



Full Length Article

Supplementary outcomes of betaine on economic and productive performance, some biochemical parameters, and lipoprotein lipase gene expression in finishing male broilers

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ABSTRACT

Egypt's population is growing with the biggest hurdle facing the Government is to secure animal protein. Broilers provide quality protein of reasonable price. This study was conducted to investigate the outcomes of dietary organic betaine (betafin S4) on productive, epigenetic make up of lipoprotein lipase gene (LPL) promoter, some blood biochemical, and economic parameters in male broilers at finishing period. Eighty one commercial Arbor Acre Plus males, 21 days old, were randomly allocated to three groups, with three replicates each in battery cages under thermo-neutral environment till 42 days. The examined groups received yellow corn-soy basal diet, supplemented with 0 (G1), 1.5 (G2) and 3.0 g (G3) betaine/kg diet, respectively. The mRNA expression levels of LPL gene were analyzed by real-time quantitative PCR. Methylation pattern on LPL gene promoter was determined by bisulfite sequencing. Doses of betaine statistically ($P \leq .05$) improved tested performance parameters; while carcass yield % and abdominal fat deposition did not achieve significant changes. The expression of LPL mRNA showed an inverse relationship with betaine dose, which illustrated as a trend toward increase in G2 and decrease in G3. Regarding serum biochemistry, both treated groups when compared to control group revealed a significant improvement ($P \leq .01$) in albumin level, simultaneously, a significant increase ($P \leq .05$) was recorded in uric acid and triglyceride levels, additionally, strong positive ($P \leq .01$) correlation between betaine dose and previously mentioned parameters was reported. Betaine is recommended in finishing male broilers as production costs were reduced by 3.97%–4.37% per kg, respectively. In conclusion, incorporation of 0.15–0.30% organic betaine to male broilers diets during finishing period improves the growth performances.

1. Introduction

The finishing stage of broilers is characterized by fast growth of muscles and weight gain supported by feeding diets with high metabolized energy concentration resulting in increased metabolic rate [1]. At this stage, greater fat deposition occurs as the extra energy is stored as triglycerides in adipose tissues [2]. Consequently, excess fat in the abdominal cavity and under the skin occurs which is unacceptable to consumers and costly for producers [1]. Using dietary manipulation to produce lean, high quality broiler meat is beneficial to consumers and profitable for producers. The feed additives used for diet manipulation would be more economic if they had dual or multiple actions. Osmolyte additive betaine; also known as oxynurine lycine or N,N,N-

Trimethylglycine, a promising methyl derivative of amino acid glycine, is a naturally-occurring byproduct of sugar beet refinement extracted from molasses [3–4]. Betaine has been reported to reduce lipid deposition in the liver and abdomen [5], and improve energy efficiency, growth, economic performance and carcass quality [6–7]. Besides, it is cost effective providing cheaper and non-hygroscopic product available all year round. As plasma protein metabolism is greatly influenced by lipo-protein lipase (LPL), additionally; less accumulation of fat may be due to decrease in lipogenesis expression or increasing the lipolytic gene expression. Betaine acts as a methyl donor in one-carbon metabolism, including DNA methylation in promoter region of the LPL gene at cytosine bases of CpG dinucleotides, which has been confirmed to be correlated with transcriptional status and intensity of expression [8].

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Epigenetically, betaine acts as a methyl donor for the re-methylation of homocysteine to methionine where Betaine Homocysteine Methyl Transferase (BHMT) enzyme catalyzes this reaction. Although the effect of betaine on fat was explored in broilers, the molecular method underlying the decrease in abdominal fat remains undetermined. This research investigated the performance, carcass yield, serum biochemistry, economic parameters and LPL gene expression in male broilers supplemented with betaine during finishing period.

2. Materials and methods

2.1. Bird management, diet, experimental design and performance measures

This study was planned following the animal research ethics in the Institute of Epigenetics and Epigenomics, Yangzhou University, China, and performed at the experimental broilers unit of Faculty of Veterinary Medicine, Suez Canal University, Egypt. The study was conducted according to ethical guidelines approved by ethics of scientific research committee, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. A thermo-neutral room (22–25 °C) containing 9 wired cages (100 cm x 100 cm) with 81 average sized 21 days old Arbor Acre Plus males broilers was used. These birds were distributed in a completely randomized design into three groups with 3 replicates of 9 birds/pen each. Broilers were offered ad-libitum access to clean water with the experimental finisher diet (Table 1) from d 21 to d 42. The finisher diet formulation is given in Table 1. Daily inspection of diets and birds was performed with shaking feeders every 2 h from 06:00 to 11:00 pm. Group1 fed with the control diet containing no betaine (G1), (G2) fed the control diet supplemented with 1.5 g betaine/kg diet or (G3) fed the control diet supplemented with 3.0 g betaine/kg of diet. Betafin, containing 98% natural betaine was used as additive.

Individual initial body weights were taken at start of the experiment (d 21) and then at weekly intervals, both individual weights and pen feed intake were determined. These measurements were used to determine live weight gain, feed conversion ratio (FCR), and performance index (PI) and on productive efficiency index (PEI) [9] on d 35 and d 42 of age. Any mortality was recorded daily and used to correct the performance measures as described by Sakomura and Rostagno [10]. On d 42, birds were weighed after an 8 h fast and then processed to remove feather and then eviscerated. At processing, the carcass neck was cut to the same length for all birds.

2.2. LPL gene expression and promoter methylation assay:

Genomic DNA was purified from abdominal adipose tissues using DNasy tissue kit (QIAGEN, GmbH, Hilden, Germany) according to

Table 1

Ingredient	kg/100 kg	Chemical constitution
Ground Yellow Corn (8.6% CP)	66	Crud protein = 19.02%
Soybean meal (46.3% CP)	24.7	ME = 3041 MCal/kg
Corn gluten (60% CP)	3.2	C/P ratio = 160
Soybean Oil	2	Ca = 1%
DiCaP	1.7	Available P = 0.3%
Lime stone	1.3	Methionine = 0.3%
Broiler Premix	0.3	Lysine = 0.2%
Salt	0.3	
Lysine	0.3	
DL-Methionine	0.2	

*Determined value.

**Every 3 kg of the high mix premix provides: Vit D3 2 mIU and vit A 12 mIU, vit E, k3, and B1: 1000 mg each, folic acid: 1000 mg, pantothenic acid: 10000 mg, nicotinic acid: 30000 mg, biotin: 50 mg, vit B2 5000 mg, vit B6 1500 mg, vit B12 10 mg, zinc 50000 mg, iron 30000 mg, manganese 60000 mg, iodine 300 mg, copper 4000 mg, cobalt and selenium 100 mg each, carrier (CaCO₃) up to 3 kg.

manufacturer instructions. Primers obtained from different exons were used for both quantitative real time PCR (qRT-PCR) and methylation of LPL gene promoter according to Xing et al. [8]. Briefly, bisulfite conversion was done using EZ DNA Gold Kit, D5005, D5006, (Zymo research, USA). Bisulfite sequencing was performed according to Sun et al. [11], 12 clones (T-Clone) were sequenced for each sample. At each CpG site, the CpG of genomic DNA template was considered as methylated when the sequence obtained was TpG.

TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) was used to isolate total RNA. The concentration and purity of the isolated RNA were measured using a UV spectrophotometer (Eppendorf, Germany). Two µg of total RNA (DNase I RNase-free) were reverse-transcribed using cDNA synthesis kit (Takara, Japan). LPL gene expression level was measured using Prime-Script RT Reagent Kit (Perfect Real Time, Takara, Japan). Relative quantification of LPL gene mRNA level was obtained using the ratio of LPL gene/GAPDH (glyceraldehyde-3-phosphatedehydrogenase).

2.3. Blood biochemistry

Blood samples from 9 randomly selected birds were collected at the end of the experiment while bleeding and the serum was separated in non-heparinized tubes using benchtop centrifuge (Centrioun scientific centrifuge, C2 series, UK) at 3000 rpm for 20 min, then stored at –20 °C until further analysis of total protein (TP), albumin (ALB) and globulin (GLB) where globulin was obtained by subtracting albumin values from total serum protein. The lipid profile including cholesterol (CHL), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), kidney markers such as uric acid (UA) and creatinine (CRE) and liver enzymes such as aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase, were determined using commercial kits (Diamond diagnostic CO., Egypt). Concentration of the biochemical constituents was calculated according to the manufacturer instructions.

2.4. Economic parameters

The following equations were used for cost calculations in Egyptian pounds (EGP) according to Tandogan and Cicek [12]. Total variable costs (TVC): included cost of (chicks + feed + protein + betaine + veterinary medication + disinfectants), total fixed costs (TFC): included cost of (labor + rent + depreciation), and total cost: was the summation of TVC and TFC. Equipment depreciation was calculated according to Nworgu [13] based on 5 years and production of 5 cycles per year. Net profit was calculated by subtracting total cost from total return according to Bokkers and Boer [14], and was calculated per kg live body weight according to Rossiter [15]. Net profit calculations were per kg live weight in EGP and USD according to currency exchange rate based on Central Bank of Egypt [16].

2.5. Statistical analysis

The results were shown as Mean ± SEM. Means of experimental groups were compared by using analysis of variance (ANOVA), followed by post hoc testing using LSD test to determine significance of differences. Results were considered significant and highly significant when *P*-values were less than .05 and .01, respectively. Correlation between parameters was also performed using IBM SPSS software version 16, NY, USA (Inc., 1989–2010).

3. Results

3.1. Production performance

No statistical difference was observed in mortality. At d 35, although broilers revealed high numerical enhancement in terms of live

Table 2

Parameters	Period	G1	G2	G3
Initial W (g)	d 21	852 ± 13	858 ± 13	877 ± 11
Live BW (g)	d 35	1809 ± 67	1945 ± 13	1935 ± 43
	d 42	2407 ± 42 ^b	2545 ± 33 ^a	2541 ± 44 ^a
Weight gain (g)	d 35	957 ± 74	1088 ± 26	1058 ± 54
	d 42	1523 ± 49 ^b	1687 ± 39 ^a	1664 ± 22 ^a
Feed intake (g)	d 35	1841 ± 16.7	1913 ± 32	1857 ± 53
	d 42	2984 ± 17	3000 ± 34	2902 ± 54
FCR	d 35	1.9 ± 0.1	1.8 ± 0.04	1.8 ± 0.1
	d 42	2.0 ± 0.1 ^a	1.8 ± 0.1 ^{ab}	1.7 ± 0.0 ^b
PI	d 35	0.95 ± 0.1	1.1 ± 0.0	1.1 ± 0.1
	d 42	1.2 ± 0.1 ^b	1.4 ± 0.1 ^a	1.5 ± 0.0 ^a
PEI	d 35	4.5 ± 0.5	5.3 ± 0.2	5.2 ± 0.2
	d 42	5.8 ± 0.3 ^b	6.8 ± 0.3 ^a	6.9 ± 0.2 ^a

Differences between means within a row having different superscripts are statistically significant ($P \leq .05$).

weight, WG, PI and PEI with some preferences for G2 but this increase was statistically non-significant. Higher feed intake was observed in G2 and G3 correlated with their weight gain to improve the FCR. Compared to G1, both G2 and G3 revealed higher ($P \leq .05$) final live body weight, WG, PI and PEI at d 42. Data also showed that feed: gain ratio was significantly decreased ($P \leq .05$) in the betaine supplemented groups compared to the control treatment (Table 2).

3.2. Carcass quality and abdominal fat

Carcass quality traits presented in (Table 3) showed non-statistical differences in terms of the dressed carcasses weight (g) and yield %. Carcass yield (including body weight without head, feet, or neck), giblets, abdominal fat pad, liver, gizzard and heart weights, were proportionate to fasted live weight; while only differences were in neck weight. Betaine was not recorded to decrease carcass yield in comparison to control group. Although with non-significant differences in giblets weight, betaine lowered the fat content in many tissues without affecting the weight of vital organs (liver and heart). Concerning neck weight, results revealed statistical increase in neck weight in betaine treated groups.

3.3. LPL mRNA expression

Results showed in Fig. 1 elucidated that the expression of LPL mRNA showed an inverse relationship with betaine dose, whereas, dietary betaine supplementation showed different patterns of a trend toward increase and decrease in the LPL mRNA expression levels in G2 and G3, respectively, but the difference was not significant compared to G1.

3.4. Blood biochemistry

The biochemical analysis is presented in (Table 4). The effect of

Table 3

Parameters	G1	G2	G3
Dressed wt g	1821 ± 38	1891 ± 41	1932 ± 49
Yield %	72.5 ± 0.5	73 ± 0.5	73 ± 0.4
Giblets	6.8 ± 0.1	7.2 ± 0.1	7.1 ± 0.2
Liver relative wt.	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.1
Heart relative wt.	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Fat relative wt.	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
Gizzard relative wt.	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
Neck relative wt.	1.2 ^b ± 0.1	1.5 ^a ± 0.1	1.5 ^a ± 0.1

Differences between means within a row having different superscripts are statistically significant ($P \leq .05$).

Relative weight: weight of organ/live weight.

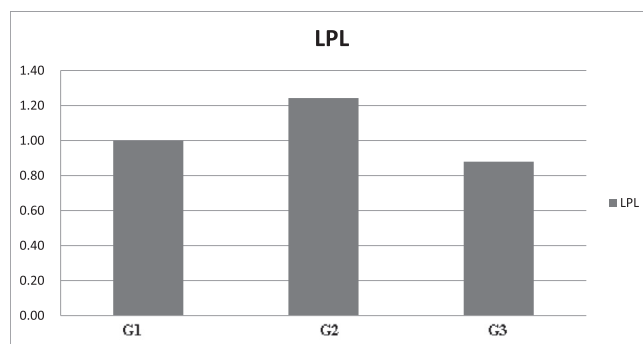


Fig. 1. Real-time quantitative PCR (qRT-PCR) of LPL mRNA expression in abdominal adipose tissues.

betaine supplementation on TP and its fractions revealed a significant impact as follows: G3 showed a significant increase in TP ($P \leq .05$) and ALB ($P \leq .01$) levels with a trend toward increase in GLB level; while, G2 illustrated a significant increase in ALB level with a trend toward increase in the other levels compared to G1. Results also showed an increase in albumin/globulin (A/G) ratio in both G2 and G3 which was significant ($P \leq .05$) in G3 and non-significant in G2. The data recorded non-significant difference in ALT between the treated groups, while alkaline phosphatase showed an increasing trend in G2. Additionally a significant increase in TG was recorded in both betaine fed groups compared to control. A trend toward increase in serum cholesterol in G3 was noticed as well.

Regarding dose response relationship with serum proteins and correlation coefficient between different serum biochemistry (Tables 4 and 5), data revealed positive significant correlation with TP and ALB ($r = 0.356$ and 0.469 at $P \leq .05$ and $P \leq .01$, respectively). Different forms of interrelationship between TP and its fraction were recorded within the treated groups, whereas G1 and G2 revealed a positive correlation between TP and ALB which was significant at ($P \leq .05$), while in G3, TP showed strong positive correlation ($P \leq .01$) with GLB. Moreover, AST level showed non-significant change in G2, while with increasing betaine concentration in G3, a significant decrease ($P \leq .05$) was observed when compared to G1 which could be strengthened by the inverse relationship ($r = -0.312$) reported between dose of betaine and AST.

There was a significant increase ($P \leq .01$) of uric acid (UA) in both betaine treatments compared to G1 with a strong positive correlation ($r = 0.608$ at $P \leq .01$) between betaine dose and UA (Table 4). The relationship between serum UA and TP was demonstrated as a positive correlation in G2 ($r = 0.659$ at $P \leq .01$) and G3 ($r = 0.488$ at $P \leq .05$). Moreover, TG showed a linear increase with betaine supplementation dose as illustrated by the strong positive correlation ($r = 0.806$ at $P \leq .01$) (Table 5). There was a positive significant correlation between TG and GLB ($r = 0.691$ at $P \leq .05$) in G3 (Table 4).

3.5. Economic parameters

The total cost per kg live body weight was 24.95 EGP, 23.47 EGP and 23.34 EGP for G1, G2 and G3 respectively. Therefore, supplementation of broilers diet with 1.5 g/kg diet and 3 g/kg diet reduced production costs per kg with 5.93% and 6.45%, respectively. Moreover, the net profit per kg live body weight was 1.53 EGP (\$ 0.087) and 1.66 EGP (\$ 0.094) for G2 and G3, respectively, while for G1 it was 0.05 EGP (\$ 0.003).

4. Discussion

The addition of betaine within normal environmental temperature, helps chronically heat stressed birds to recover their feed intake and energy that reflected positively on final BW, BW gain and FCR [17]. The

Table 4

Parameters		G1	G2	G3	Correlation coefficient with betaine dose response
Protein profile (g/dL)	Total protein *	4.796 ^b ± 0.24	5.686 ^{ab} ± 0.32	6.018 ^a ± 0.42	0.326*
	Albumin**	1.931 ^b ± 0.1	2.77 ^a ± 0.22	3.202 ^a ± 0.22	0.469**
	Globulin	2.88 ± 0.20	3.03 ± 0.29	3.08 ± 0.39	0.059
	ALB/GLB ratio ^a	0.698 ± 0.039 ^b	1.14 ± 0.14 ^{ab}	1.81 ± 0.46 ^a	0.307**
Liver enzymes (U/L)	ALT	5.44 ± 1.82	6.66 ± 0.88	7.33 ± 0.74	0.214
	AST ^a	78.22 ^{ab} ± 3.80	88.22 ^a ± 2.51	65 ^b ± 7.59	-0.312-
	ALP	505.58 ± 70.89	551.05 ± 31.39	391.67 ± 73.16	-0.249-
Kidney markers (mg/dL)	UA ^{ab}	3.013 ^b ± 0.31	8.316 ^a ± 0.10	8.316 ^a ± 0.14	0.608**
	CRE	2.379 ± 0.16	2.122 ± 0.18	2.112 ± 0.14	-0.218-
Lipid profile (mg/dL)	CHL	163.78 ± 14.16	141.87 ± 9.63	171.77 ± 19.96	0.069
	TG ^a	51.93 ^c ± 2.97	73.517 ^b ± 2.01	101.21 ^a ± 7.67	0.806**
	HDL	129.21 ± 7.52	107.11 ± 6.60	113.86 ± 13.03	-0.209-
	LDL	45.41 ± 11.90	23.07 ± 5.89	42.82 ± 13.25	0.008

Differences between means within a row having different superscripts are statistically significant * at (P ≤ .05) and ** at (P ≤ .01).

results of performance was in contrary to Sun et al. [6] who used dietary betaine in norm-thermal environment and found that supplementation of betaine to replace up to 25% of total dietary methionine had no impact on the growth performance. On the other hand, the present study was partially in agreement with He et al. [18] who found non-significant improvement by using 0.2 and 0.4% dietary betaine respectively. It was assumed that in hot countries betaine might be efficiently used to resolve the negative power of high ambient temperature in broilers. The results proposed that at a proper dose, betaine might reserve some quantity of dl-methionine and dietary energy to relief the growth process of heat-stressed broilers [19].

Lean meat production with low abdominal fat can be achieved with dietary manipulation by regulating lipid metabolism through the associated key enzymes [20]. It was predicted that inclusion of dietary betaine would significantly increase carcass yield % due to its methyl-group donor effects which would increase methionine, cysteine [21], and glycine availability for protein synthesis and also lead to reduction in fat deposition within the carcass by modifying metabolic pathways. However, this mode of action was not detected in the present study.

Results of increase in dressed carcasses weight (g) and yield % –economically- maximize the benefits of using betaine. That supports

the role of dietary manipulation with chosen feed additive and its dose in remodeling the carcass to meet customer wants [17]. Total body fat can be judged essentially through estimation of abdominal fat [22] which could decrease slightly through addition of betaine to the finishing diet [8]. The numerical decrease in the fat pad, in G3 as compared to G1 and G2 may be referred to betaine osmolyte role in reducing intestinal energy costs, leading to a lighter weight gut fat [23]. This strategy may also spare energy in terms of added oil.

Chicken LPL is an essential enzyme in the regulation of fat decomposition in the adipose tissues of chickens [24]. It is the main enzyme that hydrolyzes circulating triglycerides and might increase lipid uptake [25]. Although, LPL mRNA expression in abdominal adipose tissue might be modified in part by age and nutritional states of chicken [26], the results fairly supported the assumption of Sato and Akiba [26] who stated that the lipoprotein hydrolysis rate was dependent mainly on plasma lipoproteins biochemical characteristics and the substrate of LPL, but not on LPL mRNA expression. Betaine 0.1% might reduce LPL mRNA expression in abdominal adipose tissue through altering promoter CpG methylation distribution pattern [8].

Health, metabolism and nutrition status can be monitored through testing of serum biochemical indices, which help in detecting health

Table 5

parameters	ALT	AST	ALP	TP	ALB	GLB	UA	CRE	CHL	TG	HDL	LDL	
Control ^a	ALT	1	-0.118-	0.219	-0.693 ^a	0.155	-0.661-	-0.229-	-0.192-	-0.417-	0.192	-0.644-	-0.147-
	AST		1	-0.602-	0.614	0.317	0.183	-0.238-	-0.168-	-0.570-	-0.136-	-0.079-	-0.137-
	ALP			1	-0.554-	-0.200-	-0.242-	0.266	0.593	0.163	0.308	0.135	0.016
	TP				1	0.485 ^a	0.433	0	-0.026-	-0.030-	-0.098-	0.112	-0.318-
	ALB					1	0.36	0.056	0.057	0.098	0.101	0.308	0.239
	GLB						1	0.038	-0.120-	-0.173-	-0.331-	-0.074-	-0.493-
	UA							1	0.678 ^a	0.744 ^a	-0.022-	0.441	0.914 ^a
	CRE								1	0.393	-0.277-	0.329	0.688
	CHL									1	0.033	0.646 ^a	0.843 ^a
	TG										1	0.371	-0.476-
	HDL											1	0.447
	LDL												1
	Betaine groups ^b	ALT	1	-0.096-	-0.566-	0.058	0.029	0.048	0.128	-0.056-	-0.131-	0.497	-0.543-
AST		0.334	1	-0.159-	-0.552-	-0.399-	-0.386-	0.307	0.405	0.42	-0.257-	0.187	0.258
ALP		-0.478-	-0.171-	1	0.444	0.371	0.279	-0.092-	0.161	0.052	0.261	0.565	-0.385-
TP		-0.354-	0.35	0.353	1	0.478 ^a	0.403	0.659 ^{ab}	0.263	0.189	0.228	0.394	0.008
ALB		-0.670 ^a	0.422	0.309	0.413	1	0.09	0.082	-0.077-	0.428	0.039	0.408	0.491
GLB		-0.010-	0.163	0.239	0.836 ^{ab}	0.19	1	0.49	0.426	-0.011-	0.299	0.296	-0.263-
UA		-0.591-	0.28	0.229	0.488 ^a	0.498 ^a	0.301	1	0.723 ^a	0.625	-0.153-	0.449	0.491
CRE		-0.144-	0.48	-0.568-	0.134	0.303	-0.141-	0.588	1	0.563	0.137	0.542	0.297
CHL		0.074	0.197	-0.206-	0.211	-0.048-	0.309	-0.204-	0.386	1	-0.405-	0.797 ^{ab}	0.698 ^a
TG		0.148	0.147	0.291	0.456	-0.125-	0.691 ^a	-0.113-	-0.301-	0.263	1	-0.301-	-0.090-
HDL		0.175	0.5	-0.563-	0.214	0.11	0.153	0.154	0.831 ^{ab}	0.739 ^a	-0.075-	1	0.15
LDL		0.411	-0.222-	0.071	-0.068-	-0.449-	0.281	-0.759 ^a	-0.422-	0.722 ^a	0.476	0.039	1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

^b Upper diagonal (above 1) represented G2 (betaine 1.5 g/kg). Lower diagonal (below 1) represented G3 (betaine 3 g/kg).

disorders during the preclinical stage [27]. Therefore, these indices were used to assess the effect of betaine supplementation on performance and feed metabolism. Serum protein fractions help in transportation of lipids, vitamins, minerals, and hormones, with a role in osmotic pressure balance [28]. Increased concentrations of TP and globulin in serum might provide a favorable role of betaine on nutrient utilization, since betaine has a methyl donor function and could replace methionine in this function and the dietary methionine could be available for vital functions such as protein synthesis and immune modulation [19].

The different relationship between TP and its fractions could be attributed to the increased gamma fractions level including immunoglobulins and those synthesized in the cells of the reticuloendothelial system. TP concentration might be within physiological range, while the A/G ratio changes [29]. Metabolic status and health could be evaluated through measuring A/G ratio which could have clinical importance [30].

Testing the liver function enzymes and kidney markers was necessary, as they are the primary sites of betaine catabolism where a series of transmethylation reactions take place with the transfer of a methyl group from betaine to homocysteine [4]. The concentration of betaine could improve bone metabolism due to activity of alkaline phosphatase that could catalyze phosphomonoesters and nucleoside phosphates hydrolysis [30]. Principally AST in heart and liver tissue, which was low under normal conditions, but at high levels together with ALT in serum could be considered as sensitive markers of liver cell damage or for identifying and increase of hepatocytes permeability [27].

The increase in uric acid (UA), which is the main product of avian nitrogenous waste [31], and its correlation with betaine dose was contrary to Juráni et al. [32] who found that birds fed a diet with 640 mg/kg of betaine had a decreased concentration of serum uric acid which could be attributed to differences between doses of betaine used. This could explain the increased level of UA in treated groups, whereas, the increased level of serum TP indicated an efficient nutrition utilization especially the protein element which was reflected in the level of serum UA.

Blood lipid metabolites including levels of CHL, TG, lipoprotein fractions and fatty acids profile, are sensitive indicators of rate of fat metabolism concentration in chicken [33]. Inconsistency to the present results, Bogusławska et al. [34] observed increased plasma TG (18%) of betaine-fed pigs which could affect cholesterol metabolism, furthermore, Martins et al. [35] reported significant increase ($P > .05$) in blood serum concentrations of triglycerides in response to dietary betaine supplementation in broilers. Betaine might activate liver lipid mobilization and improve hepatic lipoprotein secretion as indicated by El-Shinnawy [36] which supported the strong positive significant correlation between TG and GLB obtained in G3. The trend of serum cholesterol observed in G3 was partially in agreement with Bogusławska et al. [34] who reported that betaine supplementation can lead to higher serum cholesterol. Additionally, Hayes et al. [37] indicated that betaine might affect cholesterol partitioning or might enhance the transportation of cholesterol in pigs. The lipotropic effect in betaine had lead to increase of serum total cholesterol and LDL in heat-stressed broilers [3,38]. In addition, betaine can activate lipolysis and inhibit lipogenesis through gene expression and the related activity of lipolytic-lipogenic-related proteins [3].

The results of the present study are in agreement with Martins et al. [35] who reported that supplementing broilers diets with betaine reduced production costs per kg live body weight. Improving profitability of broiler farms will shorten the capital cycle allowing for further investments in the industry. These findings agreed with Jurani et al. [32] who reported an improved economic performance attributed to improving FCR.

5. Conclusions

Incorporation of 0.15% and 0.30% organic betaine to male broilers diets during finishing period improves the growth performances. Using of betaine in a dose of 3 g/kg diet showed an improvement in serum protein profile with AST level, while both doses represented higher significant levels of uric acid and triglyceride compared to control. The use of betaine as a feed additive was economically feasible for the finishing period in male broilers. Effect of betaine supplementation on LPL gene expression of abdominal fat pad was not obvious and further studies are needed to investigate that effect.

Competing interests

None.

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