Influence of Dietary Protein Level on the Broiler Chicken's Response to Methionine and Betaine Supplements[1]

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ABSTRACT Two experiments were conducted to compare broiler chicken responses to methionine and betaine supplements when fed diets with low protein and relatively high metabolizable energy levels (17%, 3.3 kcal/g) or moderate protein and lower metabolizable energy levels (24%, 3.0 kcal/g), resulting in different levels of carcass fat. In Experiment 1, the basal diets were formulated with corn, soybean meal, poultry by-product meal, and poultry oil. In Experiment 2, glucose monohydrate was also added, so that identical amino acid profiles could be maintained in the 17 and 24% protein diets. On average, feeding the 17 vs. 24% protein diet decreased 21-d body weight gain by 20%, increased feed conversion ratio (FCR) by 13%, and increased abdominal fat pad weight by 104%. Methionine and betaine supplements improved the performance of chicks fed the 24% protein diet in both experiments, as indicated by body weight gain and FCR. Only supplementary methionine increased performance of chicks fed 17% protein diets, and then only in Experiment 2. Neither methionine nor betaine decreased abdominal fat pad size in either experiment. Methionine supplementation decreased relative liver size and increased breast muscle protein. Both methionine and betaine increased sample feather weight, but when expressed as a percentage of body weight, no significant differences were detected. It is concluded that increasing carcass fat by manipulating percentage dietary protein level or amino acid balance does not influence betaine's activity as a lipotropic agent.

(*Key words*: broilers, protein, betaine, methionine)

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INTRODUCTION

The interrelationship between the methionine requirement of broiler chicks and dietary protein level has been studied since the 1940s (Marvel et al., 1944). An important aspect of the protein and methionine interrelationship is the ability of both to act as lipotrophic agents. The production of lean carcasses has become especially important as producers have changed from selling live birds to selling meat (with abdominal fat pads being sold to renderers at much reduced costs). Typical least-cost feed formulations can give low-protein rations that result in increased levels of carcass fat.

The observation that protein increases carcass lean tissue and decreases carcass lipids is very well known, although the mechanism is not. Methionine may act as a lipotrophic agent through its role as an amino acid in balancing protein, or through its role as a methyl donor and involvement in choline, betaine, folic acid, and vitamin B_{12} metabolism (Schaefer et al., 1949; Young et al., 1955; March and Biely, 1956; Creek and Vasaitis, 1963; Wong et al., 1977; Tillman and Pesti, 1986; Chen et al., 1993).

Saunderson and MacKinlay (1990) presented data suggesting that betaine may be a more effective lipotrophic agent than choline for poultry. A subsequent report by Virtanen and Rosi (1995) presented data showing improved performance when methionine or betaine were added to a corn and soybean meal-based broiler diet. In their studies, both methionine and betaine improved body weights, breast meat yields, and feed conversion ratios (FCR) and decreased abdominal fat pad weights. Contrary to the results of Virtanen and Rosi (1995), Rostagno and Pack (1996) concluded that methionine, but not betaine, could reduce the carcass fat of broilers. There were a number of fundamental differences between these reports, including the basal diets, genetic stocks used, and length of the experiments.

A potentially important factor for the activity of any lipotrophic agent is the initial degree of fatness of the birds; lipotrophic agents should be more effective in fat birds. The objective of the experiments described herein

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Abbreviation Key: FCR = feed conversion ratio.

was to determine whether methionine and betaine would have increased lipolytic activities in birds made fat by feeding high-energy, low-protein diets. Two dietary approaches were taken: in Experiment 1, diets were balanced with common ingredients, so that the amino acid profiles of the diets were different. In Experiment 2, glucose monohydrate was used as a diluent, so that amino acid profiles were identical in the 17 and 24%-protein diets.

MATERIALS AND METHODS

Three-hundred eighty-four Ross × Hubbard Hi-Y (Experiment 1) and Peterson × Arbor Acres (Experiment 2) commercial male broiler chicks were obtained from commercial hatcheries. Eight chicks were randomly assigned to each pen in wire-floor Petersime battery brooders in a temperature-controlled room. The temperature was 35 C for the first 3 d, and then it was decreased by 2 C every 3 d until 24 C was reached. Water and feed were supplied for consumption ad libitum.

Diets that contained low protein and relatively high energy were based on corn, soybean meal, poultry by-product meal, and glucose monohydrate (Table 1). In Experiment 1, the proportions of corn and soybean meal changed, so that the 17% protein diet had a higher proportion of corn, and thus a higher proportion of methionine per unit of protein, than the 24% protein diet. In Experiment 2, the 17% protein diet was achieved by diluting the 24% protein diet with glucose monohydrate. Thus, the proportion of methionine per unit protein was exactly the same for both diets. The diets designed to produce relatively low levels of carcass fat (24% protein) had choline levels in excess of the chick's requirement (1,503 vs. 1,200 mg/kg; NRC, 1994), whereas the diet designed to produce high levels of carcass fat (17% protein) had choline levels below the chick's requirement (1,126 and 1,064 mg/kg for Experiments 1 and 2, respectively, vs. 1,200 mg/kg; NRC, 1994), based on ingredient composition tables (NRC, 1994). These values are difficult to compare with those determined by the method of Menten and Pesti (1998). In any case, there was adequate choline present in the diets to avoid both perosis and gross leg abnormalities.

At 10 and 21 d of age, chicks and residual feed were weighed by pen. Feed consumption data were adjusted to account for deaths on a chick-day basis. At 21 d of age, chicks were killed by carbon dioxide asphyxiation. Three

TABLE 1. Composition of the experimental diets

	Experiment 1		Experiment 2	
Ingredients	Lean	Fattening	Lean	Fattening
Group yellow corn, %	57.200	70.780	56.890	40.300
Soybean meal (48% dehulled), %	35.500	18.600	35.570	25.200
Poultry fat (stabilized), %	1.700	4.820	1.720	4.540
Poultry by-product meal, %	3.000	3.000	3.000	2.120
Glucose monohydrate, %	—	—	0.150	24.970
Defluorinated phosphate, %	1.400	1.600	1.430	1.740
Limestone, %	0.600	0.600	0.640	0.530
Iodized sodium chloride, %	0.400	0.400	0.400	0.400
Vitamin premix,[1] %	0.050	0.050	0.050	0.050
Trace mineral premix,[2] %	0.075	0.075	0.075	0.075
Cupric sulfate pentahydrate, %	0.075	0.075	0.075	0.075
Composition by calculation[3]				
Protein, %	24.00	17.00	24.00	17.00
ME_n, kcal/g	3.00	3.30	3.00	3.30
Calcium, %	0.90	0.90	0.90	0.90
Available phosphorus, %	0.45	0.45	0.45	0.45
Lysine, %	1.28	0.82	1.28	0.91
Methionine, %	0.37	0.28	0.37	0.26
Methionine + cystine, %	0.75	0.56	0.75	0.53
Arginine, %	1.57	1.04	1.57	1.11
Choline, %	1,503	1,126	1,503	1,064
Composition by analysis				
Protein,[4] %	25.62	17.38	24.03	18.08
Choline,[5] mg/kg	528	774	529	727
Methionine,[6] %	0.37	0.29	0.35	0.27
Cystine,[6] %	0.39	0.33	0.39	0.28

[1]Vitamin premix provides the following per kilogram: Vitamin A, 5,500 IU from all *trans*-retinyl acetate; cholecalciferol, 1,100 IU; vitamin E, 11 IU from all rac-α-tocopherol, acetate; riboflavin, 4.4 mg; Ca pantothenate, 12 mg; nicotinic acid, 44 mg; vitamin B_{12}, 6.6 μg; vitamin B_6, 2.2 mg; menadione, 1.1 mg (as MSBC); folic acid, 0.55 mg; d-biotin, 0.11 mg; thiamine, 1.1 mg (as thiamine mononitrate); ethoxyquin, 125 mg.

[2]Trace mineral premix provides the following in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5; I, 1.5.

[3]Based on NRC (1994) tables.

[4]Method of Etheridge et al. (1998).

[5]Method of Menten and Pesti (1998).

[6]AOAC (1995) Method 994.12.

TABLE 2. Influence of dietary protein and energy levels and methionine or betaine supplements on the hatching to 21-d performance of male broiler chickens (Experiment 1)[1,2]

Methyl source		Body weight gain			Feed conversion ratio			Fat pad (at 21 d)		
Met	Bet	17% protein	24% protein	Mean[3]	17% protein	24% protein	Mean	17% protein	24% protein	Mean
(%)		(g)			(g intake/g gain)			(% of body weight)		
0	0	497 ± 31^{z}	599 ± 33^{y}	548^{b}	1.70 ± 0.02^{x}	1.55 ± 0.04^{y}	1.63	2.10 ± 0.42	0.93 ± 0.31	1.625
1.5	0	475 ± 26^{z}	682 ± 29^{x}	579^{a}	1.76 ± 0.07^{x}	1.43 ± 0.06^{z}	1.60	2.10 ± 0.51	0.92 ± 0.29	1.595
0	0.65	506 ± 47^{z}	691 ± 31^{x}	599^{a}	1.74 ± 0.02^{x}	1.54 ± 0.05^{y}	1.64	2.12 ± 0.43	1.08 ± 0.31	1.640
Mean[3]		493^{n}	657^{m}		1.73^{m}	1.51^{n}		2.11^{m}	0.98^{n}	
ANOVA		(df)	(Pr > |T|)			(Pr > |T|)			(Pr > |T|)	
Protein level		1	0.0001			0.0001			0.0001	
$-CH_3$ source		1	0.0258			0.1565			0.3434	
Protein × $-CH_3$		1	0.0139			0.0026			0.5726	
Control vs. Met		1	0.0860			0.2212			0.9303	
Control vs. Bet		1	0.0080			0.4693			0.2247	
Met vs. Bet		1	0.2595			0.0601			0.1929	

[a–b; m,n; x–z]Values with no common superscript differ significantly ($P < 0.05$) when tested with Duncan's new multiple range test following analysis of variance.

[1]Mean ± SE of four replicate pens of eight chicks each for body weight and feed conversion ratio, and three chicks each for percentage fat pad.

[2]The 17% protein diet contained 3.3 kcal ME/g; the 24% protein diet contained 3.0 kcal ME/g.

[3]Mean = main effect means.

birds per pen were randomly chosen for tissue sampling. Livers, breast muscles, abdominal fat pads, and right legs were removed and weighed. Liver and breast muscle lipids were measured by the method of Folch et al. (1957), and crude protein was measured using a Leco CNS-2000 analyzer.[3] Tibia ash was measured by the method of the AOAC (1995). Liver betaine was measured by the spectrophotometric method of Barak and Tuma (1979). Tissue moisture was measured by difference before and after freeze drying.

At the end of Experiment 2, the Number 1 and 2 primary feathers from one wing were removed from each bird for weighing. The birds were hand defeathered, weighed for feather weight by difference, frozen, cubed, ground, and measured for protein fat and moisture by the methods cited above.

The study was conducted as a completely randomized design, with a factorial arrangement of fixed treatment effects consisting of two diets (17 vs. 24% protein) and three supplements (control, +0.15% DL-methionine, and +650 mg betaine/kg), and the interaction of diet by supplement. The methionine supplement was the difference between the NRC (1994) requirement for methionine plus cystine (0.9%) and the estimated basal diet content (0.75%). The betaine supplement should have provided adequate methyl groups for all diets based on the NRC (1994) choline requirement of 1,200 mg/kg diet. Orthogonal (single degree of freedom) contrasts were used to compare the control vs. plus-methionine, control vs. plus-betaine, and plus-methionine vs. plus-betaine treatments. All analyses were conducted using the general linear models (GLM) procedure of SAS® (SAS Institute Inc., 1985).

[3]Leco Corp., St. Joseph, MI 49085.

RESULTS AND DISCUSSION

In both experiments, feeding the higher-protein, lower-energy level diet resulted in excellent growth rates, FCR and birds with abdominal fat pads approximately half as large as those from chicks fed the lower-protein and higher-energy diet (Tables 2 and 3). The predicted dietary influences of protein and energy level were achieved.

Chicks fed the 24% protein diets in both experiments, and the 17% protein diet in Experiment 2, were methionine- or labile methyl group-deficient, as indicated by growth and FCR responses to methionine and betaine. However, chicks fed the 17% protein diet in Experiment 1 did not appear to be methionine- or methyl group-deficient. The lack of any deficiency was probably due to the higher proportion of corn, a good source of methionine, and a lower proportion of soybean meal, a poor source of methionine in that diet. Thus, there were higher proportions of methionine and methionine-plus-cystine in the 17% protein diet in Experiment 1. Although the diet was marginally low in choline, labile methyl group donors did not improve bird performance. The abdominal fat pads of the betaine-supplemented birds appeared to be larger than those of the methionine-supplemented chicks, but neither group was different from the control; perhaps a significant difference may have been due to chance.

The dietary methionine supplements decreased relative liver size (Table 4), but betaine did not. The decrease in liver size appears to be related to changes in liver lipid levels. Although there were no significant ($P < 0.05$) changes in liver lipid levels in Experiment 1, methionine decreased liver lipids in Experiment 2, especially in the chicks made fat by feeding the low-protein, high-energy diet (Table 5). Any differences in responses between the two experiments may be due to the different formula-

TABLE 3. Influence of dietary protein and energy levels and methionine or betaine supplements on the hatching to 21-d performance of male broiler chickens (Experiment 2)[1,2]

Methyl source		Body weight gain			Feed conversion ratio			Fat pad (at 21 d)		
Met	Bet	17% protein	24% protein	Mean[3]	17% protein	24% protein	Mean	17% protein	24% protein	Mean
(%)		(g)			(g intake/g gain)			(% of body weight)		
0	0	440 ± 32[y]	573 ± 14[x]	507[c]	1.87 ± 0.13	1.69 ± 0.06	1.78[a]	1.87 ± 0.50	0.98 ± 0.24	1.42
1.5	0	599 ± 25[x]	597 ± 34[x]	598[a]	1.60 ± 0.02	1.51 ± 0.10	1.56[c]	1.81 ± 0.39	0.89 ± 0.26	1.36
0	0.65	469 ± 38[y]	617 ± 34[x]	543[b]	1.80 ± 0.06	1.53 ± 0.05	1.67[b]	1.94 ± 0.44	1.08 ± 0.31	1.47
Mean[3]		502[n]	596[m]		1.76[m]	1.58[n]		1.87[m]	0.98[n]	
ANOVA		(df)	(Pr > |T|)			(Pr > |T|)			(Pr > |T|)	
Protein level		1	0.0001			0.0001			0.0001	
$-CH_3$ source		1	0.0005			0.0001			0.1220	
Protein × $-CH_3$		1	0.0015			0.1152			0.9746	
Control vs. Met		1	0.0001			0.0001			0.2620	
Control vs. Bet		1	0.0696			0.0101			0.3401	
Met vs. Bet		1	0.0090			0.0124			0.0408	

[a–c;m–n;x–y]Values with no common superscript differ significantly ($P < 0.05$) when tested with Duncan's new multiple range test following analysis of variance.

[1]Mean ± SE of four replicate pens of eight chicks each for body weight and feed conversion ratio, and three chicks each for percentage fat pad.

[2]The 17% protein diet contained 3.3 kcal ME/g; the 24% protein diet contained 3.0 kcal ME/g.

[3]Mean = mean effect means.

tions, but they may also be due to the different genetic stocks that were used (Ross × Hubbard Hi-Y in Experiment 1 vs. Peterson × Arbor Acres in Experiment 2).

Increasing dietary protein level increased breast muscle protein concentrations by about 2% (e.g., 90.8 vs. 92.9%) in each experiment (Table 6). Interestingly, the methionine, but not the betaine, supplement increased muscle protein levels in Experiment 2, although not in Experiment 1. In Experiment 1, there was a decrease in muscle protein level in the methionine-supplemented chicks fed the 17% protein diet. The changes in muscle protein levels were accompanied by changes in muscle lipid levels. Breast muscle lipids were decreased by increasing dietary protein level in both experiments: 1.32 vs. 1.17% ($P < 0.0001$) in Experiment 1, and 1.46 vs. 1.21% ($P < 0.0001$) in Experiment 2. A decrease in breast muscle lipids due to methionine supplementation approached significance in Experiment 2 ($P = 0.064$), but not in Experiment 1 ($P = 0.327$).

By increasing dietary protein and decreasing dietary energy levels (Experiment 2; Table 7), total carcass protein concentration was increased by about 13% (41% vs. 54%), whereas total carcass lipids were reduced by about 7% (17% vs. 10%). The methionine supplement significantly increased the proportion of carcass protein ($P = 0.002$), and so intuitively, should have decreased the proportion of other components. However, the methionine supplement decreased carcass lipids, but the decrease did not reach the same level of probability ($P = 0.100$) due to differences in the magnitude of the variability between

TABLE 4. Influence of dietary protein and energy levels and methionine or betaine supplements on the relative liver weight (liver weight/body weight × 100) of 21-d-old male broiler chickens (Experiments 1 and 2)[1,2]

Methyl source		Experiment 1			Experiment 2		
Met	Bet	17% protein	24% protein	Mean[3]	17% protein	24% protein	Mean[3]
(%)							
0	0	3.13 ± 0.42	3.06 ± 0.45	3.09[a]	4.00 ± 0.81[x]	3.11 ± 0.34[y]	3.56[a]
1.5	0	2.83 ± 0.45	2.75 ± 0.30	2.79[b]	3.35 ± 0.67[y]	3.07 ± 0.42[y]	3.21[b]
0	0.65	3.03 ± 0.29	2.91 ± 0.25	2.97[a]	3.86 ± 0.69[x]	3.36 ± 0.36[y]	3.61[a]
Mean[3]		3.00	2.91		3.74[m]	3.18[n]	
ANOVA		(df)	(Pr > |T|)			(Pr > |T|)	
Protein level		1	0.1069			0.0001	
$-CH_3$ source		1	0.0001			0.0002	
Protein × $-CH_3$		1	0.9148			0.0128	
Control vs. Met		1	0.0001			0.0010	
Control vs. Bet		1	0.0735			0.5957	
Met vs. Bet		1	0.0060			0.0002	

[a–b;m–n;x–y]Values with no common superscript differ significantly ($P < 0.05$) when tested with Duncan's new multiple range test following analysis of variance.

[1]Mean ± SE of four replicate pens of three chicks each.

[2]The 17% protein diet contained 3.3 kcal ME/g; the 24% protein diet contained 3.0 kcal ME/g.

[3]Mean = main effect means.

TABLE 5. Influence of dietary protein and energy levels and methionine or betaine supplements on the percentage liver fat (liver fat weight/liver weight × 100) of 21-d-old male broiler chickens (Experiments 1 and 2)[1,2]

Methyl source		Experiment 1			Experiment 2		
Met	Bet	17% protein	24% protein	Mean[3]	17% protein	24% protein	Mean[3]
(mg/kg)		(%)					
0	0	4.37 ± 0.16	4.29 ± 0.15	4.33	5.87 ± 0.96	4.70 ± 1.07	5.29
1.5	0	4.33 ± 0.15	4.17 ± 0.13	4.25	4.95 ± 0.50	4.04 ± 0.38	4.50
0	0.65	4.16 ± 0.11	4.03 ± 0.13	4.10	4.45 ± 0.25	4.12 ± 0.23	4.29
Mean[3]		4.29	4.16		5.09[a]	4.29[a]	
ANOVA		(df)	(Pr > |T|)			(Pr > |T|)	
Protein level		1	0.2925			0.0001	
$-CH_3$ source		1	0.2426			0.0002	
Protein × $-CH_3$		1	0.9555			0.1947	
Control vs. Met		1	0.5768			0.0015	
Control vs. Bet		1	0.0983			0.0001	
Met vs. Bet		1	0.2939			0.3676	

[a]Values with no common superscript differ significantly ($P < 0.05$) when tested with Duncan's new multiple range test following analysis of variance.

[1]Mean ± SE of four replicate pens of three chicks each.

[2]The 17% protein diet contained 3.3 kcal ME/g; the 24% protein diet contained 3.0 kcal ME/g.

[3]Mean = main effect means.

individuals for the two parameters and variation in the chemical procedures used.

Liver betaine levels demonstrate that the betaine supplements affected tissue levels of betaine, especially when the lower-protein level diet was fed (Table 8). Dietary protein level appeared to have an effect on liver betaine levels, although the differences were not quite significant at the 0.05 level ($P = 0.055$). This result may be a matter of faster growth resulting in increased use and catabolism of betaine by the chick.

Increasing the dietary protein level also increased the total feather weight (~4 g/bird or 16%; Table 8) and the weights of the sample feathers (~9 mg or 13%). The methionine supplements had very similar effects (~3.5 g/bird or 15%, and ~6 mg or 8%, respectively). The effect of betaine was less than that of methionine and significant for sample, but not total, feather weights. Methionine supplements were particularly helpful in increasing total feather growth of birds fed the lower-protein diet. Betaine appears to be helpful in sparing methionine for growth of the primary feathers. Perhaps methionine-deficient birds partition extra methionine (spared by betaine in this case) to the primary flight feathers before general feather growth.

Tibia ash was decreased by increasing dietary protein level in both experiments: 42.2 vs. 41.7 ($P = 0.036$) in Experiment 1 and 41.5 vs. 40.5 ($P = 0.007$) in Experiment 2. This decrease in mineralization is probably a result of

TABLE 6. Influence of dietary protein and energy levels and methionine or betaine supplements on the breast muscle protein concentration (breast muscle protein weight/breast muscle weight × 100) of 21-d-old male broiler chickens (Experiments 1 and 2)[1,2]

Methyl source		Experiment 1			Experiment 2		
Met	Bet	17% protein	24% protein	Mean[3]	17% protein	24% protein	Mean[3]
(mg/kg)		(%)					
0	0	91.1 ± 0.6[y]	92.7 ± 0.8[x]	91.9	93.6 ± 0.5	95.4 ± 0.4	94.5[ab]
1.5	0	90.4 ± 1.0[z]	93.0 ± 1.0[x]	91.7	94.8 ± 1.1	96.1 ± 0.7	95.5[a]
0	0.65	91.0 ± 0.8[y]	92.9 ± 0.7[x]	92.0	92.9 ± 0.8	95.4 ± 1.4	94.1[b]
Mean[3]		90.8[n]	92.9[m]		93.7[a]	95.6[b]	
ANOVA		(df)	(Pr > |T|)			(Pr > |T|)	
Protein level		1	0.0001			0.0001	
$-CH_3$ source		1	0.1022			0.0002	
Protein × $-CH_3$		1	0.0132			0.1361	
Control vs. Met		1	0.0813			0.0022	
Control vs. Bet		1	0.8375			0.2351	
Met vs. Bet		1	0.0523			0.0001	

[a–b;m–n;x–z]Values with no common superscript differ significantly ($P < 0.05$) when tested with Duncan's new multiple range test following analysis of variance.

[1]Mean ± SE of four replicate pens of three chicks each.

[2]The 17% protein diet contained 3.3 kcal ME/g; the 24% protein diet contained 3.0 kcal ME/g.

[3]Mean = main effect means.

TABLE 7. Influence of dietary protein and energy levels and methionine or betaine supplements on the carcass composition of (percentage of defeathered carcass) 21-d-old male broiler chickens (Experiment 2)[1,2]

Methyl source		Carcass protein			Carcass fat		
Met	Bet	17% protein	24% protein	Mean[3]	17% protein	24% protein	Mean[3]
		(%)					
0	0	44.0 ± 2.8	55.6 ± 2.8	50.0[b]	16.9 ± 2.4	10.7 ± 1.3	13.7[ab]
1.5	0	45.6 ± 2.8	59.7 ± 3.2	52.0[a]	16.4 ± 1.6	9.5 ± 1.5	13.3[b]
0	0.65	42.6 ± 2.8	57.6 ± 3.0	50.5[b]	18.3 ± 1.6	9.9 ± 1.2	13.9[a]
Mean[3]		44.1[m]	57.5[m]		17.2[m]	10.0[n]	
ANOVA		(df)	(Pr > |T|)			(Pr > |T|)	
Protein level		1	0.0001			0.0001	
–CH_3 source		1	0.0032			0.0741	
Protein × –CH_3		1	0.1440			0.0813	
Control vs. Met		1	0.0016			0.1004	
Control vs. Bet		1	0.6987			0.5362	
Met vs. Bet		1	0.0062			0.0284	

[a–b;m–n]Values with no common superscript differ significantly (P < 0.05) when tested with Duncan's new multiple range test following analysis of variance.

[1]Mean ± SE of four replicate pens of three chicks each.

[2]The 17% protein diet contained 3.3 kcal ME/g; the 24% protein diet contained 3.0 kcal ME/g.

[3]Mean = main effect means.

the influence of protein level on FCR, because the chicks fed the lower-protein level diet had higher feed (and therefore calcium, phosphorus, and vitamin D, etc.) intake per unit of gain.

The results of Experiments 1 and 2 are similar to those of Rostagno and Pack (1996), but contrast with those of Saunderson and MacKinlay (1990) and Virtanen and Rosi (1995). There are many dissimilar conditions between the studies, so that an obvious reason for the differences in responses is not clear. Corn and soybean meal-based diets were used in all of the experiments, although Saunderson and MacKinlay (1990) added isolated soybean protein to some of their diets. Because of the unique formulations in each of the studies, the proportions of methionine, cystine, and choline were different. Different strains of birds were used in each of the studies, although in three of the reports (Experiment 1; Virtanen and Rosi, 1995; Rostagno and Pack, 1996), the same male parent line was used. Chicks in Experiments 1 and 2 were younger, but heavier, than those of Saunderson and MacKinlay (1990), but only about half the age of the birds studied by Rostagno and Pack (1996) or Virtanen and Rosi (1995). Many other factors may have been different, such as maternal diet, nutrient carryover into eggs, brooding temperatures, etc. In a series of seemingly identical experiments, Tillman and Pesti (1986) observed responses to methionine but not sulfate three times, and sulfate but not methionine three times, and concluded that there were some unidenti-

TABLE 8. Influence of dietary protein and energy levels and methionine or betaine supplements on the liver betaine (Experiment 1) and feathering (Experiment 2) of 21-d-old male broiler chickens[1,2]

Methyl source		Liver betaine			Total feather weight			Sample feather weight[3]		
Met	Bet	17% protein	24% protein	Mean[4]	17% protein	24% protein	Mean[4]	17% protein	24% protein	Mean[4]
(%)		(μg/g)			(g/bird)			(mg)		
0	0	7.6 ± 0.7	8.1 ± 1.6	7.9[b]	21.5 ± 5.3[y]	27.5 ± 4.7[x]	24.5[b]	67 ± 5	78 ± 2	73[b]
1.5	0	9.5 ± 2.6	8.5 ± 1.8	9.0[b]	29.2 ± 4.8[x]	27.1 ± 7.2[x]	28.2[a]	76 ± 2	82 ± 4	79[a]
0	0.65	13.1 ± 3.3	9.2 ± 1.4	11.1[a]	21.1 ± 5.1[y]	28.8 ± 3.9[x]	25.0[b]	71 ± 2	81 ± 1	76[a]
Mean[4]		10.1	8.5		23.9[n]	27.8[m]		71[n]	80[m]	
ANOVA		(df)	(Pr > |T|)			(Pr > |T|)			(Pr > |T|)	
Protein level		1	0.0551			0.0043			0.0001	
–CH_3 source		1	0.0044			0.0486			0.0027	
Protein × –CH_3		1	0.0595			0.0066			0.1268	
Control vs. Met		1	0.2085			0.0232			0.0007	
Control vs. Bet		1	0.0012			0.7839			0.0409	
Met vs. Bet		1	0.0295			0.0495			0.0769	

[a–b;m–n;y–z]Values with no common superscript differ significantly (P < 0.05) when tested with Duncan's new multiple range test following analysis of variance.

[1]Mean ± SE of four replicate pens of three chicks each.

[2]The 17% protein diet contained 3.3 kcal ME/g; the 24% protein diet contained 3.0 kcal ME/g.

[3]Weight of the two primary feathers cloest to the axial wing feather on one wing.

[4]Mean = main effect means.

fied factors influencing the chicks' responses to nutrients involved in methionine and one-carbon metabolism. The several experiments on relative responses to methionine and betaine supplements (present Experiments 1 and 2; Saunderson and MacKinlay, 1990; Virtanen and Rosi, 1995; Rostagno and Pack, 1996) support the conclusion that some unidentified factor is allowing betaine to reduce carcass fat in some experiments, but not in others. Experiments 1 and 2 demonstrate that the factor is not just the initial fatness of the birds. These experiments were powerful enough to demonstrate the chicks' body composition changes to dietary protein level; still, there was no demonstrable reduction in body lipids due to betaine supplementation.

Clearly, methionine has consistent effects, not only improving performance, but also increasing meat yields (Hickling et al., 1990; Schutte and Pack, 1995a,b; Pesti et al., 1999). There is a real challenge to determine the conditions and factors that allow betaine to decrease carcass fat and reduce the chick's need for methionine supplements.

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