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Effect of Dietary Betaine and Folic Acid Supplementation on Performance, Egg Folate Content and Egg Production of Japanese Quail

Research Article

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ABSTRACT

A study was conducted to investigate the effects of different betaine (BET) (0.0, 0.5 or 1.0 g/kg) and folic acid (FA) (0.0, 4.0 or 8.0 mg/kg) levels on performance, egg FA concentration and egg production of laying quails using 288 forty-two day-old Japanese quail in a 3 × 3 factorial arrangement of treatment based on randomized complete design. The results showed that none of the performance traits, except feed intake, was affected by dietary treatments. No effect of FA and BET supplementation was observed for egg specific gravity but egg shape index was affected throughout the experiment. No significant differences were observed for egg white, pH, weight and ratio, but Haugh unit (HU) was affected significantly by FA supplementation ($P<0.01$). High levels of FA supplementation (8 mg/kg) decreased the HU ($P<0.01$). Moreover there was a significant interaction between the FA and BET for HU ($P<0.05$) and increasing the BET level did not changed the HU at the low and medium levels of betaine, while decreased the HU at the highest level of FA (8 mg/kg). No effects of FA or BET were observed for egg yolk pH, egg yolk index and egg yolk percentage, but egg yolk FA content (EYFC) and egg yolk color was affected significantly by FA supplementation ($P<0.01$). EYFC increased from a low of 843.87 µg/kg for birds consuming the basal diet with no added FA, up to a high of 1456.25 µg/kg for birds consuming diets with 8 mg/kg of FA.

KEY WORDS betaine, egg indices, folic acid, laying performance, quail.

INTRODUCTION

Folate or folic acid (FA) is a general name for a group of compounds with a pteroylglutamic acid backbone that have different oxidation states (i.e., folic acid, 5-methyltetrahydrofolate). These components function in one-carbon transfer reactions (Selhub and Rosenberg, 1996; Bagley and Shane, 2005). FA is an essential vitamin that is serving as a cofactor and co-substrate for biological methylation reactions such as those involved in amino acid and nucleic acid synthesis (Bagley and Shane, 2005). Adequate FA status in humans is reported to reduce the risk of neural tube defects in babies (Czeizel and Dudas, 1992; De Wals *et al.* 2007), stroke (Yang *et al.* 2006), certain cancers (Kim, 1999), and inflammatory diseases in adults (Wang *et al.* 2001). Adequate FA nutrition is necessary in human nutrition. Its deficiency results in an increase of plasma homocysteine concentrations, with the latter being linked to an increased risk for cardiovascular (Boushey *et al.* 1995; Refsum *et al.* 1998), Alzheimer (Morris *et al.* 2007), and osteoporosis diseases (Mclean *et al.* 2004). Therefore, enrichment of poultry products can increase this vitamin consumption in human. The enrichment of eggs with FA has been explored as an additional vehicle to provide FA into the human diet. More than 80% of the total FA composition of eggs is 5-methyltetrahydrofolate (5-MTHF) (Selhub and Rosenberg, 1996). Unlike the synthetically derived folic acid (FA), 5-MTHF is a naturally occurring derivative of

FA and therefore unlikely to interfere with normal FA metabolism. Previous studies have shown that dietary FA supplementation can fortify the egg FA. Dietary supplementation by FA can produces the eggs with 45 to 50 µg of dietary FA equivalents or approximately 10% of the recommended dietary allowance for adults (Food and Nutrition Board, 1998). However, further increase of FA level in eggs have not been possible because of saturable processes during intestinal FA absorption which reach a maximum plateau (Said *et al*. 2000; Said, 2004; Inoue *et al*. 2008).

By dietary FA (6.0-12.0 mg/kg) supplementation, Husseiny *et al*. (2008) observed no changes of egg production. Keshavarz (2003) reported that certain manipulations of the combination of methionine, choline, folic acid, and vitamin B12 have the potential to reduce egg weight and improve shell quality without affecting egg production during the latter stages of the egg production cycle.

Betaine (BET) and other methyl group donors such as choline and methionine play an important role in methylation reactions. Natural BET is obtained from several plants and organisms (Boch *et al*. 1994) and it is commonly extracted and purified from beetroot. It has three chemically active methyl groups bound to the nitrogen atom of a glycine molecule and classified as a methyl-ammonia (Kidd *et al*. 1997). BET is considered the readily active methyl-group donor (Kettunen *et al*. 2001). Previous studies suggested that BET might have a methionine sparing effects in non-ruminant animals (Gudev *et al*. 2011). BET has been well established as a labile methyl donor and as an osmoprotectant, which is synthesized via the oxidation of BET aldehyde formed by the irreversible oxidation of choline in the mitochondria of the liver (Figure 1) and renal tissues (Ratriyanto *et al*. 2009).

There is a growing interest to using BET and other methyl group donors in the poultry diets (Farrokhyan *et al*. 2014; Hosseintabar *et al*. 2015; Jafari-Golrokh *et al*. 2016). Supplemental dietary BET improved weight gain and feed conversion in some poultry studies (Mathews and Southern, 2000; Hassan *et al*. 2005) whereas other studies showed minimal or no effect of BET on broilers performance (Zulkifi *et al*. 2004). Addition of 600-ppm BET to laying hen diets has improved the laying performance (Park *et al*. 2006). Under heat stress, addition of BET to the poultry feeds (broilers, turkeys and meat ducks) has improved the performance. Furthermore, BT promotes the intestinal microbes against osmotic variations and improves the microbial fermentation activity, which in turn, may enhance the nutrient digestibility in non-ruminant animals (Ratriyanto *et al*. 2009). The liver is an important site for sulfur amino acid metabolism. Methionine, which is the first-limiting amino acid in commercial poultry diets, is a methyl group donor (transmethylation). Methionine is converted to homocysteine, which lies at the crossroads of sulfur amino acid metabolism.

Formation of cysteine may occur if homocysteine proceeds through the irreversible transsulfuration pathway; alternatively, homocysteine may be converted back to methionine after addition of a methyl group by FA-vitamin B_{12}-dependent methionine synthase (MS) or BET - homocysteine methyltransferase (BHMT).

The methyl group provided by BHMT is derived from betaine, a product of choline oxidation and the methyl provided by (MS) is derived from 5- methyl tetrahydrofolate (Pillai *et al*. 2006).

Transmethylation is very important in amino acids metabolism, so continued evaluation of sulfur amino acid metabolism is an important part of working toward optimum diet formulation and enrichment poultry feed. This study was carried out to establish dietary supplementation of BET and FA will be able to affect the egg FA content, performance and egg production of Japanese quail.

MATERIALS AND METHODS

General

A total of 288 forty-two day-old female quails (*Coturnix coturnix Japonica*) were used in this experiment. The birds were divided into nine treatment groups and 4 replicate (8 birds each) for each treatment. The cage dimensions were 60 × 60 cm, providing 36 cm^2 per bird. Cages were equipped with nipple drinkers and trough feeders. Birds were reared under semi controlled environmental conditions in the experimental room with windows and received additional artificial light to provide 16 hour of light and 8 hour of dark. The birds in different experimental treatments were fed by each experimental diets form week 7 to week 12 of age (for 6 weeks).

Diets

The basal diets were based on corn-soybean meal to cover the nutrients requirements of laying quails (NRC, 1994). Diets were supplemented with BET at levels of 0, 50 or 100 mg/kg and folic acid at levels of 0, 4 or 8 mg/kg for each BET level ingredients. The chemical compositions of the basal diets are shown in Table 1.

Experimental approach

One week before the study, 288 birds (42 day-old healthy female quails) were randomly assigned to 9 treatments, with 4 replicates and 8 birds in each wire cage. At day 49 of age, cages were randomly assigned to receive the dietary treatments. The diets were introduced 7 days before the egg collection period. Experimental diets were fed for a 6-week period.

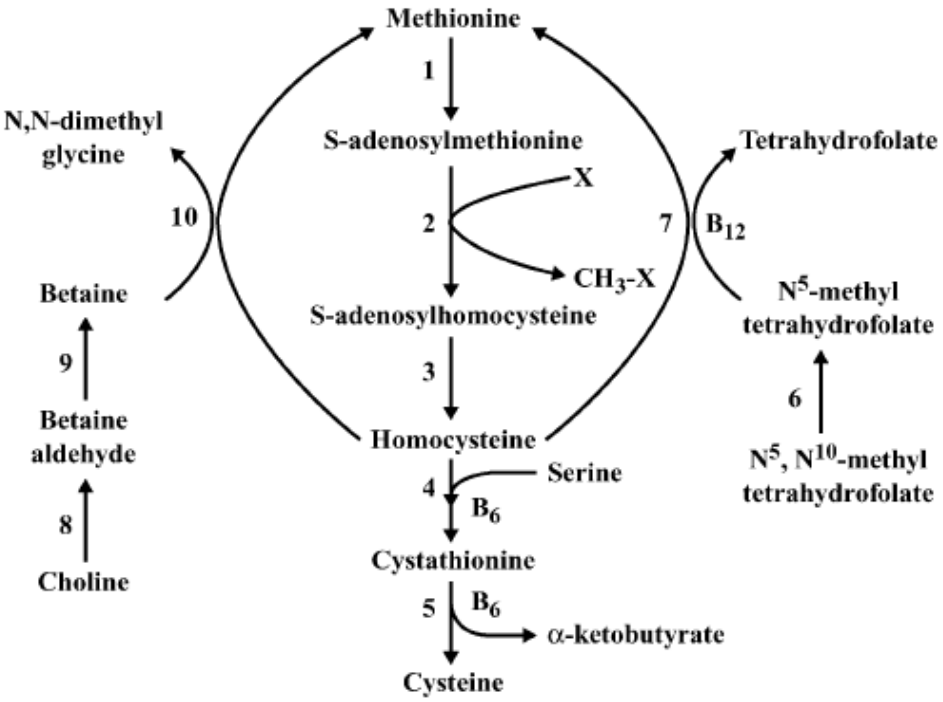

Figure 1 Metabolism of sulfur amino acids, choline and betaine

Feed intake (FI), egg production (EP) and egg mass (EM) for each cage unit were measured weekly and feed conversion ratio (FCR) calculated. FCR was calculated as grams of feed consumed by quails per gram of egg produced. The number of eggs and egg weight (EW) were recorded daily throughout the experiment by using an electronic scale (0.01 g sensitivity) and calculated as percentage hen-day EP. Random samples of three eggs from each treatment were collected weekly to measure the egg quality. Length and width of the egg were measured with Vernier caliper sensitive. Shape Index (SI) was estimated using the following equation:

Egg shape index (ESI)= [egg width / egg length] × 100

Measurements of the internal components were obtained by carefully making an opening around the sharp end of the egg, large enough to allow passage of both the albumen and the yolk through it without mixing their contents together. The yolk was then carefully separated from the albumen and placed in a petri dish for weighting. Simultaneously, associated albumen was placed on another petri dish and weighed.

After each weighing, the petri dishes were washed by clean water and wiped dry before next weighing. The yolk and albumen diameter and albumin height of the egg were measured by Vernier caliper sensitive. The yolk and albumen height were measured by Haugh micrometer.

The yolk and albumen pH were measured by sensitive digital pH Metter. The eggshell weight (ESW) with membrane was obtained by carefully placing the opened part in the shell and weighing on the electronic scale. Eggshell thickness (EST, µm) was determined by measuring the thickness mean values taken at three spots on the egg (air cell, equator, and sharp end) using a dial pipe gauge (Mitutoyo, 0.01-20 mm, Japan). Eggshell ratio (ESR) was estimated from the expression:

ESR (%)= (shell weight/egg weight) × 100.

Shell weight unit surface area (SWUSA) (g/cm2) was determined by dividing the shell weight to shell surface area. Egg yolk index (%) was calculated by the expression:

EYI= [yolk height (cm)/yolk diameter (cm)] × 100
Egg yolk percent (EYP) (%)= 100 × (yolk weight/egg weight)
Egg weight ratio (EWR) (%)= (egg weight weight/egg weight) × 100
Albumen index (%)= albumen height (mm) / average of albumen length and width) mm × 100

The egg yolk color (EYC) was recorded according to the yolk color fans (scores 1-15). Egg specific gravity (ESG) was determined by using the saline flotation method as described by Hempe *et al*. (1998).

Table 1 Composition and calculated analysis of the basal diet

Ingredient %	Amount (%)
Yellow corn (8.5% crude protein (CP))	54.26
Soybean meal (44% crude protein (CP))	34.81
Limestone	5.25
Di-calcium phosphate	1.45
Sodium bicarbonate	0.17
Sodium chloride	0.20
Vegetable oil (8.800 kcal/kg of metabolizable energy (ME))	3.22
DL-methionine	0.15
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
Calculated nutrient composition	
Energy (MJ ME/kg)	12.133
Protein %	20.00
Lysine %	1.00
Methionine %	0.49
Phenylalanine %	0.81
Arginine %	1.29
Histidine %	0.44
Threonine %	0.78
Tryptophan %	0.20
Leucine %	1.51
Valine %	1.01
Linoleic acid %	1.00
Calcium %	2.50
Phosphorus %	0.35
Potassium %	0.41
Sodium %	0.15
Chlorine %	0.14
Potassium %	0.41

[1] Provided (per kg of diet): vitamin A: 8255 IU; vitamin D_3: 3500 IU; vitamin E: 30.0 IU; vitamin K_3 (menadione with sodium bisulfite): 0.55 mg; vitamin B_{12}: 0.011 mg; Riboflavin: 4.50 mg; Pantothenic acid: 8.24 mg; Niacin: 22.55 mg; Choline: 1201.80 mg; Biotin: 0.100 mg; Thiamine (B_1; mononitrate): 2 mg; Pyridoxine (B_6; HCl): 3 mg; DL-methionine: 900 mg and Ethoxyquin (antioxidant): 125 mg.
[2] Provided (per kg of diet): MnO (manganese oxide): 70 mg; ZnO (zinc oxide): 66 mg; $FeSO_4$ (ferrous sulfate): 50 mg; $CuSO_4$ (copper sulfate): 8.8 mg; $NaSeO_3$ (sodium selenite): 0.3 mg; $Ca(IO_3)_2$ premix (calcium iodate premix contained 2.0 g of calcium iodate and 43 g of iodized salt): 0.33 mg and Iodized salt: 0.67 mg.

Determination of dietary FA content

The extraction of the FA-supplemented diets was performed as described by (Wilson and Horne, 1984; Tamura *et al*. 1997). In brief, feed samples were homogenized with a 50 m*M* N-(2-cyclohexylamino) ethanesulfonic acid - HEPES buffer with 2% ascorbic acid and 0.2 M 2-mercaptoethanol (pH 7.8) and stored at −80 ˚C until analysis.

The extracts were analyzed for FA via reverse-phase HPLC 1100 series made by Agilent Company USA with fluorescence detection, using the method of (Vahteristo *et al*. 1997). An external standard curve with purified FA was used to quantify feed FA concentration.

Extraction and analysis of egg yolk FA content (EYFC)

The extraction and analysis of the EYFC FA was performed as described previously (House *et al*. 2002). In brief, eggs were weighed, placed in boiling water for 10 min, cooled, and the yolks were separated, weighed, and retained for analysis by storing at −80 ˚C. Egg FA in the form of 5-MTHF, the major form of FA in eggs, was extracted into an acrobat buffer (pH 7.8).

The extracts were analyzed for 5-MTHF via reverse-phase HPLC 1100 series made by Agilent company USA with fluorescence detection, using the method of Vahteristo *et al*. (1997). An external standard curve with purified 5-MTHF was used to quantify EYFC.

Statistical analysis

A completely randomized design with 3 level of FA supplementation (0, 0.4 and 0.8 mg/kg of crystalline FA) and 3 level of BET (0, 0.5 and 1 g/kg of betaine) supplementation in a 3 × 3 factorial arrangement was used. Data were subjected to ANOVA, using the PROC GLM procedure of SAS software (SAS, 2008). When evidence of heterogeneity of variance was present, data were log-transformed before analysis.

RESULTS AND DISCUSSION

The results of folic acid and BET effect on laying performance and egg indices of quails are shown in Table 2. None of the performance traits (FCR, EP, EW, EM, ESI and ESG) was affected by dietary treatments.

Table 2 The effects of folic acid and betaine on laying performance and egg indices of quails

Folic acid (mg/kg)	Betaine (g/kg)	FI (g)	FCR	EP (hen/day)	EW (g)	EM (g/day)	ESI (%)	ESG (g/cm^3)
	0.0	30.26	2.68	0.90	11.27	1.00	77.24	1.07
0.0	0.5	29.39	2.61	0.89	11.29	9.84	76.79	1.07
	1.0	30.31	2.66	0.87	11.36	9.95	77.43	1.07
	0.0	30.01	2.71	0.85	11.08	9.46	77.52	1.09
4.0	0.5	30.04	2.63	0.88	11.39	9.99	77.46	1.07
	1.0	30.27	2.72	0.88	11.12	9.70	77.79	1.08
	0.0	29.96	2.65	0.88	11.28	9.77	78.36	1.07
8.0	0.5	29.68	2.66	0.87	11.16	9.56	78.06	1.07
	1.0	30.10	2.68	0.88	11.23	9.82	77.52	1.07
SEM		0.26	0.03	0.02	0.18	0.33	0.33	0.00
Main effects								
Folic acid (mg/kg)								
0.0		29.99	2.65	0.89	11.31	9.93	77.15	1.07
4.0		30.11	2.69	0.87	11.20	9.72	77.59	1.08
8.0		29.91	2.66	0.88	11.22	9.72	77.98	1.07
SEM		0.15	0.01	0.01	0.10	0.19		0.00
Betaine (g/kg)								
0.0		29.70	2.68	0.88	11.21	9.74	77.71	1.08
0.5		30.08	2.63	0.88	11.28	9.80	77.58	1.07
1.0		30.23	2.69	0.88	11.24	9.83	77.44	1.08
SEM		0.152	0.01	0.01	0.10	0.19	0.33	0.00
Source of variation					**P-value**			
Folic acid effect		0.66	0.36	0.69	0.73	0.59	0.23	0.18
Betaine effect		0.059	0.09	0.96	0.90	0.95	0.85	0.48
Folic acid × betaine effect		0.51	0.67	0.98	0.78	0.68	0.82	0.56

FI: feed intake; FCR: feed conversation rate; EP: egg production (hen/day); EW: egg weight and EM: egg mass; ESI: egg shape index and ESG: egg special gravity.
SEM: standard error of the means.

The FI had a trend and was numerically increased by dietary BET (P=0.059).

Although there were no significant difference between the treatments for FI but BET fed birds had the higher FI at any levels of folic acid.

There were no significant differences between the treatments for feed conversation ratio, egg production percent, egg weight and egg mass throughout the study period (P>0.05).

No changes of eggshell indices was observed by dietary supplementation of FA and BET (Table 3). EST decreased numerically (P=0.07) by increasing FA supplementation level. There were no difference between the treatments for ESW, ESR and SWUSA. No significant differences were observed for EW pH and EWR, but HU was affected significantly by folic acid supplementation (P<0.01). High level of FA supplementation (8 mg/kg) decreased the HU. Moreover there was a significant interaction between the FA and BET for HU (P<0.05) and increasing the BET level did not changed the HU at the low and medium levels of betaine, while decreased the HU at the highest level of FA (8 mg/kg).

No effects of FA or BET were observed for quail EY pH, EYI and EYP, but EYFC and egg yolk color (EYC) were affected significantly by folic acid supplementation (Table 4) (P<0.01).

EYFC increased from a low level of 843.87 µg/kg for birds consuming the basal diet with no added folic acid, up to a high level of 1456.25 µg/kg for birds consuming diets with 4 mg/kg of folic acid.

Performance and egg indices

The earlier studies (Keshavarz, 2003; Turker *et al*. 2004; Zhan *et al*. 2006) have reported that BET and any methyl group donors containing-diets significantly improves the EW and FCR in broilers and laying hens because of their role in sulfur amino acids metabolism as a methyl group donors (Kyeong Seon *et al*. 1955). The results of our study showed that addition of supplemental FA and BET to laying quail diets did not affect the performance, as reflected by insignificant differences in FI (g), FCR, EP (hen/day), EW (g) and EM (g).

In the present study, the vitamins contained in the basal diet possibly have met the quail requirements (NRC, 1994), and thus this diet was sufficient for normal EP. Accordingly, Husseiny *et al*. (2008) indicated no effects of feeding supplemented diets with FA (6.0-12.0 mg/kg) on egg production. In a different way, some previous studies (House *et al*. 2002; Hebert *et al*. 2005) have been reported that supplemental FA to laying hen diets significantly increased the FI. Keshavarz (2003) found that reducing dietary FA resulted in decreased EW.

Table 3 The effects of folic acid and betaine supplementation on egg shell and white indices

Folic acid (mg/kg)	Betaine (g/kg)	EST (µ m)	ESW (g)	ESR (%)	SWUSA (g/cm^2)	EW pH	EWW (g)	EWR (%)	HU (mm)
	0.0	1.50	1.03	**8.63**	0.46	9.11	7.33	61.61	94.73^{ab}
0.0	0.5	1.82	1.04	8.66	0.47	9.14	7.31	61.62	94.48^{ab}
	1.0	1.41	1.04	8.64	0.47	9.13	7.32	60.67	95.25^{ab}
	0.0	1.40	1.05	8.74	0.47	9.11	7.19	59.72	94.82^{ab}
4.0	0.5	1.31	1.01	8.40	0.47	9.13	7.51	62.52	95.81^{a}
	1.0	1.49	1.03	8.65	0.46	9.14	9.95	55.88	65.16^{ab}
	0.0	1.37	0.99	8.48	0.46	9.10	7.09	61.21	93.90^{b}
8.0	0.5	1.33	1.00	8.59	0.46	9.11	7.18	61.74	94.51^{ab}
	1.0	1.31	1.04	8.66	0.47	9.14	7.35	60.70	92.21^{c}
SEM		0.13	0.16	0.11	0.003	0.02	0.14	1.69	0.52
Main effects									
Folic acid (mg/kg)									
0.0		1.58	1.04	8.66	0.47	9.13	7.32	61.30	95.26^{a}
4.0		1.40	1.03	8.53	0.47	9.12	7.21	59.37	94.94^{a}
8.0		1.34	1.01	8.58	0.46	9.12	7.21	61.22	93.54^{b}
SEM		0.07	0.00	0.32	0.001	0.01	0.08	0.97	0.30
Betaine (g/kg)									
0.0		1.42	1.02	8.62	0.47	9.11	7.20	60.58	94.48
0.5		1.49	1.02	8.55	0.47	9.12	7.33	61.94	94.94
1.0		1.40	1.04	8.59	0.46	9.14	7.21	59.08	94.21
SEM		0.07	0.00	0.32	0.001	1.01	0.08	0.97	0.30
Source of variation						**P-value**			
Folic acid		0.07	0.14	0.38	0.59	0.85	0.56	0.29	0.0002
betaine		0.72	0.33	0.76	0.98	0.31	0.45	0.11	0.23
Folic acid × betaine		0.21	0.19	0.19	0.23	0.97	1.10	0.44	0.04

EST: egg shell thickness; ESW: egg shell weight; ESR: egg shell ratio; SWUSA: shell weight unit surface area; EWpH: egg white pH; EWW: egg white weight; HU: Haugh unit and EWR: egg white ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The lack of changes in performance indices of recent study by BET was in agreement with the results of Waldroup and Fritts (2005) who reported that 100 to 500 mg/kg BET supplementation had not affected the male broilers performance. Enting *et al*. (2007) showed that dietary addition of 1.0 and 2.0 g/kg BET improved the FCR during 0 to 14 days of age. Moreover, Honarbakhsh *et al*. (2007) evaluated the effects of different exogenous BET levels (0.0, 0.075, 0.150 and 0.225%) on broiler chickens (Ross) and observed the increased body weight, FI and improved FCR during the grower and finisher periods. In addition, Sayed and Downing (2011) found that addition of BET at an inclusion rate of 500 mg/L in drinking water improved the body weight (BW) of broiler chickens under heat stress. Tollba *et al*. (2007) showed that dietary supplemental BET for 12 or 16 weeks to Fayoumi laying hens improved FCR and body weight (BW) without significant effect on FI. Baghaei *et al*. (2009) and Maghoul *et al*. (2009) have found that 10, 20 and 30% BET replacement for choline had no effect on FI and weight gain (WG), FCR of broilers. Jahanian and Rahmani (2008) found that dietary BET inclusion (0.0, 50 and 100% in substitution for choline) had no effect on FI. In this respect, BET supplementation (800 mg/kg diet) enhanced growth at 21 days and FCR at 42 days of age in broilers (Rao *et al*. 2011).

BET significantly improved BW and FCR of Matrouh poultry strain at a level of 1.0 g/kg diet (Ezzat *et al*. 2011). Some other researches showed the improved FCR by dietary BET supplementation (Tollba *et al*. 2007; Enting *et al*. 2007; Honarbakhsh *et al*. 2007).

The result of current experiment regarding the EW is in agreement with that of Hebert *et al*. (2005) who researched the supplementation of 2.0, 4.0, 8.0, 16, 32, 64 and 128 mg/kg of FA in 2 Leghorn strains of laying hens and observed no difference in EW during a 3-wk experimental period. Tactacan *et al*. (2011) noted no effect on EW when Shaver White laying hens were fed diets supplemented with 10 or 100 mg of FA/kg diet. Khalifah and Shahein (2006) determined that the inclusion of 0.0 to 32 mg FA/kg in the Baheij chicken strain did not affected the EW. Bunchasak and Kachana (2009) suggested that FA (0.31 to 10.31 mg/kg diet) added to a corn-soy based diet had no effect on EP of older laying hens (64-72 weeks of age). Our results are in disagreement with those obtained by those of Tollba and El-negar (2008) who observed that increasing BET supplementation (2000 ppm/kg) to the diet caused the higher EP and EM. In addition, Gudev *et al*. (2011) reported that EP and EM were significantly improved when BET supplemented to laying diets up to 1.5 g/kg for hens during heat stress.

Table 4 The effect of folic acid and betaine supplementation on egg yolk indices

Folic acid (mg/kg)	Betaine (g/kg)	EYpH	EYW (gr)	EYP (%)	EYI (%)	EYC	EYFC (µg/100 g)
	0.0	9.11	3.66	**30.74**	45.41	4.00	896.05
0.0	0.5	9.14	3.72	30.76	43.05	3.97	919.52
	1	9.13	3.70	30.65	44.53	4.12	843.87
	0	9.11	3.66	30.18	41.47	4.16	752.92
4.0	0.5	9.13	3.62	29.80	45.73	4.08	1033.55
	1	9.14	3.79	35.21	43.07	4.20	1022.45
	0	9.10	3.63	31.00	42.87	3.89	1152.37
8.0	0.5	9.11	3.55	30.24	42.57	3.81	1456.25
	1	9.14	3.73	30.68	41.47	3.89	1245.67
SEM		0.02	0.08	1.29	1.21	0.11	84.55
Main effects							
Folic acid (mg/kg)							
0.0		9.13	3.69	30.63	44.33	4.15^{a}	886.48^{b}
4.0		9.12	3.69	31.73	43.42	4.03^{ab}	1022.98^{b}
8.0		9.12	3.63	30.64	42.30	3.86^{b}	1284.77^{a}
SEM		0.01	0.51	0.74	0.70	0.06	48.81
Betaine (g/kg)							
0.0		9.11	3.65	30.55	43.25	4.02	1000.00
0.5		9.12	3.63	30.27	43.78	3.95	1136.00
1.0		9.14	3.74	32.18	43.02	4.07	1037
SEM		0.01	0.51	0.74	0.70	0.06	48.81
Source of variation				**P-value**			
Folic acid		0.85	0.66	0.49	0.13	0.01	0.001
betaine		0.31	0.27	0.15	0.73	0.48	0.14
Folic acid × betaine		0.97	0.70	0.13	0.09	0.98	0.48

EYpH: egg yolk pH; EYW: egg yolk weight; EYP: egg yolk percent; EYI: egg yolk index egg yolk color and EYFC: egg yolk folate content.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Supplementation of FA showed no effect on ESI in recent experiment. The results agree with Ezzat *et al.* (2011) who reported that Matrouh poultry strains egg shape index was not affected by FA (1.0 mg/kg diet) supplementation under Egyptian summer condition.

Eseceli *et al.* (2010) investigated the effect of FA inclusion (10 mg/ton diet) to the ration of laying hens and found that supplemental folic acid did not have any significant effect on ESI and ESG. Benkova *et al.* (2009) increased the dietary content of FA from 0.5 mg/kg to 3.5 mg/kg of feed in laying hens and did not observed any significant effect on ESI.

Egg shell and white indices

No effects of FA or BET were observed on ESW, ESR, EST and SWUSA in recent experiment. The EST decreased from 1.58 µm for control to 1.40 µm and 1.34 µm by increasing the dietary FA levels to 4.0 and 8.0 mg/kg, respectively.

The results of this study are in agreement with those of Husseiny *et al.* (2008) that reported the egg quality parameters did not changed by FA supplementation. Similar results were observed for EST by Hebert *et al.* (2005) and Khalifah and Shahein (2006).

The results of current experiment showed that EW pH, egg white weight (EWW) and egg white ratio (EWR) did not affected by FA and BET supplementation. There was no interaction between FA and BET for HU. However, HU decreased from 95.26 mm for control to 94.94 mm and 95.54 mm for 4.0 and 8.0 mg/kg FA supplemented treatments, respectively. The result of this study are in disagreement with Ezzat *et al.* (2011) that showed that supplementation of Matrouh poultry strain diets by BET (1.0 g/kg diet) and FA (1.0 mg/kg diet) together from 24-36 weeks of age under Egyptian hot summer significantly increased the HU. Gudev *et al.* (2011) determined the effect of 0.7 and 1.5 g/kg supplemental BET on egg performance of 2-year-old laying hens. The results of their study indicated no changes in EYI, EYK and HU. Gudev *et al.* (2011) supplemented basal diets of 2-year-old laying hens with 0.7 and 1.5 g/kg BET and detected no changes for egg quality (albumen weight, albumen index, egg yolk weight (EYW), EYI, EYC, HU, ESG).

Egg yolk indices

EY pH, EYW, EYP and EYI were not affected by BET or FA supplementation in current experiment. Supplementing FA at 4.0 and 8.0 mg/kg to the diet decreased the EYC.

Results of this study are in agreement with those of Benkova *et al*. (2009) who fed laying hens with diets supplemented with 1.5 to 3.5 mg of FA/kg and indicated higher yolk color by dietary supplementation. In an opposite way, Hassanien *et al*. (2010) showed no effects of dietary FA on BW, EP, FI, FCR, EM, EW, EYC, ESI and EYI of laying hens.

Moreover, EYFC increased by supplementing FA at 4.0 and 8.0 mg/kg in our experiment and increased form 886.48 for control to 1002.98 and 1284.77 for 4.0 and 8.0 mg/kg FA, respectively. The results of the present study for EYFC are in agreement with the findings of some other researchers (Sherwood *et al*. 1993; House *et al*. 2002; Hebert *et al*. 2005; Hoey *et al*. 2009; Tactacan *et al*. 2010) that showed the possibility of higher FA content in animal products by synthetic folic acid. Our current study regarding the production of enriched quail eggs by FA supports the previous findings in laying hens by feeding the supplemented dietary FA (Sherwood *et al*. 1993; House *et al*. 2002; Hebert *et al*. 2005; Tactacan *et al*. 2010). The present studies provide strong evidence of the sensitivity of quail egg FA content to dietary FA levels. The results of this study are in disagreement by House *et al*. (2002) and Hebert *et al*. (2005) that reported previously, the addition of FA above 4.0 mg/kg in the diet of laying hens yielded no further significant increase in egg FA content. On the other hand, supplementation of 4.0 mg of FA/kg of diet yielded 90% maximal egg FA enrichment. In a disagreement way, House *et al*. (2002) and Sherwood *et al*. (1993) reported that egg FA concentrations responded to increasing levels of dietary FA supplementation and maximal egg FA levels were achieved when dietary FA concentrations was 8.0 mg/kg of diet. House *et al*. (2002) observed that egg FA concentrations increased above a plateau value when the level of FA in the diet was 32 mg/kg of diet.

Plasma serves as the precursor pool for egg yolk deposition. Miller and White (1986) have identified the existence of a binding protein-mediated mechanism for the transfer of riboflavin into the egg yolk and there may be a similar mechanism for FA. Plasma FA concentrations may serve as the limiting factor for egg FA concentrations (Sherwood *et al*. 1993), as both compartments appear to saturate at dietary FA concentrations between 2.0 and 4.0 mg/kg of diet. Plasma FA concentrations are higher in birds consuming 128 mg of folic acid/kg diet than those consuming 4 mg/kg; however, this increase is not sufficient to translate into higher egg FA concentrations.

Therefore, a greater understanding of the factors regulating the absorption of dietary FA and its appearance in the systemic circulation in the form of 5-methyltetrahydrofolate is needed.

In studies with rats and *in vitro* intestinal cell model systems, FA is absorbed from the gut via a membrane-bound FA transport system that accepts both oxidized and reduced forms of the monoglutamated forms of FA has been shown to be saturable in a number of model systems and as such represents a potential control point for plasma FA concentrations (Said, 2004).

However, the 5- methyltetrahydrofolate form is believed to be the primary circulating form of FA in the plasma. In the enterocyte, FA is reduced via a 2-step process involving the enzyme dihydrofolate reductase to yield tetrahydrofolate (Henderson, 1990). Tetrahydrofolate is then transported to the liver, where it is converted to 5-methyltetrahydrofolate form. Attachment to binding proteins within hepatocytes and transport via the systemic circulation is the putative mechanism whereby FA is transported to the developing yolk (Henderson, 1990). Therefore, multiple control points exist to regulate plasma FA levels, and further studies are required to elucidate the key control point (s), especially in quails.

5-methyltetrahydrofolate participates in a reaction involving the FA-dependent remethylation of the sulfur AA homocysteine to yield methionine. Increasing the crystalline FA level from the 0.0 to 8.0 mg/kg in the basal diet resulted in a significant reduction in plasma homocysteine concentrations in the laying quails of current experiment. This reduction may indicate that the Japanese quail have a higher requirement for FA as compared to the other hens and, indeed, a higher requirement than that given by NRC (1994).

CONCLUSION

In conclusion, dietary supplementation of betaine and FA have no effects on egg yolk pH, index and percent, but EYFC and color. Supplementation of 8 mg/kg FA decreases the Haugh unit, but enriches the EYFC from 886.48 to 1284.77 µg/100 g (1.45 times).

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REFERENCES

Baghaei M.A., Ashayerizadeh M., Eslami M., Bojarpour H., Roshanfekr H. and Mirzadeh K.H. (2009). Betaine (Betafin-Reg) replacement for methionine in diet on growth performance and carcass characteristics of broiler chickens. *Res. J. Biol. Sci*. **4,** 1037-1040.

Bagley P. and Shane B. (2005). Encyclopedia of Dietary Supplements. Academic Press, London, United Kingdom.

Benkova J., baumgarner J., monlar F. and peskovicova D. (2009). Effect of folic acid addition into feed mixture of laying hens on its content in eggs. *Slovak J. Anim. Sci*. **42,** 124-128.

Boch J., Kempf B. and Bremer E. (1994). Osmoregulation in bacillus subtilis: Synthesis of the osmoprotectent glycine betaine from exogenously provided choline. *J. Bacteriol*. **176,** 5371-5634.

Boushey C.J., Beresford S.A., Omenn G.S. and Motulsky A.G. (1995). A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *J. American Med. Assoc*. **274,** 1049-1057.

Bunchasak C. and Kachana S. (2009). Dietary folate and vitamin B12 supplementation and consequent vitamin deposition in chicken eggs. *Trop. Anim. Health Prod*. **41,** 1583-1589.

Czeizel A.E. and Dudas I. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *New England J. Med*. **327,** 1832-1835.

De Wals P., Tairou F., Van Allen M.I., Uh S., Lowry R.B., Sibbald B., Evans J.A., Vanden Hof M.C., Zimmer P., Crowley M., Fernandez B., Lee N.S. and Niyonsenga T. (2007). Reduction in neural tube defects after folic acid fortification in Canada. *New England J. Med*. **357,** 135-142.

Enting H., Eissen J., Mozos J., Los D., Alamo A.G. and Ayala P.P. (2007). TNI betaine improves broiler chicken performance and carcass quality under heat stress conditions. Pp. 297-300 in Proc. 16th European Symp. Poult. Nutr. Strasbourg, France.

Eseceli H., Degirmencioglu N. and Bilgic M. (2010). The effect of inclusion of chromium yeast (co-factor II, Alltech Inc) and folic acid to the ration of laying hens on performance, egg quality, egg yolk cholesterol, folic acid and chromium levels. *J. Anim. Vet. Adv*. **9,** 184-391.

Ezzat W., Shoeib M.S., Mousa S.M.M., Baelish A.M.A. and Ibrahiem Zenato A. (2011). Impact of betaine, vitamin D and folic acid supplementation to the diet on productive and reproductive performance of Matrouh poultry strain under Egyptian summer condition. *Egypt Poult. Sci*. **31,** 521-537.

Farrokhyan P., Bouyeh M., Lartey F. and Seidavi A.R. (2014). The effects of dietary L-carnitine and gemfibrozil on performance, carcass characteristics, cholesterol and triglycerides in broiler chicks. *Avian Biol. Res*. **7(3),** 160-166.

Food and Nutrition Board. (1998). Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. National Academy Press, Washington, DC., USA.

Gudev D., Popova-Ralcheva S., Yanchev I., Moneva P., Petkov E. and Ignatova M. (2011). Effect of betaine on egg performance and some blood constituents in laying hens reared indoor under natural summer temperatures and varying levels of air ammonia. *Bulgarian J. Agric. Sci*. **17,** 859-866.

Hassanien H.H.M., Ismaeill Z.S.H. and Yakout H.M. (2010). Effect of dietary folic acid supplementation on laying hens productive performance and immunity. *Egypt Poult. Sci*. **31,** 713-729.

Hassan R.A., Attia Y.A. and El-Ganzory E.H. (2005). Growth, carcass quality and serum constituents of slow growing chicks as affected by betaine addition to diets containing different levels of choline. *Int. J. Poult. Sci*. **4,** 840-850.

Hebert K., House J.D. and Guenter W. (2005). Effect of dietary folic acid supplementation on egg folate content and the performance and folate status of two strains of laying hens. *Poult. Sci*. **84,** 1533-1538.

Hempe J.M., Lauxen R.C. and Savage J.E. (1998). Rapid determination of egg weight and spesific gravity using a computerized data collection system. *Poult. Sci*. **67,** 902-907.

Henderson G.B. (1990). Folate-binding proteins. *Ann. Rev. Nutr*. **10,** 319-335.

Hoey L., Mc Nulty L.H., Mc Cann E.M.E., Mc Crackern K.J., Scott J.M., Marc B.B., Molloy A.M., Graham C. and Pentieva K. (2009). Laying hens can convert high doses of folic acid added to the feed into natural folates in eggs providing a novel source of food folate. *British J. Nutr*. **101,** 206-212.

Honarbakhsh S., Zaghari M. and Shivazad M. (2007). Can exogenous betaine be an effective osmolyte in broiler chicks under water salinity stress? *Asian-Australasian J. Anim. Sci*. **20,** 1729-1737.

Hosseintabar B., Dadashbeiki M., Bouyeh M., Seidavi A.R., van den Hoven R. and Gamboa S. (2015). Effect of different levels of L-carnitine and lysine-methionine on broiler blood parameters. *Rev. MVZ Cordoba*. **20(3),** 4698-4708.

House J.D., Braun K., Ballance D.M., O'Connor C.P. and Guenter W. (2002). The enrichment of eggs with folic acid through Supplementation of the laying hen diet. *Poult. Sci*. **81,** 1332-1337.

Husseiny O.M., Soliman A.Z., Omara I.I. and El-Sherif H.M.R. (2008). Evaluation of dietary methionine, folic acid and cyanocobalamine (B) and their interactions in laying hen performance. *Int. J. Poult. Sci*. **7,** 461-469.

Inoue K., Nakai Y., Ueda S., Kamigaso S., Ohta K., Hatakeyama M., Hayashi Y., Otagiri M. and Yuasa H. (2008). Functional characterization of PCFT/HCP1 as the molecular entity of the carrier-mediated intestinal folate transport system in the rat model. *American J. Physiol. Gastrointest. Liver Physiol*. **294,** 660-668.

Jafari-Golrokh A.R., Bouyeh M., Seidavi A.R., Van Den Hoven R., Laudadio V. and Tufarelli V. (2016). Effect of different dietary levels of atorvastatin and L-carnitine on performance, carcass characteristics and plasma constitutes of broiler chickens. *J. Poult. Sci*. **53(3),** 201-207.

Jahanian R. and Rahmani H.R. (2008). The effect of dietary fat level on the response of broiler chicks to betaine and choline supplements. *J. Biol. Sci*. **8,** 362-367.

Keshavarz K. (2003). Effects of reducing dietary protein, methionine, choline, folic acid, and vitamin B_{12} during the late stages of the egg production cycle on performance and eggshell quality. *Poult. Sci*. **82,** 1407-1414.

Kettunen H., Tiihonen K., Peuranen S., Saarinen M.T. and Remus J.C. (2001). Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. *Comp. Biochem. Physiol. Part A: Mol. Int. Physiol*. **130,** 759-769.

Khalifah M.M. and Shahein E.H.A. (2006). Effect of dietary folic acid supplementation on production and hatching performance in baheij chicken strain. *Egyptian Poult. Sci. J*. **26,** 843-855.

Kyeong Seon R., Kevin D.R., Gene M.P. and Ronald R.E. (1995). The folic acid requirements of starting broiler chicks fed diets based on practical ingredient. 1. Interrelationships with dietary choline. *Poult. Sci*. **74,** 1447-1455.

Kidd M.T., Ferket P.R. and Garlich J.D. (1997). Nutritional and osmoregulatory functions of betaine. *Worlds Poult. Sci*. **53,** 125-139.

Kim Y.I. (1999). Folate and carcinogenesis: Evidence, mechanisms, and implications. *J. Nutr. Biochem*. **10,** 66-88.

Maghoul M.A., Moghadam H.N., Kermanshahi H. and Mesgaran M.D. (2009). The effect of different levels of choline and betaine on broilers performance and carcass characteristics. *J. Anim. Vet. Adv*. **8,** 125-128.

Mathews J.O. and Southern L.L. (2000). The effect of dietary betaine in *Eimeria acervulina*-infected chicks. *Poult. Sci*. **79,** 60-65.

Miller M.S. and White H.B. (1986). Isolation of avian riboflavin-binding protein. *Methods Enzymol*. **122,** 227-234.

Mc Lean R.R., Jacques P.F., Selhub J., Tucker K.L., Samelson E.J., Broe K.E., Hannan M.T., Cupples L.A. and Kiel D.P. (2004). Homocysteine as a predictive factor for hip fracture in older persons. *New England J. Med*. **350,** 2042-2049.

Morris M.S., Jacques P.F., Rosenberg I.H. and Selhub J. (2007). Folate and vitamin B_{12} status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *American J. Clin. Nutr*. **85,** 193-200.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Park J.H., Kang C.W. and Ryu K.S. (2006). Effects of feeding betaine on performance and blood hormone in laying hens. *Korean J. Poult. Sci*. **33,** 323-328.

Pillai P.B., Fanatico A.C., Beers K.W., Blair M.E. and Emmert J.L. (2006). Homocysteine remethylation in young broilers fed varying levels of methionine, choline, and betaine. *Poult. Sci*. **85,** 90-95.

Rao S.V., Raju M.V., Panda A.K., Poonam S. and Sunder G.S. (2011). Effect of supplementing betaine on performance, carcass traits and immune responses in broiler chicken fed diets containing different concentrations of methionine. *Asian-Australasian J. Anim. Sci*. **24,** 662-669.

Ratriyanto A., Mosenthin R., Bauer E. and Eklund M. (2009). Metabolic, osmoregulatory and nutritional functions of betaine in monogastric animals. *J. Anim. Sci*. **22,** 1461-1476.

Refsum H., Ueland P.M., Nygard O. and Vollset S.E. (1998). Homocysteine and cardiovascular disease. *Ann. Rev. Med*. **49,** 31-62.

Said H.M. (2004). Recent advances in carrier-mediated intestinal absorption of water-soluble vitamins. *Ann. Rev. Physiol*. **66,** 419-446.

Said H.M., Chatterjee N., Haq R.U., Subramanian V.S., Ortiz A., Matherly L.H., Sirotnak F.M., Halsted C. and Rubin S.A. (2000). Adaptive regulation of intestinal folate uptake: Effect of dietary folate deficiency. *American J. Physio. Cell Physiol*. **279,** 1889-1895.

SAS Institute. (2008). SAS®/STAT Software, Release 9.2. SAS Institute, Inc., Cary, NC. USA.

Sayed M.A.M. and Downing J. (2011). The effects of water replacement by oral rehydration fluids with or without betaine supplementation on performance, acid- base balance, and water retention of heat-stressed broiler chickens. *Poult. Sci*. **90,** 157-167.

Selhub J. and Rosenberg I.H. (1996). Folic acid. Pp. 206–219 in Present Knowledge in Nutrition. E.E. Ziegler and L.J. Filer, Eds. ILSI Press, Washington, DC., USA.

Sherwood T.A.R.L., Saylor W.W. and White H.B.I.I.I. (1993). Folate metabolism and deposition in eggs by laying hens. *Arch. Biochem. Biophys*. **307,** 66-72.

Tactacan G.B., Jing M., Thiessen S., Rodriguez-Lecompte J.C., O'Connor D.L., Guenter W. and House J.D. (2010). Characterization of folate-dependent enzymes and indices of folate status in laying hens supplemented with folic acid or 5-methyltetrahydrofolate. *Poult. Sci*. **89,** 688-696.

Tactacan G.B., Rodriguez-Lecompte J.C., Karmin O. and House J.D. (2011). The adaptive transport of folic acid in the intestine of laying hens with increased supplementation of dietary folic acid. *Poult. Sci*. **91,** 121-128.

Tamura T., Mizuno Y., Johnston K.E. and Jacob R.A. (1997). Food folate assay with protease, á-amylase, and folate conjugase treatments. *J. Agric. Food Chem*. **45,** 135-139.

Tollba A.A.H., Shabaan S.A.M. and Wagdy A.Z. (2007). Improvement of Fayoumi laying hens performance under hot climate conditions: 2 - Betaine, folic acid and choline. *Egyptian Poult. Sci. J*. **27,** 21-35.

Tollba A.A.H. and El-Nagar A.H.I. (2008). Increasing stocking density of Egyptian laying hens by using: 3–increasing protein level and betaine supplementation. *Egyptian Poult. Sci. J*. **28,** 745-766.

Turker M., Alp M. and Kocabacli N. (2004). Performance of broiler chicks fed on methionine diets supplemented with betaine. Pp. 8-13 in Proc. 17th Poult. Congr. Istanbul, Turkey.

Vahteristo L.T., Ollilainen V. and Varo P.J. (1997). Liquid chromatographic determination of folate monoglutamates in fish meat, egg, and dairy products consumed in Finland. *J. AOAC Int*. **80,** 373-378.

Waldroup P.W. and Fritts C.A. (2005). Evaluation of separate and combined effects of choline and betaine in diets for male broilers. *Poult. Sci*. **4,** 442-448.

Wang H.X., Wahlin A., Basun H., Fastbom J., Winblad B. and Fratiglioni L. (2001). Vitamin B_{12} and folate in relation to the development of Alzheimer's disease. *Neurology*. **56,** 1188-1194.

Wilson S.D. and Horne D.W. (1984). High-performance liquid chromatographic determination of the distribution of naturally occurring folic acid derivatives in rat liver. Ann. Biochem. Rev. **142,** 529-535.

Yang Q.L., Botto L.D., Erickson J.D., Berry R.J., Sambell C., Johansen H. and Friedman J.M. (2006). Improvement in stroke mortality in Canada and the United States, 1990 to 2002. *Circulation*. **113,** 1335-1343.

Zhan X.A., Li J.X., Xu Z.R. and Zhao R.Q. (2006). Effects of methionine and betaine supplementation on growth perform-

ance, carcase composition and metabolism of lipids in male broilers. *British Poult. Sci*. **47,** 576-580.

Zulkifi I., Mysahra S.A. and Jin L.Z. (2004). Dietary supplementation of betaine (betafin [R]) and response to high temperature stress in male broiler chickens. *Asian-Australasian J. Anim. Sci*. **17,** 244-249.