

The Effect of Supplementing Methionine plus Cystine to a Low-Protein Diet on the Growth Performance and Fat Accumulation of Growing Broiler Chicks

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ABSTRACT: This experiment was conducted to determine the effects of a low-protein diet supplemented with DL-methionine plus L-cystine (Met + Cys) on the growth performance and fat accumulation of female broiler chicks during the growing period (3-6 wks old). A low-protein diet (17% CP; 3,200 ME kcal/kg) was supplemented with Met + Cys (1.1:1.0) at levels 0.75, 0.94, 1.25, 1.31 or 1.50% of diet, respectively. Another diet with 21% CP and 3,200 ME kcal/kg served as the control group. All essential amino acids were adjusted to meet the National Research Council (1984) requirement for chicks. Feed and water were given *ad libitum*. Body weight of the chicks fed the low-CP diets supplemented with Met + Cys were heavier than those of the control birds. Feed conversion ratio and feed intakes were not significantly different between and among the treatment groups. Similarly, abdominal fat content was not significantly different among the various treatments except that of the chicks fed the low CP diet with 1.25%

Met + Cys which was higher than that of the control group. Fatty acid synthetase (FAS), acetyl-CoA carboxylase (ACC) activities and carcass protein content were not influenced by dietary treatments. Carcass fat content was lowest in chicks fed low CP diet with 0.75% Met + Cys and highest in the group that received 1.50% Met + Cys supplementation. Liver triglyceride increased as Met + Cys supplementation level increased. Various lipid fraction concentrations (cholesterol ester, free cholesterol, and phospholipid) in the serum went up as Met + Cys increased up to 1.25% after which it levelled off. Results of this experiment suggest that it is possible to reduce dietary protein level from 21% to 17% for growing broiler chicks by the supplementation of Met + Cys when other EAA were sufficient.

(Key Words: Methionine + Cystine, Protein Levels, Broiler, Carcass Composition, Growth Performance, Fat Accumulation)

INTRODUCTION

There have been varying and continuing efforts to decrease the price of animal feeds particularly protein which is an expensive dietary constituent. Among those who have made such attempts were Bartov et al. (1974); Diambra and McCartney, (1985); Fancher and Jensen, (1989); and Jackson et al. (1982). Generally speaking, body fat content in broilers increases as the dietary protein level decreases. However, Lipstein and Borstein

(1975) indicated that adequate supplementation of the first limiting amino acid enables the usual protein levels in broiler finisher diets to be lowered without affecting performance parameters such as weight gain and feed utilization, and carcass composition. Met has been demonstrated as the first limiting amino acid in broilers and is commonly used in commercial feeds. It is generally accepted that Met supplementation improves dietary amino acid balance and thus promotes greater protein build-up and less fat deposition in chicks (Bomgaard and Baker, 1973). Amino acids that may be supplemented to a low CP diet while at the same time improving the growth performance and reducing the body fat of chicks, have been examined. It has been indicated by the recent report of Ohta et al. (1993) that when Met was supplemented to diets in combination with Cys, the performance in broilers was better than when Met was supplemented alone. The

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metabolic relationship between Met and Cys had been well known since long time ago. Behrends and Waibel (1980) reported that dietary Met was used to synthesize Cys when dietary Cys was deficient in growing turkeys, and the physiological requirement for Met must be determined with dietary Cys at or in excess of its maximum usable level. Subsequently, Smith et al. (1983) reported that rats fed diets supplemented with Cys adapted metabolically to store energy as glycogen, while those fed diets with Met tended to store energy as lipid. There were few reports for the effect of dietary Met + Cys on lipid metabolism in broiler chicks. This study thus seeks to determine the effect of Met + Cys supplementation to a low CP diet on the growth performance, body composition, hepatic lipogenic enzyme activities as well as on various liver and serum lipid fractions.

MATERIALS AND METHODS

Animals and diets

Sixty one-day-old female broilers of commercial strain (Chunky) were used in this experiment. They were raised in a flood pen littered with wood shavings from 1 d to 3 wks of age. Then they were weighed individually and

divided into six groups of ten chicks each. Chicks were afterwards transferred and raised in wire cages (2 chicks per cage) up to 6 wks of age during which they were fed with the experimental diets. They were raised in an environmentally controlled windowless house and given feed and water *ad libitum*.

A commercial starter diet [crude protein (CP): 24% and Metabolizable Energy (ME): 2,900 kcal/kg] was given to all chicks until 3 wks of age. From 3 to 6 wks of age, experimental diets were fed. The low protein diet (17% CP; 3,200 ME Kcal/kg) was supplemented with DL-methionine (Met) plus L-cystine (Cys) (Met + Cys, 1.1:1.0 by weight). Mixtures of Met + Cys were graded at level 0.75, 0.94, 1.31 and 1.50% of the diet. A diet with 21% CP and 3,200 ME kcal/kg was used as feed for the control group. All essential amino acids (EAA) in both the 21% CP and the 17% CP diets were adjusted to meet the requirement of chicks as recommended by the National Research Council (NRC; 1984) (table 1b). The amino acid and the ME values of the ingredients used were also taken from NRC (1984). Dietary CP was analyzed using the Association of Official Analytical Chemists (AOAC; 1980) procedures. The chicks were weighed weekly while feed intake was measured daily.

Table 1a. Composition of experimental diets

Ingredients	control (21% CP)	17% CP diet (% Met + Cys in diet)				
		0.75	0.94	1.25	1.31	1.50
..... (% in diet)						
Corn and fish meal (11% CP)	71.40	68.90	68.90	68.90	68.90	68.90
Soybean meal	14.95	10.27	10.27	10.27	10.27	10.27
Soybean protein	3.00	1.30	1.30	1.30	1.30	1.30
Corn starch	0.28	7.61	7.61	7.61	7.61	7.61
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00
Premix ¹	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Choline	0.013	0.013	0.013	0.013	0.013	0.013
Dicalcium phosphate	0.30	0.30	0.30	0.30	0.30	0.30
Calcium carbonate	1.58	1.58	1.58	1.58	1.58	1.58
Cellulose	1.80	2.57	2.40	2.20	2.00	1.83
Amino acids	0.15	0.80	0.97	1.16	1.34	1.53
Calculate analysis						
Metabolizable energy	3,200	3,200	3,200	3,200	3,200	3,200
Crude protien (%)	21.15	17.80	17.97	18.16	18.34	18.50

¹ Supplied per kilogram of diet: 14,000 IU vitamin A retinyl acetate, 2,800 ICU cholecalciferol, 2 mg vitamin B₁, 13.4 mg vitamin B₂, 5.9 mg vitamin B₆, 39.6 mg niacin, 5.8 mg vitamin K₃, 38.4 mg Ca-pantothenic acid, 20 mg vitamin E dl- α -tocopheryl acetate, 4 μ g vitamin B₁₂, 960 μ g folic acid, 140 μ g biotin, 72.2 mg Fe, 7 mg Cu, 1.89 mg I, 144 mg Mg, 85 mg Zn and 0.2 mg Co.

Table 1b. Quantities of amino acids which added to the experimental diets

	control (21% CP)	17% CP diet (% Met + Cys in diet)				
		0.75	0.94	1.25	1.31	1.50
	 (% in diet)				
Methionine	—	0.046	0.146	0.246	0.340	0.400
Cystine	0.047	0.124	0.213	0.303	0.390	0.476
Lysine	—	0.070	0.070	0.070	0.070	0.070
Arginine	—	0.240	0.240	0.240	0.240	0.240
Threonine	—	0.020	0.020	0.020	0.020	0.020
Glycine	0.100	0.280	0.280	0.280	0.280	0.280
Total of % CP	21.15	17.80	17.97	18.16	18.34	18.50

General procedure

At the end of the experiment, all chicks were weighed individually. Thereafter, five chicks of each group with body weight close to the group mean were killed. Liver and abdominal fat were rapidly excised and weighed. A part of the livers was placed in an ice-cold saline to measure the lipogenic enzyme activities. Another part of the livers and serum were stored at -30°C for subsequent analysis of the various lipid fractions. The different lipid fractions were separated by thin layer chromatography on silica gel chromarod using hexane/diethyl ether/formic acid (90:12:0.2) and hexane:benzene (1:1; v/v) as developing solvents. They were quantitated by IATROSCAN with hydrogen as gas flow (Tanaka et al., 1979). The remaining five chicks of each group were killed and placed in plastic bags, and frozen at -30°C . The frozen carcasses (without blood, feces and feathers) were cut into small pieces. They were then ground through a 5 mm screen and were made to pass through the mincer repeatedly for four times to obtain uniform mixing. Protein, fat and moisture contents of carcass were determined using the AOAC (1980) method.

Preparation of liver homogenates

Livers were homogenized in 0.25 M sucrose solution containing 1 mM ethylenediaminetetraacetate-2Na (EDTA-2Na). The homogenates were then centrifuged (Model RS-18, Tomy Seiko) at 9,800 rpm at 4°C for 10 minutes. The supernatants were recentrifuged (Model 65P, RP 40705 rotor, Hitachi koki) at 40,000 rpm at 4°C for 60 min. and the resulting clear supernatants (cytosolic fraction) were used for assaying lipogenic enzyme activities.

Enzyme assay

Acetyl-CoA Carboxylase was assayed by H^{14}CO_2 -fixation method (Qureshi et al., 1980). Fatty acid

synthetase was assayed by $1\text{-}^{14}\text{C}$ -acetyl-CoA incorporation method (Hsu et al., 1965). The protein content of solutions used for enzyme assay was determined by the method of Lowry et al. (1951) using albumin as the standard. Enzyme activities were expressed as nanomole of substrate converted product per minute per mg protein at 37°C .

Statistical analyses

All data were statistically analyzed using the one-way analysis of variance (Yoshida, 1975). Significant differences among treatments were determined by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Table 2 shows body weight, body weight gain, feed conversion ratio (FCR), feed intake, abdominal fat and liver weights. The body weight of chicks fed low CP diets with Met + Cys at various levels was heavier than those fed the control diet (21% CP diet). Chicks fed the low CP diet with 1.25% Met + Cys showed the heaviest body weight among the treatment groups; they were significantly heavier than those fed the control diet and those given the low CP diet with 0.94% Met + Cys. FCR values were not significantly different among the treatment groups, however, the group fed the low CP diet with 1.25% Met + Cys scored the best FCR value while the control group gave the lowest value. There was no significant difference in feed intake (g/day) among the treatment groups. The abdominal fat weight of chicks fed low CP diets with Met + Cys was higher than that of the control group, with those chicks fed the low CP diet with 1.25% Met + Cys registering the highest score. Liver weight values were varying. Just the same, the chicks fed the low CP diet with 1.50% Met + Cys had significantly

heavier liver than those fed the other diets.

It has been reported that chicks fed a low CP diet with Met + Cys supplementation could have growth performance that equals those whose diets' CP content was based on NRC recommendation. The body weights gain, FCR, feed intake, and weights of the liver and abdominal fat of chicks fed the experimental diets were in agreement with the observation of Ohta et al. (1993) who reported that body weight gains, feed intake, feed efficiency and abdominal fat content per unit of body weight (AF/BW) increased with increasing dietary total sulfur amino acids (TSAA), and then leveled off. Although feed intake was not a significantly different among the treatment groups, it had an effect on both body weight and FCR. Feed intake was parallel to the body weight of chicks ($Y = 108.81 + 13.445X$, $r = 0.97$, $p < 0.001$; where Y = body weight and X = feed intake). In contrast, it had an inverse relationship with FCR ($Y = 3.155 - 0.008X$, $r = -0.86$, $p < 0.05$; where Y = FCR and X = feed intake). There was no correlation between feed intake and abdominal fat. It would thus seem that

abdominal fat weight in chick was not influenced by feed intake. It is in agreement with the finding of Takahashi et al. (1994) who reported that the effect of Met supplementation on abdominal fat deposition was independent from feed intake. Body weight and FCR, however, were found to be depended on feed intake. It can be assumed that Met supplementation improves dietary amino acid balance (Bomgardt and Baker, 1973), however, an excess of this amino acid induced toxicity (Sauberlich, 1961, Benevenga and Haper, 1967). Rose (1937) pointed out that Cys improved growth only when Met was supplied in sub optimal amounts. The present study seems to confirm with these observations. It must be pointed out, however, that there must be a proper balance of amino acid before the performance of a low CP diet could be improved even if Met + Cys were supplemented. It is possible that amino acids that were in excess of what is required for life maintenance and growth were converted into energy that enhanced abdominal fat deposition. This is what was suggested by Summers and Leeson (1985).

Table 2. Effect of experimental diets on body weights, body weight gain, feed conversion ratio, feed intake, abdominal fat and liver weight of chicks

	control (21% CP)	17% CP diet (% Met + Cys in diet)					P ¹
		0.75	0.94	1.25	1.31	1.50	
Initial body weight (g)	553.88 ±33.52	552.00 ±30.93	548.00 ±34.00	552.00 ±28.55	550.00 ±29.15	548.00 ±31.72	NS
Final body weight (g)	1,748.00 ±41.82 ^a	1,800.00 ±54.12 ^{ab}	1,764.00 ±69.35 ^a	1,929.00 ±28.96 ^b	1,826.00 ±36.82 ^{ab}	1,812.30 ±50.04 ^{ab}	< 0.05
Body weight gain (g)	1,194.12 ±21.13 ^a	1,248.00 ±28.32 ^{ab}	1,216.00 ±30.11 ^a	1,377.00 ±16.12 ^b	1,276.00 ±24.20 ^{ab}	1,264.30 ±34.21 ^{ab}	< 0.05
Feed conversion ratio	2.16 ±0.08	2.13 ±0.11	2.12 ±0.07	2.03 ±0.05	2.13 ±0.04	2.10 ±0.02	NS
Feed intake (g/day)	122.61 ±8.61	124.95 ±4.91	123.40 ±4.60	135.18 ±2.68	128.91 ±1.23	125.52 ±6.12	NS
Abdominal fat (% of live weight)	2.14 ±0.24 ^a	2.24 ±0.16 ^{ab}	2.57 ±0.14 ^{ab}	2.85 ±0.09 ^b	2.42 ±0.18 ^{ab}	2.54 ±0.36 ^{ab}	< 0.05
Liver weight (% of live weight)	2.37 ±0.23 ^{ab}	2.32 ±0.11 ^{ab}	2.21 ±0.10 ^a	2.27 ±0.15 ^{ab}	2.11 ±0.08 ^a	2.58 ±0.14 ^b	< 0.05

Data are presented as the mean and SE of 10 chicks per treatment.

¹ Probability of a significant. NS = not significant.

^{ab} Values reported present an average ± SE. Means within a row followed by a common superscript are not different.

Table 3 shows the effect of experimental diets on carcass composition in broilers. Differences were not significant among experimental diets for carcass protein

content of chicks, although it showed the lowest value in chicks fed the low CP diet with 1.25% Met + Cys. Carcass fat content gradually increased parallel to the

gradual level of Met+ Cys supplementation to the low-CP diet i.e., it was significantly less in chicks fed the diet with 0.75% Met + Cys than the those fed the diet with 1.50% Met + Cys. Carcass moisture content was lowest and highest in the chicks fed a low-CP diet with 1.50% and 0.94% Met+ Cys supplementation, respectively.

Table 3. Effect of experimental diets on body composition of chicks

	control (21% CP)	17% CP diet (% Met + Cys in diet)					P ¹
		0.75	0.94	1.25	1.31	1.50	
..... (%)							
Protein	16.63 ± 0.33	16.41 ± 0.28	16.55 ± 0.44	15.17 ± 0.17	16.51 ± 0.17	16.07 ± 0.17	NS
Fat	16.63 ± 0.89 ^{ab}	15.51 ± 0.60 ^a	16.28 ± 0.87 ^{ab}	18.08 ± 0.53 ^{ab}	17.32 ± 0.69 ^{ab}	18.93 ± 0.54 ^b	< 0.05
Moisture	63.59 ± 0.49 ^{ab}	64.89 ± 0.33 ^{bc}	65.94 ± 0.81 ^c	63.15 ± 0.50 ^a	63.33 ± 0.57 ^{ab}	62.55 ± 0.20 ^a	< 0.05

Data are presented as the mean and SE of 5 chicks per treatment.

¹ Probability of a significant treatment effect; NS = not significant.

^{a,b,c} Values reported represented an average ± SE. Means within a row followed by a common superscript are not different.

This experiment further indicates that the increasing levels of Met+ Cys in the low CP diet had the effect of increasing the carcass fat content. This is in agreement with the report of Rosebrugh and Steele (1985). Likewise, the inverse relationship between carcass fat and moisture contents was confirmed by the data of this experiment ($Y = 78.08 - 0.83X$, $r = -0.82$, $p < 0.05$; where $X =$ carcass fat, $Y =$ moisture) although the moisture content pattern was irregular relative to the Met + Cys levels of

supplementation.

The effects of experimental diets on ACC and FAS activities in the liver of chicks are presented in table 4. There were no significant differences in the ACC and the FAS activities in the liver between and among the experimental diets. It could be noted, however, that the FAS activity tended to decrease gradually as the level of Met + Cys increased.

Table 4. Effect of experimental diets on activities of acetyl-coA carboxylase (ACC) and fatty acid

	control (21% CP)	17% CP diet (% Met + Cys in diet)					P ¹
		0.75	0.94	1.25	1.31	1.50	
..... (nmole/min/mg protion)							
Acetyl-CoA carboxylase	1.16 ± 0.06	0.81 ± 0.07	1.01 ± 0.09	1.09 ± 0.10	1.12 ± 0.13	1.11 ± 0.14	NS
Fatty acid synthetase	2.08 ± 0.22	2.03 ± 0.29	1.79 ± 0.20	1.97 ± 0.31	1.37 ± 0.31	1.27 ± 0.27	NS

Data are presented as the mean and SE of 5 chicks per treatment.

¹ Probability of a significant treatment effect; NS = not significant.

^{a,b} Values reported represent an average ± SE. Means within a row followed by a common superscript are not different.

ACC is the main lipogenic enzyme in the liver of chicks. Yeh and Leveille (1969) and Tanaka et al. (1983) have reported that the hepatic lipogenesis and the activities of the associate enzymes in chickens were decreased by increased dietary protein. Masaro (1965) reported that a 95% reduction in fatty acid synthesis could be induced by fasting and that a decline in ACC activity could bring down fatty acid synthesis to less than 50%. He thus suggested that other regulatory systems could be involved in the control of lipogenesis. In as much as ACC and FAS activities have influence on body

fat deposition, the quantity of body fat in chicks is directly proportional to these enzymes' activities. Since both the abdominal fat weight and the carcass fat contents of the birds used in this experiment were not in direct proportion to the activities of ACC and FAS, it would seem that there are other mechanisms which affect fat biosynthesis specifically when the dietary protein level is reduced but supplemented with Met + Cys.

The effects of experimental diets on the contents of various lipid fractions in the liver and serum of chicks are presented in table 5. The live triglyceride content was

significantly higher in chicks fed the low CP diet with 1.50% Met + Cys compared to the values obtained in the rest of the treatments. Free cholesterol content in the liver was the highest in chicks fed the low CP diet with 1.50% Met + Cys. It was significantly different from that of the birds given the low CP diet with 1.25% Met + Cys level. Liver phospholipid content tended to be or significantly higher in chicks fed the control diet than those given the low CP diets supplemented with various Met + Cys levels, with the chicks fed the low CP diet with 1.50% Met +

Cys having the lowest hepatic phospholipid value. Of all the groups fed the experimental diets, the chicks fed low CP diet with 1.25% Met + Cys yielded a significantly higher free and esterified cholesterol concentrations in the serum. In contrast, cholesterol levels were significantly lower in the chicks fed the low CP diet with 1.31 or 1.50% Met + Cys. Serum triglyceride and phospholipid concentrations were significantly higher in the treatments fed the low CP diet with 0.94% or 1.25% Met + Cys than the other experimental groups.

Table 5. Effect of experimental diets on contents of various lipid fractions in liver and serum of chicks

	control (21% CP)	17% CP diet (% Met + Cys in diet)					P ¹
		0.75	0.94	1.25	1.31	1.50	
Liver (mg/g liver)							
Triglyceride	3.34 ± 0.91 ^{ab}	3.18 ± 0.44 ^{ab}	2.27 ± 0.38 ^a	3.65 ± 1.05 ^{ab}	6.06 ± 1.75 ^b	15.08 ± 0.98 ^c	< 0.05
Free cholesterol	3.48 ± 0.06 ^{ab}	3.61 ± 0.08 ^{ab}	3.51 ± 0.06 ^{ab}	3.36 ± 0.09 ^a	3.41 ± 0.08 ^{ab}	3.71 ± 0.11 ^b	< 0.05
Phospholipid	11.17 ± 0.32 ^c	9.45 ± 0.44 ^{ab}	10.05 ± 0.33 ^{bc}	10.92 ± 0.35 ^c	9.53 ± 0.44 ^{ab}	8.68 ± 0.36 ^a	< 0.05
Serum (mg/100 ml)							
Cholesterol ester	168.99 ± 4.41 ^c	149.34 ± 4.67 ^b	158.03 ± 8.19 ^{bc}	195.03 ± 5.79 ^d	132.32 ± 4.21 ^a	134.88 ± 5.85 ^a	< 0.05
Triglyceride	11.93 ± 0.76 ^{ab}	13.54 ± 0.72 ^b	19.72 ± 1.24 ^d	15.79 ± 1.55 ^c	10.36 ± 0.91 ^a	10.36 ± 0.11 ^a	< 0.05
Free cholesterol	37.42 ± 2.39 ^c	37.97 ± 1.55 ^c	41.51 ± 2.17 ^{cd}	43.36 ± 0.49 ^d	30.02 ± 0.55 ^d	24.97 ± 1.21 ^a	< 0.05
Phospholipid	109.42 ± 2.38 ^d	114.47 ± 2.99 ^d	149.78 ± 13.68 ^c	157.48 ± 5.81 ^c	98.29 ± 4.64 ^{ab}	81.71 ± 4.89 ^a	< 0.05

Triglyceride is produced by esterification of acyl-CoA and glycerol-3-phosphate. Paik and Yearic (1978) have proposed that apart from *de novo* lipogenesis, fat deposition in adipose tissues depends on a change in the relative activities of lipoprotein lipase (LPL) which mediates the uptake of fatty acid from lipoprotein in the blood and the hormone sensitive lipase (HSL) which mediates the output of fatty acid from triglyceride in adipose tissues. Triglyceride is transported from the liver via the blood to extrahepatic tissues by very low density lipoprotein (VLDL). Its concentration in the liver and in the serum of chicks was influenced by dietary treatments in this experiment. But no relationship existed between body fat and triglyceride in both the liver and the plasma when the data were calculated by regression equation.

Nonetheless, it would be interesting to study in future experiments that the effect of dietary Met + Cys on triglyceride-body fat relationship in as much as triglyceride was significantly affected by these amino acids. Phospholipid is formed by the condensation between 1, 2 diacylglycerol and CDP-choline. CDP-choline is derived via phosphocholine from choline. Triglyceride is also synthesized from 1, 2-diacylglycerol. Thus, there seems to be a contradictory negative relationship between phospholipid synthesis and triglyceride synthesis. If triglyceride synthesis is high, phospholipid would be low. The results of the present experiment, however, deviated from this general relationship as demonstrated by the triglyceride and phospholipid contents in the liver. That is, triglyceride

and phospholipid concentration in the serum were paralled to each other as shown by this regression equation ($Y = 0.65 + 0.11X$, $r = 0.89$, $p < 0.05$; where X = phospholipid, Y = triglyceride). This may be due to hepatic secretions of lipoproteins into the blood. The relationship among lipid fractions in the liver, serum and body fat of the chicks in this experiment is unclear at this stage. Thus, it is suggested that the effect of dietary Met+Cys on transportation of lipid fraction from the liver via blood to extrahepatic tissues such as body fat be the focus of future studies.

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