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Growth, Carcass Quality and Serum Constituents of Slow Growing Chicks as Affected by Betaine Addition to Diets Containing 1. Different Levels of Choline

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Abstract: The response of slow growing chicks to different dietary levels of choline and\or betaine was investigated during the starter-grower period from 1 to 56 d of age. Therefore, a basal all-mash corn-soybean meal diet was formulated to contain 872 mg of choline based on native one. This diet was supplemented with 0, 300 and 600 mg of choline, from feed grade choline chloride (50%), which is equal to 872, 1172 and 1472 mg total choline/kg feed. Each choline level was supplemented with, 0.0, 0.072 and 0.144% betaine. Thus there were nine experimental diets; each diet was fed to 45 chicks divided equally among 5 replicates of nine unsexed chicks each. Irrespective of betaine addition, choline supplementation at 300 mg/kg diet significantly increased BWG by 3.2% and insignificantly improved FCR by 3.3% compared to unsupplemented basal diet (872 mg choline/kg diet). Choline supplementation at either medium or high level significantly increased blood serum albumin, while response to SRBC's was linearly ($P<0.05$) increased with increasing choline supplementation. Furthermore, choline at only 1172 mg/kg diet significantly decreased relative weight of abdominal fat, while increasing choline level to 1472 mg/kg diet did not affect relative weight of abdominal fat. Irrespective of choline supplementation, betaine addition at either 0.072 or 0.144% significantly improved BWG by 4.4 and 4.8%, and FCR by 4.2 and 6.1% compared to the basal diet, respectively. Betaine addition at 0.072 and 0.144% significantly increased serum total protein, albumen, and secondary response to SRBC's, while primary responses to SRBC's was linearly increased ($P<0.05$) with increasing betaine addition. Betaine significantly decreased relative weight of abdominal fat linearly. Results indicated that betaine addition at 0.072% to 872, 1172 or 1472 mg choline- containing diet increased BWG by 3.9, 4.1 and 5.1% and improved FCR by 4.1, 4.3 and 4.8% compared to their respective controls, respectively. Also, betaine addition at 0.144% to 872, 1172 and 1472 mg/kg diet increased BWG by 5.0, 4.9 and 4.4% and improved FCR by 4.8, 4.3 and 4.1% compared to their respective controls, respectively. Betaine addition at 0.072 or 0.144% to the basal diet containing 872 mg choline resulted in similar BWG and FCR, serum total protein, albumen and primary response to SRBC's of those fed diet containing 1172 or 1472 mg choline. In conclusion, slow growing chicks gained 13.3 g/d during 1-56 d of age required a choline level of 1172 mg/kg feed. However, choline level of 872 mg choline, which derived from native one, was adequate when dietary 0.072% betaine was added.

Key word: Choline, betaine, growth performance, antibody response, meat quality

Introduction

Choline and betaine besides methionine are all sources of labile methyl groups and play an important role in methylation reactions, and the methyl group metabolism of these two compounds is interrelated (Pesti *et al*., 1980 and Kettunen *et al*., 2001a and b). Methyl groups are available after conversion of choline to betaine in the liver; however, dietary betaine is twice as efficient as the equal-molar choline in increasing liver betaine level of broiler chickens (Saarinen *et al*., 2000). On the other hand, choline and betaine have different specificity in metabolic body functions (Kettunen *et al*., 2001b).

Choline has three essential metabolic roles e.g. as a constituent of phospholipids, hepatic lipid metabolism to prevent fatty liver, and as a precursor for acetylcholine synthesis (Ghazalah, 1998 and Workel *et al*., 1999). Additionally, choline has a further non-essential metabolic function as a labile methyl group as well as prevention of perosis (Ghazalah, 1998 and Workel *et al*., 1999).

Although, Venugopal (1985) reported that most animals could produce choline by hepatic synthesis, this may not be adequate and depends on stage of growth and type of animal. Due to several important metabolic functions, choline is a common supplement to poultry feed, however, dietary requirements are based on several

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studies done several decades ago and there has been a significant change in feed formulation and bird performance (Mohamed, 1998 and Workel *et al.*, 1999). Meanwhile, the choline requirement was found also to be affected by type and strain of chicks, age, feed intake, dietary crude protein and methionine level as well as fat type (Molitoris and Baker, 1976; Pesti *et al.*, 1981; NRC, 1994; Ghazalah, 1998 and Workel *et al.*, 1999). Also, bioavailability of native choline was found to be affected by prevailing crop growth conditions, e.g. climate, cultivate, soil, location, fertilizer and spray regime and variation is also exist among crops (Workel *et al.*, 1999). Choline requirements ranged from 750 to 2000-mg/kg feed and this depends on type, strain, age and growth rate of chicks NRC (1994). The effect of choline in chicken performance was of concern in the literature i.e. Harms *et al.* (1990) and Harms and Russell (2002) reported that laying hens would responded to choline supplementation of a corn-soybean meal diet marginally in methionine, however when the diet contained enough methionine no supplemented choline is needed. Also, in the literature the results of broiler experiments are controversial e.g. in early reports an improvement in BWG of broilers was found especially when choline was added during early age in methionine inadequate diet (Pesti *et al.*, 1980; Baker *et al.*, 1983; Harms and Miles, 1984 and Tillman and Pesti, 1986). In this regard, Ghazalah (1998) found that choline supplementation at 1250 mg/kg diet resulted in the best growth performance of 28-d old broiler chicks fed fat unsupplemented diet, meanwhile addition of choline at 2500 mg/kg to diets containing 5% sunflower, cotton seed oil, palm oil or poultry fat resulted in the best growth performance during 1-28 d of age. On the other hand, Mohamed (1998); Swain and Johri (2000) and Saarinen *et al.* (2000) showed that choline was ineffective in improving growth performance of broiler chicks fed methionine adequate or inadequate diets. Nonetheless, choline level at 2300 or 3300 mg/kg feed improved cellular and humoral immune response (Swain and Johri, 2000).

Betaine is a tertiary amine formed by the oxidation of choline (Kidd *et al.*, 1997 and Wang *et al.*, 2004) and implicated in methionine sparing, osmoprotective, and fat distribution and immune responses (Saunderson and Mackinlay, 1990; Petronini *et al.*, 1992; Kettunen *et al.*, 2001a; Türker *et al.*, 2004 and Remus *et al.*, 2004). However, betaine is not present in large quantities in animal feedstuffs and dietary supplementation seems to be important to improve productivity and resistance to stress (Wang *et al.*, 2004). Betaine has an energy-sparing role by reducing maintenance requirement of the pig (Schrama and Gerrits, 2000), and improves growth, carcass yields and muscle protein (Virtanen and Rosi, 1995; Esteve- Garcia and Mack, 2000; Türker *et al.*, 2004 and Wang *et al.*, 2004). Furthermore, Miles *et al.* (1987) revealed that betaine could substitute choline in broiler and laying hen diets.

Although, the positive impact of betaine on animal performance is apparent in the literature, it replacement value for choline is subjected to controversial, thereby this study investigated whether or not betaine could replace choline in the diets for slow growing chicks containing different dietary levels of choline. It was also aimed to gain information for choline needs of slow growing chicks such as El-Salam strain.

Materials and Methods

Chicks and diets: A total of 405 one -day old unsexed chicks of El-Salam strain (Nicolas × Mamourah (Alexandria× Dokki$_4$)) were randomly distributed into 9 groups of five replicates, of 9 birds each. Thus, the chicks were reared in 45 floor pens (1×1 m) furnished with rice hulls. Water and feed were provided *ad libitum*. A basal choline unsupplemented all-mash corn-soybean meal diet was formulated to contain 872 mg of choline based on only raw materials. This diet was fed with 0.0, 300 and 600 mg of choline supplemented as feed grade choline chloride (50%). These supplementations resulted in three levels of dietary choline being 872, 1172 and 1414 mg/kg. Each choline level was supplemented with 0.0, 0.072 and 0.144% betaine as Betafin® (Batch no: 313, Danisco Animal Nutrition). The Betafin® was analyzed by HPLC according to Rajakylä and Paloposki, (1983) and found to contain 945 g\kg betaine. Thus, there were nine experimental diets resulting from a factorial arrangement of 3 levels of choline ×3 levels of betaine. The experimental diets were supplemented with anticoccidial drug (Uccma pedomix produced by Uccma company) at 1kg/ton. Each 100 g of this drug contained 12.5 g of Clopidol. This was done to avoid the effect of betaine as a coccidiostat enhancer and coccidiosis challenge. Furthermore, the basal diet containing adequate level of methionine to satisfy the requirement of the 1st limiting amino acids for protein synthesis and to ensure adequate methyl group donor to avoid the positive impacts of choline and\or betaine as a methyl group donors (Workel *et al.*, 1999 and Saarinen *et al.*, 2000).

Criteria of responses: All birds were individually weighed every 4 wk and feed intake on replicate basis was recorded every 4 wk, too. Mortality was recorded daily and was taken into account for adjusting feed intake/chick/day.

Response to SRBC'S: At the end of the 4th week of age, nine birds from each treatment were used to determine the humoral immune response (primary response) by injection intravenously with 1 ml sheep red blood cell (SRBC's) 7% suspension in sterile saline. To determine secondary response the same antigen was injected

Table 1: Composition and calculated analyses of the basal diet fed during 1-56 d of age

Ingredients, %	
Yellow corn	65.0
Soybean meal (44%CP)	15.0
Corn-gluten meal	15.2
Dicalcium phosphate	2.0
Limestone	1.5
Salt	0.4
Vit.Min.mixture (premix)[1]	0.35
DL-Methionine,%	0.10
L-lysine	0.45
Total	100.0
Calculated and analyzed composition	
ME[2] (Kcal/kg)	3100
CP[3] (%)	20.60
Ca[2] (%)	1.07
Available P[2] (%)	0.49
Methionine[2] (%)	0.54
TSAA[2, 4] (%)	0.91
Lysine[2] (%)	1.08
Choline[2] (mg/kg)	872

[1]kg of vitamin- mineral premix per ton of feed supplied each kg of diet with Vit. A 12000 IU; Vit. D_3 2000 IU; Vit. E 10mg; Vit. k_3 2mg; Vit. B_1 1mg; Vit. B_2 4mg; Vit. B_6 1.5 mg; Pantothenic acid 10mg; Vit. B_{12} 0.01mg; Folic acid 1mg; Niacin 20mg; Biotin 0.05mg; Zn 55mg; Fe 30mg; I 1mg; Se 0.1mg; Mn 55mg; ethoxyqain 3000 mg.
[2]Calculated values were according to NRC (1994) text book values for feedstuffs.
[3]Analyzed values were according to AOAC (1990).
[4]Total sulphur-containing amino acids.

3-wk later to the same birds. Seven-d later, (Yamamoto and Glick, 1982) approximately 2.0 ml of blood was drowning from each bird. It was allowed to clot to provide serum for antibody titration, which was frozen for the later use. The sera samples were inactivated by incubation at 6°C or 30 minutes before titration. The response (primary and secondary) to SRBC's was measured using micro titer technique, as described by Bachman and Mashaly (1986 and 1987) and Kai *et al.* (1988).

Carcass parameters and quality: Nine birds of each treatment were slaughtered at 56 d of age to determine carcass yield, abdominal fat and giblets which is the same of heart, gizzard and liver. Also chemical composition of muscle was determined (AOAC, 1990). Also, nine more blood samples per group were also collected at 8 wk of age for colorimetrical determination of biochemical constituents of blood serum. Blood serum was separated by centrifugation at 1510 g for 10 min and stored -18°C until analyses. Blood serum total protein (g/100 ml) was measured according to Weichselbaum (1946) and Henry *et al.* (1974). Albumin concentration (g/100ml) was determined according to Doumas *et al.* (1977). Globulin concentration (g/100 ml) was calculated as the difference between total protein and albumin. Serum total cholesterol (mg/100ml) was determined according to the method of Watson (1960). The activities (µ/L) of liver enzymes e.g. alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were assayed by the method of Reitman and Frankel (1957).

At the end of the experiment (8 wk of age), 6 birds from each group were housed in separate metabolic cages for 5 days. Birds were allowed to the experimental diets for 2 days as preliminary period followed by 3 days as a main experimental period, in which quantities of feed intake and excreta were determined. The proximate analyses of feed and dried excreta were carried out according to AOAC (1990). Digestibilities of nutrients were calculated according to Attia *et al.* (2005).

Statistical evaluation: Data were statistically analyzed using factorial 3×3 arrangement of SAS® (SAS Institute, 1985; Cary, NC, USA). Choline and betaine levels were the main effects. Mean differences were tested at $P < 0.05$ using Student-Newman Keuls test.

Results

Growth performance: Irrespective of betaine level, choline supplementation at 300 mg/kg diet significantly increased BWG during 1-28, 29-56 and 1-56 d of age by 4.3, 2.6 and 3.2% and significantly improved FCR by 2.6% during only 29-56 d of age compared to those fed unsupplemented control (Table 2). There was no significant effect of choline supplementation at 600 mg/kg feed on BWG and FCR during all experimental period, nor was there a significant impact of either 300 or 600 mg choline on FCR during 1-28 as well as 1-56 d of age. Although, choline level of 300 mg/kg feed (1172 mg choline) tended to slightly improve FCR during 1-28 and 1-56 d of age by 4.1 and 3.4%, respectively.

Irrespective of choline level, betaine addition significantly increased BWG and improved FCR of chicks by different magnitude during all experimental period. Results indicated that differences were linear in BWG during 1-28 d of age. Meanwhile, there were no significant differences in BWG between the medium and the high levels of betaine during 29-56 d as well as during 1-56 d of age. Results also showed that although betaine supplementation at 0.072 and 0.144% significantly improved FCR during 1-28, 29-56 and 1-56 d of age, there were no significant differences in FCR between the medium and the high levels of betaine, indicating that 0.072% of betaine is adequate.

Results presented in Table 2 show that BWG and FCR was significantly affected by the interaction between choline and betaine levels during all experimental periods. Betaine addition at 0.072 and 0.144% to 872, 1172 and 1472 mg choline/kg feed increased BWG by 7.1 and 10.0, 4.3 and 5.5 and 6.5 and 7.7% during 1-28d

Table 2: Effect of level of dietary choline (%) and\or betaine (%) on BWG, FCR and number of dead chicks during 1-56 d of age

Treatments		BWG, during			FCR, during			Number of dead
		1-28d	29-56 d	1-56 d	1-28d	29-56 d	1-56 d	birds
Interaction effect between choline and betaine								
Choline	Betaine							
0.0	0.0	257.9	430.1	688.0	2.57	3.49	3.15	3
0.0	0.072	276.1	439.0	715.1	2.41	3.41	3.02	2
0.0	0.144	283.6	438.9	722.5	2.36	3.40	3.00	2
300	0.0	275.2	435.0	710.2	2.42	3.42	3.04	1
300	0.072	287.0	452.2	739.2	2.33	3.31	2.93	2
300	0.144	290.3	454.6	744.9	2.31	3.29	2.91	1
600	0.0	260.1	424.8	684.9	2.56	3.51	3.15	2
600	0.072	276.9	443.0	719.9	2.42	3.37	3.00	1
600	0.144	280.2	435.1	715.3	2.39	3.42	3.02	2
SEM		14.95	27.75	27.69	0.170	0.036	0.106	---
P value		0.0001	0.0001	0.0001	0.07	0.001	0.01	---
Main effect of supplemented choline								
0.0		272.5^{b}	436.0^{b}	708.5^{b}	2.45	3.43^{a}	3.06	7
300		284.2^{a}	447.3^{a}	731.5^{a}	2.35	3.34^{b}	2.96	4
600		272.4^{b}	434.3^{a}	706.7^{b}	2.46	3.43^{a}	3.06	5
SEM		8.62	16.01	15.98	0.098	0.021	0.061	---
P value		0.0001	0.0001	0.0001	NS	0.002	NS	
Main effect of betaine								
0.0		264.4^{c}	430.0^{b}	694.4^{b}	2.52^{a}	3.47^{a}	3.11^{a}	6
0.072		280.0^{b}	444.7^{a}	724.7^{a}	2.35^{b}	3.36^{b}	2.98^{b}	5
0.144		284.8^{a}	442.9^{a}	727.7^{a}	2.39^{b}	3.37^{b}	2.98^{b}	5
SEM		8.62	16.01	15.98	0.098	0.021	0.061	
P value		0.0001	0.0001	0.0001	0.006	0.0001	0.0006	---

Means within the same column within the same treatment not sharing similar superscripts are significantly different (P<0.05); NS P >0.05.

of age, respectively. These improvements were declined during 29-56 d of age being 2.1 and 2.0, 4.0, and 4.5, 4.3 and 2.4%, respectively. Thus, for the whole experimental period, betaine addition at 0.072% to 872, 1172 or 1472 mg choline- containing diets increased BWG by 3.9, 4.1 and 5.1% and FCR by 4.1, 3.6 and 4.8%. Also, betaine addition at 0.144% to 872, 1172 and 1472 mg/kg diet increased BWG by 5.0, 4.9 and 4.4% and improved FCR by 4.8, 4.3 and 4.1%, respectively. It could be observed that betaine addition at medium and high levels to 300 or 600 mg choline supplemented-groups increased BWG and improved FCR without distinguish differences between betaine levels (Table 2). Furthermore, dietary betaine to the basal diet (872 mg choline/kg feed) resulted in similar BWG and FCR to those fed diet containing 1172 or 1472 mg choline without betaine supplementation indicating that 0.072% betaine could substitute 300 mg of choline in slow growing chicks diets (Table 2).

Choline supplementation at 300 mg/kg diet to the basal diet significantly increased BWG and improved FCR during all experimental (Table 2). Choline supplementation at 300-mg/kg diet increased BWG by 6.7, 1.1 and 3.2% during 1-28, 29-56 and 1-56 d of age. The corresponding improvement in FCR for the same periods was 5.8, 2.0 and 3.5%. On the other hand, increasing choline supplementation to 600-mg/kg diets had nil effect on BWG and FCR during all experimental periods compared to unsupplemented control. Results showed that the effect of choline and\or betaine was apparent in early phase of growth and declined with advanced age of chicks (Table 2).

Mortality was in normal range and was not influenced by the level of choline and\or betaine. There were only ten chicks died during 1-28 d of age, and another six chicks during 29-56 d of age. Thus, the total number of dead birds amounted to ~ 4% of the experimental population.

Carcass characteristics and internal organ: There were no statistical significant influences of choline and\or betaine supplementation on carcass yields and giblets (Table 3). Betaine addition at 0.072 to 600 mg choline supplemented group improved carcass yield by 2.0%. Also, choline supplementation at only 300 mg/kg feed slightly improved carcass yield by 1.8%.

It was observed that betaine at 144mg/kg diet to basal

Table 3: Effect of level of dietary choline (%) and\or betaine (%) on carcass characteristics, chemical composition and digestibility coefficients for chickens at 8 wk of age

Treatments		Carcass characteristics, %			Chemical composition of muscle, %				Digestibility coefficient			
		Dressing	Giblets	Abdominal fat	DM	CP	EE	CA	DM	CP	EE	CF
Effect of the interaction between choline and betaine												
Choline	Betaine											
0.0	0	65.5	5.43	0.22	29.5	20.6	2.50	1.30	79.5	88.0	74.6	23.0
0.0	0.072	65.9	5.36	0.18	29.1	20.8	2.47	1.22	79.6	88.7	75.0	23.1
0.0	0.144	66.2	5.40	0.16	29.0	21.0	2.40	1.25	80.0	89.0	75.8	23.1
300	0	66.7	5.38	0.19	29.5	20.7	2.45	1.30	79.8	88.6	74.9	23.0
300	0.072	66.5	5.41	0.15	29.1	21.2	2.54	1.24	80.0	89.7	75.9	23.2
300	0.144	66.9	5.37	0.16	29.0	21.4	2.50	1.27	80.2	89.8	76.0	23.2
600	0	65.7	5.42	0.21	29.5	20.9	2.49	1.31	79.5	88.0	74.5	23.0
600	0.072	67.0	5.36	0.18	29.1	20.9	2.50	1.25	79.7	88.2	75.0	23.2
600	0.144	66.8	5.39	0.17	29.1	20.8	2.51	1.26	80.0	88.5	75.2	23.2
SEM		1.35	0.474	0.042	1.43	0.66	0.29	0.13	1.56	1.96	2.36	2.23
P value		NS	NS	0.0001	NS	NS	NS	NS	NS	NS	NS	NS
Main effect of supplemented choline												
0.0		65.9	5.40	0.183[a]	29.2	20.8	2.50	1.28	79.7	88.6	75.1	23.1
300		66.7	5.39	0.167[b]	29.2	21.1	2.49	1.27	80.0	89.4	75.6	23.1
600		66.5	5.39	0.183[a]	29.2	20.9	2.50	1.27	79.7	88.2	74.9	23.1
SEM		0.78	1.16	0.024	0.83	0.38	0.17	0.075	0.90	1.13	1.36	1.29
P value		0.06	NS	0.002	NS	NS	NS	NS	NS	0.09	NS	NS
Main effect of betaine												
0		66.0	5.41	0.185[a]	29.5	20.7	2.50	1.30	79.6	88.2	74.7	23.0
0.072		66.5	5.38	0.170[b]	29.1	21.0	2.48	1.26	79.8	88.9	75.3	23.1
0.0144		66.6	5.39	0.163[c]	29.1	21.1	2.40	1.26	80.1	89.1	75.7	23.2
SEM		0.78	1.16	0.024	0.83	0.38	0.17	0.075	0.90	1.13	1.36	1.29
P value		NS	NS	0.0001	NS	NS	NS	NS	NS	NS	NS	NS

Means within the same column within the same treatment not having similar superscripts are significantly different (P<0.05). NS P >0.05.

diet or that supplemented with 600mg choline improved carcass yield slightly by 1.1% and 1.7% respective compared to their controls, respectively.

There were significant effects of choline and\or betaine on relative weight of abdominal fat (Table 3). It was found that betaine supplementation at either medium or high level decreased relative weight of the abdominal fat with the impact being depends on choline level as the impact declined in choline supplemented diet (Table 3). Irrespective of betaine addition, choline supplementation at only 300 mg/kg diet significantly deceased relative weight of abdominal fat by 8.7% compared to basal diet or those supplemented with 600 mg choline/kg feed. Whilst, increasing choline supplementation to 600mg/kg did not significantly affect relative weight of abdominal fat compared to the basal diet (Table 3).

Regardless of choline supplementation, results indicated that relative weight of abdominal fat was linearly decreased by 8.1 and 11.9% with increasing betaine level compared to unsupplemented control group. This indicates that 0.072% betaine had similar positive effect to 300 mg of choline on

Table 4: Effect of level of dietary choline (%) and\or betaine (%) on serum total protein (g/100 ml), albumen (g/100 ml), globulin (g\100 ml), total cholesterol (mg/100ml), AST and ALT (IU/ L) and response to sheep red blood cells (SRBC's)

Treatments		Serum constituents						Responses to SRBC'S	
		Total Protein	Albumin	Globulin	Cholesterol	AST	ALT	Primary	Secondary
Effect of the interaction between choline and betaine									
Choline	Bet								
0.0	0	4.00	2.00	2.00	90.0	44.4	9.00	4.15	6.35
0.0	0.072	4.45	2.30	2.15	87.0	44.1	8.60	4.20	6.50
0.0	0.144	4.36	2.30	2.06	90.0	44.2	9.00	6.35	6.40
300	0	4.46	2.30	2.16	89.0	44.0	9.00	4.26	6.53
300	0.072	4.52	2.41	2.11	88.0	43.9	8.90	4.60	6.70
300	0.144	4.62	2.46	2.16	89.0	43.8	8.60	6.53	6.62
600	0	4.55	2.35	2.20	88.0	44.2	8.80	4.50	6.60
600	0.072	4.60	2.50	2.10	89.0	43.8	8.70	4.85	6.76
600	0.144	4.60	2.48	2.12	89.0	43.7	8.50	6.60	6.59
SEM		0.344	0.291	0.131	3.66	2.63	0.946	0.313	0.353
P value		0.01	0.03	0.06	NS	NS	NS	0.0001	NS
Main effect of supplemented choline									
0.0		4.34	2.22^{b}	2.12	89.0	44.2	8.93	4.30^{c}	6.50
300		4.52	2.40^{a}	2.12	88.0	43.9	8.73	4.55^{b}	6.54
600		4.53	2.41^{a}	2.11	89.3	43.9	8.70	5.32^{a}	6.65
SEM		0.198	0.168	0.078	2.11	1.52	0.541	0.180	0.203
P value		0.08	0.02	NS	NS	NS	NS	0.0001	NS
Main effect of betaine									
0		4.27^{b}	2.20^{b}	2.07	89.0	44.2	8.87	4.90^{c}	6.42^{b}
0.072		4.53^{a}	2.39^{a}	2.14	88.7	43.9	8.83	5.13^{b}	6.62^{a}
0.144		4.58^{a}	2.44^{a}	2.14	88.7	43.9	8.67	5.32^{a}	6.65^{a}
SEM		0.198	0.168	0.078	2.11	1.52	0.541	0.180	0.203
P value		0.003	0.008	0.07	NS	NS	NS	0.0001	0.04

Means within the same column within the same treatment not having similar superscripts are significantly different (P<0.05); NS P >0.05.

decreasing abdominal fat deposition (8.1 vs. 8.7%).

There was no significant effect due to feeding different levels of betaine, choline or their interactions on chemical composition of muscle including DM, CP, EE and ash percentage (Table 3).

Also, digestibility coefficients for DM, CP, EE, CF, and NFE were not significantly influenced by of the level of choline and\or betaine (Table 3).

Serum constituents and responses to SRBC'S: There were no statistical significant influences of the level of choline and\or choline on serum cholesterol, and liver functions as judged by liver enzymes e.g. AST and ALT (Table 4). On the other hand, choline and\or betaine supplementation significantly affected serum total protein and albumin. It was found that choline as an independent variable at 300 or 600 mg/kg feed significantly increased serum total protein compared to the unsupplemented controls. Irrespective of choline supplementation, betaine at 0.072 and 0.144% increased serum total protein (P<0.003), albumen (P<0.008) and

globulin (P<0.07), compared to unsupplemented controls (Table 4).

Results showed that betaine supplementation at 72 or 144 mg/kg feed to the basal diet increased serum total protein and albumen. Also, serum total protein was responded positively when 72 or 144 mg of betaine was supplemented to the diet supplemented with 300 or 600 mg choline/kg. Nonetheless, globulin was increased (P<0.06) by 7.5 and 3% due to addition of 0.072 and 0.144% betaine to the basal diet, respectively, indicating that 72 mg /kg diet was adequate.

Results indicated that choline and\or betaine had a significant effect on primary response against SRBC's. Results revealed that there was a linear increase in responses to SRBC's with increasing level of supplemented choline or betaine (Table 4). Meanwhile, only dietary levels of betaine significantly affected secondary response, with no significant difference between the medium and the high level. The interaction between choline and between showed that betaine supplementation at 144 mg/kg to the basal diet with or without 300 or 600 mg choline /kg increased primary responses to SRBC's. Also, 72 mg betaine/kg diet slightly increased primary responses to SRBC's of group fed diet supplemented with 300 or 600 mg choline, although the 144 mg betaine had greater effect than the medium dose of betaine (Table 4).

Discussion

Growth of slow growing chicks during 1-28, 29-56 and 1-56 d of age was significantly improved due to betaine supplementation. However, this may be depended on choline or methionine contents of the experimental diets; fat level and ability of chicks to synthesis choline via hepatic synthesis (Venugopal 1985; Ghazalah, 1998 and Workel *et al.*, 1999). The present results showed that the response to betaine supplementation was diminished at 600 mg of added choline or 1472 mg choline/kg feed (Table 2). It was also observed that growth stimulating and feed utilization improving effect of betaine is greater than that of choline. For example choline supplementations at 300 mg to the basal diet improved BWG by 3.2 and FCR by 3.5%, whilst betaine addition at 0.072 and 0.144% improved BWG of the basal diet (872 mg choline) by 3.9 and 5.0% and FCR by 4.1 and 4.8%. These results agree with that by Virtanen and Rosi (1995), Augustine *et al.* (1997) and Waldenstedt *et al.* (1999) who reported that dietary betaine addition improved performance of chicks. Furthermore, Abel *et al.* (1985) reported that betaine was more efficient than choline for improving FCR. Furthermore, Miles *et al.* (1987) showed that betaine could replace choline in chicken diets.

The present results are in agreement with those of Menten *et al.* (1997) who found greater response to supplemented choline when basal diet containing low level of choline. Along the same line, Emmert *et al.* (1996) reported that choline supplementation to methionine deficient diet significantly increased growth and improved FCR. It was shown that choline addition to methionine deficient diets improved growth of chicks especially during early phase of growth (Pesti *et al.*, 1980 and Harms and Miles, 1984). In the present study, it should be noticed however, that methionine level of the basal diet was adequate to meet chick's requirements of the 1st limiting amino acid and methyl group donors. However, methyl group donors have been shown specificity in body functions, and blood uptake of betaine was faster than choline and methionine (Kettunen *et al.*, 2001a and b). Additionally, Rostagno and Pack (1995), Schutte *et al.* (1997) and McDevitt *et al.* (2000) reported that choline or betaine can reduce the requirements of methionine by furnishing methyl groups, but they can not reduce the need for dietary methionine when the diet contain inadequate level of choline. Baker and Czarneck (1985) suggested that dietary betaine, but not choline showed some efficiency in remethylating homocysteine to methionine in chicken fed methionine inadequate diets, and alleviate the growth depression caused by excessive levels of homocysteine in chickens (Hafez *et al.*, 1978).

It was also shown that the growth response to choline or betaine supplementation declined with increasing age of chicks, indicating that methyl donor requirements are higher during early growth phase than latter growth period (Table 2). Similar results were reported with ducks by Wang *et al.* (2004). Also, NRC (1994) cited data indicated that choline requirements for leghorns, broilers and turkeys and this declined with increasing age of chicks and depends on strains and type as well as growth potential of chickens.

On the other hand, the lack of responses to increasing choline level to 1472 mg/kg diet, indicating that a saturation of growth and FCR at 1172 mg of choline/kg diet showing that 1172 mg of choline is adequate for slow growing chicks. This is clear when the data was examined irrespective of betaine supplementation or when unsupplemented groups were compared (Table 2). This is slightly less than the current recommendation of 1300 mg choline /kg feed for choline requirements for Leghorn and broiler type chicks (NRC, 1994). Similar to the present findings, Tsiagbe *et al.* (1987), Mohamed (1998) and Rama Rao *et al.* (2001) concluded that efficiency of dietary choline utilization decreased with increasing choline level.

It is clear that betaine addition at 0.072 or 0.144% to 872 mg choline containing- diet yield similar growth and FCR to those supplemented with either 300 or even 600 mg of choline alone. This indicates that 72 mg of betaine/kg feed could replace 300 or even 600 mg of choline in the diets for slow growth chicks (Table 2). This is in accordance with the conclusion of Miles *et al.* (1987)

who reveals that betaine could substitute choline in broiler and laying hen diets. Furthermore, the best growth and FCR was shown of group fed diets containing 1172 mg/kg choline (300 mg of supplemented choline) and supplemented with 144 mg betaine/kg diet (Table 2). This indicating an additive effect of betaine when supplemented to slow growth chick diets containing adequate level of methionine and choline.

The improved performance due to betaine supplementation could be attributed to several reasons i.e as methyl donor group, its diverse physiological properties that could improve gut environment and thus enhance the ability of the chicks to withstand coccidial infection (Augustine *et al.*, 1997; Allen *et al.*, 1998; Kettunen *et al.*, 2001a and Remus *et al.*, 2004), reduce intestinal membrane damage, dehydration, diarrhea and mal-digestion and\or absorption (Crompton,1976; Allen *et al.*, 1998; Kettunen *et al.*, 2001b). However, choline and betaine had no apparent effect on digestibility of nutrients measured in this experiment (Table 3). Apparently in this work, there was no coccidiosis syndrome and the experimental diets contained adequate methionine level that meets NRC (1994) requirements and\or methyl donor groups for fast growing chicks i.e. broilers. However, exclusion of occurrence of sub-clinical infection of coccidiosis could not be warrant in floor pens-reared chicks. In this connection, betaine significantly enhanced primary and secondary responses to SRBC's (Table 4). In this regard, Saunderson and Mackinlay (1990) reported that accumulation of betaine in the cell protects it from osmotic stress. This allows them to continue regular metabolic activities in conditions that would normally inactivate the cell (Rudolph *et al.*, 1986; Petronini *et al.*, 1992 and Ko *et al.*, 1994).

The involvement of choline and betaine in lipid metabolism offer interesting opportunity to poultry industry to satisfy consumer desire and improve feed conversion. Results showed slight insignificant effect of choline and\or betaine on carcass yield, however, the effect of betaine was more evident (Table 3). Similarly, Saunderson and Mackinlay (1990), Virtanen and Rosi (1995), Esteve-Garcia and Mack (2000) and Wang *et al.* (2004) indicated that betaine supplementation significantly increased breast yielded.

It is interesting to report that slow growth chicks, which exhibited low deposition of abdominal fat, showed a significant effect of betaine and\or choline on relative weight of abdominal fat. However, the effect of betaine depends on dietary choline level showing the similarity in the mode of action. On the other hand, the effect of betaine on percentage abdominal fat was stronger than that of choline when probability value was compared ($P< 0.0001$ vs. 0.002), also the impact of betaine being linear while the influence of choline being quadratic in nature. In deed, this is apparent only when a decrease (11.9%) caused by addition of 0.144% betaine was compared to that of 300 mg of choline (8.7%). Nonetheless, the effect of 0.072% betaine is similar (8.1%) to that 300 mg choline/kg feed. These results are in agreement with that by Esteve-Garcia and Mack (2000) who reported that the abdominal fat pads of the betaine- supplemented chicks was less than that of the control group. Also, Saunderson and Mackinlay (1990) presented data indicating that betaine may be more effective lipotrophic agent than choline for poultry, and the methylation properties of betaine may also be important during lipid metabolism by reducing and redistributing body fat. Also, carcass with less fat could also result if choline or betaine spared methionine, leaving more of the available essential amino acid for protein synthesis. In such case, better use of dietary nutrients would leave fewer amino acids for deamination and eventual synthesis into adipose tissue (Wallis, 1999).

The decrease in abdominal fat deposition due to 300 mg of choline addition are in partial agreement with the findings, Rama Rao *et al.* (2001) who observed that dietary addition of choline at 760 but not 1520 mg/kg diet significantly decreased fatty liver and had no significant effect on abdominal fat deposition of laying hens.

Although, there was a significant effect of choline/and betaine on abdominal fat deposition, there were no significant effects of these variables on chemical composition of muscle includes DM, CP, EE and ash (Table 3). This may indicate that later deposition of intramuscularly fat compared to early deposition of abdominal fat. Similar results were reported by Schutte *et al.* (1997), and Garcia *et al.* (2000) who revealed that betaine addition had no significant effect on DM, EE, and ash of meat. In contrast to the present findings, Esteve-Garcia *et al.* (2000) observed that betaine supplementation increased protein level in the muscles. Also, Türker *et al.* (2004) indicated that betaine supplementation to broiler corn-soybean diets containing reduced level of methionine and choline resulted in similar dressing percentage, crude protein and EE percentage of meat as those fed diet containing adequate methionine, showing the beneficial effect of betaine in protein deposition.

The improvement in serum total protein, albumin and globulin due to betaine and\or choline supplementation indicating a further evidence about the role of methyl donor groups in protein metabolism. Results showed that the responses to betaine supplementation declines with increasing choline level, as the responses was greater in the diet containing only native choline (Table 4). The positive effect of betaine and choline in serum protein was confirmed by the improvement in antibody responses to SRBC's, showing the positive effect of choline and betaine on immune functions. The improvement in the primary antibody titer of chicks due

to choline and\or betaine are in general agreement with those of Remus *et al*. (2004) they concluded that betaine addition increased broilers withstand coccidial infection. In this concern, it should be kept in mind that betaine addition increased serum total protein (P<0.003), albumin (P<0.008) and globulin (P<0.07). Meanwhile, the effect of choline was small on serum protein (P<0.08) and albumin (P<0.02) and had insignificant effect on serum globulin compared to the effect of betaine. Similar to the present findings, Mathews and Southern (2000) found that betaine increased plasma protein of broilers inculcated with chronic dose of coccidiosis. Also, Swain and Johri (2000) found that choline supplementation at 2300 and 3300 mg/kg feed improved cellular and humoral immune responses. However, early study by Tsiagbe *et al*. (1987) indicated that choline supplementation of 0.125 or 0.25% to 0.13% choline containing diet did not affect total antibodies, IgG and IgM and mitogen PHA-P.

In conclusion, slow growing chicks gained 13.3 g/d during 1-56 d of age required a choline level of 1172 mg/kg feed. However, choline level of 872 mg choline, which derived from native one, was adequate when dietary 0.072% betaine was added.

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