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Growth, Carcass Quality and Serum Constituents of Slow Growing Chicks as Affected by Betaine Addition to Diets Containing 2. Different Levels of Methionine

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Abstract: The responses of slow growing chicks to different dietary levels of methionine and betaine and their interaction were investigated during the starter-grower period from 1 to 56 d of age. Therefore, a basal marginally methionine deficient all-mash corn-soybean meal diet was formulated to contain 0.32% methionine, 0.65% total sulphur amino acid (TSAA) and adequate level of choline (NRC, 1994). This diet was supplemented with 0, 0.05 and 0.10% of DL-methionine. Each methionine level was supplemented with 0.0, 0.035 and 0.07% betaine. Thus, there were nine experimental diets; each diet was fed to 40 chicks divided equally among 5 replicates of eight chicks each. Methionine level at 0.37 and 0.42% significantly improved BWG by 2.1 and 8.1% and FCR by 4.5 and 8.6% compared to the basal diet, respectively. Methionine level at 0.37 and 0.42% increased blood serum total protein, albumin, globulin, antibody response to Newcastle disease virus (NDV), and percentage carcass yield, feather weight and CP of muscle tissue compared to the basal diet. Moreover, methionine at 0.37 and 0.42% significantly decreased serum AST and ALT, and abdominal fat compared to the basal diet. Betaine supplementation at either 0.035 or 0.070% significantly improved BWG by 5.1 and 9.0%, and FCR by 8.4 and 12.0% compared to the basal diet, respectively. Betaine supplementation improved percentage carcass yield, feather weight and CP of muscle tissue, while a significant decrease in blood serum ALT was observed at 0.07% betaine. There were significant interaction between methionine level and betaine supplementation in BWG and FCR, indicating that betaine addition at 0.035% to the basal diet (0.32% methionine) resulted in similar BWG and FCR to those fed diet containing 0.37% methionine and supplemented with same level of betaine. Meanwhile, betaine supplementation at 0.07% to the basal diet resulted in similar BWG and FCR of those fed 0.37% methionine supplemented with 0.07% betaine or those fed 0.42% methionine without or with any level of betaine. In conclusion, slow growing chicks (14.6 g/d) during 1-56 d of age could be fed diet containing 0.42% methionine. However, when diet was supplemented with 0.07% betaine, methionine level could be decreased to 0.37 or even to 0.32%.

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

Introduction

Betaine (glycine betaine, trimethylglcine) is a methylated amino acid and naturally occurring product present in large quantities in aquatic invertebrates and sugar beet from which it can be extracted (Virtanen, 1995; Danisco, 2000 and Wang *et al.*, 2004). On the other hand, betaine is not present in large quantities in animal feedstuffs (Wang *et al.*, 2004). Betaine is a tertiary amine formed by the oxidation of choline (Kidd *et al.*, 1997; Wang *et al.*, 2004) and implicated in methionine sparing, osmoprotective, and fat distribution (Saunderson and Mackinlay, 1990; Petronini *et al.*, 1992 and Türker *et al.*, 2004).

Methionine, betaine and choline are all sources of labile methyl groups and play an important role in methylation reactions, and the methyl group metabolism of these three compounds is interrelated as illustrated by Kettunen *et al.* (2001a) in Fig. 1.

Methionine plays three crucial roles in metabolism of all vertebrates. First, it is an essential amino acid; second, it is a precursor of cysteine. The third role of methionine is as a key intermediate in methyl group transfer. Over 100 methylation and transmethylation reactions involve methionine, including the synthesis and metabolism of phosphatidylcholine and creatine (Larbier and Leclerco, 1992). In these methylation reactions, methionine is converted to s-adenosylmethionine, the methyl group donor, and then to homocysteine. The backup reaction, which involves either betaine or tetrahydrofolate as the provider of the methyl group necessary to convert

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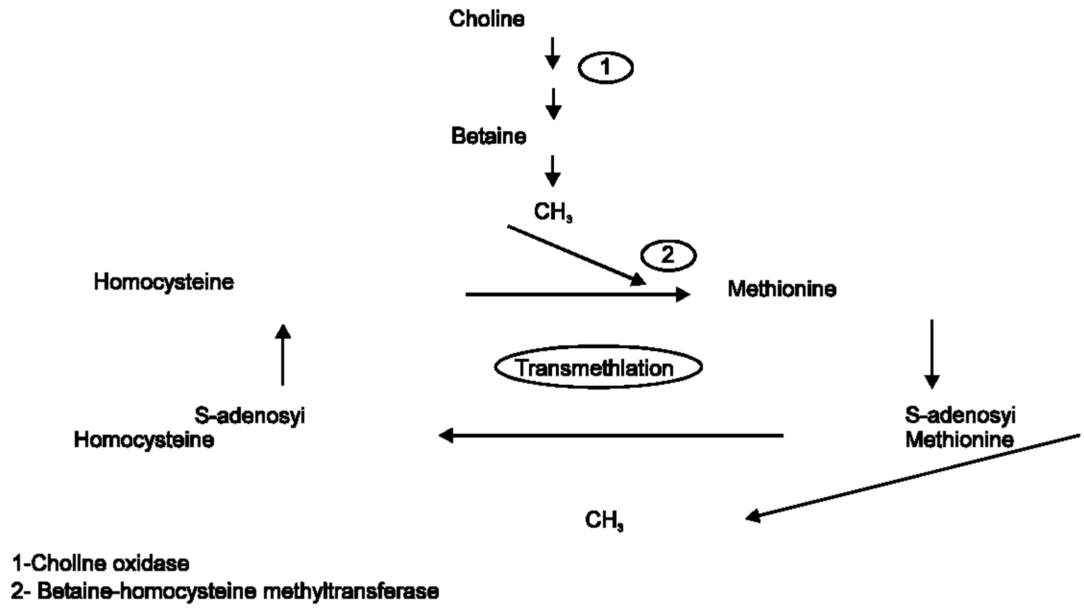

Fig. 1: A schematic presentation of metabolites involvd in the transmethylation cycle (after Kettunen *et al.*, 2001a)

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

Ingredients (%)	
Yellow corn	63.3
Soybean meal (44%CP)	33.0
Dicalcium phosphate	2.0
Salt	0.3
Limestone	1.1
Vit.Min.mixture (premix)[1]	0.3
Total	100.0
Calculated and analyzed composition	
ME[2] (kcal/kg)	2868
CP[3] (%)	19.80
Ca[2] (%)	0.97
Available P[2] (%)	0. 45
Methionine[2] (%)	0.32
Methionine[3] (%)	0.31
TSAA[2, 4] (%)	0.65
TSAA[3] (%)	0.63
Lysine[2] (%)	1.05
Choline[2] (mg/kg)	1314

[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; Choline chloride (50% choline) 500 mg; 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), while methionine was determined at BASF AG, Germany using amino acid analyser model Biochrom 20, Amershan Pharmcia, USA. Methionine and cystine were determined in samples oxidized with performic acid (Moore, 1963). [4]Total sulphur containing amino acids.

homocysteine to methionine (Finkelstein and Martin, 1984).

There are controversy in literature regarding the methionine- sparing and fat distribution effects of betaine. For example, Virtanen and Rosi (1995), Garcia *et al.* (2000) and Wang *et al.* (2004) observed that betaine was effective in improving growth and feed conversion and indicates that betaine has a methionine sparing effect in broilers and ducks. On the other hand, Schutte *et al.* (1997) and Esteve-Garcia and Mack (2000) concluded that betaine had small and non-significant effect and could not replace methionine in a methionine –deficient diet for broilers. Also, results showed a positive effect of betaine on fat distribution (Saunderson and Mackinlay 1990 and Wang *et al.*, 2004) and immune response (Allen *et al.*, 1998; Swain and Johri, 2000; Keuttunen *et al.*, 2001b and Remus *et al.*, 2004). In this regard, Rostagno and Pack (1995) and Türker *et al.* (2004) indicated that betaine supplementation to corn-soybean meal broiler diets marginally deficient in methionine and choline improved growth performance and resulted in similar percentage carcass, as well as CP and EE of meat to those of the positive control.

This work aimed to investigate whether or not betaine could spare methionine in corn-soybean meal diets contained different dietary levels of methionine fed with or without betaine supplementation utilizing native chicks of Mandarah strain. It was also aimed to gain information for methionine needs of slow growing chicks such as Mandarah chicks.

Materials and Methods

Chicks and diets: A total of 360 one -day old unsexed chicks of Mandarah strain [Alexandria (Fayoumi× Barred

Plymouth Rocks×Leghorn × RIR) × Dokki$_4$ (Fayoumi× Barred Plymouth Rocks)] were randomly distributed into 9 groups of five replicates each of 8 birds each. Thus, the chicks were reared in 45 floor pens (1×1 m) furnished rice hulls. Water and feed were provided *ad libitum*.

A basal marginally methionine deficient all-mash corn-soybean meal diet was formulated to contain 0.32% methionine and adequate level of choline based on NRC, 1994 (Table 1). This diet was fed without or with 0.05 and 0.10% DL-methionine. DL- methionine was added as a 99% pure dry powder (Degussa AG, Frankfurt, Germany). These supplementations resulted in three levels of dietary methionine being 0.32, 0.37 and 0.42%. Each methionine level was supplemented or not with 0.0, 0.035 and 0.07% betaine as Natural Betafin® (Betafin S6, 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 methionine ×3 levels of betaine. The experimental diets was 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 coccidiosis challenge and reduce the efficiency of betaine as a coccidiostat enhancer to the methylation benefit rather than its osmolytic benefits.

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.

At the end of the experiment (8 wk of age), 6 males of each treatment were slaughtered for carcass characteristics and chemical composition of muscle (AOAC, 1990). Also, nine chickens per group were randomly chosen, marked and bled at 7, 20 and 31 days of age and serum was examined for haemagglutination inhibition (HI) antibodies to (NDV) according to Anonymous (1971).

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 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 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). Digestibility of nutrients was 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). Methionine and betaine levels were the main effects. Mean differences were tested using Student-Newman-Keuls Test.

Results

Chick performance: Means of BWG, and FCR are shown in Table 2. It is clear that BWG and FCR were significantly improved linearly by increasing dietary methionine level during all experimental periods from 1 to 56 d of age. For the whole period, 0.37 and 0.42% methionine level significantly increased BWG by 2.1 and 8.1%, and improved FCR by 4.5 and 8.6% respectively compared to the basal diet (Table 2).

Betaine addition at medium or high dose significantly improved BWG and FCR during all experimental periods. For the whole experimental period, betaine supplementation at 0.035 and 0.070% significantly linearly increased BWG by 5.1 and 9.0% and improved FCR by 8.4 and 12.0% compared to the unsupplemented control respectively.

Also, results showed that BWG and FCR of chicks were significantly affected by the interaction between methionine level and betaine supplementation during all experimental periods. In general, results indicated that betaine supplementation to 0.32 or 0.37% methionine containing-diets significantly improved BWG and FCR compared to chicks fed diet containing 0.42% methionine, which showed small responses to either medium or high level of betaine supplementation. The improvement for the whole experimental period in BWG due to betaine supplementation at 0.035 and 0.07% to diet containing 0.32 and 0.37% methionine amounted to 11.7 and 19% and 4.2 and 10.7%, respectively compared to their negative controls. The corresponding improvement in FCR amounted to 21.0 and 26.0% and 1.9 and 6.8%, respectively compared to their negative controls.

As shown in Table 2, there were only seven chicks died during the starter period (1-28 d of age), and another four chicks during the grower period (29-56 d of age). Thus, the total number of dead birds amounted to 3.2% of the experimental birds, which was within an permissible range.

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

Treatments		BWG			FCR			Number of dead birds
		1-28 d	29-56 d	1-56 d	1-28 d	29-56 d	1-56 d	
Interaction effect between methionine and betaine Methionine Betaine								
0.32	0.0	245.0	440.0	685.0	2.69	3.46	3.19	1
0.32	0.035	270.8	494.3	765.1	2.52	2.52	2.52	1
0.32	0.07	295.2	519.9	815.1	2.31	2.39	2.36	1
0.37	0.0	265.0	470.0	735.0	2.58	2.67	2.63	1
0.37	0.035	274.1	491.5	765.6	2.50	2.63	2.58	---
0.37	0.07	295.1	518.5	813.6	2.36	2.50	2.45	1
0.42	0.0	299.3	517.7	817.0	2.33	2.50	2.43	1
0.42	0.035	298.3	521.9	820.2	2.35	2.51	2.45	---
0.42	0.07	304.3	510.4	814.7	2.30	2.55	2.45	1
SEM		13.42	13.22	15.37	0.099	0.062	0.061	---
P value		0.0001	0.0001	0.0001	0.001	0.001	0.0001	---
Main effect of methionine level								
0.32		270.6[c]	485.2[c]	755.8[c]	2.51[a]	2.78[a]	2.68[a]	3
0.37		278.1[b]	493.5[b]	771.6[b]	2.48[b]	2.60[b]	2.56[b]	3
0.42		300.6[a]	516.7[a]	817.3[a]	2.33[c]	2.52[c]	2.45[c]	1
SEM		7.97	7.80	8.90	0.057	0.035	0.305	---
P value		0.0001	0.0001	0.0001	0.0001	0.001	0.001	---
Main effect of betaine								
0.0		270.0[c]	476.3[c]	746.3[c]	2.53[a]	2.87[a]	2.75[a]	3
0.035		281.5[b]	502.7[b]	784.2[b]	2.46[b]	2.55[b]	2.52[b]	2
0.07		298.2[a]	516.4[a]	813.6[a]	2.32[c]	2.48[c]	2.42[c]	2
SEM		7.97	7.80	8.90	0.057	0.035	0.305	---
P value		0.0001	0.0001	0.0001	0.0001	0.001	0.0001	---

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

Blood constituents and immune response to NDV: There was a significant effect of methionine level on serum total protein, albumin, globulin, AST, and ALT (Table 3). Chicks fed diet containing 0.37 and 0.42% methionine showed significant higher total protein, albumin, and globulin, but lower AST and ALT than those fed the basal diet. While, the decrease was linear only in serum ALT. Nonetheless, serum cholesterol was not significantly affected by methionine and/or betaine.

Betaine supplementation had no significant effect on most of serum constituents except for ALT where a significant decrease was shown of birds fed diet containing 0.07% betaine compared to the control group (Table 3). There was no significant interaction between level of methionine and betaine supplementation on total protein, albumin, globulin, and AST of serum (Table 3). However, only significant effect was shown in ALT. Results indicated a progressive decrease in serum ALT with increasing betaine supplementation to groups fed diets containing 0.32 and 0.37% methionine level. Meanwhile, the contrary was shown of groups fed diet containing 0.42% methionine.

Only methionine level had a significant effect on antibody titers to NDV at 7 days of age. The differences being significant only between the control group and those fed diet contained 0.42% methionine level (Table 3). Also, there was a slight insignificant improvement in antibody titers to NDV at 20 and 30 d of age due to supplementation with either methionine or betaine (Table 3).

There was no significant interaction between methionine and betaine supplementation in antibody titer to NDV.

Carcass characteristics and chemical composition of muscle tissue: Methionine and betaine addition significantly increased carcass yield percentage, feather weight percentage as well as CP percentage of muscle tissue (Table 4). The effect being significantly linear in percentage feather weight and CP of muscle tissue for methionine level, and feather weight for betaine.

Abdominal fat was significantly decreased due to methionine addition compared to the basal diet, with no difference between the two supplemented levels. Meanwhile, abdominal fat was significantly increased linearly with increasing the level of betaine (Table 4). There was a significant interaction between the level of methionine and betaine supplementation on percentage feather and abdominal fat. Results indicated that betaine supplementation to 0.32 and 0.37% methionine containing-diets increased percentage feather, while the

Table 3: Effect of level of dietary methionine (%) and/or betaine (%) on serum total protein (g/100 ml), albumin (g/100 ml), globulin (g\100 ml), total cholesterol (mg/100ml), AST and ALT (IU/ L) and immune response to Newcastle disease virus (NDV)

Treatments		Serum constituents						Antibody titer to NDV		
		Total Protein	Albumin	Globulin	Cholesterol	AST	ALT	HI- 7	HI -20	HI –30
Effect of the interaction between methionine and betaine										
Methionine	Betaine									
0.32	0	4.10	2.09	2.01	89.0	45.3	9.10	2.20	2.79	3.00
0.32	0.035	4.20	2.15	2.05	88.0	45.0	8.60	2.43	2.83	3.09
0.32	0.070	4.23	2.19	2.04	87.0	44.6	8.30	2.43	2.88	3.10
0.37	0	4.45	2.22	2.23	88.0	44.0	8.50	2.46	2.85	3.00
0.37	0.035	4.52	2.32	2.20	85.0	43.6	8.30	2.50	3.00	3.12
0.37	0.070	4.63	2.40	2.23	87.0	43.1	8.00	2.55	3.03	3.14
0.42	0	4.57	2.32	2.22	85.0	42.8	8.00	2.53	3.00	3.10
0.42	0.035	4.62	2.50	2.12	86.0	41.9	8.25	2.57	3.02	3.15
0.42	0.075	4.60	2.43	2.17	88.0	42.4	8.30	2.59	3.00	3.12
SEM		0.09	0.098	0.046	1.15	0.53	0.15	0.09	0.11	0.09
P value		NS	NS	NS	NS	NS	0.01	NS	NS	NS
Main effect of Methionine										
	0.32	4.18[b]	2.14[b]	2.03[b]	88.0	45.0[a]	8.67[a]	2.35[b]	2.83	3.06
	0.37	4.53[a]	2.31[a]	2.22[a]	86.7	43.6[b]	8.27[b]	2.50[ab]	2.96	3.09
	0.42	4.60[a]	2.42[a]	2.17[a]	86.3	42.4[c]	8.18[b]	2.56[a]	3.01	3.12
SEM		0.054	0.056	0.025	0.66	0.31	0.088	0.054	0.062	0.054
P value		0.0001	0.004	0.0001	NS	0.001	0.005	0.02	NS	NS
Main effect of betaine										
	0	4.37	2.21	2.15	87.3	44.0	8.53[a]	2.40	2.88	3.03
	0.035	4.45	2.32	2.12	86.3	43.5	8.38[ab]	2.50	2.95	3.12
	0.070	4.49	2.34	2.15	87.3	43.4	8.20[b]	2.52	2.97	3.12
SEM		0.054	0.056	0.025	0.66	0.31	0.088	0.054	0.062	0.054
P value		NS	NS	NS	NS	NS	0.03	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

effect being only linear in-group fed 0.37% methionine. Also, betaine supplementation to any level of methionine increased abdominal fat deposition, while the effect being only linear in-group fed 0.32% methionine. Level of either methionine and/or betaine did not significantly affect percentages giblets and moisture, EE and ash of meat.

Digestibility coefficients: There were no significant influences of the level of either methionine or betaine or the interaction between them on digestibility of DM, CP, EE and CF (Table 4).

Discussion

In literature, there are some evidences indicating that either choline (Pesti *et al.*, 1981) or betaine (Virtanen, 1995; Virtanen and Rosi, 1995 and Wang *et al.*, 2004) can spare methionine and increase its availability as an essential amino acid for protein synthesis. Our results support research that betaine can replace methionine (Türker *et al.*, 2004 and Wang *et al.*, 2004) and suggest

Table 4: Effect of level of dietary methionine (%) and/or betaine (%) on carcass characteristics, chemical composition and digestibility coefficients for chickens at 56 d of age

Treatments		Carcass characteristics, %				Chemical composition of muscle, %				Digestibility coefficient			
		Dressing	Giblets	Feather	Abdominal fat	Moisture	CP	EE	Ash	DM	CP	EE	CF
Effect of the interaction between methionine and betaine													
Meth	Betaine												
0.32	0	65.0	5.50	3.07	0.10	70.5	20.3	2.60	1.30	79.9	88.2	74.8	23.1
0.32	0.035	66.5	5.35	3.83	0.30	71.2	21.2	2.50	1.25	79.9	89.2	76.6	23.8
0.32	0.070	66.7	5.40	3.93	0.60	71.5	21.7	2.47	1.28	80.1	89.9	77.0	23.9
0.37	0	67.0	5.35	3.88	0.04	70.5	21.5	2.27	1.20	80.7	89.0	76.4	23.7
0.37	0.035	68.0	5.25	4.00	0.30	70.7	22.0	2.50	1.28	80.2	90.0	77.3	23.9
0.37	0.070	68.5	5.32	4.70	0.31	70.7	22.5	2.35	1.22	80.1	90.0	77.5	23.8
0.42	0	68.0	5.22	4.65	0.06	70.3	22.7	2.17	1.13	80.0	89.8	76.6	23.6
0.42	0.035	69.0	5.40	4.73	0.32	70.3	22.7	2.33	1.22	80.3	90.1	77.0	23.6
0.42	0.07	68.0	5.37	4.80	0.30	70.8	22.8	2.40	1.30	79.7	90.0	76.8	23.5
SEM		0.55	0.123	0.092	0.035	0.69	0.32	0.114	0.051	0.58	0.58	1.05	0.82
P value		NS	NS	0.0001	0.0001	NS	NS	NS	NS	NS	NS	NS	NS
Main effect of Methionine													
0.32		66.1[b]	5.42	3.61[c]	0.32[a]	71.1	21.1[c]	2.52	1.28	80.0	89.1	76.1	23.6
0.37		67.8[a]	5.31	4.19[b]	0.22[b]	70.6	22.0[b]	2.37	1.23	80.3	89.7	77.1	23.8
0.42		68.3[a]	5.33	4.73[a]	0.23[b]	70.5	22.6[a]	2.30	1.22	80.0	90.0	76.8	23.6
SEM		0.32	0.068	0.053	0.020	0.40	0.19	0.066	0.031	0.32	0.32	0.60	0.48
P value		0.001	NS	0.0001	0.0002	NS	0.0001	0.06	NS	NS	NS	NS	NS
Main effect of betaine													
0		66.7[b]	5.36	3.87[c]	0.07[c]	70.4	21.3[b]	2.34	1.21	80.2	89.0	75.9	23.4
0.035		67.9[a]	5.33	4.19[b]	0.31[b]	70.7	22.0[a]	2.44	1.25	80.1	90.0	77.0	23.8
0.07		67.7[a]	5.36	4.48[a]	0.40[a]	71.0	22.3[a]	2.41	1.27	80.0	90.0	77.1	23.7
SEM		0.32	0.068	0.053	0.020	0.40	0.19	0.066	0.031	0.32	0.32	0.60	0.48
P value		0.02	NS	0.0001	0.0001	NS	0.001	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 $P>0.05$.

that betaine at 0.07% could replace 0.05% methionine in the diets for slow growth type chicks. Betaine supplementation to 0.32 or 0.37% methionine containing-diets significantly improved growth and FCR compared to chicks fed diet containing 0.42%, which showed small responses to each level of betaine supplementation. This revealed that the response to betaine is methionine level dependent as the responses declined as the level of methionine increased (Table 2). Miles *et al.* (1987) fed broilers maize-soy diets devoid of supplemental methionine, choline and betaine and showed that adding 1.1 g/kg choline or 1.06-g/kg betaine improved growth, but no further growth resulted from adding compounds. They added that birds grew the fastest when the basal diet was supplemented with 2.4 g/kg DL-methionine either alone or in combination with choline or betaine. They concluded in accordance with the results of Pesti *et al.* (1981) and Steve-Garcia and Mack (2000) that betaine may not substitute methionine and chicks fed low-methionine diets containing 500 g/kg isolated soybean protein, responded positively to either methionine or choline or betaine. Along the same line, Chavez and Kratzer (1974) studied the dermatitis in turkeys fed soybean meal and reported that homocysteine or betaine enhanced growth, but could not alleviate a foot pad dermatitis caused by methionine deficiency, indicating that betaine could not replace methionine in all of its functions. However, Wang *et al.* (2004) showed that betaine significantly improved growth and FCR of ducks fed methionine adequate diets.

The improvement in growth performance due to betaine may be due to its effect as methyl donor as already mentioned and 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; Swain and Johri, 2000; Keuttunen *et al.*, 2001b and Remus *et al.*, 2004). For example, betaine has been shown to stabilize cell membranes through interaction with membrane phospholipid during dehydration (Rudolph *et al.*, 1986), and to reduce fecal water content and increase the digestibility of several nutrients (Virtanen, 1995). These properties could reduce intestinal membrane damage, dehydration, diarrhea and mal-digestion and/or absorption (Crompton, 1976; Allen *et al.*, 1998 and Kettunen *et al.*, 2001a). Therefore, betaine may have contributed to the improved performance of chicks directly, by partial inhibition of coccidial invasion and development, and, indirectly by support of intestinal structure and function. In this experiment, there was no apparent occurrence of coccidiosis syndrome and the experimental diets contained adequate choline level that meet NRC (1994) requirements and/or methyl donor groups. However subclinical infection of coccidial could not be totally excluded in floor pens-reared chicks. Additionally, betaine numerically enhanced antibody responses to NDV (Table 3). The improvement in antibody titer of chicks due to methionine and/or betaine are in general agreement with those of Swain and Johri (2000) and Remus *et al.* (2004) they concluded that betaine supplementation increased immune response. Methionine is also required for some components of the antibody response, and might be required for thymus-derived T-cell helper function (Tsiage *et al.*, 1987).

Evidently, the present results indicate that methionine requirement for slow growing chicks (14.6 g/d) is ~0.42% as judged by BWG and FCR, too. The results also showed that the effect of methionine and/or betaine is more effective during early growth period (1-28 d of age) than the late growth period (29-56 d of age). For example the responses to increasing methionine level from 0.32 to 0.37 or 0.42% was 2.8 and 11.1% during 1-28 d of age and declined to 1.7 and 6.5% during 29-56 d of age (Table 2). This indicates that the requirements for methyl group donors are higher during early growth phase than letter growth period. Similar results were reported with ducks by Wang *et al.* (2004). Methionine may act as a lipotrophic agent through its role as an amino acid in balancing protein, or through its role as a methyl donor and involvement in choline, betaine, folic acid, and vitamin B_{12} metabolism (Wong *et al.*, 1977; Tillman and Pesti, 1986 and Chen *et al.*, 1993). Saunderson and Mackinlay (1990) presented data suggesting that betaine may be more effective lipotrophic agent than choline for poultry. A subsequent report by Virtanen and Rosi (1995) showed improved performance when methionine or betaine was added to a corn and soybean meal- based broiler diets.

The involvement of methionine and betaine in lipid metabolism offer interesting opportunity in meat production of poultry due to recent trend in producing lean birds to satisfy consumer desire and improve feed conversion. The present results indicated that methionine was more effective in increasing dressing percentage and decreasing abdominal fat than betaine. Moreover, betaine significantly increased abdominal fat deposition and confirmed the results by Esteve-Garcia and Mack (2000) who reported that abdominal fat pads of the betaine-supplemented birds appeared to be larger than those of the methionine-supplemented chicks. Also, results with pigs (Haydon *et al.*, 1995) indicated that betaine increased back fat thickness and decreased longissimus dorsi area. On the other hand, Saunderson and Mackinlay (1990) and Wang *et al.* (2004) indicated betaine supplementation significantly decreased abdominal fat, and increased breast yielded. Ingredient compositions, methionine and choline contents of the experimental diets, strain and spices of animals may explain this contradiction in responses to betaine as lipotropic agent. It is well known that betaine, as a methyl donor, donates a methyl group to dimethlyethanolamine to for trimethlyethanolamine, which is choline (Fernández *et al.*, 2000 and Wang *et al.*, 2004). Choline can then be used directly for the synthesis of lecithin, which facilitates the transport of fat through the body (Saunderson and Mackinlay, 1990 and Wang *et al.*, 2004). When methionine inadequate diet was fed, methionine supplementation is used for protein synthesis, while betaine is efficient as a methyl donor involved in carnitine synthesis, which is of fatal importance for beta-oxidation of long chain fatty acids (Wang *et al.*, 2004).

The significant improvement in dressing percentage due to increasing level of methionine and/or betaine (Table 4), indicating a saturation of responses when diet supplemented with 0.035% betaine or 0.05% methionine (0.37% methionine level). Also, results reported by Saunderson and Mackinlay (1990), Virtanen and Rosi (1995) and Wang *et al.* (2004) indicated that both methionine and betaine added to a corn and soybean meal-based broiler and ducks diets improved breast meat yielded. Esteve-Garcia and Mack (2000) reported that breast yielded increased significantly ($P \geq 0.05$) at 0.6 and 1.2g/kg of added DL- methionine, while betaine increased carcass yielded significantly ($P \geq 0.05$). They attributed the increase in carcass yielded of betaine-supplemented group to its osmotic effects and increasing water retention. It is clear from the present results that, betaine improved carcass yielded of either inadequate or adequate methionine containing-diets (Table 4). Similar finding was reported by Wang *et al.* (2004) and Waldroup and Fritts (2005)

However, there was a linear increase in crude protein of meat with each increase in methionine level. The present results agree with those reported by Schutte *et al.* (1997) and Garcia *et al.* (2000) who observed that methionine supplementation significantly increased the proportion of carcass protein. On the other hand, methionine supplementation did not affect chemical composition of meat of broilers (Aggoor, 1998 and Yalcin *et al.*, 1999) and ducks (Attia, 2003).

On the other hand, the decrease in abdominal fat deposition due to methionine supplementation and the increase in it due to betaine supplementation, indicated that methionine is an effective lipotrophic agent where betaine increased energy availability for deposition of fat in only the abdominal cavity (Table 4). Recent results (Kettunen *et al.*, 2001a) indicated that 20% of label methyl donor of betaine was found in the protein fraction, showing the important of betaine for transmethylation and the following use of regenerated methionine for protein synthesis. While, one third of the label from methionine was found in protein fraction showing greater importance of methionine in protein synthesis than betaine. The lack of responses to methionine and/or betaine supplementation on EE of meat, although there was a response in abdominal fat may be due to relative late deposition of intramuscular fat (Koohmaraie *et al.*, 1995 and Frenández *et al.*, 2000).

Results by Schutte *et al.* (1997) and Garcia *et al.* (2000) revealed that betaine supplementation insignificantly increased moisture, EE, and ash of meat. However, crude protein was significantly increased by betaine supplementation, although the response to betaine was saturated at 0.035%. This could be explained by the results of Esteve-Garcia and Mack (2000) who suggested that betaine in the diet for chicks may increase water retention in flesh of carcass. Also, Türker *et al.* (2004) reported results 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. In poultry, the methylation properties of betaine may also be important during lipid metabolism by reducing and redistributing body fat (Saunderson and Mackinlay, 1990). Carcass with less fat could also result if 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).

In accordance with the present results, Tafuri *et al.* (1985) and Sonbol (1991) indicated that increasing dietary methionine level caused higher levels of total protein, albumin, and globulin of blood broilers and this correlated with improved growth. On the other hand, the lack of significant effect of methionine level on serum cholesterol agrees with the results reported by Attia (2003) who showed that increasing dietary methionine level had no significant effect on plasma cholesterol of ducks.

There was an improvement in percentage feather due to methionine and/or betaine supplementation with the response to methionine being stronger (Table 4), indicating that methionine is more effective for feather growth as a metabolic pathway for cystine synthesis. Also, Garcia *et al.* (2000) reported that methionine supplementation was particularly helpful in increasing growth of feather of birds fed low-protein diet. As indicated above, the results obtained herein declared that betaine appears to be helpful in sparing methionine for growth of feathers especially in diets marginally deficient in methionine (Table 4).

The present results confirm the protective effect of methionine and betaine on liver function (Table 3); this may be due to their lipotrophic agent, which could prevent fatty liver. These results are similar to that reported by EL-daly and Mohamed (1999) who found that concentration of serum AST and ALT decrease with increasing the level of supplemental methionine. Liver transaminases, AST and ALT are essential in protein biosynthesis (El- Ansary *et al.*, 1996) and the decrease in their concentration with increasing level of methionine supplementation reflects better liver function.

In conclusion results indicated that slow growing chickens (14.6 g/d) during 1-56 d of age could be fed diet containing 0.42% methionine and 0.75% TSAA. However, when such diet supplemented with 0.07% betaine, methionine level may be decreased to 0.37 or even to 0.32%.

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