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Impact of dietary changes on hepatic homocysteine metabolism in young broilers

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ABSTRACT

Information regarding the impact of sulfur amino acids (SAA) on hepatic homocysteine (Hcy) flux through the various metabolic pathways competing for Hcy in young broilers is lacking. An experiment was conducted to evaluate the impact of varying levels of dietary methionine (Met), choline, and betaine on hepatic Hcy flux in young broiler chickens. A standard starter basal diet was fed to chicks until 8 d of age; 12 experimental diets were given from 8-22 d. The experimental basal diet contained deficient levels of Met and cysteine (Cys); supplemental Met (0, 0.08, 0.16, and 0.24%) was added to the basal diet in combination with isomethyl levels of choline (0 or 0.25%) or betaine (0 or 0.28%). The 12 dietary treatments were replicated with three pens containing five chicks each (15 birds per treatment). Weight gain and feed efficiency increased ($P < 0.05$) with Met addition and were maximized with the addition of 0.16% digestible Met. No significant interactions ($P > 0.05$) with choline or betaine addition were noted for weight gain, feed intake, or feed efficiency, but numerical improvements for these variables were observed with the addition of choline and betaine to the Met-deficient basal diet. Analysis of liver tissue indicated that folate-dependent remethylation of Hcy predominated over betaine-dependent remethylation. Further, folate-dependent remethylation of Hcy appeared to be impacted by dietary choline and betaine levels, whereas betaine-dependent remethylation appeared to be more impacted by dietary SAA levels.

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INTRODUCTION

Methionine (Met) is a critically important amino acid for poultry, and there is a multi-million dollar industry for Met supplements, including DL-Met, which is a relatively inexpensive supplement. Compounds involved in sulfur amino acid (SAA) metabolism, including choline and betaine, may also be used as supplements to poultry diets, which further increases the value of this market. Methionine is the first-limiting amino acid in typical commercial poultry diets fed in the United States and has important metabolic roles that include initiation of protein synthesis, synthesis of polyamines, and donation of a methyl group to dozens of compounds.

Enzyme activity and SAA studies suggest that dietary modifications, specifically varying levels of Met, impact remethylation of homocysteine (Hcy; Emmert et al., 1996), which lies at a branch point in SAA metabolism (Fig. 1). Cysteine (Cys) can be synthesized from Met via transsulfuration of Hcy; alternatively Hcy may be remethylated (forming Met) by betaine-homocysteine methyltransferase (BHMT) or methionine synthase. In chickens and pigs, BHMT is found primarily in the liver with trace amounts of activity in the kidney (Emmert et al., 1996; Emmert et al., 1998), and hepatic BHMT levels of chicks and swine appear to be impacted by variation of dietary Met (Emmert et al., 1996; Emmert et al., 1998). These studies also showed that variations of dietary Cys impact hepatic BHMT activity, especially when levels of dietary choline and betaine are increased. Remethylation of Hcy may be further impacted by high intake of choline or betaine, the necessity to conserve Met, and/or the necessity to dispose of excess Hcy.

Previous data suggested that when dietary Met and Cys levels are adequate, BHMT contributes to a relatively small proportion of Hcy remethylation in the chick (Emmert et al., 1996). Conversely, it has been suggested that BHMT is an important regulator of cellular Met levels when chicks are given diets containing deficient or excess levels of Met (Emmert et al., 1996; Saunderson and MacKinlay, 1990), but these conclusions have been based on enzymatic activity data rather than actual Hcy flux. Our objective was to investigate the impact of dietary changes (varying amounts of Met, choline, and betaine) on growth performance and hepatic Hcy remethylation in young broiler chickens.

MEET THE STUDENT-AUTHOR

I am originally from Washburn, Wis., where I graduated with high honors from Washburn High School. Currently, I am a senior majoring in poultry science with a minor in communication. I plan to graduate in the summer of 2004 through the Honors Program. Several scholarships have made it possible for me to study at the University of Arkansas including the Joseph E. Fleming scholarship, the Randal Tyson Memorial scholarship, the James Whitmore Scholarship, and Poultry Science departmental scholarships. My collegiate activities have included Collegiate 4-H/FFA, Emerging Leaders, the Alpha Zeta Fraternity, the National Society of Collegiate Scholars, and the Poultry Science Club. I have also had the opportunity to serve as a Dale Bumpers College of Agricultural, Food and Life Sciences ambassador as well as an orientation leader for the University.

Fauna M. Ganson

I approached Dr. Emmert during the spring semester of 2003 in search of a research project, and he graciously involved me in his ongoing nutrition research. This project proved highly beneficial to me in terms of developing a project grant proposal, conducting scientific experiments, researching scientific journals, and defending a thesis. These skills will provide a firm background for my graduate work at the University of Wisconsin – Platteville where I will pursue a master's degree in adult education. Ultimately, I plan to enter the field of extension where my scientific background and communication skills may serve the general public.

MATERIALS AND METHODS

The University of Arkansas Institutional Animal Care and Use Committee approved all design and housing procedures. A 24-h photoperiod was maintained throughout the experiment, and water and experimental diets were freely available. Broiler chicks were obtained from a local hatchery, placed in floor pens that contained pine wood shavings, hanging tube feeders, and plasson waterers, and fed a standard starter diet (Table 1). At 8 d of age, chicks were weighed, wing-banded, and randomly placed in battery pens in an environmentally controlled room at the University of Arkansas Poultry Research Farm. Experimental diets (Table 1) were given from 8-22 d of age; the diets were similar to those used in preliminary research to assess BHMT activity (Emmert et al., 1996). Experimental diets were fortified to meet or exceed NRC (1994) recommendations, with the exception of Met and Cys. Supplemental DL-Met, choline, and betaine were added in varying combinations at the expense of cornstarch (Table 2). The experiment contained 12 dietary treatments that were replicated with three pens containing five chicks each. At assay termination, chicks and feed were weighed so that weight gain, feed intake, and feed efficiency could be calculated.

Table 1. Starter[z] and experimental diets.[y]

Ingredient	Starter diet	Experimental diet
Corn	58.75	55.78
Soybean meal	28.80	----
Poultry byproduct meal	5.00	----
Peanut meal	----	32.10
Soybean oil	----	6.00
Poultry fat	4.00	----
Dicalcium phosphate	1.49	2.00
Limestone	0.96	1.00
NaCl	0.44	0.40
Vitamin mix	0.20[x]	0.20[v]
Trace mineral mix	0.10[w]	0.15[v]
Choline Cl (60%)	----	0.12
DL-methionine	0.19	----
L-lysine•HCl	0.07	----
Amino acids[u]	----	1.44
Cornstarch	----	to 100

[z] Fed until day 8 posthatching

[y] Diet supports maximum growth when fortified with Met and Cys (Emmert and Baker, 1997). The diet contained 0.23% digestible Met and 0.25% digestible Cys, and was fed from 8 to 22 d posthatching.

[x] Provides per kilogram of diet: vitamin A, 9,900 IU; cholecalciferol, 3,300 ICU; vitamin E, 13 IU; vitamin B_{12}, 0.013 mg; riboflavin, 6.6 mg; niacin, 66mg; d-pantothenic acid, 16.5 mg; choline, 660 mg; menadione, 1.1 mg; folacin, 1.1 mg; thiamin, 1.1 mg; pyridoxine, 3.3 mg; d-biotin, 0.11 mg; Se, 0.20 mg; ethoxyquin, 125 mg

[w] Provides per kilogram of diet: Mn ($MnSO_4$•H_20) 100 mg; Zn ($ZnSO_4$•$7H_20$) 100 mg; Fe ($FeSO_4$•$7H_20$) 50 mg; Cu ($CuSO_4$•$5H_20$) 10 mg; I ($Ca(IO_3)2$•H_2O) 1mg.

[v] Han and Baker (1993)

[u] Included crystalline lysine, threonine, valine, isoleucine, and tryptophan

A stable isotope technique used by Storch et al. (1988, 1991) and van Gueldner et al. (1999) was utilized (with slight modifications). At assay termination, birds were killed by carbon dioxide asphyxiation, and liver tissue was collected and immediately frozen in liquid nitrogen. Tissue samples were stored at -80°C prior to analysis. Subsamples of tissue were weighed and homogenized, then suspended in potassium buffer. After centrifugation, designated amounts of Hcy and a stable isotope of betaine were added to the supernatant alone or in different combinations and incubated for 0 to 10 min. Addition of 2 ml of 2 N HCl terminated the reactions at the appropriate time, and the solutions were subjected to liquid chromatography-mass spectrometry (LC-MS) analysis. Results of LC-MS analysis were used to estimate Hcy flux. In our assay Met generated through BHMT has a higher atom mass unit than Met generated through Met synthase (153 a.m.u. versus 150 a.m.u.); Met remethylated through betaine- and folate-dependent pathways is thus distinguishable.

The experiment was analyzed as a completely randomized design, and the General Linear Models (GLM) procedure of SAS® was used to conduct one-way ANOVA on all data. Differences among treatment means were established using the least-significant difference multiple-comparison procedure.

RESULTS AND DISCUSSION

Weight gain and feed efficiency increased ($P<0.05$) with Met addition (Table 3). The experimental basal diet yielded a gain of 428 g and a feed efficiency of 582 g/kg; weight gain and feed efficiency were maximized with the addition of 0.16% DL-Met. This is surprising considering that the addition of 0.16% DL-Met to the basal diet gave a value that appears to fall below the Met and Cys requirements for broilers of this age (NRC, 1994). However, similar observations have been previously noted in our laboratory (unpublished data).

Choline and betaine addition did not significantly impact growth performance ($P>0.05$; Table 3). However, choline and betaine additions yielded numerical improvements in weight gain and feed efficiency when added to the basal diet that was deficient in Met and Cys (data not shown). These data agree with previous research in broilers showing that there was little growth response to choline or betaine addition to a diet significantly deficient in Met and Cys (Emmert and Baker, 1997; Emmert et al., 1996).

Figure 2 illustrates native (formed via Met synthase) and enriched (formed via BHMT) Met formation. Flux

Table 2. Description of dietary treatments.[z]

Treatment	Supplemental met	Supplemental choline[y]	Supplemental betaine[y]
	(%)	(%)	(%)
1	0	0	0
2	0	0.25	0
3	0	0	0.28
4	0.08	0	0
5	0.08	0.25	0
6	0.08	0	0.28
7	0.16	0	0
8	0.16	0.25	0
9	0.16	0	0.28
10	0.24	0	0
11	0.24	0.25	0
12	0.24	0	0.28

[z] Experimental diet contained 0.23% digestible methionine and 0.25% digestible cysteine, representing approximately 60% of the digestible SAA requirement.

[y] Choline and betaine additions were isomethyl.

of Hcy through BHMT was not substantially impacted by betaine, choline, or SAA level. Rather, Met-synthase was more responsive than BHMT to dietary changes in choline and betaine levels as well as more responsible for regulating Hcy levels in birds fed deficient or excess levels of SAA.

Figure 3 illustrates the relative BHMT contribution to Met synthesis. It has been previously estimated from data with rats and humans that there is an approximately equal distribution of Hcy remethylation via Met synthase or BHMT (Finkelstein and Martin, 1984; Mudd et al., 1970). However, in this study, BHMT contribution typically ranged from 16.3% to 38.0%, with the exception of the diet containing 0.16% DL-Met addition and no choline or betaine; under these conditions the BHMT contribution was 48.3%. This was the only time that BHMT remethylation approached 50%, which suggests that BHMT and Met synthase contribution is only equivalent in broilers when SAA levels are adequate and there is not an excess of choline or betaine.

In conclusion, we found no significant correlation between growth and the means of remethylation. In contrast to previous assumptions based on enzyme activity, Met synthase appears to be more active in the young broiler than BHMT. Moreover, these data suggest that Met synthase may play a more active regulatory role in maintaining hepatic Met levels of broilers fed diets containing deficient or excess SAA.

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Table 3. Impact of dietary treatment on growth performance.[z]

Supplement	%	n	Gain (g)	Feed intake (g)	Gain:feed (g/kg)
Methionine	0	9	428c[y]	736b	582c
	0.08	9	533b	810a	660b
	0.16	9	573a	802a	715a
	0.24	9	561ab	810a	692ab
Pooled SEM[x]			13.3	13.2	16.6
Choline	0	24	532	797	666
	0.25	12	508	774	655
Pooled SD[x]			37.2	35.3	53.6
Betaine	0	24	516	783	657
	0.28	12	540	803	673
Pooled SD			37.2	35.3	53.6
Main effects					
Methionine			P < 0.0001	P < 0.0001	P < 0.0006
Choline			P = 0.08	P = 0.08	P = 0.54
Betaine			P = 0.07	P = 0.13	P = 0.43

[z] Values are means of three replicate pens containing five chicks.
[y] Means within columns (within supplement) lacking a common superscript differ (P < 0.05).
[x] SEM = standard error of the mean; SD = standard deviation.

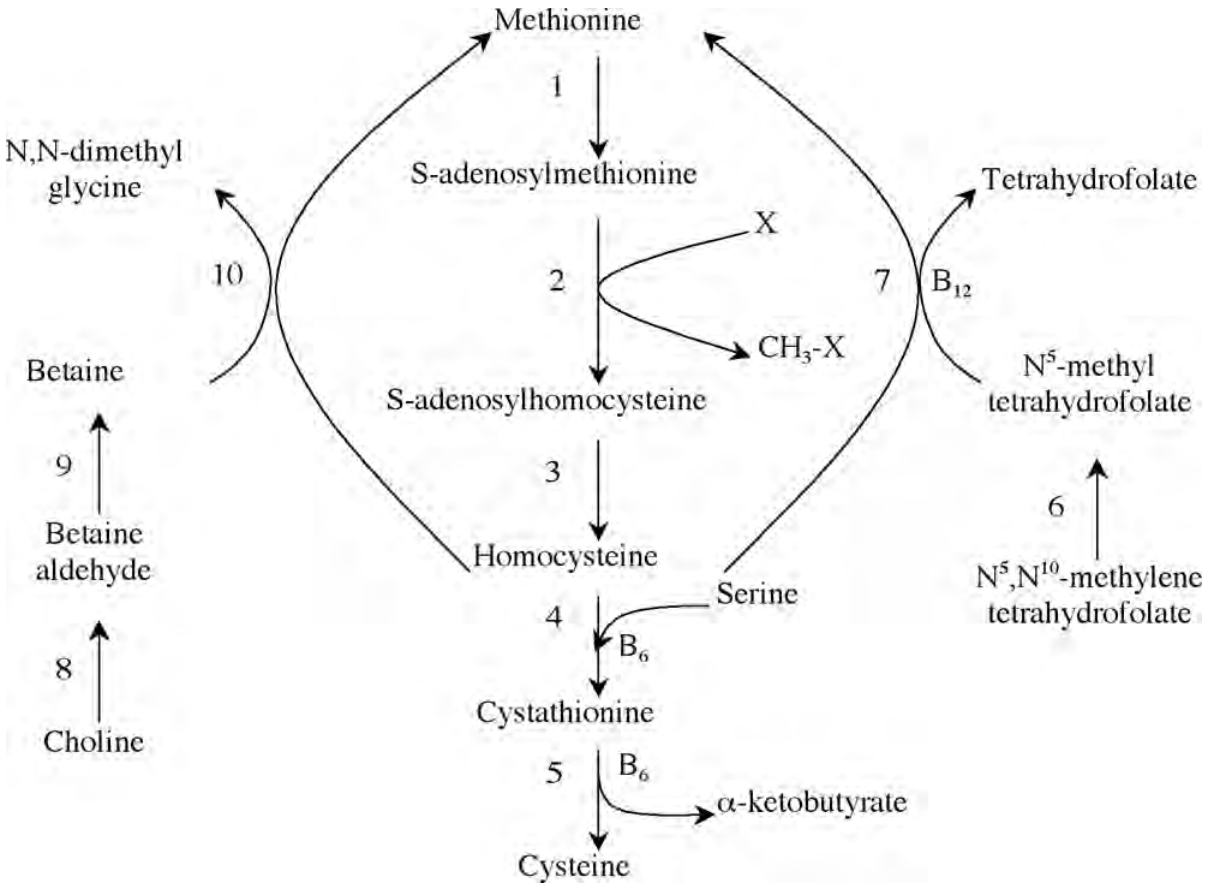

Fig. 1. Metabolism of sulfur amino acids, choline, and betaine. Numerals indicate the following enzymes: 1) methionine adenosyltransferase; 2) various enzymes; 3) S-adenosylhomocysteine hydrolase; 4) cystathionine synthase; 5) cystathionine lyase; 6) N5,N10-methylenetetrahydrofolate reductase; 7) methionine synthase; 8) choline dehydrogenase; 9) betaine aldehyde dehydrogenase; 10) betaine-homocysteine methyltransferase.

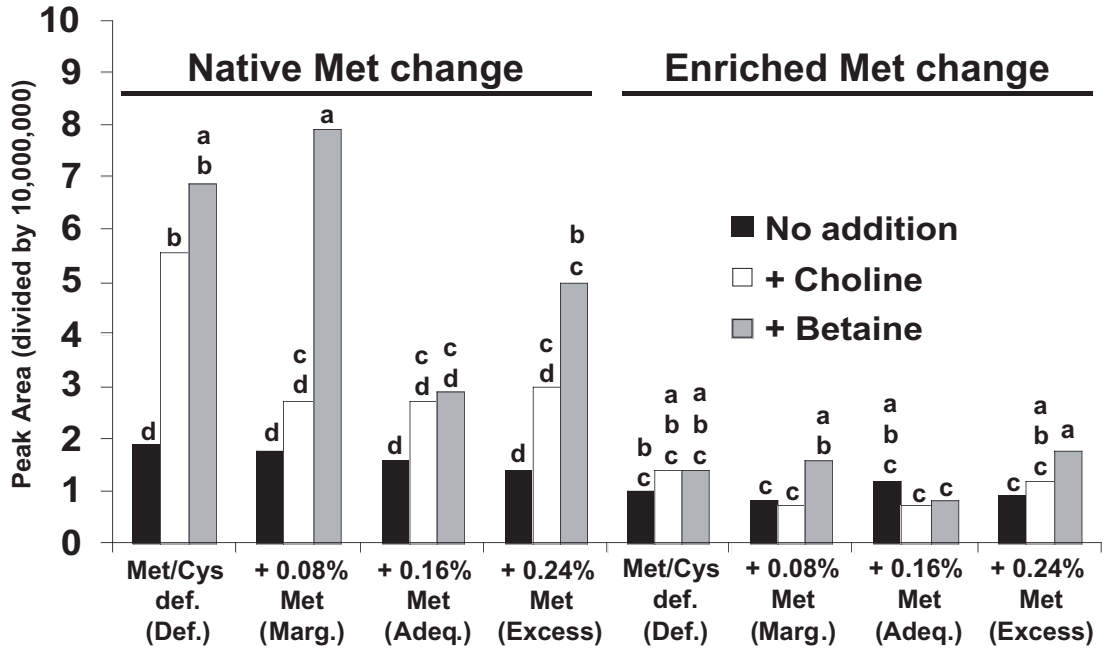

Fig. 2. Formation of enriched and native methionine. The data represent the amount of methionine formed by remethylation via methionine synthase (native methionine) or BHMT (enriched methionine). Data were generated by subtracting the 0 incubation time peak area (from LC-MS analysis) for enriched or native methionine from the 10 minute incubation peak area (values were then divided by 10,000,000). Superscripts denote differences ($P < 0.05$) as measured within the type of methionine formation (native or enriched).

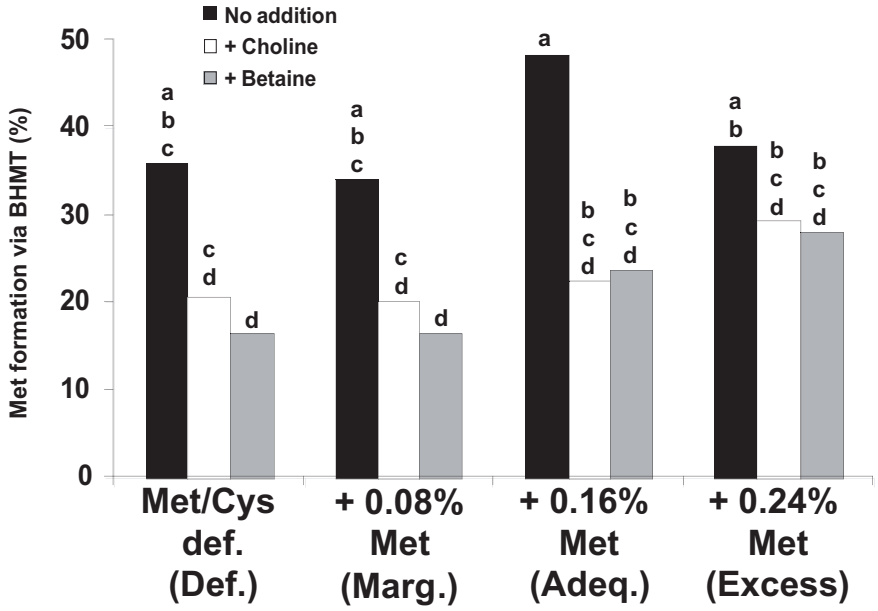

Fig. 3. Relative contribution of BHMT to Hcy remethylation. The data represent the percent of methionine formed by remethylation via BHMT. Superscripts denote differences ($P < 0.05$) among treatments.