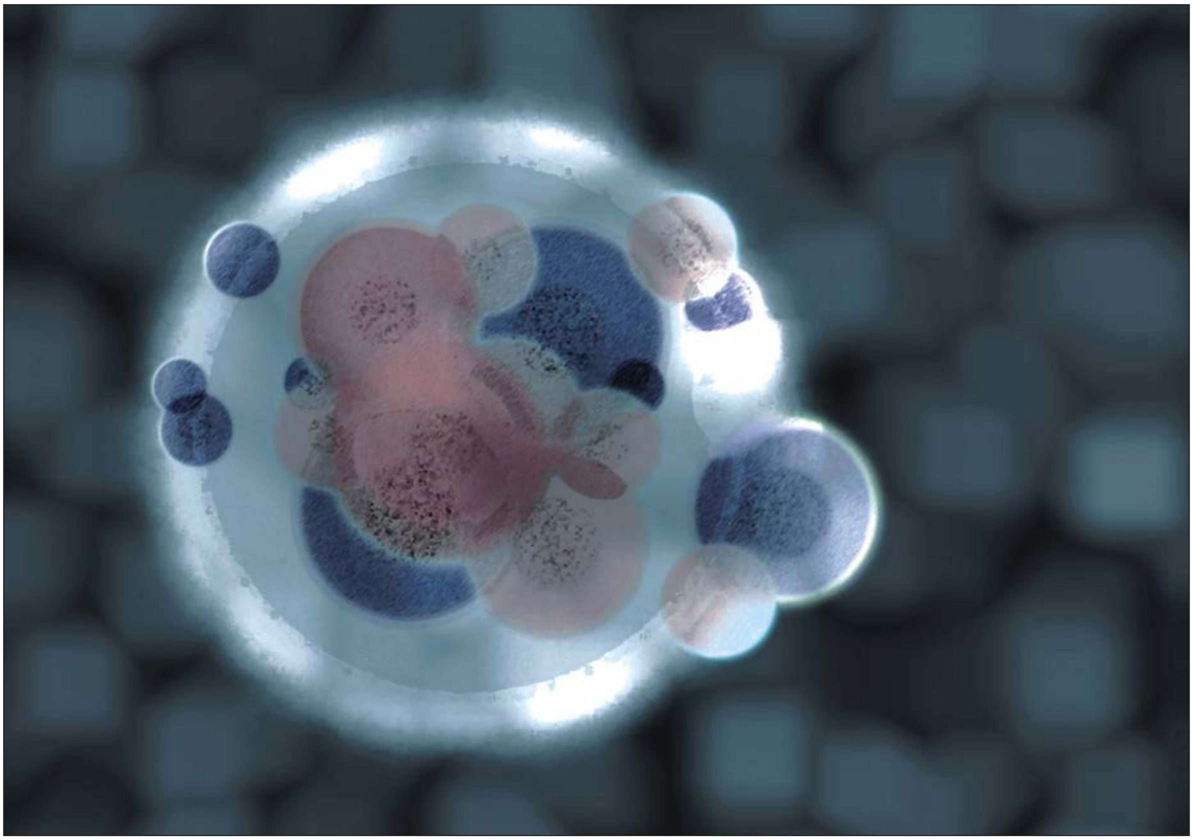

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The Effect of Dietary Fat Level on the Response of Broiler Chicks to Betaine and Choline Supplements

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Abstract: This study was conducted to investigate the effect of dietary betaine (Betafine) supplementation as a replacement for choline on broiler performance and carcass characteristics. Three betaine replacement levels (0, 50 and 100% in substitution for choline) were used in two various basal diets (without or containing 30 g kg^{-1} oil) in a 2×3 factorial arrangement with four replicates of 10 birds. Two hundred-forty day-old broiler chicks were fed with the experimental diets from 1 to 49 days of age and at 49 days of age, two birds from each replicate were selected randomly for blood sampling and comparison of carcass characteristics. Dietary betaine inclusion had no effect on feed intake, but the significant differences in body weight (BW) gain (at 1-3 and 3-5 weeks of age) and feed conversion ratio (at 3-5 weeks of age) were observed among the experimental diets. Replacing choline with betaine increased ($p<0.05$) dressing and breast meat percentages and reduced ($p<0.01$) abdominal fat percent, but had no significant effect on thigh and liver weight percentages. Plasma levels of cholesterol and low density lipoproteins (LDL) were not affected by dietary substitution of betaine for choline. Dietary betaine replacement caused a significant decrease in plasma triglycerides ($p<0.05$) and very low density lipoproteins ($p<0.01$) and significant increase in ($p<0.05$) high density lipoproteins (HDL). These findings indicate that although dietary betaine inclusion instead of choline had little benefit in terms of performance parameters, but resulted favourable changes in abdominal fat and breast meat percentages.

Key words: Broiler chick, betaine, choline, carcass characteristic, blood lipoprotein

INTRODUCTION

Betaine, the common term for trimethylglycine, is a naturally occurring amino acid derivative found in a variety of feedstuffs of plant and animal origin. Betaine can also be synthesized endogenously through oxidation of excess choline (Olthof and Verhoef, 2005). Betaine has two primary metabolic roles: it is a methyl group donor and it is an osmolyte that assists in cellular water homeostasis (Petronini *et al*., 1992). Due in large measure to its osmoregulatory functions (Kidd *et al*., 1997; Matthews *et al*., 1997), betaine appears to be of benefit in chicks infected with coccidiosis (Augustine *et al*., 1997; Matthews *et al*., 1997). In addition, Jukes (1940) and McGinnis *et al*. (1942) reported a positive antiperotic action for betaine; however, it seems that betaine and ethanolamine fed jointly are somewhat more effective than betaine alone (McGinnis *et al*., 1942). It is well understood that choline may act as a methyl group donor but, in order to this function, it needs to be converted to betaine in the mitochondria (Schutte *et al*., 1997). In the function as a methyl group donor, betaine, choline and methionine had strong interrelationships as depicted in Fig. 1 (Pillai *et al*., 2006).

Many studies have examined the interrelationships between choline and methionine and between betaine and methionine to determine if these compounds can spare the needs of the chicks for methionine, with considerable variation in results (West *et al*., 1951; Baker *et al*., 1983; Miles *et al*., 1983). While some studies (Virtanen and Rosi, 1995; Virtanen and Rumsey, 1996) suggested that the response to betaine was greater than that obtained from the addition of methionine, others have failed to demonstrate that the dietary methionine content could be reduced by betaine supplementation (Rostagno and Pack, 1996; McDevitt *et al*., 2000). However, several studies suggest that the addition of betaine may improve breast meat yield (Schutte *et al*., 1997; Wallis, 1999; McDevitt *et al*., 2000). Betaine is indirectly involved in the synthesis of carnitine, which is required for transporting long chain fatty acids across the inner mitochondrial membrane for oxidation (McDevitt *et al*., 2000) and

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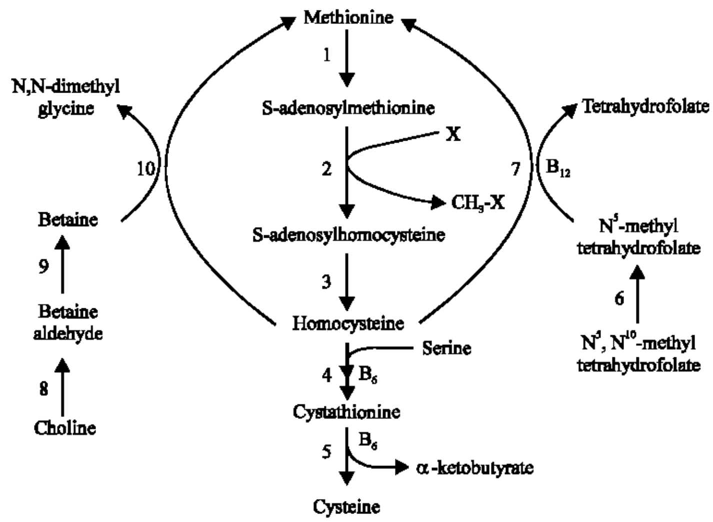

Fig. 1: Schematic overview of metabolism and interrelationships of sulfur containing amino acids, choline and betaine. Numerals indicate the following enzymes: (1) methionine adenosyltransferase, (2) various enzymes, (3) S-adenosylhomocysteine hydrolase, (4) cystathionine β-synthase, (5) cystathionine γ-lyase, (6) N^5, N^{10}-methylene-tetrahydrofolate reductase, (7) methionine synthase, (8) choline dehydrogenase, (9) betaine aldehyde dehydrogenase and (10) betaine- homocysteine methyltransferase (Pillai *et al*., 2006)

therefore may result in a leaner carcass; however, there is little evidence in this regard. To our knowledge, there is no study that investigates the effect of dietary fat level on the broiler chicken's response to dietary betaine supplementation. The objective of present study, therefore, was to examine the choline sparing effect of betaine and to evaluate the effect of betaine in enhancement of broiler breast meat yield in the presence of varying dietary fat levels.

MATERIALS AND METHODS

Diets and management guidelines: This study was carried out in experimental farm of Isfahan University of Technology, Isfahan, Iran. The commercial basal diets were formulated to meet the nutritional requirements (National Research Council, 1994) of male broiler chicks in different phases of growth. The dietary treatments consisted of two various basal diets (without or containing 30 g kg^{-1} sunflower oil) and three betaine replacement levels (0, 50 and 100%) for choline, were fed to chicks from 1 to 49 days of age. The experimental diets were formulated to be isocaloric and isonitrogenous in each starter (first 3 weeks), grower (second 2 weeks) and finisher (last 2 weeks) growth stages. To making experimental diets, the choline-free premix was used and choline was supplied as a dry 60% choline chloride product in the level of 1000 mg kg^{-1} of diet, while betaine was added as a crystalline 97% product (Betafine). Two hundred-forty day-old Ross×Ross broiler chickens were purchased from a local hatchery and randomly assigned to floor pens in a 2×3 factorial arrangement with four replicates and ten birds in each. Feed and water were provided for ad libitum consumption and chicks had access to 24 h lighting schedule during the experiment. Temperature was 33°C during the first week of age and was reduced by 3°C/week until the birds were 4 weeks old. Prior to formulating the diets, the ingredients used were analyzed for crude protein, ether extract, crude fibre and ash contents according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1995), then the metabolizable energy of ingredients was calculated by use of National Research Council (1994) recommended formula. The composition of experimental diets is given in Table 1.

Recording and chemical analysis: Feed consumption and body weight gain were recorded by phase and mortality was recorded daily. Any bird that died was weighed and weight was included in body weight gain data and used to adjust feed/gain ratio. On day 49, two birds from each replicate (close to mean body weight of that pen) were selected randomly for blood parameter measurements and comparison of carcass characteristics. After slaughtering, the thigh, breast, liver and abdominal fat pad were removed and weighed separately. The livers were stored at -20°C until analysis for fat content. The plasma samples were analyzed for cholesterol, triglycerides, LDL (low density lipoproteins), VLDL (very

Table 1: Composition and nutritive value (g kg^{-1}) of experimental diets

	Starter (1-21 day)		Grower (21-35 day)		Finisher (35-49 day)	
Ingredients (g kg^{-1})	Without oil	Containing 30 g kg^{-1} oil	Without oil	Containing 30 g kg^{-1} oil	Without oil	Containing 30 g kg^{-1} oil
Yellow corn	604.40	494.80	663.80	554.30	703.80	594.70
Soybean meal	328.90	315.20	284.90	271.20	260.00	247.40
Fish meal	30.00	30.00	15.00	15.00	—	—
Wheat bran	—	93.60	1.80	95.30	2.90	93.10
Sunflower oil	—	30.00	—	30.00	—	30.00
Ground limestone	11.00	11.10	12.40	12.50	11.90	12.00
Dicalcium phosphate	14.00	13.60	11.20	10.80	10.70	10.40
Common salt	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin premix[1]	2.50	2.50	2.50	2.50	2.50	2.50
Mineral premix[2]	2.50	2.50	2.50	2.50	2.50	2.50
DL-Methionine	1.20	1.20	0.40	0.40	0.20	0.20
Variable[3]	2.50	2.50	2.50	2.50	2.50	4.20
Nutrient composition (g kg^{-1})						
ME_n (MJ kg^{-1})	12.24	12.24	12.47	12.47	12.62	12.62
Crude protein	210.30	210.30	186.30	186.30	169.60	169.60
Ether extract	34.50	62.30	35.10	62.90	35.20	62.90
Lysine	12.10	12.00	10.40	10.30	9.10	9.00
Methionine	4.80	4.80	3.50	3.50	3.10	3.10
TSAA	8.20	8.20	6.70	6.70	6.10	6.10
Arginine	13.70	13.80	12.00	12.10	10.90	11.00
Threonine	8.20	8.10	7.20	7.10	6.50	6.50
Tryptophan	2.90	2.90	2.60	2.50	2.40	2.30
Calcium	9.40	9.40	8.60	8.60	7.70	7.70
Non-phytate P	4.20	4.20	3.40	3.40	2.90	2.90
Sodium	1.50	1.60	1.40	1.50	1.40	1.40

[1]Choline-free premix; provided per kilogram of diet: vitamin A (from vitamin A acetate), 8700 IU; cholecalciferol, 2300 IU; vitamin E (from DL-α-tocopheryl acetate), 16 IU; vitamin B_{12}, 0.31 mg; riboflavin, 6.6 mg; niacin, 28 mg; calcium pantothenate, 35 mg; menadione (from menadione dimethyl-pyrimidinol), 1.50 mg; folic acid, 0.80 mg; thiamine, 3 mg; pyridoxine, 2.50 mg; biotin, 30 mg; ethoxyquin, 125 mg.
[2]Provided per kilogram of diet: Mn (from $MnSO_4.H_2O$), 80 mg; Zn (from $ZnO.H_2O$), 75 mg; Fe (from $FeSO_4.7H_2O$), 50 mg; Cu (from $CuSO_4.5H_2O$), 10 mg; I (from $Ca(IO_3)_2.H_2O$), 1 mg.
[3]Variable amounts of choline (from 60% choline chloride product), betaine (from Betafine, 97% purity) and washed builders sand

low density lipoproteins) and HDL (high density lipoproteins) according to standard procedures of AOAC (1995).

Statistical analysis: Data were analyzed according to General Linear Model (GLM) procedures of SAS (2002) as a factorial experiment with the basal diet and replacement level as main effects. The data in percentage were first transformed to its Arc sin% and then analyzed; however, the normal data are presented for relevance. The Duncan's multiple range tests (Duncan, 1955) was used to compare treatment means. Means were separated at the p<0.05 significant level.

RESULTS

Weight gain and feed consumption: As shown, dietary inclusion of 30 g kg^{-1} sunflower oil caused the highly significant increase (p<0.01) in weight gain during 1-3 and 3-5 weeks of age and also significant increase (p<0.05) in body weight gain (BWG) during 5-7 and 1-7 weeks of age (Table 2). Replacing betaine with choline increased BWG only in the first two growth stages. Dietary betaine inclusion led to higher increase in body weight gain in oil-supplemented birds than non-supplemented groups, but the basal diet type by replacement level interaction did not reach to significant level. Feed consumption within the given periods was not affected by dietary treatments (Table 2). Dietary inclusion of sunflower oil resulted in numerical but insignificant (p>0.05) increase in feed consumption.

Dietary betaine replacement had a significant effect (p<0.01) on feed to gain ratio at 3-5 weeks of age, but not in other experimental periods (Table 2). Dietary oil inclusion resulted in significant improvements (p<0.05) of FCR values at 3-5 and 5-7 weeks of age, but the average FCR in entire trial period was not affected by oil supplementation of experimental diets. The mortality was scare and not affected by dietary treatments (data not shown).

Carcass components: As noted (Table 3), the dressing and breast meat percentages were affected by both dietary inclusion of oil and betaine. The breast meat percentage showed a linearly significant increase (p<0.05) with increasing dietary betaine concentration. Dietary betaine substitution caused the significant (p<0.01) decrease in abdominal fat percentage; however, this beneficial effect was completely obtained by 50% betaine replacement. Similarly, betaine supplementation resulted in significant (p<0.05) decrease in liver fat percentage.

Table 2: Effect of betaine substitution for choline in various basal diets on daily body weight gain, daily feed intake (g/day per bird) and feed conversion ratio (g feed/g gain) in different stages

Replacement level (%)	Diets without oil			Diets containing 30 g kg^{-1} oil				Probability		
	0	50	100	0	50	100	SE	Basal diet	Replacement level	Basal diet× Replacement
BWG (1-21 day)	22.62	29.01	29.49	28.96	31.83	32.07	0.956	**	*	NS
BWG (21-35 day)	58.91	61.46	62.08	62.51	64.11	65.48	1.068	**	*	NS
BWG (35-49 day)	69.03	70.40	70.11	70.63	72.03	72.65	0.879	*	NS	NS
BWG (1-49 day)	48.39	50.11	50.41	50.45	52.54	53.21	1.060	*	NS	NS
FI (1-21 day)	40.46	42.99	42.67	43.19	46.66	47.01	2.381	NS	NS	NS
FI (21-35 day)	114.75	115.60	117.18	120.15	118.76	119.92	4.539	NS	NS	NS
FI (35-49 day)	169.27	173.99	172.64	171.57	172.90	173.58	6.296	NS	NS	NS
FI (1-49 day)	98.49	101.16	101.09	101.86	103.33	104.00	3.721	NS	NS	NS
FCR (1-21 day)	1.47	1.48	1.45	1.49	1.47	1.47	0.029	NS	NS	NS
FCR (21-35 day)	1.95	1.88	1.89	1.92	1.85	1.83	0.022	*	**	NS
FCR (35-49 day)	2.45	2.47	2.46	2.43	2.40	2.39	0.026	*	NS	NS
FCR (1-49 day)	2.04	2.02	2.01	2.02	1.97	1.95	0.043	NS	NS	NS

BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio, NS: Not significant,*p<0.05, **p<0.01

Table 3: Effect of betaine substitution for choline in various basal diets on relative weights (g/100 g live body weight) of carcass components measured at 49 days of age

Replacement level (%)	Diets without oil			Diets containing 30 g kg^{-1} oil				Probability		
	0	50	100	0	50	100	SE	Basal diet	Replacement level	Basal diet× Replacement
Carcass	70.11	71.05	70.93	71.08	72.57	73.12	0.997	**	*	NS
Breast	18.63	18.81	19.45	19.07	19.80	20.57	0.641	*	*	NS
Thigh	19.82	19.63	20.32	19.73	20.70	19.95	0.596	NS	NS	NS
Abdominal fat	2.25	2.10	2.17	2.46	2.24	2.16	0.072	*	**	NS
Liver	2.23	2.20	2.18	2.34	2.35	2.28	0.082	NS	NS	NS
Liver fat (g/100 g DM)	13.87	13.10	12.98	14.03	13.18	12.57	0.462	NS	*	NS

NS: Not significant, *p<0.05, **p<0.01

Table 4: Effect of betaine substitution for choline in various basal diets on some blood metabolites (mg dL^{-1}) measured at 49 days of age

Replacement level (%)	Diets without oil			Diets containing 30 g kg^{-1} oil				Probability		
	0	50	100	0	50	100	SE	Basal diet	Replacement level	Basal diet× Replacement
Cholesterol	138.42	142.63	142.97	134.25	136.51	138.03	3.298	NS	NS	NS
Triglycerides	49.41	49.36	48.39	53.21	52.63	49.00	0.871	**	*	NS
LDL	95.34	96.14	94.87	94.76	97.01	98.83	2.611	NS	NS	NS
VLDL	10.72	9.79	9.53	11.81	10.67	9.53	0.448	NS	**	NS
HDL	28.33	31.50	32.79	30.20	34.45	33.73	1.432	NS	*	NS

NS: Not significant, *p<0.05, **p<0.01

Blood parameters: As shown in Table 4, plasma cholesterol level tended to decrease by dietary inclusion of sunflower oil, although this effect did not reach to significant level (p = 0.0757). Dietary betaine replacement caused the significant increase in plasma levels of triglycerides (p<0.05) and VLDL (p<0.01), while the plasma LDL was not affected by betaine supplementation. The effect of betaine in lowering triglycerides and VLDL levels was more obvious in oil-supplemented than that of non-supplemented groups. The plasma HDL level was increased (p<0.05) by dietary betaine inclusion.

DISCUSSION

Dietary inclusion of 30 g kg^{-1} sunflower oil caused the highly significant increase (p<0.01) in weight gain during 1-3 and 3-5 weeks of age and also significant increase (p<0.05) in body weight gain during 5-7 and 1-7 weeks of age. Also, dietary betaine inclusion resulted in higher increase in body weight gain in oil-supplemented birds than non-supplemented groups. Since the demand of broiler chicks for betaine is increased by growth rate as well as stress and because the chicks are under stress conditions during the first weeks of age; so it is obvious that the betaine requirements of chicks is high during this period of life. The needs for choline not only did not decrease by age, but followed an increasing pattern. Because most cells and body components enlarge with age and choline is present in all membrane structures, so it is clear that the choline and in turn, betaine requirements are high during the growth stage; however, it seems that the choline supplement should not completely replace with betaine, in order to achieving the special benefits of choline. In agreement with our results, Matthews *et al.* (1997) reported that betaine increased average daily gain of coccidiosis-infected chicks; however, the same authors observed that dietary betaine decreased average daily gain and feed intake in uninfected chicks. Waldroup and Fritts (2005) reported that the addition of 1000 mg kg^{-1} of choline to diet of chicks that had not been fed betaine or to the diet of chicks that had been fed 1000 mg kg^{-1} of betaine from day of age, had no effect on chick's body weight; however, when 1000 mg kg^{-1} choline was added to the diet of chicks that had not been fed betaine until 35 day of age, there was a significant reduction in body weight at both 42 and 49 day of age.

In overall, feed consumption was not affected by dietary treatments. Dietary inclusion of sunflower oil caused to numerical increase in feed consumption which is probably due to palatability of dietary oils.

Dietary betaine replacement had a significant effect ($p<0.01$) on feed to gain ratio at 3-5 weeks of age, but not in other experimental periods. In the study of Baker and Czarnecki (1985), betaine and not choline, showed some efficacy in enhancing the conversion of homocysteine to methionine. In addition, some studies indicated that feed efficiency was more improved by betaine than by choline. In their study with broiler chicks, Schutte *et al.* (1997) observed that feed conversion efficiency was significantly improved with addition of 0.4 g kg^{-1} betaine to the practical diets, whereas was not improved with the corn-soybean meal diets. Similarly, betaine supplementation to the diets already containing 0.5 or 1.0 g kg^{-1} added methionine had no significant effect on weight gain and feed conversion ratio. In our trial, the beneficial effect of betaine substitution was observed only in the grower stage. It seems that the exponential growth rate in this period has been increased the betaine demands of chicks.

The breast meat percentage showed a linearly significant increase ($p<0.05$) with increasing dietary betaine amount (2.44 and 6.15% increase by 50 and 100% replacement, respectively). Dietary betaine substitution caused to significant ($p<0.01$) decrease in abdominal fat percentage (more than 8%); however, this beneficial effect was completely obtained by 50% betaine replacement for choline. Similarly, betaine supplementation resulted in significant ($p<0.05$) decrease in liver fat percentage. These results are in agreement with Rostagno and Pack (1996), Remus (2001) and Waldroup and Fritts (2005), who also showed that breast meat percentage was increased as diets containing different levels of betaine were fed. Otherwise, researchers have reported the reduction in abdominal fat as the result of dietary betaine supplementation. These effects are probably due to betaine effects on lipid metabolism in organism. These findings confirm the observations of Wang *et al.* (2004) who reported similar results in respect to the role of betaine in lipid metabolism. It appears that betaine plays this role via carnitine synthesis.

The plasma level of cholesterol decreased by dietary inclusion of sunflower oil, although this trend was not significant ($p = 0.0757$). It seems that the above change is resulted from the unsaturated nature of dietary fat source. Dietary betaine replacement caused significant increase in plasma levels of triglycerides ($p<0.05$) and VLDL ($p<0.01$). The effect of betaine in lowering triglycerides and VLDL levels was more obvious in oil-supplemented than that of non-supplemented groups. These results indicate that the presence of oil in diets promote chick's response to dietary betaine supplements, that is, the chick's demand for betaine is increased with diets high in fat content. The plasma HDL level was increased by dietary betaine inclusion. The reason for this observation is unclear; however, it seems that the betaine accelerates lipid oxidation and whereby increase the substrate needed for cholesterol synthesis. In their work with rats, Olson *et al.* (1958) observed that the choline-supplemented animals tended to have somewhat higher total lipid, phospholipids and high-density lipoprotein levels than controls. Choline deficiency in the young adult rat caused to marked alteration in fat transport, with a decrease in the level of cholesterol and phospholipids, a reduction in the high-density β-lipoproteins and virtual disappearance of the low-density β-lipoproteins.

There is a noticeable variation in the published literature regarding to the efficacy of betaine and choline for methylation. It is reported that the betaine methylates homocysteine to methionine roughly three times more efficient than choline (Saunderson and McKinlay, 1990). Conversely, other experiments showed that betaine, choline and methionine appear to be equivalent as sources of methyl group (Rostagno and Pack, 1996). Garcia Neto *et al.* (2000) using a chick growth model concluded that the sparing of methionine by methylation of homocysteine to methionine is increased by adding choline or betaine to chick diets.

CONCLUSION

In conclusion, the results of the present study indicate little positive benefit in terms of body weight gain and feed conversion ratio from the addition of betaine to corn-soybean meal-based diets. Betaine supplementation improved body weight gain and feed conversion ratio at the initial stages of growth but not in finisher stages; therefore, the bird's age should be considered when these supplements are decided to use in practical diets. The birds marketed at younger ages might be more responsive to these dietary supplements. Further studies are needed to evaluate potential effect of age on the response to choline and betaine supplements.

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