tissue, which constitutes the largest pool of amino acids in the body, exhibits a rapid rate of growth that greatly surpasses whole body growth rate of the poult. Studies involving the measurement of muscle protein turnover of domestic species are sparce, despite the obvious relationship between turnover rate, nutrient utilization and tissue deposition. Accurate measurements of the rates of protein synthesis and degradation might provide an insight into the possibility of manipulating protein deposition through nutritional intervention.

The research reported in this dissertation was designed to meet the following objectives:

1. to distinguish between the relative deficiencies of several essential amino acids in a low protein diet fed to young turkeys;

2. to determine the effect of dietary protein level on protein turnover and composition of the pectoralis and gastrocnemius muscles of poults; and

3. to assess the possibility of manipulating muscle protein turnover with the addition of various essential and/or dispensable amino acids to a low protein diet.

REVIEW OF LITERATURE

Amino Acid Deficiencies of Low Protein Diets

Attempts to minimize the cost of turkey production have generally centered on the efficacy of feeding low protein diets adequately supplemented with crystalline amino acids. Maximum benefit can be achieved with the lowering of dietary protein during the starting period when levels of 28 to 30% are commonly used. The primary obstacle at present is the reduced growth rate and feed efficiency that invariably accompanies the feeding of low protein starter diets.

The commercial use of low protein levels in poult diets necessitates increased accuracy regarding the required levels of amino acid supplementation to achieve maximum performance. Considerable effort has been expended on the determination of the lysine requirement of the young turkey fed low protein diets based on known deficiencies of this amino acid in cereal grains. Similarly, the inadequacy of the methionine or total sulfur amino acid (TSAA) content of soybean meal has prompted numerous studies investigating the optimum level of supplementation.

Several researchers have asserted that the lysine requirement varies as a function of the dietary protein level rather than as an absolute percent of the diet (Kratzer et al., 1950; Balloun and Phillips, 1957; Balloun, 1962). Anderson and Dobson (1959) claimed that lysine and methionine requirements increased directly with the dietary essential amino acid content as opposed to crude protein levels.

Employing least-cost diet formulation, Couch et al. (1969) estimated the requirements of turkeys to 3 weeks of age at 1.18% lysine, .41% methionine, and .71% TSAA when soybean meal was the primary protein

source. Including meat and bone scraps and poultry by-product meal in the formulation increased requirements to 1.43, .50, and .85% for lysine, methionine and TSAA, respectively. Potter and Shelton (1979) concluded that dietary protein levels fed to 4 weeks of age could be reduced from 30 to 27% provided the 1.10% TSAA requirement was met with adequate methionine supplementation. A considerably lower estimate of the TSAA requirement (.73% of the diet) for the 3-week-old turkey was obtained using semi-purified diets (D'Mello, 1976). Hurwitz et al. (1983) proposed a .93% TSAA requirement for the 1- to 4-week-old turkey. A lysine level between 1.36 and 1.44% was suggested. Current National Research Council (NRC, 1984) recommendations place the requirements for lysine, methionine, and TSAA at 1.70, .53, and 1.05%, respectively, for the turkey from hatch to 4 weeks of age.

Few investigators have achieved complete success with lysine and methionine addition to low protein diets for young turkeys. Klain et al. (1954) first reported that depressed growth persisted with supplementation of 22 and 25% protein diets with lysine and methionine equivalent to that obtained in a 29% protein diet. A methionine-supplemented 28% protein diet remained superior to a 20% protein corn-soybean meal diet with .2% DL-methionine and .3% L-lysine HCl for 3-week-old turkeys (Baldini et al., 1954). The inadequacy of a 20% protein diet containing added methionine, lysine and glycine for turkeys to 6 weeks of age was later reported by Waibel et al. (1962). A 22% protein diet benefited from the addition of .1% methionine or a combination of .1% methionine, .57% lysine and .2% tryptophan when fed to 8 weeks of age (Ferguson et al., 1956). However, growth equal to a 29% protein diet was

achieved only by increasing crude protein to 25% and adding .1% methionine. Lysine and tryptophan were without effect at the higher protein level. Balloun and Phillips (1957) concluded that lysine supplementation could reduce the protein requirement of poults from hatch to 6 weeks of age by only 2%.

In contrast, Fisher et al. (1956) reported superior growth with a 20% protein diet supplemented with .05% methionine hydroxyanalogue and .4% lysine HCl in comparison to a control 28% protein diet. Balloun (1962) found that the dietary protein level could be reduced to 24% with adequate methionine and lysine supplementation without sacrificing performance. Potter and Shelton (1976) asserted that reducing dietary protein from 30 to 27 or 24% warranted methionine supplementation while additional lysine elicited no response from turkeys to 7 weeks of age. In a more recent study (Rosebrough et al., 1982), supplementing a 23% protein diet with lysine and methionine at levels equal to NRC (1984) recommendations produced growth and feed efficiency equal to a control 30% protein diet.

Some researchers attribute the inferior performance of turkeys fed low protein diets despite lysine and methionine supplementation to a deficient concentration of protein per se (Klain et al., 1954; Stas and Potter, 1982). An alternate proposal is the possibility of further limiting amino acids beyond lysine and methionine in low protein diets (Fitzsimmons and Waibel, 1962; Waibel et al., 1962; Askelson and Balloun, 1965; Atkinson et al., 1976).

Methionine, threonine and lysine have been identified as the first, second and third limiting amino acids in soybean meal for the rat (Berry

et al., 1962). Attempts to determine further deficiencies were unsuccessful. The individual removal of amino acids from a mixture added to an 18% protein peanut meal diet indicated that methionine, lysine and threonine were limiting in that order on the basis of weight and feed efficiency depressions of broiler chicks (Anderson and Warnick, 1965). Using both corn and peanut meal to formulate an 18% protein diet, lysine was found to be more deficient than methionine and threonine, respectively. In a similarly designed experiment (Anderson and Warnick, 1966), lysine, methionine, isoleucine and threonine were identified as limiting, in descending order of severity, in an 18% protein diet composed primarily of cottonseed meal. Warnick and Anderson (1968) ascertained deficiencies of TSAA, threonine, valine and lysine in that order for 14-day-old broiler chicks fed a 14% protein diet containing soybean meal as the sole protein source. Lysine and methionine were identified as the first and second limiting amino acids for turkeys in a 22% protein diet formulated from practical ingredients (Atkinson et al., 1976). A valine deficiency in addition to inadequate lysine and methionine contents of a 20% protein diet was observed by Novacek et al. (1969) for young turkeys.

The inferior performance of 4-week-old turkeys noted with a 20% protein corn-soybean meal diet containing added lysine and methionine was not corrected by a supplemental mixture of arginine, valine, glycine, phenylalanine, tryptophan, threonine and leucine (Fitzsimmons and Waibel, 1962). A dietary protein level of 24% supported maximum performance if supplemented with a mixture of lysine, arginine, methionine, cystine, leucine and threonine (Tuttle and Balloun, 1974). Additional deficiencies were proposed for a 22% protein diet on the basis of inferior growth

despite fortification with the aforementioned amino acids. More recently, Stas and Potter (1982) were able to obtain equal performance from corn-soybean meal diets containing either 30% protein or a combination of 22% protein containing .3% supplemental DL-methionine and an essential amino acid mixture. Individual removal of amino acids comprising the mixture indicated deficiencies of valine, lysine, threonine and isoleucine in the 22% protein diet for 3-week-old turkeys.

Amino Acid Availability of Soybean Meal

Chick growth bioassays have been employed extensively to evaluate the amino acid availability of poultry feedstuffs. Availability estimates can be influenced by the experimental protocol employed according to Robel and Frobish (1977), who observed a positive correlation between the duration of the bioassay and the availability of lysine, TSAA, and tryptophan in soybean meal.

Smith (1968) observed that, in general, the amino acid availability of soybean meal exceeded 85%, with the exception of isoleucine (65%), valine (58%), and histidine (76%). Availabilities in excess of 92% were later reported (Nwokolo et al., 1976) attributable to a correction for endogenous amino acids in fecal samples.

Although availability estimates between soybean meal samples are relatively constant, considerable variation exists in the amino acid composition expressed on a percentage basis (Ivy et al., 1971). However, the total lysine, threonine, serine, cystine, methionine and leucine contents of soybean meal appear to be very similar between samples regardless of protein content (Nelson et al., 1976).

Amino Acid Interactions

Determination of the requirements for various amino acids can be influenced by interrelationships among amino acids present in the diet. The lysine-arginine interaction has been studied extensively in broilers. An increase in the arginine requirement with lysine supplementation was first reported by Anderson and Dobson (1959). The growth depression commonly associated with an arginine deficiency in chick diets can be attributed in part to a reduced utilization of lysine for protein synthesis according to Stutz et al. (1972). Further investigation of the interaction by D'Mello and Lewis (1970a,c) revealed that each increment of .25% lysine above the chick's requirement increased the arginine requirement by .10% of the diet. Using a lysine-deficient diet, these authors were also able to demonstrate an adverse effect of excess arginine on the lysine requirement although the reciprocal antagonism was far more potent. Wilburn and Fuller (1975) found that an increase in the lysine content of a 22.5% protein diet from 1.30 to 1.93% concomitantly elevated the arginine requirement from the basal level of 1.53 to 1.75% in a corn-soybean meal diet. Similarly, an increase in dietary arginine necessitated a concurrent increase in lysine supplementation to maintain maximum growth, inferring a reciprocal antagonism between lysine and arginine.

The importance of the lysine-arginine antagonism in turkey nutrition has been largely ignored to date. D'Mello and Emmans (1975) investigated the relationship between lysine and arginine requirements of the poult from 1 to 3 weeks of age. With dietary lysine at 1.30%, maximum response to arginine occurred with a level of 1.50%. A further increase in

arginine to 1.75% resulted in a growth depression. Increasing lysine to 1.55% required a concomitant elevation in arginine to 1.75%. Because the levels of lysine and arginine employed in this study did not greatly exceed requirements, the effects of excess amounts of either amino acid could not be established. Supplementing a 23% protein diet with lysine to 2.11% acted to depress growth of poults in a later study (Rosebrough et al., 1982). However, arginine status was not implicated in the response. Adding excess amounts of lysine or arginine to egg protein has been shown to decrease nitrogen retention in rats (Eggum et al., 1981).

The branched chain amino acid antagonism has been demonstrated conclusively in the broiler chick (D'Mello and Lewis, 1970b,c). Increased isoleucine requirements were observed in the face of excess dietary leucine. With 1.40% dietary leucine, maximum weight gain occurred with isoleucine at .68% of the diet. Leucine levels of 2.15 and 2.90% precipitated growth inhibitions that were only partially overcome by isoleucine up to 1.08% of the diet. Similar results were reported for the effects of excess leucine in diets containing inadequate valine. Minimum requirements for leucine and valine were placed at 1.40 and .77%, respectively. As leucine was increased to 2.40 and 3.40%, valine requirements were elevated to .89 and 1.01%, respectively. Plasma amino acid profiles mirrored the growth response, with decreases in both isoleucine and valine observed with leucine supplementation. Valine appeared to be more sensitive than isoleucine to excess dietary leucine. Supplemental isoleucine and leucine acted additively to further depress performance and plasma valine levels obtained with valine-deficient di-

ets. Reduced nitrogen retention has been demonstrated in rats fed excess amounts of leucine, isoleucine or valine (Eggum et al., 1981).

Novacek et al. (1969) first reported that a growth retardation associated with isoleucine and leucine supplementation of a 20% protein diet fed to young turkeys could be corrected by valine addition. The branched chain amino acid requirements of the turkey to 3 weeks of age were later evaluated by D'Mello (1975). Performance on semi-purified diets supplemented with various combinations of leucine, isoleucine and valine was compared with that obtained on a practical corn-soybean meal diet. A level of 1.21% valine was adequate in diets containing 1.42% leucine while an increase in leucine to 1.72% necessitated an elevation in valine to 1.36%. Maximum weight gain was not achieved with 2.02% leucine regardless of valine content due to a superimposed isoleucine deficiency precipitated by excess leucine. Isoleucine requirement was estimated at .88%.

More recently, Tuttle and Balloun (1976) found that a combination of 1.37% dietary isoleucine and valine was required to overcome the growth retardation produced by raising the leucine content from 1.96 to 3.46% in a 22% protein diet fed to poults. Plasma amino acid profiles revealed a decrease in valine and isoleucine when leucine was present in excess. Current requirements for leucine, isoleucine and valine for the turkey from hatch to 4 weeks of age are estimated at 1.9, 1.1 and 1.2%, respectively (NRC, 1984).

The interaction between threonine and tryptophan was examined in a study involving 1- to 3-week-old broiler chicks (D'Mello and Lewis, 1970c). Dietary threonine in excess of the requirement resulted in a large decrease in growth that was partially overcome by tryptophan

supplementation. Increasing threonine from .80 to 2.38% of the diet resulted in an elevation in the tryptophan requirement from .17 to .20%. Conversely, a level of 1.5% tryptophan in purified diets fed to chicks resulted in a decrease in growth that was partially alleviated by threonine supplementation (Davis and Austic, 1982a). Reducing dietary tryptophan to .9% obviated threonine addition to the basal diet. Other additions shown to increase the chick's requirement for threonine included 3% serine, 6% of a mixture of branched chain amino acids and a 6% mixture of the essential amino acids excluding threonine. In contrast, .9% phenylalanine or 5% supplements of arginine or glycine had no effect on dietary threonine adequacy. In a subsequent experiment (Davis and Austic, 1982b), plasma and liver-free threonine concentrations were depressed by 3% dietary serine, possibly due to increased threonine catabolism. However, reduced feed intake caused by a serine-induced threonine imbalance appeared to be solely responsible for the observed growth depression.

Methodology Employed in Protein Turnover Determination

The techniques that have been developed for the determination of protein turnover rates consist of two major types that differ in applicability to the measurement of synthesis or degradation. Neither method is without problem and both misrepresent actual turnover rates to different degrees.

Labeled amino acids (AA) have been applied to the study of turnover rates for both specific proteins and entire tissues. Problems associated with the methods applied to skeletal muscle have resulted in recommendations against the direct measurement of protein degradation. Errors

result from reutilization of labeled AA which reduces their rate of removal from the tissue. Amino acids derived from protein breakdown may be preferentially incorporated into newly synthesized protein leading to underestimates of protein turnover. Young and co-workers (1973) estimated reutilization of amino acids derived from degradation at 50% and 10 to 30% in the liver and muscle of fed rats, respectively. Substantial increases were noted in the fasted animal. An increase in reutilization of ^{14}C-tyrosine was induced with a protein-free diet in a later study by Garlick et al. (1975). However, no differences were observed between fasted and fed rats. In the ideal situation, isotope flow through the precursor pool should be rapid relative to its rate of incorporation into proteins in order to reduce reutilization. Techniques involving the infusion of an essential AA suffer from high rates of incorporation and reutilization relative to release from the tissue. Infusion of a nonessential AA has the advantage of low rates of reutilization relative to removal rates. However, the initial incorporation rate is low and difficult to measure. Thus, fractional degradation rates (FDR) are generally inferred from the difference between fractional synthesis (FSR) and growth rates (FGR). Bates and Millward (1981) estimated the FGR of rats from changes in weight gain over several days preceding the experiment on the assumption that growth in protein mass was proportional to that of the whole body. The FGR of chicks was determined as the average FGR over 48 hr preceding the synthesis measurement (Maruyama et al., 1978; MacDonald and Swick, 1981). The FGR of muscle was estimated from the linear regression of total muscle protein on body weight of other chicks in the treatment groups. The major error associated with the estimation

of FDR from FSR and FGR is the measurement of protein synthesis over a few minutes and growth rate over several days. The validity of the estimate relies on the assumption that the measured synthesis rate is representative of the entire day.

In vitro techniques have been employed with some success to measure changes in protein turnover in response to AA, glucose and hormone addition to the media (Goldberg and Odessey, 1972; Fulks et al., 1975; Li and Goldberg, 1976). The method involves incubation of an isolated muscle with trace amounts of a labeled AA. Incorporation or release of the label is determined for the measurement of synthesis or degradation, respectively. The advantage to in vitro determination of turnover is the ability to impose strict controls on the muscle environment while altering a single parameter of interest. However, the idiopathic acceleration in protein degradation that accompanies isolation of the muscle regardless of the incubation medium limits this technique to delineation of control mechanisms as opposed to absolute protein turnover rates. Fulks et al. (1975) reported a two-fold increase in protein degradation over synthesis despite obtaining muscles from growing rats.

Perhaps the most commonly used labeling technique, constant infusion relies on the assumption that prolonged exposure to a labeled AA produces a steady state metabolism of the label in all potential precursor pools. Thus, the rate of flow of the label through the AA pool into protein synthesis, oxidation, and other metabolic processes, defined as the AA flux, reaches an equilibrium during the infusion period. The method involves the intravenous infusion of trace amounts of a labeled AA in physiological saline over a period of 6 hr. Muscles are excised and blood

samples obtained at the conclusion of the infusion period for the determination of plasma and tissue free and tissue protein-bound specific radioactivity (SR). Labeled tyrosine has been the predominant AA used because it is neither synthesized nor degraded in muscle tissue (Garlick et al., 1973; Millward et al., 1973; Garlick et al., 1975; Millward et al., 1975; Bates and Millward, 1981; Glick et al., 1982). However, the technique was developed using lysine (Waterlow and Stephen, 1968), and proline has since been successfully employed by correcting for muscle hydroxyproline as an estimate of collagen content (Laurent et al., 1978a,c).

The primary source of error encountered with the constant infusion technique is the uncertainty surrounding the SR of the true precursor AA pool. Because of the difficulty involved in the direct measurement of AA-tRNA activity, either the plasma or tissue labeled free AA pool has been used to calculate protein synthesis rate. Monitoring of tissue and plasma free radioactivity at intervals during the infusion demonstrates a lower SR in the tissue pool (Garlick et al., 1973). The discrepancy between the intracellular and extracellular pools has been attributed to the constant dilution of the tissue pool with the unlabeled AA from protein degradation during the 6 hr infusion period. The divergence in free radioactivity between the pools has been used as an index of label reutilization which increases with prolonged exposure to a label. The greater proportionality between the SR of intracellular and protein-bound labeled AA has been reported to result in less variation in estimates of protein synthesis than with extracellular SR designated as the precursor pool using a variety of tissues and isotopic techniques (Fern and Garlick,

1973; Li et al., 1973; Rourke, 1975b). Conversely, in vitro assessment suggests that extracellular SR is the predominant precursor AA source for protein synthesis (Hider et al., 1969). An additional error inherent in the calculation of FSR obtained by constant infusion is the reliance on the measurement of AA flux. Over-estimation of the rate of protein synthesis results from the inclusion of AA leaving the precursor pool for pathways other than synthesis.

A modification of the constant infusion technique was developed by Harney et al. (1976) which employs the incorporation of a labeled AA into purified diets offered for a period of 6 hr. A comparison of dietary and IV infusions revealed similar results for both methods. Valid estimates of FSR in pectoral and gastrocnemius muscles of chicks were obtained by feeding a labeled AA diet for 3 days (Maruyama et al., 1978). A drawback to dietary infusion is the necessity for quantitative collection of all unabsorbed label, including that recovered in the gastrointestinal tract (GIT) as well as spilled or uneaten feed.

Another isotopic technique that is being used to advantage for the measurement of protein synthesis is the injection of large amounts of a single AA, or massive injection. The administered solution contains a mixture of the unlabeled AA and the labeled AA of high SR. The large dose minimizes errors in the determination of the SR of the free AA at the site of protein synthesis, since all precursor pools are rapidly flooded and exhibit essentially equal SR. The SR of the intracellular and AA-tRNA pools differed by less than 10% (Henshaw et al., 1971), while the difference between plasma and tissue free pools was considerably smaller than observed with constant infusion.

The dose chosen depends on the size of the free AA pool, the rate of incorporation of the label, and the mode of administration. Subcutaneous injection necessitated a larger dose than for IV administration because of the slow rate of absorption (Conde and Scornik, 1977). Intravenous and IP injections of the label appeared to give similar results (Henshaw et al., 1971). However, IP administration resulted in slower absorption and produced a linear increase in the SR of the tissue-free pool (Macdonald and Swick, 1981), while the converse occurred with an IV injection (McNurlan et al., 1979). Essential AA can be employed at lower dosages (10 to 30 μCi/100 g body weight) than non-essential AA or bicarbonate (100 to 400 μCi/100 g body weight) because of their greater rates of incorporation into protein (Millward, 1970a,b; Rourke, 1975a; McNurlan et al., 1979; McNurlan and Garlick, 1980, 1981; MacDonald and Swick, 1981). Lower doses were inadequate to cause homogeneous SR of all precursor pools.

Leucine is the most commonly used AA in massive injection studies (Conde and Scornik, 1977; McNurlan et al., 1979; McNurlan and Garlick, 1980, 1981). Incubation of isolated rat muscle with leucine has been shown to stimulate protein synthesis through enhanced polysome formation and to inhibit protein degradation (Buse and Reid, 1975; Fulks et al., 1975; Goldberg and Chang, 1978). Conversely, massive doses of leucine were found to exert no effect on the incorporation of AA into protein in vivo (Conde and Scornik, 1977; McNurlan et al., 1979). Similarly, large amounts of valine do not alter protein synthesis rate (MacDonald and Swick, 1981).

The length of the experimental period varies from 10 min for liver proteins (McNurlan et al., 1979; McNurlan and Garlick, 1980, 1981) to 20 min for muscle proteins (MacDonald and Swick, 1981) due to the difference in turnover rates of these tissues. Additional animals are killed at 2 min (liver) and 5 min (muscle) in order to determine the average SR of the intracellular pool over the time course of the experiment. The short experimental period allows for the inclusion of the more rapidly turned over proteins as well as the removal of AA reutilization errors which tend to over-estimate the FSR. Computation of FSR is also simplified as the reliance on AA flux is obviated due to the abbreviated experimental period.

The massive injection technique is a modification of the trace injection method, with major differences being the magnitude of the dose and the length of the experimental period. The small dosages employed using the latter technique result in low rates of incorporation requiring considerable extension of the experimental period to several days (Goldberg, 1968, 1969; Laurent and Sparrow, 1977). Because problems of reutilization are magnified as exposure to the label is extended, investigators have adopted the use of a "chase", or unlabeled AA injection, subsequent to label injection (Goldberg, 1969; Laurent and Sparrow, 1977). Feeding a high protein diet has also been employed in an attempt to minimize label reutilization.

The analytical determination of muscle RNA and DNA generally accompanies the measurement of protein synthesis in order to elucidate the controlling mechanisms involved. Short-term changes in protein synthesis are initiated by alterations in the efficiency of protein synthesis con-

trolled by disaggregation of ribosomes (Millward et al., 1973). Conversely, changes in FSR produced by long-term exposure to a diet result from variations in the capacity for protein synthesis controlled by the turnover of RNA. The capacity for protein synthesis is defined as the total amount of RNA, while synthetic efficiency refers to the rate of protein synthesis per unit RNA (Millward, 1978). Protein capacity varied between muscle types while efficiency is constant for SCWL roosters (Laurent et al., 1978a).

Determination of muscle RNA and DNA also aids in overcoming the confounding effect of changes due to development rather than nutritional status. Relating the rates of protein synthesis to the size and number of DNA-units renders protein turnover estimates independent of developmental changes. The DNA-unit size refers to the protein:DNA ratio while the DNA-unit number is equivalent to total muscle DNA (Millward and Waterlow, 1978). The DNA-unit size varies between muscle types (Millward, 1978), while total muscle DNA is constant with age for poultry (MacDonald and Swick, 1981).

Urinary 3-methylhistidine (3MeHIS) content has been proposed as an alternative to radioisotopes for the measurement of skeletal muscle turnover, based on the discrete localization of this AA in actin and myosin molecules. While applicable only to the direct measurement of protein degradation, the rate of protein synthesis can be calculated from the sum of FDR and FGR. The major advantage to the use of 3MeHIS is the lack of reutilization of this AA as a substrate for protein synthesis. Quantitative excretion of 3MeHIS has been verified in rats, chicks, humans and rabbits following the injection of the labeled compound (Cowgill and

Freeburg, 1957; Young et al., 1972; Long et al., 1975; Harris et al., 1977). The sheep and pig appear to differ in 3MeHIS metabolism (Rangeley and Lawrie, 1976; Harris and Milne, 1977), prohibiting the use of 3MeHIS as an index of myofibrillar turnover in these species.

Implicit in the use of 3MeHIS as an indicator of protein degradation is the absence of 3MeHIS in feedstuffs used in diet formulation. Thus, dietary inclusion of animal proteins is prohibited based upon findings of substantial increases in 3MeHIS excretion by humans consuming meat (Bilmazes et al., 1978). Pure samples of wheat protein, soy protein, casein and gelatin were devoid of 3MeHIS (Hibbert and Lawrie, 1972; Rangeley and Lawrie, 1976). Data on other commonly used feedstuffs are lacking.

Skeletal muscle 3MeHIS concentration in rats was unaltered by changes in diet composition (Haverberg et al., 1975a; Nishizawa et al., 1977b) or prolonged fasting (Dunn et al., 1982). Thus, differences in 3MeHIS excretion can be interpreted as reflections of alterations in protein catabolism rather than artifacts of changes in total 3MeHIS pool. However, Fisher et al. (1975) reported a correlation between dietary histidine content and pectoral muscle concentration of free 3MeHIS for adult SCWL males.

The use of urinary 3MeHIS as a valid index of muscle protein degradation is complicated by the uncertainty surrounding actual turnover rates of muscle tissue proteins. Reports of equal turnover rates for actin and myosin (Funabiki and Cassens, 1972; Perry, 1974; Lobley and Lovie, 1979) have contrasted with claims of a more rapid rate of turnover for myosin molecules (Koizumi, 1974; Swick and Song, 1974). Similarly,

both homogeneous (Koizumi, 1974; Lobley and Lovie, 1979) and heterogeneous (Low and Goldberg, 1973; Wikman-Coffelt et al., 1973) turnover rates have been observed for light and heavy meromyosin chains. Turnover rates of sarcoplasmic proteins have been found to exceed (Perry, 1974) or equal (Laurent et al, 1978a; Lobley and Lovie, 1979) that of myofibrillar proteins. Heterogeneity in synthesis rates of sarcoplasmic and myofibrillar proteins among muscle types were observed by Laurent et al. (1978a) who examined cardiac, gizzard, pectoral and gastrocnemius muscles of adult SCWL chickens. Differences in techniques used to measure turnover rates of muscle protein fractions may be responsible for contradictory findings.

A greater impediment to the employment of urinary 3MeHIS is the identification of significant quantities of this AA in tissues other than skeletal muscle. Actin molecules have been identified in skeletal, smooth, and cardiac muscle, fibroblasts, blood platelets, histones and the brain of several species and appear to be highly conserved across tissues and species (Gershey et al, 1969; Byvoet, 1971; Yang and Perdue, 1972; Elzinga et al., 1973). Actin and myosin have been isolated from the brush border of intestinal epithelial cells in the chicken (Tilney and Mooseker, 1971). An analysis of 11 rat tissues (Haverberg et al., 1975b) showed that the total amount of protein-bound 3MeHIS in non-skeletal muscle tissue and organs (diaphragm, heart, liver, stomach, kidney, lung, spleen, testis, brain, and serum), representing 20% of body weight, equaled 9.6 µmol versus 118 µmol for skeletal muscle at 45% of body weight. Nishizawa et al. (1977b) estimated the contribution of rat skeletal muscle at 90% of the total body 3MeHIS pool. The second largest

source of 3MeHIS was identified as the skin and hair, estimated at 26.5% of body weight and accounting for 8.5% of the total body pool. Of the remaining tissues analyzed (brain, testis, spleen, kidney, liver, lung, heart, stomach, and intestine), only the GIT was cited as a potentially significant source at 1.1% of the total body pool.

The contribution of non-skeletal sources to daily 3MeHIS excretion depends not only on the absolute amounts relative to the total 3MeHIS pool but also on the turnover rate of a particular tissue. To determine the proportion of urinary 3MeHIS attributable to pool size and turnover rates of non-skeletal tissues, Nishizawa et al. (1977a) administered [methyl-^{3}H]-methionine IP to young rats. Results indicated that 10.4 and 6.2% of the daily 3MeHIS excretion was derived from the GIT and skin, respectively. Using a similar technique in a later study, 9.8 and 6.8% of the daily urinary 3MeHIS content could be accounted for by the GIT and skin, respectively (Millward et al., 1980). Rapid turnover rates for the GIT (9.7%/day) and the skin (2.6%/day) relative to hind leg muscles (1.1%/day) were cited as the basis for their signficant contributions. The proportion of whole body protein synthesis occurring in the GIT has been estimated at 18.8% (McNurlan and Garlick, 1980), with the small intestine alone accounting for 14% in a 100-g rat (McNurlan et al., 1979) using a massive infusion of [1-^{14}C]leucine. Fractional synthesis rates in the small intestine were reduced in response to starvation (McNurlan et al. 1979) and a protein-free diet (McNurlan and Garlick, 1981).

Effect of Diet on Protein Turnover in Rats

A. Starvation. A 2-day fast resulted in a decrease in the fractional rate of protein synthesis coincident with a 13% reduction in muscle tissue

weight of 60-g rats (Garlick et al., 1975). Similarly, a progressive decline in protein synthesis rates to 42 and 28% with respect to control values was observed for 100-g rats at 2 and 4 days of a fast, respectively (Millward et al., 1976). A transient decrease in fractional degradation rates to 77% at 2 days was followed by a marked increase to 210% of control levels by day 4 of starvation. Refeeding of fasted rats produced an immediate 2-fold increase in the rate of protein synthesis, surpassing those of weight and age controls, while degradation rate was maintained at a low level during the initial period of repletion. By 8 days of refeeding, both synthesis and degradation rates were increased to the extent that fractional growth rate was indistinguishable from controls.

B. Protein-free diets. Decreases in both synthesis and degradation rates result from the feeding of protein-free diets to rats using either urinary 3MeHIS content or decay of ^{14}C-tyrosine as the index of turnover. Feeding a protein-free diet to young rats for 14 days progressively decreased 3MeHIS excretion as fractional degradation and synthesis rates declined from 4%/day to 1.7 and 0.75%/day, respectively (Funabiki et al., 1976). The larger decrease in protein synthesis relative to degradation was corroborated by a concomitant reduction in body weight to 85% of control values. Subsequent repletion for 8 days resulted in a marked elevation in 3MeHIS excretion and, thus, protein degradation to 2.8%/day. Perhaps of greater significance was the enhanced fractional synthesis rate to 5.7%/day, contributing to an increase in body weight to 134% of initial weights after 8 days of repletion.

The greater resistance to change observed for fractional degradation rate relative to synthesis rate was confirmed by Garlick et al. (1975),

using a constant infusion of ^{14}C-tyrosine. A decrease in the rate of protein breakdown to 5.7%/day by day 21 of a protein-free regime was inadequate to compensate for the larger reduction in protein synthesis to 4.3%/day. A similar relationship between synthesis and degradation rates for rats fed protein-free diets at intervals spanning the period from weaning to adulthood was reported by Millward et al. (1975).

Feeding a protein-free diet to rats for one day revealed the rapid adaptability of protein synthesis rate, which exhibited an immediate decrease to 52% of control levels (Millward et al., 1976). Intervals of 9 and 30 days on a protein-free regime further reduced synthesis rates to 27 and 14% of control values, respectively. Again, degradation rate appeared to be less sensitive to the absence of dietary protein, with reductions to 89, 46 and 22% of controls after 1, 9 and 30 days, respectively.

C. Protein restriction. Good agreement among studies employing 3MeHIS or radioisotopes to monitor protein turnover have been obtained for the response of rats to protein-deficient diets. In general, decreases in both synthesis and degradation have been reported.

The imposition of a severe protein restriction reduced 3MeHIS excretion of growing rats from 1.3 to .26 μmol per day by day 14, followed by a rapid increase to control levels by day 28 of repletion (Haverberg et al., 1975a). Thus, inadequate dietary protein acted to reduce muscle protein degradation while repletion resulted in an adaptation to increased AA availability despite the persistence of low body weights at 28 days, with repleted rats attaining weights of only 62% of control rats. A decrease in muscle protein catabolism concomitant with body weight loss

in response to a protein deficiency was confirmed by Nishizawa et al. (1977b) and Burini et al. (1981) who also monitored urinary 3MeHIS content.

Weanling rats fed low protein diets to maturity exhibited reduced rates of protein synthesis and degradation in comparison to controls (Millward et al., 1975). However, synthesis exceeded degradation, corresponding to a positive growth response of 5.6%/day versus 6.2%/day for control rats at weaning. Efficiency of muscle growth was improved in malnourished rats, denoted by 41% of protein synthesis retained for growth compared to only 22% for controls at weaning. A reduction in fractional synthesis rates from 11.7 to 5.8%/day for rats fed protein-deficient diets was later reported by Millward et al. (1979).

Incubation of soleus (oxidative) and extensor digitorium longus (glycolytic) muscles obtained from fasted rats with glucose or an AA mixture in addition to [U-^{14}C] tyrosine acted to increase protein synthesis and decrease protein degradation (Li and Goldberg, 1976). However, levels observed for muscles from fed rats were not achieved. Conversely, Smith et al. (1982) observed increases in both synthesis and degradation rates in the same muscles obtained from rats fed diets varying in casein content from 5 to 15%. Enhanced rates of protein synthesis and degradation noted for muscles incubated with [U-^{14}C] tyrosine were positively correlated with body and muscle weight responses to dietary protein supply.

D. Energy restriction. Millward et al. (1976) demonstrated reduced rates of protein synthesis and degradation to 55 and 82% of control levels, respectively, with a low energy diet, monitored with a constant infusion

of labeled tyrosine. Further evaluation of an energy deficiency demonstrated a decline in FSR from 11.7 to 4.3%/day (Millward et al., 1979).

An energy deficiency failed to alter 3MeHIS excretion expressed as a percentage of leg muscle weight of rats in a study by Burini et al. (1981). The resistance of leg muscles to changes in diet composition, in contrast to marked differences in body weight noted in this and other studies (Millward et al., 1976; Hayase and Yoshida, 1980) has been attributed to the protective effect of locomotor activity. Muscular activity appears to be the fundamental determinant of muscle mass, acting independently of hormonal and nutritional alterations. Thus, hypertrophy of leg muscle occurs with exercise even in fasted animals (Goldberg et al., 1975). Similarly, stretch was shown to stimulate wing muscle hypertrophy in SCWL roosters (Laurent et al., 1978c). Therefore, the use of the hind limb muscle weights as representative of muscle protein turnover may effectively mask changes in total skeletal muscle mass, resulting in misinterpretation of urinary 3MeHIS data.

E. Combined protein/energy restriction. A combined protein-energy deficiency produced a transient increase in 3MeHIS output as a percentage of rat body weight that persisted for 11 days on a deficient regime (Haverberg et al., 1975a). Protein catabolism then decreased from days 11 to 18 despite introduction of a repletion diet on day 14. Adaptation to the use of body fat stores appears to be a likely explanation for decreased catabolism and conservation of myofibrillar protein after 11 days of protein-energy depletion. Similarly, a 3-day adaptive period to the repletion diet was required to increase protein degradation to control levels. Diminished protein breakdown, denoted by decreases in urinary

3MeHIS, also resulted from a protein-energy deficient diet fed to rats for 20 days (Burini et al., 1981).

Overfeeding appears to induce alterations in protein turnover similar in direction to those observed with a dietary protein or energy deficiency. Rats fed in excess of 135% of ad libitum protein and energy intake responded with a 14% decrease in protein synthesis rate of the gastrocnemius muscle (Glick et al., 1982). An increase in absolute muscle protein content suggested a concomitant decline in the fractional rate of protein degradation.

F. Protein quality. Feeding a poor quality protein elicits similar responses to those observed with an absolute protein deficiency. High growth rates of young rats achieved with good quality proteins such as egg albumin and casein were accompanied by elevated 3MeHIS excretion while protein degradation decreased with the feeding of equal amounts of imbalanced protein sources (Omstedt et al., 1978). A strong positive correlation ($r = .78$) was observed between urinary 3MeHIS and net protein utilization (NPU). However, significant differences in body weight gain and NPU between casein- and albumin-fed rats were unaccompanied by corresponding alterations in 3MeHIS excretion. Similarly, 3MeHIS output remained constant with imbalanced protein diets despite marked differences in weight gain among treatments. A lack of sensitivity inherent in the use of 3MeHIS as an index of protein degradation may be responsible for the poor correlation between weight gain and urinary 3MeHIS. Alternatively, a 5-day experimental period may have been insufficient to elicit significant depressions in the rate of protein degradation. Extension of the experimental period to 15 days resulted in marked differences in

weight gain, gastrocnemius weight and absolute muscle protein content for rats fed 10% protein diets containing whole egg protein, gluten or gelatin as the sole protein source, reflecting dietary AA balance (Hayase and Yoshida, 1980). Again, wide variation in AA balance was required to produce a significant change in the rate of protein degradation. In contrast, the addition of .51% cystine to a purified diet deficient in total sulfur AA was sufficient to increase protein synthesis in rat gastrocnemius muscle (Harney et al., 1976). These researchers employed a continuous dietary infusion of [U-^{14}C]tyrosine in the determination of synthesis rate, reflecting the sensitivity of radioisotopic techniques.

Protein Turnover in Poultry

A dearth of information is available on protein turnover in poultry to date. Characterization of chick muscle protein composition throughout development showed that pectoral muscle acquires its adult form by 4 weeks in the SCWL chick, as nitrogen partitioning between the various fractions reaches a steady level (Robinson, 1952). The sarcoplasmic and actomyosin fractions remain constant beyond 2 weeks of age, with actomyosin constituting 50% of the total muscle protein at hatch. Collagen, elastin and reticulin fall from 15% of total protein at hatch to a steady state level of 5% by 4 weeks of age. Tropomyosin remains constant at 1% of muscle protein beyond the 16th day of incubation.

The capacity of the pectoral muscle for growth in the Rhode Island Red is large, denoted by a 10-fold increase in muscle weight by 2.5 weeks of age versus only a 2-fold increase in body weight gain (Dickerson, 1960). Sarcoplasmic and myofibrillar proteins accounted for 32.7 and 52.5% of total muscle protein, respectively, by 4 weeks of age, at which

time the composition of the pectoral muscle resembled that of an adult. Broiler gastrocnemius protein concentration was unaffected by lysine supplementation of a low protein diet, although absolute protein content increased to a maximum with 0.5 and 0.25% supplemental lysine at 14 and 28 days (Akinwande and Bragg, 1974).

Laurent et al. (1978a) were unable to distinguish between myofibrillar and sarcoplasmic turnover rates within cardiac, gizzard and anterior (ALD) and posterior latissimus dorsi (PLD) muscles of adult SCWL males using a constant infusion of ^{14}C-proline. Fractional synthesis rates of sarcoplasmic and myofibrillar proteins in red muscle exceeded that for white muscle. Similarly, synthesis rates in the heart and gizzard were significantly higher than rates obtained for the PLD, a white muscle.

A subsequent study (Laurent et al., 1978b) monitoring the incorporation of ^{14}C-proline into hydroxyproline of the ALD and PLD muscles of adult SCWL males, confirmed the essentially inert nature of muscle collagen. Fractional synthesis rates were equal at .88%/day regardless of the muscle type (oxidative versus glycolytic, respectively). Hypertrophy induced a temporary increase in the rate of collagen synthesis, albeit minor in comparison to the increase in total muscle protein synthesis.

Laurent and Sparrow (1977) estimated the rates of protein synthesis and degradation of the ALD muscle of the adult SCWL from the rate of loss of protein-bound ^{3}H-leucine. Reutilization of the label was minimized by the administration of a "chase" in the form of L-[4,5-^{3}H] leucine and the feeding of a high protein diet. Hypertrophy was associated with a

marked increase in fractional synthesis rate from 4.8 to 7.3%/day. Degradation rate appeared to be unchanged with hypertrophy, but may have been confounded with label reutilization. Further investigation into response of SCWL cockerels to hypertrophy using a constant infusion of ^{14}C-proline demonstrated similar changes in protein synthesis although of a greater magnitude (Laurent et al., 1978c). An increase from 15.9 to 32.4%/day in fractional synthesis rate of the ALD muscle was observed within 24 hr. Changes in the fractional synthesis rate with hypertrophy involved equal increases for the sarcoplasmic and myofibrillar fractions. Protein degradation increased slightly from 17.0 to 24.6%/day by 28 days of imposed hypertrophy.

A comparison between crossbred (New Hampshire x SCWL) and Rock Cornish chicks yielded fractional growth rates of 6.4 and 10.1% at 14 days of age, respectively (Maruyama et al., 1978). No differences in protein synthesis rates for leg and breast muscles were observed at 14 days of age, using a continuous dietary infusion of [U-^{14}C] tyrosine incorporated into an agar gel. Fractional degradation rate of leg muscles tended to decrease with an increase in the fractional rate of growth. Protein synthesis in leg muscle was independent of age while synthesis rate decreased from 38.0 to 21.5%/day in breast muscle of crossbred chicks from 7 to 14 days of age. Protein degradation declined in both muscle types with age.

Dietary AA content influenced turnover in both muscle types to varying degrees. Feeding lysine at 50% of the requirement failed to effect protein synthesis but protein degradation doubled in both leg and breast muscles. Protein synthesis decreased in leg muscle with a diet

containing all essential AA at 75% of requirements, an energy restricted diet or a diet restricted in both protein and energy. Degradation rates increased slightly in leg muscle and markedly in breast muscle on all three dietary regimes. Breast muscle appeared to be highly resistant to changes in fractional synthesis rate with the exception of an increase in synthesis in response to a combined protein-energy restriction. Thus, growth depressions arising from deficient diets were not associated with a fall in the efficiency or capacity for protein synthesis.

The large decrease in protein retention of Rock Cornish chicks from 62.5 to 10% with a dietary deficiency appears to be related to alterations in protein degradation rather than protein synthesis. Conversely, a decrease in the rate of degradation seemingly played a more important role in the achievement of a high fractional growth rate with only small changes in synthesis rate.

MacDonald and Swick (1981) questioned the conclusions of Maruyama and co-workers (1978) on the basis of synthesis rate comparisons with age rather than weight controls. Using a massive dose of [1-^{14}C] valine, developmental changes in breast muscle turnover were determined for SCWL cockerels from hatch to 7 weeks of age. Both synthesis and degradation rates stabilized beyond 4 weeks of age, with a 50% decrease in both between 1 and 2 weeks of age. Surprisingly, rates of synthesis and degradation were indistinguishable at 6 and 7 weeks, indicating no net protein accretion in breast muscle. Thus, they contended that the apparent insensitivity of breast muscle in terms of fractional synthesis rate noted previously (Maruyama et al., 1978) could be explained on the basis of arrested developmental changes in response to the introduction of a

dietary deficiency at 1 week of age. Reanalysis of the data using weight comparisons showed reduced fractional synthesis rates in breast muscle of restricted chicks with smaller changes in degradation rate.

In an attempt to avoid the confounding effects of developmental changes, MacDonald and Swick (1981) introduced a protein-free diet at 4 weeks of age. Fractional synthesis rates decreased 50% in pectoral muscle of SCWL chicks within 7 days, while degradation rates were unaffected. By 17 days, degradation rates of protein-depleted birds increased relative to values noted at 4 weeks while synthesis rates remained slightly depressed compared to control chicks of the same weight. Both synthesis and degradation were elevated in birds fed protein-free diets in comparison to control chicks of the same age, with differences in degradation rates predominating even in the absence of developmental changes in protein turnover. Refeeding the control diet for 5 days acted to increase both synthesis and degradation in pectoral muscle with respect to weight and age controls.

Data reported by MacDonald and Swick (1981) are in general agreement with trends observed for protein-depleted rats, while those of Maruyama et al. (1978) suggest a markedly different regulation of pectoral protein turnover in response to a milder dietary restriction. Further studies are warranted to elucidate controlling mechanisms of protein accretion in pectoral muscles of poultry.

STUDY I

RELATIVE DEFICIENCIES OF AMINO ACIDS AND NITROGEN *PER SE* IN LOW PROTEIN DIETS FOR YOUNG TURKEYS

INTRODUCTION

In recent years, attempts to reduce the cost of turkey production have increasingly centered on the efficacy of lowering the dietary protein level through the use of crystalline amino acid (AA) supplementation. Accurate identification of limiting AA as well as levels of required supplementation has limited the practical implementation of low protein diets for growing turkeys.

Stas and Potter (1982) reported equal growth of poults from 8 to 20 days of age with a control 30% protein diet and a 22% protein diet supplemented with an AA mixture. Lysine, threonine, valine and isoleucine were identified as limiting AA in a 22% protein diet composed primarily of corn and soybean meal with .3% supplemental methionine based on growth depressions associated with their removal from the AA mixture. Unexpectedly, weight gain was not reduced to the level of the basal 22% protein diet, suggesting that a nitrogen deficiency *per se* was confounded with the essential AA insufficiency of the low protein diet.

The present study was designed to distinguish between the relative deficiencies of lysine, threonine, valine and crude protein *per se* in a low protein diet composed of yellow corn and soybean meal with .3% supplemental methionine for young turkeys. Glutamic acid served as a non-specific nitrogen source in that efficient utilization of this amino acid for growth of chicks has been reported (Blair *et al.*, 1972; Allen and Baker, 1974).

EXPERIMENTAL PROCEDURES

Three similar trials were performed using a total of 1,296 sexed poults of a medium-size variety obtained from the university flock. Poults were placed in Petersime starter batteries at one day of age and fed a common practical-type starter diet (Table 1) to 8 days of age. At that time, poults were allotted to experimental groups on the basis of body weight to ensure similar average pen weights at the start of each experiment. Experimental diets were introduced at 8 days of age and fed to 20 days in Experiments 1 and 2 and to 19 days of age in Experiment 3. Each diet was offered ad libitum to two pens of nine males and two pens of nine females in each trial. Group body weight and feed consumption data were recorded at 15 and 20 days of age for the initial two experiments while comparable values were obtained on days 14 and 19 for Experiment 3.

The composition of the 22% protein basal diet is presented in Table 2. Substitution of 10.08 and 20.15% soybean meal for an equal amount of yellow corn yielded the 26 and 30% protein diets, respectively. A mixture of AA equivalent to that provided by the difference between the 22 and 30% protein diets was added to the 22% protein diet (Table 3). Additional diets were formulated by individually removing lysine, threonine, or valine from the AA mixture and by adding glutamic acid to each to form a 5 x 2 design exclusive of the 26 and 30% protein diets. All AA were added as the L-isomer with the exception of DL-methionine. Lysine and arginine were supplied as the hydrochloride. The AA mixture and glutamic acid replaced equal aliquots of glucose monohydrate where required. Vitamin

and minerals were present in all diets to exceed requirements for poults as recommended by NRC (1984).

Combined performance data for the three trials were subjected to analysis of variance. Significant differences between diet means were calculated using the method of least significant differences.

RESULTS

Dietary protein level exerted a significant influence on weight gain of poults from 8 to 19 or 20 days of age (Table 4). By increasing the protein content from 22 to 26 and 30%, body weight gains increased 15.6 and 23.1% ($P<.001$), respectively. Poults responded to the 26% protein diet with an 11.5% improvement ($P<.001$) in feed efficiency. No further increase was noted with the 30% protein diet.

The addition of 4% glutamic acid to the 22% protein diet failed to improve either weight gain or feed efficiency, regardless of dietary AA status. By adding the AA mixture to the 22% protein diet, body weight gains increased 18.4% ($P<.001$), a value not significantly different from the 23.1% increase obtained by adding 8% protein from the addition of soybean meal in place of ground yellow corn to form the 30% protein diet. Feed efficiency was similar for the 26 and 30% protein diets and the AA supplemented 22% protein diet.

The removal of lysine, threonine or valine from the AA mixture produced smaller body weight gain and feed efficiency ($P<.001$) than that of the fully supplemented diet (Table 4). Differences in body weight gain among the AA deficient diets were not significant, thus, the data were pooled for the comparison between these diets and the basal 22% protein diet with and without added glutamic acid. A signficant ($P<.05$) increase in body weight gain of 6.7 g was observed with the addition of the AA mixture, albeit devoid of lysine, threonine or valine. Furthermore, feed efficiency was significantly higher for the AA deficient diets in comparison to the 22% protein diet.

DISCUSSION

Increasing the protein content of the corn-soybean meal diet from 22 to 26 or 30% increased body weight gain as expected due to the apparent AA deficiencies in the 22% protein diet. Removal of lysine, threonine or valine from the AA supplemented 22% protein diet markedly reduced growth rate of poults in support of previous findings by Stas and Potter (1982). Regardless of the AA deleted, the resultant growth depressions were essentially equal, indicating that lysine, threoinine, and valine were equally limiting. Lysine and threonine contents of the basal diet were calculated to be deficient at 76 and 89% of NRC recommendations, respectively (Table 5).

D'Mello and Emmans (1975) demonstrated the interdependence of lysine and arginine requirements for poults. In the present study, the removal of lysine from the AA-supplemented 22% protein diet produced a diet containing 2.23% arginine and 1.29% lysine, with the result that an absolute lysine insufficiency may have been exacerbated by an arginine excess.

The apparent valine deficiency of the 22% protein diet is surprising in view of its presence at 1.25%, or 104% of NRC requirements. Using a factorial design, D'Mello (1975) established a requirement of 1.21% valine for poults from 7 to 21 days of age, provided the leucine content did not exceed 1.42% in semi-purified diets. With an increase in the dietary leucine level to 1.72%, a valine content of 1.36% was required to maintain optimum growth. In the present work, the AA-supplemented 22% protein diet contained 2.46% leucine. Thus, deletion of valine without the concomitant removal of leucine from the AA mixture may have precipi-

tated an aliphatic AA antagonism, thus producing the observed severe growth depression.

Although the deletions of a single AA resulted in marked depressions in growth and feed efficiency, a reduction to the level of performance obtained with the basal diet was not observed. Furthermore, the differences in weight gain between the 22% protein diet and AA deficient diets were approximately equal, regardless of the AA removed. Reconciliation of the superior weight gain on the basis of the presence of a limiting AA in the AA mixture beyond lysine, threonine or valine is attractive in view of the sulfur AA content of the basal diet at 95% of NRC recommendations (Table 5).

The addition of 4% glutamic acid to the basal or to the AA supplemented diets failed to improve performance, indicating that nitrogen per se was not the limiting factor to growth, assuming efficient use of this AA as a non-specific nitrogen source as suggested by others (Blair et al., 1972; Allen and Baker, 1974). From these results it is postulated that the remaining AA in the added mixture are influencing the lysine, threonine or valine adequacy of the 22% protein corn-soybean meal diet with .3% added methionine.

SUMMARY

Three trials were conducted to determine the relative deficiencies of lysine, threonine, valine and nitrogen per se in a low protein diet composed primarily of corn, soybean meal and .3% added DL-methionine fed to young turkeys. Diets containing 22, 26 and 30% protein served as controls. A mixture of amino acids (AA) was added to the 22% protein diet. Additional diets were formulated by individually removing lysine, threonine or valine from the AA mixture and by adding 4% glutamic acid to each to form a 5 x 2 design exclusive of the 26 and 30% protein diets. Each diet was fed ad libitum to two pens of nine male poults and two pens of nine female poults of a medium-size variety from 8 to 19 or 20 days of age in each experiment.

Body weight gains and feed consumptions were increased ($P<.001$) by supplementing the 22% protein diet with the AA mixture or by increasing the protein level to 26 or 30%. Removal of either lysine, threonine or valine from the AA mixture produced body weight gains and feed efficiencies which were smaller ($P<.001$) than the fully supplemented diets but greater ($P<.05$) than the unsupplemented diet. The addition of 4% glutamic acid to the diets failed to improve performance, indicating that nitrogen per se is not the limiting factor for growth. From these results, it is postulated that the remaining AA in the added mixture are influencing the lysine, threonine or valine adequacy of the 22% protein corn-soybean meal diet containing .3% added methionine.

Table 1. Composition of starter diet fed to 7 days of age

Ingredient	g/kg
Ground yellow corn	426.224
Stabilized fat	20
Dehulled soybean meal	445
Menhaden fish meal	50
Poultry meal blend	25
Defluorinated phosphate	19
Ground limestone	5
Sodium chloride	4
DL-Methionine	3
Trace mineral mix[a]	.5
Vitamins and feed additives[b]	2.276
Total	1000.000
Calculated composition	
Protein (%)	29.8
Energy (kcal ME/kg)	2919
Methionine (%)	.82
TSAA (%)	1.25
Lysine (%)	1.83

[a]Supplied per kg of diet: 75 mg manganese, 50 mg zinc, 35 mg iron, 5 mg copper, 1.1 mg iodine, and .4 mg cobalt from manganese oxide, zinc oxide, ferrous sulfate, copper oxide, calcium iodate, and cobalt carbonate, respectively, and calcium carbonate as a diluent.

[b]Supplied per kg of diet: 13,200 IU vitamin A, 6,600 ICU vitamin D_3, 11 IU vitamin E, 3.5 mg menadione dimethylpyrimidinol bisulfite, 1.1 mg thiamine HCl, 5.5 mg riboflavin, 16.5 mg D-calcium pantothenate, 66 mg niacin, 500 mg choline chloride, 14.6 µg vitamin B_{12}, 1.1 mg folic acid, .11 mg biotin, 1.1 mg pyridoxine HCl, 125 mg ethoxyquin, 44 mg bacitracin, and .2 mg selenium.

Table 2. Composition of the basal 22% protein diet

Ingredient	g/kg
Ground yellow corn	413.38
Glucose monohydrate	95
Stabilized fat	60
Dehulled soybean meal	380
Defluorinated phosphate	37.5
Iodized salt	4
Trace mineral mix[a]	1
DL-Methionine	3
Vitamins and feed additives[b]	6.12
Total	1000.00

[a]Supplied per kg of diet: 150 mg manganese, 100 mg zinc, 70 mg iron, 10 mg copper, 2.2 mg iodine, and .8 mg cobalt from manganese oxide, zinc oxide, ferrous sulfate, copper oxide, calcium iodate, and cobalt carbonate, respectively, and calcium carbonate as a diluent.

[b]Supplied per kg of diet: 14,300 IU vitamin A, 7,150 ICU vitamin D_3, 55 IU vitamin E, 5.5 mg menadione dimethylpyrimidinol bisulfite, 3.3 mg thiamine HCl, 11 mg riboflavin, 22 mg D-calcium pantothenate, 110 mg niacin, 2,200 mg choline chloride, 15.4 µg vitamin B_{12}, 2.2 mg folic acid, .22 mg biotin, 5.5 mg pyridoxine HCl, 125 mg ethoxyquin, .2 mg selenium, and 55 mg erythromycin thiocyanate.

Table 3. Amino acid composition of the 22 and 30% protein diets (%)[1]

Amino acid	22% protein	30% protein	Difference
Arginine	1.59	2.23	.64
Glycine	1.02	1.41	.39
Serine	1.25	1.76	.50
Histidine	.58	.81	.23
Isoleucine	1.12	1.57	.44
Leucine	1.92	2.46	.54
Lysine	1.29	1.89	.60
Methionine	.66	.76	.10
Cystine	.34	.46	.12
Phenylalanine	1.00	1.33	.33
Tyrosine	.95	1.26	.31
Threonine	.89	1.20	.31
Tryptophan	.30	.42	.12
Valine	1.25	1.69	.44

[1]Calculated from values given by NRC (1984).

Table 4. Average body weight gain, feed consumption and feed efficiency for turkeys from 8 to 19 or 20 days of age

Protein (%)	Amino acid supplementation	Body weight gain (g) Glutamic acid 0	Body weight gain (g) Glutamic acid 4	Feed consumption (g) Glutamic acid 0	Feed consumption (g) Glutamic acid 4	Feed efficiency Glutamic acid 0	Feed efficiency Glutamic acid 4
22	None	173.6[1]	178.9	283.3	284.4	.6124	.6295
	Added[2]	205.6	205.4	303.0	302.6	.6783	.6777
	Added minus lysine	181.0	186.0	279.3	286.7	.6481	.6485
	Added minus threonine	185.9	182.5	290.2	286.1	.6406	.6385
	Added minus valine	182.1	180.2	277.3	277.7	.6560	.6483
26	None	200.7	---	293.7	---	.6830	---
30	None	213.7	---	311.2	---	.6856	---
Difference required for significance		9.7		11.7		.0206	

[1]Each value represents the average of 12 pens or 108 poults per average.

[2]Amino acid mixture contains .64% arginine, .39% glycine, 50% serine, .23% histidine, .44% isoleucine, .54% leucine, .59% lysine, .10% methionine, .12% cystine, .33% phenylanine, .31% tyrosine, .31% threonine, .12% tryptophan, and .44% valine.

Table 5. Comparison of NRC requirements with the calculated amino acid composition of the basal 22% protein diet expressed as a percent of diet

Amino acid	NRC (1984) requirement (0-4 weeks) (%)	22% protein diet (%)	Percentage of requirement
Arginine	1.6	1.59	99
Glycine and serine	1.0	2.27	227
Histidine	.58	.58	100
Isoleucine	1.1	1.12	102
Leucine	1.9	1.92	101
Lysine	1.7	1.29	76
Methionine and cystine	1.05	1.00	95
Methionine	.53	.66	125
Phenylalanine and tyrosine	1.8	1.95	108
Phenylalanine	1.0	1.00	100
Threonine	1.0	.89	89
Tryptophan	.26	.30	115
Valine	1.2	1.25	104

STUDY II

INFLUENCE OF BASIC AND BRANCHED CHAIN AMINO ACID INTERACTIONS ON THE LYSINE AND VALINE REQUIREMENTS OF YOUNG TURKEYS

INTRODUCTION

The successful implementation of low protein diets for young turkeys requires accurate identification and supplementation of limiting amino acids. A reduction in the protein level of a corn-soybean meal diet to 22% results in deficiencies of lysine, threonine, valine and isoleucine based on retarded performance with their removal from an essential amino acid supplement (Stas and Potter, 1982; Jackson et al., 1983). Growth depressions were approximately equal with lysine, threonine or valine deletion regardless of their presence at different levels relative to requirements (NRC, 1984), inferring influences of the remaining amino acids in the supplement.

Previous reports have shown that interactions among amino acids can influence requirements. The interrelationship between lysine and arginine has been demonstrated for the broiler chick (Anderson and Dobson, 1959; D'Mello and Lewis, 1970a,c). Although D'Mello and Emmans (1975) observed little or no interdependence between the lysine and arginine requirements of the poult, the levels evaluated did not greatly exceed NRC (1984) recommendations. Thus, the effects of excess quantities of either amino acid could not be established from their study.

Investigations of interactions among the branched chain amino acids have shown that dietary leucine content influenced both the valine and isoleucine requirement of the broiler chick (D'Mello and Lewis, 1970b,c). An increase in dietary leucine from 1.42 to 1.72% necessitated a concurrent elevation in dietary valine from 1.21 to 1.36% to achieve maximum weight gain and feed efficiency of young turkeys (D'Mello, 1975). Current

estimates of the leucine and valine requirements of the poult are placed at 1.9 and 1.2%, respectively (NRC, 1984).

The present study was designed to differentiate between deficiencies of lysine and valine in a 22% protein diet composed primarily of corn and soybean meal. The first experiment was conducted to elucidate the effects of increases in dietary arginine and lysine above requirements on the performance of turkeys from 7 to 19 days of age. The second experiment was performed to investigate the effects of excess dietary leucine on the valine and isoleucine adequacy of a 22% protein diet.

EXPERIMENTAL PROCEDURES

Experiment 1. Three trials were performed employing a total of 1,152 sexed Large White poults. A 30% protein starter diet was fed from hatch to 7 days of age (Table 1). Poults were then separated into similar weight groups and randomly distributed into Petersime starter batteries. Each experimental diet was fed ad libitum from 7 to 19 days of age to two pens of eight males and two pens of eight females in each trial with the exception of the 22% protein diet which was fed to four pens of each sex. Body weight gains and feed consumptions were obtained for periods between 7 and 13 or 19 days of age by pens.

The composition of the basal 22% protein diet is shown in Table 2. Replacement of 20.15% ground yellow corn with an equal amount of soybean meal yielded a 30% protein diet. Mean determined protein contents (N x 6.25) of the 22% and 30% protein diets were 21.3 and 29.4%, respectively. On the basis of calculated amino acid contents (NRC, 1977), a mixture was formulated to contain the difference in essential amino acid content between the 22 and 30% protein diets (Table 6). Supplementation of the basal diet with the amino acid mixture produced a diet equal in essential amino acid content to the 30% protein diet with the exception of lysine and arginine. Increments of lysine (0, .60, and 1.20%) and arginine (0, .64, and 1.28%), respectively, were added to the amino acid mixture in a factorial arrangement. These levels were chosen so that each increment represented the lysine and arginine contents of 8% dietary protein derived by substituting dehulled soybean meal for yellow corn in the 22% protein diet (NRC, 1984). All amino acids were added as the L-isomer with the

exception of DL-methionine. Lysine and arginine were added as the hydrochloride.

Statistical analyses included an analysis of variance of the data from the combination of the three trials. In addition, a subset of the data comprised of the factorial treatments was reanalyzed for the detection of significant interactions between the test amino acids. Significant differences between factor means were determined using Duncan's Multiple Range Test. Comparisons between factor and control means were made by the method of Least Significant Difference.

Experiment 2. Three trials were conducted using a total of 1,344 sexed Large White poults. Management of poults and collection of data were as described for Experiment 1. Each diet was fed to two pens of eight males and two pens of eight females in each trial.

Formulation of the control 22 and 30% protein diets was the same as that described for Experiment 1 (Table 2). Mean determined protein contents (N x 6.25) of the 22 and 30% protein diets were 20.4 and 28.9%, respectively. Additional diets encompassed various levels of supplemental leucine, isoleucine, and valine inclusion in the amino acid mixture added to the 22% protein diet (Table 6). Increments of leucine (0, .54, and 1.08%), isoleucine (0 and .44%), and valine (0 and .44%) were arranged factorially. Each increment of the supplemental amino acids on test was chosen to equal their respective amounts in 8% dietary protein derived by substituting dehulled soybean meal for ground yellow corn in the 22% protein diet (NRC, 1984). Statistical analyses were similar to those delineated for Experiment 1.

RESULTS

Experiment 1. The analysis of variance of the factorial treatments is presented in Table 7. Decreasing supplemental lysine from .60 to 0% caused an 8.6% reduction ($P<.001$) in body weight gain (Table 8). A similar reduction in dietary lysine achieved by decreasing absolute protein from 30 to 22%, decreased weight gain by 10.5%. The effect of a lysine deficit on feed utilization was less pronounced, with lysine deletion producing only a 2.4% depression contrasted to a difference of 8.4% between the 30 and 22% protein diets.

The addition of 1.28% arginine to the amino acid mixture significantly ($P<.05$) improved weight gain and feed efficiency. The only interaction of significance (Table 7) between lysine and arginine was that for feed consumption ($P<.001$). Although feed intake increased as arginine was added to diets containing no supplemental lysine, arginine addition had no effect on feed consumption at higher levels of dietary lysine (Figure 1).

Experiment 2. The analysis of variance for the factorial treatments is presented in Table 9. Each increment of added leucine significantly ($P<.001$) depressed weight gain and feed efficiency (Table 10). Although a level of .54% leucine was sufficient to decrease feed efficiency of female poults, 1.08% supplemental leucine was required for an adverse affect on male poults, producing a significant ($P<.01$) leucine x sex interaction. Dietary isoleucine content had no effect on weight gain or feed consumption(Table 10), but feed efficiency was improved with isoleucine supplementation ($P<.05$). However, this improvement was due to a large increase in feed efficiency by poults in Trial 3 of the series

only, questioning the validity of the response. The addition of .44% valine to the amino acid mixture resulted in an improvement in weight gain of 10.3%, accompanied by increases in both feed intake and feed efficiency ($P<.001$). Weight gain and feed efficiency were 4.9 and 5.3% higher, respectively, for poults fed diets lacking added valine in comparison to the basal 22% protein diet.

A significant interaction ($P<.01$) between leucine and valine for body weight gain resulted from the linear decrease in gain with each increment of added leucine in the absence of supplemental valine (Figure 2). Growth was independent of supplemental leucine with valine-supplemented diets. The response of poults to a combination of excess leucine and deficient valine was similar for feed consumption (Figure 3), resulting in a significant interaction ($P<.01$). Maximum weight gain and feed intake were not observed with diets containing 1.25% valine despite the removal of leucine from the amino acid mixture.

A reciprocal antagonism resulted in a significant ($P<.001$) interaction between isoleucine and valine for weight gain (Figure 4). A 2.5% increase in weight gain was realized by the concomitant removal of isoleucine and valine relative to the same diet containing .44% added isoleucine. Conversely, added valine was detrimental in the absence of supplemental isoleucine, as evidenced by a 3.2% reduction in weight gain. This relationship between isoleucine and valine was more pronounced for female than male poults, producing a significant ($P<.05$) isoleucine x valine x sex interaction.

A significant interaction ($P<.05$) for feed intake resulted from a depression in consumption with a level of .44% supplemental isoleucine

in diets devoid of added valine (Figure 5). Dietary isoleucine did not signficantly alter feed intake when valine was added to the amino acid mixture. Again, males were more tolerant of an imbalance between isoleucine and valine than were females, resulting in an isoleucine x valine x sex interaction ($P<.05$) for feed consumption. The isoleucine x valine interaction ($P<.05$) for feed efficiency arose from improved feed utilization in response to isoleucine supplementation of diets containing added valine while feed efficiency remained unchanged with isoleucine addition to diets lacking supplemental valine (Figure 6).

DISCUSSION

Lysine deletion from the amino acid mixture resulted in significant depressions in the performance of poults in agreement with previous reports (Stas and Potter, 1982; Jackson et al., 1983). The response of the poult to various combinations of lysine and arginine appears to differ from that of the broiler chick with little or no interdependence between lysine and arginine requirements in evidence (D'Mello and Emmans, 1975). However, the highest levels of lysine and arginine employed in their study (1.55 and 1.75%, respectively) did not greatly exceed requirements, such that the effects of excess quantities of either amino acid could not be established. The absence of an interaction between lysine and arginine for body weight gain or feed efficiency is indicative of independent responses to these amino acids within the constraints of the present experiment. The inferior growth and feed efficiency of poults fed diets containing 1.29% lysine was not exacerbated by arginine supplementation up to 179% of the NRC (1984) requirement. Thus, the difference in the lysine content of corn-soybean meal diets containing 22 and 30% protein was responsible for the observed retardations in performance independently of arginine content. Additional deficiencies in the 22% protein diet beyond lysine were also apparent, evidenced by improvements of 1.9% in weight gain and 6.0% in feed efficiency, attributable to the supplement devoid of lysine.

Increasing the dietary leucine content above the requirement of 1.9% (NRC, 1984) adversely affected poult performance. Elevation in leucine from the basal level of 1.92% to 2.46 and 3.00% in diets containing no supplemental valine produced stepwise decreases in weight gain and feed

efficiency, indicative of increased valine requirements with each increment of leucine. Thus, leucine content had a direct influence on the dietary valine requirement consistent with reports concerning the deleterious effects of excess leucine on the valine adequacy for broilers (D'Mello and Lewis, 1970b,c) and poults (D'Mello, 1975). Weight gain from the amino acid supplemented diet devoid of added valine and leucine was 292 g in comparison to only 268 g for the basal 22% protein diet, a difference of 8.2%. The superior gain for the former can be explained by the presence of lysine and threonine in the amino acid mixture (Stas and Potter, 1982; Jackson et al., 1983).

Although studies by D'Mello and Lewis (1970b,c) indicated adverse effects of leucine on the isoleucine requirements of chicks, supplemental leucine had no affect on the isoleucine adequacy of the 22% protein diet fed in the present experiment. Stas and Potter (1982) observed a small decrease in weight gain of poults with a reduction in isoleucine to the basal level of 1.12%. The present results infer that this decrease was due to valine in the amino acid mixture.

The relationship between isoleucine and valine has not been adequately investigated. In the present study, a reciprocal antagonism between these amino acids was demonstrated when either was present at or near the requirement level. Thus, a marginal valine deficiency was exacerbated by isoleucine supplementation. Conversely, isoleucine requirements for growth increased with valine addition. Previous studies have shown that plasma valine concentration declines with isoleucine supplementation of broiler (D'Mello and Lewis, 1970c) and poult (D'Mello, 1975) diets.

Despite the removal of excess leucine or isoleucine from the amino acid mixture to the requirement level, maximum weight gain or feed efficiency was not achieved with diets containing 1.25% valine. Thus, the improved performance of poults in response to valine supplementation indicates a true valine deficiency in the 22% protein diet. Reported valine requirements of poults vary considerably and may be largely attributable to the dietary leucine contents used in the investigations. Low levels of dietary leucine can be achieved with the use of purified ingredients and, in consequence, result in minimum estimates of the valine requirement (Kelly, 1970). High levels of dietary leucine necessitate increased valine to obtain an amino acid balance capable of supporting maximum growth (Warnick and Anderson, 1973). D'Mello (1975) reported valine and leucine requirements of 1.21 and 1.42%, respectively, for the young turkey using semi-purified diets. A level of 1.36% valine was required for maximum weight gain when dietary leucine was increased to 1.72%.

In light of the relatively high leucine content of commonly used feedstuffs, formulation of practical diets low in leucine is not feasible. Even a marked reduction in dietary protein to 22%, as in the present study using corn and soybean meal as the sole protein sources, produced a diet containing 1.92% leucine. The design of the present experiment prohibits concise estimation of the valine requirement of the poult from 7 to 19 days of age. However, a level in excess of 1.2% (NRC, 1984) appears to be warranted on the basis of the inferior performance of poults fed diets containing 1.25% valine and no added leucine or isoleucine.

In summary, both lysine and valine are deficient in a 22% protein diet composed primarily of corn and soybean meal and supplemented with

.3% DL-methionine. Furthermore, while the lysine content of the 22% protein diet was more limiting for growth than was the valine content, valine adequacy declined with the addition of leucine or isoleucine to the basal diet.

SUMMARY

Two experiments were conducted to determine the influence of amino acid interactions on the lysine and valine adequacy of a low protein diet fed to Large White turkeys from 7 to 19 days of age. Corn-soybean meal diets containing 22 and 30% protein served as controls in both experiments. Test diets involved supplementation of the 22% protein diet with several essential amino acid mixtures.

Experiment 1 involved varying the levels of lysine (0, .60 and 1.20%) and arginine (0, .64 and 1.28%) included in the amino acid mixture. A decrease in supplemental lysine from .60 to 0% reduced weight gain and feed efficiency by 8.6 and 2.4%, respectively. The lysine deficiency was not exacerbated by arginine supplementation up to 179% of the requirement.

The effects of altering the leucine (0, .54 and 1.08%) and isoleucine (0 and .44%) contents of the amino acid mixture on the valine requirement were investigated in Experiment 2. Leucine and isoleucine supplementation of diets containing 1.25% valine acted independently to reduce weight gain and feed intake, but the depressions were reversed with the addition of .44% valine. Maximum weight gain and feed efficiency were not achieved with diets containing 1.25% valine and no added leucine or isoleucine, indicative of a true valine deficiency in the basal 22% protein diet. However, lysine appeared to be more deficient than valine in a 22% protein corn-soybean meal diet based on greater decreases in weight gain associated with lysine removal from the amino acid mixture.

Table 6. Composition of the amino acid mixture

Amino acid[1]	Percent added
Arginine	.64†
Cystine	.12
Glycine	.39
Histidine	.23
Isoleucine	.44*
Leucine	.54*
Lysine	.60†
Methionine	.10
Phenylalanine	.33
Serine	.50
Threonine	.31
Tryptophan	.12
Tyrosine	.31
Valine	.44*

[1]All amino acids were added as the L-isomer with the exception of DL-methionine. Lysine and arginine were added as the hydrochloride.

†Variable in Experiment 1. For levels employed, see text.

*Variable in Experiment 2. For levels employed, see text.

Table 7. Analysis of variance of body weight gain, feed consumption and feed efficiency for turkeys from 7 to 19 days of age (Experiment 1)[1]

Source of variation	df	Mean squares		
		Body weight gain	Feed consumption	Feed efficiency (x 10^{-6})
Diet (D)	8	2214***	3277***	1277***
Lysine (L)	2	7515***	9430***	2559***
Arginine (A)	2	869*	462	1176*
L x A	4	236	1608***	687
Sex (S)	1	26822***	47973***	1752*
Trial (T)	2	4268***	203	23060***
S x T	2	115	643	1185*
D x S	8	90	92	214
Error	86	183	301	321
D x T	16	123	195	221
D x T x S	16	329	365	343
Within D/T/S	54	158	313	330

[1]Analysis excludes 22 and 30% protein control diets.

*P<.05; ***P<.001.

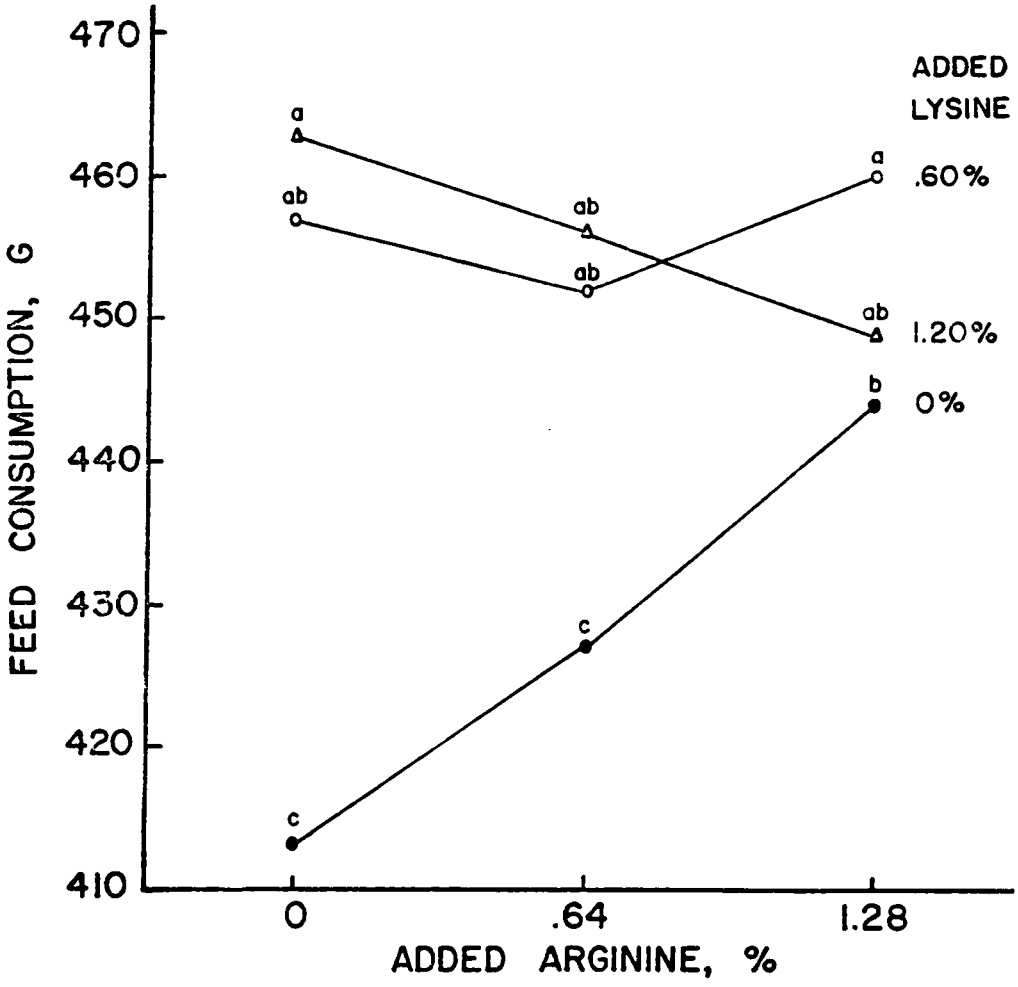

Figure 1. Plot of lysine x arginine interaction for 7- to 19-day feed consumption (Experiment 1).

Table 8. The effect of dietary lysine, arginine, and protein levels on the performance of turkeys from 7 to 19 days of age (Experiment 1)

Variable	Body weight gain (g)	Feed consumption (g)	Feed efficiency
Lysine (%)	***	***	***
0 (1.29)[1]	277[b2]	428[b]	.647[b]
.60 (1.89)	303[a]	456[a]	.663[a]
1.20 (2.49)	301[a]	456[a]	.660[a]
Arginine (%)	*		*
0 (1.59)	289[b]	444	.651[b]
.64 (2.23)	292[b]	445	.656[ab]
1.28 (2.87)	299[a]	451	.663[a]
Dietary protein (%)	***		***
22	263[b]	432	.609[b]
30	294[a]	442	.665[a]

[1]Number in parenthesis indicates total calculated content of diet.

[2]Means represent average of male and female poults. Means with like superscripts do not differ significantly (P>.05).

*P<.05; ***P<.001.

Table 9. Analysis of variance of body weight gain, feed consumption and feed efficiency of turkeys from 7 to 19 days of age (Experiment 2)[1]

Source of variation	df	Mean squares		
		Body weight gain	Feed consumption	Feed efficiency
				(x 10^{-6})
Diet (D)	11	3642***	4543***	1969***
Leucine (L)	2	2441***	721	5001***
Isoleucine (I)	1	77	175	1243*
Valine (V)	1	30622***	41895***	7643***
L x I	2	12	44	301
L x V	2	908**	1728**	191
I x V	1	2565***	2463**	1585*
L x I x V	2	37	225	90
Sex (S)	1	43573***	86054***	1081
Trial (T)	2	554*	5684***	14324***
S x T	2	107	694	322
D x S	11	155	327	421
Error	116	161	323	302
D x T	22	273	423	406
D x T x S	22	101	214	310
Within D/T/S	72	145	326	268

[1]Analysis excludes 22 and 30% protein control diets.

*P<.05; **P<.01; ***P<.001.

Table 10. The effect of dietary leucine, isoleucine, valine and protein levels on the performance of turkeys from 7 to 19 days of age (Experiment 2).

Variable	Body weight gain (g)	Feed consumption (g)	Feed efficiency
Leucine (%) [1]	*** [2]		***
0 (1.92)	302[a]	450	.671[a]
.54 (2.46)	296[b]	447	.662[b]
1.08 (3.00)	288[c]	442	.651[c]
Isoleucine (%)			*
0 (1.12)	295	448	.658[b]
.44 (1.56)	296	445	.664[a]
Valine (%)	***	***	***
0 (1.25)	281[b]	429[b]	.654[b]
.44 (1.69)	310[a]	463[a]	.669[a]
Dietary protein (%)	***	*	***
22	268[b]	432[b]	.621[b]
30	303[a]	454[a]	.667[a]

[1] Number in parenthesis indicates total calculated content of diet.

[2] Means represent average of male and female poults. Means with like superscripts do not differ significantly (P>.05).

*P<.05; ***P<.001.

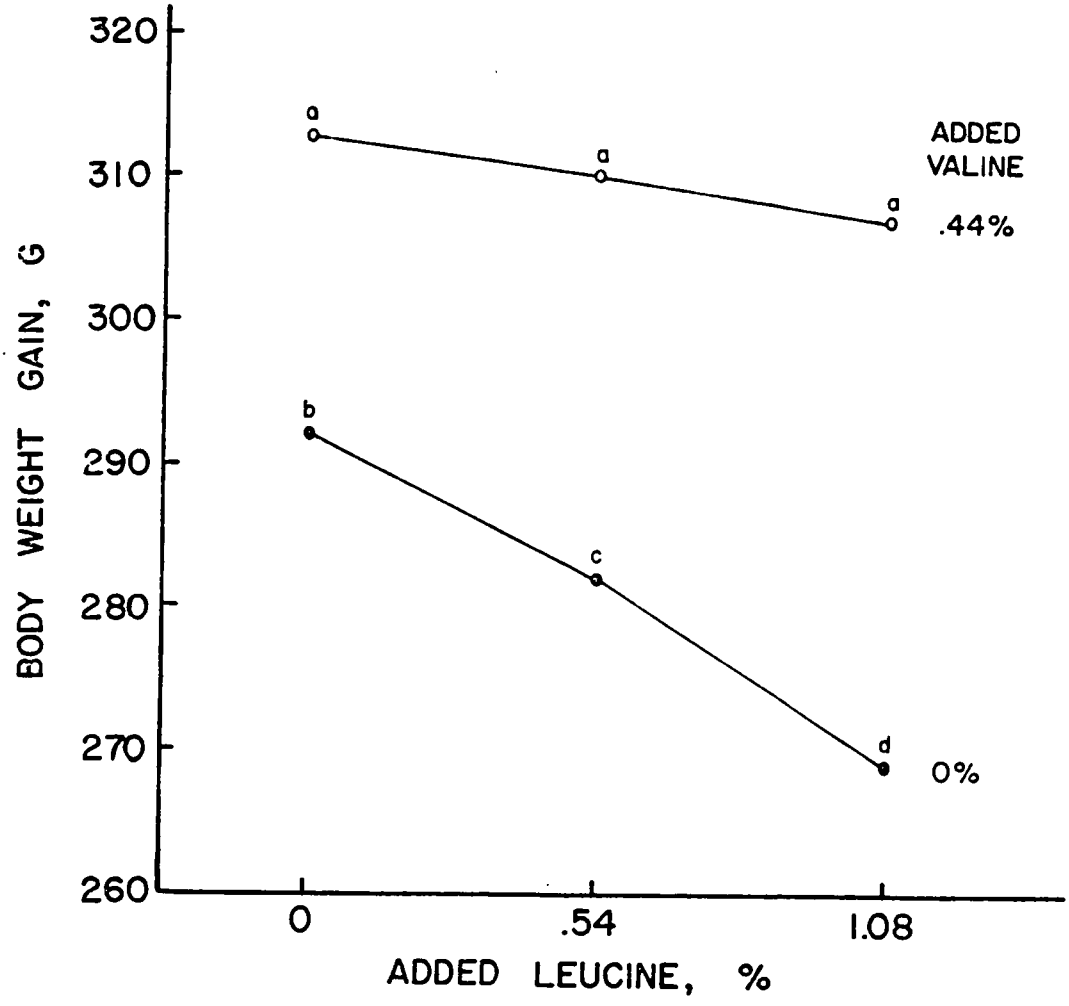

Figure 2. Plot of leucine x valine interaction for 7- to 19-day body weight gain (Experiment 2).

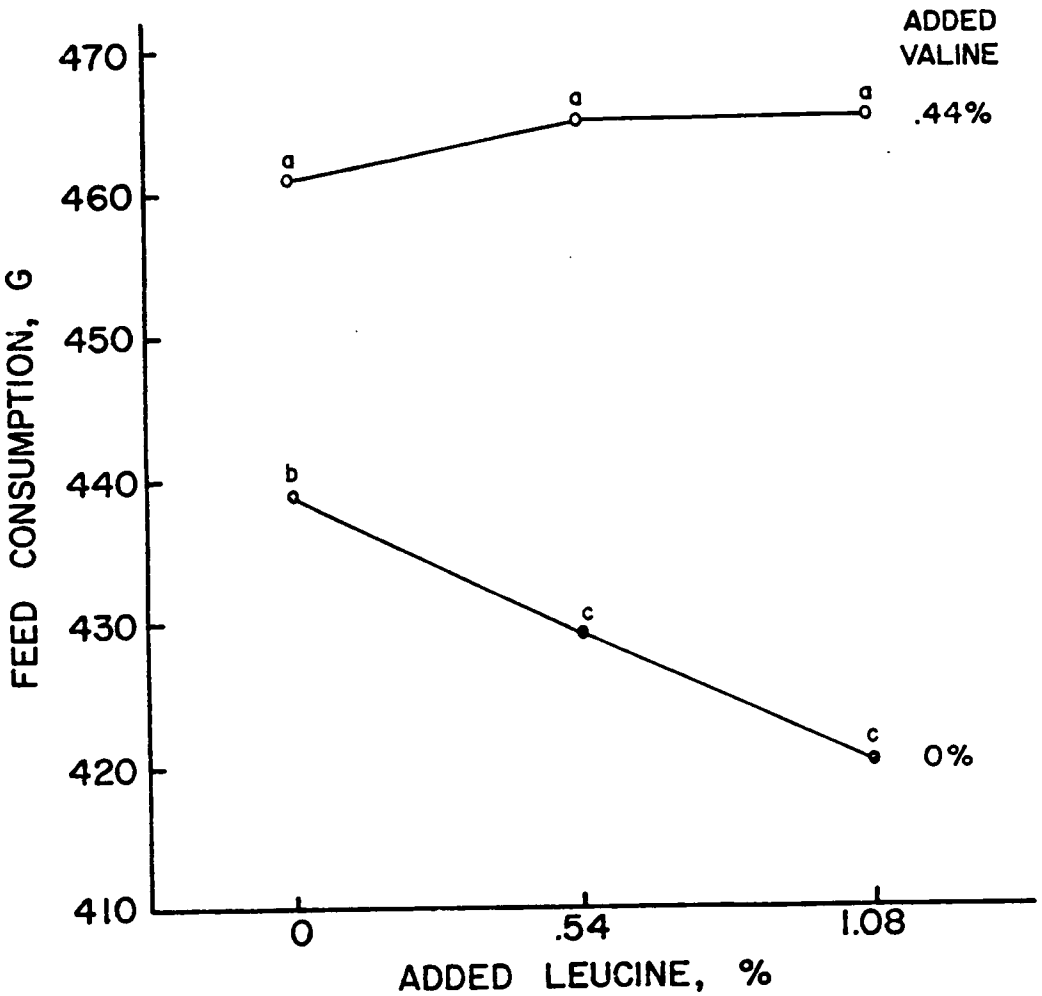

Figure 3. Plot of leucine x valine interaction for 7- to 19-day feed consumption (Experiment 2).

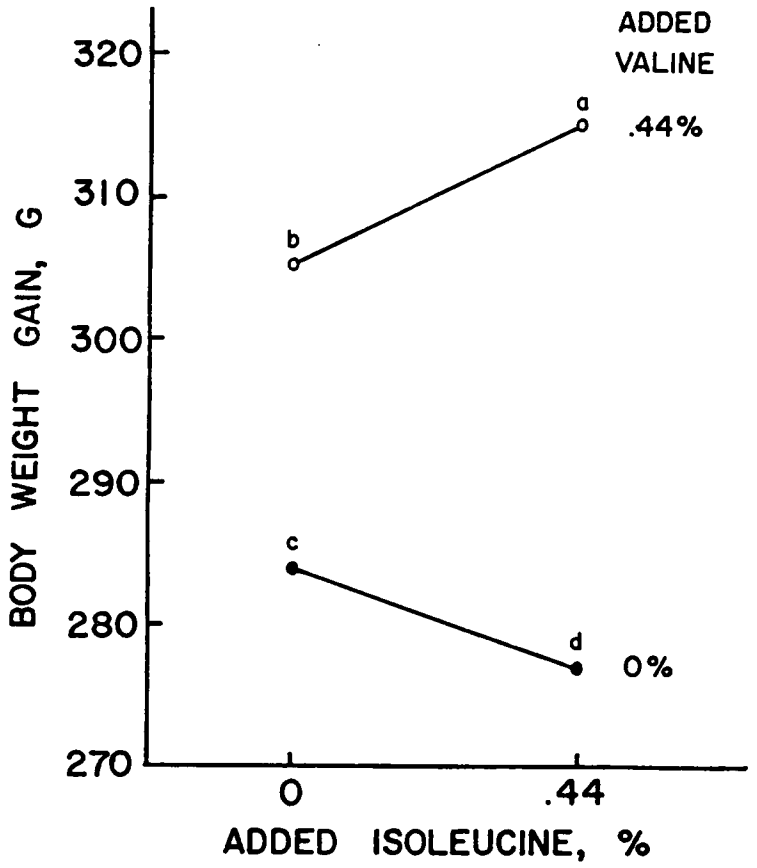

Figure 4. Plot of isoleucine x valine interaction for 7- to 19-day body weight gain (Experiment 2).

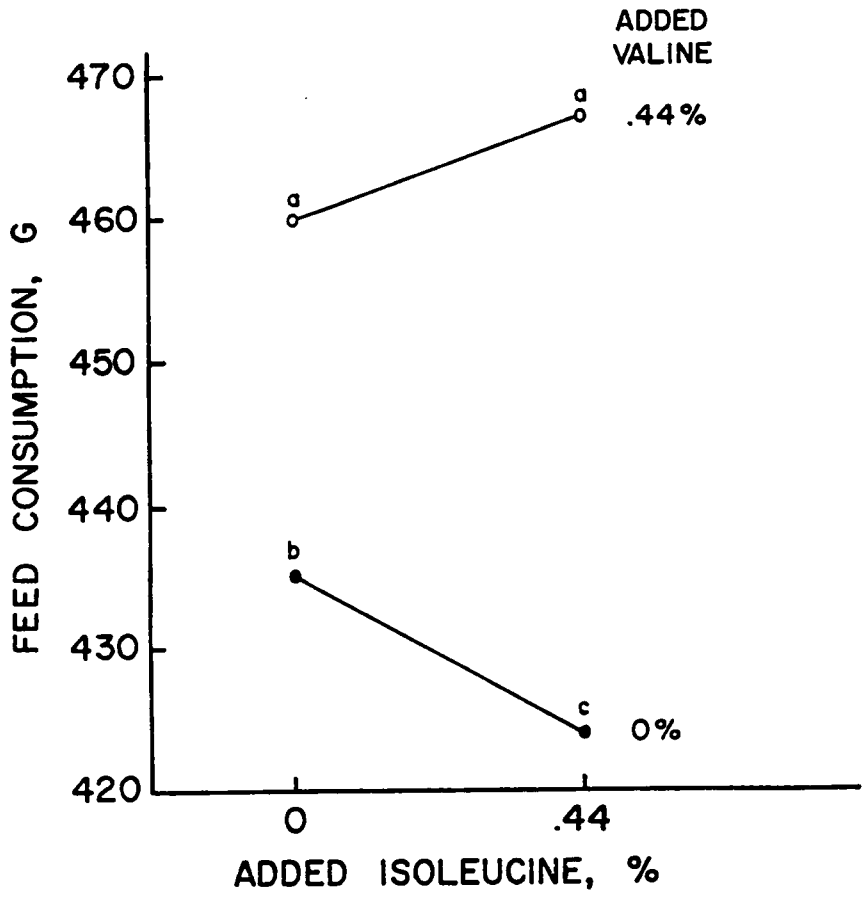

Figure 5. Plot of isoleucine x valine interaction for 7- to 19-day feed consumption (Experiment 2).

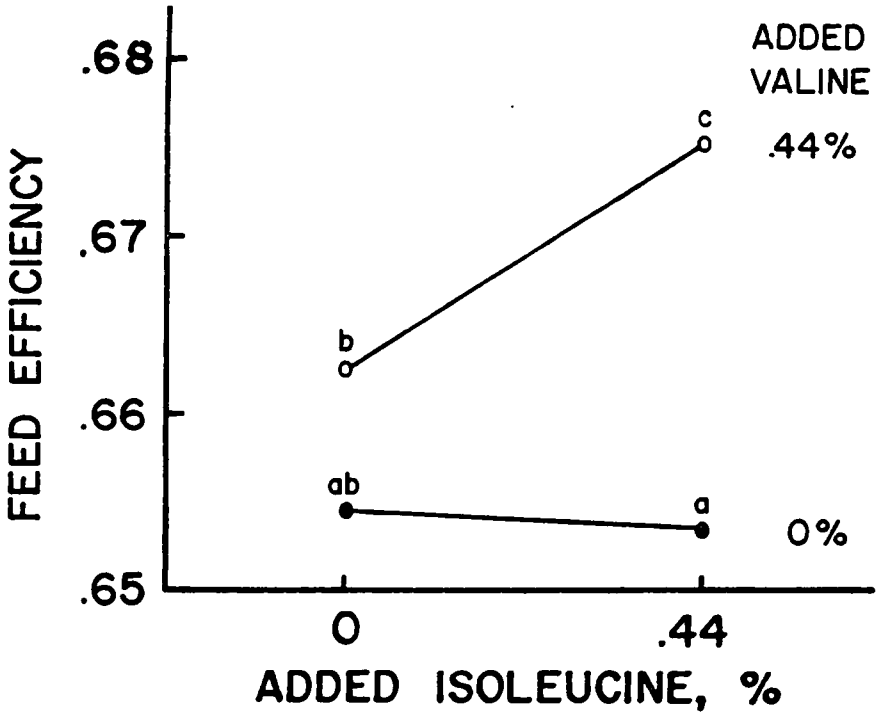

Figure 6. Plot of isoleucine x valine interaction for 7- to 19-day feed efficiency (Experiment 2).

STUDY III

EFFECT OF DIETARY PROTEIN LEVEL AND METHIONINE AND LYSINE SUPPLEMENTATION ON MUSCLE PROTEIN METABOLISM IN YOUNG TURKEYS

INTRODUCTION

Numerous studies have demonstrated the poor response of young turkeys to a reduction in dietary protein level (Klain et al., 1954; Baldini et al., 1954; Atkinson et al., 1976; Potter and Shelton, 1976; Rosebrough et al., 1982, Stas and Potter, 1982). Poor performance from low protein diets is credited to deficiencies of essential amino acids. Methionine is generally considered to be the first limiting amino acid in a corn-soybean meal diet (Ferguson et al., 1956; Fitzsimmons and Waibel, 1962; Potter and Shelton, 1976). A reduction in the protein level of a corn-soybean meal diet containing added methionine from 30 to 22% has been shown to produce deficiencies of lysine, threonine and valine (Stas and Potter, 1982; Jackson et al., 1983). Lysine appeared to be more limiting than valine based on the results of Study II. Others have reported improved performance from lysine supplementation of low protein diets for poults (Fitzsimmons and Waibel, 1962; Tuttle and Balloun, 1974; Rosebrough et al., 1982).

In view of the poor amino acid balance of a low protein diet, changes in protein metabolism might be expected to compensate for an inadequate dietary amino acid profile. Skeletal muscle accounts for the largest pool of body protein and, thus, represents the most economically important tissue of the commercial turkey. Few studies relating diet and muscle metabolism in poultry are available. Using a crystalline amino acid diet, Maruyama et al. (1978) observed no change in the fractional synthesis rate of the breast and leg muscles of chicks in response to a lysine deficiency while degradation rate increased. MacDonald and Swick (1981) found that feeding chicks a protein-free diet from 4 to 5 weeks of age markedly re-

duced the rate of pectoral protein synthesis. In the rat, decreases in protein synthesis and degradation rates in leg muscles in response to a restriction in protein intake have been observed (Millward et al., 1975; Smith et al., 1982; Sampson and Jansen, 1984).

The present study was undertaken to determine the influence of dietary protein on the composition and protein turnover of the pectoralis and gastrocnemius muscles in turkeys from 7 to 21 days of age. Methionine and lysine were added to a low protein diet to investigate the ability of limiting amino acids to offset the detrimental effects of a protein deficiency.

EXPERIMENTAL PROCEDURES

Animals and Housing. Large White turkeys were obtained from the university flock at hatch and housed under continuous lighting in Petersime starter batteries for the duration of the experiment. A standard starter diet was fed to 7 days of age (Table 1). Poults were then separated into similar weight groups and randomly assigned to battery cages. Each experimental diet was fed ad libitum from 7 to 22 days of age to five pens of eight male poults. Treatments included 30 and 22% protein diets containing .3 and 0% added DL-methionine, respectively (Table 11). The latter diet was supplemented with .4% DL-methionine or a combination of .4% DL-methionine and .6% lysine supplied in the form of L-lysine HCl. The levels of added methionine and lysine were chosen to equal their respective amounts in the control 30% protein diet. All diets were formulated to be isoenergetic by varying the amounts of glucose monohydrate and stabilized fat. Individual body weights were obtained at 7, 14 and 21 days of age. Feed consumption was determined at 14 and 21 days of age on a pen basis.

Injection Procedure. Preliminary experiments were conducted using poults at 7 and 21 days of age to determine the rise in plasma radioactivity following the subcutaneous injections of .5 and .7 μCi per 100 g body weight, respectively. For 7-day-old poults, an emulsion was prepared by homogenizing .1 ml of uniformly labeled ^{14}C-tyrosine (100 μCi/ml, 415 mCi/mmol) with 9.9 ml pure sesame oil to obtain a final concentration of 1 μCi per ml (Kang et al., 1985a). The concentration of ^{14}C-tyrosine was increased to 2 μCi per ml for 21-day-old poults.

Blood samples were collected in heparinized syringes at 10, 20, 30, 45 and 60 min following the injection at both ages. Whole blood samples were centrifuged at 2,000 g for 10 min in a table-top Sorvall centrifuge (Model GLC-3, Dupont Instruments) to prepare plasma. Aliquots of .5 ml plasma were diluted with an equal volume of cold 10% (w/v) TCA and allowed to stand 1 hr at room temperature before centrifugation. The TCA precipitate was washed twice with 5% (w/v) TCA and the washes were added to the supernatant. The protein pellet was discarded. The combined supernatant and washes were neutralized with .52N NaOH. A 1 ml aliquot of the final solution was added to 10 ml Beckman Ready-Solv EP scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA 92634) to quantitate the level of radioactivity in the nonprotein pool of plasma. Plastic scintillation vials were counted on a Beckman LS 7500 scintillation counter (Beckman Instruments, Inc., Irvine, CA 92713).

Prior to the introduction of the experimental diets at 7 days of age, 16 poults were selected at random for the determination of muscle protein synthesis. Poults were injected subcutaneously along the back at a dose of .5 µCi per 100 g body weight. All injections were carried out between 9:00 and 14:00 hr.

Isotopic studies were repeated on days 21 and 22, with four poults from each treatment injected subcutaneously on each day. The large number of poults involved created handling difficulties within the time constraints allowed, thus necessitating a 2-day interval to complete the requisite injections. The procedure followed was as described for day 7 although the dose was increased to .7 µCi per 100 g body weight. Prepa-

ration of the emulsion involved homogenizing .2 ml of [U-^{14}C] tyrosine with 9.8 ml sesame oil to yield a mixture containing 2 µCi per ml.

Blood samples were collected from all turkeys in heparinized syringes via a wing vein at 30 min post-injection. Poults were then killed immediately by cervical dislocation. The pectoralis thoracica and gastrocnemius muscles from both the right and left sides were dissected, wrapped in aluminum foil, weighed and immersed in liquid nitrogen. The entire procedure was completed within 10 min of obtaining a blood sample. Muscles were stored frozen at -20 C until analytical procedures were performed. The right side of each muscle type was used for the determination of muscle specific activity and tyrosine concentration. Composition assays were carried out using the contralateral side of each muscle. Protein concentration was also determined in the muscles of 16 and 8 non-infused poults per treatment at 5 and 20 days of age, respectively.

Analytical Procedures. For the determination of the contents of protein, RNA and DNA in the muscles, pectoralis and gastrocnemius samples weighing approximately 500 mg were homogenized in 4 ml .6N KCl using a Brinkmann polytron. Muscle protein concentration was determined using a microbiuret protein assay (Bailey, 1965) with bovine serum albumin serving as the standard protein source. Triplicate aliquots of the sample homogenate were diluted 1:10 in distilled water and precipitated with 5 ml 10% trichloroacetic acid (TCA) by centrifuging at 10,000 g for 10 min. The supernatant was discarded and the pellet redissolved in 3.61% (w/v) NaOH. After the addition of Benedict's Reagent (Appendix B), sample tubes were incubated for 15 min at room temperature and then read at 330 nm on

a Gilford Spectrophotometer (Model 250, Gilford Instruments Laboratories, Inc., Oberlin, OH 44074).

Muscle RNA content was analyzed according to the orcinol procedure (Schneider, 1957), using calf liver RNA as the working standard. Muscle homogenates were treated with 10% (v/v) perchloric acid (PCA), incubated on ice for 10 min, and centrifuged at 10,000 g for 10 min. The supernatant containing small molecular weight compounds was discarded. The pellet was solubilized in 4 ml distilled water. RNA was precipitated a second time by the addition of an equal volume of 10% PCA. The tubes were incubated at 70 C for 20 min in a water bath, and recentrifuged, and the supernatant containing hydrolyzed RNA was retained. Triplicate .2 ml samples of the supernatant were diluted 1:5 in distilled water and an equal volume of orcinol reagent (Appendix B) added to each tube. Absorbance was determined at 670 nm after incubation of the tubes for 1 hr in a boiling water bath.

Muscle DNA concentration was assayed using the diphenylamine method (Burton, 1968) with calf thymus DNA as the standard source. Sample homogenates were treated with 10% PCA and incubated at 70 C for 20 min in a water bath to release and hydrolyze the muscle DNA. Proteins were precipitated by centrifugation at 10,000 g for 10 min and the supernatant was retained for DNA determination. Triplicate .5 ml aliquots were diluted 1:2 in .5N PCA. Two ml diphenylamine reagent (Appendix B) were added to each tube. Following incubation of the tubes at room temperature for 20 hr, absorbance was determined at 600 nm.

Plasma samples were prepared from whole blood as described previously and counted to determine the level of radioactivity in the nonpro-

tein plasma pool. Frozen muscle samples were homogenized in 8 volumes of 5% TCA using a Brinkmann-Polytron. Following centrifugation at 10,000 g for 15 min, the supernatant containing the free amino acids was decanted and the protein pellet was washed twice with 2 volumes of cold 5% TCA. The washes were added to the supernatant, neutralized with 2N NaOH, and designated as the precursor free amino acid pool. A 1 ml aliquot was dispersed in 10 ml scintillation fluid and counted as described above. The TCA precipitate was redissolved in 1N NaOH by incubating samples at 60 C overnight in a water bath. After neutralization with 1N HCl, 1.5 ml of the solution was added to 10 ml scintillation fluid to determine the specific activity of the protein-bound amino acid pool.

The concentration of protein-bound tyrosine in both the pectoralis and gastrocnemius muscles was determined in a preliminary experiment. Muscle samples were obtained from four poults subjected to each treatment and homogenized in 5 volumes of cold 10% TCA. The resultant protein pellet was hydrolyzed in 6N HCl for 24 hr at 110 C and dissolved in sodium citrate buffer, pH 2.2, for the determination of tyrosine concentration using a Beckman Automatic Amino Acid Analyzer (Model 121, Beckman Instruments, Inc., Fullerton, CA 92634). Protein-bound tyrosine concentration was unaltered by diet or muscle type. Thus, a mean concentration of 30.5 nmol/mg tissue was employed in the calculation of protein synthesis rates. The concentration of tissue free tyrosine was considerably more variable and unrelated to diet or muscle type. Therefore, duplicate samples obtained from injected poults were treated as previously described and the supernatant analyzed for free tyrosine concentration.

Calculation of Fractional Turnover Rates. Fractional synthesis rate (k_s) was calculated using the equation of Garlick et al. (1973):

$$\frac{S_B}{S_I} = \frac{R}{(R-1)} \cdot \frac{(1 - e^{-k_s t})}{(1 - e^{-Rk_s t})} - \frac{1}{(R-1)}$$

where:

S_B = specific activity of protein-bound tyrosine (dpm/μmol tyrosine)

S_I = specific activity of tissue free tyrosine (dpm/μmol tyrosine)

R = ratio of bound to free tyrosine

t = time of infusion

The rate of protein deposition at the time of infusion was estimated from the regression of total muscle protein content determined when poults were 5 and 7 or 20, 21 and 22 days of age. The fractional deposition rate was calculated from the proportion of muscle protein gain relative to the initial muscle protein content. The difference between the fractional rates of synthesis and deposition was used to represent the fractional rate of protein degradation.

Statistical Analyses. An analysis of variance was performed on the experimental results where applicable. Diet means were compared with the use of Duncans's New Multiple Range Test (Steel and Torrie, 1980). Differences between muscles or developmental stages were detected with Student's t-test.

RESULTS

Pre-experimental Muscle Composition. The muscle characteristics of turkeys at the commencement of the experiment are shown in Table 12. Both protein and DNA concentrations were similar for the pectoralis and gastrocnemius muscles at 7 days of age. However, the RNA concentration of the pectoralis muscle was significantly greater ($P<.001$) than that of the gastrocnemius muscle. Total protein, RNA and DNA contents of the breast exceeded ($P<.001$) the respective constituents of the gastrocnemius as a result of the four-fold difference in weight between the muscle types ($P<.001$).

The gastrocnemius muscle exhibited an increased ratio of protein:DNA ($P<.05$) and a decreased RNA:protein ratio ($P<.001$) in comparison to the pectoralis muscle. No difference in RNA:DNA was observed. Significantly higher rates of protein synthesis ($P<.001$) were detected in the pectoralis muscle, although neither DNA nor RNA activity were altered by muscle type. Protein deposition in the pectoralis also exceeded that of the gastrocnemius, both on an absolute and fractional basis. A lower fractional rate of protein degradation in the breast was translated into a higher absolute rate, reflecting the greater pectoral protein content.

Performance. Dietary manipulation had a significant influence ($P<.001$) on body weight gain, feed consumption and feed efficiency of turkeys from 7 to 21 days of age (Table 13). Body weight gain was 68% higher on the 30% protein diet in comparison to the unsupplemented 22% protein diet. Feed consumption was increased only 22%, resulting in a considerable im-

provement in feed efficiency of 38% for the high protein diet over the same time period.

Adding .4% methionine to the 22% protein diet elicited increases of 23 and 17% in weight gain and feed efficiency, respectively, with no significant difference in feed consumption. A further addition of .6% lysine acted to depress growth of poults to the level observed with the unsupplemented 22% protein diet although feed consumption and feed efficiency were not significantly affected. Performance equal to that observed with the high protein diet was not attained by supplementing the 22% protein diet with methionine or a combination of methionine and lysine.

Differences in body weight gain among treatments for injected poults (Table 14) were similar to those described above with the exception of the turkeys fed the diet containing added lysine. The latter treatment resulted in a high incidence of leg abnormalities and turkeys exhibiting leg problems were excluded from the injection study. Thus, the poults selected tended to be heavier than the treatment mean, resulting in equal weight gain with diets containing added methionine or methionine and lysine.

Muscle Composition. Good agreement between body and muscle weight data was observed at 21 days of age for both muscle types (Table 14). Increases of 86 and 64% in the weights of the pectoralis and gastrocnemius muscles, respectively, were noted with an elevation in dietary crude protein ($P<.001$). Smaller improvements were obtained by supplementing the 22% protein diet with methionine alone or a combination of methionine and lysine.

Expression of the pectoralis muscle weight as a percent of body weight revealed similar differences among treatments, with the 30% protein diet outperforming the 22% protein diet with and without added methionine ($P<.001$). However, proportional muscle weights were equal for the diet containing both added lysine and methionine and the 30% protein diet. The situation for the gastrocnemius muscle differed slightly, with only the unsupplemented 22% protein diet constituting a smaller proportion of body weight than the remaining treatments ($P<.05$).

The protein concentration of the breast muscle was unaffected by treatment (Table 15). The only difference noted in RNA concentrations was between the 22 and 30% protein diets where RNA concentration decreased from 3177 to 2631 µg per g tissue with an increase in dietary protein ($P<.05$). Similarly, an elevation in DNA concentration was obtained from the unsupplemented 22% protein diet in comparison to the other treatments ($P<.01$).

The protein content of the gastrocnemius muscle for poults fed the lysine-supplemented diet was significantly higher ($P<.05$) than that of the 30% protein diet and the low protein diet containing added methionine. The RNA and DNA concentrations were unaffected by dietary treatment.

Because only small alterations in the concentrations of muscle constituents were observed due to treatment, differences in total protein, RNA and DNA contents largely reflected variation in muscle weight (Table 16). Total protein contents of the pectoralis and gastrocnemius muscles were greatest for the 30% protein diet ($P<.001$). Lysine supplementation resulted in an increased protein content over the 22% protein diet with

and without added methionine. The sole addition of methionine proved to be of benefit in terms of pectoralis muscle protein content only.

The 30% protein diet resulted in significantly higher total RNA ($P<.001$) for both muscle types relative to the other treatments. Methionine alone was inadequate to increase total RNA above the level observed for the unsupplemented 22% protein diet. However, RNA content increased in response to a combined lysine and methionine addition. Total DNA contents of the gastrocnemius and pectoralis muscles were not signficantly altered by treatment.

The protein:DNA ratio was significantly lower ($P<.05$) in the pectoralis muscle of turkeys fed the unsupplemented 22% protein diet (Table 17). No other differences among treatments were observed. Both an increase in crude protein level and supplementation of the low protein diet with lysine and methionine depressed the RNA:protein ratio below that observed with the 22% protein diet ($P<.05$). No significant difference was obtained for the pectoralis muscle ratio of RNA:DNA despite a tendency towards a reduction with the basal 22% protein diet. Examination of the same parameters for the gastrocnemius muscle revealed no significant differences among treatments.

Protein Turnover. The rate of breast muscle protein synthesis was significantly greater ($P<.05$) with the 30% protein diet in contrast to the 22% protein diet supplemented with methionine and lysine (Table 18). Numerical reductions only in fractional synthesis rate were observed with the low protein diet with and without added methionine. Diet had no significant effect on the fractional rate of protein synthesis in the gastrocnemius muscle. Significant differences among means could not be

determined for the rates of deposition and degradation. However, the rate of protein deposition was markedly reduced by amino acid supplementation in both muscles. Protein accretion apparently ceased in the presence of methionine alone. Dietary protein level had no consistent effect on the fractional deposition rate or degradation rate. Degradation rates in both muscles were unaltered by the combination of added methionine and lysine while methionine alone produced the highest rates of protein degradation.

Feeding the low protein diet resulted in a significant decrease ($P<.001$) in the absolute rate of protein synthesis in the pectoralis muscle regardless of amino acid supplementation (Table 19). Deposition was highest with the 30% protein diet followed by the basal 22% protein diet. A further decrease in protein accretion was observed by adding limiting amino acids to the low protein diet, with methionine eliciting the larger decline in deposition rate. Degradation rate was reduced by decreasing dietary protein. Intermediate rates of degradation were noted with amino acid supplementation of the 22% protein diet.

The absolute rate of protein synthesis in the gastrocnemius was not significantly altered by diet. Deposition rate declined with the low protein diet regardless of amino acid supplementation. A cessation in protein deposition was produced with methionine addition in conjunction with the highest degradation rate. The rate of protein degradation decreased in response to low dietary protein.

Pectoral RNA activity was depressed by feeding the 22% protein diet ($P<.01$) commensurate with alterations in the total amount of protein synthesized daily (Table 20). The DNA activity for poults fed the 30% protein diet was superior ($P<.05$) to that observed for the 22% protein

diet containing added lysine and methionine or unsupplemented. Methionine alone tended to increase the DNA activity to the level of the high protein diet. Both RNA and DNA activities in the gastrocnemius muscle were insensitive to changes in crude protein level or amino acid supplementation.

Differences Between Muscles. Protein concentration was equal for both muscles despite differences in other components (Table 21). The breast muscle contained a significantly higher ($P<.001$) concentration of RNA than the gastrocnemius muscle while the converse was true for DNA concentration ($P<.001$). The lower DNA content of the pectoralis translated into an increased ratio of protein:DNA and a higher RNA:DNA ratio in comparison to the gastrocnemius ($P<.001$). Total muscle contents of protein, RNA and DNA were considerably greater for the pectoralis, reflecting the six-fold difference in weight between the muscles.

The fractional rates of protein synthesis, deposition and degradation were equal in both muscles. However, absolute rates in the pectoralis greatly exceeded those observed for the gastrocnemius. Although RNA activity was unaffected by muscle type, breast muscle exhibited a higher DNA activity ($P<.001$).

Developmental Changes. A comparison of 7-day-old poults with those fed the 30% protein diet for 14 days revealed that a five-fold increase in body weight was accompanied by increases of ten- and six-fold in the weights of the pectoralis and gastrocnemius, respectively (Tables 22 and 23). Thus, both muscles constituted greater proportions of total body weight at 21 days ($P<.001$).

The composition of the pectoralis muscle varied significantly ($P<.001$) with age (Table 22). Protein concentration increased from 142 to 169 mg per g tissue over the 2-week period. Conversely, the concentrations of RNA and DNA declined ($P<.001$) from 3534 to 2631 µg per g tissue and from 778 to 180 µg per g tissue, respectively. The ratios of RNA:protein and protein:DNA significantly decreased and increased, respectively, with age ($P<.001$). An elevation in the RNA:DNA ratio was also noted at 21 days of age ($P<.001$). Total protein, RNA and DNA contents of the pectoralis muscle were elevated commensurate with increases in muscle weight ($P<.001$).

The gastrocnemius muscle contained an increased amount of protein per g tissue at 21 days of age ($P<.05$)(Table 23). Although both RNA ($P<.05$) and DNA ($P<.001$) concentrations declined with age, the decreases were considerably less than observed for breast muscle tissue. Total protein, RNA and DNA contents of the gastrocnemius increased over the 2-week period ($P<.001$). Developmental changes also included an increase in protein:DNA ($P<.01$) and a decrease in RNA:protein ($P<.01$). The ratio of RNA:DNA was unaffected by age.

The fractional rate of protein synthesis declined with age in both muscles, from 59 to 36% in the pectoralis ($P<.001$) and from 40 to 28% in the gastrocnemius ($P<.05$). The fractional deposition rate decreased in the pectoralis but apparently doubled in the gastrocnemius over the 2 weeks. The converse was observed for the fractional rate of protein degradation. The total amount of protein synthesized ($P<.001$), degraded and deposited on a daily basis increased markedly, however, as a result of the larger tissue mass at 21 days. The RNA activities of the

pectoralis and gastrocnemius muscles were unchanged. The DNA activity of the pectoralis increased from 7 to 21 days of age ($P<.001$), whereas age had no effect on the corresponding value obtained for the gastrocnemius.

DISCUSSION

Performance Decreasing dietary protein from 30 to 22% produced large decreases in body weight gain, feed consumption and feed efficiency. The inferior performance of turkeys in response to low protein diets has been well documented (Baldini et al., 1954; Klain et al., 1954; Atkinson et al., 1976; Stas and Potter, 1982). The partial alleviation of depressions in weight gain and feed efficiency with methionine supplementation to equal NRC (1984) recommendations confirmed methionine as the primary limiting amino acid of the low protein diet in general agreement with other reports (Ferguson et al., 1956; Fitzsimmons and Waibel, 1962; Potter and Shelton, 1976).

Feed intake remained depressed despite methionine supplementation, indicating further deficiencies in the low protein diet. Earlier studies implicated lysine, threonine and valine as limiting amino acids in a 22% protein diet composed of corn and soybean meal as the sole protein sources (Stas and Potter, 1982; Jackson et al., 1983). Deficits of both lysine and valine were confirmed in Study II, with the lysine deficiency being of a greater magnitude. However, lysine addition to the 22% protein diet in the present study to provide a total of 1.9% lysine not only failed to improve performance but actually depressed weight gain in comparison to the methionine supplement alone. The addition of small amounts of lysine to low protein diets containing adequate methionine has been shown to be beneficial to poult performance (Baldini et al., 1954; Rosebrough et al., 1982). However, an increase in dietary lysine above requirements to 2.11%, as in the study of Rosebrough et al. (1982), was a detriment to body weight without significantly affecting feed intake.

The reduction in feed intake commonly associated with an amino acid imbalance (Akinwande and Bragg, 1974; Leslie and Summers, 1975) was not observed in the present study. Hence, while the poor response of turkeys to lysine supplementation may be due to an imbalance precipitated by excessive lysine, a deficiency of further limiting amino acids is a more likely explanation. Threonine has been identified as the second most limiting amino acid beyond methionine in soybean meal for the rat (Berry et al., 1962). Both threonine and valine were more deficient than lysine in a low protein diet containing soybean meal as the sole protein source for chicks (Warnick and Anderson, 1968).

Effect of Diet on Muscle Metabolism. The large difference in body weight between turkeys fed the 30 and 22% protein diets was translated into even larger differences in the weight of the pectoralis muscle. The breast muscle constituted almost 15% of body weight for turkeys fed the high protein diet in comparison to only 12% for those offered a low protein level. Alterations in the proportional weights of the gastrocnemius were less dramatic, reflecting fundamental differences in the functional capacity of each muscle. Timson et al. (1983) examined the influence of a drop in dietary protein from 18 to 14% on the anterior latissimus dorsi muscle of the leghorn chick from hatch to 4 weeks of age. A 40% decrease in the weight of the muscle was attributed to a substantial decline in fiber size rather than number.

Muscle fiber growth is the result of increments in the number of nuclei per fiber and in the cytoplasmic mass per nuclei (Howarth, 1972). The increase in the number of nuclei results from division of the satellite cell nuclei and fusion of the daughter cells with existing myofibers.

Total DNA content of a muscle, termed DNA-unit number, can be used as an indicator of the number of nuclei on the basis of a constant amount of DNA per nucleus in somatic cells within a species (Moss et al., 1964). A later study (Moss, 1968) revealed that the cross-sectional area of both the pectoralis and gastrocnemius muscles of growing chicks increased in proportion to total muscle DNA content. The volume of cytoplasm per nuclei is roughly estimated by the ratio of protein to DNA or the DNA-unit size (Cheek et al., 1971). This terminology overcomes the difficulty of defining cell size in a multinucleated tissue such as skeletal muscle. Inaccuracies in the use of the protein:DNA ratio and total DNA values arise from the potential for variation in the number of non-muscle cells due to dietary treatment and possible differences in composition among cell types. Myonuclei constitute a relatively fixed proportion of the total number of nuclei within a muscle (Moss, 1968). Seventy percent of all muscle nuclei are associated with contractile cells while the remainder originate from fibroblasts, adipocytes and vascular cells (Laurent et al., 1978a).

In the present study, the 22% protein diet produced a large decrease in DNA-unit size of the pectoralis and a smaller, non-significant, decrease in DNA-unit number. Feeding a protein-free diet to leghorn chicks from 4 to 5 weeks of age had no effect on DNA-unit number of breast muscle whereas DNA-unit size was considerably smaller (MacDonald and Swick, 1981). A reduction in pectoralis weight of growing chicks restricted to 70% of ad libitum intake was ascribed solely to a decrease in DNA-unit size (Cornejo and Pokniak, 1983). A similar dietary regime markedly reduced sartorius growth of chicks due to concurrent decreases in both

DNA-unit size and number (Montgomery et al., 1964). A similar observation was reported for the mixed leg muscles of rats fed protein-deficient diets (Millward et al., 1975). However, a decrease in DNA-unit number rather than size was responsible for the reduced gastrocnemius weight of rats fed protein-deficient diets (Howarth, 1972) and of chicks subjected to feed restriction (Cornejo and Pokniak, 1983). Our results show that neither the DNA-unit size or number in the gastrocnemius muscle of poults was altered significantly by dietary protein, despite a considerable difference in muscle weight. However, small decreases in both size and number with the imposition of a protein deficiency tended to be masked by a large amount of experimental variation.

Few studies in the literature address the effect of diet on the muscle composition of poultry. In the present study, the protein concentrations of the pectoralis and gastrocnemius muscles were not changed by dietary protein level. The pectoral concentrations of both RNA and DNA were inversely related to muscle size and dietary protein level. Young and Alexis (1968) claimed that the decrease in RNA concentration observed with an increase in dietary protein is reflective of increased muscle fiber growth. Similarly, the concentration of DNA in the pectoralis muscle of chickens has been shown to decrease as muscle weight increases (Moss et al., 1964). Conversely, dietary protein had little influence on the concentrations of RNA and DNA in the gastrocnemius muscle. Protein deprivation had no effect on RNA concentration in the leg muscles of growing rats (Millward et al., 1975).

The absence of a detectable change in the RNA:DNA ratio of either muscle indicates that the cellular RNA and DNA pools expanded at essen-

tially the same rate as dietary protein was altered in agreement with the rat study of Smith et al. (1982). The RNA:DNA ratio decreased in the pectoralis muscle of leghorns fed a protein-free diet for 7 days, indicating a more rapid decline in muscle RNA than DNA (MacDonald and Swick, 1981). A similar response was observed in the leg muscles of growing rats subjected to a 48-hour fast (Li and Goldberg, 1976).

Muscle RNA content has been confirmed as a reliable indicator of ribosomal RNA (rRNA) in several studies. Alterations in rRNA concentration due to diet or age closely paralleled RNA concentration in the hind leg of growing rats (Young and Alexis, 1968). Zak et al. (1967) found that approximately 85% of the RNA associated with the myofibrils of chick embryonic cardiac tissue was extractable as ribosomes. Hence, total muscle RNA appears to be directly related to the total amount of translational apparatus and the capacity for protein synthesis. An alternative measure of the capacity for protein synthesis is the concentration of RNA or the RNA:protein ratio which can be directly related to the fractional rate of protein synthesis. Millward et al. (1975) claimed that alterations in ribosome concentration were quantitatively the most important component of changes in the rate of protein synthesis.

The efficiency of protein synthesis has been defined as the rate of peptide bond formation per unit of ribosomes and was represented by muscle RNA activity (Millward et al., 1974). Polysomal profiles have previously been shown to be related to the synthetic activity of hind leg muscles of growing rats (Young and Alexis, 1968). A greater proportion of polysomes versus monomers and dimers elicited with a high protein diet corresponded to an increase in in vitro protein synthesis activity of

ribosomes. More recently, a decrease in the efficiency of protein synthesis has been related to the disaggregation of polysomes and a resultant fall in the initiation of protein synthesis (Li and Goldberg, 1976).

The fractional rate of protein synthesis in the pectoralis was not altered by dietary protein level as a result of reciprocal changes in the RNA:protein ratio and RNA activity. Thus, an increase in the efficiency of protein synthesis with the 30% protein diet was apparently offset by a reduced cellular capacity for protein synthesis. An increase in the total synthetic capacity as well as a higher synthetic efficiency contributed to the two-fold increase in the amount of protein synthesized daily in the breast muscle of poults fed the 30% protein diet. Thus, poults fed the 22% protein diet produced only 47% as much total protein on a daily basis as those fed high protein although they possessed 64% as much pectoral RNA. The situation differed for the gastrocnemius muscle in that 66% as much protein was synthesized from 60% as much RNA with the 22% protein diet, corroborating the lack of an effect of dietary protein on the efficiency of protein synthesis. The cellular capacity for protein synthesis in the gastrocnemius was also unaffected by dietary protein, resulting in equal fractional rates of synthesis. Reductions in the fractional synthesis and degradation rates with protein malnutrition has invariably been observed in the skeletal muscles of growing rats (Waterlow and Stephen, 1968; Haverberg et al., 1975a; Li and Goldberg, 1976; Nishizawa et al., 1977b; Burini et al., 1981; Smith et al., 1982). Decreases in synthetic capacity and/or efficiency have been implicated in reduced protein synthesis. The lack of response in fractional synthesis

rate in the present study may be related to a less severe dietary protein restriction.

Few studies have evaluated the effect of diet on the synthetic capability of poultry muscles. Feeding a protein-free diet adversely affected the rate of protein synthesis in the breast muscle of leghorns (MacDonald and Swick, 1981). Maruyama et al. (1978) observed no response in the synthesis rate of the pectoralis muscle of chicks to a decrease in the level of essential amino acids to 75% of requirements in a purified diet. A low level of essential amino acids depressed the fractional synthesis rate in leg muscles primarily as a result of a reduction in synthesis capacity. Protein degradation was doubled in the breast but remained relatively stable in the leg while deposition declined in both muscles. Our results depict a similar response in the leg muscles of turkeys. However, a decrease in the fractional rate of degradation was observed in the pectoralis as dietary protein was reduced to 22%. The lack of a concurrent decline in fractional deposition rate is surprising in view of the retardation in muscle growth associated with the low protein diet. However, the reductions in the absolute deposition rates of 32 and 41% in the breast and leg muscles, respectively, are sufficient to account for the decreases in total muscle protein content obtained with the 22% protein diet.

Methionine supplementation of the 22% protein diet acted to increase weights of both the pectoralis and gastrocnemius muscles although both remained considerably below those found in poults fed the high protein diet. The increased pectoral weight was attributable to a greater

DNA-unit size rather than number, as DNA concentration decreased to the amount obtained with the high protein diet.

An increase in the weight of the gastrocnemius muscle in response to methionine supplementation was unrelated to the concentrations of protein, RNA and DNA. Hayase and Yoshida (1980) found that feeding diets composed of gelatin, gluten or whole egg had no effect on gastrocnemius protein concentration of growing rats, although muscle weight varied directly with protein quality. No difference in DNA-unit number or size could be ascertained to explain the increase in muscle weight with methionine supplementation. Parallel increases in body weight gain and DNA-unit size of the gastrocnemius muscle of broilers were noted with the addition of the first limiting amino acid to a 15% protein diet (Akinwande and Bragg, 1974).

The fractional rate of protein synthesis was not altered in either the pectoralis and gastrocnemius muscles by methionine supplementation. Consistent with this observation is the absence of any changes in synthetic capacity or efficiency. Harney et al. (1976) found that the addition of cystine to a TSAA-deficient diet increased the protein synthesis rate in the gastrocnemius muscle of rats. Maruyama et al. (1978) reported no difference in synthetic rate, efficiency or capacity by decreasing the level of lysine to 35% of requirements in a crystalline amino acid diet fed to young chicks. Rather, a low rate of protein deposition in both muscles was attributable to an increase in the fractional rate of degradation. The converse was observed in the present study, with a lower degradation rate associated with the basal 22% protein diet in comparison to the same diet supplemented with methionine. The larger muscle weight

and protein content obtained with the latter diet conflicts with the apparent cessation of protein deposition. Thus, the degradation rates obtained with methionine supplementation apparently overestimate of the true values. A major drawback to the determination of the degradation rate is its dependence on the accuracy of the estimates for protein synthesis and deposition. The large amount of variation associated with the observed synthesis values, coupled with the calculation of deposition over a 2-day period severely limits the reliability of degradation estimates.

Lysine supplementation proved to be of little benefit in terms of muscle metabolism. Muscle weights were unchanged by lysine addition, consistent with the absence of an effect on DNA-unit number or size in either the pectoralis or gastrocnemius. A decline in the fractional protein synthesis rate for the pectoralis muscle was associated with a decrease in the capacity for synthesis. However, the total amount of protein synthesized daily remained at the level achieved with methionine addition as a result of the increase in total pectoral capacity for protein synthesis. Synthetic efficiency in the pectoralis muscle was unaffected by amino acid supplementation and remained depressed in comparison to the 30% protein diet. Neither the capacity nor efficiency in the gastrocnemius muscle was influenced by lysine in agreement with the lack of change in the fractional and absolute synthesis rates. The fractional and absolute rates of protein deposition were somewhat improved in both muscles with lysine addition, although remaining considerably below that observed for the basal 22% protein diet. Protein degradation was unchanged on a fractional basis but increased in the pectoralis muscle when

expressed on an absolute basis. Others have found that a large difference in dietary protein quality was necessary to elicit an alteration in the degradation rate of skeletal muscle (Omstedt et al., 1978; Hayase and Yoshida, 1980).

Differences Between Muscles. The composition of the gastrocnemius and pectoralis muscles differed at both 7 and 21 days of age with the exception of protein concentration. The RNA concentration was higher in the breast tissue in direct contrast to the findings of Hentges et al. (1983). In their study, the RNA concentration of wing muscles containing both red and white fibers exceeded that of wing muscles containing only white fibers. DNA-unit number was lower in the gastrocnemius muscle at both ages. Although gastrocnemius DNA-unit size was higher than for the pectoralis at 7 days, the converse was true at 21 days of age, which may explain the more rapid growth rate of the pectoralis muscle.

Major differences in fractional synthesis rate were observed only at 7 days of age when the rate for the pectoralis was higher than for the gastrocnemius. This difference was due solely to a greater capacity. Fractional rates were equal at 21 days of age despite the persistence of a higher capacity for protein synthesis exhibited by the pectoralis muscle. A greater total capacity for protein synthesis in the breast muscle was reflected in the greater absolute amount of protein synthesized daily at both ages tested. Maruyama et al. (1978) observed a higher rate of protein synthesis in the breast muscle of one-week-old chicks primarily due to a superior synthetic capacity in comparison to that for leg muscles. Little difference between breast and leg muscles was discernible at 2 weeks of age. Similarly, the fractional synthesis rate of the breast

muscle of turkeys exceeded that of the leg muscles only at one week of age (Kang et al., 1985b).

The fractional rate of protein deposition was higher in the pectoralis at one week of age but no difference between muscles was discernible at 3 weeks, in agreement with other results for turkeys (Kang et al., 1985b) and broilers (Kang et al., 1985a). However, the present value of 7.8% for protein deposition in the leg was considerably lower than 17.9% reported by Kang et al. (1985b). This discrepancy results in an overestimate of degradation and a subsequently higher rate in the gastrocnemius than in the pectoralis. Degradation rate was essentially equal for both muscles at 3 weeks of age, concurring with the results for broilers (Kang et al., 1985a). However, slightly higher rates were observed in the leg muscles of poults beyond 2 weeks of age (Kang et al., 1985b).

Developmental Changes. The pectoralis muscle increased in size at approximately twice the rate of the total body over the one to 3-week period. The weight of the pectoralis muscle determined for various breeds of chickens comprised an increasing proportion of body weight with age (Simmonds et al., 1964; Maruyama et al., 1978). The gastrocnemius muscle of poults also increased in weight faster than total body weight although the difference was considerable less dramatic than that noted for the breast.

Pectoralis muscle growth was primarily a result of an increase in DNA-unit size, as evidenced by the 5-fold expansion from one to 3 weeks of age. DNA-unit number increased by a factor of 2 over the same time period. The converse was observed for the gastrocnemius, with DNA-unit

number increasing faster than size. Elevations in DNA-unit size as well as DNA-unit number with age or an increase in muscle mass have been a general finding for rats (Millward et al., 1975), chickens (Moss et al., 1964; MacDonald and Swick, 1981) and turkeys (Kang et al., 1985b). Developmental increases in the ratio of RNA:DNA and DNA activity observed in the present study attest to the considerable expansion of the DNA-unit size.

In accordance with increasing DNA-unit size, the concentrations of DNA and RNA declined in both muscles over the 2-week period. Moss et al. (1964) established an inverse relationship between breast DNA concentration and the age of various breeds of chickens. Decreases in both RNA and DNA concentrations with age were observed in the wing muscles of broilers and leghorns (Hentges et al., 1983). Rat studies have generated similar results (Devi et al., 1963; Young and Alexis, 1968; Millward et al., 1975).

Protein comprised an increasing proportion of muscle mass with age. An increase in pectoral protein concentration of chicks from hatch to 4 weeks of age has been reported previously (Robinson, 1952; Dickerson, 1960; Simmonds et al., 1964; Akinwande and Bragg, 1974). Total protein and RNA contents increased over time in conjunction with muscle mass. However, because protein accumulation exceeded the increase in RNA content, muscle hypertrophy produced a decrease in the ratio of RNA:protein. The reduction in cellular capacity for protein synthesis elicited a corresponding decline in the fractional synthesis rate, from 59 to 36% in the pectoralis and from 40 to 28% in the gastrocnemius. Synthetic efficiency was unaltered. The fractional rate of protein de-

position in the pectoralis was reduced while degradation rate increased slightly. The converse was observed in the gastrocnemius. Developmental changes in the breast and leg muscles of turkeys from one to 8 weeks of age were examined by Kang et al. (1985b). Synthesis rates of 56, 29 and 17% were observed in the breast muscle at 1, 2 and 4 weeks of age, respectively. Comparable values for leg muscles were 38, 31 and 20%. A reduced capacity rather than efficiency was responsible for the fall in synthesis rate in agreement with the present results. However, they observed stepwise decreases in fractional rates of deposition and degradation at each time period for both breast and leg muscles. Age-related decreases in the fractional synthesis rates of the breast muscle of chicks has been well documented (Maruyama et al., 1978; MacDonald and Swick, 1981; Kang et al., 1985a; Lauterio et al., 1983). The fractional synthesis rate in the leg muscles of leghorn chicks remained stable from one to two weeks of age in a study by Maruyama et al. (1978). Deposition rate increased due to a reduction in the fractional rate of degradation. Bergen et al. (1983) reported no changes in the synthesis and degradation rates of breast and leg muscles for broilers from 4 to 7 weeks of age although protein deposition exhibited small declines.

SUMMARY

The effect of diet on protein metabolism in the pectoralis and gastrocnemius muscles was investigated in young Large White turkeys. Diets containing either 22 or 30% protein were fed from one to three weeks of age. In addition, the 22% protein diet was supplemented with .4% methionine or a combination of .4% methionine and .6% lysine to evaluate the capacity of limiting amino acids to alleviate the adverse effects of a protein deficiency. Each diet was offered ad libitum to 4 pens of 8 male poults.

A reduction in dietary protein from 30 to 22% caused parallel decreases in body weight and pectoralis weight. The reduced weight of the pectoralis for poults fed the low protein diet was attributed to a decrease in DNA-unit size rather than number. Pectoral RNA and DNA concentrations increased with a decrease in dietary protein whereas total RNA and protein contents decreased. Fractional synthesis rate was unaffected by dietary protein as a result of reciprocal changes in synthetic capacity and efficiency. Performance remained inferior with the 22% protein diet regardless of amino acid supplementation. Methionine addition effected an increase in pectoral DNA-unit size and muscle weight consistent with a decrease in DNA concentration. Synthetic activity did not respond to added methionine. The failure of lysine supplementation to alter body weight or the weight and composition of the pectoralis muscle suggests that lysine may not be the most limiting amino acid beyond methionine in a low protein diet. That lysine addition was detrimental to poult performance was evidenced by a decrease in the fractional rate of synthesis in the pectoralis muscle.

The composition of the gastrocnemius muscle was relatively insensitive to dietary manipulation despite significant differences in gastrocnemius weight among treatments. Among the parameters unaffected by diet were the concentrations of RNA and DNA, the ratio of RNA:DNA and the DNA-unit size and number. Protein concentration was increased only by a combination of methionine and lysine. Total protein and RNA contents increased in conjunction with muscle mass. The fractional synthesis rate remained relatively stable regardless of diet, in agreement with the lack of change in synthetic capacity or efficiency.

Developmental changes included an expansion of muscle protein relative to both the RNA and DNA pools in the pectoralis and gastrocnemius muscles. Thus, an increase in muscle size from one to three weeks of age corresponded to a reduction in cellular synthetic capacity and a resultant drop in the fractional synthesis rate in both muscles. Synthetic efficiency was not altered with age.

Table 11. Composition of experimental diets

Ingredient	Diet 1	2	3	4
			(g/kg)	
Ground yellow corn	211.88	413.38	413.38	413.38
Glucose monohydrate	95	141	134	122
Stabilized fat	60	17	20	24.55
Dehulled soybean meal	581.5	380	380	380
Defluorinated phosphate	37.5	37.5	37.5	37.5
Iodized salt	4	4	4	4
Trace mineral mix[1]	1	1	1	1
Vitamins and feed additives[2]	6.12	6.12	6.12	6.12
DL-Methionine	3	--	4	4
L-Lysine HCl	--	--	--	7.45
Total	1000.00	1000.00	1000.00	1000.00
Calculated composition				
Protein (%)[3]	30.06	22.07	22.07	22.07
Energy (kcal ME/kg)	2949	2949	2951	2948
Methionine (%)	.76	.36	.76	.76
TSAA (%)	1.20	.70	1.10	1.10
Lysine (%)	1.90	1.31	1.31	1.90

[1]Supplied per kilogram of diet: 150 mg manganese, 100 mg zinc, 70 mg iron, 10 mg copper, 2.2 mg iodine, and .8 mg cobalt from manganese oxide, zinc oxide, ferrous sulfate, copper oxide, calcium iodate, and cobalt carbonate, respectively, and calcium carbonate as a diluent.

[2]Supplied per kilogram of diet: 14,300 IU vitamin A, 7,150 ICU vitamin D_3, 55 IU vitamin E, 5.5 mg menadione dimethylpyrimidinol bisulfite, 3.3 mg thiamine HCl, 11 mg riboflavin, 22 mg D-calcium pantothenate, 110 mg niacin, 2,200 mg choline chloride, 15.4 µg vitamin B_{12}, 2.2 mg folic acid, .22 mg biotin, 5.5 mg pyridoxine HCl, 125 mg ethoxyquin, .2 mg selenium, and 55 mg erythromycin thiocyanate.

[3]Determined protein contents (N x 6.25) of the 22 and 30% protein diets were 22.37 and 30.63%, respectively.

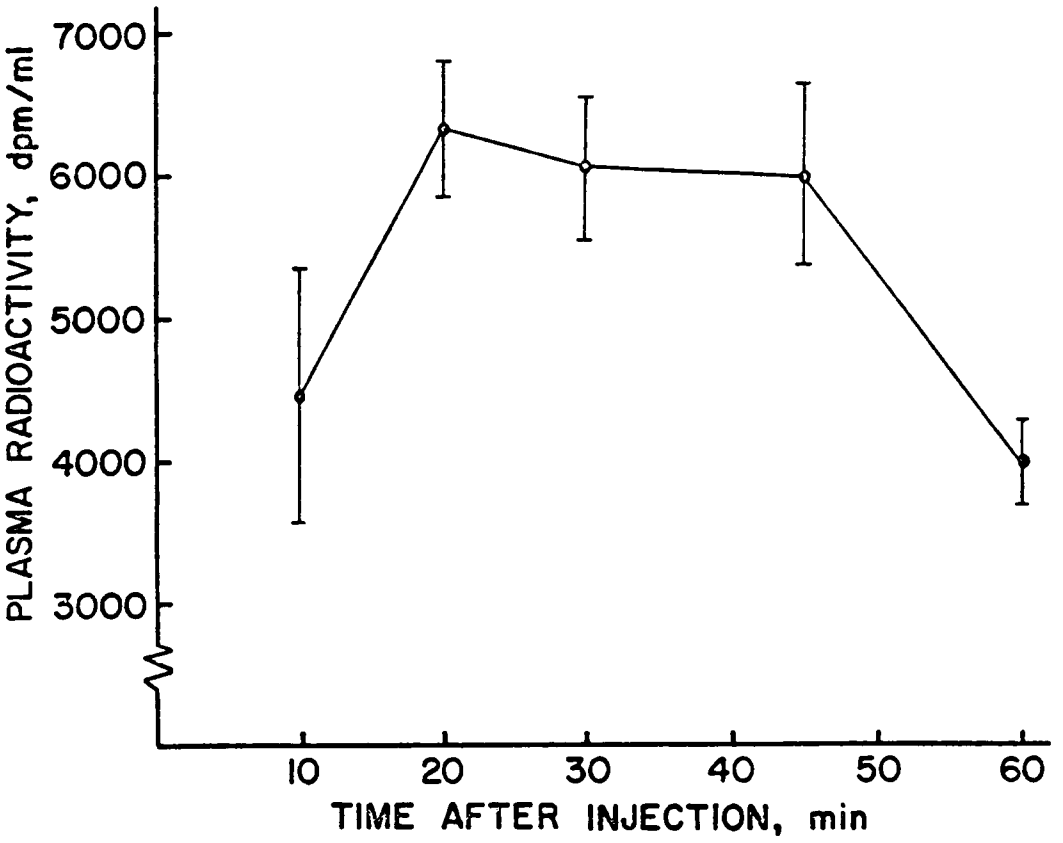

Figure 7. Plasma radioactivity over a 1-hour period following injection of .5 μCi ^{14}C-tyrosine per 100 g body weight at 1 week of age

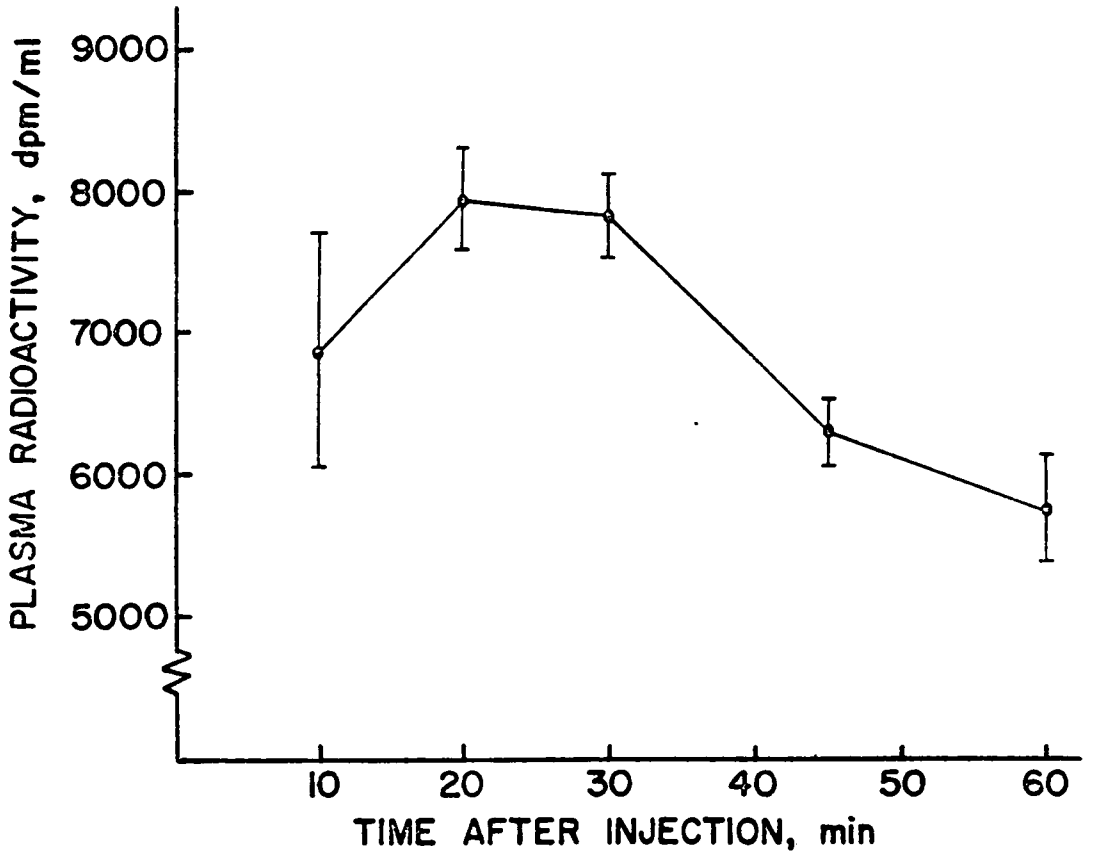

Figure 8. Plasma radioactivity over a 1-hour period following injection of .5 μCi ^{14}C-tyrosine per 100 g body weight at 3 weeks of age

Appendix Table 12. Ratios of specific radioactivities and concentrations of protein-bound to free tyrosine in the skeletal muscles of 3-week-old turkeys (Study IV)[1]

Diet	Pectoralis		Gastrocnemius	
	S_B/S_I[2]	R[3]	S_B/S_I	R
30% Protein	.00518 (.00122)[4]	240 (67)	.00360 (.00098)	234 (76)
22% Protein	.00334 (.00081)	183 (44)	.00183 (.00059)	210 (43)
22% Protein plus MET	.00018 (.00005)	142 (53)	.00003 (.00001)	180 (47)
22% Protein plus EAA	.00203 (.00069)	198 (13)	.00266 (.00085)	240 (135)
22% Protein plus MET and NEAA	.00046 (.00010)	150 (21)	.00026 (.00008)	188 (21)
22% Protein plus EAA and NEAA	.00281 (.00081)	219 (41)	.00235 (.00050)	243 (47)

[1]Values represent the mean of 8 poults.

[2]Ratio of specific radioactivity of protein-bound to free tyrosine.

[3]Ratio of muscle protein-bound to free tyrosine.

[4]Value in parenthesis indicates standard deviation.

Table 13. Performance of turkeys from 1 to 3 weeks of age[1]

Diet	Body weight gain[2]	Feed consumption	Feed efficiency
	-g-		
30% CP	399[a3]	575[a]	.693[a]
22% CP	238[c]	472[b]	.503[c]
22% CP plus MET[4]	293[b]	498[b]	.589[b]
22% CP plus MET, LYS[5]	262[c]	479[b]	.547[bc]
Standard deviation	19.9	20.9	.035
Significance	***	***	***

*** P<.001.

[1]Values represent means of 4 pens with 8 poults per pen.

[2]Initial body weight at one week of age was 124 g (±2).

[3]Numbers within a column bearing similar superscripts are not significantly different (P>.05).

[4]Contains .4% supplemental methionine.

[5]Contains .4% supplemental methionine and .6% supplemental lysine.

Table 14. Body weight and muscle characteristics of turkeys at 3 weeks of age[1]

Variable	Diet				SD[2]	Significance
	30% Protein	22% Protein	22% Protein plus MET	22% Protein plus MET, LYS		
Body weight, g	574[a3]	379[c]	414[bc]	432[b]	37.2	***
Pectoralis weight, g	84.4[a]	45.3[c]	56.0[b]	61.2[b]	7.01	***
% of body weight	14.7[a]	11.9[c]	13.5[b]	14.1[ab]	.77	***
Gastrocnemius weight, g	12.6[a]	7.7[c]	8.9[b]	9.3[b]	1.04	***
% of body weight	2.2[a]	2.0[b]	2.2[a]	2.1[a]	.11	*

*P<.05; ***P<.001.

[1]Values represent the mean of 8 poults used in the infusion study.

[2]SD - standard deviation.

[3]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

Table 15. Muscle concentrations of protein, RNA and DNA for turkeys at 3 weeks of age[1]

Variable	Diet: 30% Protein	22% Protein	22% Protein plus MET	22% Protein plus MET, LYS	SD[2]	Significance
Protein, mg/g wet tissue						
Pectoralis	169	166	167	183	14	--
Gastrocnemius	165b[3]	174ab	161b	192a	19	*
RNA, µg/g wet tissue						
Pectoralis	2631b	3177a	2838ab	2973ab	332	*
Gastrocnemius	2226	2212	2321	2325	267	--
DNA, µg/g wet tissue						
Pectoralis	180b	264a	171b	204b	49	**
Gastrocnemius	374	488	391	381	121	--

*P<.05; **P<.01.

[1]Values represent the mean of 8 poults.

[2]SD - standard deviation.

[3]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

Table 16. Total muscle content of protein, RNA and DNA for turkeys at 3 weeks of age[1]

Variable	Diet: 30% Protein	22% Protein	22% Protein plus MET	22% Protein plus MET, LYS	SD[2]	Significance
Protein, mg/muscle						
Pectoralis	14240[a3]	7477[d]	9304[c]	11183[b]	1119	***
Gastrocnemius	2090[a]	1333[c]	1429[c]	1781[b]	275	***
RNA, mg/muscle						
Pectoralis	222[a]	143[c]	159[bc]	182[b]	30	***
Gastrocnemius	28.1[a]	16.9[c]	20.8[bc]	21.6[b]	4.0	***
DNA, mg/muscle						
Pectoralis	15.3	12.1	9.8	12.6	4.1	--
Gastrocnemius	4.7	3.7	3.5	3.5	1.2	--

*** $P<.001$.

[1] Values represent the mean of 8 poults.

[2] SD - standard deviation.

[3] Numbers within a row bearing similar superscripts are not significantly different ($P>.05$).

Table 17. Muscle ratios of protein: DNA, RNA:DNA and RNA:protein for turkeys at 3 weeks of age[1]

Variable	Diet: 30% Protein	22% Protein	22% Protein plus MET	22% Protein plus MET, LYS	SD[2]	Significance
Protein:DNA						
Pectoralis	988[a3]	650[b]	1050[a]	964[a]	255	*
Gastrocnemius	486	386	427	557	162	--
RNA:DNA						
Pectoralis	16	12	17	15	3.6	--
Gastrocnemius	7	5	6	7	2.2	--
RNA:Protein ($\times 10^{-2}$)						
Pectoralis	1.6[b]	1.9[a]	1.7[ab]	1.6[b]	.2	*
Gastrocnemius	1.4	1.3	1.5	1.2	.2	--

*P<.05.

[1]Values represent the mean of 8 poults.

[2]SD - standard deviation.

[3]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

Table 18. The fractional rates of muscle protein synthesis, deposition and degradation for turkeys at 3 weeks of age

Variable	Diet				SD[1]	Significance
	30% Protein	22% Protein	22% Protein plus MET	22% Protein plus MET, LYS		
Protein synthesis, %/day[2]						
Pectoralis	36.2[a3]	32.7[ab]	30.4[ab]	25.4[b]	7.0	*
Gastrocnemius	28.0	30.1	26.7	23.7	8.3	-
Protein deposition, %/day[4]						
Pectoralis	12.8	17.3	1.4	6.7	-	NA
Gastrocnemius	15.2	13.4	-1.9	8.9	-	NA
Protein degradation, %/day[4,5]						
Pectoralis	23.4	15.4	29.0	18.7	-	NA
Gastrocnemius	12.8	16.7	28.6	14.8	-	NA

*P<.05; NA - not applicable.

[1]SD - standard deviation.

[2]Values represent the mean of 8 poults.

[3]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

[4]Values represent the mean of 16 poults.

[5]Determined from the difference between protein synthesis and deposition.

Table 19. The absolute rates of muscle protein synthesis, deposition and degradation for turkeys at 3 weeks of age[1]

Variable	Diet: 30% Protein	22% Protein	22% Protein plus MET	22% Protein plus MET, LYS	SD[1]	Significance
Protein synthesis, mg/day[2]						
Pectoralis	5161[a3]	2406[b]	2939[b]	2829[b]	845	***
Gastrocnemius	601	397	385	429	187	-
Protein deposition, mg/day[4]						
Pectoralis	1508	1029	132	666	-	NA
Gastrocnemius	262	154	-29	143	-	NA
Protein degradation, mg/day[4,5]						
Pectoralis	3653	1377	2807	2163	-	NA
Gastrocnemius	339	243	414	286	-	NA

*** $P<.001$; NA - not applicable.

[1] SD - standard deviation.

[2] Values represent the mean of 8 poults.

[3] Numbers within a row bearing similar superscripts are not significantly different ($P>.05$).

[4] Values represent the mean of 16 poults.

[5] Determined from the difference between protein synthesis and deposition.

Table 20. Muscle DNA and RNA activities for turkeys at 3 weeks of age[1]

Variable	Diet: 30% Protein	22% Protein	22% Protein plus MET	22% Protein plus MET, LYS	SD[2]	Significance
DNA activity[3]						
Pectoralis	357[a4]	214[b]	321[ab]	245[b]	99	*
Gastrocnemius	151	122	111	118	69	-
RNA activity[5]						
Pectoralis	23[a]	17[b]	19[b]	16[b]	4	**
Gastrocnemius	22	24	19	20	8	-

*P<.05; **P<.01.

[1]Values represent the mean of 8 poults.

[2]SD - standard deviation.

[3]Defined as g protein synthesized per g DNA per day.

[4]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

[5]Defined as g protein synthesized per g RNA per day.

Table 21. Differences in weight and composition between the pectoralis and gastrocnemius muscles of turkeys at 3 weeks of age[1]

Variable	Pectoralis muscle	Gastrocnemius muscle	Significance
Weight, g	61.7 (7.0)[2]	9.6 (1.0)	***
% of body weight	13.5 (.8)	2.1 (.1)	***
Protein, mg/g wet muscle	171 (14)	173 (19)	-
mg/muscle	10551 (1119)	1659 (275)	***
RNA, µg/g wet muscle	2905 (332)	2271 (267)	***
mg/muscle	177 (30)	22 (4)	***
DNA, µg/g wet muscle	205 (49)	409 (121)	***
mg/muscle	12.4 (4.1)	3.9 (1.2)	***
Protein:DNA	913 (255)	464 (162)	***
RNA:DNA	15.2 (3.6)	6.1 (2.2)	***
RNA:protein (x 10^{-2})	1.7 (.2)	1.3 (.2)	***
Protein synthesis, %/day	31.2 (7.0)	27.4 (8.3)	-
mg/day	3346 (845)	454 (187)	***
Protein deposition, %/day	9.0	8.9	NA
mg/day	834	132	NA
Protein degradation, %/day[3]	22.2	18.5	NA
mg/day	2512	322	NA
DNA activity[4]	283 (99)	126 (69)	***
RNA activity[5]	19 (4)	21 (8)	-

*** P<.001;NA - not applicable.

[1]Mean body weight of 32 infused poults was 450 g (±37).

[2]Values in parenthesis indicate standard deviation.

[3]Determined from the difference between protein synthesis and deposition.

[4]Defined as g protein synthesized per g DNA per day.

[5]Defined as g protein synthesized per g RNA per day.

Table 22. Developmental changes in the pectoralis muscle characteristics of turkeys from 1 to 3 weeks of age

Variable	Age		Significance
	7 days[1]	21 days[2]	
Weight, g	8.6 (1.9)[3]	84.4 (7.1)	***
% of body weight	6.9 (1.2)	14.7 (.4)	***
Protein, mg/g wet tissue	142 (15)	169 (7)	***
mg/muscle	1247 (381)	14240 (996)	***
RNA, µg/g wet tissue	3534 (369)	2631 (328)	***
mg/muscle	30.5 (7.3)	222.2 (34.5)	***
DNA, µg/g wet tissue	778 (140)	180 (43)	***
mg/muscle	6.6 (1.6)	15.3 (4.4)	***
Protein:DNA	190 (43)	988 (249)	***
RNA:DNA	4.7 (1.0)	15.5 (4.7)	***
RNA:protein (x 10^{-2})	2.5 (.4)	1.6 (.2)	***
Protein synthesis, %/day	58.7 (11.4)	36.2 (7.0)	***
mg/day	768 (280)	5161 (1092)	***
Protein deposition, %/day	39.8	12.8	NA
mg/day	277	1508	NA
Protein degradation, %/day[4]	18.9	23.4	NA
mg/day	491	3653	NA
DNA activity[5]	114 (34)	357 (115)	***
RNA activity[6]	24 (6)	23 (3)	-

***P<.001; NA - not applicable.

[1]Values represent the mean of 16 poults with a mean body weight of 124 g (±11).

[2]Values represent the mean of 8 poults with a mean body weight of 574 g (±39).

[3]Values in parenthesis indicate standard deviation.

[4]Determined from the difference between protein synthesis and deposition.

[5]Defined as g protein synthesized per g DNA per day.

[6]Defined as g protein synthesized per g RNA per day.

Table 23. Developmental changes in the gastrocnemius muscle characteristics of turkeys from 1 to 3 weeks of age

Variable	Age		Significance
	7 days[1]	21 days[2]	
Weight, g	2.2 (.3)[3]	12.6 (1.3)	***
% of body weight	1.8 (.1)	2.2 (.1)	***
Protein, mg/g wet tissue	147 (20)	165 (16.8)	*
mg/muscle	326 (67)	2091 (379)	***
RNA, µg/g wet tissue	2599 (342)	2226 (288)	*
mg/muscle	5.7 (1.0)	28.1 (5.3)	***
DNA, µg/g wet tissue	661 (210)	374 (112)	***
mg/muscle	1.5 (.5)	4.7 (1.4)	***
Protein:DNA	248 (103)	486 (191)	**
RNA:DNA	4.4 (1.8)	6.5 (2.4)	–
RNA:protein (x 10^{-2})	1.8 (.4)	1.4 (.2)	**
Protein synthesis, %/day	40.4 (12.6)	28.0 (10.0)	*
mg/day	132 (50)	601 (298)	***
Protein deposition, %/day	7.8	15.2	NA
mg/day	22	262	NA
Protein degradation, %/day[4]	32.6	12.8	NA
mg/day	110	339	NA
DNA activity[5]	99 (37)	151 (108)	–
RNA activity[6]	24 (9)	22 (10)	–

*P<.05; **P<.01; ***P<.001; NA - not applicable.

[1]Values represent the mean of 16 poults with a mean body weight of 124 g (±11).

[2]Values represent the mean of 8 poults with a mean body weight of 574 g (±39).

[3]Values in parenthesis indicate standard deviation.

[4]Determined from the difference between protein synthesis and deposition.

[5]Defined as g protein synthesized per g DNA per day.

[6]Defined as g protein synthesized per g RNA per day.

STUDY IV

EFFECT OF ESSENTIAL AND NON-ESSENTIAL AMINO ACID ADDITION TO A LOW PROTEIN DIET ON MUSCLE PROTEIN METABOLISM IN YOUNG TURKEYS

INTRODUCTION

The results of Study III demonstrated marked differences in the performance and muscle protein turnover of poults in response to amino acid supplementation of a 22% protein diet. Both methionine and lysine proved to be detrimental to the rates of protein deposition in the pectoralis and gastrocnemius muscles. However, conflicting results concerning the mechanism of the change in protein metabolism were obtained. Methionine addition produced an increase in protein degradation whereas lysine acted to depress the rate of protein synthesis.

Because lysine failed to improve the performance of poults in Study III, additional deficiencies in the 22% protein diet were indicated. Warnick and Anderson (1968) found that threonine, valine and lysine were the most limiting amino acids beyond methionine in a low protein diet fed to broiler chicks. Similarly, the results of Studies I and II suggest deficiencies of lysine, threonine and valine in a 22% protein diet for poults. The primary objective of the present study was to evaluate the efficacy of added methionine, lysine, threonine and valine on the performance and muscle protein metabolism of turkeys from one to three weeks of age.

Essential amino acid (EAA) supplementation of poult diets containing less than 24% protein has repeatedly been shown to be inadequate as a means to achieve acceptable performance (Baldini et al., 1954; Ferguson et al., 1956; Tuttle and Balloun, 1974; Atkinson et al., 1976). The data of Stas and Potter (1982) suggest a primary deficit of non-essential amino acids (NEAA) rather than EAA in a 22% protein diet composed primarily of corn and soybean meal. Glutamic acid has generally been used as a non-specific nitrogen source in low protein diets based on reports of its

efficacy in purified diets for poultry (Blair et al., 1972; Allen and Baker, 1974). Increasing the crude protein level from 18 to 22% with the addition of glutamic acid failed to improve the performance of chicks (Askelson and Balloun, 1965) leading to the conclusion that dietary nitrogen per se was not limiting to growth. Similarly, the results of Study I indicated no benefit from the addition of 4% glutamic acid to a 22% protein diet for young turkeys. However, a nitrogen deficiency may have been fulfilled by the EAA supplement, obviating any further addition of glutamic acid. The ability of various EAA to serve as non-specific nitrogen sources has been demonstrated by Allen and Baker (1974). Thus, in the present study, a mixture of NEAA were added in lieu of glutamic acid alone in a further attempt to differentiate between the benefits of limiting EAA and nitrogen per se in a 22% protein diet for young turkeys.

EXPERIMENTAL PROCEDURES

Animals and Housing. Large White turkeys obtained from the university flock were housed under continuous lighting in Petersime starter batteries from hatch to 22 days of age. A standard starter diet was fed to 7 days of age (Table 1). Poults were individually weighed on day 7 and assigned to cages such that the mean initial body weight was similar for all pens. Four pens of eight male turkeys per pen were fed each experimental diet ad libitum from 7 to 22 days of age.

In addition to the 22 and 30% protein diets employed in Study III, treatments involved supplementing the 22% protein diet with .4% DL-methionine (MET) or an essential amino acid (EAA) mixture (Table 24). A non-essential amino acid (NEAA) mixture was combined with the latter two supplements to provide a total of six dietary treatments. The composition of the EAA mixture was .4% DL-methionine, .31% L-threonine, .22% L-valine and .745% L-lysine HCl (which supplied .596% L-lysine). These amino acids were chosen to fulfil deficiencies observed previously in a 22% protein corn-soybean meal diet for young turkeys (Stas and Potter, 1982; Jackson et al., 1983). The NEAA mixture was formulated from isonitrogeneous aliquots of aspartic acid, glutamic acid, serine, glycine, and alanine (.61, .67, .48, .34, and .41%, respectively), to yield an equivalent crude protein content (N x 6.25) of 2%. Diets were maintained isocaloric by varying the levels of glucose monohydrate and stabilized fat. Individual body weights were recorded at 7, 14 and 21 days of age. Feed consumption was determined at 14 and 21 days on a pen basis.

Injection Procedure. At 7 days of age, 16 poults selected at random were injected subcutaneously along the back with [U-^{14}C] tyrosine in a sesame oil emulsion at a dose of .5 μCi per 100 g body weight according to the procedure of Kang et al. (1985a). The injection procedure was conducted again at 21 and 22 days of age with four poults from each treatment injected with radioactive tyrosine at a dose of .7 μCi per 100 g body weight on each day. All injections were carried out between 9:00 and 15:00 hr. Poults were killed by cervical dislocation 30 min post-injection. Preparation of the emulsions as well as the collection of blood samples and excision of the pectoralis and gastrocnemius muscles were as described for Study III. The right side of each muscle was used in the determination of muscle specific activity and tyrosine concentration, while tissue protein, RNA and DNA contents were quantitated using the left side. In addition, the pectoralis and gastrocnemius muscles were excised from 16 poults on day 5 and 5 poults per dietary treatment on day 20 and analyzed for protein. The protein contents determined in these samples were subsequently used to estimate the fractional rate of protein deposition at the time of infusion.

Analytical Procedures. Pectoralis and gastrocnemius samples weighing approximately 500 mg were homogenized in 8 volumes of .6N KCl. The resultant homogenates were used for the determination of protein, RNA and DNA concentrations as delineated for Study III. Bovine serum albumen served as the standard protein source in the microbiuret assay (Bailey, 1965) employed in the quantitation of muscle protein concentration. Tissue RNA content was analyzed according to the orcinol procedure of Schneider (1957) using calf liver RNA as the working standard. The con-

centration of DNA was determined by the diphenylamine method (Burton, 1968) with calf thymus DNA as the standard source.

The procedures employed in the determination of the specific activities of blood and muscle samples have been described fully in Study III. Muscle-free tyrosine concentration was determined for each injected poult. Muscle samples were homogenized in 5 volumes of cold 10% TCA. Following centrifugation, the supernatant was decanted and assayed for tyrosine using a Beckman Automatic Amino Acid Analyzer (Model 121, Beckman Instruments, Inc., Fullerton, CA 92634). A concentration of 30.5 nmol bound tyrosine per mg tissue was employed in the calculation of protein synthesis rate as no differences between muscle type or among dietary treatments were detected previously. The fractional synthesis rate was calculated using the ratio of the specific activities of bound to free tyrosine according to Garlick et al. (1973). The rate of protein deposition was determined from the rate of increase in muscle protein content over a 2-day period preceding the infusion for one-week-old poults and over days 20, 21 and 22 days of age for 3-week-old poults. The fractional rate of protein degradation was calculated as the difference between protein synthesis and deposition.

Statistical Analyses. All data save those for degradation and deposition rates were subjected to an analysis of variance. Significant differences among diet means were detected with Duncan's New Multiple Range Test (Steel and Torrie, 1980). Differences due to muscle type or age were determined using Student's t-test.

RESULTS

Pre-experimental Muscle Composition. The muscle composition of turkeys prior to the introduction of the experimental diets on day 7 is shown in Table 25. The concentration of protein in the gastrocnemius muscle was significantly higher ($P<.001$) than in the pectoralis muscle. The converse was true for RNA concentration ($P<.001$) while DNA concentration did not differ between muscles. The total contents of protein, RNA and DNA were considerably lower ($P<.001$) for the gastrocnemius muscle, reflecting the large difference in muscle weights ($P<.001$).

Although the ratio of protein:DNA was greater for the gastrocnemius ($P<.05$), the ratios of RNA:DNA and RNA:protein were higher in breast muscle tissue ($P<.001$). Significantly higher rates of protein synthesis ($P<.001$) and deposition were obtained for the pectoralis. The fractional degradation rate was higher in the gastrocnemius while the converse was true for the absolute rate of degradation. DNA activity was similar for both muscles whereas the gastrocnemius exhibited an increased RNA activity ($P<.05$).

Performance. Significant alterations ($P<.001$) in body weight gain, feed consumption, and feed efficiency in response to dietary treatments are shown in Table 26. A difference of 59% in body weight gain from 7 to 21 days of age was achieved by varying dietary crude protein from 22 to 30%. The addition of MET or the EAA mixture to the 22% protein diet increased weight gains by 34 and 52%, respectively. Elevating dietary crude protein by 2% with the NEAA supplement improved growth in combination with added MET only.

The large decrease in feed intake produced by reducing dietary protein from 30 to 22% was alleviated by MET supplementation of the latter diet. Because poults did not respond to MET addition with maximum gains, feed efficiency values were intermediate to those obtained for the 22 and 30% protein diets. A combination of MET and NEAA did not increase feed intake or feed efficiency over those noted with MET alone.

The high growth rate observed with the EAA-supplemented diet was achieved at the expense of increased feed consumption as intake was 9% greater than with the 30% protein diet. Thus, poorer feed efficiency resulted from EAA addition in comparison to the high protein diet. The further addition of the NEAA mixture tended to reduce intake somewhat, resulting in a significant improvement in feed efficiency. However, the 30% protein diet remained superior to the low protein diet containing both the EAA and NEAA supplements.

Mean body weights of poults used in the infusion study (Table 27) followed similar trends to those described above. A difference of 48% in body weight ($P<.001$) was observed with a decrease from 30 to 22% protein. Methionine and EAA supplements improved weight by 23 and 37%, respectively. An increase in crude protein by adding the NEAA mixture elicited no further increases in body weight over added MET or EAA alone. However, the greater variation associated with means obtained from individual *versus* pen values acted to mask a 30 g difference in weight between the diets containing added MET with and without the NEAA.

Muscle Composition. Muscle weights varied in response to dietary manipulation commensurate with changes in body weight (Table 27). Reducing dietary protein from 30 to 22% exerted a greater influence on muscle

weight than on body weight, regardless of amino acid supplementation. Pectoralis and gastrocnemius weights were depressed 44 and 41%, respectively, as dietary protein was decreased from 30 to 22% ($P<.001$). Methionine and EAA supplementation of the low protein diet produced muscle weights that were intermediate to those obtained with the two protein levels. Further addition of the NEAA had no significant effect on the weight of the pectoralis or gastrocnemius muscles.

Expression of pectoralis weights as a proportion of body weight demonstrated similar differences among dietary treatments ($P<.001$). Smaller, but significant ($P<.01$), alterations were noted for the gastrocnemius muscle in response to dietary protein and amino acid supplementation. Although MET addition failed to effect the proportional gastrocnemius weight, the EAA supplement elicited a significant elevation. No response in the proportional weight of the gastrocnemius was observed with NEAA supplementation.

The concentration of protein, RNA and DNA in the gastrocnemius muscle were not significantly altered by dietary manipulation (Table 28). Protein concentration of the pectoralis muscle varied significantly ($P<.01$) with diet. Protein content was greatest in the muscles of poultry receiving the 30% protein diet and the 22% protein diet containing the EAA mixture. Methionine supplementation of the low protein diet, alone or in combination with the NEAA mixture, yielded an equivalent protein concentration to that of the basal 22% protein diet. Similarly, the NEAA supplement was ineffective in increasing breast muscle protein concentration when fed in conjunction with the EAA mixture.

A decrease of 18% in the RNA concentration of the pectoralis resulted from increasing dietary protein to 30% ($P<.01$). Amino acid supplementation of the low protein diet, whether in the form of MET or the EAA mixture, had no influence on RNA concentration in comparison to the basal 22% protein diet.

Pectoral DNA concentration was significantly ($P<.001$) higher for the unsupplemented 22% protein diet than the other dietary treatments. The DNA concentration for the 30% protein diet was indistinguishable from those of the low protein diets containing added amino acids.

Total protein, RNA and DNA contents of the pectoralis muscle varied as a function of both muscle weight and concentration of each component (Table 29). For turkeys receiving the low protein diet supplemented with MET or the EAA mixture, total protein contents were 72 and 87%, respectively, of the control 30% protein diet ($P<.001$). NEAA supplementation failed to influence the amount of breast muscle protein. Total pectoral RNA was depressed for turkeys receiving the basal 22% protein diet in comparison to the other treatments ($P<.001$). Methionine supplementation elevated RNA content significantly from 134 to 178 mg while NEAA addition produced a further increase to 208 mg. The amount of RNA in the breast muscle of turkeys receiving the 30% protein diet was indistinguishable from the RNA content observed with the 22% protein diet containing amino acids in any combination. The DNA content of the pectoralis was greater with the high protein diet than with the 22% protein diet with or without MET ($P<.05$). The amount of DNA produced with the EAA supplement was intermediate to that of the high and low protein diets, although not

significantly different from either. Again, no response was obtained from the NEAA mixture.

Differences in the total protein and RNA contents of the gastrocnemius muscle largely reflected changes in muscle weight. Total muscle protein was 70% higher for turkeys fed the basal 30% protein diet than those receiving the 22% protein diet ($P<.001$). An increase of 27% in protein content was obtained with MET addition to the low protein diet. Equal protein contents were obtained with the 30% protein diet and the 22% protein diet containing the EAA supplement. The NEAA mixture did not influence the amount of protein in the gastrocnemius muscle. A 59% increase in total RNA content of the gastrocnemius was produced by increasing dietary protein from 22 to 30% ($P<.001$). No improvement was elicited with MET supplementation. Conversely, RNA content responded to EAA addition with a significant increase such that the total amount of RNA was equal to that obtained with the 30% protein diet. NEAA supplementation had no effect on the RNA content of the gastrocnemius. The total amount of gastrocnemius DNA was not altered by diet manipulation.

The ratio of protein:DNA in the pectoralis muscle was significantly decreased ($P<.001$) by reducing the dietary protein level (Table 30), but increased to the control value by supplementing the low protein diet with any combination of the amino acids. Differences among treatments in the ratio of RNA:DNA, although significant ($P<.01$), were less distinct. Adding both MET and NEAA to the low protein diet resulted in a superior RNA:DNA ratio than that obtained with the 30% protein diet. The ratios of RNA:DNA obtained from the remaining combinations of amino acids were

equivalent to that noted with the 30% protein diet but higher than that with the unsupplemented 22% protein diet. No difference between the 30 and 22% protein diets was detectable. The RNA:protein ratio of the pectoralis was depressed by increasing dietary protein or supplementing the low protein diet with a combination of the EAA and NEAA mixtures ($P<.01$). The addition of MET, EAA or MET and NEAA proved to be essentially equal to the basal 22% protein diet.

The ratios of protein:DNA, RNA:DNA, and RNA:protein observed for the gastrocnemius muscle were not significantly altered by diet. However, protein:DNA tended to be lower for the unsupplemented 22% protein diet than the remaining treatments, similar to the observation for the pectoralis.

Protein Turnover. Dietary treatment had no significant effect on the fractional rate of protein synthesis in the pectoralis muscle (Table 31). The fractional deposition rate was highest for the control 30% protein diet and the EAA-supplemented diet but declined sharply for the remaining treatments. A decrease in dietary protein acted to increase the fractional degradation while amino acid addition reversed the trend. Degradation rate was lowest when the EAA mixture was added to the 22% protein diet. The fractional rate of protein synthesis in the gastrocnemius muscle was significantly altered by diet ($P<.001$). Adding MET or MET and NEAA to the 22% protein diet depressed the rate of protein synthesis from 32% for the 30% protein diet to 21 and 19%, respectively. Reducing the dietary protein level or supplementing the low protein diet with EAA alone or combined with the NEAA mixture produced values which were essentially equal to the rate for the 30% protein diet. The inability to assign

significance to the means for protein deposition and degradation renders interpretation of the results for the gastrocnemius difficult at best. The rate of protein deposition for the gastrocnemius was maximized with EAA addition to the low protein diet. Dietary protein level appeared to have little influence on deposition although adding the NEAA mixture to the EAA-supplemented diet halved the fractional rate of protein deposition. Methionine addition produced deposition rates that were somewhat lower than that obtained with the 30% protein diet. The fractional degradation rate was decreased by supplementing the low protein diet with MET, EAA or MET and NEAA. Dietary protein level had no effect on degradation.

Expressing the rate of protein synthesis on an absolute weight basis demonstrated a significantly ($P<.01$) higher rate in the breast muscle of turkeys fed the 30% protein diet (Table 32). No other differences among treatments were observed. Deposition was highest for poults receiving the 30% protein diet and the EAA-supplemented diet. Methionine addition failed to increase protein deposition relative to the basal 22% protein diet whereas increases of 86 and 66% were obtained by adding the NEAA mixture jointly with MET or EAA, respectively. The 30% protein diet produced the highest rate of protein degradation. The low degradation rate observed with the 22% protein diet was not altered by amino acid addition with the exception of the combined EAA and NEAA mixtures which increased degradation.

The absolute rate of protein synthesis in the gastrocnemius muscle was highest for the 30% protein diet ($P<.001$). In comparison, the total amount of protein synthesized was significantly reduced for turkeys re-

ceiving the low protein diet supplemented with the EAA alone or with the NEAA mixture. Feeding the basal 22% protein diet or a supplement of MET or MET and NEAA produced the lowest rates of protein synthesis. Changes in the absolute rate of protein deposition paralleled the results for the pectoralis. Degradation rates were lowest when MET was added to the 22% protein diet alone or in combination with the NEAA mixture. The increase in degradation noted with EAA addition relative to the basal 22% protein diet was accentuated by the NEAA supplement although the 30% protein diet elicited the highest absolute rate of degradation.

Both the DNA and RNA activities in the pectoralis were noticeably higher with the 30% protein diet although a large amount of experimental variation precluded the detection of significant differences among treatments (Table 33). Differences in the DNA activity obtained for the gastrocnemius due to treatment were highly significant ($P<.001$) but indistinct. Adding the EAA mixture to the 22% protein diet resulted in a DNA activity that was intermediate to the 30% protein diet and the remaining treatments but not significantly different from either. A higher level of DNA activity was observed for the 30% protein diet in comparison to the low protein diet containing the amino acids or supplements of MET, MET and NEAA, or EAA and NEAA. The RNA activity resulting from the 30% protein diet was significantly greater than that from the 22% protein diet with added MET ($P<.05$). The NEAA mixture elicited no further increase over MET alone. Although a reduction in dietary protein tended to reduce RNA activity, this difference was not significant. Similarly, adding the EAA mixture to the 22% protein diet alone or in combination with NEAA had no effect on gastrocnemius RNA activity.

Differences Between Muscles. The concentrations of protein and RNA were significantly higher (P<.001) in the pectoralis muscle than in the gastrocnemius (Table 34). Conversely, DNA concentration was greater for the latter muscle (P<.001). The large differences in total protein, RNA and DNA contents between muscles (P<.001) occurred primarily as a result of the six-fold distinction in muscle weight (P<.001). Breast muscle exhibited significantly higher (P<.001) ratios of protein:DNA, RNA:DNA and RNA:protein than the gastrocnemius muscle.

The mean protein synthesis rate for the breast was 36.8% versus 28.3% for the gastrocnemius at 21 days of age (P<.001). Similarly, the absolute rate of synthesis in the pectoralis exceeded that in the gastrocnemius (P<.001). The fractional rate of degradation appeared to be higher in the pectoralis whereas the fractional deposition rates were essentially equal between muscles. Absolute rates of deposition and degradation were 6- and 10-fold greater for the pectoralis. Pectoral DNA activity was increased commensurate with synthesis rates (P<.001) while RNA activity was similar between muscles.

Developmental Changes. A comparison between turkeys at one week of age and the 3-week-old turkeys fed the control 30% protein diet demonstrates developmental changes in muscle characteristics. A five-fold increase in body weight was accompanied by an 11-fold increase in pectoralis weight (P<.001) (Table 35). Associated with the marked growth of the pectoralis was a 15% increase in protein concentration (P<.001). The breast muscle concentrations of RNA and DNA decreased by 34 and 68%, respectively, with age (P<.001). Total protein, RNA and DNA contents were elevated (P<.001) as a result of the large increase in muscle weight.

The ratios of protein:DNA and RNA:DNA were 72 and 51% higher for the older turkeys ($P<.001$) whereas the RNA:protein ratio declined from 2.6 at one week to 1.5 at 3 weeks ($P<.001$).

Although the rate of protein synthesis in the breast muscle declined from 58.7 to 44.0% ($P<.05$) over the 2-week period, the large increase in muscle mass resulted in a five-fold increase in the absolute amount of protein synthesized ($P<.001$). An increase in the fractional degradation rate was apparently related to a substantial fall in the fractional deposition rate, from 48% at one week to only 15% at 3 weeks. However, both the deposition and degradation increased over time when expressed on an absolute weight basis. DNA activity was elevated at three weeks ($P<.001$) while RNA activity was not altered significantly.

The growth rate of the gastrocnemius muscle was only slightly greater than the whole body growth rate, with a six-fold increase ($P<.001$) observed for the former from one to three weeks of age (Table 36). Both protein and RNA concentrations remained unchanged at 3 weeks whereas the concentration of DNA was reduced by 57% ($P<.001$). Again, the increased muscle mass produced substantial elevations in the total amounts of protein, RNA and DNA in the gastrocnemius ($P<.001$). The ratios of protein:DNA and RNA:DNA were increased ($P<.001$) over the 2-week period, consistent with the decrease in DNA concentration. The RNA:protein ratio declined slightly from 1.6 to 1.3 over the same period ($P<.05$).

The fractional rates of protein synthesis and deposition did not undergo a developmental fall as observed for the pectoralis. Similarly, degradation rate remained stable. However, the total amount of protein synthesized was increased ($P<.001$) as was total protein deposition and

degradation due to the greater muscle weight at 3 weeks of age. The DNA activity increased from 84 g at one week to 173 at 3 weeks ($P<.01$). No change in RNA activity was observed.

DISCUSSION

Performance. A decrease in dietary protein level resulted in depressed weight gain, feed consumption and feed efficiency of poults at 3 weeks of age, concurring with the results of Study III. The positive growth response to MET supplementation of the 22% protein diet was due equally to increases in feed intake and feed efficiency. The NEAA supplement produced additional small elevations in weight gain, feed intake and feed efficiency, inferring the fulfillment of a crude protein deficit.

Although added MET proved beneficial to body weight gain, the further addition of lysine, threonine and valine was necessary to restore control growth. Because the improvement in weight gain was achieved at the expense of an increased feed intake, feed efficiency with the EAA-supplemented diet was inferior to that of the 30% protein diet. The NEAA mixture in combination with EAA augmented feed efficiency only, possibly due to the achievement of maximum growth with the EAA addition alone.

Few investigators have been completely successful with amino acid supplementation of low protein diets for turkeys. Atkinson et al. (1976) found that poults fed a 22% protein diet supplemented with .3% methionine and .2% lysine from hatch to 18 days were 16% smaller than those fed a control 30% protein diet. Supplementing a 20% protein diet with methionine, lysine, arginine, valine, glycine and phenylalanine resulted in only 92% of the body weight and feed efficiency of poults fed a 28% protein diet for 4 weeks (Fitzsimmons and Waibel, 1962). Additions of arginine, cystine, leucine, threonine and lysine to a 22% protein diet did not produce weight gains equal to those for poults fed a 28% protein

diet to 5 weeks of age (Tuttle and Balloun, 1974). Increasing dietary protein to 24% in combination with similar EAA additions resulted in performance equal to controls.. Because dietary protein was elevated by manipulating the levels of corn and soybean meal inclusions, distinguishing the influence of limiting EAA from crude protein per se was not possible. In the present study, the amelioration in body weight gain and feed efficiency with an increase in dietary protein to 24% by adding the NEAA mixture can be interpreted as a response to nitrogen alone.

The positive effect of NEAA addition appears to be at variance with the lack of response to added glutamic acid in Study I. One explanation for the differing results may be a more efficient utilization of a mixture of amino acids over glutamic acid alone. However, data refuting this hypothesis was presented by de Moraes et al. (1984) who found that chicks performed equally well when crystalline amino acid diets were supplemented with glutamic acid or an isonitrogeneous mixture of glutamic acid, aspartic acid, alanine and serine. Alternatively, the EAA mixture added to the 22% protein diet in Study I may have provided a source of nitrogen for the synthesis of NEAA, obviating an additional supply in the form of glutamic acid. The ability of various EAA to displace glutamic acid as a non-specific nitrogen source, albeit less efficiently, was demonstrated by Allen and Baker (1974).

Thus, while lysine supplementation adversely affected the performance of poults in Study III, the combination of threonine and valine with lysine elicited weight gains equal to that obtained with a 30% protein diet. An increase in crude protein to 24% jointly with the EAA addition further improved the performance of poults by increasing feed efficiency.

The remaining difference in feed efficiency of 5% between the 30% protein diet and the fully supplemented diet was apparently due to further marginal deficiencies in the latter diet. Arginine, histidine and isoleucine would appear to be likely candidates based on their presence at or near stated requirements (NRC, 1984).

Effect of Diet on Muscle Metabolism. Changes in muscle weight in response to diet reflected similar alterations in the body weight of infused turkeys. Poults fed the unsupplemented 22% protein diet were the smallest in weight and exhibited the lowest pectoralis and gastrocnemius muscle weights, both on an absolute and proportional basis. The EAA mixture proved to be superior to MET addition in terms of absolute muscle weight. An increase in crude protein with the NEAA mixture tended to increase both the breast and leg muscle weights proportionally with body weight.

The low breast muscle weight of the poults fed the unsupplemented 22% protein diet resulted from decreases in both DNA-unit size and number. Methionine supplementation effectively restored DNA-unit size while the number of DNA-units responded only to the addition of the EAA mixture. Feeding a protein-free diet to chicks from 4 to 6 weeks of age resulted in considerable decreases in DNA-unit size and number of the pectoralis muscle (MacDonald and Swick, 1981) while feed restriction of chicks depressed DNA-unit size only (Cornejo and Pokniak, 1983).

Despite alterations in gastrocnemius muscle weight with diet, no change in the DNA-unit was statistically detectable although reductions in both size and number were indicated from an 8% decrease in dietary crude protein. The large amount of experimental error associated with determinations for the gastrocnemius muscle acted to mask any dietary

effects both in the present study and Study III. Previous studies have demonstrated the adverse effects of a dietary protein or EAA deficiency on the size and number of DNA-units in the leg muscles of chicks (Montgomery et al., 1964; Akinwande and Bragg, 1974) and rats (Howarth, 1972; Millward et al., 1975).

Alterations in muscle weight bore little relation to muscle composition in response to dietary manipulation. Gastrocnemius component concentrations were unchanged by diet. Published results vary according to the severity of the dietary restriction. The concentrations of DNA and protein in the gastrocnemius muscle of rats tended to increase as dietary protein was reduced from 24 to 6% (Howarth, 1972). The converse was true for RNA concentration. Feed restriction of chicks from 10 days of age to adulthood resulted in a decrease in protein concentration and an increase in DNA concentration of the sartorius muscle (Montgomery et al., 1964). In contrast to the aforementioned studies, dietary amino acid balance had little effect on leg muscle composition. The concentrations of protein, RNA and DNA were not altered in the gastrocnemius muscle of broilers by supplementing a low protein diet with the first limiting amino acid (Akinwande and Bragg, 1974). The protein concentration in the gastrocnemius muscle of rats was unaffected by cystine supplementation of a diet marginal in methionine (Harney et al., 1976) or by feeding gelatin, gluten or whole egg diets (Omstedt et al., 1978).

Pectoral muscle composition was considerably more sensitive to diet. A drop in crude protein from 30 to 22% had the greatest impact, resulting in concomitant increases in RNA and DNA concentrations and a decrease in protein concentration. The EAA mixture restored control protein and DNA

levels although the further addition of NEAA was required to reduce RNA to the control concentration. An increase in carcass protein retention was noted with the supplementation of chick diets with a mixture of EAA or glutamic acid, with the former being a more potent stimulus to carcass protein deposition (Velu et al., 1972).

Diet had no discernible effect on the fractional rate of protein synthesis in the pectoralis muscle. However, fractional rates were reduced numerically in response to amino acid supplementation. Cellular protein synthetic capacity and efficiency appeared to be inversely related as observed in Study III. Cellular capacity was lowest and efficiency highest with the 30% protein diet. The large amount of variation associated with DNA activity precluded any significant change in response to diet, although DNA activity was substantially higher with the 30% protein diet, as observed previously.

Little change in the fractional synthesis rate in the pectoralis muscle of chicks was observed with the imposition of a protein-free diet for a 2-week period (MacDonald and Swick, 1981). At the same time, degradation rate was elevated from 14% in the control chicks to 23% in the birds deprived of protein. Feeding a lysine-deficient diet to young chicks had no influence on the fractional synthesis rate of the breast muscle whereas the fractional degradation rate doubled (Maruyama et al., 1978). Reducing the level of EAA in the diet to 75% of requirements elicited a similar response, resulting in a decrease in the rate of protein deposition from 18 to 13%. The fractional degradation rate of the pectoralis muscle was apparently increased in the present report in response to a dietary protein deficiency, resulting in a correspondingly

poorer rate of protein deposition. Amino acid supplementation reduced the degradation rate, with the lowest value observed for the diet containing the EAA mixture. However, protein deposition rates did not recover with the exception of the EAA-supplemented diet. Thus, a decrease in dietary protein primarily acted to increase the fractional degradation rate, thereby limiting muscle growth. Amino acid addition to the low protein diet produced small decreases in both the synthesis and degradation rates of the pectoralis muscle.

While few differences in the fractional synthesis rate were discernible for the pectoralis muscle, the absolute rate of protein synthesis was halved by a drop in crude protein. The total amount of protein synthesized was improved by either EAA or NEAA supplementation, in accordance with large increases in total synthetic capacity. However, an impairment in the efficiency of protein synthesis was inferred from the persistence of lower absolute synthesis rates in comparison to the 30% protein diet. The absolute rate of protein degradation was also depressed with low dietary protein and remained so despite the addition of amino acids. MacDonald and Swick (1981) observed decreases in the absolute rates of synthesis and degradation in the pectoralis muscles of chicks on a protein-free regime, even in the absence of significant changes in fractional rates.

The gastrocnemius muscle exhibited a marked decrease in fractional synthesis rate in response to MET supplementation of the low protein diet. The drop was ascribed to a decrease in the efficiency of protein synthesis rather than capacity. Adding the NEAA mixture to the diet had no influence on synthetic capabilities. The fractional degradation rate was

similarly reduced by MET addition such that the fractional deposition rate was not adversely affected in comparison to the remaining treatments. This effect of MET supplementation on protein synthesis and degradation was not observed in Study III and, thus, requires further investigation to determine the veracity of the response. Little change in synthetic characteristics were obtained from the remaining dietary treatments. However, the EAA supplement resulted in a lower rate of degradation than that for the 30% protein diet, concurring with the observation for the pectoralis. In that any reduction in protein degradation represents a considerable reduction in energy expenditure, additional research is warranted to corroborate this finding.

Imposing a low protein regime on growing rats has invariably reduced the rates of protein synthesis and degradation in leg muscles (Waterlow and Stephen, 1968; Haverberg et al., 1975a; Millward et al., 1975; Nishizawa et al., 1977b; Burini et al., 1981; Smith et al., 1982). Decreases in synthetic capacity (Howarth, 1972) and efficiency (Millward et al., 1975) have alternately been credited with the decline in synthesis rate. Dietary protein quality has been implicated in protein turnover of rat muscles as well. The poor amino acid balance of wheat gluten was detrimental to the fractional synthesis rate of leg muscles in comparison to a casein diet (Sampson and Jansen, 1984). The fractional degradation rate also declined from feeding poor quality feedstuffs such as gelatin and gluten, evidenced by a decrease in 3-methylhistidine excretion (Omstedt et al., 1978; Hayase and Yoshida, 1980). Although weight gain was directly related to amino acid balance, differences in the fractional

degradation rate were detectable only with drastic alterations in protein quality.

The difference in response noted between rats and poults may be attributed to species variation or, alternately, to the imposition of more moderate dietary restrictions in the studies involving poultry according to MacDonald and Swick (1981). In an earlier study by Maruyama et al. (1978), reducing all EAA to 75% of requirements depressed synthesis rates in the leg muscle without altering degradation rates. The situation was reversed by a lysine deficiency, where the fractional degradation rate was doubled and synthesis rate remained stable. The conflicting results of this and the present study necessitate additional research to elucidate the impact of diet on muscle protein metabolism in poultry.

A 37% decrease in total synthetic capacity of the gastrocnemius muscle was obtained with a decrease in crude protein level. The absolute rate of protein synthesis fell 52%, however, as a result of a concurrent decline in synthetic efficiency. Changes in the capacity for protein synthesis in the gastrocnemius muscles of rats fed a protein-free diet were more important determinants of synthesis rate than alterations in synthetic efficiency (Millward et al., 1974). Both synthetic efficiency and capacity remained depressed with MET supplementation, producing the lowest daily rate of protein synthesis. Although degradation decreased as well, the reduction was insufficient to prevent a 50% decline in deposition. The EAA mixture increased synthetic capacity to a level indistinguishable from the 30% protein diet. A lower efficiency of synthesis prevented the attainment of maximum protein synthesis. However, the increase in synthesis coupled with a relatively low rate of

degradation resulted in an equivalent deposition rate to that of the 30% protein diet. Increasing crude protein to 24% produced conflicting results but, in general, failed to improve the rate of protein deposition.

In summary, the addition of methionine, lysine, threonine and valine to the 22% protein diet afforded a low rate of protein turnover while maintaining acceptable levels of protein deposition in both the pectoralis and gastrocnemius muscles. No further benefit of an elevation in crude protein was observed, save an increase in overall feed efficiency. The potential for improvement in the performance of poults fed the EAA-supplemented diet may lie in the provision of additional limiting amino acids beyond those evaluated in the present study.

Differences Between Muscles. The differences between the pectoralis and gastrocnemius muscles at one and 3 weeks of age generally conformed to the observations in Study III. The considerably greater weight of the pectoralis was associated with a higher concentration of RNA and a correspondingly larger ratio of RNA:DNA. Protein concentration of the gastrocnemius exceeded that of the pectoralis at one week of age while the converse was true at 3 weeks of age. In contrast, no difference between muscle type was discernible at either stage of development in Study III.

Pectoral DNA-unit number was greater than that of the gastrocnemius at both ages. Sunde et al. (1984) reported no difference between the DNA-unit size of the breast and leg muscles of one-week-old poults while we obtained a greater value for the gastrocnemius. Pectoral DNA-unit size was higher at 3 weeks of age in both studies.

The concentration and activity of DNA were similar between muscles at one week of age. An increased DNA concentration in the gastrocnemius was accompanied by a decreased DNA activity. DNA activity was higher in the breast muscle of poults from one to 8 weeks of age in a study by Kang et al. (1985b).

The greater fractional and absolute rates of protein synthesis in the pectoralis at both ages were due to increased cellular and total synthetic capacities, respectively. The increased efficiency of synthesis noted at one week of age for the gastrocnemius was not observed in Study III and is, thus, likely due to experimental error. Furthermore, the difference in the total capacity for protein synthesis between the muscles was sufficient to account for the lower synthesis rate. The high fractional rate of protein deposition noted at one week for breast muscle declined at 3 weeks to equal the level attained for the leg muscle.

Higher rates of synthesis and deposition in breast muscle at one week of age conforms with published results (Maruyama et al., 1978; Kang et al., 1985a; Kang et al., 1985b). However, synthesis rates were similar for both muscles beyond the first week of age with the exception of the study by Bergen et al. (1983) where rates in the breast remained elevated in comparison to the leg at 4, 5 and 6 weeks of age. Equal rates of deposition at 3 weeks of age concurs with the available literature involving chicks (Maruyama et al., 1978; Kang et al., 1985a; Bergen et al., 1983) and poults (Kang et al., 1985b).

The results for protein degradation are complicated by overestimations of the deposition rate at one week of age and the synthesis rate at 3 weeks of age for the pectoralis. Kang et al. (1985a) found no dif-

ference in the fractional degradation rate between the breast and leg muscles of broilers aged one to six weeks. Similarly, fractional degradation rates were equal in the wing muscles of broilers despite a comparison between muscles containing solely white fibers or a mixture of red and white fibers (Hentges et al., 1983). In a study involving turkeys, degradation rates were equal in breast and leg muscles at one week of age, followed by greater rates in the leg muscles at 2, 4 and 8 weeks of age (Kang et al., 1985b). Conversely, Bergen et al. (1983) reported higher fractional rates in comparison to leg muscles at 4, 5 and 6 weeks of age. Breast muscle exhibited a higher degradation rate than leg muscles in broilers at 2 weeks of age (MacDonald and Swick, 1981). The contradictory results are related to the use of a calculated value for protein degradation rate as opposed to a direct determination. Thus, the higher and lower rates of degradation in the gastrocnemius muscle at one and 3 weeks of age, respectively, in the present study should be viewed with caution.

Developmental Changes. In general, alterations in pectoralis muscle characteristics from one to 3 weeks of age paralleled the results of Study III. The large increase in pectoralis weight was accompanied by changes in muscle composition, including an increase in protein concentration and decreases in RNA and DNA concentrations. The increase in breast muscle protein concentration from hatch to 4 weeks of age in Rhode Island Red chickens was the result of essentially equal increases in sarcoplasmic and myofibrillar protein (Dickerson, 1960). However, myofibrillar protein comprised a greater proportion of breast muscle protein than sarcoplasmic protein throughout development.

Both pectoral DNA-unit number and size expanded approximately four-fold over the 2-week period in general agreement with previous findings for chickens (Moss et al., 1964) and turkeys (Kang et al., 1985b). Pectoral DNA-unit size increased from approximately 220 at one week of age for chicks to 350 and 520 at 2 and 4 weeks of age, respectively (MacDonald and Swick, 1981). A comparable value obtained in the present study was considerably larger at 685 for 3-week-old turkeys in agreement with the higher DNA activity for the poult (Sunde et al., 1984) in comparison to the chick (MacDonald and Swick, 1981).

The age-related decline in the fractional synthesis rate of the pectoralis was attributable to a decline in synthetic capacity rather than efficiency. Synthesis rate in the breast muscle of chicks decreased more than 40% from one to two weeks of age, with rates of 42 and 20% noted at the respective ages (MacDonald and Swick, 1981). Synthesis rates appeared to be stable from 2 to 7 weeks of age. Similar observations for chicks have been reported by others (Maruyama et al., 1978; Lauterio et al., 1983). A decline in the cellular synthetic capacity was responsible for significant decreases in the fractional synthesis rate in the breast muscle of poults, with no change in the efficiency of synthesis (Kang et al., 1985b). Synthesis rates for the breast were 56, 29 and 16% at 1, 2 and 4 weeks of age, respectively. While the synthesis rate at one week of age concurs with the present results, our value of 44% at 3 weeks is considerably higher. Thus, the apparent increase in fractional degradation rate over time in contrast to previous reports (Maruyama et al., 1978; MacDonald and Swick, 1981; Kang et al., 1985b) may be explained, in part, by an overestimation of the fractional synthesis rate at 3 weeks

of age. Similarly, the fractional rate of protein deposition at one week of age is considerably higher than the values of 33% for broilers (Kang et al., 1985a), 16% for leghorns (MacDonald and Swick, 1981) and 34% for turkeys (Kang et al., 1985b). However, the consistency of the results for synthesis deposition and degradation in both Study III and the present study lends veracity to the findings.

Observations for the gastrocnemius differed somewhat from the findings in Study III. The six-fold increase in muscle weight produced a change in DNA concentration only. The increase in protein concentration and decrease in RNA concentration obtained previously were not evident in the present experiment. The concentrations of both DNA and RNA in the skeletal muscle of growing rats declined as the animals aged, coincident with rapid muscle growth (Devi et al., 1963; Young and Alexis, 1968). However, Akinwande and Bragg (1974) reported an increase in DNA concentration and a decrease in RNA concentration of the gastrocnemius muscle of broilers from 2 to 4 weeks of age.

Both DNA-unit size and number increased with age in relatively equal proportions. The values for DNA-unit size concur with published results for the turkey (Sunde et al., 1984). Growth of the hind leg muscles of growing rats was due to coincident increases in DNA-unit size and number with the former being quantitatively more important (Millward et al., 1975).

The fractional rate of protein synthesis in the gastrocnemius failed to decline over the 2-week period despite a decrease in synthetic capacity. Synthetic efficiency was similar at both ages. Both the fractional deposition and degradation rates remained stable in contrast to the re-

sults of Study III. Kang et al. (1985b) reported small decreases in the rates of synthesis, deposition and degradation in leg muscles of poults from one to two weeks of age. Comparatively larger decreases were noted from 2 to 4 weeks of age. No age-related fall in synthesis rate was observed in the leg muscles of chicks from one to two weeks of age, approximating 24% at both ages (Maruyama et al., 1978). Degradation rate declined from 18 to 14%. Thus, protein metabolism in the gastrocnemius muscle is relatively stable in comparison to the marked changes occurring in the pectoralis, reflecting basic differences in the functional capacities of the two muscles.

SUMMARY

The present study was designed to evaluate the effects of essential (EAA) and non-essential amino acid (NEAA) supplementation of a 22% protein diet on the performance and muscle protein metabolism of male turkeys from one to three weeks of age. A corn-soybean meal diet was supplemented with either .4% methionine (MET) or a mixture of .4% methionine, .6% lysine, .31% threonine and .22% valine (EAA). An isonitrogeneous mixture of NEAA formulated to supply an equivalent crude protein content of 2% was added to each of the supplemented diets to evaluate the nitrogen adequacy of the 22% protein diet. A 30% protein diet supplemented with .3% methionine served as a control. Sixteen poults were sacrificed at 7 days of age to obtain initial observations on the composition and protein turnover of the pectoralis and gastrocnemius muscles. Dietary effects were determined in four poults from each treatment at 21 and 22 days of age. The fractional rate of protein synthesis was ascertained using the tyrosine emulsion technique. The rate of protein deposition was determined from the rate of increase in muscle protein content over a 2-day period preceding the infusion for one-week-old poults and over days 20, 21 and 22 for 3-week-old poults. The fractional rate of degradation was calculated as the difference between protein synthesis and deposition.

Maximum performance was obtained with the control 30% protein diet. The EAA supplement elicited growth equal to the high protein diet although feed efficiency remained inferior. The latter was improved with the NEAA mixture, indicating a crude protein deficiency in the low protein diet. Methionine addition produced intermediate results.

Changes in muscle weight due to diet, albeit directly related to body weight, were independent of muscle composition. Protein synthesis in the pectoralis was not significantly altered by diet although rates tended to be lowered by amino acid addition. Cellular synthetic capacity and efficiency were inversely related. Degradation rate was increased by a drop in crude protein and decreased by EAA addition. The low rate of degradation for the latter diet allowed a rate of deposition equal to the 30% protein diet.

A decrease in the fractional synthesis rate of the gastrocnemius was obtained with the addition of MET, due to a drop in synthetic efficiency. Degradation rate also declined, preventing a decrease in deposition rate relative to the unsupplemented diet. Deposition was maximized with EAA supplementation as a result of a low degradation rate in comparison to the 30% protein diet. A decrease in protein degradation represents a considerable reduction in energy expenditure. The low rate of protein turnover in both muscles exhibited by poults fed the EAA supplement merits further attention in light of the maintenance of maximum protein deposition rates.

Table 24. Composition of experimental diets

Ingredient	Diet 1	2	3	4	5	6
Ground yellow corn	211.88	413.38	413.38	413.38	413.38	413.38
Glucose monohydrate	95	141	134	112.7	92.02	70.63
Stabilized fat	60	17	20	28.55	36.88	45.52
Dehulled soybean meal	581.5	380	380	380	380	380
Defluorinated phosphate	37.5	37.5	37.5	37.5	37.5	37.5
Iodized salt	4	4	4	4	4	4
Trace mineral mix[1]	1	1	1	1	1	1
Vitamins and feed additives[2]	6.12	6.12	6.12	6.12	6.12	6.12
DL-Methionine	3	--	4	4	4	4
L-Lysine HCl	--	--	--	7.45	--	7.45
L-Threonine	--	--	--	3.1	--	3.1
L-Valine	--	--	--	2.2	--	2.2
Amino acid mixture[3]	--	--	--	--	25.1	25.1
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated composition						
Protein (%)[4]	30.06	22.07	22.07	22.07	22.07	22.07
Energy (kcal ME/kg)	2949	2949	2951	2950	2950	2950
Methionine (%)	.76	.36	.76	.76	.76	.76
TSAA (%)	1.20	.70	1.10	1.10	1.10	1.10
Lysine (%)	1.90	1.31	1.31	1.90	1.31	1.90
Threonine (%)	1.20	.89	.89	1.20	.89	1.20
Valine (%)	1.69	1.25	1.25	1.47	1.25	1.47

[1]Supplied per kilogram of diet: 150 mg manganese, 100 mg zinc, 70 mg iron, 10 mg copper, 2.2 mg iodine, and .8 mg cobalt from manganese oxide, zinc oxide, ferrous sulfate, copper oxide, calcium iodate, and cobalt carbonate, respectively, and calcium carbonate as a diluent.

[2]Supplied per kilogram of diet: 14,300 IU vitamin A, 7,150 ICU vitamin D_3, 55 IU vitamin E, 5.5 mg menadione dimethylpyrimidinol bisulfite, 3.3 mg thiamine HCl, 11 mg riboflavin, 22 mg D-calcium pantothenate, 110 mg niacin, 2,200 mg choline chloride, 15.4 µg vitamin B_{12}, 2.2 mg folic acid, .22 mg biotin, 5.5 mg pyridoxine HCl, 125 mg ethoxyquin, .2 mg selenium, and 55 mg erythromycin thiocyanate.

[3]Contains .61% aspartic acid, .67% glutamic acid, .48% serine, .34% glycine and .41% alanine to yield an equivalent crude protein content of 2%.

[4]Determined protein contents (N x 6.25) of the 22 and 30% protein diets were 20.71 and 30.00%, respectively.

Table 25. Muscle characteristics of turkeys at 1 week of age[1]

Variable	Pectoralis muscle	Gastrocnemius muscle	Significance
Weight, g	7.0 (1.6)[2]	2.1 (.1)	***
% of body weight	6.2 (1.1)	1.8 (.1)	***
Protein, mg/g wet tissue	148 (10)	167 (17)	***
mg/muscle	1035 (258)	342 (39)	***
RNA, µg/g wet tissue	3815 (353)	1934 (306)	***
mg/muscle	26.8 (6.9)	4.0 (.7)	***
DNA, µg/g wet tissue	804 (143)	774 (100)	-
mg/muscle	5.5 (.7)	1.6 (.2)	***
Protein:DNA	189 (34)	219 (41)	*
RNA:DNA	4.9 (1.1)	3.4 (.7)	***
RNA:protein (x 10^{-2})	2.6 (.3)	1.6 (.3)	***
Protein synthesis, %/day	58.7 (12.8)	34.2 (10.1)	***
mg/day	581 (152)	128 (47)	***
Protein deposition, %/day	47.7	12.2	NA
mg/day	253	34	NA
Protein degradation, %/day[3]	11.0	24.7	NA
mg/day	328	94	NA
DNA activity[4]	108 (27)	84 (38)	-
RNA activity[5]	22 (6)	32 (13)	*

*P<.05; ***P<.001; NA - not applicable.

[1]Mean body weight of the 16 infused poults was 111 g (±8).

[2]Values in parenthesis indicate standard deviation.

[3]Determined from the difference between protein synthesis and deposition.

[4]Defined as g protein synthesized per g DNA per day.

[5]Defined as g protein synthesized per g RNA per day.

Table 26. Performance of turkeys from 1 to 3 weeks of age[1]

Diet	Body weight gain[2]	Feed consumption	Feed efficiency
	-g-		
30% CP	370[a3]	520[b]	.713[a]
22% CP	232[d]	440[c]	.528[c]
22% CP plus MET[4]	310[c]	508[b]	.610[d]
22% CP plus EAA[5]	363[ab]	565[a]	.643[c]
22% CP plus MET and NEAA[6]	342[b]	544[ab]	.628[d]
22% CP plus EAA and NEAA	363[ab]	535[ab]	.678[b]
Standard deviation	13.2	20.7	.011
Significance	***	***	***

***P<.001.

[1]Values represent means of 3 pens with 8 poults per pen.

[2]Initial body weight at one week of age was 113 (±2).

[3]Numbers within a column bearing similar superscripts are not significantly different (P>.05).

[4]MET - .4% supplemental methionine.

[5]EAA - .4% methionine, .6% lysine, .31% threonine, and .22% valine.

[6]NEAA - .61% aspartic acid, .67% glutamic acid, .48% serine, .34% glycine and .41% alanine.

Table 27. Body weight and muscle characteristics of turkeys at 3 weeks of age[1]

Variable	Diet[2]							
	30% CP	22% CP	22% CP plus MET	22% CP plus EAA	22% CP plus MET and NEAA	22% CP plus EAA and NEAA	SD[3]	Significance
Body weight, g	538[a4]	364[d]	447[c]	500[b]	477[bc]	511[ab]	32	***
Pectoralis weight, g	78.3[a]	43.5[d]	60.4[c]	65.9[bc]	66.0[bc]	70.3[b]	6.8	***
% of body weight	14.5[a]	11.9[c]	13.5[b]	13.2[b]	13.8[ab]	13.8[ab]	.9	***
Gastrocnemius weight, g	12.3[a]	7.3[d]	9.5[c]	11.2[ab]	10.2[bc]	11.3[ab]	1.1	***
% of body weight	2.3[a]	2.0[c]	2.1[bc]	2.2[ab]	2.1[ab]	2.2[ab]	.1	**

**P<.01; ***P<.001.

[1]Values represent the mean of 8 poults used in the infusion study.

[2]For description of diets, see Table 22, footnotes 4, 5 and 6.

[3]SD - standard deviation.

[4]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

Table 28. Muscle concentrations of protein, RNA and DNA for turkeys at 3 weeks of age[1]

Variable	Diet[2] 30% CP	22% CP	22% CP plus MET	22% CP plus EAA	22% CP plus MET and NEAA	22% CP plus EAA and NEAA	SD[3]	Significance
Protein, mg/g wet tissue								
Pectoralis	174[a4]	160[b]	161[b]	179[a]	161[b]	171[ab]	10	**
Gastrocnemius	154	153	149	161	147	143	14	--
RNA, µg/g wet tissue								
Pectoralis	2531[c]	3081[ab]	2960[ab]	3008[ab]	3150[a]	2753[bc]	340	**
Gastrocnemius	2037	2152	1770	2163	1895	1950	280	--
DNA, µg/g wet tissue								
Pectoralis	257[b]	379[a]	284[b]	280[b]	259[b]	273[b]	39	***
Gastrocnemius	336	468	395	374	382	414	99	--

** P<.01; *** P<.001.

[1] Values represent the means of 8 poults.

[2] For description of diets, see Table 22, footnotes 4, 5 and 6.

[3] SD - standard deviation.

[4] Numbers within a row bearing similar superscripts are not significantly different (P>.05).

Table 29. Total muscle content of protein, RNA and DNA for turkeys at 3 weeks of age[1]

Variable	Diet[2] 30% CP	22% CP	22% CP plus MET	22% CP plus EAA	22% CP plus MET and NEAA	22% CP plus EAA and NEAA	SD[3]	Significance
Protein, mg/muscle								
Pectoralis	13634[a4]	6996[d]	9756[c]	11802[b]	10604[bc]	12034[b]	1401	***
Gastrocnemius	1892[a]	1113[d]	1414[c]	1802[ab]	1500[c]	1623[bc]	234	***
RNA, mg/muscle								
Pectoralis	197[ab]	134[c]	178[b]	198[ab]	208[a]	193[ab]	26	***
Gastrocnemius	25.0[a]	15.7[c]	16.9[c]	24.2[a]	19.6[bc]	22.0[ab]	3.7	***
DNA, mg/muscle								
Pectoralis	20.0[a]	16.5[b]	16.8[b]	18.4[ab]	17.0[b]	19.2[ab]	2.6	*
Gastrocnemius	4.0	3.4	3.8	4.2	3.9	4.6	1.0	-

*P<.05; ***P<.001.

[1]Values represent the means of 8 poults.

[2]For description of diets, see Table 22, footnotes 4, 5 and 6.

[3]SD - standard deviation.

[4]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

Table 30. Muscle ratios of protein:DNA, RNA:DNA and RNA:protein for turkeys at 3 weeks of age[1]

Variable	Diet[2]						SD[3]	Significance
	30% CP	22% CP	22% CP plus MET	22% CP plus EAA	22% CP plus MET and NEAA	22% CP plus EAA and NEAA		
Protein:DNA								
Pectoralis	685[a4]	434[b]	588[a]	646[a]	628[a]	631[a]	88	***
Gastrocnemius	481	332	408	442	399	383	103	--
RNA:DNA								
Pectoralis	10[bc]	8[c]	11[ab]	11[ab]	12[a]	10[bc]	1.9	**
Gastrocnemius	6	5	5	6	5	5	1.4	--
RNA:Protein (x 10^{-2})								
Pectoralis	1.5[d]	1.9[ab]	1.8[abc]	1.7[bcd]	2.0[a]	1.6[cd]	.3	**
Gastrocnemius	1.3	1.4	1.2	1.4	1.3	1.4	.2	--

** P<.01; *** P<.001.

[1] Values represent the means of 8 poults.

[2] For description of diets, see Table 22, footnotes 4, 5 and 6.

[3] SD - standard deviation.

[4] Numbers within a row bearing similar superscripts are not significantly different (P>.05).

Table 31. The fractional rates of muscle protein synthesis, deposition and degradation for turkeys at 3 weeks of age

Variable	Diet[1] 30% CP	22% CP	22% CP plus MET	22% CP plus EAA	22% CP plus MET and NEAA	22% CP plus EAA and NEAA	SD[2]	Significance
Protein synthesis, %/day[3]								
Pectoralis	44.0[a4]	41.6[a]	32.2[b]	33.7[a]	34.3[b]	34.7[a]	10.7	--
Gastrocnemius	36.6[a]	31.9[a]	20.7[b]	30.2[a]	19.4[b]	31.1[a]	8.1	***
Protein deposition, %/day[5]								
Pectoralis	15.3	7.3	4.8	14.4	9.4	7.1	-	NA
Gastrocnemius	12.9	10.3	8.8	16.0	11.4	7.8	-	NA
Protein degradation, %/day[5,6]								
Pectoralis	28.7	34.3	27.4	19.3	24.9	27.6	-	NA
Gastrocnemius	23.7	21.6	11.9	14.2	8.0	23.3	-	NA

***P<.001; NA - not applicable.

[1]For description of diets, see Table 22, footnotes 4, 5 and 6.

[2]SD - standard deviation.

[3]Values represent the means of 8 poults.

[4]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

[5]Values represent the means of 13 poults.

[6]Determined from the difference between protein synthesis and deposition.

Table 32. The absolute rates of muscle protein synthesis, deposition and degradation for turkeys at 3 weeks of age

Variable	Diet[1] 30% CP	22% CP	22% CP plus MET	22% CP plus EAA	22% CP plus MET and NEAA	22% CP plus EAA and NEAA	SD[2]	Significance
Protein synthesis, mg/day[3]								
Pectoralis	5760[a4]	2982[b]	3148[b]	3863[b]	3638[b]	4137[b]	1177	**
Gastrocnemius	680[a]	352[c]	300[c]	526[b]	289[c]	502[b]	119	***
Protein deposition, mg/day[5]								
Pectoralis	1666	463	436	1379	860	769	-	NA
Gastrocnemius	195	97	107	228	143	113	-	NA
Protein degradation, mg/day[5,6]								
Pectoralis	4094	2519	2712	2484	2778	3368	-	NA
Gastrocnemius	485	255	193	298	146	389	-	NA

** P<.01; *** P<.001; NA - not applicable.

[1] For description of diets, see Table 22, footnotes 4, 5 and 6.

[2] SD - standard deviation.

[3] Values represent the means of 8 poults.

[4] Numbers within a row bearing similar superscripts are not significantly different (P>.05).

[5] Values represent the means of 13 poults.

[6] Determined from the difference between protein synthesis and deposition.

Table 33. Muscle DNA and RNA activities for turkeys at 3 weeks of age[1]

Variable	Diet[2] 30% CP	22% CP	22% CP plus MET	22% CP plus EAA	22% CP plus MET and NEAA	22% CP plus EAA and NEAA	SD[3]	Significance
DNA activity[4]								
Pectoralis	303	184	193	219	217	229	79	--
Gastrocnemius	173[a5]	100[bc]	84[bc]	132[ab]	78[bc]	120[bc]	41	***
RNA activity[6]								
Pectoralis	29	23	18	20	18	22	8	--
Gastrocnemius	28[a]	23[ab]	17[b]	22[ab]	16[b]	23[ab]	7	*

*P<.05; ***P<.001.

[1]Values represent the means of 8 poults.

[2]For description of diets, see Table 22, footnotes 4, 5 and 6.

[3]SD - standard deviation.

[4]Defined as g protein synthesized per g DNA per day.

[5]Numbers within a row bearing similar superscripts are not significantly different (P>.05).

[6]Defined as g protein synthesized per g RNA per day.

Table 34. Differences in weight and composition between the pectoralis and gastrocnemius muscles of turkeys at 3 weeks of age[1]

Variable	Pectoralis muscle	Gastrocnemius muscles	Significance
Weight, g	64.0 (6.8)[2]	10.3 (1.1)	***
% of body weight	13.4 (.9)	2.2 (.1)	***
Protein, mg/g wet muscle	168 (10)	151 (14)	***
mg/muscle	10804 (1401)	1557 (234)	***
RNA, µg/g wet muscle	2914 (340)	1994 (280)	***
mg/muscle	185 (26)	21 (4)	***
DNA, µg/g wet muscle	289 (39)	395 (99)	***
mg/muscle	18.0 (2.6)	4.0 (1.0)	***
Protein:DNA	602 (88)	407 (103)	***
RNA:DNA	10.4 (1.9)	5.4 (1.4)	***
RNA:protein (x 10^{-2})	1.8 (.3)	1.3 (.2)	***
Protein synthesis, %/day	36.8 (10.7)	28.3 (8.1)	***
mg/day	3843 (1177)	443 (119)	***
Protein deposition, %/day	10.0	11.3	NA
mg/day	929	147	NA
Protein degradation, %/day[3]	26.8	17.0	NA
mg/day	2914	296	NA
DNA activity[4]	221 (79)	115 (41)	***
RNA activity[5]	21 (8)	22 (7)	--

***P<.001; NA - not applicable.

[1]Mean body weight of the 48 infused poults was 473 (±32).

[2]Values in parenthesis indicate standard deviation.

[3]Determined from the difference between protein synthesis and degradation.

[4]Defined as g protein synthesized per g DNA per day.

[5]Defined as g protein synthesized per g RNA per day.

Table 35. Developmental changes in the pectoralis muscle characteristics of turkeys from 1 to 3 weeks of age

Variable	Age		Significance
	1 week[1]	3 weeks[2]	
Weight, g	7.0 (1.6)[3]	78.3 (8.0)	***
% of body weight	6.2 (1.1)	14.5 (.9)	***
Protein, mg/g wet muscle	148 (10)	174 (7)	***
mg/muscle	1035 (258)	13634 (1586)	***
RNA, µg/g wet muscle	3815 (353)	2531 (375)	***
mg/muscle	26.8 (6.9)	196.9 (27.0)	***
DNA, µg/g wet muscle	804 (143)	257 (27)	***
mg/muscle	5.5 (.7)	20.0 (2.4)	***
Protein:DNA	189 (34)	685 (78)	***
RNA:DNA	4.9 (1.1)	10.0 (2.0)	***
RNA:protein ($\times 10^{-2}$)	2.6 (.3)	1.5 (.2)	***
Protein synthesis, %/day	58.7 (12.8)	44.0 (12.7)	*
mg/day	581 (152)	5760 (1481)	***
Protein deposition, %/day	47.7	15.3	NA
mg/day	253	1166	NA
Protein degradation, %/day[4]	11.0	28.7	NA
mg/day	328	4094	NA
DNA activity[5]	108 (27)	303 (88)	***
RNA activity[6]	22 (6)	29 (8)	-

*P<.05; **P<.01; ***P<.001; NA - not applicable.

[1]Values represent the mean of 16 poults with a mean body weight of 111 g (±8).

[2]Values represent the mean of 8 poults with a mean body weight of 538 g (±42).

[3]Values in parenthesis indicate standard deviation.

[4]Determined from the difference between protein synthesis and deposition.

[5]Defined as g protein synthesized per g DNA per day.

[6]Defined as g protein synthesized per g RNA per day.

Table 36. Developmental changes in the gastrocnemius muscle characteristics of turkeys from 1 to 3 weeks of age

Variable	Age		Significance
	1 week[1]	3 weeks[2]	
Weight, g	2.1 (.1)[3]	12.3 (1.7)	***
% of body weight	1.8 (.1)	2.3 (.2)	***
Protein, mg/g wet muscle	167 (17)	154 (16)	-
mg/muscle	342 (39)	1892 (383)	***
RNA, µg/g wet muscle	1934 (306)	2037 (155)	-
mg/muscle	4.0 (.7)	25.0 (3.9)	***
DNA, µg/g wet muscle	774 (100)	336 (79)	***
mg/muscle	1.6 (.2)	4.0 (.7)	***
Protein:DNA	219 (41)	481 (128)	***
RNA:DNA	3.4 (.7)	6.3 (1.4)	***
RNA:protein (x 10^{-2})	1.6 (.3)	1.3 (.2)	*
Protein synthesis, %/day	36.9 (11.3)	36.6 (9.7)	
mg/day	128 (47)	680 (172)	***
Protein deposition, %/day	12.2	12.9	NA
mg/day	34	195	NA
Protein degradation, %/day[4]	24.7	23.7	NA
mg/day	94	485	NA
DNA activity[5]	84 (38)	173 (54)	**
RNA activity[6]	32 (13)	28 (9)	-

*P<.05; **P<.01; ***P<.001; NA - not applicable.

[1]Values represent the mean of 16 poults with a mean body weight of 111 g (±8).

[2]Values represent the mean of 8 poults with a mean body weight of 538 g (±42).

[3]Values in parenthesis indicate standard deviation.

[4]Determined from the difference between protein synthesis and deposition.

[5]Defined as g protein synthesized per g DNA per day.

[6]Defined as g protein synthesized per g RNA per day.

CONCLUSIONS

Several experiments were conducted to identify the amino acid deficiencies of a 22% protein diet composed of corn and soybean meal for poults from one to 3 weeks of age. Additional studies were designed to investigate the effect of supplementing the low protein diet with limiting essential (EAA) and non-essential amino acids (NEAA) on poult performance and muscle protein turnover.

Methionine was confirmed as the first limiting amino acid in the 22% protein diet. Adding a mixture of EAA to achieve levels equivalent to those in a 30% protein diet resulted in maximum weight gains and feed efficiency. Individual deletion of the EAA from the mixture indicated deficiencies of lysine, threonine and valine. Lysine was more deficient than valine in the 22% protein diet on the basis of greater decreases in weight gain associated with lysine removal from the mixture. However, lysine addition to a methionine-supplemented low protein diet adversely affected performance, suggesting that lysine is not the second most limiting amino acid. Although the lysine adequacy of the diet was not influenced by dietary arginine level, the valine deficit was exacerbated by high dietary leucine and isoleucine levels. A level of 1.25% valine in the 22% protein diet was inadequate to support optimum growth, inferring a higher valine requirement for young turkeys than current recommendations.

Growth limiting deficits of the 22% protein diet were fulfilled by a combination of supplemental methionine, lysine, threonine and valine although feed efficiency remained depressed. A crude protein deficiency was superimposed upon an EAA deficit in light of improvements in growth.

and feed efficiency associated with NEAA addition to the 22% protein diet. Further improvement in the performance of poults may lie in the provision of additional limiting amino acids beyond those evaluated in the present research.

The weights of the pectoralis and gastrocnemius muscles varied directly with body weight. Increases in pectoralis weight in response to diet occurred primarily as a result of expansions in DNA-unit size although small increases in DNA-unit number were also observed. Changes in the DNA-unit size and number of the gastrocnemius could not be demonstrated despite consistent alterations in muscle mass.

In general, alterations in the fractional rate of protein deposition in both muscles were attributable to fluctuations in degradation rather than synthesis rates. Reciprocal alterations in pectoral synthetic efficiency and capacity in response to dietary protein level and amino acid balance prevented a decrease in the fractional rate of protein synthesis. However, a tendency towards a reduction in sythesis rate was exhibited with EAA supplementation of the 22% protein diet. The fractional rate of protein synthesis in the gastrocnemius was relatively stable regardless of diet, consistent with little change in synthetic capacity and efficiency. A decrease in the synthesis rate noted with methionine supplementation was not consistently observed and, thus, requires further investigation to ascertain the veracity of the response.

Protein deposition was markedly reduced with methionine and lysine supplementation in comparison to the 30% protein diet. However, the fractional and absolute rates of protein deposition were maximized by the combined addition of methionine, lysine, threonine and valine, concurring

with body weight gain results. Decreases in the fractional rate of degradation were primarily responsible. Absolute synthesis and degradation rates fell 33 and 39%, respectively, in the pectoralis with EAA addition to the low protein diet in comparison to the control diet. Comparable reductions for the gastrocnemius amounted to 23 and 39%, respectively. Thus, while equal rates of deposition were obtained with the 30% protein diet and the EAA-supplemented diet, the latter represents a considerable reduction in energy expenditure for protein turnover. Further, it appears that a more economical approach to the feeding of young turkeys is feasiable through the supplementation of a low protein diet with limiting EAA.

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APPENDIX A

Appendix Table 1. Analysis of variance of body weight gain, feed consumption, and feed efficiency for turkeys from 8 to 19 or 20 days of age (Study I)

Source of variation	Degrees of freedom	Mean squares		
		Body weight gain	Feed consumption	Feed efficiency ($\times 10^{-6}$)
Diets	11	2,045***	1,439***	6,375***
22 vs 26 and 30% protein	1	8,882***	4,735***	26,660***
26 x 30% protein	1	1,019**	1,819**	41
Glutamic acid (GA)	1	27	23	61
Amino acid supplementation (AAS)	1	2,910***	305	21,612***
Removal of lysine, threonine or valine (R)	1	9,163***	7,171***	17,672***
Within lysine, threonine or valine (W)	2	62	678*	1,001
GA x AAS	1	140	0	1,844
GA x R	1	0	11	29
GA x W	2	226	201	103
Experiments (E)	2	1,287***	2,752***	3,630**
Sex (S)	1	17,956***	26,934***	8,606***
E x S	2	290	61	1,953
Diet x S	11	162	231	834
Replicate	1	15	70	1,024
Error	115	144	209	652

*P<.05; **P<.01; ***P<.001.

Appendix Table 2. Body weight gain of turkeys from 1 to 3 weeks of age (Study III)[1,2]

Diet	Age (days) 7-14	14-21
	-g-	
30% CP	154[a3]	245[a]
22% CP	97[c]	141[c]
22% CP plus MET[4]	121[b]	172[b]
22% CP plus MET, LYS[5]	119[b]	143[c]
Standard deviation	14.2	14.8
Significance	***	***

***P<.001.

[1]Values represent means of 4 pens with 8 poults per pen.

[2]Initial body weight at 7 days of age was 124 g (±2).

[3]Numbers within a column bearing similar superscripts are not significantly different (P>.05).

[4]Contains .4% supplemental methionine.

[5]Contains .4% supplemental methionine and .6% supplemental lysine.

Appendix Table 3. Feed consumption of turkeys from 1 to 3 weeks of age (Study III)[1]

Diet	Age (days) 7-14	14-21
	-g-	
30% CP	221	354[a2]
22% CP	196	276[bc]
22% CP plus MET[3]	210	288[b]
22% CP plus MET, LYS[4]	209	270[c]
Standard deviation	16.9	9.5
Significance	NS	***

NS non-significant; *** P<.001.

[1]Values represent means of 4 pens with 8 poults per pen.

[2]Numbers within a column bearing similar superscripts are not significantly different (P>.05).

[3]Contains .4% supplemental methionine.

[4]Contains .4% supplemental methionine and .6% supplemental lysine.

Appendix Table 4. Feed efficiency of turkeys from 1 to 3 weeks of age (Study III)[1]

Diet	Age (days) 7-14	14-21
30% CP	.694[a2]	.692[a]
22% CP	.492[c]	.511[b]
22% CP plus MET[3]	.576[b]	.599[ab]
22% CP plus MET, LYS[4]	.567[b]	.531[b]
Standard deviation	.027	.061
Significance	***	**

**P<.01; ***P<.001.

[1]Values represent means of 4 pens with 8 poults per pen.

[2]Numbers within a column bearing similar superscripts are not significantly different (P>.05).

[3]Contains .4% supplemental methionine.

[4]Contains .4% supplemental methionine and .6% supplemental lysine.

Appendix Table 5. Body weight gain of turkeys from 1 to 3 weeks of age (Study IV)[1,2]

Diet	Age (days) 7-14	14-21
	-g-	
30% CP	137[ab3]	233[a]
22% CP	94[d]	138[d]
22% CP plus MET[4]	121[c]	189[c]
22% CP plus EAA[5]	139[ab]	224[ab]
22% CP plus MET and NEAA[6]	129[c]	212[b]
22% CP plus EAA and NEAA	143[a]	220[ab]
Standard deviation	5.7	9.5
Significance	***	***

***P<.001.

[1]Values represent means of 3 pens with 8 poults per pen.

[2]Initial body weight at 7 days of age was 113 (±2).

[3]Numbers within a column bearing similar superscripts are not significantly different (P>.05).

[4]MET - .4% supplemental methionine.

[5]EAA - .4% methionine, .6% lysine, .31% threonine and .22% valine.

[6]NEAA - .61% aspartic acid, .67% glutamic acid, .48% serine, .34% glycine and .41% alanine.

Appendix Table 6. Feed consumption of turkeys from 1 to 3 weeks of age (Study IV)[1]

Diet	Age (days) 7-14	14-21
	-g-	
30% CP	194[c2]	326[ab]
22% CP	182[d]	258[c]
22% CP plus MET[3]	199[c]	309[b]
22% CP plus EAA[4]	216[a]	348[a]
22% CP plus MET and NEAA[5]	202[bc]	341[ab]
22% CP plus EAA and NEAA	212[ab]	323[ab]
Standard deviation	6.6	18.3
Significance	***	***

***P<.001.

[1]Values represent means of 3 pens with 8 poults per pen.

[2]Numbers within a column bearing similar superscripts are not significantly different (P>.05).

[3]MET - .4% supplemental methionine.

[4]EAA - .4% methionine, .6% lysine, .31% threonine, and .22% valine.

[5]NEAA - .61% aspartic acid, .67% glutamic acid, .48% serine, .34% glycine and .41% alanine.

Appendix Table 7. Feed efficiency of turkeys from 1 to 3 weeks (Study IV)[1]

Diet	Age (days) 7-14	14-21
30% CP	.709[a 2]	.715[a]
22% CP	.519[d]	.534[c]
22% CP plus MET[3]	.607[c]	.612[b]
22% CP plus EAA[4]	.644[bc]	.643[b]
22% CP plus MET and NEAA[5]	.640[bc]	.622[b]
22% CP plus EAA and NEAA	.672[ab]	.682[a]
Standard deviation	.023	.019
Significance	***	***

***P<.001.

[1]Values represent means of 3 pens with 8 poults per pen.

[2]Numbers within a column bearing similar superscripts are not significantly different (P>.05).

[3]MET - .4% supplemental methionine.

[4]EAA - .4% methionine, .6% lysine, .31% threonine and .22% valine.

[5]NEAA - .61% aspartic acid, .67% glutamic acid, .48% serine, .34% glycine and .41% alanine.

Appendix Table 8. Sample calculations of the fractional synthesis rate using data generated from the pectoralis muscle of 7-day-old poults

Poult No.[1]	DPM/g tissue[2]		Muscle nonprotein tyrosine concentration (μmol/g tissue)	S_B[3]	S_I[4]	S_B/S_I	R^s[5]	K_S (%/day)
	Protein fraction	Nonprotein fraction						
19	1104	6007	.330	36.2	18203	.00199	92	61.3
21	1346	4821	.285	44.1	16916	.00261	107	57.2
24	1325	4743	.332	43.4	14286	.00304	92	66.6
25	909	3826	.360	29.8	10628	.00280	85	69.9
26	2626	8604	.315	86.1	27314	.00315	97	64.5
27	1012	4152	.210	33.2	19771	.00063	145	35.9
30	1093	10076	.335	35.8	30078	.00119	91	58.2
31	728	4066	.297	23.9	13690	.00173	103	54.9

[1]Poults were injected with .5 μCi per 100 g body weight at 7 days of age.

[2]Disintegrations per minute calculated from counts per minute x efficiency of counting.

[3]Specific radioactivity of protein bound tyrosine (dpm/μmol).

[4]Specific radioactivity of free tyrosine (dpm/μmol).

[5]Ratio of protein-bound to free tyrosine.

Appendix Table 9. Ratios of specific radioactivities and concentrations of protein-bound to free tyrosine in the skeletal muscles of 1-week-old turkeys (Study III)[1]

Muscle	S_B/S_I[2]	R[3]
Pectoralis	.00432 (.00070)[4]	128 (31)
Gastrocnemius	.00237 (.00063)	162 (33)

[1]Values represent the mean of 16 poults.

[2]Ratio of specific radioactivity of protein-bound to free tyrosine.

[3]Ratio of muscle protein-bound to free tyrosine.

[4]Value in parenthesis indicates standard deviation.

Appendix Table 10. Ratios of specific radioactivities and concentrations of protein-bound to free tyrosine in the skeletal muscles of 3-week-old turkeys (Study III)[1]

Diet	Pectoralis		Gastrocnemius	
	S_B/S_I[2]	R[3]	S_B/S_I	R
30% Protein	.00203 (.00039)[4]	184 (26)	.00112 (.00042)	213 (55)
22% Protein	.00223 (.00057)	221 (93)	.00132 (.00042)	204 (32)
22% Protein plus MET	.00016 (.00004)	136 (47)	.00069 (.00022)	174 (33)
22% Protein plus MET, LYS	.00026 (.00006)	178 (72)	.00082 (.00016)	201 (41)

[1] Values represent the mean of 8 poults.

[2] Ratio of specific radioactivity of protein-bound to free tyrosine.

[3] Ratio of muscle protein-bound to free tyrosine.

[4] Value in parenthesis indicates standard deviation.

Appendix Table 11. Ratios of specific radioactivities and concentrations of protein-bound to free tyrosine in the skeletal muscle of 1-week-old turkeys (Study IV)[1]

Muscle	S_B/S_I[2]	R[3]
Pectoralis	.00279 (.00051)[4]	93 (22)
Gastrocnemius	.00076 (.00019)	153 (44)

[1]Values represent the mean of 16 poults.

[2]Ratio of specific radioactivity of protein-bound to free tyrosine.

[3]Ratio of muscle protein-bound to free tyrosine.

[4]Value in parenthesis indicates standard deviation.

Appendix Table 12. Ratios of specific radioactivities and concentrations of protein-bound to free tyrosine in the skeletal muscles of 3-week-old turkeys (Study IV)[1]

Diet	Pectoralis		Gastrocnemius	
	S_B/S_I[2]	R[3]	S_B/S_I	R
30% Protein	.00518 (.00122)[4]	240 (67)	.00360 (.00098)	234 (76)
22% Protein	.00334 (.00081)	183 (44)	.00183 (.00059)	210 (43)
22% Protein plus MET	.00018 (.00005)	142 (53)	.00003 (.00001)	180 (47)
22% Protein plus EAA	.00203 (.00069)	198 (13)	.00266 (.00085)	240 (135)
22% Protein plus MET and NEAA	.00046 (.00010)	150 (21)	.00026 (.00008)	188 (21)
22% Protein plus EAA and NEAA	.00281 (.00081)	219 (41)	.00235 (.00050)	243 (47)

[1]Values represent the mean of 8 poults.

[2]Ratio of specific radioactivity of protein-bound to free tyrosine.

[3]Ratio of muscle protein-bound to free tyrosine.

[4]Value in parenthesis indicates standard deviation.

APPENDIX B

Benedict's Reagent

Dissolve 86.5 g sodium citrate and 50 g sodium carbonate in approximately 300 ml warm distilled water. Dissolve 13.53 g copper sulfate in 50 ml distilled water and add to citrate/carbonate solution. Dilute to a final volume of 500 ml with distilled water.

Orcinol Reagent

Dissolve 100 mg ferric chloride and 100 mg orcinol in 100 ml concentrated hydrochloric acid.

Diphenylamine Reagent

Dissolve 1.5 g diphenylamine in 100 ml glacial acetic acid. Add 1.5 ml concentrated sulfuric acid and .5 ml 1.6% acetaldehyde.

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INFLUENCE OF DIETARY AMINO ACID ADEQUACY ON PERFORMANCE AND MUSCLE PROTEIN TURNOVER IN POULTS

by

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(ABSTRACT)

Several experiments were conducted to identify the limiting amino acids in a 22% corn-soybean meal diet for poults from one to three weeks of age. Additional studies were designed to investigate changes in muscle composition and protein turnover in response to essential (EAA) and non-essential amino acid (NEAA) supplementation of the low protein diet. Developmental changes in muscle metabolism as well as differences between muscles were also examined. Protein synthesis was determined using a ^{14}C-tyrosine emulsion technique. Protein degradation was calculated as the difference between synthesis and deposition rates. A 30% protein diet served as a control in all experiments.

Methionine was confirmed as the first limiting amino acid. Individual deletion of EAA from a mixture added to the 22% protein diet indicated deficiencies of lysine, threonine and valine. Although lysine was more deficient than valine, the valine deficit was exacerbated by high dietary leucine and isoleucine levels. A dietary level of 1.25% valine was inadequate to support optimum growth, inferring a higher requirement than current recommendations.

The addition of methionine, lysine and threonine and valine to the 22% protein diet supported maximum growth but feed efficiency remained depressed. Improvements in performance associated with NEAA

supplementation indicated a crude protein deficiency in the 22% protein diet.

The weights of the pectoralis and gastrocnemius muscles varied directly with body weight. Increases in pectoralis weight were primarily the result of expansions in DNA-unit size. Changes in DNA-unit size and number of the gastrocnemius could not be demonstrated despite consistent alterations in muscle mass.

A supplement of methionine and lysine significantly decreased pectoral synthesis rate although protein synthesis tended to decline with any combination of added amino acids. The fractional synthesis rate in the gastrocnemius was relatively stable regardless of diet although a decrease was noted with methionine supplementation. Therefore, alterations in the fractional rate of protein deposition in both muscles were primarily attributable to fluctuations in degradation. Protein deposition was markedly reduced with methionine and lysine supplementation. However, the fractional and absolute rates of protein deposition were maximized by the combined addition of methionine, lysine, threonine and valine, concurring with body weight gain results. Thus, while equal rates of deposition were obtained with the 30% protein diet and the EAA-supplemented 22% protein diet, the latter represents a considerable reduction in energy expenditure for protein turnover.