METHIONINE BIOASSAYS AND METHIONINE-CHOLINE-SULFATE RELATIONSHIPS IN PRACTICAL-TYPE DIETS FOR YOUNG TURKEYS

by

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INTRODUCTION

Currently, four compounds with methionine activity are available for methionine supplementation of poultry diets. DL-Methionine and methionine hydroxy analogue calcium salt have been utilized as sources of methionine supplementation by the poultry industry for the past several years. Recently, two liquid forms, methionine hydroxy analogue free acid and the sodium salt of DL-methionine, have become available as methionine supplements. The need to estimate the relative biopotencies of each product has become important in order to determine the relative amounts of supplemental methionine products required to provide equal performance in poultry diets.

A methionine bioassay is a method by which the relative biopotencies of several methionine products can be determined within an experment. The slope ratio procedure is a method by which the relative potency of a compound with respect to a standard can be determined based upon multiple-linear regression of performance parameters on various independent variables such as level of added methionine, methionine-intake or intake of a methionine product. Either DL-methionine or L-methionine can serve as the standard. In addition to obtaining an estimate of the potency of a product, fiducial limits should be calculated about the estimate, enabling the detection of differences among the potencies of products.

Several studies have been conducted to examine the bioefficacies of the various methionine products, and several potency estimates have been obtained. In only a few of these studies have the authors calculated fiducial limits about the potency estimates. Without these fiducial limits, the validity of the resulting potency estimates is questionable. The objectives of the experiments discussed in the first part of this thesis were threefold: 1) to estimate the relative biopotencies of the sodium salt of DL-methionine, methionine hydroxy analogue calcium salt, and methionine hydroxy analogue free acid with respect to DL-methionine on an equimolar basis in a practical-type diet for young turkeys using a slope ratio bioassay, 2) to calculate fiducial limits around the potency estimates obtained in order to establish how big a difference can be detected among methionine products, and 3) to apply an exponential model to the data in order to contrast this procedure to the slope ratio procedure.

Practical-type diets composed primarily of ground yellow corn and dehulled soybean meal are first limiting in sulfur amino acids, and thus require synthetic methionine supplementation. In addition to other functions, methionine serves as a donor of a labile methyl group and sulfur. Choline is a supplier of methyl groups, and inorganic sulfate is a source of sulfur. Choline and sulfate are considerably less expensive than methionine and, if they could spare methionine, diets for poultry could be formulated more economically. Several studies have been conducted to determine if choline or sulfate is capable of sparing methionine in practical-type diets for poultry. The results have been inconclusive, and some studies indicate that a requirement for sulfate *per se* exists for broilers and poults. In a recent study with broilers, Miles *et al*.(1983) have reported thatboth choline and sulfate must be added to the diet together in order to spare a maximum amount of methionine.

The objective of the experiments in the second part of this thesis were to determine whether choline or sulfate can spare methionine, or if both choline and sulfate must be present for any sparing of methionine in practical-type diets for young turkeys.

SERIES I - METHIONINE BIOASSAYS

REVIEW OF LITERATURE

Methionine

Several researchers (Donovan *et al.*, 1955; Ferguson *et al.*, 1956; Waibel; 1959; Fitzsimmons and Waibel, 1962) have demonstrated that methionine is the first limiting amino acid in practical-type diets for poults and chicks. The stated NRC (1977) requirements for methionine and total sulfur amino acids (TSAA) are .53 and 1.05%, respectively, in a poult diet which contains 28% protein and 2,800 kcal metabolizable energy per kg from 0 to 4 weeks of age. Almquist (1952), in reviewing the literature on the amino acid requirements of chickens and turkeys stated, that the minimum methionine requirement can be estimated only with adequate levels of vitamin B_{12}, cystine, and choline present in the diet. Ferguson *et al.* (1957) demonstrated a significant methionine x energy interaction in poults. This interrelationship was further supported by Baldini *et al.* (1957) who reported that the methionine requirement of the poult, expressed as percentage of the diet, is related to the productive energy level of the diet. Studies by Baker and Bray (1972), and Potter (1977), have shown that practical-type diets for poultry are more deficient in TSAA than in methionine *per se*. Potter (1977) stated that the TSAA requirement for poults is at least 1.12% for the first 7 or 8 weeks of life.

Methionine has several important metabolic functions in the avian body. The sulfur atom is donated to serine through an irreversible step to form cysteine, which is important in feathering (Machlin *et al.*,

1954) and protein structure. Methionine has also been demonstrated to serve as a donor of labile methyl groups. Langer and Kratzer (1964a) demonstrated this function in the young turkey by feeding a diet deficient in labile methyl groups and observing a growth response from added methionine. In the same study, they found that control of methionine biosynthesis in the poult appears to be mediated through the repression or induction of necessary enzymes by methionine in the liver. In a separate experiment, Langer and Kratzer (1964b) examined the effect of methionine on liver concentrations of dimethylethanolamine (DMEA), the precursor of choline and showed that when methionine was added to the diet, no accumulation of DMEA was oberved in the liver, illustrating the role of methionine in choline biosynthesis in the poult. Another function of methionine in poults is in the prevention of footpad dermatitis (Chavez and Kratzer, 1974).

The alpha hydroxy analogue forms of methionine, methionine hydroxy analogue calcium salt (MHAC) and methionine hydroxy analogue free acid (MHAA), have been studied metabolically to determine what conversions must take place before they assume methionine activity. Gordon and Sizer (1965) proposed a mechanism whereby MHAC and MHAA are converted to the alpha-keto methionine analogue, and then aminated to methionine before their conversion into cysteine in the liver. The branched chain amino acids (BCAA) serve as amino group donors in the amination step in their model. Work conducted by Featherston and Horn (1974), and Katz and Baker (1975), disputed that the BCAA are the principle amino donors, since BCAA added to crystalline amino acid (CAA) diets did not improve MHAC or MHAA performance. Katz and Baker (1975) found that this conversion takes place primarily in the liver and kidney, and that the amination step is not rate limiting. They also stated that the methionine to cystine ratio in the diet was an important factor. When methionine and cystine were present in a 60:40 ratio, MHAC performance was better than when the ratio was 40:60.

Methionine Bioassays

Several studies have been conducted over the past thirty years in an effort to determine the relative potencies of DL-methionine (DL), MHAC and MHAA with respect to L-methionine when evaluated in crystalline amino acid, semipurified, and practical-type diets. Schmidt (1981) extensively reviewed the literature and reported results that are quite variable from study to study because of different responses to added methionine.

Recently, several studies have been conducted to further determine the biopotencies or efficacies of methionine products. Reid *et al*. (1982) fed diets containing added DL, MHAC, and MHAA to laying hens, and regressed egg output on TSAA intake to examine the biopotency of the analogue products with respect to DL. They reported no significant differences among the products tests based on statistical analyses of the slopes, intercepts, and regression lines. They failed, however, to give any estimates as to the relative biopotencies of the analogue products. Expression of the regression coefficients as ratios show that the relative potency with respect to DL was 96.3 and 98.0%

for MHAC and MHAA, respectively. Fiducial limits were not calculated for the potency estimates, so any small differences among products (probably up to 10 or 20%) could not be detected.

Studies with chickens employing crystalline amino acid diets have been conducted recently to compare methionine products. Relative potencies of 101, 81, and 76% determined for the sodium salt of DL-methionine (MENA), MHAC, and MHAA, respectively, based on regression of body weight gain on methionine intake of methionine source were reported by van Weerden _et_ _al_. (1982). The latter two values appear to be lower than comparable values reported from experiments using corn-soybean meal diets. Again, no fiducial limits were calculated about the potency estimates in this study, so no indication is given as to whether or not the values are different from those determined with practical-type diets. The authors suggested that the type of basal diet may affect the efficacy of MHAC and MHAA, however, a more appropriate explanation was given that differences between treatments are difficult to detect when methionine is marginal in the diet or when the growth response is small.

Boebel and Baker (1982) found similar results to those of van Weerden _et_ _al_. (1982). Using a crystalline amino acid diet, MHAC and MHAA were found to be 87 and 78% as potent as DL, respectively. A mixture of free acid polymers consisting of trimers and higher polymers of MHAA was fed in the same experiment. The mixture of polymers was found to be 69% as potent as DL, indicating that these higher order polymers may not possess full methionine activity. In the same study, a semipurified diet consisting of 23.53% feather meal was used for the determination of the relative potency of the same products. All methionine hydroxy analogue products were inferior to DL and the polymer product was inferior to both MHAC and MHAA. The relative potencies obtained using this diet were 84, 77 and 54% for MHAC, MHAA, and the polymer mixture with DL as the standard, respectively. The authors concluded that the type of diet does not affect the efficiency of the methionine hydroxy analogues. They reasoned that the analogues are converted to methionine in the same way, regardless of the type of diet employed. The differences in potency estimates obtained are due to the degree of methionine inadequacy or the methionine-cystine ratio.

Thomas _et_ _al_. (1983) observed both a methionine source x choline or cystine interaction with broilers using a semi-purified diet. In addition, DL and MENA were significantly better than the analogue products in promoting growth and decreasing feed conversion. The authors concluded that the level of cystine, choline, and the degree of methionine deficiency in the diet may influence results obtained in methionine bioassay.

In a study using turkey poults, Harms _et_ _al_. (1977) reported the relative efficiency of MHAC to DL to be 82.4 and 82.8% based on body weight and feed efficiency, respectively. The calculations of the relative potencies were determined using the weight gain method where the increase in body weight gains over the basal diet from only the highest level of methionine supplementation are expressed as a ratio.

The weight gain of the test product (MHAC) is divided by the weight gain of the standard product (DL) to obtain a potency estimate. Applying the slope ratio procedure to the data, which includes all levels of methionine supplementation, the relative potency of MHAC to DL was found to be 93.4 and 100.8% based on body weight and feed efficiency, respectively.

The above studies failed to calculate fiducial limits about the determined potency estimates. Fiducial limits must be calculated about an estimate in order that true differences among products can be detected. Parsons (1978) determined the relative potencies of DL and MHAC to L-methionine in a corn-soybean-gelatin diet for poults using a slope ratio bioassay. He reported that DL was 106.7% as potent as L with 95% upper and lower fiducial limits of 80 and 143%, respectively. MHAC was found to be 93.2% as potent as L, with limits of 65 and 128%. By establishing fiducial limits about the calculated potencies, an indication was given as to how large a difference could be detected within the bioassay.

Schmidt (1981) conducted four methionine slope ratio bioassays using six-week body weights of turkeys. In one experiment, MHAC was found to be significantly less potent than DL with a potency estimate of 74.5%, and 95% fiducial limits of 57.6 and 92.7%. In two experiments, MHAA was significantly less efficient than DL with calculated potencies of 72.2 % (48.3, 98.5) and 76.5% (59.6, 94.9). Using the weighted means procedure outlined by Finney (1964), the four experiments were combined. MHAC was found to be 89.1% (71.5, 106.7) and MHAA 84.1% (66.5, 101.7) as potent as DL. The author concluded that MHAC and MHAA are likely to be less efficient in promoting growth of turkeys. In addition to using a slope ratio bioassay, Schmidt (1981) also applied a non-linear model to the six-week body weights of male turkeys from 48 pens in one experiment. The extra one degree of freedom associated with the calculated curve reduced the residual variation among diets 9.3% from that obtained using the slope ratio bioassay. The relative potencies of MHAC and MHAA to DL were 84.1 and 86.8%, respectively. The author concluded that five criteria of a good methionine bioassay are: 1) a practical-type diet similar to those in commercial use without supplemental methionine, 2) the use of diets containing graded levels of each test and standard material where L- or DL-methionine is used as the standard, 3) a large growth response from the added methionine, 4) a linear response to added methionine with no significant curvature or intersection effects of the regression lines, and 5) fiducial limits calculated for each determined relative potency estimate.

OBJECTIVES

A 28% protein corn-soybean meal diet was used in two methionine bioassays to estimate the relative biopotencies of the sodium salt of DL-methionine, methionine hydroxy analogue calcium salt, and methionine hydroxy analogue free acid with respect to DL-methionine on an equimolar basis using a slope ratio bioassay. Fiducial limits about each potency estimate were calculated in order to establish how big a difference could be determined among methionine products. In addition, an exponential model was applied to the data in order to contrast this procedure to the slope ratio procedure.

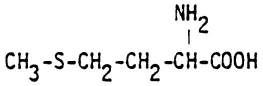
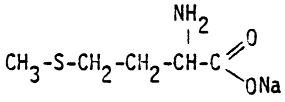
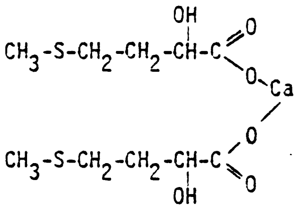
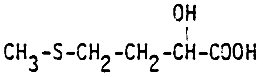
EXPERIMENTAL PROCEDURE

Methionine Products Under Test

Table 1 contains the structures and molecular weights of five sources of methionine. The DL-methionine (DL) is a dry racemic mixture of L- and D-methionine, and is assumed to be 99% methionine. The sodium salt of DL-methionine (MENA) is a liquid racemic mixture, with a sodium atom replacing the acid hydrogen on the carboxyl carbon. It is assumed to be 40% methionineequivalent after accounting for the 6.17% sodium and 53.83% water contents. Both DL-methionine and its sodium salt are manufactured by Degussa Corporation, Teterboro, New Jersey. The liquid form is marketed under the trade name Liquimeth.

The alpha hydroxy analogues of DL-methionine are also manufactured in both the dry and liquid form. The primary difference between the analogue products and DL-methionine is the replacement of the amino group with a hydroxyl group at the alpha carbon (Table 1). The calcium salt of methionine hydroxy analogue (MHAC) is in a dry form consisting of two molecules of methionine bonded to a calcium atom which replaces the acid hydrogen on the carboxyl groups. It is assumed to be 82.8% methionine hydroxy analogue after accounting for the 10.2% calcium content and 7% inert material. The liquid form of the analogue is methionine hydroxy analogue free acid (MHAA) which is commercially available as Alimet. The free acid form contains 12% water, giving it a value of 88% methionine hydroxy analogue free acid. The states of the free acid molecules in the commercial form are 65% monomer, 20% dimer, 10% trimer, and 5% higher order polymers. The

Table 1. Structures and molecular weights of methionine compounds

Source	Structure	Molecular weight
L-Methionine	 $CH_3\text{-}S\text{-}CH_2\text{-}CH_2\text{-}\overset{NH_2}{\overset{\vert}{C}H}\text{-}COOH$	149.2
DL-Methionine (DL)	Racemic mixture	149.2
L-Methionine, sodium salt* (MENA)		171.2
Methionine hydroxy analogue calcium salt* (MHAC)		338.4
L-Methionine hydroxy analogue free acid* (MHAA)	 $CH_3\text{-}S\text{-}CH_2\text{-}CH_2\text{-}\overset{OH}{\overset{\vert}{C}H}\text{-}COOH$	150.2

*All occur as racemic mixures.

polymers are formed when the hydroxyl goup on the alpha carbon of one molecule bonds to the acid hydrogen of another molecule, releasing water. Both of the methionine hydroxy analogue products are racemic mixtures of the D- and L-isomers, and are manufactured by Monsanto Company, St. Louis, Missouri.

Birds

For each of these two experiments, 432 male and 432 female Large White turkeys were obtained from Wampler Turkey Hatchery, Harrisonburg, Virginia. The poults had been vent-sexed, injected with an antibiotic-vitamin-electrolyte mixture, debeaked, toe-clipped, and the males desnooded. Upon arrival at the Turkey Research Center battery room, the poults of a sex were randomized into 48 pens of two Petersime starter batteries with 9 poults per pen, resulting in a total of 96 pens and the use of four batteries. The poults of each pen were then wingbanded and group weighed to determine a starting average body weight at one day of age. At three weeks of age in Experiment 1 and at three weeks two days of age in Experiment 2, the males were transferred to Oakes grower batteries and the females to Petersime grower batteries where they remained until seven weeks of age when the experiments were terminated. Water and assigned diets were provided *ad libitum* throughout the seven-week experiments. Room temperature was recorded twice daily in both the starter and grower rooms to monitor the environmental temperatures throughout each experimental period.

Diets

The composition of the basal diet used in these two experiments is presented in Table 2. Three identical basal mixtures representing 99.0% of the total basal diet were prepared with all ingredients present except glucose monohydrate. The calculated analysis of 28% protein meets or exceeds the stated NRC (1977) requirement for poults from 0 to 4 or from 0 to 8 weeks of age. The basal diet containing .43% methionine and .37% cystine is deficient in methionine and in total sulfur amino acids for these age periods.

Table 3 contains the experimental design for the two experiments. Glucose or glucose and increments of .0606% DL-methionine, .15% sodium salt of DL-methionine, .0724% methionine hydroxy analogue calcium, or .0682% methionine hydroxy analogue acid were added directly to equal portions of the basal mixture to form diets containing 0, .06, .12, .18, .24 and .30% added methionine equivalent. These 24 diets formed a 4 x 6 factorial with four sources and six levels of methionine of which the four diets at the 0% level of added methionine equivalent were of identical formulation. Each diet was fed to two pens of males and two pens of females from one day to seven weeks of age.

Data Collection

Feed and mortality records were kept so that average feed consumption of the poults in each pen could be determined on a weekly basis. The poults were group weighed by pens at 2, 4, 6 and 7 weeks of age, and average body weights, body weight gains, feed consumptions and feed efficiencies were determined for each pen on both a cumulative and a period basis.

Table 2. Composition of basal diet used in Experiments 1 and 2

Ingredients	Grams per kilogram
Ground yellow corn	474.56
Glucose monohydrate (cerelose)	10
Stabilized fat	20
Dehulled soybean meal	360
Meat and bone meal	120
Defluorinated phosphate	5
Iodized salt	2
Trace mineral mix[1]	1
Vitamin and feed additive premix[2]	2.94
L-Lysine HCl	3.5
L-Threonine	1
Total	1,000.00
Calculated analyses (%)[3]:	
Protein	28.0
Calcium	1.48
Phosphorus, total	1.04
Phosphorus, available	.80
Lysine	1.85
Methionine	.43
Cystine	.37
Metabolizable energy (kcal/kg)	2,904

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[1] Supplied the following amounts of trace minerals in mg/kg complete diet: 150 manganese, 101 zinc, 70 iron, 10 copper and 2.6 iodine from manganous oxide, zinc oxide, ferrous sulfate (heptahydrate), anhydrous copper sulfate, and potassium iodate, respectively.

[2] Supplied the following amounts of vitamins and feed additives in mg/kg complete diet unless otherwise stated: 13,228 IU vitamin A, 3,312 ICU vitamin D_3, 11 IU vitamin E, 4.4 menadione sodium bisulfite complex, 1.1 thiamine HCl, 5.5 riboflavin, 16.5 calcium pantothenate (D), 66.1 niacin, 1,000 choline chloride, .015 vitamin B_{12}, 1.1 folic acid, .11 biotin, 2.2 pyridoxine HCl, 125 ethoxyquin, 44.1 bacitracin, and .2 selenium.

[3] Values obtained from the National Research Council (1977) publication "Nutrient Requirements of Poultry".

Table 3. Design of Experiments 1 and 2

Level of methionine equivalent (%)	Source of methionine			
	DL	MENA	MHAC	MHAA
		Diet number		
.00	1	7	13	19
.06	2	8	14	20
.12	3	9	15	21
.18	4	10	16	22
.24	5	11	17	23
.30	6	12	18	24

Slope Ratio Assay

All body weight, body weight gain, feed consumption, and feed efficiency data for each period in each experiment were subjected to analysis of variance. The independent variables of interest in the model were level of methionine, source of methionine, replicate, and sex. The diet x replicate and/or sex interactions were combined for a measure of the error mean square.

All data were subjected to a slope ratio assay as described by Finney (1964) using multiple linear regression. Regression lines were determined using the following multiple regression model:

$$Y = b_a + b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5$$

where b_a is the difference between the average of Y value resulting from feeding no added methionine and the b_0 intercept, b_0 is the Y value determined from the regression omitting the values obtained from feeding no added methionine, b_1 through b_4 are the slopes and X_1 through X_4 are the percentages of added methionine equivalent from the four methionine compounds, DL, MENA, MHAC, and MHAA, respectively, and b_5 is the difference between males and females, with X_5 being coded 1 and -1 for males and females, respectively. The dependent variable Y equals the body weight (kg), body weight gain (kg), feed consumption(kg), or feed efficiency (kg/kg).

To statistically validate the assays prior to calculating relative potencies, each of the calculated regression lines was tested for significant blank, intersection, and curvature components. The blank effect was determined by the sums of squares associated with b_0.

Several partial regression models omitting the zero level of added methionine were determined to test for the quadratic, cubic and quartic curvature components. The sum of squares for intersection were then determined by difference.

The relative potency (R) for each methionine product was determined by the following ratio:

$$R = \frac{b_t}{b_s}$$

where b_t is the slope of the test compound, and b_s is the slope of the standard (DL). For each relative potency, the variance V(R), and 95% fiducial limits were determined. The variance of a relative potency is calculated by the following outlined by Finney (1964):

$$V(R) = \frac{s^2}{b_s^2} (v_{22} - 2Rv_{12} + R^2 v_{11})$$

where s is the square root of the mean square error for the experiment, b_s is the slope of the standard (DL), R is the calculated relative potency of the test compound, and v_{11}, v_{22} and v_{12} are the variances and covariances obtained by the inversion of the (X'X) matrix.

The fiducial limits about each potency were calculated using Fieller's theorem as outlined by Finney (1964):

$$R_L, R_U = \frac{R - \frac{g v_{12}}{v_{11}} \pm \frac{ts}{b_s}\left[v_{22} - 2Rv_{12} + R^2 v_{11} - g\left(v_{22} - \frac{v_{12}^2}{v_{11}}\right)\right]^{\frac{1}{2}}}{(1-g)}$$

where $g = \frac{t^2 s^2 v_{11}}{b_s^2}$

The average relative potency ($\bar{R}$) of each compound from the two experiments was determined by the weighted means procedure (Finney, 1964) at common ages. Prior to combining the data, a test of the homogeneity of the two estimates of each test compound was made as defined by Finney (1964):

$$X^2_{[1]} = (w_1 R^2_1 + w_2 R^2_2) - \frac{(w_1 R_1 + w_2 R_2)^2}{w_1 + w_2}$$

where $w_i = \frac{1}{V(R_i)}$

The weighted means are obtained by weighing the individual estimates by the inverse of their variance:

$$\bar{R} = \frac{\dfrac{R_1}{V(R_1)} + \dfrac{R_2}{V(R_2)}}{\dfrac{1}{V(R_1)} + \dfrac{1}{V(R_2)}}$$

where $V(\bar{R}) = \dfrac{1}{w_1 + w_2}$

The 95% fiducial limits around each average relative potency were then calculated by:

$$\bar{R}_L, \bar{R}_U = \bar{R} \pm t\,(V(\bar{R}))^{\frac{1}{2}}$$

Nonlinear Regression:

Exponential regressions of all data for all periods of each experiment were fit to the level of added methionine from the four sources to obtain estimates of the relative potencies of each compound. The goal was to determine whether the curvilinear model was more precise than the linear model (including all data) used in the slope ratio assay. The regression curves and coefficients used in the determination of the relative potencies were obtained by the following exponential regression model:

$$Y = b_0 + b_6\,\{1 - e^{(-b_1x_1 - b_2x_2 - b_3x_3 - b_4x_4)}\} + b_5x_5$$

where b_0 equals the Y intercept (lower asymptote), b_1 through b_4 equals some increment of body weight, body weight gain, feed consumption or feed efficiency resulting from each one percent added methionine, b_5 equals the difference between males and females, and b_6 equals the difference between the Y intercept and the upper asymptote. The variables X_1 through X_4 equals the percent of added methionine equivalent from DL, MENA, MHAC, and MHAA, respectively, and X_5 equals the codes 1 and -1 for males and females, respectively. The dependent variable Y equals body weight (kg), body weight gain (kg), feed consumption (kg), or feed efficiency (kg/kg). The relative potency of each compound was calculated as a ratio of one regression coefficient to another. Comparison to the slope ratio procedure was made by examining the standard errors of the regression coefficients and calculating the reduction of residual variation among diets.

RESULTS

The relative potency estimate for each methionine compound was determined for all periods based on regression analysis of body weight, body weight gain, feed consumption, and feed efficiency data (Tables 1,2,3 and 4, Appendix). For the purpose of this thesis, only the six-week body data will be discussed in detail. This parameter and age was chosen to be the most representative of the results obtained in both methionine bioassays. Additionally, previous methionine bioassays conducted in this laboratory have utilized six-week body weight data in the determination of relative potencies for methionine compounds. This allows the comparison and combination of the data obtained in this study with those of other studies at this age. The relative potencies obtained from both slope ratio and nonlinear analysis of feed consumption and feed efficiency data are similar to those obtained using the body weight data.

Slope Ratio Assay - Experiment 1

Table 4 contains the average body weights, feed consumptions and feed efficiencies for turkeys at six weeks of age. The average body weight of the turkeys fed diets containing .30% added methionine was 32% greater than those fed diets containing no added methionine. The growth responses from the added methionine at the higher levels were not as great as those from an equal addition of methionine to the more deficient diets.

The analysis of variance of the six-week body weight data is presented in Table 5. Multiple linear regression of body weight on

Table 4. Six-week body weights and 0 to 6-week feed consumptions and feed efficiencies of turkeys (Experiment 1)

Added methionine (%)	Source of methionine[1]			
	DL	MENA	MHAC	MHAA
		Body weight, kg		
.00	1.438[2]	1.432	1.370	1.428
.06	1.586	1.570	1.526	1.554
.12	1.647	1.732	1.670	1.680
.18	1.748	1.744	1.780	1.774
.24	1.842	1.867	1.770	1.730
.30	1.855	1.846	1.919	1.840
		Feed consumption, kg		
.00	2.446	2.331	2.219	2.324
.06	2.460	2.446	2.346	2.467
.12	2.478	2.563	2.520	2.515
.18	2.584	2.597	2.588	2.620
.24	2.662	2.745	2.583	2.615
.30	2.725	2.634	2.725	2.644
		Feed efficiency		
.00	.5631	.5908	.5898	.5878
.06	.6198	.6180	.6247	.6046
.12	.6402	.6520	.6401	.6439
.18	.6533	.6450	.6647	.6535
.24	.6687	.6573	.6621	.6378
.30	.6567	.6770	.6810	.6734

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[1] The sources of methionine are DL-methionine (DL), the sodium salt of DL-methionine (MENA), methionine hydroxy analogue as the calcium salt (MHAC), and methionine hydroxy analogue as the free acid (MHAA).

[2] Each value represents the average of four pens of turkeys (two pens of each sex); each pen contained nine birds.

Table 5. Analysis of variance of six-week body weights in grams (Experiment 1)

Source	Degrees of freedom	Sums of squares	Mean square	F-ratio
Sex	1	1,751,220		
Replicate	1	22,632	22,632	4.04*
Sex x replicate	1	20,358	20,358	3.63
Diets	23			
Regression[1]	4	2,140,294	535,074	95.41***
Blank	1	71,780	71,780	12.80***
Intersection	6	24,242	4,040	.72
Curvature	12	99,656	8,305	1.48
or				
Regression[2]	5	2,242,012	448,402	79.95***
Regression[3]	4	2,140,294	535,074	95.41***
Reduction	1	101,718	101,718	18.14***
Residual	18	93,960	5,220	.93
Error	69	386,993	5,608	
Total	95	4,517,175		

*P<.05.

***P<.001.

[1] Multiple linear regression omitting zero levels of supplementation.

[2] Exponential regression.

[3] Multiple linear regression including zero levels of supplementation.

percent added methionine from 0 to .30% produced a significant blank component, indicating that the common regression line for all compounds does not remain linear to zero supplementation. A valid bioassay was obtained when the body weight data from the unsupplemented groups were omitted. Regression analysis of body weight in kilograms on percent added methionine from .06 to .30% gave the final equation: $Y = 1.514 + 1.228X_1 + 1.288X_2 + 1.263X_3 + 1.103X_4 + .135X_5$. Results of the analysis of variance indicate that the final regression equation was highly significant (F = 95.41) and the tests for intersection and curvature of the regression lines were not significant. Figure 1 contains the plot of average six-week body weights on percent added methionine (.06 to .30%) with the corresponding regression lines for DL, MENA, MHAC and MHAA. For each 1% added methionine, body weight was increased 1.228, 1.288, 1.263, and 1.103 kg from DL, MENA, MHAC, and MHAA, respectively. The growth response of the standard (DL) at the .30% level was 17% over the .06% level.

When the regression coefficients were expressed as a ratio with DL as the standard, the relative potencies for MENA, MHAC, and MHAA were 104.9, 102.8 and 89.8%, respectively. The corresponding variances and 95% fiducial limits are presented in Table 6. Each of the fiducial limits for MENA, MHAC, and MHAA encompass 100%, indicating that no signficant differences among the products were detected in this experiment.

Slope Ratio Bioassay - Experiment 2

The average body weights, body weight gains, feed consumptions

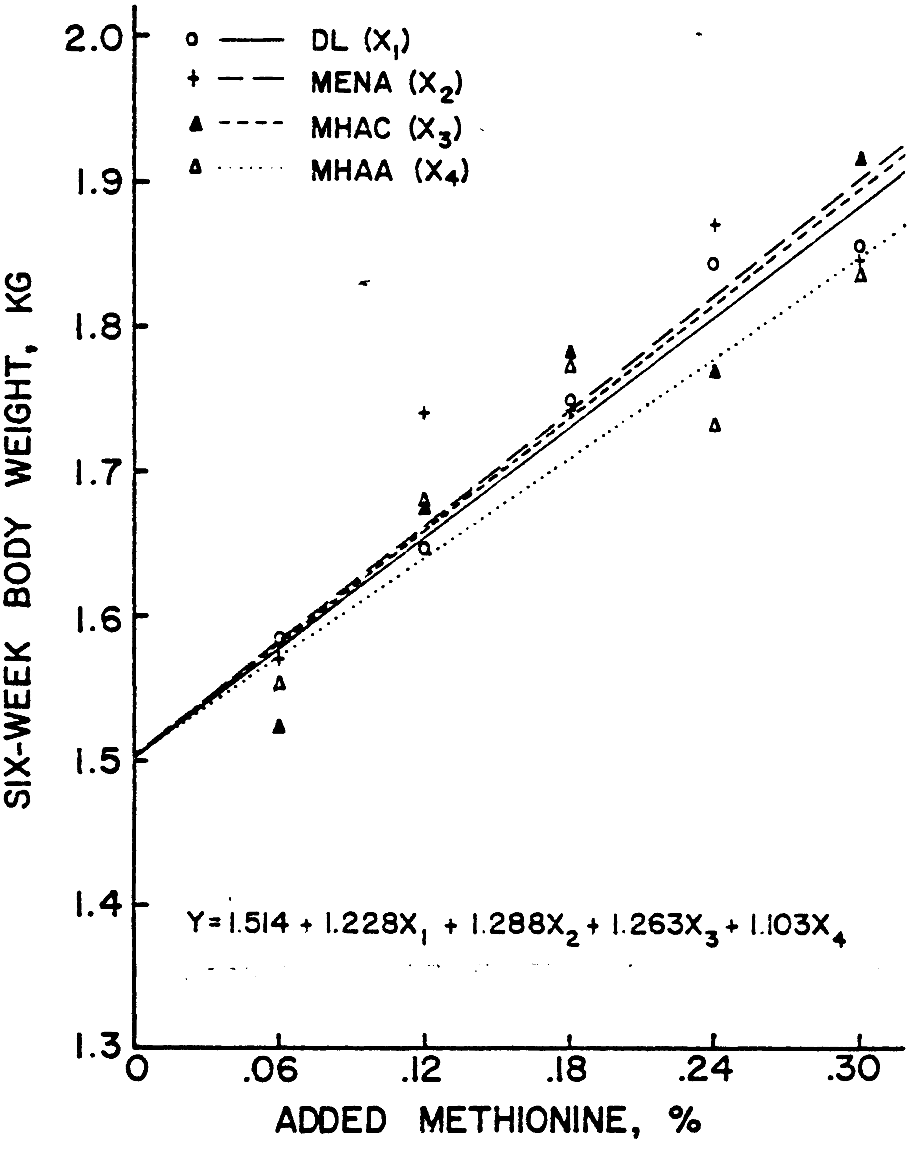

Figure 1. Plot of average six-week body weights on level of added methionine with associated regression lines using slope ratio analysis (Experiment 1)

Table 6. Relative potency, variance and 95% fiducial limits for methionine compounds based on six-week body weights using slope ratio bioassay with DL-methionine as standard (Experiment 1)

Source of methionine	Relative potency R	V(R)	Fiducial limits, 95%	
			Lower	Upper
DL	100.0			
MENA	104.9	.0099	86.7	127.4
MHAC	102.8	.0097	84.7	125.0
MHAA	89.8	.0085	72.4	110.2
DL and MENA	100.0	-	-	-
MHAC and MHAA	94.0	.0042	81.6	107.9

and feed efficiencies of turkeys at six weeks of age are presented in Table 7. The average body weight of turkeys fed diets containing .30% added methionine was only 11% greater than that of turkeys fed diets containing no added methionine. When compared to Experiment 1, this small growth response provides a much less sensitive bioassay. The growth response from .24 to .30% added methionine was smaller than that from the 0 to .06% level of supplementation.

The analysis of variance of the six-week body weights is presented in Table 8. A significant blank component was obtained, therefore, the data from the unsupplemented methionine groups was omitted from the bioassay. The regression equation of six-week body weights on percent added methionine from .06 to .30% was:

$Y = 1.464 + .352X_1 + .359X_2 + .324X_3 + .442X_4 + .114X_5.$

This regression component was significant at the .01% level with an F-value of 14.48. Tests for intersection and curvature of the regression lines were non-significant (Table 8), making the bioassay procedure acceptable for use. The plot of the average six-week body weights versus percent added methionine in Figure 2 shows a linear growth response over the levels of added methionine.

The potencies of MENA, MHAA, and MHAA relative to DL determined from the ratio of regression coefficients were 102.2, 92.3, and 125.8%, respectively. The corresponding variances and 95% fiducial limits for each compound are presented in Table 9. The extreme width of the fiducial limits around each potency estimate of about 50 to 200% is due to the small growth response. The variances of the relative

Table 7. Six-week body weights and 0 to 6-week feed consumptions and feed efficiencies of turkeys (Experiment 2)

Added methionine (%)	Source of methionine[1] DL	MENA	MHAC	MHAA
	Body weight, kg			
.00	1.451[2]	1.375	1.338	1.443
.06	1.464	1.488	1.480	1.492
.12	1.517	1.528	1.516	1.451
.18	1.541	1.568	1.497	1.560
.24	1.564	1.513	1.556	1.608
.30	1.547	1.567	1.559	1.580
	Feed consumption, kg			
.00	2.394	2.294	2.286	2.460
.06	2.436	2.414	2.415	2.505
.12	2.459	2.519	2.510	2.335
.18	2.517	2.542	2.457	2.588
.24	2.516	2.502	2.521	2.579
.30	2.497	2.579	2.485	2.557
	Feed efficiency			
.00	.5802	.5728	.5592	.5630
.06	.5754	.5909	.5885	.5713
.12	.5908	.5824	.5798	.5950
.18	.5858	.5924	.5852	.5795
.24	.5963	.5805	.5931	.6007
.30	.5962	.5824	.6037	.5948

[1] The sources of methionine are DL-methionine (DL), the sodium salt of DL-methionine (MENA), methionine hydroxy analogue as the calcium salt (MHAC), and methionine hydroxy analogue as the free acid (MHAA).

[2] Each value represents the average of four pens of turkeys (two pens of each sex); each pen contained nine birds.

Table 8. Analysis of variance of six-week body weights in grams (Experment 2)

Source	Degrees of freedom	Sums of squares	Mean squares	F-ratio
Sex	1	1,236,015	1,236,015	
Replicate	1	14,875	14,875	3.11
Sex x replicate	1	858	858	.18
Diets	23			
Regression	4	274,621	68,655	14.34***
Blank	1	28,935	28,935	6.04*
Intersection	6	40,300	6,717	1.40
Curvature	12	44,087	3,674	.77
or Regression[2]	5	304,076	60,815	12.70***
Regression[3]	4	274,621	68,655	14.34***
Reduction	1	29,455	29,455	6.15*
Residual	18	83,867	4,659	.97
Error	69	330,319	4,787	
Total	95	1,970,010		

*P<.05.

***P<.001.

[1] Multiple linear regression omitting zero levels of supplementation.

[2] Exponential regression.

[3] Multiple linear regression including zero levels of supplementation.

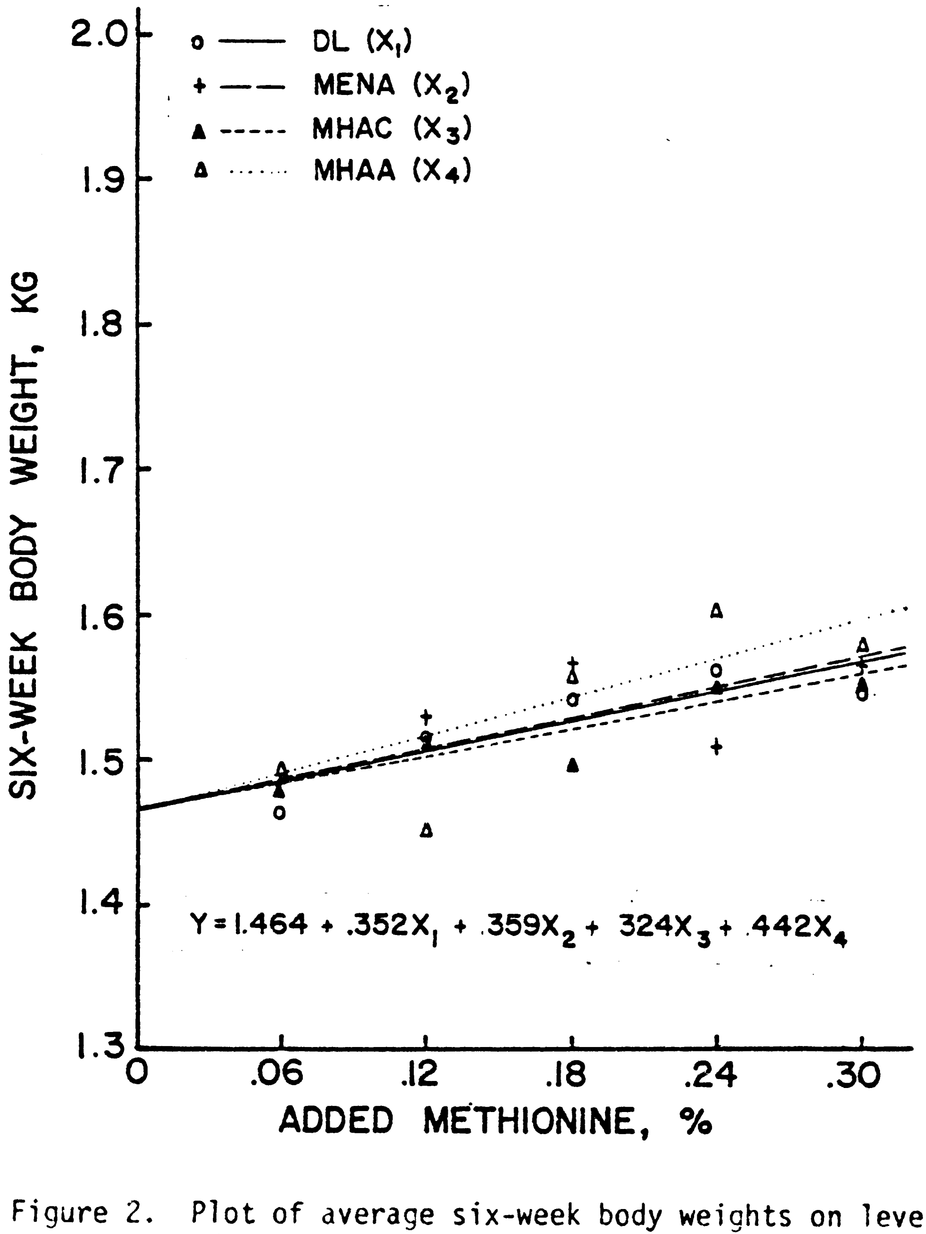

Figure 2. Plot of average six-week body weights on level of added methionine with associated regression lines using slope ratio analysis (Experiment 2)

Table 9. Relative potency, variance, and 95% fiducial limits for methionine compounds based on six-week body weights using slope ratio bioassay with DL-methionine as standard (Experiment 2)

Source of methionine	Relative potency R	V(R)	Fiducial limits, 95%	
			Lower	Upper
DL	100.0			
MENA	102.2	.0999	47.4	226.9
MHAC	92.3	.0908	36.6	203.8
MHAA	125.8	.1298	70.0	285.0
DL and MENA	100.0	-	-	-
MHAC and MHAA	107.7	.0518	68.8	174.4

potency estimates are much larger than the corresponding variances in Experiment 1, reflecting the smaller growth response from added methionine in Experiment 2 compared to that in Experiment 1. Due to the width of the 95% fiducial limits, MENA, MHAC, and MHAA did not significantly differ from DL. Because of the small growth response and thus the wide fiducial limits associated with the relative potency estimates in Experiment 2, this slope ratio bioassay was much less sensitive than that in Experiment 1.

Combination of Potencies - Experiments 1 and 2

In order to obtain an average relative potency estimate for each compound based on the results of Experiments 1 and 2, the individual estimates for each compound from the two experiments were subjected to the weighted means procedure as outlined by Finney (1964). The estimated relative potency of each methionine source in Experiments 1 and 2 must be in agreement except for differences in experimental error. This agreement is checked by a test for homogeneity of the two estimates obtained for each compound from Experiments 1 and 2. The Chi-square values determined for each compound using the procedure of Finney (1964) are presented in Table 10. Each of the values was non-significant showing that the individual relative potencies are in agreement, and the method for combining estimates by the weighted means procedure was valid.

The average relative potencies for each compound with respect to DL on an equimolar basis are presented in Table 10 with the associated 95% fiducial limits. Overall, MENA was 104.7%, MHAC 101.8%, and

Table 10. Relative potency and 95% fiducial limits using the slope ratio bioassay for methionine compounds based on combined data from Experiments 1 and 2

Source of methionine	Experiment	Test[1] for homogeneity (χ^2)	Relative potency (R)	Fiducial limits, 95%	
				Lower	Upper
DL			100		
MENA	1	.006	104.9	86.7	127.4
	2		102.2	47.4	226.9
	Average		104.7	86.1	123.3
MHAC	1	.154	102.8	84.7	125.0
	2		92.3	36.6	203.8
	Average		101.8	83.5	120.1
MHAA	1	.405	89.8	72.4	110.2
	2		125.8	70.0	285.0
	Average		92.5	74.9	110.1
DL and MENA	1		100.0		
	2	-	100.0	-	-
	Average		100.0		
MHAC and MHAA	1	.335	94.0	81.6	107.9
	2		107.7	68.8	174.4
	Average		95.0	82.5	107.5

[1] Value required for significance at .05 level = 3.841.

MHAA 92.5% as potent as DL in promoting growth in young turkeys at six weeks of age. These final values were not significantly different from one other, based upon the 95% fiducial limits among the combined estimates. In the calculation of the average relative potencies, most importance was attached to the individual estimates from Experiment 1, the more sensitive bioassay. The combined estimates strongly favor the individual estimates obtained for each compound in the first experiment (Table 10). The 95% fiducial limits about each of the average relative potencies are smaller than the individual estimates of Experiments 1 or 2, indicating that the combined potency for each compound is the more reliable value.

Nonlinear Analysis - Experiment 1

The exponential regression of six-week body weight data on percent added methionine (all levels) resulted in the equation:

$$Y = 1.416 + .568[1-e^{(-5.07X_1 - 5.59X_2 - 5.09X_3 - 4.55X_4)}] + .135X_5.$$

This regression was significant at the .001% level, with an F-value of 79.95 (Table 5). Figure 3 contains the plot of average six-week body weights as a function of percent added methionine for DL, MENA, MHAC, and MHAA with the associated regression lines. When expressed as a ratio of regression coefficients with respect to DL, the relative potencies were 110.3% for MENA, 100.4% for MHAC, and 89.7% for MHAA.

The multiple linear regression of six-week body weight on percent added methionine (0 level included) yielded the regression equation

$$Y = 1.464 + 1.459X_1 + 1.519X_2 + 1.494X_3 + 1.334X_4 + .135X_5.$$

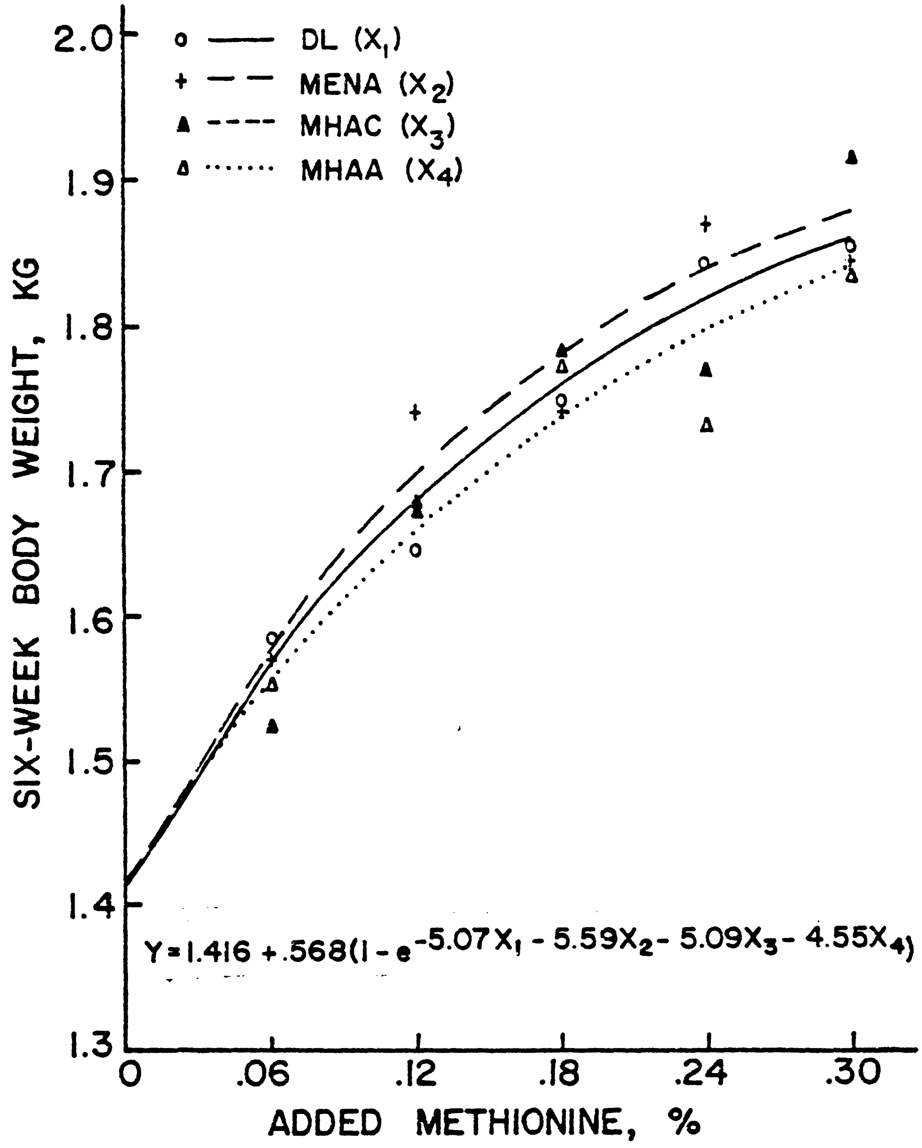

Figure 3. Plot of average six-week body weights on level of added methionine with associated regression lines using exponential analysis (Experiment 1)

In comparing the exponential versus the linear regression model, the reduction in the residual variation among diets was measured. The results from the analyses of variance given in Table 5 show that the extra one degree of freedom in the exponential model decreased the residual variation by 4.8%. This reduction was highly significant ($P<.001$) giving an F-value of 18.14. Table 11 contains the regression coefficients with their associated standard errors and the relative potency for each compound as determined by both the exponential and linear models. The standard errors associated with the coefficients of the exponential model are much larger when expressed as a percentage of the coefficient than those of the linear model. This difference can be explained by the computational formulas, where the standard error of the exponential coefficient is obtained by using the covariances and partial derivations of the parameters, whereas the standard error of the linear coefficient is determined by using the variance. If a linear growth response results from each level of added methionine, the slope ratio procedure is preferred, as it gives a more solid estimate of the reliability of the relative potency estimates.

Nonlinear Analyses - Experiment 2

When exponential regression analysis of six-week body weights on percent added methionine (0 to .30%) was used, the final equation obtained was:

$$Y = 1.404 + .179\ [1-e^{(-7.63X_1\ -\ 8.82X_2\ -\ 6.72X_3\ -\ 8.57X_4)}] + .114X_5.$$

This regression equation was highly significant ($F = 63.59$) at the .001% level (Table 8). The plot of average six-week body weight as a

Table 11. Comparison of the relative potencies and regression coefficients with associated standard errors using slope ratio *versus* exponential analysis of six-week body weight data (Experiment 1)

Source of methionine	Regression coefficients		Relative potency	
	Linear (kg)	Nonlinear	Linear	Nonlinear
DL	1.228 ±.129	5.07 ±1.33	100.0	100.0
MENA	1.228 ±.129	5.59 ±1.51	104.9	110.3
MHAC	1.263 ±.129	5.09 ±1.33	102.8	100.4
MHAA	1.103 ±.129	4.55 ±1.15	89.8	89.7

function of percent added methionine for DL, MENA, MHAC, and MHAA with the corresponding regression lines is presented in Figure 4. The relative potencies determined by the ratio of regression coefficients with DL as the standard were 115.6, 88.1, and 112.3% for MENA, MHAC, and MHAA, respectively (Table 12). No significant differences were seen among the four methionine compounds.

Multiple linear regression analysis of six-week body weight data on percent added methionine using all levels of supplementation yielded the equation:

$Y = 1.432 + .498X_1 + .506X_2 + .471X_3 + .589X_4 + .114X_5.$

The analysis of variance presented in Table 8 shows that the residual variation was reduced 10.7%, a significant reduction at the .05% level (F = 6.15). The regression coefficients with their corresponding standard errors and the relative potency for each compound using both the exponential and linear regressions are presented in Table 12. As in Experiment 1, the relative potency for MENA was numerically larger, and the potencies for MHAC and MHAA were numerically smaller using the exponential model versus the linear model. The exponential analysis also increased the standard errors of the regression coefficients. Therefore, if a linear response in growth is obtained over the levels of methionine supplementation, the slope ratio assay is preferred.

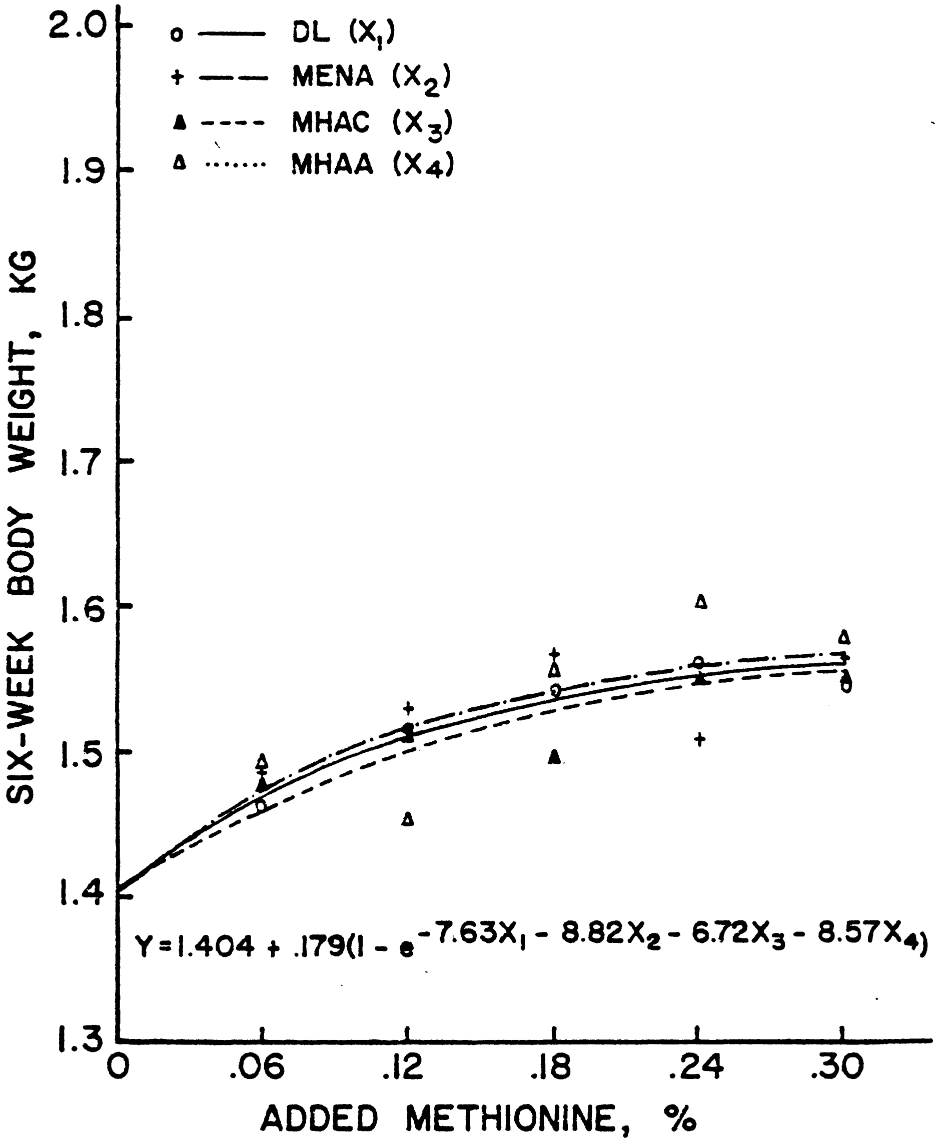

Figure 4. Plot of average six-week body weights on level of added methionine with associated regression lines using exponential analysis (Experiment 2)

Table 12. Comparison of the relative potencies and regression coefficients with associated standard errors using slope ratio *versus* exponential analysis of six-week body weight data (Experiment 2)

Source of methionine	Regression coefficients		Relative potency (R)	
	Linear	Nonlinear	Linear	Nonlinear
DL	.352 ±.114	7.63 ±3.79	100.0	100.0
MENA	.359 ±.114	8.82 ±4.64	102.2	115.6
MHAC	.324 ±.114	6.72 ±3.20	93.3	88.1
MHAA	.442 ±.144	8.57 ±4.45	125.8	112.3

DISCUSSION

Based on a slope ratio bioassay of the six-week body weights in Experiments 1 and 2, the average relative potency estimates of MENA, MHAC, and MHAA to DL are 104.7, 101.8, and 92.5%, respectively. None of the methionine products were significantly different from 100%, as the potency of MENA ranged from 86.1 to 123.3%, MHAC from 83.5 to 120.1%, and MHAA from 74.9 to 110.1%, based upon 95% fiducial limits. The average relative potencies and corresponding 95% fiducial limits are calculated by combination of the data using the weighted means procedure (Finney, 1964), which gives a more valid relative potency estimate than a potency estimate determined within an experiment. Based upon the combined data, differences required for significance from DL were 18.6, 18.3, and 17.6% for MENA, MHAC, and MHAA, respectively. These differences vary in magnitude among compounds as the relative potencies are estimates of a continuous rather than a discrete random variable. Comparison of the DL-methionine products (DL and MENA) with the analogue products (MHAC and MHAA) shows that the analogue products were numerically less potent (95%) than the DL-products, but not significantly different with lower and upper 95% fiducial limits of 82.5 and 107.5%, respectively.

The potency estimates obtained using slope ratio procedure in Experiments 1 and 2 are not significantly different from those in other studies employing practical-type diets for turkeys (Harms, 1977; Parsons, 1978; Schmidt, 1981). The potency estimate obtained for MHAC was numerically higher than the estimate (87%) obtained by

Boebel and Baker (1982) and the estimate (81%) obtained by van Weerden *et al*. (1982). The value obtained for MHAA is numerically higher than those respective values of 78 and 76% obtained by Boebel and Baker (1982) and by van Weerden *et al*. (1982) using crystalline amino acid diets with broilers. The relative potency estimate for MENA in this practical-type diet for turkeys is in agreement with that estimate (101%) determined using a crystalline amino acid diet (van Weerden *et al*. 1982). Because of the large variances associated with potency estimates in any one assay, the potency of either MHAC or MHAA to DL has yet to be shown statistically different due to type of diet used.

A slope ratio bioassay for methionine products is a method by which the relative potency of a product can be estimated through multiple linear regression analyses of performance parameters and ratio of regression coefficients. Furthermore, fiducial limits around a potency estimate can be calculated in order that true differences among products can be detected, and that some measure of precision can be associated with the potency estimate. Parsons (1978) and Schmidt (1981) have calculated fiducial limits about their potency estimates obtained with practical-type diets for poults. The need of a method to estimate and detect differences among methionine products in practical-type poultry rations is important commerically. Addition of a synthetic methionine source to the diet is now based upon the molecular purity of the product. The potencies of methionine products on an equimolar basis requires further consideration. The more potent a methionine product, the molecular quantity needed for supplementation is decreased, which is important in the least cost formulation of poultry diets.

The choice of which performance parameter to use in the determination of relative potency estimates using the slope ratio procedure is important. Researchers have based statements about the bioefficacy of methionine products on analysis of egg output, body weight or weight gain, and feed conversion or efficiency. The six-week body weight data was chosen in this study because the regression equations from both experiments accounted for the largest percentage of the variance (F = 106 and 53 in Tables 1 and 3 respectively, Appendix).

Schmidt (1981) outlined five requirements of a good methionine bioassay. One requirement is that a large growth response must be obtained from the added methionine. The importance of a large growth response can be illustrated in the variance formula of a relative potency, the components of which are the standard deviation, potency estimate, variance and covariance, and the slope of the standard (growth response). The goal in a good methionine bioassay is to, through experimental design, reduce the variance associated with a potency estimate, by manipulating the components of the variance equation which is:

$$V(R) = \frac{s^2}{b_s^2} (v_{22} - 2Rv_{12} + R^2 v_{11})$$

It is obvious that for a given potency estimate, (R) is fixed in the equation. The error term associated with an experiment (s) is reduced by having a large number of birds per pen. The coefficient of variation of bird weights within a pen is about 10%. Theoretically, the standard error among pen averages could be reduced from 10 to 1% by increasing the pen size from 1 to 100 birds per pen, if bird variation were the only factor affecting the magnitude of the standard error "s". However, environmental factors also increase the magnitude of "s" as the size of the pen increases. The smallest standard error among body weights of birds by pen means is obtained with about 100 to 300 birds per pen, and is about 1.5% of the mean (Potter, 1965).

Large standard errors are usually associated with battery experiments in which only 9 birds are used per pen. The standard errors per pen mean associated with each of these two bioassays (74.9 and 69.2 g) were quite large (about 5% of the mean body weight) compared to the standard errors obtained by Schmidt (1981) (55.6 and 53.4 g) who conducted two similar studies within the same facilities. The larger variances decreased the sensitivity and increased the difference detectable in the present two experiments. The large standard errors in Experiments 1 and 2 are believed to be associated with a relative large incidence of leg problems involving a twisted femur at two weeks of age (55 and 50 birds of the 864 started in Experiments 1 and 2, respectively). Although these birds were removed from the experiments, other birds with slightly twisted legs and resulting poorer growth remained.

The standard error can be reduced by using larger pens in floor facilities rather than battery facilities. In such facilities the number of birds per pen can be increased. Another method of reducing the standard error would be to start the bioassay at one-week of age after removing all unhealthy birds, and then randomizing the birds by their individual weights among treatments.

The slope of the standard (b_s) has a very large influence on the variance of a potency estimate. This influence is dramatically illustrated in Experiments 1 and 2, where linear growth responses of 17% and 6%, respectively, were obtained from .30 over .06% added methionine. Consequently, the variances associated with the potency estimates for MENA, MHAC, and MHAA in Experiment 1 were approximately one-tenth as large as those in Experment 2. The difference in the magnitude of the growth response between experiments is difficult to explain. The same shipments of corn, soybean meal, and meat and bone meal were used in the preparation of the basal diets. Differences in potencies between methionine products must be approximately 17% for significance in Experiment 1 while a difference of 55% was required in Experiment 2. The importance of a large growth response is an explanation why significant differences have been obtained in CAA diets. When CAA diets are used, growth responses to added methionine are large due to the severe TSAA deficiency.

The design of a methionine bioassay utilizing a practical-type diet is important in order to achieve a larger growth response from the added methionine. A large growth response is often difficult to achieve, as methionine levels are marginally deficient and therefore differences among treatments are small and difficult to detect. Boebel and Baker (1982) and van Weerden _et al_. (1982) offer this reason as to why results in CAA diets appear to contradict those obtained in corn-soybean meal diets. One possible way to increase the growth response obtained is to lower the protein level of the basal diet. The lower protein level will increase the deficiency of methionine or TSAA in the diet, thereby placing more pressure on the methionine products to supply active methionine to correct the deficiency. A 28% protein basal diet was used in this study, but this level could safely be reduced to 24 or 25% protein to increase the magnitude of the TSAA deficiency (Potter and Shelton, 1979). In such a case, lysine and threonine may be added to the low protein basal diet in order to help avoid a deficiency of these amino acids.

By reducing the level of protein and thus increasing the methionine deficiency of the basal diet, more and higher levels of methionine supplementation can be utilized. The wider the interval between the lowest and highest level of supplementation, the smaller the variance (v_{11} and v_{22}) and covariance (v_{12}) components of the variance equation. By doubling the amount of supplemental methionine i.e. .1 to .2% and still obtaining linearity, the difference detectable between methionine products can be reduced to one-half. By quadruplicating the number of pens or number of experiments, the difference detectable between methionine products can be reduced to one-half. Each level of added methionine must give a linear response, a requirement of the slope ratio bioassay (Finney, 1964).

Recent studies have shown that various diet parameters may have an effect on the efficiency of the analogue products. Boebel and Baker

(1982) postulate that the diet-type (CAA, semi-purified or practical) does not affect the relative potencies obtained for the analogue products, as they are metabolized the same way regardless of diet-type. They stated that the methionine-cystine ratio of the diet as a factor. Katz and Baker (1975) in studying the utilization of MHAC previously pointed out the importance of the methionine-cystine ratio. Thomas et al. (1983) observed a source of methionine x choline and source of methionine x cystine interaction in studies with body weight gains of broiler chicks and concluded that the level of cystine or choline in a diet may affect the magnitude of the increase in body weight from added methionine. With a lower level of cystine or choline in the diet, the need for methionine increases because methionine must be used for the synthesis of cystine and choline rather than of protein. The level of methionine deficiency and the amount of dietary cystine or choline may influence the magnitude of the growth response.

Determining differences in relative potency estimates of methionine compounds within an experiment employing a practical-type diet is difficult. Utilizing the weighted means procedure (Finney, 1964) allows replication of methionine bioassays over time. The combining of all data gives a more valid relative potency estimate of a compound, which is illustrated in the average relative potency for each compound based upon the combined data of Experiments 1 and 2. The 95% fiducial limits associated with the average relative potency esimate are narrower than those obtained for an individual estimate in either experiment. The combination of results from several bioassays by the weighted means procedure is a valid and valuable method in determining the best estimate of the relative potency of a methionine product.

The prediction of six-week body weight in response to methionine supplementation by a nonlinear regression model was more accurate than the multiple linear regression model using the slope ratio procedure. The extra one degree of freedom associated with the exponential model accounted for 4.8 and 10.7% more of the residual variation among the diets in Experiments 1 and 2, respectively. The predicted response from the exponential model is logical. As the increments of methionine are added to a very deficient diet to satisfy the methionine or the TSAA requirement, the response in growth to each successive increment of supplementation decreases and produces a curvilinear response. Although the responses to higher levels of methionine decrease it is important to realize that the potency of the product does not change. The nonlinear model, in contrast to the multiple-linear model, is not restricted to the linear portion of the growth curve, thus more levels of methionine may be included in the bioassay. The major drawback of nonlinear analysis is that no statistically valid method is known for calculating fiducial limits around a relative potency estimate. Therefore, no measure of validity can be assigned to the potency estimate or to the difference detectable. If a linear growth response is obtained to methionine supplementation, the slope ratio procedure is a more sensitive method of estimating the relative potency of a methionine product. The relative potencies calculated from the nonlinear model were in agreement with those obtained utilizing the slope ratio procedure in both Experiments 1 and 2. With respect to DL, MENA had potencies of 110 and 115.6%, MHAC 100.4 and 88.1%, and MHAA 89.7 and 112.3% in Experiments 1 and 2, respectively.

The application of the nonlinear model more accurately predicts the response of six-week body weight from supplemental methionine. However, fiducial limits around the relative potency estimates have not been determined because appropriate procedures are not available to calculate the limits.

The application of the slope ratio procedure permits the calculation of a variance and fiducial limits for a relative potency estimate. Based upon measured variation in poultry diets, a difference of approximately 15% or more between potency estimates of methionine products must be observed in order to detect a significant difference using practical-type diets. Much larger differences must be observed in some bioassays where the increase in growth from added methionine, such as in Experiment 2, is small (6 compared to 17%). Therefore in such experiments, it is more appropriate to conclude that the bioassay was not sensitive enough to detect differences in potencies of products rather than that potencies of the products are not different significantly.

Statistical procedures have been developed to combine or average the estimates of relative potencies of a product. When estimates with similar variances are combined, the difference detectable can be reduced to one-half that detectable in a single experiment. Thus, by combining the relative potency results of two methionine products with their variances over several experiments, the relative potency of such methionine products with a relatively small biological potency difference (under 15% or perhaps as small as 4 or 2%) can be obtained for the poultry and feed industries.

SERIES II - METHIONINE - CHOLINE - SULFATE RELATIONSHIPS

REVIEW OF LITERATURE

Choline

In a study with turkey poults from 0 to 28 days of age, Jukes (1940a) showed that choline was effective in preventing perosis when added at .2% of the diet. Choline also promoted growth when added to a simplified diet in the same study. Further studies by Jukes (1940b) supported this finding in turkeys, and choline was also found to prevent perosis in chickens. In these experiments choline was found to improve growth when supplemented at the .1% level and to prevent perosis when added at the .2% level. In addition, supplemental betaine and methionine were ineffective in preventing perosis, indicating that methyl groups were not involved. Other studies (Evans et al., 1942; Evans, 1943; Vohra et al., 1960) have supported the role of choline in preventing perosis and increasing growth. Since then, work has indicated that the entire choline molecule is required for phospholipid formation (phosphotidyl choline), an important component of normal cartilage matrix of the bone (Scott et al., 1976). Choline is also a component of the neurotransmitter acetylcholine as well as sphingomyelin. Several researchers (Almquist and Grau, 1944; Langer and Kratzer, 1964a,b; Leach et al., 1966) have shown that choline functions as a supplier of labile methyl groups.

Choline and Methionine Relationships

Early work by Marvel et al. (1944) showed that methionine and/or choline additions to a corn-soybean meal diet improved growth in chicks and overcame a deficiency in the basal ration. They concluded that if a deficiency of both methionine and choline existed in the basal these two compounds were interchangeable. Since choline in comparison to methionine was at a relatively low price, they noted the economic importance of this possible relationship.

Knowing that choline is associated with fat metabolism, Quillin et al. (1961) studied the effect of choline on the methionine requirement in high-fat diets (10 or 12% added fat) for broiler chicks from 0 to 8 weeks of age. The results showed that methionine supplementation produced a significant growth response only at low levels of choline and that the addition of choline approached a significant growth response only with diets low in methionine. The authors concluded that methionine and choline exerted sparing effects on each other in high-fat broiler diets. Regression of the percent methionine required in the ration on added choline showed that one gram of choline was equivalent to approximately 2.3 to 2.4 g of DL-methionine as methyl donors in the highfat diets. Pesti et al. (1979) also expected that a smaller amount of choline is required to replace a quantity of DL-methionine, since choline has three methyl groups and a smaller molecular weight than methionine. They also concluded that investigations on the efficiency of utilization of the methyl carbons must be conducted before establishing a precise minimum choline requirement in a diet.

The amount of sulfur amino acids and methyl donors in corn-soybean meal diets fed to broiler chicks and turkey poults were investigated by Pesti et al. (1979). Broilers fed a 23% protein diet with added cystine, homocystine, or $2(CaSO_4)\cdot H_2O$ gave no growth response. A growth response was obtained however when choline or betaine was added to the basal diet, indicating a methyl group deficiency. When the basal diet was fed with added choline or betaine (.23%), no response was obtained to methionine, indicating that the diet contains an adequate amount of total sulfur amino acids for chicks when sufficient dietary methyl donors were present. The impact of this research indicates that if a diet is deficient in labile methyl group status only, choline or betaine supplementation can be used instead of methionine to correct the deficiency. A 28% protein diet fed to poults resulted in a deficiency of TSAA, as the response to methionine was greater than that to choline or betaine. To further investigate this finding, Pesti _et_ _al_. (1980) conducted experiments to test the hypothesis that metabolically labile methyl groups, and not methionine _per_ _se_ are limiting the growth of starting broiler chicks fed corn-soybean-grease diets formulated to meet NRC (1977) recommendations (except for methionine). Addition of choline or betaine to a 23% protein diet containing .32% methionine, .42% cystine, 1300 mg choline per kg and 7.97% white grease significantly improved ($P<.05$) weight gain and feed conversion over the basal or added DL-homocystine diet. The authors concluded that the above hypothesis was true, as the results indicated that the methyl group utilization was not impaired by the lack of a carbon skeleton of methionine. The results obtained agree with those of Quillin _et_ _al_. (1961) in that high fat diets are deficient in dietary methyl groups. Pesti _et_ _al_. (1980), using three mathematical models, suggested that it may be practical to add higher levels of choline (700 to 800 mg/kg) and lower levels of methionine to broiler diets, as choline is 35 to 45% lower in cost. Recent work by Thomas et al. (1983) has shown that choline exerts a slight sparing effect on methionine.

The above studies have indicated that choline has the capacity to spare methionine in poultry diets (primarily broilers) if the diet is deficient in methyl group donors and not methionine per se. Other studies have indicated that methionine can spare choline. Slinger et al. (1962) in studying the interrelationship of methionine and choline in turkeys found no significant interaction between the two compounds. Feeding semipurified diets to broiler chicks, Derilo and Balnave (1980) found that methionine replaced part of the dietary requirement for choline at low dietary choline concentrations. Also, increasing choline when the diet was deficient in TSAA may decrease performance indicating that choline is unable to replace methionine. Dean (1982) found that supplementing a 23% corn-soybean meal diet with methionine (.1%) can eliminate the need for supplemental choline in ducklings. Choline's failure to improve growth in the presence of .1% added methionine indicated that the reverse was not true. Baker et al. (1983) reported that in feeding a crystalline amino acid or a practical corn-soybean meal diet to broiler chicks, methionine spares choline when choline is deficient and choline spares methionine when methionine is deficient in the diet. An important finding was that when either compound was present at the physiological requirement in the presence of excess levels of the other, an excess of one nutrient does not reduce the dietary need for the second.

Inorganic Sulfate

Sulfur from either inorganic sulfate or from the sulfur amino acids serves as an important element in the body compounds. Injection of labeled sodium sulfate-S^{35} intraperitoneally in rats has shown that it is utilized primarily in the synthesis of the chondroitin matrix of cartilage (Dziewiatkowski, 1951). Sulfate in smaller quantities is used in the synthesis of taurine in the body (Martin, 1972). Machlin *et al*. (1953) found S^{35} from sulfate in the albumen and yolk as organic sulfur when it was added to the diets of laying hens. They also found that the sulfur of sulfate was incorporated into cystine, but not into methionine. A later study confirmed this process (Machlin *et al*., 1954) in young chickens, although the incorporation of the sulfur of sulfate into cystine was found to be an inefficient process. In the same study, high concentrations of labeled sulfur were found in the gizzard lining, feathers, and muscle.

Several researchers have conducted studies to examine the importance of sulfate as a possible dietary essential for chickens fed purified diets. The first investigators to show a growth response from sodium sulfate were Gordon and Sizer (1955), using a casein-gelatin diet. Addition of .5% sulfate to this low cystine and inorganic sulfur-free diet gave a response in body weight gain and feed efficiency. The authors concluded that the sulfur requirement of the chicken can be met in part with inorganic sulfate because the addition of DL-methionine or methionine hydroxy analogue calcium salt did not completely satisfy the sulfur requirement. Additionally, they found that inorganic sulfate cannot replace cystine or methionine for protein synthesis. Machlin (1955) using a modification of the Gordon and Sizer (1955) diet observed a 5 to 45% growth response from chicks fed .5% added sodium sulfate. They concluded that this growth response was due to a physiological requirement for sulfate per se and that the utilization of sulfate sulfur for synthesis of cystine or methionine was of probably no nutritional value to the chick. Martin (1972) attributed the growth response from sulfate supplementation to the theory that it is, in part, due to the conversion of sulfate to taurine. Feeding a crystalline amino acid diet, Soares (1974) found that added potassium sulfate significantly stimulated growth when the TSAA were at suboptimal levels and that the low cystine level was a contributive factor to the relative efficacy of sulfate.

The question to whether sulfate is required per se or that it functions through sparing the dietary requirement of TSAA was partially answered by several workers. Miller (1974) observed a significant increase ($P<.05$) in chick growth only when the CAA diet was deficient in methionine and that sulfate was not required per se. Contrary to this, Sasse and Baker (1974a) found that sulfate spares cystine rather than methionine; however, they agreed that sulfate is not an essential dietary element when feeding young chicks a CAA diet. In another study, Sasse and Baker (1974b) demonstrated that cystine can furnish less of the TSAA need (spare methionine) when sulfate was present in the diet (50%) than when sulfate was absent (55%). A quantitative measure of the amount of sulfate that can spare cystine in diets containing less than 200 ppm sulfate has been established by Sasse and Baker (1974a,b) and Soares (1974). Approximately 15% of the physiological requirement for cystine can be spared by sulfate in cystine-deficient diets. Soares (1974) states that 9% of total dietary sulfur can be supplied by sulfate.

In addition to work with purified diets, extensive research has been conducted to examine the role of inorganic sulfate in practical-type diets. The results obtained in these studies have been quite variable. Ross and Harms (1970) found consistent increases in weight gains with .05 and .5% added sodium sulfate, yet these growth responses were small. Later, Ross and co-workers (1972) fed a semipurified diet using isolated soy protein (2 types). They found a significant increase in body weight gain due to added sulfate when treatments were pooled among diets according to methionine level. A significant growth response was obtained even at the highest level of methionine supplementation, indicating a requirement for sulfate _per se_. A response to .10% added sodium sulfate independent of the TSAA level and in the presence of adequate methionine levels was reported by van Weerden _et al_. (1976). These results are in contrast to the work done using purified diets mentioned previously by Sasse and Baker (1974a) and Soares (1974) who stated that a response to sulfate occurs only when the diet is deficient in TSAA, primarily cystine. Marquardt and Campbell (1975) failed to obtain a response from supplemental potassium sulfate when feeding chicks a 23.4% protein diet made up of 90% raw faba beans. Soares _et al_. (1974) stated that if sulfate is used to replace 25% of added TSAA in practical rations, then approximately 6% of the total sulfur of such diets can be supplied as inorganic sulfate.

Research of inorganic sulfate in turkey diets has been done to a much lesser degree and with inconsistent findings. Sloan and Harms (1972) and Kuhl and Sullivan (1974) have reported growth responses to supplemental sulfate in poults diets, indicating that the turkey poult can utilize sulfate from sodium sulfate to spare its TSAA requirement. Other workers have observed no responses from supplemental sulfate in practical-type diets for turkey poults (Kuhl and Sullivan, 1973; Potter, 1977).

Methionine, Choline, and Sulfate Relationships

Miles _et al_. (1983) conducted a study to examine the interrelationships of methionine, choline and sulfate in practical-type diets fed to broiler chicks. They reported that when choline and sulfate were added to the diet in the absence of methionine, a larger growth response was obtained when either was added alone. They concluded that supplementation of sulfate in the presence of choline had a sparing action on the TSAA or that for sulfate _per se_ was required. Significant choline x methoinine and sulfate x choline x methionine interactions were observed, yet weight gains were not significantly different from those obtained when methionine was added alone. Their final conclusion was that sulfate must be present in the diet in order for choline to spare a maximal amount of methionine.

OBJECTIVES

Two experiments were conducted to determine if choline and/or sulfate can spare methionine in a 21% protein corn-soybean meal diet for young turkeys.

EXPERIMENTAL PROCEDURE

Birds

For Experiment 3, 432 male and 432 female medium-type turkeys hatched at the Turkey Research Center were used. At four weeks of age, the males were randomized into Oakes and the females into Petersime grower batteries such that each of 96 pens contained 9 poults per pen. For Experiment 4, 432 male and 384 female Large White turkeys hatched at the center were used. At three weeks of age, the males were randomized into Oakes grower batteries with 9 poults per pen in 48 pens, and the females into Petersime grower batteries with 8 poults per pen in 48 pens.

In each experiment, after randomization, the poults of each pen were group weighed to determine the starting average body weight. Water and assigned diets were provided ad libitum until eight weeks of age in Experiment 3 and until seven weeks of age in Experiment 4, when the experiments terminated. Room temperature was recorded twice daily to monitor the environmental temperatures during the experiments.

Diets

The formulation of the basal diet used in these two experiments is presented in Table 13. The calculated analyses of 21.4% protein, .67% TSAA, and 1,113 mg choline per kg for the basal diet are considerabaly below the NRC (1977) requirements for poults 0 to 8 weeks of age.

The experimental design for these two experiments is presented in Table 14. Three variables were added to the diet to form 24 diets in a 6 x 2 x 2 factorial. The variables were DL-methionine at

Table 13. Composition of basal diet used in Experiments 3 and 4

Ingredients	Grams per kilogram
Ground yellow corn	608.97
Glucose monohydrate (cerelose)	11
Stabilized fat	10
Dehulled soybean meal	320
Defluorinated phosphate	38
Iodized salt	3
Trace mineral mix[1] (sulfate free)	2
Vitamin and feed additive premix[2] (choline free)	1.03
L-Lysine HCl	6
Total	1,000.00
Calculate analyses (%)[3]:	
Protein	21.4
Calcium	1.32
Phosphorus, total	1.05
Phosphorus, available	.80
Lysine	1.64
Methionine	.35
Cystine	.32
Sulfur	.11
Choline (mg/kg)	1,113
Metabolizable energy (kcal/kg)	2,952

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[1] Supplied the following amounts of trace minerals in mg/kg complete diet: 150 manganese, 101 zinc, 70 iron, 10 copper, and 2.6 iodine from manganese carbonate, zinc oxide, ferric citrate, cupric oxide, and potassium iodate, respectively.

[2] Supplied the following amounts of vitamins and feed additives in mg/kg complete diet unless otherwise stated: 13,202 IU vitamin A, 3,312 ICU vitamin D_3, 11 IU vitamin E, 4.4 menadione sodium bisulfite complex, 1.1 thiamin HCl, 5.5 riboflavin, 16.5 calcium pantothenate (D), 66.1 niacin, .015 vitamin B_{12}, 1.1 folic acid, .11 biotin, 2.2 pyridoxine HCl, 125 ethoxyquin, 0.2 selenium and 26.4 penicillin.

[3] Values obtained from the National Research Council (1977) publication Nutrient Requirements of Poultry".

Table 14. Design of Experiments 3 and 4

Level of added DL-Methionine %	Potassium sulfate[1] (%)			
	0		.10	
	Choline chloride[2], (%)		Choline chloride, (%)	
	0	.20	0	.20
	Diet number			
.00	1	2	3	4
.06	5	6	7	8
.12	9	10	11	12
.18	13	14	15	16
.24	17	18	19	20
.30	21	22	23	24

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[1] Because the potassium sulfate was assumed 99% pure, it required .10 ÷ .99 = .1010% potassium sulfate which provided .06% sulfate or .02% sulfur.

[2] Because the choline chloride came from choline chloride 60%, it required .20 ÷ .60 = .33% choline chloride which provided .33 x .868 = .29% choline.

0, .06, .12, .18, .24 and .30%, choline chloride at 0 and .20%, and potassium sulfate at 0 and .10%. The potassium sulfate was assumed to be 99% pure, and choline was added to the diets in the form of choline chloride containing 86.8% choline. For each experiment, three basal mixes, totaling 2,175.8 pounds per mix, were prepared with all ingredients present except 1.10% glucose monohydrate. The trace mineral and vitamin mixes were free of sulfate and choline. Individual diets totaling 120 pounds were prepared by blending the proper amounts of the dietary variables and glucose monohydrate with equal quantities of the three basal mixes. Each diet was fed to two pens of males and two pens of females from four to eight weeks of age and three to seven weeks of age in Experiments 3 and 4, respectively.

At weekly intervals, the poults were group weighed by pens and the feed consumption for each pen was determined. Mortality records were kept so that the average feed consumption and feed efficiency for the poults in each pen could be determined. Average body weights and body weight gains for each period were also determined.

Statistical Analysis

All body weight, body weight gain, feed consumption and feed efficiency data for each period in each experiment were subjected to analysis of variance. The independent variables of interest in the model were levels of added DL-methionine, choline chloride, potassium sulfate, sex, and replicate. The sex x replicate and diet x sex and/or replicate interactions were combined for a measure of error mean square with 70 degrees of freedom.

RESULTS - EXPERIMENT 3

For the purpose of this thesis, only cumulative body weight gains, feed consumptions, and feed efficiencies will be presented and discussed. The results obtained for this period are representative of those obtained at other periods in the two experiments.

Average body weight gain, feed consumption, and feed efficiency of the medium-type turkeys fed each diet from four to eight weeks of age are presented in Table 15. The mean, standard deviation, coefficient of variation, and difference required for significance are presented in Table 5 of the Appendix. The percent increase of each performance parameter over the basal diet for each period of all turkeys fed each level of added DL-methionine, choline chloride, and potassium sulfate are presented in Tables 6, 7 and 8 of the Appendix, respectively. Table 16 contains the analyses of variance for body weight gains, feed consumptions and feed efficiencies.

DL-Methionine significantly affected the above performance parameters, while supplemental choline and sulfur failed to do so. No significant interactions among these three dietary variables were observed.

Added DL-Methionine

Body weight gains from four to eight weeks of age were significantly increased ($P<.001$) from adding DL to the diets (Table 16). From diets containing 0, .06, .12, .18, .24, and .30% added DL, body weight gains were 1,073, 1,191, 1,208, 1,306, 1,235 and 1,252 g, respectively (Table 17). In comparison to the unsupplemented groups

Table 15. Average body weight gains, feed consumptions, and feed efficiencies of medium-type turkeys fed each diet from four to eight weeks of age[1] (Experiment 3)

Level of added DL-Methionine (%)	Potassium sulfate (%)			
	0		.10	
	Choline chloride (%)		Choline chloride (%)	
	0	.20	0	.20
		Body weight gain (g)		
.00	1017	1092	1117	1065
.06	1158	1197	1210	1201
.12	1207	1243	1173	1211
.18	1330	1308	1295	1290
.24	1212	1244	1255	1230
.30	1257	1202	1315	1232
		Feed consumption (g)		
.00	2377	2436	2540	2390
.06	2538	2610	2536	2619
.12	2477	2623	2468	2531
.18	2716	2659	2647	2691
.24	2506	2578	2616	2527
.30	2554	2512	2602	2557
		Feed efficiency		
.00	.4259	.4464	.4382	.4453
.06	.4538	.4580	.4750	.4577
.12	.4857	.4728	.4743	.4772
.18	.4886	.4903	.4868	.4783
.24	.4840	.4818	.4767	.4859
.30	.4909	.4775	.5044	.4808

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[1] Each value calculated from values representing two pens of each sex.

Table 16. Analyses of variance of four to eight-week body weight gains, feed consumptions, and feed efficiencies of medium-type turkeys (Experiment 3)

Source	Degrees of freedom	Mean squares		
		Body weight gain	Feed consumption	Feed efficiency
Sex	1	1,718,536***	3,478,792***	2,917,997***
Replicate	1	14,625	1,629	254,162***
Diets	23			
Methionine	5	98,312***	98,302**	577,658***
Choline	1	155	4,237	17,631
Sulfate	1	2,628	3,165	10,230
Methionine x choline	5	5,309	15,937	46,332
Methionine x sulfate	5	4,345	6,802	19,818
Choline x sulfate	1	9,779	19,958	13,184
Methionine x choline x sulfate	5	2,609	13,456	22,054
Error	70	4,215	11,038	18,556
Total	95			

**P<.01.

***P<.001.

Table 17. Effects of dietary variables on the four to eight-week body weight gain, feed consumption, and feed efficiency of medium-type turkeys (Experiment 3)

Dietary variable	Body weight gain (g)	Feed consumption (g)	Feed efficiency
DL-Methionine (%)			
.00	1073[a]	2436	.4390
.06	1191	2576	.4611
.12	1208	2525	.4775
.18	1306	2678	.4860
.24	1235	2557	.4821
.30	1252	2556	.4884
Difference			
.06 over .00	118***	140**	.0221**
.12 over .00	135***	89*	.0385***
.18 over .00	233***	242	.0470***
.24 over .00	162***	121**	.0431***
.30 over .00	179***	121**	.0494***
Choline chloride (%)			
.0	1212[b]	2548	.4737
.2	1210	2561	.4710
Difference	-2	13	-.0027
Potassium sulfate (%)			
.0	1206[b]	2549	.4713
.1	1216	2560	.4731
Difference	10	11	.0021
Difference required for significance (P=.05)			
16 *vs* 16 pens	45.8	74.2	.0096
48 *vs* 48 pens	26.5	42.9	.0055

[a] Each value represents the average of 16 pens (8 of each sex) with nine poults per pen.

[b] Each value represents the average of 48 pens (24 of each sex) with nine poults per pen.

*P<.05.

**P<.01.

***P<.001.

(0% level), these values represent 11.0, 12.6, 21.7, 15.1, and 16.7% increases in body weight gain from the respective methionine additions.

The increases in feed consumption and feed efficiency from added DL were similar to the increases in body weight gain, except the values on the average were approximately one-third and one-half the magnitude when expressed on a percentage basis, respectively. Feed consumption for the four to eight-week period were 2,436, 2,576, 2,525, 2,678, 2,557 and 2,556 g from feeding diets containing 0, .06, .12, .18, .24 and .30% added methionine, respectively (Table 17). These values represent a 5.8, 3.6, 9.9, 5.0 and 5.0% increase in feed consumption resulting from the respective methionine addition. Adding DL significantly increased feed efficiencies for the period or were .4390, .4611, .4775, .4860, .4821 and .4884 for diets containing 0, .06, .12, .18, .24 and .30% added DL, respectively (Table 17). Plots of percent increase in body weight gain, feed consumption, and feed efficiency on percent added DL are presented in Figure 5. Those turkeys fed diets containing .18% added DL had consistently higher values for each parameter throughout the experiment, and this apparent difference is attributed to bird variation.

Added Choline

The addition of choline to the diet failed to affect body weight gains, feed consumptions and feed efficiencies (Table 16). From feeding diets containing 0 or .20% added choline chloride, body weight gains were 1,212 and 1,210 g, feed consumptions were 2,548 and 2,561 g, and feed efficiencies were .4739 and .4710, respectively (Table 17).

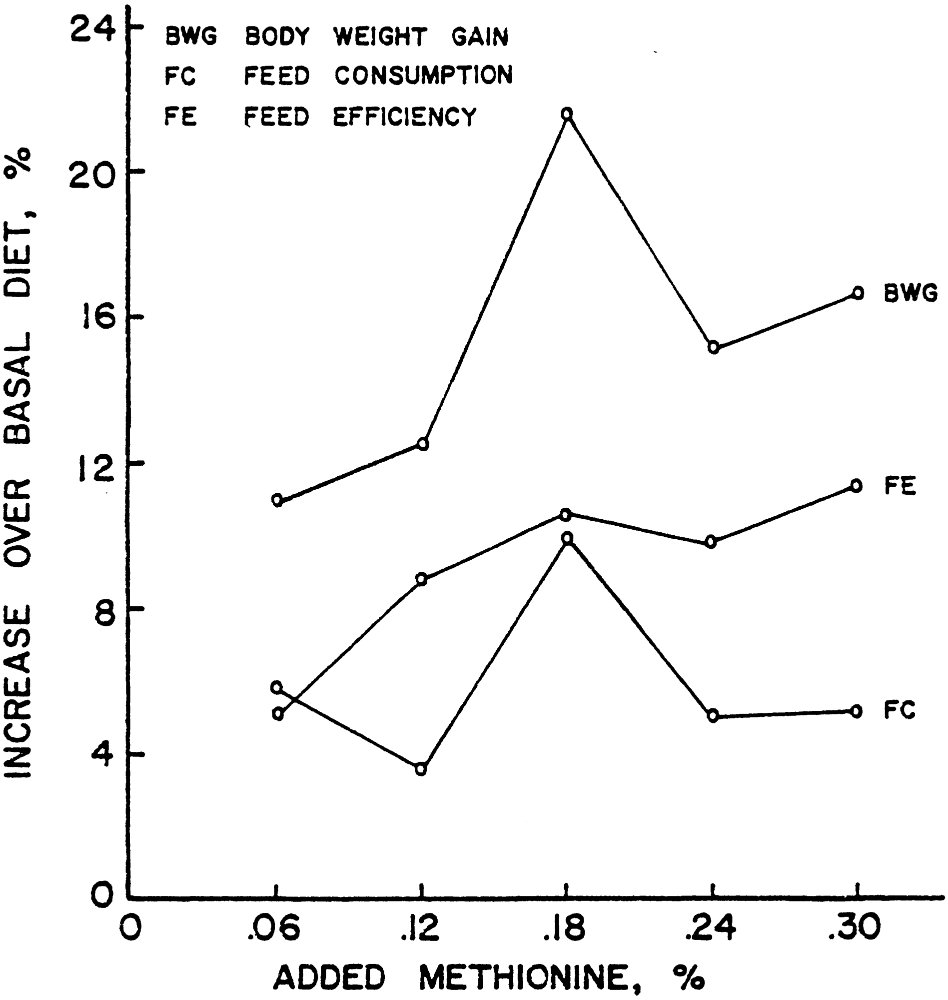

Figure 5. Plot of percent increase in average four to eight-week body weight gain, feed consumption, and feed efficiency on level of added DL-methionine of medium-type turkeys (Experiment 3)

Perosis problems did not arise, indicating an adequate amount of choline in the basal diet to prevent this problem.

Added Sulfate

Adding sulfur to the diet in the form of potassium sulfate failed to affect body weight gains, feed consumptions, and feed efficiencies of turkeys. From diets containing 0 or .1% added potassium sulfate, body weight gains from four to eight weeks of age were 1,206 and 1,216 g, feed consumptions were 2,549 and 2,560 g, and feed efficiencies were .4713 and .4734, respectively (Table 17).

Interactions

No significant interactions among the dietary variables were observed in the data from this experiment. Added choline and/or sulfur failed to significantly affect these performance parameters, even in the absence of DL-methionine.

A plot of four to eight-week body weight gains on level of added methionine at each level of choline and/or sulfate is presented in Figure 6 to illustrate the consistency of body weight gain from added methionine regardless of choline and sulfate supplementation.

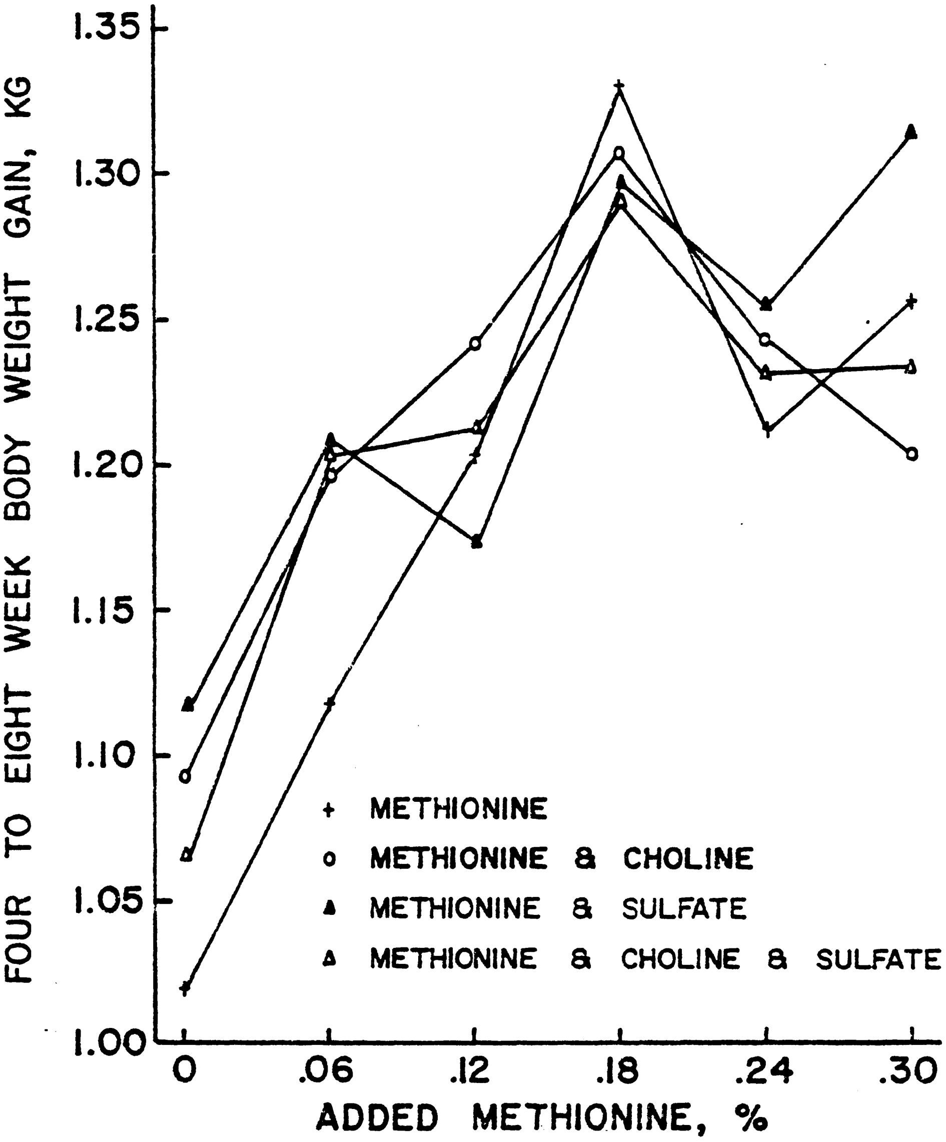

Figure 6. Plot of average four to eight-week body weight gains on level of added DL-methionine at each level of choline and/or sulfate of medium-type turkeys (Experiment 3)

RESULTS - EXPERIMENT 4

Average body weight gains, feed consumptions, and feed efficiencies of the Large White turkeys fed each diet from three to seven weeks of age are presented in Table 18. Table 9 of the Appendix contains the mean, standard deviation, coefficient of variation, and difference required for significance among all data. The body weights, body weight gains, feed consumptions, and feed efficiencies for each period of all turkeys fed each level of added DL, choline chloride, and potassium sulfate are presented in Tables 10, 11 and 12 of the Appendix. The analyses variances for body weight gains, feed consumptions and feed efficiencies are presented in Table 19.

Added DL-Methionine

Body weight gains from three to seven weeks of age were significantly increased ($P<.001$) from adding DL-methionine to the diets. Body weight gains were 1,411, 1,537, 1,587, 1,607, 1,588 and 1,631 from diets containing 0, .06, .12, .18, .24 and .30% added methionine (Table 20). These values represent 8.9, 12.5, 13.9, 12.5, and 15.6% increase in body weight gain over the basal diet. The plot of percent increase in body weight gain on level of added methionine is presented in Figure 7.

Feed consumptions and feed efficiencies were significantly increased from added methionine (Tables 19 and 20). Feeding diets containing 0, .06, .12, .18, .24 and .30% added methionine gave feed consumptions of 2,908, 3,045, 3,060, 3,046, 3,035 and 3,068 g,

Table 18. Average body weight gains, feed consumptions, and feed efficiencies of Large White turkeys fed each diet from three to seven weeks of age[1] (Experiment 4)

Level of added DL-Methionine (%)	Potassium sulfate (%)			
	0		.10	
	Choline chloride (%)		Choline chloride (%)	
	0	.20	0	.20
		Body weight gain (g)		
.00	1398	1418	1394	1434
.06	1540	1550	1488	1569
.12	1621	1583	1565	1580
.18	1529	1653	1646	1601
.24	1614	1598	1578	1560
.30	1624	1676	1596	1628
		Feed consumption (g)		
.00	2884	2927	2886	2935
.06	3102	3060	2959	3061
.12	3178	3036	2999	3028
.18	2944	3107	3084	3051
.24	3064	3065	3023	2988
.30	3066	3121	3046	3039
		Feed efficiency		
.00	.4837	.4834	.4818	.4878
.06	.4962	.5054	.5030	.5118
.12	.5093	.5210	.5215	.5208
.18	.5191	.5312	.5332	.5240
.24	.5260	.5209	.5212	.5223
.30	.5293	.5365	.5237	.5346

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[1] Each average calculated from values representing two pens of each sex.

Table 19. Analyses of variance of three to seven-week body weight gains, feed consumptions, and feed efficiencies of Large White turkeys (Experiment 4)

Source	Degrees of freedom	Mean squares		
		Body weight gain	Feed consumption	Feed efficiency
Sex	1	1,297,356***	1,604,447***	2,619,213***
Replicate	1	92,845***	303,772***	5,385
Diets	23			
Methionine	5	100,796**	56,674*	489,718***
Choline	1	10,933	5,582	44,419
Sulfate	1	4,430	34,436	9,460
Methionine x choline	5	3,147	7,923	7,706
Methionine x sulfate	5	3,137	10,405	6,983
Choline x sulfate	1	346	113	5,475
Methionine x choline x sulfate	5	7,384	18,649	12,969
Error	70			
Total	95			

***P<.001.

Table 20. Effects of dietary variables on the three to seven-week body weight gain, feed consumption, and feed efficiency of Large White turkeys (Experiment 4)

Dietary variable	Body weight gain (g)	Feed consumption (g)	Feed efficiency
DL-Methionine (%)			
.00	1411[a]	2908	.4842
.06	1537	3045	.5041
.12	1587	3060	.5182
.18	1607	3046	.5269
.24	1588	3035	.5226
.30	1631	3068	.5310
Difference			
.06 over .00	126***	137***	.0199***
.12 over .00	176***	152***	.0340***
.18 over .00	196***	138***	.0427***
.24 over .00	177***	127**	.0384***
.30 over .00	220***	160***	.0468***
Choline chloride (%)			
.0	1550[b]	3020	.5123
.2	1571	3035	.5166
Difference	21	15	.0043
Potassium sulfate (%)			
.0	1567[b]	3046	.5135
.1	1553	3008	.5155
Difference	-14	-38	.0020
Difference required for significance (P=.05)			
16 vs 16 pens	40.1	79.1	.0077
48 vs 48 pens	23.2	45.7	.0044

[a] Each value represents the average of 16 pens (8 of each sex) with nine male and eight female poults per pen.

[b] Each value represents the average of 48 pens (24 of each sex) with nine male and eight female poults per pen.

** P<.01.

*** P<.001.

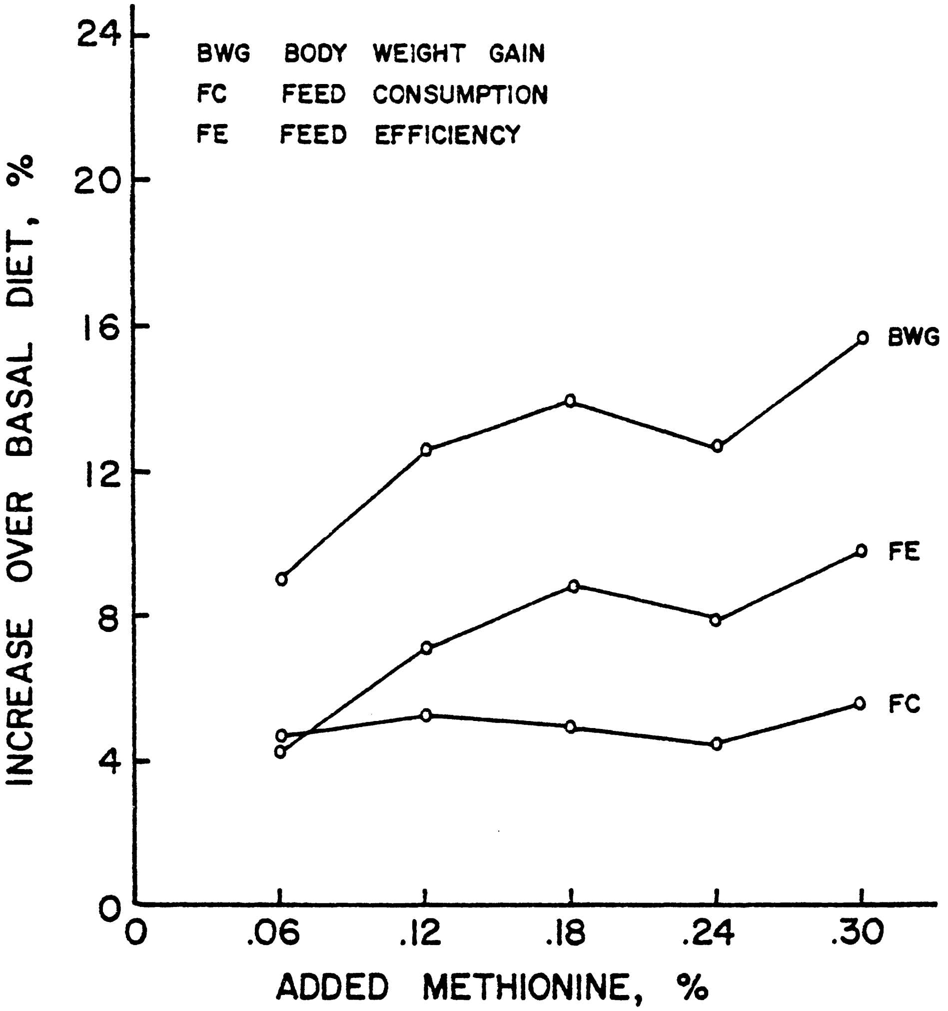

Figure 7. Plot of percent increase in average three to seven-week body weight gain, feed consumption, and feed efficiency on level of added DL-methionine of Large White turkeys (Experiment 4)

respectively (Table 20). These values represent a 4.7, 5.2, 4.8, 4.4 and 5.5% increase of feed consumed over the unsupplemented group, all significant at the .01% level. Feed efficiencies were significantly increased (P<.001) by adding DL-methionine from three to seven weeks of age. Feed efficiencies were .4842, .5041, .5182, .5269, .5226 and .5310 from feeding diets containing 0, .06, .12, .18, .24 and .30% added methionine (Table 20). When expressed as percentages, the values represent a 4.1, 7.0, 8.8, 7.9 and 9.7% increase in feed efficiency over the basal diet from the respective methionine additions. Plots of increases in feed consumption and feed efficiency from added DL-methionine are presented in Figure 7.

Added Choline

The addition of choline to the diet failed to affect body weight gains, feed consumptions and feed efficiencies of Large White turkeys, from three to seven weeks of age. A 2.9 and 1.9% increase (P<.05) in body weight gain and feed efficiency from four to five weeks was obtained, but the effect of added choline was not significant at other age periods during this experiment. No perosis problems occurred indicating the level of choline in the basal diet was also adequate to prevent perosis in this experiment.

Added Sulfate

Sulfur supplementation of the diet failed to affect body weight gains, feed consumptions and feed efficiencies of Large White turkeys. From three to seven weeks of age, feeding diets containing 0 or .10% potassium sulfate, body weight gains were 1,567 and 1,553 g, feed consumptions were 3,046 and 3,008 g, and feed efficiencies were .5135 and .5155, respectively (Table 20).

Interactions

No significant interactions were observed among the dietary variables from the data obtained in this study. Figure 8 contains a plot of body weight gain on level of added methionine from three to seven weeks of age at each level of choline chloride and/or potassium sulfate. The increase in body weight gain was consistent from added methionine, regardless of the addition of choline or sulfate.

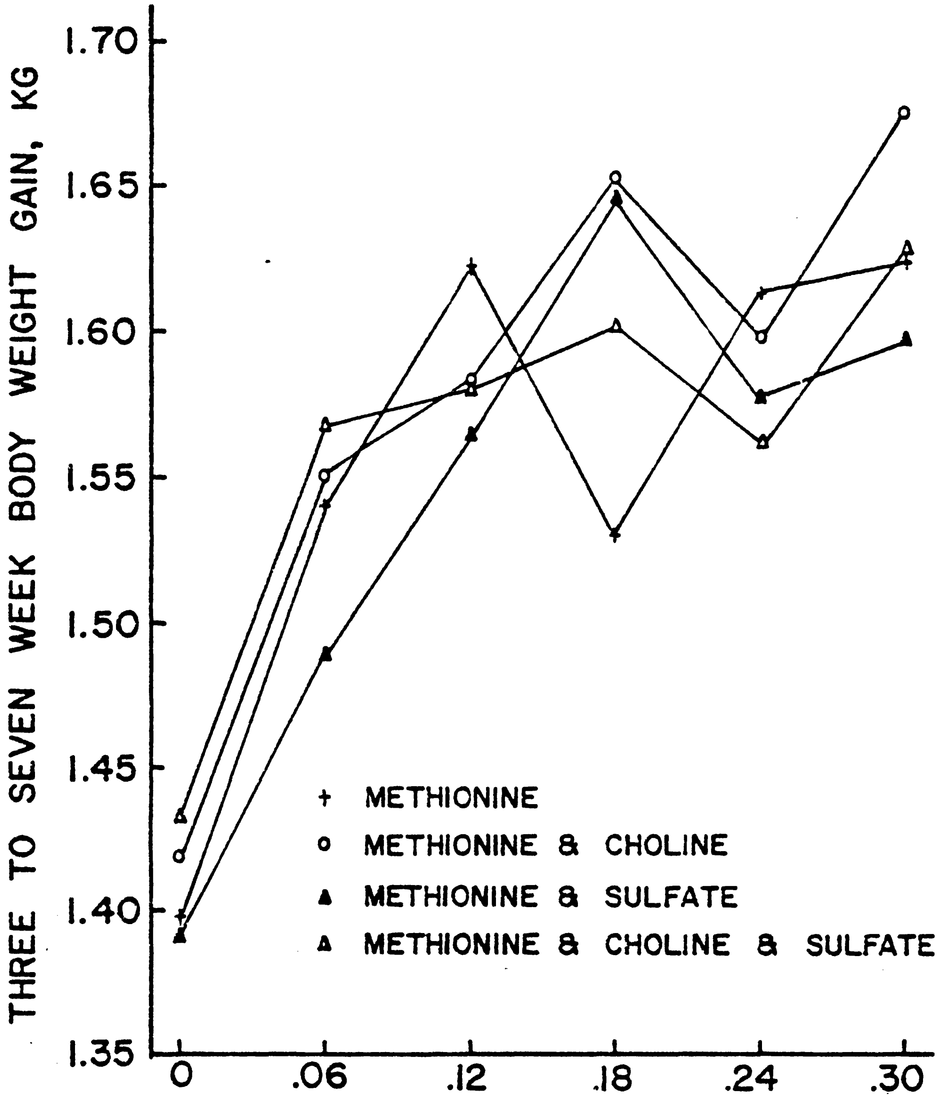

Figure 8. Plot of average three to seven-week body weight gains on level of added DL-methionine at each level of choline and/or sulfate of Large White turkeys (Experiment 4)

DISCUSSION

The failure of supplemental choline or sulfate to significantly affect the body weight gains, feed consumptions, and feed efficiencies in the presence and absence of supplemental methionine indicates that these two variables did not spare methionine in practical-type diets for young turkeys in this study. Results of the study indicate that methionine is required *per se* in a 21% protein corn-soybean meal diet for young turkeys and that the basal diet contained adequate amounts of choline and sulfate even though the diet was deficient in choline by NRC(1977) standards.

When studying the possible effects of choline on methionine, the primary focal point is methyl group transfer within the avian body. Vitamin B_{12}, folacin, and pyridoxine have all been shown to be involved in one-carbon transfers but were not considered factors in this study, as each was present in adequate amounts. The failure of choline addition to significantly promote growth, even in the absence of methionine, confirms that the basal diet was deficient in SAA or methionine *per se*. Pesti *et al*. (1979) reported that a 28% protein corn-soybean meal diet for poults was deficient in TSAA. The corn-soybean meal basal diet used in Experiments 3 and 4 contained 21% protein. The protein level was reduced in order to lower the choline level of the basal by decreasing the quantity of dehulled soybean meal. The results obtained point to a lack of methionine and carbon-skeletons supplied by methionine for cystine synthesis rather than a labile methyl group deficiency *per se*, as methionine supplementation consistently promoted significant improvement in body weight gain, feed consumption and feed efficiency over all levels of supplementation.

The basal diet used in these studies was not deficient in choline as no perosis problems occurred, even though the level was below the stated NRC (1977) requirements. Researchers have reported that the dietary requirement for choline to prevent perosis is higher than that to obtain maximal growth (Jukes, 1940a,b; Evans et al., 1942). The absence of a choline deficiency is further illustrated by the failure of choline to improve body weight gain or feed efficiency at adequate methionine levels in either experiment.

In Experiment 4, choline supplementation statistically improved($P<.05$) body weight gain and feed efficiency from four to five weeks of age indicating a slight sparing effect on methionine with choline replacing methionine as a methyl group donor. Baker et al. (1983) has demonstrated the fact that choline can exert a slight sparing effect on methionine when the level of methionine is deficient in the diet. This effect was not observed in the first experiment. When methionine levels were near or in excess of requirement levels, choline failed to affect the performance parameters in both experiments. Interpretation of this result indicates that actually methionine can exert a slight sparing effect on the dietary requirement for choline, though this would be impractical from an economic standpoint. Dean (1982) and Baker et al. (1983) have also reported a similar effect in ducklings and broilers, respectively.

Previous studies showing a choline sparing effect on methionine in practical-type rations have employed high-fat rations greater than 4% added fat or grease (Quillin _et al._, 1961; Pesti _et al._, 1979). Quillin _et al._ (1961), in reviewing the literature concerning choline and fat metabolism, stated that choline prevented fatty infiltration of the liver. The question brought forth is, does a diet containing at least 4% added fat need to be used to demonstrate a choline-methionine interrelationship? High-fat rations would increase the requirement for labile methyl groups (choline) thus a growth response from choline would be observed. The basal diet used in this study contained only 1% added fat. This low level of fat may, in part, explain the failure of obtaining a response to supplemental choline while other studies have shown positive results.

The failure to obtain either a significant methionine x choline interaction or a response from choline indicates that it does not spare methionine in practical-type diets for young turkeys. This type of diet is deficient in TSAA _per se_, not in labile methyl groups or choline. Baker _et al._ (1983) concluded that no measurable interdependence on the dietary requirments of choline or methionine occurs, due to the marginal deficiencies of methionine or choline in the diet.

The addition of inorganic sulfate to the diet failed to give a significant increase in body weight gain, feed consumption, or feed efficiency. The lack of response to sulfate, both in the absence and presence of adequate methionine indicates that the basal diet was adequate in sulfate, and that sulfate failed to spare methionine. This finding is in agreement with those of Kuhl and Sullivan (1973), Sasse and Baker (1974a) and Potter (1977). Miles et al. (1983) reported in studies using broiler chicks that sulfate per se is required. This statement can be questioned, as when sulfate was added to the basal diet in the absence of choline and methionine, a growth depression occurred.

The lack of response to sulfate supplementation of the basal diet indicates that the basal may not be deficient in sulfate or that a requirement for sulfur per se exists which is fulfilled by methionine rather than a direct requirement for the sulfate moiety. The trace mineral mix used in these experiments was sulfate-free, yet sulfur was contributed to the ration through the defluorinated phosphate, corn, soybean meal and additional intake through the water. Sasse and Baker (1974a,b) concluded that two conditions must be met in order to show consistent growth responses to sulfate. These conditions are 1) cystine (not methionine) must be first limiting in a sulfur amino acid deficient diet, and 2) that dietary sulfate must be less than 200 ppm. Since cystine is first-limiting in practical-type diets for poultry, the level of dietary sulfate becomes critical. In commercial practice, the sulfate level of rations is well above 200 ppm; many of the minerals are added as sulfate salts such as iron sulfate, copper sulfate and zinc sulfate.

Sulfate supplementation failed to promote growth, feed consumption or feed efficiency significantly and no methionine x sulfate interactions were observed. These findings point to the conclusion that sulfate does not replace methionine or sulfur amino acids in a practical-type diet for turkeys.

From two experiments using broilers, Miles et al. (1983) concluded that sulfate must be present in the diet in order for choline to spare a maximum amount of methionine. The authors obtained a significant sulfate x choline interaction, as supplementation with sulfate in the presence of choline resulted in a greater growth response than when either was added alone. In the same studies, Miles et al. (1983) also obtained a significant growth response from added choline, indicating a deficiency of lable methyl groups in the basal diet, while a growth depression occurred when sulfate was added alone. The results from Experiments 3 and 4 failed to show any two-way or threeway interactions among methionine, choline or sulfate. Choline or sulfate did not replace methionine or SAA when the two were added to the diet together. A growth depression ($P>.05$) was observed in Experiment 3, similar to that observed by Miles et al. (1983). The failure to reproduce these results found with broilers may in part be due to the age period during which the studies were conducted. The significant interactions observed by Miles et al. (1983) were based on body weight gains and feed conversion data of broilers from 0 to 2 weeks of age. At this age, chicks are growing rapidly, and increased amounts of choline to prevent perosis (Jukes, 1940 a,b) and sulfate or methionine as sources of sulfur for synthesis of the chondroitin matrix of cartilage (Dziewiatkowski, 1951), are required. Hence, supplementation of sulfate and choline may be beneficial in sparing methionine in the young bird due to its physiological state, as the need for methionine to be metabolized into choline or sulfur is decreased, leaving more methionine available for protein synthesis. Miles et al. (1983) was unable to detect any significant interactions based upon the same parameters at three weeks of age. Experiments 3 and 4 were conducted using poults from 3 to 8 weeks of age. At this age period, the bird has a major requirement for methionine *per se* for protein synthesis, therefore choline or sulfate supplementation may be of no value.

The difficulty of trying to detect the effects of choline or sulfate supplementation on methionine in practical-type poultry diets parallels that in trying to detect differences in the biopotencies of methionine products in bioassays. Practical-type diets contain only marginal deficiencies of the nutrients under test, allowing much smaller growth responses, thus any differences are difficult to detect, and this may account for the variation of results among studies. It has been well established that choline can replace methionine as a donor of labile methyl groups. Studies with chemically defined diets must be conducted in order to quantitaie the level at which the requirement for methionine *per se* is met and when it begins to function as a methyl methyl group donor. If this level can be determined, choline may be used to replace methionine which is metabolized for labile methyl groups and be of economic value to the industry. The results from this study using a practical-type diet for young turkeys indicate that choline and/or sulfate are unable to spare methionine *per se*.

SUMMARY AND CONCLUSIONS

Two methionine bioassays were conducted in order to determine the relative potencies of MENA, MHAC, and MHAA with respect to DL on an equimolar basis using the slope ratio procedure. Increments of .06% methionine from each of the four sources were added in the place of glucose monohydrate to a practical corn-soybean meal basal diet (28% protein) to form diets containing 0 to .30% added methionine. The resulting twenty four diets were fed to poults from one day to seven weeks of age in each experiment.

On the average, MENA was 104.7 (86.1, 123.3) MHAC 101.8 (83.5, 120.1), and MHAA 92.5% (74.9, 110.1) as potent as DL (with 95% fiducial limits). Based upon the combined six-week body weight data from both experiments (weighted means procedure, Finney, 1964), no differences were detected among the biopotencies of the methionine products.

The extra one degree of freedom associated with the non-linear model decreased the residual variation among diets 4.8 and 10.7% in Experiments 1 and 2, respectively. The lack of an appropriate error term for a relative potency estimate with the non-linear model makes it inferior to the slope ratio procedure. In using the slope ratio procedure, a large growth response (greater than 20%) must be obtained in order to increase the sensitivity and to detect differences in methionine products within an experiment. The combination of data by the weighted means procedure gives a more reliable potency estimate than an estimate from an individual experiment.

Two additional experiments were also conducted to examine methionine, choline and sulfate interrelationships in a practical-type diet for turkeys. In a factorial arrangement, DL-methionine, choline chloride and potassium sulfate were added to a 21% protein diet composed primarily of ground yellow corn and dehulled soybean meal. The experiments were conducted using medium-type or Large White turkeys from 4 to 8 and 3 to 7 weeks of age, respectively. Average body weight, body weight gain, and feed consumption and feed efficiency were determined weekly for each pen of turkeys.

Cumulative body weight gains, feed consumptions and feed efficiencies were significantly improved ($P<.001$) by the addition of DL-methionine to all diets in both experiments. Sulfate supplementation failed to affect the above parameters in either experiment even in the absence of methionine indicating that the basal was deficient in methionine *per se*. Choline failed to spare methionine. Addition of choline to the diet produced an increase ($P<.05$) in growth and feed efficiency only between 4 and 5 weeks of age in the second experiment, and no significant effect in the first. Results indicate that a practical corn-soybean meal diet (21% protein) contains an adequate amount of sulfate and that sulfate is unable to spare methionine. When choline and sulfate were added to the diet together, they were unable to spare methionine in a practical-type diet for young turkeys 3 to 8 weeks of age.

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APPENDIX

Table 1. Regression coefficient from 96 pens of poults (48 of each sex) fed diets containing five levels of methionine (.06, .12, .18, .24 and .30%) from four sources on a molar basis based on a slope ratio bioassay where regression includes an X value for "blank" (Experiment 1)

Week	Intercept	Blank	Sex	DL-Meth	MENA	MHAC	MHAA	SE	F
	b_0	b_a	b_5	b_1	b_2	b_3	b_4		
				Body weight, g					
2	252.2	-4.7	-20.2	201.8	190.2	197.5	162.5	24.67	37.29
4	733.2	-37.9	-95.5	594.1	632.9	557.5	530.7	53.51	107.09
6	1649.4	-97.1	-270.1	1228.2	1287.9	1262.9	1103.4	129.16	106.24
7	2235.4	-139.7	-384.0	1189.1	1275.7	1292.3	1202.3	171.90	93.62
				Body weight gain, g					
0-2	191.6	-5.1	-19.8	202.3	189.8	195.6	163.2	24.51	37.65
0-4	672.6	-38.3	-95.0	594.5	632.4	555.7	531.4	52.74	110.08
0-6	1588.8	-97.5	-269.7	1228.4	1287.5	1261.1	1104.3	128.53	107.22
0-7	2175.0	-140.0	-383.7	1189.2	1275.6	1289.9	1203.2	171.25	94.28
2-4	481.0	-33.3	-75.3	392.2	442.5	360.1	368.2	44.56	83.38
4-6	916.2	-59.0	-174.7	633.4	655.1	705.1	573.1	100.35	61.40
6-7	586.1	-42.7	-113.9	-38.8	-12.8	29.1	98.4	97.12	16.51
				Feed consumption, g					
0-2	274.7	-5.7	-26.1	145.7	131.0	131.2	94.7	28.23	20.89
0-4	1052.5	-47.6	-127.1	489.9	540.7	433.7	458.3	75.12	60.65
0-6	2567.4	-87.9	-361.7	1106.3	1124.9	997.3	983.9	184.60	63.22
0-7	3619.5	-124.3	-536.4	1247.0	1363.8	1197.4	1160.0	242.22	71.84
2-4	777.7	-41.8	-101.0	344.2	409.9	302.7	363.7	56.83	65.25
4-6	1514.8	-40.3	-234.6	616.8	585.6	563.9	525.3	144.66	38.19
6-7	1052.1	-36.5	-174.8	141.0	237.9	200.3	176.3	102.31	35.21
				Feed efficiency					
0-2	.6976	-.0075	-.0008	.3239	.3267	.3430	.3341	.0417	31.87
0-4	.6390	-.0103	-.0098	.2473	.2510	.2477	.2132	.0370	24.34
0-6	.6186	-.0209	-.0145	.1967	.2123	.2383	.1813	.0350	27.88
0-7	.6006	-.0224	-.0152	.1144	.1180	.1527	.1347	.0304	21.26
2-4	.6181	-.0116	-.0140	.2171	.2272	.2117	.1738	.0511	10.75
4-6	.6056	-.0273	-.0191	.1568	.1795	.2273	.1533	.0554	11.28
6-7	.5581	-.0249	-.0209	-.1212	-.1386	-.0803	-.0038	.0767	1.86

Table 2. Relative potency of methionine products to DL-methionine based on slope ratio bioassay of data from 96 pens of poults (48 of each sex) fed five levels of methionine (.06, .12, .18, .24 and .30%) from four sources on a molar basis where regression includes an X value for "blank" (Experiment 1)

Weeks	MENA	MHAC	MHAA	SE as % of standard
		Body weight, g		
2	94.3	97.9	80.5	12.2
4	106.5	93.8	89.3	9.0
6	104.9	102.8	89.8	10.5
7	107.3	108.7	101.1	14.5
		Body weight gain, g		
0-2	93.8	96.7	80.7	12.1
0-4	106.4	93.5	89.4	8.9
0-6	104.8	102.7	89.9	10.5
0-7	107.3	108.5	101.2	14.4
2-4	112.8	91.8	93.9	11.4
4-6	103.4	111.3	90.5	15.8
6-7	***	***	***	***
		Feed consumption, g		
0-2	89.9	90.0	65.0	19.4
0-4	110.4	88.5	93.5	15.3
0-6	101.7	90.1	88.9	16.7
0-7	109.4	96.0	93.0	19.4
2-4	119.1	87.9	105.7	16.5
4-6	94.9	91.4	85.2	23.5
6-7	168.7	142.1	125.0	72.6
		Feed efficiency		
0-2	100.9	105.9	103.1	12.9
0-4	101.5	100.2	86.2	15.0
0-6	107.9	121.1	92.2	17.8
0-7	103.1	133.5	117.7	26.6
2-4	104.7	97.5	80.1	23.5
4-6	114.5	145.0	97.8	35.3
6-7	***	***	***	***

***Negative coefficient for standard.

Table 3. Regression coefficient from 96 pens of poults (48 of each sex) fed diets containing five levels of methionine (.06, .12, .18, .24 and .30%) from four sources on a molar basis based on a slope ratio bioassay where regression includes an X value for "blank" (Experiment 2)

Weeks	Intercept	Blank	Sex	DL-Meth	MENA	MHAC	MHAA	SE	F
	b_0	b_a	b_5	b_1	b_2	b_3	b_4		
				Body weight, g					
2	225.4	-4.2	-20.5	113.8	122.7	107.0	151.1	24.28	22.07
4	685.0	-29.0	-88.9	302.4	300.1	243.4	349.9	58.12	44.62
6	1576.8	-61.6	-226.9	351.6	359.4	324.4	442.2	113.88	53.09
7	2167.3	-53.9	-337.6	472.9	467.6	313.0	502.2	147.68	63.55
				Body weight gain, g					
0-2	164.4	-3.5	-19.6	113.9	125.6	111.6	155.3	23.89	22.44
0-4	623.9	-28.3	-88.0	302.5	303.2	248.2	354.3	57.83	44.65
0-6	1515.5	-60.7	-226.0	353.6	362.8	330.3	447.9	113.96	52.70
0-7	2106.1	-52.9	-336.7	473.4	471.4	318.6	507.7	147.74	63.21
2-4	459.5	-24.8	-68.4	188.6	177.6	136.6	198.9	42.17	43.79
4-6	891.8	-32.5	-138.1	50.5	58.5	82.1	93.7	73.82	40.11
6-7	590.6	7.8	-110.7	120.4	108.7	-11.6	59.6	81.61	20.52
				Feed consumption, g					
0-2	240.2	-4.3	-19.4	88.7	104.9	79.9	130.6	31.97	10.00
0-4	999.1	-31.2	-104.0	294.5	320.1	250.3	356.9	86.59	24.87
0-6	2584.2	-63.9	-323.4	337.6	497.6	296.7	515.4	184.74	37.88
0-7	3681.0	-73.4	-510.5	370.4	531.9	234.1	494.1	243.62	50.34
2-4	758.9	-26.9	-84.6	205.7	215.1	170.3	226.3	62.76	28.50
4-6	1585.1	-32.8	-219.4	42.6	178.0	47.1	158.6	116.52	39.84
6-7	1097.0	-9.5	-187.0	32.2	33.3	-63.7	-22.9	100.60	37.36
				Feed efficiency					
0-2	.6850	-.0056	-.0242	.2031	.2027	.2229	.2502	.0406	19.05
0-4	.6243	-.0109	-.0227	.1136	.0966	.0919	.1271	.0302	15.58
0-6	.5863	-.0101	-.0148	.0597	.0251	.0639	.0584	.0239	9.81
0-7	.5717	-.0029	-.0124	.0720	.0458	.0528	.0640	.0227	7.35
2-4	.6054	-.0132	-.0231	.0822	.0580	.0456	.0803	.0362	8.38
4-6	.5624	-.0092	-.0105	.0173	-.0278	.0397	.0058	.0312	2.82
6-7	.5372	.0152	-.0079	.0970	.0896	.0137	.0685	.0535	1.11

Table 4. Relative potency of methionine products to DL-methionine based on slope line bioassay of data from 96 pens of poults (48 of each sex) fed five levels of methionine (.06, .12, .18, .24 and .30%) from four sources on a molar basis where regression includes an X value for "blank" (Experiment 2)

Weeks	MENA	MHAC	MHAA	SE as % of standard
		Body weight, g		
2	107.8	94.0	132.8	21.3
4	99.2	80.5	115.7	19.2
6	102.2	92.3	125.8	32.4
7	98.9	66.2	106.2	31.2
		Body weight gain, g		
0-2	110.3	98.0	136.3	21.0
0-4	100.2	82.0	117.1	19.1
0-6	102.6	93.4	126.7	32.2
0-7	99.6	67.3	107.2	31.2
2-4	94.2	72.4	105.5	22.4
4-6	115.8	162.6	185.5	146.2
6-7	90.3	-9.6	49.5	67.8
		Feed consumption, g		
0-2	118.3	90.1	147.2	36.0
0-4	108.7	85.0	121.2	29.4
0-6	147.4	87.9	152.7	54.7
0-7	143.6	63.2	133.4	65.8
2-4	104.6	82.8	110.0	30.5
4-6	417.8	110.6	372.3	273.5
6-7	103.4	-198.1	-77.3	312.4
		Feed efficiency		
0-2	99.8	109.7	123.2	20.0
0-4	85.0	80.9	111.9	26.6
0-6	42.0	107.0	97.8	40.0
0-7	63.6	73.3	88.9	31.5
2-4	70.6	55.5	97.7	44.0
4-6	-161.1	229.5	33.5	180.3
6-7	92.4	14.1	70.6	57.0

Table 5. Mean, standard deviation, coefficient of variation and difference required for significance among data for body weights, body weight gains, feed consumptions and feed efficiencies based on 70 degrees of freedom for error (Experiment 3)

Age, weeks	Mean	Standard deviation	Coefficient of variation	Difference required for significance 16 vs 16 pens[1]		48 vs 48 pens[2]	
	g	g	%	g	%	g	%
			Body weights				
5	631.6	32.7	5.18	23.1	3.7	13.3	2.1
6	905.9	43.1	4.76	30.4	3.4	17.6	1.9
7	1260.0	63.2	5.02	44.6	3.5	25.8	2.0
8	1597.6	78.7	4.92	55.6	3.5	32.1	2.0
			Body weight gains				
4-5	244.9	13.5	5.51	9.5	3.9	5.5	2.2
4-6	519.2	27.3	5.26	19.3	3.7	11.1	2.1
4-7	873.3	48.1	5.51	34.0	3.9	19.6	2.2
4-8	1210.9	64.9	5.36	45.8	3.8	26.5	2.2
5-6	274.3	19.0	6.92	13.4	4.9	7.8	2.8
6-7	354.1	35.5	10.00	25.1	7.1	14.5	4.1
7-8	337.6	32.1	9.51	22.7	6.7	13.1	3.9
			Feed consumptions				
4-5	426.9	21.2	4.96	15.0	3.5	8.6	2.0
4-6	978.8	49.0	5.00	34.6	3.5	20.0	2.0
4-7	1715.3	74.4	4.34	52.5	3.1	30.4	1.8
4-8	2554.6	105.1	4.11	74.2	2.9	42.9	1.7
5-6	551.9	31.0	5.61	21.9	4.0	12.6	2.3
6-7	736.5	35.8	4.87	25.3	3.4	14.6	2.0
7-8	839.3	43.5	5.18	30.7	3.7	17.7	2.1
			Feed efficiencies				
4-5	.5726	.0170	2.95	.0120	2.1	.0069	1.2
4-6	.5293	.0172	3.26	.0121	2.3	.0070	1.3
4-7	.5073	.0172	3.39	.0121	2.4	.0070	1.4
4-8	.4723	.0136	2.88	.0096	2.0	.0055	1.2
5-6	.4956	.0270	5.44	.0191	3.8	.0110	2.2
6-7	.5778	.0347	9.26	.0245	5.1	.0142	3.0
7-8	.4009	.0271	6.76	.0191	4.8	.0110	2.8

[1] The differences required for significance between averages of methionine levels are calculated by:

$$\text{Difference} = \frac{st\sqrt{2}}{\sqrt{n}} = \frac{s(1.997)\sqrt{2}}{\sqrt{16}} = \frac{s(1.997)}{\sqrt{8}} = .706s$$

[2] The differences required for significance between averages of choline chloride levels on averages of potassium sulfate levels are calculated by:

$$\text{Difference} = \frac{st\sqrt{2}}{\sqrt{n}} = \frac{s(1.997)\sqrt{2}}{\sqrt{48}} = \frac{s(1.997)}{\sqrt{24}} = .408s$$

Table 6. Effects of varying levels of methionine in diets of poults on body weight, body weight gain, feed consumption and feed efficiency (Experiment 3)

Age,	DL-methionine, %[1]					
weeks	0	.06	.12	.18	.24	.30
			Body weight, g			
5	599.3	617.7	619.4	665.0	648.5	639.6
6	840.4	884.1	895.4	961.4	928.2	925.9
7	1158.8	1228.0	1249.9	1343.0	1295.7	1284.5
8	1463.6	1575.2	1585.4	1703.2	1626.0	1632.3
			Body weight gain, g			
4-5	208.6	233.8	242.4	267.8	257.8	258.9
4-6	449.8	500.2	518.3	564.1	537.6	545.2
4-7	768.1	844.2	872.9	945.8	905.1	903.8
4-8	1072.9	1191.3	1208.3	1306.0	1235.3	1251.6
5-6	241.1	266.4	275.9	296.3	279.6	286.3
6-7	318.4	343.9	354.6	381.7	367.5	358.5
7-8	304.8	347.1	335.4	360.2	330.2	347.8
			Feed consumption, g			
4-5	409.3	426.6	423.3	446.4	432.1	423.8
4-6	941.1	982.0	970.4	1025.4	982.3	971.9
4-7	1640.6	1727.6	1699.3	1795.5	1718.5	1710.3
4-8	2435.8	2575.9	2524.7	2678.1	2556.7	2556.4
5-6	531.9	555.3	547.1	579.0	550.2	548.1
6-7	699.5	745.7	728.9	770.0	736.2	738.4
7-8	795.2	848.3	825.5	882.5	838.2	846.1
			Feed efficiency			
4-5	.5098	.5480	.5720	.5990	.5964	.6105
4-6	.4775	.5087	.5337	.5491	.5464	.5603
4-7	.4665	.4873	.5126	.5250	.5254	.5272
4-8	.4390	.4611	.4775	.4860	.4821	.4884
5-6	.4526	.4784	.5039	.5106	.5066	.5214
6-7	.4523	.4590	.4840	.4920	.4966	.4832
7-8	.3821	.4080	.4054	.4066	.3934	.4098

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[1] Each value represents the average of 16 pens (8 of each sex) with 9 poults per pen.

Table 6 (cont.). Effects of varying levels of methionine in diets of poults on body weight, body weight gain, feed consumption and feed efficiency (Experiment 3)

Age, weeks	Increase over basal, g					Increase over basal, %				
	.06	.12	.18	.24	.30	.06	.12	.18	.24	.30
			Body weight							
5	18.4	20.1	65.7***	49.2***	40.3***	3.1	3.4	11.0	8.2	6.7
6	43.7**	55.0***	121.0***	87.8***	85.5***	5.2	6.5	14.4	10.4	10.2
7	69.2**	91.1***	184.2***	136.9***	125.7***	6.0	7.9	15.9	11.8	10.8
8	111.6***	121.8***	239.6***	162.4***	168.7***	7.6	8.3	16.4	11.1	11.5
			Body weight gain, g							
4-5	25.2***	33.8***	59.2***	49.2***	50.3***	12.1	16.2	28.4	23.6	24.1
4-6	50.4***	68.5***	114.3***	87.8***	95.4***	11.2	15.2	25.7	19.5	21.2
4-7	76.1***	104.8***	177.7***	137.0***	135.7***	9.9	13.6	23.1	17.8	17.7
4-8	118.4***	135.4***	233.1***	162.4***	178.7***	11.0	12.6	21.7	15.1	16.7
5-6	25.3***	34.8***	55.2***	38.6***	45.2***	10.5	14.4	22.9	16.0	18.7
6-7	25.5*	36.2**	63.3***	49.1***	40.1***	8.0	11.4	19.9	15.4	12.6
7-8	42.3***	30.6**	55.4***	25.4**	43.0***	13.9	10.0	18.2	8.3	14.1
			Feed consumption, g							
4-5	17.3*	14.0	37.1***	22.8**	14.5	4.2	3.4	9.1	5.6	3.5
4-6	40.9*	29.3	84.3***	41.2*	30.8	4.3	3.1	9.0	4.4	3.3
4-7	87.0**	58.7*	154.9***	77.9**	69.7*	5.3	3.6	9.4	4.7	4.2
4-8	140.1***	88.9*	242.3***	120.9**	120.6**	5.8	3.6	9.9	5.0	5.0
5-6	23.4*	15.2	47.1***	18.3	16.2	4.4	2.9	8.9	3.4	3.0
6-7	46.2***	29.4*	70.6***	36.7**	38.9**	6.6	4.2	10.1	5.2	5.6
7-8	53.1***	30.3*	87.3***	43.0**	50.9**	6.7	3.8	11.0	5.4	6.4
			Feed efficiency							
4-5	.0382***	.0622***	.0892***	.0866***	.1007***	7.5	12.2	17.5	17.0	19.8
4-6	.0312***	.0562***	.0716***	.0689***	.0828***	6.5	11.8	15.0	14.4	17.3
4-7	.0208**	.0461***	.0585***	.0589***	.0607**	4.5	9.9	12.5	12.6	13.0
4-8	.0221**	.0385***	.0470***	.0431***	.0494***	5.0	8.8	10.7	9.8	11.3
5-6	.0258**	.0513***	.0580***	.0540***	.0688***	5.7	11.3	12.8	11.9	15.2
6-7	.0067	.0317*	.0397**	.0443***	.0309*	1.5	7.0	8.8	9.8	6.8
7-8	.0259**	.0233*	.0245**	.0113	.0277**	6.8	6.1	6.4	3.0	7.2

[*] P<.05.

[**] P<.01.

[***] P<.001.

Table 7 . Effects of adding choline chloride to diets of poults on body weight, body weight gain, feed consumption and feed efficiency (Experiment 3)

Age, weeks	Choline chloride, %[1]		Difference	Percent increase
	0	.2		
Body weight, g				
5	630.4	632.8	2.4	0.4
6	902.9	908.9	6.0	0.7
7	1256.2	1263.8	7.6	0.6
8	1598.8	1596.3	-2.5	-0.2
Body weight gain, g				
4-5	243.7	246.1	2.4	1.0
4-6	516.2	522.2	6.0	1.2
4-7	869.5	877.1	7.6	0.9
4-8	1212.2	1209.6	-2.6	0.2
5-6	272.5	276.1	3.6	1.3
6-7	353.3	354.9	1.6	0.5
7-8	342.7	332.5	-10.2	-3.0
Feed consumption, g				
4-5	426.1	427.7	1.6	0.4
4-6	974.8	982.9	8.1	0.8
4-7	1707.8	1722.8	15.0	0.9
4-8	2547.9	2561.2	13.3	0.5
5-6	548.7	555.2	6.5	1.2
6-7	733.0	739.9	6.9	0.9
7-8	840.2	838.4	-1.8	-0.2
Feed efficiency				
4-5	.5707	.5747	.0042	0.7
4-6	.5284	.5302	.0018	0.3
4-7	.5073	.5073	.0000	0.0
4-8	.4737	.4710	-.0027	-0.6
5-6	.4955	.4957	.0002	0.0
6-7	.4788	.4769	-.0019	-0.4
7-8	.4054	.3964	-.0090	-2.2

[1] Each value represents the average of 48 pens (24 of each sex) with 9 poults per pen.

Table 8. Effects of adding potassium sulfate to diets of poults on body weight, body weight gain, feed consumption and feed efficiency (Experiment 3)

Age, weeks	Potassium sulfate, %[1] 0	 .1	Difference	Percent increase
		Body weight, g		
5	629.0	634.2	5.2	0.8
6	902.4	909.4	7.0	0.8
7	1257.2	1262.8	5.6	0.4
8	1591.5	1603.6	12.1	0.8
		Body weight gain, g		
4-5	243.1	246.7	3.6	1.5
4-6	516.5	521.9	5.4	1.0
4-7	871.3	875.3	4.0	0.5
4-8	1205.7	1216.1	10.4	0.9
5-6	273.4	275.2	1.8	0.7
6-7	354.8	353.4	-1.4	-0.4
7-8	334.4	340.8	6.4	1.9
		Feed consumption, g		
4-5	425.2	428.6	3.4	0.8
4-6	975.7	982.0	6.3	0.6
4-7	1709.0	1721.6	12.6	0.7
4-8	2548.8	2560.3	11.5	0.5
5-6	550.5	553.3	2.8	0.5
6-7	733.3	739.6	6.3	0.9
7-8	839.8	838.8	-1.0	-1.0
		Feed efficiency		
4-5	.5707	.5745	.0038	0.7
4-6	.5281	.5304	.0023	0.4
4-7	.5078	.5068	-.0010	-0.2
4-8	.4713	.4734	.0021	0.4
5-6	.4953	.4959	.0006	0.1
6-7	.4805	.4751	-.0054	-1.1
7-8	.3971	.4047	.0076	1.9

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[1] Each value represents the average of 48 pens (24 of each sex) with 9 poults per pen.

Table 9. Mean, standard deviation, coefficient of variation and difference required for significance among data for body weights, body weight gains, feed consumptions and feed efficiencies based on 70 degrees of freedom for error (Experiment 4)

Age, weeks	Mean	Standard deviation	Coefficient of variation	Difference required for significance 16 vs 16 pens[1]		48 vs 48 pens[2]	
	g	g	%	g	%	g	%
			Body weights				
4	632.4	31.87	5.04	22.5	3.6	13.0	2.1
5	990.2	45.84	4.63	32.4	3.3	18.7	1.9
6	1444.2	61.06	4.23	43.1	3.0	24.9	1.7
7	1935.5	69.89	3.61	49.3	2.5	28.5	1.5
			Body weight gains				
3-4	257.0	12.33	4.80	8.7	3.4	5.0	2.0
3-5	614.9	26.19	4.26	18.5	3.0	10.7	1.7
3-6	1068.9	43.87	4.10	31.0	2.9	17.9	1.7
3-7	1560.1	56.83	3.64	40.1	2.6	23.2	1.5
4-5	357.9	19.82	5.54	14.0	3.9	8.1	2.3
5-6	454.0	25.94	5.71	18.3	4.0	10.6	2.3
6-7	491.3	25.06	5.10	17.7	3.6	10.2	2.1
			Feed consumptions				
3-4	459.7	19.73	4.30	14.0	3.0	8.1	1.8
3-5	1098.0	44.23	4.03	31.2	2.8	18.0	1.6
3-6	1969.3	80.33	4.08	56.7	2.9	32.8	1.7
3-7	3027.2	112.1	3.70	79.1	2.6	45.7	1.5
4-5	638.3	28.39	4.45	20.0	3.1	11.6	1.8
5-6	871.2	41.08	4.72	29.0	3.3	16.8	1.9
6-7	1057.9	40.98	3.87	28.9	2.7	16.7	1.6
			Feed efficiencies				
3-4	.5588	.1088	3.36	.0133	2.4	.0077	1.4
3-5	.5591	.0102	1.83	.0072	1.3	.0042	0.7
3-6	.5420	.0088	1.63	.0062	1.1	.0036	0.7
3-7	.5145	.0109	2.13	.0077	1.5	.0044	0.9
4-5	.5594	.0182	3.25	.0128	2.3	.0074	1.3
5-6	.5202	.0168	3.24	.0119	2.3	.0068	1.3
6-7	.4633	.0206	4.46	.0145	3.1	.0084	1.8

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[1] The differences required for significance between averages of methionine levels are calculated by:

$$\text{Difference} = \frac{st\sqrt{2}}{\sqrt{n}} = \frac{s(1.997)\sqrt{2}}{\sqrt{16}} = \frac{s(1.997)}{\sqrt{8}} = .706s$$

[2] The differences required for significance between averages of choline chloride levels on averages of potassium sulfate levels are calculated by:

$$\text{Difference} = \frac{st\sqrt{2}}{\sqrt{n}} = \frac{s(1.997)\sqrt{2}}{\sqrt{48}} = \frac{s(1.997)}{\sqrt{24}} = .408s$$

Table 10. Effects of varying levels of methionine in diets of poults on body weight, body weight gain, feed consumption and feed efficiency (Experiment 4)

Age, weeks	DL-Methionine, %[1] 0	.06	.12	.18	.24	.30
			Body weight, g			
4	608.4	621.6	639.5	636.0	642.1	646.6
5	924.8	977.0	1005.5	1003.3	1011.7	1019.2
6	1333.7	1427.9	1471.1	1473.0	1463.0	1496.8
7	1781.5	1908.5	1966.5	1980.2	1964.5	2011.6
			Body weight gain, g			
3-4	237.9	250.0	259.9	263.1	265.1	266.0
3-5	554.3	605.4	625.8	630.4	634.7	638.5
3-6	963.2	1056.4	1091.5	1100.1	1086.1	1116.1
3-7	1411.0	1537.0	1587.1	1607.3	1587.5	1630.9
4-5	316.4	355.4	366.0	367.2	369.6	372.5
5-6	409.0	450.9	465.6	469.7	451.4	477.6
6-7	447.8	480.6	495.6	507.2	501.4	514.8
			Feed consumption, g			
3-4	449.9	454.8	463.6	461.1	463.5	465.4
3-5	1063.8	1099.3	1107.5	1100.1	1106.8	1110.7
3-6	1897.2	1978.9	1995.0	1979.6	1968.6	1996.5
3-7	2908.0	3045.3	3060.2	3046.4	3035.1	3067.9
4-5	613.9	644.5	643.9	639.0	643.2	645.3
5-6	833.3	879.5	887.5	879.5	861.8	885.8
6-7	1010.9	1066.5	1065.2	1066.9	1066.5	1071.4
			Feed efficiency			
3-4	.5282	.5510	.5602	.5703	.5717	.5715
3-5	.5202	.5502	.5645	.5722	.5730	.5745
3-6	.5070	.5334	.5466	.5550	.5513	.5585
3-7	.4842	.5041	.5182	.5269	.5226	.5310
4-5	.5144	.5502	.5677	.5734	.5738	.5766
5-6	.4897	.5123	.5242	.5335	.5235	.5382
6-7	.4411	.4499	.4651	.4745	.4696	.4796

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[1] Each value represents the average of 16 pens (8 of each sex) with 9 male and 8 female poults per pen.

Table 10 (cont.). Effects of varying levels of methionine in diets of poults on body weight, body weight gain, feed consumption and feed efficiency (Experiment 4)

Age,	Increase over basal, g					Increase over basal, %				
weeks	.06	.12	.18	.24	.30	.06	.12	.18	.24	.30
				Body weight						
4	13.2	31.1**	27.6*	33.7**	38.2**	2.2	5.1	4.5	5.5	6.3
5	53.0**	80.7***	78.5***	86.9***	94.4***	5.7	8.7	8.5	9.4	10.2
6	94.2***	137.4***	139.3***	129.3***	163.1***	7.1	10.3	10.4	9.7	12.2
7	127.0***	185.3***	198.7***	183.0***	230.1***	7.1	10.4	11.2	10.3	12.9
				Body weight gain						
3-4	12.1*	22.0***	25.2***	27.2***	28.1***	5.1	9.2	10.6	11.4	11.8
3-5	51.1***	71.5***	76.1***	80.4***	84.2***	9.2	12.9	13.7	14.5	15.2
3-6	93.2***	128.3***	136.9***	122.9***	152.9***	9.7	13.3	14.2	12.8	15.9
3-7	126.0***	176.1***	196.3***	176.5***	219.9***	8.9	12.5	13.9	12.5	15.6
4-5	39.0***	49.6***	50.8***	53.2***	56.1***	12.3	15.7	16.1	16.8	17.7
5-6	41.9***	56.6***	60.7***	42.4***	68.6***	10.2	13.8	14.8	10.4	16.8
6-7	32.8***	47.8***	59.4***	52.6***	67.0***	7.3	10.7	13.3	12.0	15.0
				Feed consumption						
3-4	4.9	13.7	11.2	13.6	15.5*	1.1	3.0	2.5	3.0	3.4
3-5	35.5*	43.7**	36.3*	43.0**	46.9**	3.3	4.1	3.4	4.0	4.4
3-6	81.7**	97.8***	82.4**	71.4**	99.3***	4.3	5.2	4.3	3.8	5.2
3-7	137.3***	152.2***	138.4***	127.1**	159.9***	4.7	5.2	4.8	4.4	5.5
4-5	30.6**	30.0**	25.1**	29.3**	31.4	5.0	4.9	4.1	4.8	5.1
5-6	46.2**	54.2***	46.2**	28.5	52.5***	5.5	6.5	5.5	3.4	6.3
6-7	55.6***	54.3***	56.0***	55.6***	60.5***	5.5	5.4	5.5	5.5	6.0
				Feed efficiency						
3-4	.0228**	.0320***	.0421***	.0435***	.0433***	4.3	6.1	8.0	8.2	8.2
3-5	.0300***	.0443***	.0520***	.0528***	.0543***	5.8	8.5	10.0	10.1	10.4
3-6	.0264**	.0396***	.0480***	.0443***	.0515***	5.2	7.8	9.5	8.7	10.2
3-7	.0199***	.0340***	.0427***	.0384***	.0468***	4.1	7.0	8.8	7.9	9.7
4-5	.0358***	.0533***	.0590***	.0594***	.0622***	9.0	10.4	11.5	11.5	12.1
5-6	.0226***	.0345***	.0438***	.0338***	.0485***	4.6	7.0	8.9	6.9	9.9
6-7	.0088	.0240**	.0334***	.0285***	.0385***	2.0	5.4	7.6	6.5	8.7

*P<.05.

**P<.01.

***P<.001.

Table 11. Effects of adding choline chloride to diets of poults on body weight, body weight gain, feed consumption and feed efficiency (Experiment 4)

Age, weeks	Choline chloride, %[1]		Difference	Percent increase
	0	.2		
Body weight, g				
4	630.0	634.7	4.7	0.7
5	982.8	997.6	14.8	1.5
6	1435.4	1453.1	17.7	1.2
7	1924.2	1946.9	22.7	1.2
Body weight gain, g				
3-4	255.4	258.6	3.2	1.3
3-5	608.1	621.6	13.5*	2.2*
3-6	1060.7	1077.1	16.4	1.5
3-7	1549.5	1570.8	21.3	1.4
4-5	352.8	362.9	10.1*	2.9*
5-6	452.6	455.5	2.9	0.6
6-7	488.7	493.8	5.1	1.0
Feed consumption, g				
3-4	458.5	461.0	2.5	0.5
3-5	1093.6	1102.5	8.9	0.8
3-6	1063.1	1975.4	12.3	0.6
3-7	3019.5	3034.8	15.3	0.5
4-5	635.1	641.5	6.4	1.0
5-6	869.5	872.9	3.4	0.4
6-7	1056.4	1059.4	3.0	0.3
Feed efficiency				
3-4	.5566	.5610	.0044	0.8
3-5	.5553	.5629	.0076***	1.4***
3-6	.5396	.5443	.0047**	0.9*
3-7	.5123	.5166	.0043	0.8
4-5	.5542	.5645	.0103**	1.9**
5-6	.5197	.5207	.0010	0.2
6-7	.4615	.4651	.0036	0.8

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[1] Each value represents the average of 48 pens (24 of each sex) with 9 male and 8 female poults per pen.

Table 12. Effects of adding potassium sulfate to diets of poults on body weight, body weight gain, feed consumption and feed efficiency (Experiment 4)

Age, weeks	Potassium sulfate, %[1]		Difference	Percent increase
	0	.1		
Body weight, g				
4	633.9	630.8	-3.1	-0.5
5	995.2	985.3	-10.1	-1.0
6	1452.7	1435.8	-17.1	-1.2
7	1943.3	1927.7	-15.6	-0.8
Body weight gain, g				
3-4	257.5	256.5	-1.0	-0.4
3-5	618.8	610.9	-7.9	-1.3
3-6	1076.3	1061.5	-14.8	-1.4
3-7	1566.9	1553.4	-13.5	-0.9
4-5	361.3	354.5	-6.8	-1.9
5-6	457.6	450.5	-7.1	-1.6
6-7	490.6	491.9	1.3	0.3
Feed consumption, g				
3-4	462.7	456.7	-6.0	-1.3
3-5	1105.9	1090.2	-15.7	-1.4
3-6	1984.3	1954.3	-30.0	-1.5
3-7	3046.1	3008.2	-37.9	-1.2
4-5	643.2	633.4	-9.8	-1.5
5-6	878.4	864.1	-14.3	-1.6
6-7	1061.8	1054.0	-7.8	-0.7
Feed efficiency				
3-4	.5560	.5616	.0056	1.0
3-5	.5586	.5596	.0010	0.2
3-6	.5416	.5424	.0008	0.1
3-7	.5135	.5155	.0020	0.4
4-5	.5603	.5584	-.0017	-0.3
5-6	.5200	.5205	.0005	0.1
6-7	.4609	.4657	.0048	1.0

[1] Each value represents the average of 48 pens (24 of each sex) with 9 male and 8 female poults per pen.

METHIONINE BIOASSAYS AND METHIONINE-CHOLINE-SULFATE RELATIONSHIPS IN PRACTICAL-TYPE DIETS FOR YOUNG TURKEYS

by

Michael Everett Blair

ABSTRACT

Two experiments were conducted with a total of 1,728 Large White turkeys to determine the relative potencies of four methionine compounds on an equimolar basis. A 28% protein basal diet composed primarily of ground yellow corn, dehulled soybean meal, and meat and bone meal was supplemented with DL-methionine (DL), sodium salt of DL-methionine (MENA), methionine hydroxy analogue calcium salt (MHAC), or methionine hydroxy analogue free acid (MHAA) at the .06, .12, .18, .24, or .30% level of added methionine. Each of these 20 diets was fed to two pens of poults of each sex (9 birds per pen), and the basal diet was fed to eight pens of each sex from one day to seven-weeks of age in each experiment.

From the combined six-week body weight data, MENA was 104.7 (86.1, 123.3), MHAC 101.8 (83.5, 120.1), and MHAA 92.5% (74.9, 110.1) as potent as DL (with 95% fiducial limits) by the slope ratio procedure. No differences were detectable among the methionine products. The extra one degree of freedom associated with the nonlinear procedure accounted for 4.8 and 10.7% of the residual variation in Experiments 1 and 2, respectively. Relative potencies

were obtained for MENA of 110.3 and 115.6, MHAC of 100.4 and 88.1, and MHAA of 89.7 and 112.3% in Experiments 1 and 2, respectively.

Two additional experiments were conducted using a total of 1,680 poults to study the relationships of methionine, choline, and sulfate in practical-type diets. A 6 x 2 x 2 factorial design was used involving increments of .06% DL-methionine from 0 to .30%, 0 or .20% choline chloride, and 0 or .10% potassium sulfate. The variables were added to a 21% protein basal diet containing 61% ground yellow corn and 32% dehulled soybean meal to which no supplemental choline or sulfate was added. Each of the 24 diets was fed to two pens of medium-type turkeys of each sex (9 birds per pen) from four to eight-weeks of age in the first experiment, and to two pens of Large White turkeys of each sex (9 and 8 birds per pen for males and females, respectively) from three to seven-weeks of age in the second experiment.

From the addition of .06% and .12% DL-methionine, body weight gains were increased 11.0 and 16.5% in the first experiment, and 8.9 and 13.6% in the second experiment, respectively. In addition, methionine increased feed consumption about 1/3 these amounts, and feed efficiencies 1/2 these amounts. The addition of choline or sulfate failed to significantly increase these parameters, even in the absence of methionine. No significant interactions were observed among the dietary variables in either experiment.