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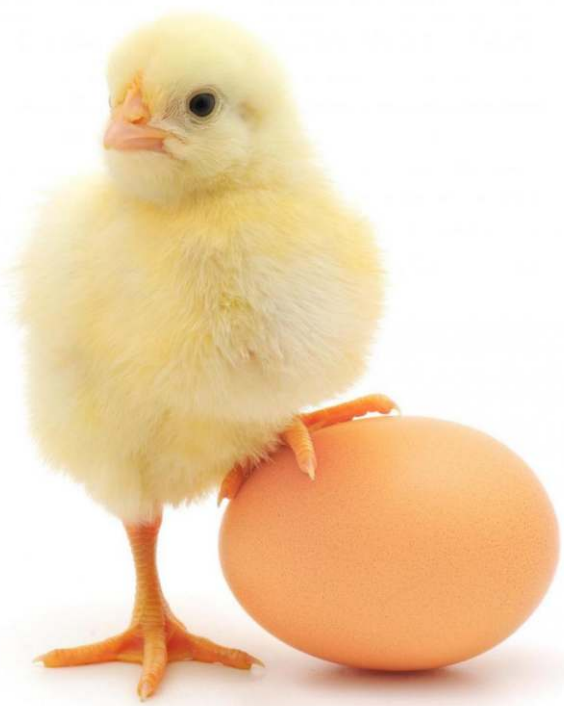

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Evaluation of Separate and Combined Effects of Choline and Betaine in Diets for Male Broilers

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Abstract: A study was conducted to evaluate the effects of supplementation of corn-soybean meal based diets with betaine and choline on live performance and breast meat yield of male broiler chicks. Diets formulated to meet nutrient levels of top broiler companies were supplemented with either 0 or 1000 mg/kg betaine in combination with either 0 or 1000 mg/kg choline. Betaine supplementation was carried out either for the full 49 d feeding trial or initiated at 35 d of age. Each dietary treatment was assigned to eight replicate pens of 60 male chicks of a commercial strain. At 42 and 49 d of age samples of birds were processed under commercial conditions and dressing percentage and breast yield determined. The results of this study indicate little or no positive benefit in terms of body weight gain, feed conversion, or mortality from the addition of betaine to nutritionally adequate corn-soybean meal based diets for broiler chicks in situations where disease challenge from coccidiosis is not a major issue. Improvements in dressing percentage were obtained from betaine supplementation and in breast yield from choline supplementation at 42 d of age but not at 49 d of age. Therefore, age of bird might be a consideration for using these supplements as birds processed at younger ages might be more responsive to these nutrients. Further studies are needed to evaluate the potential effect of age on response to choline and betaine.

Key words: Broilers, betaine, choline, processing yield, breast meat

Introduction

Many studies have examined the interrelationship between choline and methionine (Marvel *et al.*, 1944; Clandinin *et al.*, 1946; Gerry *et al.*, 1948; Sunde *et al.*, 1951; West *et al.*, 1951; Featherston and Stephenson, 1960; Quillen *et al.*, 1961; Pesti *et al.*, 1980; Baker *et al.*, 1983; Miles *et al.*, 1983; Tillman and Pesti, 1986; Garcia-Neto *et al.*, 2000) and between betaine and methionine (Rostagno and Pack, 1996; Schutte *et al.*, 1997; Kidd *et al.*, 1997; Virtanen and Rumsey, 1996; Esteve-Garcia and Mack, 2000; McDevitt *et al.*, 2000) to determine if these compounds can spare the needs of the chick for methionine, with considerable variation in results. While some studies (Virtanen and Rosi, 1995; Virtanen and Rumsey, 1996) suggest that the response to betaine was greater than that obtained from the addition of methionine, others have failed to demonstrate that the methionine content of the diet could be reduced by supplementation with betaine (Rostagno and Pack, 1996; Schutte *et al.*, 1997; Esteve-Garcia and Mack, 2000; McDevitt *et al.*, 2000). However, several studies suggest that addition of betaine may improve breast meat yield (Schutte *et al.*, 1997; Wallis, 1999; Esteve-Garcia and Mack, 2000; McDevitt *et al.*, 2000; Remus, 2001). A recent study from our laboratory (Waldroup *et al.*, 2005) concluded that addition of 1000 mg/kg betaine, 1000 mg/kg choline, or the combination of 500 mg/kg of betaine and 500 mg/kg of choline, fed continuously from one day of age, resulted in a significant improvement in breast meat yield of male broilers, independent of the level of methionine in the diet. From this study it was difficult to determine the relative contributions of betaine and choline to this response. The present study was designed to evaluate the individual and combined contributions of betaine and choline to enhancement of breast meat yield in male broilers grown for further processing.

Materials and Methods

Diets were formulated using corn and soybean meal of known crude protein and moisture content to meet the nutrient levels of the top five companies in a popular agricultural survey The nutrient standards used for formulation are shown in Table 1 and the composition of the diets for the various age periods is shown in Table 2. For each age period, a large batch of the basal diet was prepared and aliquots used for mixing the various test diets. Diets were fortified with complete vitamin and trace mineral premixes obtained from local poultry companies.

Dietary treatments consisted of a 2 x 3 factorial arrangement of treatments with two choline levels (0 or 1000 mg/kg supplemental choline) and three betaine treatments (0, 1000 mg/kg from 0 to 49 d, 0 to 35 followed by 1000 mg/kg to 49 d). Because much of the development of breast muscle takes place during the latter stages of growth it might be possible to obtain the same improvements by supplementing the finisher diets with betaine, thus the reason for feeding only from 35 to 49 days in the latter treatment. Choline was supplied as

Table 1: Nutrient content of diets from top broiler companies

Nutrient	Unit	Starter 0-16 d	Grower 16-30 d	Finisher 1 30-42 d	Finisher 2 42d-mkt
ME	Kcal/lb	1362.00	1394.00	1416.00	1460.00
Crude protein	%	20.93	19.16	17.02	16.08
Lysine	%	1.23	1.09	0.93	0.88
TSAA	%	0.96	0.85	0.76	0.76
Tryptophan	%	0.25	0.22	0.20	0.18
Arginine	%	1.52	1.31	1.17	1.08
Threonine	%	0.85	0.76	0.68	0.64
Nonphytate P	%	0.43	0.37	0.34	0.33
Calcium	%	0.83	0.85	0.76	0.76
Sodium	%	0.23	0.20	0.21	0.20

Table 2: Composition (g/kg) and nutrient content of diets formulated to standards of top broiler companies

Ingredient	Starter 0-14 d	Grower 14-35 d	Finish 135-42 d	Finish 2 42-56 d
Yellow corn	621.21	655.37	718.36	722.44
Soybean meal (48% CP)	312.75	276.01	218.11	198.36
Dicalcium phosphate	20.39	17.27	15.27	14.77
Poultry oil	16.67	23.32	20.69	36.73
Ground limestone	8.70	10.86	10.16	10.42
Feed grade salt	5.24	4.67	4.76	4.68
Constant ingredients[1]	4.25	4.25	4.25	4.25
L-Lysine HCl (98%)	1.88	1.27	1.30	1.29
L-Threonine	0.90	0.57	0.65	0.64
L-Arginine	1.93	1.02	1.39	1.16
DL-Methionine (98%)	3.08	2.39	2.06	2.26
Variable[2]	3.00	3.00	3.00	3.00
TOTAL	1000.00	1000.00	1000.00	1000.00
Nutrient composition[3]ME kcal/lb (C)	1362.00	1394.00	1416.00	1460.00
Crude protein, % (C)	20.93	19.17	17.02	16.08
Crude protein, % (A)	21.45	20.16	17.84	16.43
Lysine, %(C)	1.24	1.09	0.93	0.88
Lysine, % (A)	1.26	1.11	0.96	0.92
TSAA, %(C)	0.96	0.85	0.76	0.76
TSAA, % (A)	1.01	0.93	0.84	0.82
Threonine, %(C)	0.85	0.76	0.68	0.64
Threonine, % (A)	0.88	0.79	0.72	0.67
Nonphytate P, %(C)	0.43	0.37	0.34	0.33
Calcium, %(C)	0.84	0.85	0.76	0.75
Sodium, %(C)	0.23	0.20	0.21	0.20

[1]Contains 0.5 g/kg BMD-50 (Alpharma, Inc., Fort Lee NJ 07024), 0.75 g/kg Coban 60 (Elanco Animal Health division of Eli Lilly & Co., Indianapolis IN 46285) 2.0 g/kg of vitamin premix (provides per kg of diet: vitamin A 7714 IU; cholecalciferol 2204 IU; vitamin E 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione 1.5 mg; folic acid 0.9 mg; thiamin 1.54 mg; pyridoxine 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg), 1.0 g/kg trace mineral mix (provides per kg of diet: Mn (from $MnSO_4{\bullet}H_20$) 100 mg; Zn (from $ZnSO_4{\bullet}7H_2O$) 100 mg; Fe (from $FeSO_4{\bullet}7H_2O$) 50 mg; Cu (from $CuSO_4{\bullet}5H_20$) 10 mg; I from $Ca(IO_3)_2{\bullet}H_20$), 1 mg.

[2]Variable amounts of choline, betaine, and washed builders sand.

[3]C = calculated from NRC (1994) adjusted to crude protein and moisture content of ingredients used in formulation; A = Analyzed by Ajinomoto Heartland Lysine LLC, Chicago IL.

a dry 60% choline chloride product while betaine was added as a crystalline 97% product. Choline and betaine analyses of the mixed diets were conducted by a commercial laboratory specializing in these assays. The basal diet in each period was analyzed for crude protein and amino acid content.

The house used in this study was an insulated steel truss building of commercial design with a 3 ft curtain sidewall containing 48 pens with 56 ft^2 per pen. New softwood shavings over concrete floors served as litter.

Table 3: Choline content (mg/kg) of experimental diets

Feeding period	Basal diet		+ 1000 mg/kg Choline	
	Calculated[1]	Analyzed[2]	Analyzed[2]	Difference[3]
Starter	1239	1235	2140	905
Grower	1160	1220	2080	860
Finisher 1	1041	1135	2013	898
Finisher 2	990	1115	1933	818

[1]Calculated from NRC (1994). [2]Analyzed by Danisco Animal Nutrition, St. Louis MO. [3]Difference between analyzed level of basal diet and choline supplemented diets.

Table 4: Betaine content (mg/kg) of experimental diets

Feeding period	Basal diet	+ 1000 mg/kg betaine[1]
Starter	Bdl[2]	1018
Grower	Bdl	1051
Finisher 1	Bdl	1036
Finisher 2	bdl	1072

[1]Analyzed by Danisco Animal Nutrition, St. Louis MO
[2]Bdl = below detection limits.

Temperature was controlled by thermostatically controlled gas brooders, adjustable sidewall curtains, and exhaust fans. Each pen was equipped with two tube feeders and one automatic water fount. For the first seven days supplemental feeders and waterers were used. Incandescent lights supplemented natural daylight to provide 23 hr light daily.

Male broilers of a commercial strain were obtained from a local hatchery where they had been vaccinated *in ovo* for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. Sixty chicks were assigned at random to each pen. Care and management of the birds followed recommended guidelines (FASS, 1999). Each of the six treatments was fed to eight pens of chicks, randomly distributed throughout the house.

Body weight by pen was obtained at 14, 35, 42, and 49 d with feed consumption during each period determined. Birds were checked twice daily; any bird that died or was removed to alleviate suffering was weighted and the weight used to adjust feed conversion (FCR; grams of feed per gram of gain). At 42 and 42 d five birds per pen, selected from among 15 birds marked by a toe slit at day-old, were processed to determine dressing percentage and parts yield. After a 12 hr fast the birds were placed in coops and transported approximately one mile to the processing plant. They were weighed and processed with automated evisceration as described by Fritts and Waldroup (2005). Due to the automatic evisceration it was not possible to determine abdominal fat content. The eviscerated carcasses were weighed and placed in a chiller with air agitation for one hour. The carcasses were removed, hung on shackles, and allowed to drain for 30 min. The carcasses were then deboned by trained technicians to determine parts yield.

Pen means served as the experimental unit for statistical analysis. Data were subjected to ANOVA using the General Linear Models procedure of the SAS Institute (1991). When significant differences among treatments were found, means were separated using repeated t-tests using the LSMEANS option of the GLM procedure. Main effects of betaine program and choline addition were examined along with the interaction of betaine program and choline addition. Data for 14 and 35 d combined the results of the birds fed no betaine with those who did not receive betaine until 35 d of age. Mortality data were transformed to $\sqrt{n+1}$ prior to analysis; data are presented as natural numbers.

Results

Analysis of the basal diet indicated that the levels of crude protein and essential amino acids were in good agreement with calculated values (Table 2). The analyzed choline content of the basal diet was also in good agreement with calculated values (Table 3). Amount of added choline was determined by difference by subtracting the total choline content of the supplemented diets from that obtained on the basal diet; added choline values were slightly under the expected value of 1000 mg/kg but averaged 874 mg/kg over the entire study. Analyzed betaine content of the test diets was in good agreement with expected values (Table 4) with an average of 1044 mg/kg over the entire study. Thus, the test diets were considered to contain the expected levels of nutrients.

The effects of betaine feeding program and choline supplementation on body weight at different ages are shown in Table 5. Feeding 1000 mg/kg of betaine from 0 to 49 or from 35 to 49 d had no significant influence on body weight at any age. There was a significant effect of choline supplementation on body weight at 42 d, with a significant reduction in BW from birds fed 1000 mg/kg of choline. However, there was a significant interaction of betaine and choline supplementation at both 42 and 49 d of age. The addition of 1000 mg/kg of choline to diet of chicks that had not been fed betaine or to the diet of chicks that had been feed 1000 mg/kg of betaine from day of age had no effect on BW of chicks; however, when 1000 mg/kg choline was added to the diet of chicks that

Table 5: Effect of supplemental betaine and choline on body weight of broilers (means of eight pens of 60 birds per treatment. Data from birds fed diets with no betaine from 0 to 35 d were combined at 14 and 35 d)

Measurement	Betaine Program[1]	Supplemental choline (mg/kg)		
		None	1000	Mean
14 d BW (kg)	None	0.463	0.459	0.461
	0-49 d	0.461	0.466	0.464
	Mean	0.462	0.463	
35 d BW (kg)	None	2.068	2.068	2.068
	0-49 d	2.023	2.025	2.024
	Mean	2.046	2.047	
42 d BW (kg)	None	2.800[bc]	2.809[ab]	2.804
	0-49 d	2.789[bc]	2.770[bc]	2.780
	35-49 d	2.876[a]	2.727[c]	2.801
	Mean	2.822[x]	2.768[y]	
49 d BW (kg)	None	3.311[b]	3.382[ab]	3.346
	0-49 d	3.402[ab]	3.328[b]	3.365
	35-49 d	3.469[a]	3.309[b]	3.389
	Mean	3.394	3.340	

Measurement	Probability > F			
	Betaine	Choline	Bet x Cho	CV
14 d BW	0.61	0.84	0.61	4.34
35 d BW	0.09	0.93	0.52	3.70
42 d BW	0.61	0.04	0.03	3.40
49 d BW	0.56	0.10	0.03	3.92

[1]Time of addition of 1000 mg/kg betaine. [abc]Means with common superscripts do not differ significantly (P<0.05).
[xy]Means with common superscripts do not differ significantly (P<0.05).

had not been fed betaine until 35 d of age, there was a significant reduction in BW at both 42 and 49 d of age.

Feed conversion from 0 to 42 d was numerically improved (P = 0.08) and from 0 to 49 d was significantly improved (P < 0.01) by feeding 1000 mg/kg betaine from day of age but not when fed from 35 d (Table 6). Overall, choline supplementation had no significant effect on feed utilization but the addition of 1000 mg/kg choline to the diet significantly reduced feed conversion at 42 d when fed in diets in which betaine feeding was initiated at 35 d. This interaction was not observed at 49 d of age.

Mortality was very low during the study (Table 7). There was no indication of any effect of betaine or choline on mortality at any age during the study, nor was their any interaction of the two nutrients. The chicks were placed on new litter and the diets contained an anticoccidial and bacitracin methylene disalicylate. Examination of dead or culled birds by a qualified poultry pathologist did not reveal any indication of problems with coccidiosis or intestinal necrosis.

The effects of betaine feeding program and choline supplementation on carcass parameters are shown in Table 8. Feeding 1000 mg/kg of betaine beginning at one day of age resulted in a significant improvement in dressing percentage at 42 d but not at 49 d. Addition of betaine had no significant effect on breast yield at 42 or 49 d of age. Addition of 1000 mg/kg of choline significantly improved breast yield at 42 d but not at 49 d.

Discussion

No attempts were made in the present study to reduce the methionine content of the diet by supplementation with choline or betaine as previous work from our laboratory (Waldroup *et al.*, 2005) and elsewhere (Rostagno and Pack, 1996; Schutte *et al.*, 1997; Esteve-Garcia and Mack, 2000; McDevitt *et al.*, 2000) demonstrated no methionine sparing effect for betaine in corn-soybean based broiler diets. No improvement in body weight over the course of the present study was observed as a result of supplementing the diets with 1000 mg/kg of betaine. A negative effect on body weight at both 42 and 49 d of age was observed when 1000 mg/kg choline was added to diets of chicks that had been fed betaine from 35 d of age after receiving no betaine supplementation for the first 35 d. This effect was not observed when choline was added to diets of chicks that had been receiving 1000 mg/kg of betaine from day old.

Feed conversion over the 49 d feeding period was significantly improved by continuous addition of 1000 mg/kg betaine, but not when fed only for 35 to 49 d of age. The negative effect of choline supplementation of diets of chicks that had been fed betaine from 35 d of age after receiving no betaine supplementation for the first 35 d was also observed on feed conversion at 42 d of age but not at 49 d. It is possible that some competition for metabolic pathways may take place

Table 6: Effect of supplemental betaine and choline on feed conversion by male broilers (means of eight pens of 60 birds per treatment. Data from birds fed diets with no betaine from 0 to 35 d were combined at 14 and 35 d)

Measurement	Betaine Program[1]	Supplemental choline (mg/kg)		
		None	1000	Mean
0-14 d FCR	None	1.200	1.192	1.196
	0-49 d	1.176	1.189	1.183
	Mean	1.188	1.191	
0-35 d FCR	None	1.613	1.605	1.609
	0-49 d	1.597	1.599	1.598
	Mean	1.605	1.602	
0-42 d FCR	None	1.765[ab]	1.740[bc]	1.753
	0-49 d	1.738[bc]	1.726[c]	1.732
	35-49 d	1.739[bc]	1.786[a]	1.762
	Mean	1.747	1.751	
0-49 d FCR	None	1.873	1.854	1.863[x]
	0-49 d	1.814	1.833	1.823[y]
	35-49 d	1.849	1.844	1.847[x]
	Mean	1.845	1.843	
Measurement	Probability > F			
	Betaine	Choline	Bet x Cho	CV
0-14 d FCR	0.37	0.96	0.60	2.96
0-35 d FCR	0.62	0.65	0.59	2.56
0-42 d FCR	0.08	0.73	0.02	2.22
0-49 d FCR	0.01	0.88	0.35	1.94

[1]Time of addition of 1000 mg/kg betaine. [abc]Means with common superscripts do not differ significantly (P<0.05).
[xy]Means with common superscripts do not differ significantly (P<0.05).

Table 7: Effect of supplemental betaine and choline on mortality of broilers (means of eight pens of 60 birds per treatment. Data from birds fed diets with no betaine from 0 to 35 d were combined at 14 and 35 d)

Measurement	Betaine Program[1]	Supplemental choline (mg/kg)		
		None	1000	Mean
0-14 d Mortality (%)	None	1.25	0.83	1.04
	0-49 d	0.00	0.00	0.00
	Mean	0.63	0.42	
0-35 d Mortality (%)	None	2.08	1.25	1.67
	0-49 d	0.83	0.00	0.42
	Mean	1.46	0.63	
0-42 d Mortality (%)	None	0.83	1.67	1.25
	0-49 d	0.83	0.83	0.83
	35-49 d	3.33	0.83	2.08
	Mean	1.67	1.11	
0-49 d Mortality (%)	None	0.83	1.67	1.25
	0-49 d	0.83	0.83	0.83
	35-49 d	3.33	0.83	2.08
	Mean	1.67	1.11	
Measurement	Probability > F			
	Betaine	Choline	Bet x CHO	CV[2]
0-14 d mortality	0.32	0.69	0.06	1.15
0-35 d mortality	0.28	0.33	0.28	1.42
0-42 d mortality	0.51	0.54	0.29	1.50
0-49 d mortality	0.51	0.54	0.29	1.50

[1]Time of addition of 1000 mg/kg betaine. [2]CV of transformed means.

Table 8: Effect of supplemental betaine and choline on carcass parameters of broilers (means of eight pens of five birds per time period)

Measurement	Betaine Program[1]	Supplemental choline (mg/kg)		
		None	1000	Mean
42 d dressing percentage	None	70.88	70.82	70.85[xy]
	0-49 d	71.91	70.96	71.44[x]
	35-49 d	70.49	70.54	70.52[y]
	Mean	71.10	70.78	
42 d breast yield	None	25.73	26.58	26.15
	0-49 d	26.30	26.44	26.37
	35-49 d	26.31	26.74	26.52
	Mean	26.11[b]	26.58[a]	
49 d dressing percentage	None	70.48	71.88	71.18
	0-49 d	69.98	70.25	70.11
	35-49 d	70.40	71.32	70.86
	Mean	70.29	71.15	
49 d breast yield	None	27.14	27.14	27.14
	0-49 d	26.92	26.40	26.66
	35-49 d	26.40	26.98	26.68
	Mean	26.81	26.84	
Measurement	Probability > F			
	Betaine	Choline	Bet x Cho	CV
42 d Dressing percentage	0.01	0.20	0.21	2.72
42 d Breast yield	0.35	0.03	0.38	6.05
49 d Dressing percentage	0.22	0.09	0.66	5.56
49 d Breast yield	0.17	0.93	0.16	6.71

[1]Time of addition of 1000 mg/kg betaine. [ab]Means with common superscripts do not differ significantly ($P<0.05$).
[xy]Means with common superscripts do not differ significantly ($P<0.05$).

between choline and betaine that may require some adjustment period; this is worthy of further investigation. In contrast to previous reports from our laboratory (Waldroup *et al*, 2005) and elsewhere (Schutte *et al.*, 1997; Wallis, 1999; Esteve-Garcia and Mack, 2000; McDevitt *et al.*, 2000; Remus, 2001) that betaine supplementation may improve breast meat yield, there was no indication of improvements in breast yield in the present study. Addition of 1000 mg/kg betaine from day of age significantly improved carcass dressing percentage at 42 d of age but not at 49 d. Addition of 1000 mg/kg of choline significantly improved breast yield at 42 d but not at 49 d.

Due in large measure to its osmoregulatory functions (Kidd *et al.*, 1997; Kettunen *et al.*, 2001a, 2001b), betaine appears to be of benefit in chicks infected with coccidiosis (Matthews *et al.*, 1997; Augustine *et al.*, 1997; Allen *et al.*, 1998; Augustine and Danforth, 1999; Matthews and Southern, 2000; Waldenstedt *et al.*, 1999). Chicks in the present study received an anticoccidial in the diet and were grown on new litter with no apparent problems with coccidial infection.

In conclusion, the results of this study indicate little or no positive benefit in terms of body weight gain, feed conversion, or mortality from the addition of betaine to corn-soybean meal based diets for broiler chicks in situations where disease challenge from coccidiosis is not a major issue. At 42 d of age improvements in dressing percentage were obtained from betaine supplementation and in breast yield from choline supplementation but not at 49 d of age. Therefore, age of bird might be a consideration for using these supplements as birds processed at younger ages might be more responsive to these nutrients. Further studies are needed to evaluate the potential effect of age on response to choline and betaine.

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