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Original Research Article

EFFECT OF DIETARY BETAINE AND LOW METHIONINE ON MULARD DUCK PERFORMANCE, BLOOD PARAMETERS AND LIPOGENESIS GENE EXPRESSION

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Abstract: The effects of dietary methionine and betaine (Bet) on productive performance, blood biochemical parameters and mRNA expression levels of fat acid synthase (FAS) and acetyl-CoA carboxylase (ACC), were investigated in Mulard ducks which raised in summer season. Three hundred one-day old healthy Mulard ducklings with similar body weight were randomly distributed into six groups with five replicates per treatment and ten ducks per replicate. Six diets were prepared as following: control diet (C); low methionine diet (ML); diet supplemented with Bet (0.25% Bet); diet supplemented with Bet (0.5% Bet); diet supplemented with 0.25% Bet and low in methionine (0.25% Bet + ML); diet supplemented with 0.5% Bet and low in methionine (0.5% Bet + ML). Regarding allover growth performance results revealed that increasing dietary Bet significantly increased (P<0.05) body gain of ducks by 13% when compared with the control diet, moreover both groups fed on 0.5% Bet and 0.5%Bet +ML diet exhibited the improved feed conversion ratio (1.88 and 1.93, respectively) when compared with control (2.13) and ML (2.29) groups. Inclusion of Bet either in control or ML diet significantly increased carcass yield, breast and thigh meat yield percent and decreased breast, thigh skin and abdominal fat percent. Nutrient digestibility was improved by adding Bet even in ML diet. Serum total lipids, triglycerides and total cholesterol constituents were significantly decreased in all group supplemented with Bet. Dietary Bet significantly decreased (P<0.05) mRNA expression of fatty acid synthase, acetyl-coA carboxylase genes, this decline was more obvious in control group with Bet than in ML with Bet. In conclusion, the role of Bet in ML diet was clear by improving productive performance of ducks, thus Bet can partially replace methionine in duck's diet.

Key words: Betaine; methionine; performance; carcass; lipogenesisgenes; Mulard ducklings

Introduction

The production prosperity of meat-type ducks can be achieved by good nutritional manipulations and the adjustment of their nutrient requirements is still required for maximizing productive performance. To enhance the production, sustain animals' health, economize feed-cost, supplementing

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feed-grade amino acids it is required (1,2). In growing chicks, previous studies found that supplementation with methionine (Met) could improve carcass quality and metabolism of protein (3). A corn-soybean meal-based broiler diet is often lack Met and supplementation of synthetic Met is an alternative way to meet the nutritional requirements of the bird. However, the unavailability and higher cost of synthetic Met often forces poultry feed nutritionists to search for alternative Met source. Consequently, reduction added DL-Met by supplementation of another dietary methyl donors may reduce Met requirement. Betaine (Bet), a trimethyl derivative of the amino acid Glycine, naturally extracted from sugar beet bulb and molasses, with low dietary Met, Bet can partially replace apart of Met, and can do its function and is as effective as Met in improving the growth performance and carcass quality of broilers (4), also, it may improve the availability of methionine (5). The main function of betaine as a donor of methyl groups like choline and methionine (6). Betaine can supply methyl groups for transmethylation reactions important for many metabolic processes like synthesis of proteins, energy metabolism (7) and also can partially substitute Met in the diet by providing a methyl group thus of homocysteine converted to methionine (8). Additionally, betaine can regulate fat distribution (9). Moreover, betaine has the potential to improve nutrient digestibility by augmenting the intestinal cells growth and health owing to its osmotic functions (10). An addition function of betaine within tissue is synthesis of carnitine, phosphatidylcholine, and creatine which play a key role inside the cells through protein and energy metabolism (11). In this regard, betaine significantly protects birds against the heat stress (12). Moreover, reduction of fat following to dietary betaine supplementation is more prominent than synthetic Met (13) as well, in adipose tissue, betaine supplementation declined the acetyl-CoA carboxylase (ACC), fat acid synthase (FAS) and malic enzyme activities as well as the FAS mRNA level in pigs decreasing the fat deposition (14). In Mulard ducks, although the effect of betaine on fat deposition was investigated, the molecular mechanism by which adipose tissue is down-regulated by betaine is poorly understood and FAS and ACC expression in Mulard ducks remain unknown. The main functions of betaine are methionine-sparing and fat-distribution effects. Accordingly, this study was conducted to investigate the effects of dietary supplementation of Bet with reducing Met on the performance, nutrient digestibility, carcass quality, fat distribution, blood parameters, mRNA expression level of FAS and ACC genes.

Material and methods

The experimental protocol was approved by Committee of Animal Welfare and Research Ethics Faculty of Veterinary Medicine, Zagazig University, Egypt.

Experimental birds and management

A total of 300Mulard ducks (one-day old) were obtained from a commercial hatchery. On arrival, they were weighed and randomly assigned to six groups, each consisting of five replicates of ten ducks each and raised for 42 days under the stress of Egyptian summer season. The temperature was kept at 33°C up to 3 days of age, and then it was reduced gradually to room temperature. Ducks were reared in naturally ventilated open house with sawdust as litter .

Experimental diets and design

The rearing period was divided into starter (up to 21 days) and finisher (22-42 days). The diets were formulated according to NRC (15) nutrition specification. The ducks were allowed free access to water and experimental diets were in wet mash form. The ingredient composition of the control diet is shown in Table (1). Six dietary treatments were prepared as following: control diet (C); low methionine diet; diet supplemented with 0.25% betaine; diet supplemented with 0.5% betaine; Diet supplemented with 0.25% betaine and low in methionine and diet supplemented with 0.5% betaine and low in methionine. In methionine low groups, no supplemented methionine was added to the diet, where the methionine level,

Table 1: Ingredient composition and calculated nutrient content of basal diets for Mulard ducks

Ingredient (%)	Starting diet (0-21)	Growing-overfeeding diet (22-42)
Corn	50	63.5
Soy bean meal (44)	37.3	21.6
Wheat bran	7	9
Soybean oil	2.5	2.6
Di-calcium phosphate	1.5	1.5
Ca carbonate	0.8	0.9
Na chloride	0.3	0.3
Premix[1]	0.5	0.5
Methionine	0.1	0.1
Calculated nutrient content		
Crude protein	21.67	16.24
Ether extract	2.65	3.07
Crude fiber	3.32	3.27
Metabolizable energy (kcal/kg)	2913	3012
Lysine	1.26	0.85
Methionine	0.42	0.36

Premix1 (per kg of diet): 10000 IU; Vitamin A, 5000 IU; vitamin D3, 16 mg; vitamin E, 11 mg; vitamin K3, 2.5 mg; Vitamin B1, 2.3mg; Vitamin B2, 7 mg; Vitamin B6: 4 mg; Vitamin B12: 14mg; Folic acid:1.3 mg; Biotin:140 mg; Calcium pantotenate: 25 mg; Nicotinic acid: 65 mg; Fe: 75 mg; Cu:9 mg; Mn:65 mg; Zn: 40 mg; I: 0.32 mg; Se: 0.15 mg.

in starter low methionine diet was 0.33% and in grower low methionine diet was 0.26%. Betaine was purchased from (Beta-key, Excentials, Netherland).

Growth performance

The body weight, gain, and feed intake of all groups of ducks were recorded weekly and feed conversion ratio (FCR), protein efficiency ratio (PER) at 21 and 42 d of age and overall performance were calculated.

Digestibility trial

At the end of feeding trail, each group was supplied by its original feed with addition of chromic oxide (0.3%, analytical marker) for 7 days (as a preliminary period) and then the excreta were collected daily on a plastic sheet for another 7 days (as a collecting period). The fecal samples were dried in hot air oven at 60 ºC for 72 h and were grounded. A complete proximate analysis was made for both feed and fecal samples for dry matter crude protein, fat and crude fibre as described by the Association of Official Analytical Chemists (AOAC) (16).

Carcass traits and meat quality

At 42 d of age five ducks from each group were selected at the end of the experiment, fasted overnight, weighed and then sacrificed to obtain weight of the dressed carcass, breast, thigh, and abdominal fat yields expressed as a percentage of live body weight. Samples were stored at -20ºC until analysis. The chemical composition for dry matter, crude protein and ether extract in breast and thigh was done according to AOAC (16). For meat quality measurements, right side of breast meat was used to determine Drip loss (%; proportionate weight loss of a sample hanging in a plastic bag for 48 h at 2°C). The same sample was used for cooking and thaw loss, after storage at −20°C, thaw loss (%; proportional weight loss of a meat sample before frozen storage at −20°C and after overnight defrosting at 4°C and cooking loss (%; proportionate weight loss of a sample after cooking in an open plastic bag in a water bath at 70°C for 40 min followed by cooling).

Serum biochemical analysis

Blood samples were collected at 42 d of age from 5 ducks per group into tubes without anticoagulant for collection of serum and with anticoagulant for haematological parameters . The separated serum was used for determination of total protein (T1949), albumin(A5503), globulin, triglyceride, (TR0100), high-density lipoprotein-cholesterol

(HDL-C), low-density lipoprotein-cholesterol (LDL-C) (MAK045) colorimetrically using kits from Sigma Aldrich, following the manufacturer's instructions.For haematological indices, total red blood cell counts (RBCs), haematocrit (HCT), haemoglobin (HGB),mean corpuscular volume (MCV), white blood cells (WBCs), lymphocytes and granulocytes were measured using an automated blood cell counter(Sysmex IV2000).

Sampling for real-time quantitative RT-PCR

Adipose tissue samples were collected immediately after slaughter, frozen in liquid nitrogen, and stored at −80°C till analysis for gene expression of fatty acid synthase, FAS, acetyl-coA carboxylase, ACC and β-Actin.

Real-Time quantitative RT-PCR for gene expression

Total RNA was isolated from abdominal adipose using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to manufacturer's instructions. Before RT-PCR, RNase-free DNase (Promega, Madison, WI) was used to remove contaminating genomic DNA from samples. The total RNA was reverse transcribed into cDNA, using QIAGEN Long Range 2 Step RT-PCR Kit, following manufacturer's directions. One µl of cDNA was mixed with 12.5 µl of 2x SYBR Green qPCR mix with ROX from Bio-Rad (USA), with addition of 5.5 µl of RNase-free water and 0.5 µl of each forward and reverse gene specific primers. The values for the specific targets were normalized using β-Actin as reference gene. The primer sequences from published were used as following of studied genes are the following: FAS gene; F: 5' TGG GAG TAA CAC TGA TGG C 3', R: 5'-GAT GGT GAG GAG TCG GAT-3' (Gene Bank ID, JQ080308), ACC gene, F: 5' TGC CTC CGA GAA CCC TAA 3' R:5'- TCC AGG CTT GAT ACC ACA 3'(Gene Bank ID, JQ080306) β-Actin, F: 5' CAA CGA GCG GTT CAG GTG T-3' R: TGG AGT TGA AGG TGG TCT CG 3'(Gene Bank ID, EF667345).

Statistical analyses

The experimental data were evaluated using One-way ANOVA procedure, post hoc comparisons were applied, whenever appropriate, using Duncan's test. All statistical measures were performed using PASW statistics 18 (SPSS Inc., USA). Statistical significance was considered at $P \leq 0.05$. Fold change was considered using the (2-ΔΔCt) method to quantify mRNA levels.

Results

The effect of replicates was non-significance, so all data was merged together.

Ducks growth performance and nutrient digestibility

A growth response to betaine (Bet) supplementation in control diet or in methionine low (ML) diet is shown in Table (2). Ducks in 0.5% Bet group had the highest ($P< 0.05$) final body weight (FBW), followed by the ducks in (0.25% Bet) and 0.5% Bet with ML groups, then 0.25% Bet with ML when compared with the control group, while ML group recorded the lowest FBW. The all over values of feed conversion ratio and protein efficiency ratio were improved in groups fed 0.5% Bet and 0.5% Bet with ML groups followed by 0.25% Bet when compared with the other groups.

Dietary supplementation of 0.25 and 0.5% Bet to Mulard ducks improved DM digestibility by 2.9 and 3.5%, respectively. The reduction of methionine content in duck's diet with Bet supplementation improved the DM especially with 0.5 % Bet. Moreover, crude protein digestibility significantly increased ($P< 0.05$) in groups supplemented with dietary Bet either in control or ML diet. In relation to fat and crude fiber digestibility, addition of Bet especially 0.5% significantly increased ($P< 0.05$) their digestibility (Table 2). In accordance with this result, crude protein (CP), crude fiber (CF) AND ether extract (EE) digestibility were higher with Bet supplementation either in methionine supplemented group or methionine deficient groups compared to the control group.

Table 2: Effect of two levels of dietary betaine supplementation in normal and low methionine diets on all over growth performance and nutrient digestibility of Mulard ducks (d 42)

	Control	Methionine low (ML)	Betaine 0.25%	Betaine 0.5%	Betaine 0.25%+ ML	Betaine 0.5%+ML	*P*-value
Allover Growth Performance							
Initial body weight, g/bird	59.50±0.24	61.00±0.44	60±0.20	59.80±0.30	60±0.40	60.5±0.40	0.55
Final body weight, g/bird	2909±11.84^{d}	2783±9.27^{e}	3211±6.19^{b}	3354±8.46^{a}	3087±6.11^{c}	3185±3.12^{b}	<0.001
Body weight gain, g/bird	2849 ±11.85^{d}	2722±9.33^{e}	3151± 6.24^{b}	3294 ±8.5^{a}	3027±6.14^{c}	3125±3.12^{b}	<0.001
Feed intake, g/bird	6083±32.65^{bc}	6228 ±34.49^{abc}	6395± 34.78^{ab}	6187±30.07^{abc}	6479±44.77^{a}	6023±40.25^{c}	<0.001
Feed conversion ratio	2.13±0.03^{b}	2.29±0.03^{a}	2.03 ± 0.02^{bc}	1.88±0.01^{d}	2.14 ±0.02^{b}	1.93±0.04^{cd}	<0.001
Protein efficiency ratio	2.25±0.03^{c}	2.10 ±0.03^{d}	2.37 ± 0.02^{b}	2.55 ±0.02^{a}	2.24±0.02^{c}	2.49±0.05^{a}	<0.001
Digestibility %							
Dry matter	89.40±0.12^{c}	87.57±0.18^{d}	92.30±0.12^{b}	92.93±0.09^{a}	91.40±0.85^{b}	92.33±0.13^{b}	<0.001
Crude protein	82.53±0.15^{c}	79.90 ±0.15^{d}	88.33±0.13^{a}	89.40±0.12^{a}	89.33±0.03^{a}	88.70±0.40^{a}	<0.001
Ether extract	68.53±0.19^{d}	67.70±0.15^{e}	73.50±0.06^{b}	75.77±0.09^{a}	70.33±0.03^{c}	70.53±0.03^{c}	<0.001
Crude fibre	32.40±0.06^{b}	28.53±0.19^{c}	32.33±0.15^{b}	33.50±0.07^{a}	32.20±0.1^{b}	32.47±0.09^{b}	<0.001

Values were means ± standard error. Values with different superscripts within a row were significantly different (P<0.05).

Carcass Characteristics and meat quality

Results related to carcass traits and meat quality are shown in Table (3). The carcass weight yield and dressing % were significantly higher (P< 0.05) in Bet supplemented groups even in ML diet when compared with control group or ML group, moreover the percentage of skin was significantly decreased by increasing of dietary Bet. The lowered abdominal fat % was found in group with 0.5% Bet followed by groups supplemented with 0.25% Bet and 0.25 and 0.5% Bet with ML diet, there were no significance difference between the latter groups, when compared with control and ML group. Concerning meat chemical composition, increasing the level of Bet from 0.25 to 0.5% significantly increased (P< 0.05) the breast and thigh dry matter and crude protein content and in contrast, the breast and thigh fat content were significantly decreased. Also, with increasing the level of Bet from 0.25 to 0.5% in ML diet, the protein content was significantly increased in thigh and breast when compared with control group or ML group. The drip loss, thaw loss and cooking loss % of whole carcass meat were significantly lowered for ducklings fed diet supplemented with Bet, moreover reduction of methionine together with betaine decreased these previous parameters, as compared to those fed the control and ML diet.

Gene expression

Data related to mRNA expression of fatty acid synthase (FAS) and acetyl-coA carboxylase (ACC) genes are shown in figure 1. The results revealed that mRNA expression of FAS gene reached its lower level (P< 0.05) in Mulard duck group fed on control diet supplemented with 0.5% Bet. In addition, inclusion of dietary 0.25% Bet, 0.25% Bet + ML or 0.25% Bet + ML significantly decreased (P<0.05) the expression of FAS gene, with no significance difference in latter groups, when compared with control and methionine low groups. In regard to ACC gene expression, the groups of ducks supplemented with dietary 0.25 and 0.5% Bet exhibited the lower expression followed by groups supplemented by dietary 0.25 and 0.5% Bet +ML in comparison with the control and ML diet.

Table 3: Effect of two levels of dietary betaine supplementation in normal and low methionine diets on carcass characteristics, meat chemical composition on dry matter basisand meat quality in Mulard ducks at slaughter (d 42)

	Control	Methionine low (ML)	Betaine 0.25%	Betaine 0.5%	Betaine 0.25%+ ML	Betaine 0.5%+ML	*P*-value
Carcass weight, g	2288 ± 11.51^{d}	2148 ±7.42^{e}	2588 ±7.25^{b}	2725±8. 61^{a}	2467±6.20^{c}	2566±3.15^{b}	< 0.001
Carcass yield g/kg	1047 ± 9.77^{d}	903 ± 8.51^{e}	1047 ± 8.71^{b}	1488 ±8.31^{a}	1236±6. 40^{c}	1337±3.15^{b}	< 0.001
Dressing %	77.32±0.01^{c}	76.38 ±0.15^{d}	77.92 ± 0.03^{b}	79.15±0.05^{a}	77.34±0.07^{c}	77.98±0.03^{b}	< 0.001
Carcass parts, %							
Breast yield	50.72 ± 0.09^{c}	47.15±0.06^{e}	51.69 ± 0.09^{b}	53.44 ±0.07^{a}	50.31 ±0.04^{d}	51.76 ± 0.07^{b}	< 0.001
Breast skinless	40.56 ±0.04^{c}	39.54 ±0.07^{d}	42.52 ± 0.09^{b}	44.65 ±0.05^{a}	40.78±0. 2^{c}	42.28 ±0.1^{b}	< 0.001
Breast skin	12.06 ±0.05^{a}	12.20 ±0.05^{a}	10.72 ±0.06^{c}	10.38 ±0.08^{d}	11.13 ±0.06^{b}	10.74 ±0.05^{c}	< 0.001
Thigh yield	30. 64 ±0.08^{d}	28.71 ±0.1^{e}	31.93 ±0.04^{b}	32.50 ±0.05^{a}	31.42 ±0.5^{c}	31.21 ±0.1^{c}	< 0.001
Thigh skinless	22.32 ±0.04^{c}	22.12 ±0.05^{c}	24.11 ±0.05^{b}	24.50 ±0.09^{a}	23.82 ±0.05^{b}	23.94 ±0.2^{b}	< 0.001
Thigh skin	9.45 ±0.1^{a}	9.58 ±0.03^{a}	7.46 ±0.07^{c}	6.41 ±0.03^{cd}	8.43±0.03^{b}	8.19 ±0.06^{b}	< 0.001
Abdominal fat, %	1.93 ±0.05^{a}	1.87 ±0.03^{a}	1.64 ±0.04^{b}	1.35 ±0.02^{c}	1.58 ±0.03^{b}	1.52±0.05^{b}	< 0.001
Chemical composition, %							
Breast DM	26.78 ± 0.08^{b}	25.7 ±0.06^{c}	26.82 ±0.05^{a}	26.89±0.03^{a}	26.42±0.04^{b}	26.75±0.04^{b}	< 0.001
Thigh DM	28.33 ± 0.07^{a}	27.66 ± 0.08^{b}	28.33 ± 0.07^{a}	28.48 ±0.05^{a}	28.31±0.05^{a}	28.31±0.04^{a}	< 0.001
Breast crude protein	73.49±0.06^{e}	71.23 ±0.03^{f}	75.80 ± 0.04^{b}	76.35±0.02^{a}	73.70±0.09^{d}	74.76 ±0.06^{c}	< 0.001
Thigh crude protein	70.42 ± 0.07^{e}	68.64±0.1^{f}	72.26 ± 0.04^{b}	72.82 ±0.07^{a}	70.87 ±0.03^{d}	71.70 ± 0.04^{c}	< 0.001
Breast ether extract	13.33 ±0.01^{b}	14.66 ±0.08^{a}	12.70 ± 0.07^{c}	10.76 ±0.1^{f}	11.56±0. 05^{d}	11.23 ±0.06^{e}	< 0.001
Thigh ether extract	16.42 ±0.09^{a}	16.48 ±0.1^{a}	15.48 ±0.12^{b}	13.16 ±0.07^{e}	14.52 ±0.11^{c}	13.60 ±0.8^{d}	< 0.001
Meat quality							
Drip loss, %	0.98 ±0.04^{a}	1.04 ±0.08^{a}	0.68 ±0.1^{b}	0.64 ±0.09^{b}	0.76 ±0.05^{ab}	0.62 ±0.06^{b}	< 0.001
Thaw loss, %	4.76 ±0.1^{b}	5.94 ±0.04^{a}	3.72 ±0.11^{c}	3.50 ±0.22^{c}	3.84 ±0.22^{c}	3.64 ±0.18^{c}	< 0.001
Cooking loss, %	13.06 ±0.18^{ab}	13.54 ±0.19^{a}	12.32 ±0.1^{c}	11.5 ±0.26^{d}	12.52±0.1^{bc}	12.06 ±0.09^{cd}	< 0.001

Values were means ± standard error. Values with different superscripts within a row were significantly different ($P < 0.05$).

Table 4: Effect of two levels of dietary betaine supplementation in normal and low methionine diets on serum and blood hematological parameters in Mulard ducks at slaughter (d 42)

	Control	Methionine low (ML)	Betaine 0.25%	Betaine 0.5%	Betaine 0.25%+ ML	Betaine 0.5%+ML	*P*-value
TP, g/ dl	4.18±0.11^{c}	3.58±0.12^{d}	4.9±0.06^{b}	5.2±0.07^{a}	4.2±0.07^{c}	4.7±0.07^{b}	< 0.001
Albumin, g/ dl	1.9±0.18^{ab}	1.48±0.12^{c}	2.1±0.07^{a}	1.84±0.09^{ab}	1.46±0.1^{c}	1.58±0.1^{c}	< 0.001
Globulin, g/ dl	2.3±0.09^{c}	2.1±0.05^{c}	2.7±0.06^{b}	3.3±0.09^{a}	2.7±0.07^{b}	3.14±0.08^{a}	< 0.001
Bet. Homocystine-transferase	1.6±0.07^{c}	1.3±0.05^{d}	2.3±0.09^{b}	2.7±0.07^{a}	2.4±0.05^{b}	2.4±0.05^{b}	< 0.001
Triglycerides, mg/dl	3.7±0.1^{a}	3.6±0.1^{ab}	3.3±0.05^{bc}	3.3±0.1^{bc}	3.4±0.06^{abc}	3.1±0.04^{c}	< 0.001
HDL-C, mg/dl	128.2±0.6^{b}	132.0±0.6^{a}	127.0±0.7^{bc}	123.4±0.5^{d}	125.6±0.7^{cd}	123.8±0.5^{d}	< 0.001
LDL-C, mg/dl	87.8±0.9^{a}	85.8±0.9^{b}	81.2±0.5^{cd}	81.0±0.7^{cd}	82.2±0.7^{c}	79.2±0.4^{d}	< 0.001
Blood hematological parameters							
RBC, M/µl	2.1±0.06^{c}	2.0± 0.07^{c}	2.7±0.06^{b}	3.1±0.07^{a}	2.9±0.04^{ab}	3.0±0.07^{ab}	< 0.001
HGB, g/dl	12.6±0.7^{c}	12.0±0. 2^{d}	15.6±0.1^{b}	17.2±0.2^{a}	16.8±0.2^{a}	17.2±0.2^{a}	< 0.001
MCV, Fl	128.8± 0.6^{d}	123.0±0.9^{e}	150±0.9^{a}	146.4±0.6^{b}	141.6±0.5^{c}	145.6±0.6^{b}	< 0.001
WBCs x10^{3}, M/µl	7.5±0.07	7.3±0.2	7.6±0.09	7.5±0.07	7.5±0.06	7.4±0.08	0.1
Lymphocytes x10^{3}, M/µl	5.6±0.1	5.7±0.07	5.3±0.2	5.3±0.6	5.2±0.9	5.6± 0.7	0.09
Granulocytes x10^{3}, M/µl	2.3±0.1	1.9±0.2	1.8±0.7	1.7±0.8	1.9±0.1	2.0±0.3	0.2

TP: Total protein; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Values are means ± standard error. Values with different superscripts within a row are significantly different ($P < 0.05$).

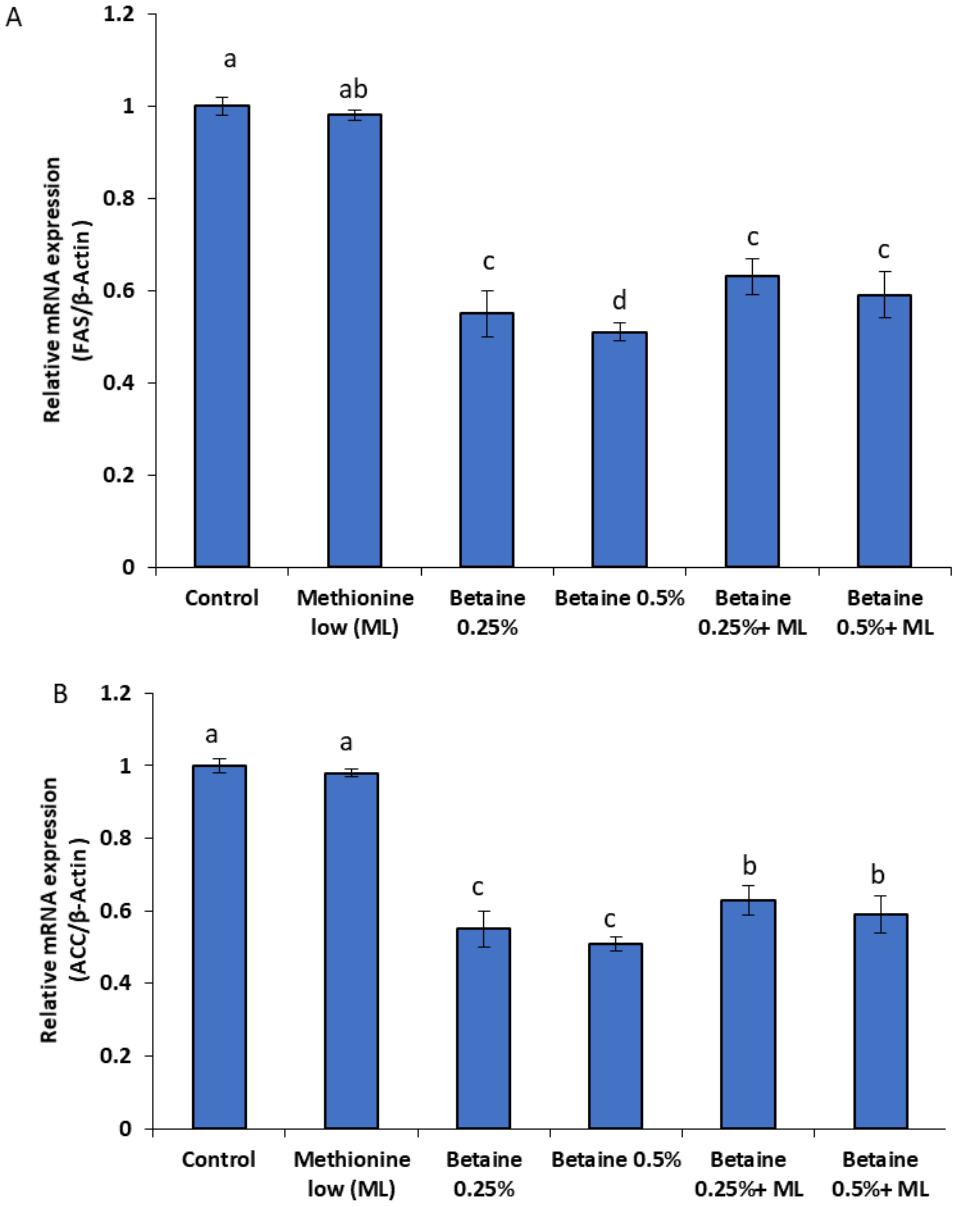

Figure 1: Effect of two levels of dietary betaine supplementation in normal and low methionine diets on the relative mRNA expression A) fatty acid synthase gene, FAS B) acetyl-coA carboxylase, ACC gene of Mulard ducks at slaughter (d 42)

Blood parameters

Data related to serum and hematological constituents are shown in Table 4. Significant interaction was found between supplementation of Bet as methyl donor either in normal diet or methionine deficient diet and total protein. With increasing levels of Bet, the value of total protein was also increased even in methionine deficient diet supplemented with betaine. As serum lipid profile is an important index of lipid metabolism, betaine revealed a lowered significant effect on triglycerides (TG) and low-density lipoprotein (LDL-C) and higher levels of high-density lipoprotein (HDL-C). In addition, increased count of RBCS was observed with addition of betaine in normal control diet or in methionine deficient diet. While there was no significance difference between WBCs, lymphocytes and granulocytes count between different groups.

Discussion

Effects on duck performance and nutrient digestibility

Dietary inclusion of betaine (Bet) up to 0.5% significantly increased gain and feed efficiency of ducks, also our results suggest that betaine can partially replace the methionine in maize-soybean diets of meat-type ducks and resulted in good productive performance. These findings were supported by Su et al. (18) that geese supplementation with betaine (1 g/d/ goose) could increase the final live body weight. Recently, yang et al. (19) reported an improvement in growth performance parameters of goose fed on Met-deficient diets supplemented with betaine (600mg/ kg diet). Likewise, addition of dietary Bet by 1000 mg/kg to broilers chicks significantly enhanced their growth performance in a 10% Met-reduced diet (11). Moreover, Chen et al. (20) suggested that supplementation of dietary Met supplementation at optimum level could increase growth performance in growing birds. In addition, our results are in agreement with reports about using different levels of Bet (0.5, 1 and 1.5g/Kg diet) for Domyati ducks (21) and (0.1, 0.2 and 0.4%) for broilers (22) significantly improved body weight gain and feed utilization (12). The improved performance due to Bet supplementation in ducks might be attributed to several factors as increasing intestinal integrity (23), stimulating growth hormone and insulin-like growth factor-I (24), increasing intestinal immunity (25). Moreover, the osmolytic function of betaine influence positively on the growth, survival and integrity of intestinal cell (26).Thus, it appears that betaine in a marginally methionine low diet could lead to corresponding growth response in ducks, and that betaine could spare a small portion of methionine which agreed with Zhan et al.(27).Observations of current study about digestibility were in agreement with the findings of FariaFilho et al. (28) concerning weight gain and feed conversion ratio of an efficient nutrient degradation and absorption. Also, in monogastric animals' fermentation process in the gastrointestinal system is affected by dietary betaine supplementation (29). The role of betaine in improvement of nutrient digestibility could attribute to its osmoprotective properties, protecting intestinal epithelium and augment the growth of beneficial intestinal microorganism (10). On other hand, poultry lacks fiber-degrading enzymes and the improvement in fiber digestibility indicates that betaine has the potential to stimulate the bacterial fermentation of dietary fiber (30). Also, El-Husseiny (31) reported that in methionine-deficient diet supplementation of 0.05-0.10% to broilers chicks fed on dietary betaine significantly increased the digestibility of organic matter, protein, fat and fiber. According to Rørvik et al. (32), the potential role of betaine on ether extract digestibility can explained by an increased bile acid secretion.

Effects on carcass characteristics and meat quality

The obtained results suggested that it would be ideal to optimize the quantity of supplemental methionine with Betaine, which has a positive influence on carcass meat yield which agreed with Waldroup et al. (33). The results in accordance with Chand et al. (34) who revealed that betaine supplementation significantly ($P< 0.05$) improved dressing percentage and in methionine deficient the breast yield of broilers was increased with Betaine supplementation (5). Similar results were reported with dietary betaine (0, 0.075, 0.150 and 0.225%) inclusion in broilers (35). Other interesting perspective of betaine inclusion in poultry' diets are decreasing carcass fat content associated with increasing the lean meat which may be satisfy consumer needs (22) and this property of betaine may be related to its methyl group donor properties (29),higher availability of methionine and cystine for protein deposition (36) and enhanced utilization dietary amino acids for protein synthesis that may result in fewer amino acids available for deamination and eventual synthesis of adipose tissue (37). Also similar to the present study, Neoh and Ng (38) reported that addition of dietary betaine improved carcass yield and breast percentage as well as reduced abdominal fat. Accordingly, variations

in hormone levels and growth factors involved in the regulation of fat synthesis and degradation, as well as lower activities of lipogenic enzymes, have been observed following dietary betaine supplementation (14). A potential explanation for decreasing abdominal fat of dietary betaine may be due to its major effect on lipid metabolism as a result of activating carnitine synthesis in liver and muscle (27) and synthesis of lecithin which was responsible for fatty acids transport to the mitochondria for oxidation, accordingly, lipid deposition was reduced (29). Awadet al. (12) reported that, followed to feeding of Domyati ducklings on dietary betaine (0.5, 1.0 and 1.5 g /kg) eviscerated carcass was significantly increased by 8.34, 13.73 and 16.92 %, and abdominal fat percentage was significantly reduced as compared to the control.

The results related to meat quality in our study revealed that betaine inclusion in control diet or in methionine low diet improved drip loss, thaw loss and cooking loss percentage. This result is in agreement with those obtained by Alirezaei et al. (11) who reported that adding Bet 1 gm/kg to a Met-deficient diet significantly improved meat quality as betaine function could lead to high water retention in broilers exposed to cyclic heat stress (39) and maintain cell integrity and function by regulating water inside and surrounding the cell (40).

Effects on expression of genes responsible for Lipogenesis

Concerning mRNA expression of fatty acid synthase gene, FAS and acetyl-coA carboxylase, ACC in abdominal adipose, addition of betaine significantly decreased their expression especially with 0.5%, also when increasing dose of betaine to 0.5%, betaine could replace part of methionine in duck's diet. The finding of our results was in accordance with the lipotropic effect of betaine because betaine supplementation might result in a decrease in the lipogenesis, as evidenced by the decrease in fatty acid synthase mRNA expression in abdominal adipose tissue and to decrease the activities of acetyl-CoA carboxylase, in finishing pigs after Betaine supplementation (14). Furthermore, betaine acts as methyl group donor for transmethylation reactions needed for synthesis of carnitine and thus altering animal fat metabolism (5). Moreover, betaine may reduce the mRNA level for fatty acid synthase and adipocyte type acid binding protein (41).

Effects on blood parameters

In this study, dietary Bet supplementation positively affect on total protein level, lipid fractions of serum and RBCs counts even in methionine deficient diet. In accordance with our results, Rao et al. (5) described that following Bet supplementation for broilers, total protein, triglycerides (TG) and total cholesterol, free fatty acids (FFA), low-density lipoprotein cholesterol and high-density lipo-protein cholesterol in serum were improved, particularly at the lower Met concentrations compared with the control group which evidence that described Bet can alter lipid metabolism (22). Additionally, Ratriyanto et al. (10), found that in laying hens, betaine enhanced hormone-sensitive lipase activity, leading to a higher levels of serum FFA through activating the hydrolysis of TG to glycerol thus lower the concentration of TG and cholesterol in serum. The results also correlated with the decreased plasma TG and LDL, as Bet is associated with the carnitine metabolisms, which in turn is involved in mitochondrial transport and the fatty acids oxidation (42).

In addition, Park and Kim (43) found that Bet supplementation resulted in more significant improvement in hematological indicators such as RBCs and platelet count than the heat stress control group (P <0.05). The higher values of hematological indices in the ducks fed a dietary Bet than those under heat stress fed a basal diet, suggested that betaine may contribute to synthesis of methionine and control of osmotic pressure surrounding cells (6,26). Thus, the protective effect of betaine for erythrocytes is clear in the current study by increasing their count, therefore protecting against anemia (hemorrhagic and hemolytic). Whereas the decrease of hemoglobin causes microcythemia. As, there is a correlation between hematocrit and packed red cells

volume; when the hematocrit means decreases, the levels of hemoglobin and hemoglobin become lower, their high value following betaine supplementation indicating preventing effect of betaine from iron deficiency anemia specially in the period of stress as heat (44).

Conclusion

It could be concluded that the dietary betaine supplementation up to 0.5% has a positive effect on the productive performance of Mulard ducks and also regulates lipogenesis gene expressions in adipose tissuesthus controlling body fat accumulation in ducks. Methionine could be partially replaced by betaine and there is a positive interaction between the two compounds which was clearly affect on performance of ducks, regulate lipid synthesis. Hence, addition of 0.5% betaine with 0.1% deficiency in methionine than NRC recommendation can be used in feeding of Mulard ducks.

Conflict of interest

The authors declare no conflict of interest.

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