Poultry Science

Betaine as an alternative feed additive to choline and its effect on performance, blood parameters, and egg quality in laying hens rations

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ABSTRACT This research aimed to evaluate how using betaine levels as a choline substitute affects productive performance, egg quality parameters**,** fatty acids profile, and antioxidant status in laying hens. One hundred and forty brown chickens, 45 wk old, were divided into 4 groups, each group of 7 replicates with 5 chickens per replicate. The first group of diets with choline has control (A) 100% choline, the second group (B) 75% choline + 25% betaine, the third group (C) 50% choline + 50% betaine, and the fourth group (D) received 100% betaine. No significant effects were observed in final body weight (**BW**), body weight gain (**BWG**), egg production (**EW**), and feed intake (**FI**) for laying hens. In the diet in which betaine was replaced choline, egg mass (**EM**) and egg weight (**EW**) increased compared to the control group ($P < 0.05$). Also, after 12 wk of feeding, the egg quality parameters were not influenced; however, yolk color was increased significantly compared with the control group. Serum total cholesterol, **LDL**-lipoprotein, HDL-lipoprotein, triglyceride, glucose, aspartate transaminase (AST), and alanine transaminase (**ALT**) were not affected by replacing choline with betaine. Furthermore, liver malondialdehyde (**MDA**) content, yolk vitamin E, and fatty acid levels were not significantly affected by replacing choline with betaine. Moreover, hens fed betaine displayed an increased antibody titer of the Newcastle disease (**ND**) virus. EW and EM were increased by 3.50% and 5.43% in 100% betaine group (D) when compared to the control group. Isthmus weight was decreased by 48.28 % in 50% choline + 50% betaine group (C) when compared to the control group. ND was increased by 26.24% in 100% betaine group when compared to the control group. In conclusion, betaine supplementation positively affected productive performance, egg quality measurements, and immunity response in Bovans brown laying hens.

Key words: betaine, choline, performance, blood parameter, laying hen

2023 Poultry Science 102:102710
https://doi.org/10.1016/j.psj.2023.102710

INTRODUCTION

By optimizing the use of raw materials, reducing production costs, and minimizing pollution by nitrogen and phosphorus caused by the environment, the formulation of diets that closely meet ideal requirements is a crucial technique to tackle these issues (Wang et al., 2004; Chen et al., 2022). The exact amino acid (**AA**) requirements vary based on breed, age, feeding procedures, housing conditions, and the accuracy with which requirements have been determined. The required intakes of AA and energy, which are interdependent, have also changed due to genetic developments and improved laying hen performance (Liu and Selle et al., 2017; Arif et al., 2022). Adding lysine and choline to the diet of laying hens may enhance the health of the immune system and digestive tract (Kidd, 2004; Vaezi et al., 2011; Abd El-Ghany and Babazadeh 2022). Additionally, it has been demonstrated that laying hens' egg production, egg

Received March 16, 2023.
Accepted April 9, 2023.

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weight, egg mass, and feed conversion efficiency can all be improved by increasing dietary lysine (Faria et al., 2002; Kakhki et al., 2016). Due to their low availability in protein sources of plant origin, methionine, cysteine, and choline, which are generally known as the total sulfur-containing AA, are the first limiting AA in experimental poultry diets (Kakhki et al., 2016). Since it is needed for cell metabolism as a methyl donor, methionine contributes to synthesize coenzymes and precursors of cysteine, methionine and choline and receives more attention than cysteine in birds (Bunchasak, 2009; Saeed et al., 2017). For feathering, high levels of methionine-1-cysteine are required for protein synthesis (Fisher et al., 1981). Additionally, the high levels of total sulfur-containing AA were required for increasing egg mass and feed conversion ratio (**FCR**) (Narváez-Solarte et al., 2005). Acetylcholine, a neurotransmitter, and the 2 main phospholipids (phosphatidylcholine [**PC**] and sphingomyelin) occurring in membranes include choline as a source of labile methyl groups (Zeisel and Blusztajn, 1994). Moreover, B vitamins has choline for forming PC, the main ingredient of very low-density lipoprotein and the principal phosphorus-containing lipid recently found in laying hen liver, blood, and eggs suggesting that choline is an essential nutrient in laying hen diets (Ridgway and Vance, 1992; Zeisel and Blusztajn, 1994).

Previous research found that choline supplementation reduced the total lipids in the liver of laying hens (Saeed et al., 2017). Furthermore, adding more choline to the diet seems to reduce the risk of producing fatty liver and benefit laying hens with fatty liver hemorrhagic syndrome (Wolford and Polin, 1975). Although Daghir et al. (1960) reported that choline did not significantly affect laying hens' egg yolk and serum cholesterol levels, Tsiagbe et al. (1988) observed that adding choline with 500 or 1000 mg/kg caused significant increases in the concentration of PC and total phospholipids in egg yolks.

Betaine (N, N, N-trimethylglycine) is a nontoxic amino acid derivative used by various plants and organisms to provide methyl groups for the synthesis of several substances, including methionine, carnitine, and creatine (Arif et al., 2022). Betaine use thus may improve poultry tolerance to heat stress (Attia et al., 2016). According to some studies, betaine has osmoprotective properties that could protect proteins and enzymes in intestinal cells from environmental stress prevent dehydration and increase body retention and are vital for reducing body temperature in heat-stressed chickens (Zulkifli et al., 2004; Lan and Kim, 2018). Moreover, Liu et al. (2019) demonstrated that dietary supplementation of betaine at 0.05%, 0.1%, and 0.2% reduced the adverse effects of heat stress on average daily feed intake and body weight (**BW**) in broilers. Betaine is known to promote the growth and survival of gut microbes (Eklund et al., 2005; Ratriyanto et al., 2017). Furthermore, Hamidi et al. (2010) reported that adding 0.12% betaine to the feed significantly increased the gain of mixed-infected coccidia, which may increase the protective effect retained in the epithelial cells, intestinal tract, and mucosa (Attia et al., 2016; Park and Kim, 2017).

Adding 0.10% betaine to poultry feed improves protein and yolk weight in a Haugh unit (Ezzat et al., 2011; Attia et al., 2016). Based on available research, there is limited evidence for using betaine and choline in organic diets. Therefore, this study aimed to determine the effects of betaine and/or choline supplementation on performance, egg quality, blood, antioxidant status, lipid peroxidation, and reproductive qualities of laying hens.

MATERIALS AND METHODS

One hundred and forty Bovans brown chickens, 45 wk old, 25 wk of production, with eggs production 76%, were housed in individual cages on a 16-h: 8-h light/dark cycle. The chickens were randomly assigned to 4 groups (7 replicates of 5 chickens each, each chicken was housed individually). The cage is Big Dutchman in regular dimensions of 40 × 35 × 60 cm^3, double-sided battery cage. An automated nipple drinker was given for each cage. The first group (control, A) was supplemented with a basal diet: 500g/ton choline; the second (B): 375g/ton choline +125g/ton betaine; the third group (C): 250g/ton choline + 250g/ton betaine; the fourth group: 500g/ton betaine (D). The primary feed composition is prepared according to the recommendations of (NRC, 1994), which are presented in Table 1.

Laying Growth Performance

Hens were weighed at 45 and 57 wk of age, whereas feed intake was recorded weekly and expressed on a cage basis. Eggs were collected and weighed daily, and the

Table 1. Composition and nutrient levels of basal diet.

Ingredients	%	Calculated nutrient levels[2]	
Yellow corn	59.10	Crude protein, %	18.14
Soybean meal, 46%	23.3	ME, Kcal/kg diet	2850
Gluten meal, 62%	4.42	Calcium, %	3.27
Soybean oil	1.92	Total phosphorus, %	0.72
Di calcium phosphate	2.02	Available phosphorus, %	0.47
DL-Methionine, 99%	0.21	Ether extract, %	4.44
Threonine, 99%	0.05	Fiber, %	2.80
Limestone	7.22	Lysine, %	0.88
NaCl	0.30	Methionine, %	0.49
Vitamin mineral premix[1]	0.42		
Sodium bicarbonate	0.24		
Potassium carbonate	0.30		
Choline chloride	0.50		
Total	100		
Chemical analysis	%		
Crude protein, %	18.12		
Ether extract, %	4.43		
Calcium, %	3.27		
Total phosphorus, %	0.71		

[1]Vitamin mineral premix (units per kilogram of feed): vitamin A, 10,000 IU; vitamin D3, 3,500 IU; vitamin E, 35 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B12, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg.

[2]Calculated according to NRC (1994) for brown Bovens laying hens.

average value of eggs during the experimental period was used to determine egg production (percentage hen-day) and egg weight. Egg mass was calculated by multiplying egg production by egg weight. The overall FCR was estimated on a cage basis as g feed/g egg.

Egg Quality

Assessment of egg and shell feature parameters including yolk color, egg weight, shell weight, shell thickening, yolk width, yolk height, yolk weight, albumin weight, albumin height, and albumin width was performed at 45 and 57 wk. For this, 40 eggs/ group placed between 08:00 am and 12:00 pm were randomly selected, weighed, and prepared for evaluation. Evaluation of yellow color was performed with Roche yellow color fan method (Vitamins and Chemicals 1988). The weight of the shell is calculated as follows: any protein present in the shell was washed, then dried, and weighed to the weight of the whole egg. The egg was marked with a specific method for each egg based on the measured weight of the egg.

Assessment of Reproductive Morphology

At the end of the experimental period (57 wk), 10 hens from each group were randomly selected, weighed, anesthetized and euthanized by decapitation. The reproductive system weight and morphology were also recorded. The oviducts were isolated and weighed and the total length of oviduct was measured. The relative mass and the lengths of the vagina, uterus, isthmus, magnum, and infundibulum were estimated. Liver samples were dissected and stored at -20°C for farther analysis.

Blood Parameters Examination

At the end of the trial, blood samples were obtained in heparinized tubes from the wing vein and centrifuged at 3000 × g for 20 min to get plasma samples, kept at -20 ° C until analysis. Plasma concentrations of total lipid, total cholesterol, triglyceride, high and low-density lipoproteins (HDL-lipoprotein and LDL-lipoprotein), albumin, total protein, and glucose levels were analyzed using a colorimetric kit (Egyptian Company for Biotechnology, Cairo, Egypt, and Wako Chemicals, VA). The content of liver malondialdehyde (**MDA**) was determined utilizing a commercial colorimetric kit (Liquizyme MDA; Biotechnology, Egypt). The absorbance was monitored using a spectrophotometer (Unico UV 2000; Spectra Lab Scientific Inc., Markham, Ontario, Canada) at a wavelength of 545 nm. Regarding antibody titre, hemagglutination inhibition antibody titers were measured using U-bottom microtiter plates according to Beard et al. (1975).

Egg Yolk Fatty Acids Profile

At the end of the experiment, 70 eggs were collected per group to measure the yolk fatty acid profile in egg yolk, including linolenic, oleic, palmitic acid by the use of Shimadzu GC-4 CM gas chromatograph (PFE), outfitted with a flame ionization detector. Calcium concentration was measured by flame atomic absorption spectrophotometer. The total concentration of vitamin E and cholesterol in egg yolk were determined by high-performance liquid chromatography as described by Catoni, (2008).

Data Analysis

The differences between the treatment and control groups were analyzed with a General Liner model using SPSS (Version 17.0). One-way ANOVA was applied to determine the effects of choline and/or betaine when birds were the statistical units for performance parameters, organ weights, and samples for biochemical and other parameters. Duncan's new multiple-range tests were used to identify which treatment conditions were significantly different from each other at a significance level of $P < 0.05$.

RESULTS

Laying Growth Performance

Effect of replacing choline with betaine on final BW, BWG, egg production, egg weight, and egg mass in laying hens is shown in Table 2. Supplementation with different levels of betaine substitutes had no significant

Table 2. Effect of replacing choline with betaine in laying growth performance.

Item	Treatment[1]				*P* value
	A	B	C	D	
IBW, g	1662.9 ± 73.1	1611.4 ± 60.8	1592.9 ± 44.1	1628.6 ± 57.9	0.86247
FBW, g	1722.9 ± 55.3	1668.6 ± 46.1	1650 ± 31.00	1684 ± 40.50	0.69058
BWG, g	60.00 ± 22.47	57.14 ± 20.20	57.14 ± 20.20	55.71 ± 29.43	0.99935
FI g/d	115.76 ± 1.20	111.9 ± 2.740	113.19 ± 1.82	112.5 ± 1.70	0.55369
EW, g	81.71 ± 1.19[b]	84.43 ± 5.37[a]	84.14 ± 0.55[a]	84.57 ± 0.75[a]	0.04910
EP, %	59.48 ± 0.50	59.89 ± 0.330	60.57 ± 0.30	60.57 ± 0.36	0.14266
EM, g egg/hen/d	48.59 ± 0.64[b]	50.57 ± 0.405[a]	50.96 ± 0.35[a]	51.23 ± 0.59[a]	0.00514

[a,b]Different letters in the same row indicate significant differences ($P < 0.05$). The values presented are the means and standard error of 60 per treatment.

[1]Abbreviations: control (A) A basal diet supplemented with 500g/ton choline (B) A basal diet supplemented with 375 g/ton choline and 125g/ton betaine (C) A basal diet supplemented with 250 g/ton choline and 250 g/ton betaine (D) A basal diet supplemented with 500g/ton betaine.

effect on final body weight, weight gain, and egg production compared to the control. On the other hand, egg weight and mass were increased significantly in all the treatment groups compared with the control group. No mortality occurred during the experiment.

Egg Euality Parameters

Table 3 presents the egg quality data at 45 wk and the end of the trial. In the initial study period (45 wk), no significant changes in egg quality characteristics were recorded. Betaine supplementation in laying hens' diets increased egg weights, yolk color score, and albumin width at 57 wk, whereas the group received 500g/ton betaine had the highest values for the egg weights, yolk color score, and albumin width. On the other hand, choline and/or betaine dietary supplementation did not significantly affect shape index, yolk width, yolk height, albumin height, shell thickness, and Haugh units through the experimental period of laying hens.

Reproductive Organ Morphology Measurements

Results for the effects of betaine and/or choline supplements on reproductive organ weights and lengths are presented in Table 4. The diet of choline and/or betaine had a significant effect in the oviduct weights, where the highest weight was in the control treatment (500g/ton choline) and (250 g/ton choline with 250g/ton betaine). The weight of the ovary, vagina, uterus, isthmus, magnum, and infundibulum were not affected. However, supplementation with choline and/or betaine did not affect the oviduct length compared to the control ($P < 0.05$).

Blood Measurement and Antibody Titer

As shown in Table 5, the administration of dietary choline and/or betaine did not significantly change the plasma parameters of AST, ALT albumin, total protein, globulin, cholesterol, LDL, HDL, and triglycerides. In addition, chickens fed betaine showed higher antibody titers against the Newcastle disease (**ND**) virus.

Chemical Characteristics of Egg Yolk and Liver

Table 6 shows the effect of choline and/or betaine supplementation on the fatty acid profile of eggs. Choline and/or betaine had no significant impact on the chemical fatty acid concentration in the egg yolk. Figure 1 presents the results of effect of choline and/or betaine supplementation on liver MDA content, yolk total cholesterol and vitamin E in laying hens, yolk total cholesterol was not influenced whereas yolk vitamin E and liver MDA content were decreased significantly by replacing choline to betaine groups.

DISCUSSION

There are many studies on the importance of using betaine and choline as feed additives to improve broiler and laying hens' immune status and productivity. In this study, the betaine and choline diet increased egg production and mass at 57 wk of age ($p < 0.05$, Table 2). Congruent and conflicting results have been certified

Table 3. Effect of replacing choline to betaine on egg quality parameters in laying hens at 45 and 57 wk of age.

	Treatment[1]				
Item	A	B	C	D	*P* value
At 45 wk of age					
Egg weight, g	61.43 ± 1.42	61.57 ± 0.89	61.71 ± 1.409	61.43 ± 1.307	0.9982
Yolk color score	7.344 ± 0.32	7.295 ± 0.34	7.1783 ± 0.25	7.1783 ± 0.25	0.9705
Shell thickness, μm	395.7 ± 19.0	398.5 ± 15.5	397.14 ± 6.44	395.71 ± 12.8	0.9987
Yolk width, cm	3.619 ± 0.14	3.504 ± 0.09	3.454 ± 0.039	3.454 ± 0.039	0.5229
Albumin width, cm	3.10 ± 0.448	3.19 ± 0.338	3.051 ± 0.355	3.051 ± 0.355	0.9927
Yolk height, mm	16.50 ± 0.38	16.64 ± 0.42	16.44 ± 0.307	16.39 ± 0.322	0.9672
Albumin height, mm	4.70 ± 0.345	4.753 ± 0.37	4.727 ± 0.310	4.87 ± 0.3790	0.9870
Shape index	75.52 ± 0.91	74.82 ± 0.78	74.95 ± 0.522	74.95 ± 0.246	0.8843
Haugh units	116.25 ± 2.4	118.02 ± 1.2	119.46 ± 2.23	119.46 ± 2.23	0.6638
At 57 wk of age					
Egg weight, g	56.43 ± 0.75[b]	59.29 ± 1.74[ab]	58.57 ± 0.896[ab]	62.14 ± 1.48[a]	0.0350
Yolk color score	7.429 ± 0.36[b]	8.286 ± 0.184[a]	8.429 ± 0.202[a]	8.714 ± 0.18[a]	0.0078
Shell thickness, μm	380 ± 14.800	391.43 ± 18.82	409.3 ± 13.29	414.3 ± 7.82	0.3154
Yolk width, cm	3.52 ± 0.074	3.53 ± 0.135	3.50 ± 0.0930	3.45 ± 0.039	0.9375
Albumin width, cm	2.77 ± 0.268[ab]	2.23 ± 0.302[ab]	1.85 ± 0.321[b]	3.05 ± 0.355[a]	0.0521
Yolk height, mm	16.06 ± 0.30	15.984 ± 0.28	16.36 ± 0.270	16.321 ± 0.35	0.7667
Albumin height, mm	4.836 ± 0.27	5.764 ± 0.757	6.209 ± 0.490	5.441 ± 0.66	0.4074
Shape index	77.2 ± 1.054	78.26 ± 1.015	76.86 ± 1.250	75.10 ± 0.56	0.1899
Haugh units	116.84 ± 0.96	119.96 ± 2.67	121.62 ± 1.59	119.46 ± 2.23	0.4084

[a,b]Different letters in the same row indicate a significant difference ($P < 0.05$). The values presented are the means and standard error of 60 per treatment.

[1]Abbreviations: control (A) A basal diet supplemented with 500g/ton choline (B) A basal diet supplemented with 375 g/ton choline and 125g/ton betaine (C) A basal diet supplemented with 250 g/ton choline and 250 g/ton betaine (D) A basal diet supplemented with 500g/ton betaine.

Table 4. Effect of replacing choline with betaine on reproductive internal organ morphology of laying hens at 57 wk.

Item	Treatment[1]				*P* value
	A	B	C	D	
Live body weight, g	1818 ± 231.6	1988 ± 156	1720 ± 95.70	1932 ± 76.6	0.62488
Oviduct weight, g/100 g BW	3.93 ± 0.399[a]	2.46 ± 0.34[b]	3.75 ± 0.283[a]	3.09 ± 0.12[ab]	0.03396
Vagina weight, g/100 g OW	1.44 ± 0.063	2.22 ± 0.55	1.56 ± 0.029	1.67 ± 0.020	0.28810
Uterus weight, g/100 g OW	35.4 ± 2.084	38.3 ± 2.65	40.03 ± 1.04	34.60 ± 2.37	0.30350
Isthmus weight, g/100 g OW	13.98 ± 3.54[a]	9.42 ± 0.48[ab]	7.23 ± 0.426[b]	8.3 ± 0.100[ab]	0.11503
Magnum weight, g/100 g OW	46.17 ± 2.11	46.07 ± 1.9	46.43 ± 1.81	51.4 ± 2.010	0.26391
Infundibulum weight, g/100 g OW	2.87 ± 0.120	3.83 ± 0.53	2.58 ± 0.485	3.86 ± 0.610	0.20568
Oviduct length, cm	74.17 ± 0.92	63.0 ± 2.88	69.00 ± 0.01	71.97 ± 2.60	0.22525
Vagina length, cm	1.667 ± 0.33	1.33 ± 0.33	1.667 ± 0.33	1.33 ± 0.330	0.80180
Uterus length, cm	6.667 ± 0.33	5.50 ± 0.50	5.333 ± 0.33	5.5 ± 0.7640	0.30194
Isthmus length, cm	16.67 ± 1.33	12 ± 1.528	12.67 ± 0.88	13.33 ± 1.85	0.18375
Magnum length, cm	38.33 ± 0.33	34.66 ± 2.9	41.67 ± 2.33	40.67 ± 3.66	0.30955
Inf. length, cm	12.67 ± 2.33	12 ± 1.155	8.83 ± 0.441	8.83 ± 0.928	0.17554

[a,b]Different letters in the same row indicate significant differences ($P < 0.05$). The values presented are the means and standard error of 60 per treatment.

[1]Abbreviations: control (A) A basal diet supplemented with 500g/ton choline (B) A basal diet supplemented with 375 g/ton choline and 125g/ton betaine (C) A basal diet supplemented with 250 g/ton choline and 250 g/ton betaine (D) A basal diet supplemented with 500g/ton betaine.

regarding choline and/or betaine and their potential effects on laying productive performance.

Although Omer et al. (2020) noted that supplementing with 0.5% betaine had no effect on the BW change and egg weight (**EW**) of laying hens. Egg production (**EP**) rate and egg mass (**EM**) were significantly ($P < 0.01$) improved in hens fed the diet supplemented with levels of betaine diet as compared to control groups (Table 2). Feed conversion can be improved due to betaine's ability to act as l-methionine donor and its many physiological properties that improve the intestinal environment and thus increase absorption (Remus and Quarles, 2000).

The accumulation of betaine in the cell protects it from osmotic stress and allows normal metabolic function under conditions where the cell is usually inactive (Saunderson and MacKinlay, 1990). These results were similar to those of (Ryuet et al., 2002). Furthermore, compared to the control group, adding betaine to laying hen diets at levels of 5.00 and 2.00 ppm significantly increased EP, EM, and FCR.

Gudev et al. (2011) showed that adding betaine to a laying diet of up to 1.5 g/kg for hens in heat stress significantly increased egg production and egg mass ($P < 0.05$). The increased secretion of blood insulin-like growth factor binding protein-3 (IGFBP-3) may have improved laying performance by increasing the half-life of blood insulin-like growth factor I and improved preservation productivity and liver tissue differentiation (Park and Kim, 2017). Moreover, betaine could reduce the requirement for additional methyl group donors such as methionine and choline. Also, it increases the liver's ability to synthesize proteins and fatty acids. These results were in agreement with those obtained by Park and Kim (2017), who reported that betaine supplement to laying hen diets up to 1.2 g/ kg did not significantly improve egg production compared to the control group. FCR improved when betaine was included in the diet of layer chickens (Eklund et al., 2005) and egg production (Zou et al., 1998). Betaine improved the productivity and laying parameters of hens under heat stress. For instance, adding 0.2% betaine to the diet increased egg

Table 5. Effect of replacing choline to betaine on antibody titers against (ND and H9), components in the blood of 57-wk-old laying hens.

Item[2]	Treatment[1]				*P* value
	A	B	C	D	
Glucose, mg/dL	169.3 ± 14.33	170 ± 2.887	152 ± 5.292	155 ± 6.110	0.3480
ALT, IU/L	35 ± 3.06	34.33 ± 1.85	35.33 ± 1.45	35.33 ± 1.20	0.9820
AST, IU/L	45 ± 4.51	46.00 ± 2.01	46 ± 6.110	41.01 ± 1.52	0.7876
Total protein, mg/dL	6.3 ± 0.20	6.30 ± 0.060	6.4 ± 0.058	6.23 ± 0.13	0.8294
Albumin, mg/dL	3.83 ± 0.03	3.80 ± 0.100	3.83 ± 0.09	3.70 ± 0.06	0.5694
Globulin, mg/dL	2.467 ± 0.16	2.50 ± 0.120	2.567 ± 0.06	2.53 ± 0.18	0.9620
Cholesterol, mg/dL	124.3 ± 3.84	117.7 ± 2.66	119 ± 1.528	117.3 ± 2.02	0.2890
Triglyceride, mg/dL	271 ± 4.933	273.3 ± 3.33	255.7 ± 28.2	234 ± 20.23	0.4198
HDL, mg/dL	19.67 ± 0.88	20.67 ± 1.33	22.33 ± 2.18	22 ± 1.155	0.5775
LDL, mg/dL	21.17 ± 0.70	102.0 ± 4.00	94.33 ± 2.60	94 ± 1.528	0.1633
H9	3.01 ± 0.58	3.333 ± 0.33	4.333 ± 0.33	40.1 ± 0.58	0.2503
ND	7.667 ± 0.67[b]	9.33 ± 0.33[a]	9.33 ± 0.33[a]	9.67 ± 0.33[a]	0.0466

[a,b]Different letters in the same row indicate significant differences ($P < 0.05$). The values presented are the means and standard error of 60 per treatment.

[1]Abbreviations: control (A) A basal diet supplemented with 500g/ton choline (B) A basal diet supplemented with 375 g/ton choline and 125g/ton betaine (C) A basal diet supplemented with 250 g/ton choline and 250 g/ton betaine (D) A basal diet supplemented with 500g/ton betaine.

[2]AST: Aspartate transaminase, ALT: alanine transaminase, HDL: high density lipoprotein, LDL: high density lipoprotein, ND: Newcastle disease.

Table 6. Effect of replacing choline with betaine on chemical fatty acid concentration in the egg yolk at the end of the experiment (57 wk).

Item	Treatments[1]				*P* value
	A	B	C	D	
Butric acid	3.143 ± 0.0289	3.177 ± 0.024	3.119 ± 0.044	3.103 ± 0.0059	0.362684
Enanthicacid	87.21 ± 0.0167	87.29 ± 0.023	87.25 ± 0.592	87.18 ± 0.5645	0.997754
Caproic acid	0.271 ± 0.0283	0.278 ± 0.022	0.277 ± 0.022	0.267 ± 0.0267	0.988286
Caprylic acid	0.269 ± 0.0318	0.273 ± 0.023	0.266 ± 0.029	0.263 ± 0.0379	0.996081
Capric acid	0.271 ± 0.0260	0.268 ± 0.050	0.266 ± 0.030	0.261 ± 0.0296	0.997558
Undecanoic acid	0.107 ± 0.0167	0.097 ± 0.014	0.103 ± 0.020	0.077 ± 0.0203	0.658836
Lauric acid	0.012 ± 0.0029	0.016 ± 0.001	0.016 ± 0.001	0.015 ± 0.0020	0.631203
Myristoleic acid	0.036 ± 0.0029	0.033 ± 0.006	0.035 ± 0.004	0.033 ± 0.0015	0.933431
Cis-10-heptadecanoic acid	0.175 ± 0.0043	0.164 ± 0.013	0.170 ± 0.024	0.183 ± 0.0088	0.839223
Stearic acid	1.444 ± 0.0059	1.447 ± 0.006	1.444 ± 0.005	1.458 ± 0.0095	0.512213
Palmetic acid	0.055 ± 0.0123	0.044 ± 0.002	0.045 ± 0.002	0.043 ± 0.0012	0.592888
Palmetoleic acid	0.069 ± 0.0002	0.069 ± 0.000	0.064 ± 0.002	0.067 ± 0.0017	0.184612
Oliecacid	0.437 ± 0.0035	0.436 ± 0.002	0.438 ± 0.003	0.437 ± 0.0031	0.990976
Eliadic acid	0.073 ± 0.0029	0.073 ± 0.002	0.073 ± 0.002	0.073 ± 0.0028	0.999998
Linoleic acid	0.506 ± 0.0035	0.506 ± 0.002	0.513 ± 0.005	0.505 ± 0.0037	0.529247
Linolenic acid	0.208 ± 0.0034	0.209 ± 0.002	0.208 ± 0.003	0.209 ± 0.0029	0.989752
Cis-11,14-eicosadienoic acid	0.522 ± 0.0017	0.523 ± 0.002	0.522 ± 0.001	0.522 ± 0.0042	0.961035
Cis-11,14,17-eicosatrienoic acid	2.155 ± 0.0033	2.156 ± 0.003	2.155 ± 0.005	2.157 ± 0.0052	0.976000
Cis-5,8,11,14,17eicosapentaenoic acid	0.519 ± 0.0123	0.510 ± 0.003	0.512 ± 0.002	0.511 ± 0.0027	0.733147
Cis-11-ecosenoic acid	0.868 ± 0.0029	0.868 ± 0.002	0.862 ± 0.004	0.875 ± 0.0073	0.330022
Heneicosanoic acid	0.274 ± 0.0301	0.238 ± 0.101	0.251 ± 0.009	0.263 ± 0.0299	0.704976
Behenic acid	0.135 ± 0.0026	0.135 ± 0.002	0.136 ± 0.002	0.135 ± 0.0029	0.989468
Cis-13,16-docosadienoic acid	0.522 ± 0.0410	0.555 ± 0.066	0.555 ± 0.066	0.555 ± 0.0664	0.972303
Cis-11-ecosenoic acid	0.328 ± 0.0029	1108 ± 1108.1	0.33 ± 0.0029	0.328 ± 0.0029	0.441101

The values presented are the means and standard error of 60 per treatment.

[1]Abbreviations: control (A) A basal diet supplemented with 500 g/ton choline (B) A basal diet supplemented with 375 g/ton choline and 125 g/ton betaine (C) A basal diet supplemented with 250 g/ton choline and 250 g/ton betaine (D) A basal diet supplemented with 500 g/ton betaine.

production and eggshell quality in heat-stressed chickens (Ryu et al., 2002).

In heat-stressed birds, supplementing other dietary nutrients with betaine improved laying features. For instance, Fayoumi laying hens fed in hot conditions significantly improved after consuming betaine, folic acid, and choline supplements (Tolba et al., 2007). Egg performance, laying performance, and health of layer chickens under heat stress were increased after vaccination with 400 mg/kg betaine (Hao et al., 2017). When betaine is added to the chickens' diet, the serum increases several hormones, including lutein, estradiol, triiodothyronine, thyroxine, progesterone, and follicle hormone (Zou et al., 1998).

Betaine also increases melatonin concentrations, serum estradiol and daily egg mass in chickens, which increases egg production (Zou et al., 1998). Betaine supplementation of 0.10 to 0.21% increases the digestibility of the ether extract by 9.4% in laying hens and quail (Ezzat et al., 2011; Ratriyanto et al., 2018). Rørvik et al. (2000) showed that increased bile acid excretion was associated with better digestibility of ether extracts. Also, significantly increased glycine availability in the diet has been associated with an improvement in ether extract digestibility (Han and Thacker, 2011). Glycine's availability is improved by betaine which creates bile salts by connecting with the bile acids that the liver produces (Eklund et al., 2005; Powell et al., 2009). In addition, higher amounts of short-chain fatty acids in the intestinal tract can increase the absorptive capacity of the intestinal epithelium, resulting in better digestion (Butzner et al., 1994). However, microbial activity is thought to interfere with the excretion of bile acids required for the digestion of lipids, thus affecting the digestibility of ether extracts; this observation is consistent with the findings of several studies showing that betaine does not affect ether extract digestibility (Attia et al., 2016; Ratriyanto et al., 2017 (. Betaine can directly participate in biochemical processes such as lipid metabolism, amino acid transport and regeneration, and the biosynthesis of purines and pyrimidines (Abu Ahmad et al., 2019; Zhang et al., 2019). As presented here, the use of betaine supplementation in laying hens' diets increased egg weights at 57 wk, where the treatment of complete replacement with betaine (500g/ton) recorded the highest rates of egg weights compared to other treatments during the experiment and also the highest rates of increase albumin width than other treatments. On the other hand, the change in the yolk color recorded the lowest values in the control treatment (500g/ton choline) than the other treatments. Thus, egg quality was improved after betaine supplementation under ambient temperature. Additionally, adding 0.10% betaine to chicken feed has increased yolk weight, albumen and the Haugh unit (Ezzat et al., 2011; Attia et al., 2016). Betaine inhibits hepatic fat accumulation while increasing mitochondrial content and activity, suggesting a betaine role in regulating fat metabolism (Zhang et al., 2019). According to Ratriyanto et al., (2017), the effects of the retention of protein and energy metabolism may be due to the addition of betaine 0.06-0.12% increased egg weight in quail and increased egg yolk and egg white weight in birds raised under natural tropical conditions (Ratriyanto et al., 2018). Another observation suggested that adding 0.14% betaine to the laying diet caused the yolk to develop more fat (Ratriyanto

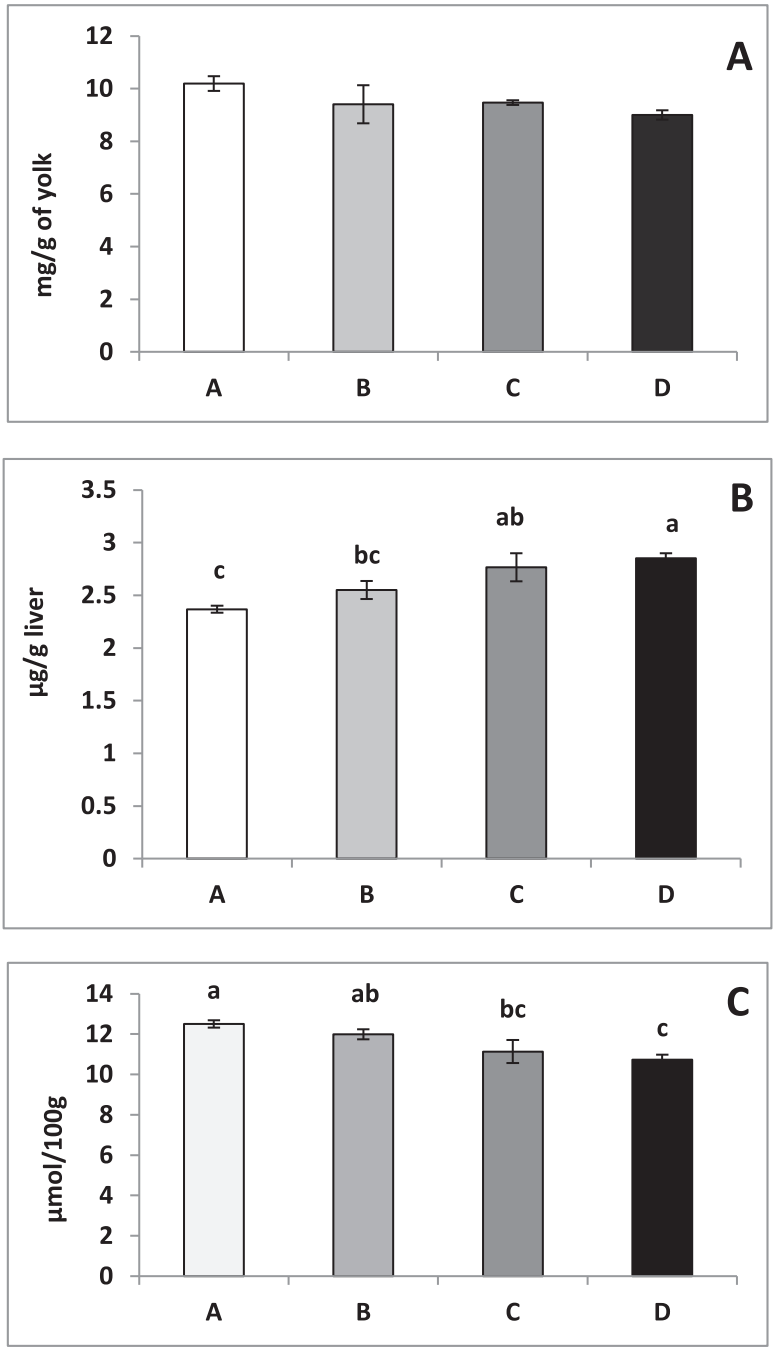

Figure 1. Effect of replacing choline with betaine on total cholesterol (A), vitamin E (B) and liver MDA content (C) in egg yolk. [a,b,c]Different letters in the same row indicate significant differences ($P < 0.05$). The values presented are the means and standard error of 60 per treatment. Abbreviations: control (A) A basal diet supplemented with 500g/ton choline (B) A basal diet supplemented with 375 g/ton choline and 125g/ton betaine (C) A basal diet supplemented with 250 g/ton choline and 250 g/ton betaine (D) A basal diet supplemented with 500g/ton betaine.

et al., 2018). Regarding the increase in lipase and HDL activity, the improved blood profile may be related to the improved egg quality, total protein and globulin, triglycerides and the reduced total cholesterol and its derivatives (LDL) (Ezzat et al., 2011; Awad et al., 2014). Herein, the supplementation of choline and/or betaine showed a significant increase in the weights of the oviduct, where the highest weight was in the control treatment (500g/ton choline) and (250 g/ton choline with 250g/ton betaine), it also led to a significant increase in the weights of the isthmus during the treatments. Betaine supplementation did not affect ovaries, vagina, uterus, magnum, and cage weights, despite an increase in oviduct length compared to control. The increasing estrogen and progesterone levels in the blood can be attributed to the augmentation in laying performance with betaine. These hormones regulate ovulation and oviduct development and regulate the ovulation rate (Etches, 1996; Attia et al., 2016). Attia et al. (2016) observed that supplementation of betaine at 0.10% in laying hens led to an increase in oviduct weight and length and an increase in the large ovarian follicles weight, which contributed to an increase in egg production. The present study reported no significant change

for the biometric parameters of ALT, AST, total protein, albumin, globulin, triglycerides, cholesterol, and its derivatives (HDL and LDL) after the use of choline and/or betaine supplement. The present findings are consistent with Øverland et al. (1999) who found that the betaine supplement did not affect plasma triglycerides. Moreover, this study showed that chickens fed betaine have higher antibody titers to the ND virus. Betaine showed beneficial effects on immune function of broilers against Brucella abortus and Newcastle-modified live or dead virus vaccine (Gonzalez-Vega-Aguire et al., 1995). Farooqui et al. (2005) noted that the immune response was suppressed by vaccines against the respiratory virus and bursa fabricius gland weight was increased by supplementation with 0.15% betaine; supplementing birds with a 0.10% betaine increases resistance to heat stress and vitamin C. Similarly, betaine (1 g/kg) supplementation at 27 and 35 d of age significantly increased primary antibody titers to ND virus (NDV) and infectious bronchitis virus in heat-stressed broilers (Ghasemi et al., 2020). However, betaine supplementation (0.121%) had no significant effect on the humoral immune response of 42-day-old broiler chickens (Tsiagbe et al., 1988). Also, injection of betaine and choline into oocytes did not affect immunoglobulin IgM and IgG, or total antibody titers in hatched chicks (Gholami et al., 2015) Betaine has been shown to stimulate the humoral immune response by regulating cytokine production in liver macrophages and inhibiting prostaglandin synthesis (Zhang et al.,1996), as well as increasing nitric oxide release from heterophils and macrophages (Klasing et al.,2002). Tsiagbe et al. (1988) observed that choline addition in the chicken feed did not affect either humoral or cellular immune responses when supplemented with methionine or alone,. Conversely, significant responses to choline addition were observed in chicks' antibody titers (Swain and Johri, 2000). In the current study, dietary choline and/or betaine did not differ significantly in yolk chemical fatty acids profile concentration. Dietary supplementation of choline and/or betaine in liver MDA content, yolk total cholesterol vitamin E and fatty acid levels were not significantly affected by replaced choline to betaine groups. Although some studies have suggested that betaine significantly impacts the concentration of fatty acids and the level of MDA content. Betaine's lipotropic activity also affects the amount of body fat. Betaine increases the creation of lecithin by providing methyl groups, which enables fat to be transported throughout the body (Saunderson and MacKinlay, 1990). Betaine also increases choline's availability for synthesizing very low-density lipoprotein, which reduces liver fat absorption and promotes hepatic fat excretion (Yao and Vance, 1989; Hassan et al., 2005). Moreover, it is associated with increased synthesis of methylated substances, carnitine, and creatine, in the liver and muscle via betaine (Zhan et al., 2006). The internal membrane of mitochondria, where fatty acid oxidation occurs, is traversed by carnitine to transport long-chain fatty acids (Wang et al., 2004). Betaine can also improve antioxidant defense, reduce lipid peroxidation, and improve poultry quality (Alirezaei et al., 2012). In the current study, total antioxidant capacity and MDA were not significantly affected by dietary supplementation of betaine choline, and their combination. Antioxidant enzymes are generally the first defense in an animal cell's antioxidant system providing protective response to oxidative stress (Altan et al., 2003). However, oxidative stress increases the release of reactive oxygen species, which induces lipid peroxidation reactions and increases the level of MDA in tissues and plasma (Sahin et al., 2002).

CONCLUSIONS

In conclusion, this study shows that supplementing hens with 500 g/ton of betaine increases performance (egg weight and egg mass). Furthermore, using betaine as a substitute for choline at rates of 500 g/ton improved the stage's production efficiency and increased egg production and weight, as well as egg quality and bird immune efficiency against various pathogens.

ACKNOWLDGMENTS

This work was supported by the Science and Technology Plan Project of Guangzhou (202206010168); the Science and Technology Program of Guangdong Academy of Agricultural Sciences (202106TD, R2019PY-QF008); Introduction of Talents Program from Guangdong Academy of Agricultural Sciences (R2021YJ-YB3012; Guiding Agreement of Young Scholar from Guangdong Academy of Agricultural Sciences R2021QD-024; the Open Project from Guangdong Key Laboratory of Animal Breeding and Nutrition (2021SZ03). The acknowledgment was extended to Researchers Supporting Project (RSPD2023R731), King Saud University (Riyadh, Saudi Arabia).

ETHICAL STATEMENT: The experiment was accepted by the Ethics Committee of the Local Experimental Animals Care Committee and performed under the guidelines of the Department of Poultry Production, Faculty of Agriculture, Kafrelsheikh University, Egypt (Number 4/2016 EC).

DISCLOSURES

The authors declare no conflicts of interest.

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