COMBINATION OF BETAINE AND SOME OTHER METHYL DONOR COMPOUNDS TO IMPROVE GROWTH PERFORMANCE, LIPID METABOLISM, AND LIVER FUNCTION OF BROILERS.

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SUMMARY

The effect of betaine, Dl-methionine, and choline on the growth performance, lipid profile, liver function, lipid peroxidation, and carcass characteristics of broilers was examined. A total number of 240 broiler chicks were divided into four different groups (control and three groups treated with 1g betaine , 0.5g betaine + 0.5 g DL Methionine or 0.33g betaine + 0.33 g DL- Methionin +0.33 g choline / liter via drinking water). The results indicate that weight gain of broilers treated with betaine is significantly higher than control while combination of betaine by methionine or choline did not cause any significant deference in body weight gain. Also, the methyl donor compounds significantly reduced the percentage of abdominal fat in carcass. Treatment with betaine or mixture of betaine and methionine and choline led to significant reduction in total lipids, triglycerides, and LDL cholesterol. Betaine and other methyl donor compounds reduce the level of lipid peroxidation in liver tissues as indicated by observed reduction in malondialdhyde (MDA) contents in liver tissues or plasma. In addition, the activity of liver enzymes, AST, ALT and ALP in serum was reduced by betaine treatments which reflect the protective effect of betaine and other methyl donor compounds on the liver tissues. Histological observations support the performance data where the growth performance of the treatment groups was significantly improved without any undesirable effects on the liver histology. It could be concluded that treatment with betaine, methionin and choline improves broiler growth performance, regulate lipid metabolism and protect liver tissues.

Keywords: *broilers, betaine , methyl donor compounds, carcass characteristics, liver tissues, blood.*

INTRODUCTION

Betaine,the common term ,is a naturally occurring amino acid derivative found in a variety of feedstuffs of planet and animal origin. Betaine has tow primary metabolic roles: it is a methyl group donor and it is an osmolyte that assitsts in cellular water homeostasis (petronine *et al*;1992).

Betaine, choline and methionine can serve as sources of methyl (-CH3) group. It is well understood that choline may acts as methyl group donor but, in order to function as a methyl group donor, it needs to be converted to betaine in the mitochondria (Molitoris and Baker, 1976). Many studies have examined the interrelationship between choline and methionine to determine if these compounds can spare the needs of the chick for methionine with considerable variation in results.while some studies (Virtanen and Rossi,1995) suggest that the response of broiler growth to betaine was greater than the obtained from the addition of methionine , other have failed to demonstrate that the methionine content of the diet could be reduced by supplementation with betaine (Rostangose and Pack, 1996,Mc Devit *et al*, 2000). However, several studies suggest that addition of betaine may improve breast meat yield (schutte *et al*.., 1997, Mc Devite *et al* ., 2000). Betaine is indirectly involved in the synthesis of carnitine , which is required for transporting long chain fatty acids across the inner mitochondrial membrane for oxidation (Mc Devitt et al., 2000) and therfore may result in a baner carcass.

The objective of this study was to evaluate the effect of betaine as methyl donor and its combination with DL-methionine and choline on broiler performance, carcass traits, liver function , lipid profile , and liver and plasma lipid peroxidation.

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MATERIALS AND METHODS

Design and management:

A total number of two hundred and fourty day old broiler chicks) were randomly distributed into four treatment groups (60 birds\ group with three replicates) . Each group was nearly equal in average body weight.The first group was a control group. Additionally 1.0 g\ L of betaine, 1g\L mixture of betaint+ DL methonine and 1 g\L mixture of (betaine+ DL.methionine + choline) administration via drinking water for group two, three and four respectively.

A commercial basal diet was formulated to meet the nutritional requirements of the birds (NRC,1994).The composition of the experimental diet are presented in Table (1).

Feed and water were available ad libitum during the experimental period (six weeks). During the experimertal Period, birds were raised under similar environmental, hygienic and managerial conditions. Individual live body weight of chicks was recorded at 1,3 and 6 weeks of age. Feed consumption was determined per sub group in the same periods.Feed conversion efficiency was calculated (feed consumption g \ live weight gain, g).

Table (1) . Composition of the experimental basal diets.

Ingredient	%
Yellow corn	61.0
Soybean meal (44%)	28.0
Broiler concentrate[1]	10.0
Vitamin and minerals Premix[2]	1.0
Total	100
Calculated Values	
ME Kcal/Kg.	2882.9
Crude Protein(%)	23.28
C/P ratio	132
Methionine (%)	0.46
Lysine (%)	1.23
Ca %	0.98
Available P %	0.61

[1] *Broiler concentrate containing 48% CP, 2088 ME kcal/kg diet, 1.53% Meth., 2.78% lysine, 10.0% calcium, 3.63% avilable phosphorus, 3% NaCl, 0.72% crude fiber and 6.45 crude fat.*

[2] *Provide Per kilogram of diet: vitamin A(as all-trans-retinyl acetate); 5.500 IU; vitamin E (all rac-a tocopheryl acetate); 11IU ; menadione (as menadione sodium bisufite); 1.1 mg; Vit D3, 1.100 ICU; riboflavin, 4.4 mg vitamin B12, 12.1 ug; vitamin B6, 2.2 mg ; thiamin (as thiamin mononitrate); 2.2 mg; folic acid, 0.55mg d-biotin, 0.11 mg. Trace mineral (mg/kg diet): Mn , 60; Zn, 50; Fe, 30; Cu, 5; Se.0.07.*

Body and organs examination :

Ten unsexed chickens per treatment were randomly chosen and sacrified at 42 days old to determine carcass taits and giblets weight. Immediately after slaughtering, representative samples of breast muscles and livers were taken, fixed in 10% formaline and prepared by the ordinary histological techniques. Other samples of fresh liver were put into saline solution to determine liver lipid peroxidation.

Blood biochemical parameters:

Blood samples were collected in heparinized tubes from ten birds \ group mentioned above. These samples were centrifuged at 3000 rpm /min for 15 minutes, clear plasma was separated, then stored in a deep freezer at -20°C until the time of biochemical analysis. Plasma samples were subjected to biochemical analysis using the specific kits produced by Boeheringer and Merck companies. Plasma was submitted for determination of Albumin, total lipids ,triglycerides, cholesterol,LDL and HDL .,as well as activities of aspartate aminotransaminase (AST) and alanine transaminase(ALT) .

Albumin (g\L) was determined by the method described by Doumas *et al*, (1971). Total lipids (g\L) was determined according to knight *et al* , (1972) and cholesterol (mg\dL) according to Richmond, (1973), Triglycerides (mg\dL) was also estimated by the method of Trinder, (1969) . Activites of alanine transaminase (A L T) (U\L) und aspartate transaminase (AST) (U\L) were colorimetrically determined by using commercial kits according to Reitman and Frankel, (1957). Alkaline phosphatase (ALP) was also determined as described by Brooks and Purdy , (1972).

Malondialdehyde (MDA) was determined in blood plasma and intact liver homogenate by the thiobarbituric acid reaction (Dorman, *et al*, 1995).

Statistical analysis:

Data were statistically analyzed according to ANOVA procedures of SAS (SAS institute , 2002). Means differences were compared using Duncan's multiple range test (Duncan,1955).

RESULTS AND DISCUSSION

Betaine and other methyl donor compounds improve growth performance of broilers.

As shown in Table (2) significant increase in body weight at 6 weeks of age in case of betaiene treatment followed by betaine + DL methione + choline treatment as compared with untreated control group. Also significant increase (P>0.05) in body weight gain at the same period was recorded.

Concering feed consumption, (betaine+ DL methione+ choline) treatment had significant effect on feed to gain at 3 periods (Table 2). Significant improvement (p>0,05) of average FCR in the above treatment at 2-3 weeks of age. Our results are in agreement with those obtained with schutte *et al..*, 1997 who reported that feed efficiency was more improved by be betaine than by choline , while , Lowry and Baker, (1987) showed contrast results. Mathews *et al*, 1997) reported that betaine increased average daily gain and feed intake of coccidiosis infected chicks but decreased average daily gain and feed intake in uninfected chicks.

Table (2): growth preformance of broilers treated with different methyl donor compounds.

Item	Age	Control	Betaine	Betaine+DL-Methionine	Betaine+DL-M+choline
Body weight (g)	1week	143.78 ± 2.35^a	147.69 ± 2.62^a	145.45 ± 2.98^a	144.12 ± 2.89^a
	3week	486.33 ± 11.84^a	521.59 ± 13.57^b	519.51 ± 12.12^b	517.59 ± 12.68^b
	6week	1591.87+42.47^b	1777.96+43.55^a	1662.64+30.63^{ab}	1685.79+39.87^{ab}
Daily weight gain (g)	1-3 weeks	24.45 ± 0.82^a	26. 67 ± 0.91^a	26.67 ± 0.91^a	26.87 ± 0.92^a
	3-6 weeks	53.32 ± 2.07^a	58.25 ± 1.78^a	53.95 ± 1.56^a	55.81 ± 2.00^a
	1-6 weeks	41.88 ± 1.16^b	46.52 ± 1.22^a	43.38 ± 0.87^{ab}	44.12 ± 1.14^{ab}
Daily feed consumption (g)	1-3 weeks	50.79 ± 1.57^a	48.79 ± 1.00^b	50.39 ± 1.10^a	48.17 ± 0.94^b
	3-6 weeks	114.58 ± 1.60^b	123.92 ± 1.79^a	114.37 ± 1.53^b	114.29 ± 0.99^b
	1-6 weeks	89.06 ± 1.59^b	93.87 ± 1.43^a	88.78 ± 1.35^b	87.85 ± 0.97^c
Daily feed conversion (feed (g)/gain(g))	1-3 weeks	2.25 ± 0.08^a	2.01 ± 0.09^b	2.01 ± 0.07^b	1.95 ± 0.08^b
	3-6 weeks	2.52 ± 0.19^a	2.29 ± 0.09^a	2.27 ± 0.08^a	2.27 ± 0.08^a
	1-6 weeks	2.29 ± 0.11^a	2.13 ± 0.07^a	2.11 ± 0.05^a	2.10 ± 0.07^a

**Means having different letters at the same row are differ significantly.*

=(P<0.01); NS=Not Significant.

Betaine and other methyl donor compounds enhance carcass characteristics.

Carcass composition data are presented in (Table 3). Betaine supplementation alone or in addition to choline or DL methionine resulted in a slight but non significant increase in caracass and giblets. Relative to carcass,the effect of either betaine or its combinationen with D-L methionine and choline on abdominal fat was a reduction of abdominal fat deposition comparing with the control group. These results are in agreement with those of Jahanian, and Kahman, (2008). Mc Devit *et al*,(2000) found a significant reduction in relative visceral mass by betaine and D-L methionin, which suggests that the effect of betaine observed can be due to a reduction of relative weight of the intestines. Besides a possible methionine sparting effect , betaine may also interact with the lipid metabolism by stimulating the oxidative catabolism of fatty acid via its role in carnithine synthesis . Thus offering a potential for reduced carcass fatness in commercial production (Schutte, *et al*, 1997).

Table (3): Carcass characteristics of broilers treated with different methyl donner compounds.

Treatments	Pre-slaughter	Carcass %	Liver %	Heart %	Gizzard	Giblets %	Abdominal fat %
Control	1855 ± 67.64[a]	71.29 ± 4.51[b]	2.89 ± 0.11[a]	0.56 ± 0.02[a]	1.69 ± 0.12[a]	5.14 ± 0.16[a]	1.23 ± 0.37[a]
Betaine	1804 ± 62.86[a]	68.34 ± 1.78[b]	2.61 ± 0.14[a]	0.51 ± 0.02[a]	1.30 ± 0.12[a]	4.43 ± 0.26[a]	0.97 ± 0.10[b]
Betaine + DL-Methionine	1847 ± 112.08[a]	69.44 ± 1.10[b]	2.67 ± 0.13[a]	0.61 ± 0.01[a]	1.09 ± 0.08[a]	4.37 ± 0.21[a]	0.73 ± 0.10[c]
Betaine +DL-Methionine + Choline	1764 ± 105.08[a]	73.62 ± 4.83[a]	2.74 ± 0.34[a]	0.70 ± 0.11[a]	1.33 ± 0.28[a]	4.78 ± 0.72[a]	0.68 ± 0.12[d]

**Means having different letters at the same column are significantly different at (P<0.01);*

Methyl donor compounds regulate lipid metabolism in broilers

Lipid profile in plasma was measured to investigate the effect of methyl donor treatments on lipid metabolism in broilers. Data in Table (4) indicate that betaine treatment caused a significant reduction in plasma total lipids, triglycerides, total cholesterol, LDL-cholesterol and significant increase in HDL cholesterol. Broilers treated with betaine + DL- methionine showed total lipids, total cholesterol, HDL-cholesterol and LDL cholesterol similar to untreated control. Treatment with (betaine + Methionine) caused significant reduction in triglycerides. The mixture of betaine, methionine and choline is the most effective methyl donor treatment in the reduction of plasma total lipids, triglycerides, total cholesterol, and LDL-cholesterol. Treatment with a mixture of betaine, methionine and choline did not cause any significant change in HDL-cholesterol. Data clearly demonstrate that betaine and other methyl donor compounds regulate lipid metabolism in broilers. The observed reduction in lipids in plasma could be due to the ability of betaine to reduce the transcription of lipogensis genes especially fatty acid synthase as mentioned by Xing *et al*, (2011). The inhibition of lipid biosynthesis in plasma and adipose tissues explain the reduction of abdominal fats and improvement of feed conversion ratio of broiler chicks. Also Rudolph *et al*, (2007) indicated that methyl donor compounds especially betaine regulate lipid metabolism via regulation of transcription of fatty acid synthesis genes.

Table (4): lipid profile of broilers treated with different methyl donor compounds

Treatments	Total lipids (g/L)	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
Control	2.6 ± 0.08[a]	119.53 ± 4.64[a]	98.55 ± 5.61[a]	71.98 ± 1.73[a]	26.57 ± 6.5[a]
Betaine	2.2 ± 0.08[b]	102.00 ± 4.14[c]	91.39 ± 9.08[b]	76.12 ± 1.5[b]	17.77 ± 6.16[b]
Betaine + Methionine	2.56 ± 0.12[a]	106.59 ± 3.81[b]	101.18 ± 4.58[a]	73.20 ± 1.53[a]	27.97 ± 6.00[a]
Betaine + Methionine + Choline	2.03 ± 0.17[c]	99.41 ± 2.86[c]	83.37 ± 5.67[c]	74.90 ± 0.59[ab]	10.94 ± 4.49[c]

**Each value represents means of 5 replicates ± SD.*

**Different letters reefer to significant differences.*

Methyl donor compounds inhibit lipid peroxidation in broiler liver:

The level of lipid peroxidation was measured by determination of malanadialdhyde (MDA) in plasma and liver homogenate. The obtained results are presented in Table (5). The level of lipid peroxidation was significantly reduced by treatment with betiane combined with other methyl donor compounds. Treatment with betaine + methionine + choline showed the most effective treatment in the reduction of the level of MDA in blood and liver homogenate. The results clearly demonstrate that betaine acts as a methyl donor and as antioxidant by participating in the generation of methionine from homocysteine through the catalytic action of betaine-homocysteine methyltransferase. Previous studies have shown that betaine is effective at preventing a variety of toxic injuries to the liver, such as those induced by niacin, carbon tetrachloride CCl_4, and hyperosmolarity (Lu *et al*, (2001) & Lu *et al*, (2008)). Betaine appears to confer this protection by reducing peroxidation in the liver Lu *et al*, (2008). Powel *et* al, (2010) found that MDA levels decreased in the 1% betaine group when compared with the model group. Although the increase in the dosage of betaine did not increase the antioxidant activity in a dose-dependent manner. They suggested that betaine may increase the antioxidant activity of the rat hepatocytes.

Table (5): Level of plasma lipid peroxidation in broilers treated with betaine and other methyl donor compounds

Treatments	Lipid peroxidation	
	MDA (□mol/dl plasma)	MDA (□mol/g liver)
Control	118.11 ± 3.77^{a}	20.06 ± 0.071^{a}
Betaine	116.46 ± 5.54^{a}	19.36 ± 0.98^{ab}
Betaine + Methionine	100.10 ± 3.56^{b}	20.47 ± 0.34^{a}
Betaine + Methionine + Choline	92.40 ± 7.58^{c}	18.62 ± 1.01^{b}

**Each value represents means of 5 replicates ± SD.*

**Different letters refer to significant differences.*

Methyl donor compounds improve broiler liver function:

Liver function of different groups was evaluated by measuring the activity of liver enzymes and albumin in the plasma. Data in Table (6) indicate that all examined methyl donor treatments exhibited a significant reduction in the level of the activities of ALT, AST and ALP in plasma. Treatment with (betaine and both Betaine + Methionine) have significantly similar effect on the level of liver enzymes in plasma. The mixture of Betaine, methionine and choline is the most effective methyl donor treatment in the reduction of the liver enzymes in plasma of broiler chicks. Data also showed that methyl donor effect of betaine and other methyl donor compounds could be contributed to their ability strengthen the hepatocellular membrane by its membrane stabilizing action or to a counteraction of free radicals by their antioxidant property (Ganesan *et al* (2010). Also, glycine betaine can induce antioxidant defensive enzyme which reduce lipid peroxidation of hepatocyte membranes and reduce the leakage of liver enzyme to plasma.

Table (6): Protective effect of methyl donor enriched compounds on the liver function of broilers.

Treatments	ALT (IU/dl)	AST (IU/dl)	ALP(IU/dl)	Albumin (g/L)
Control	32.9 ± 2.19^{a}	62.83 ± 4.30^{a}	129.42 ± 4.66^{a}	2.94 ± 0.14^{a}
Betaine	26.38± 3.19^{b}	56.87 ± 2.33^{b}	116.14 ± 4.00^{b}	2.92 ± 0.12^{a}
Betaine + Methionine	28.27 ± 3.52^{b}	57.76 ± 4.99^{b}	130.15 ± 6.59^{a}	2.95 ± 0.05^{a}
Betaine + Methionine + Choline	23.48 ± 3.92^{c}	52.55 ± 4.42^{c}	107.88 ± 4.41^{c}	2.91 ± 0.04^{a}

**Each value represents means of 5 replicates ± SD.*

**Different letters refer to significant differences.*

Methyl donor compounds improve histology of breast muscles and liver.

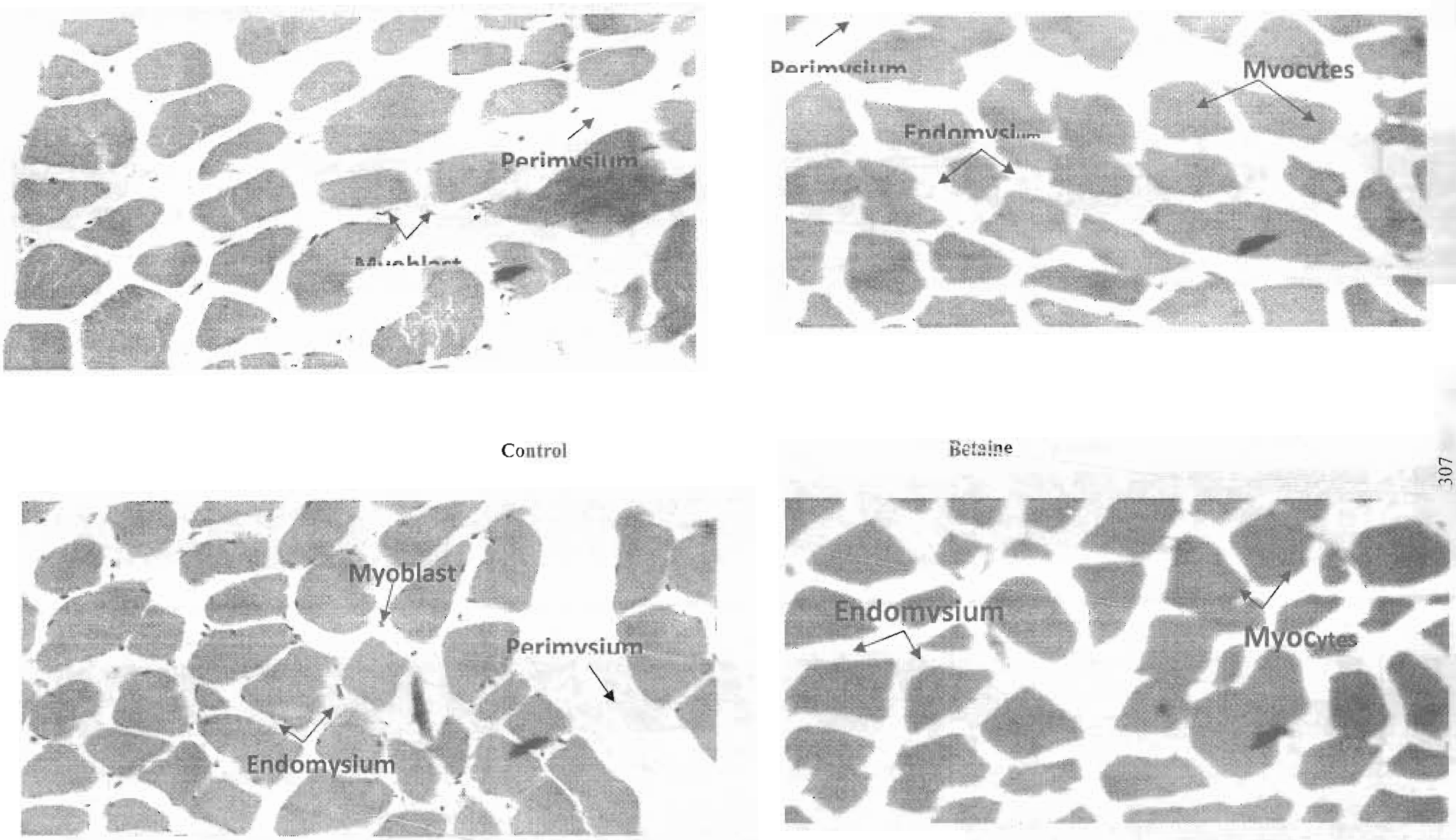
A- Breast muscle (BM) histology:

Histological examination of BM sections from different treatment groups showed pronounced changes as illustrated in Fig.1. . It is clear from sections that the number and size of myocytes were numerically increased in a dose dependent manner. In this respect, the control group (Fig.1) reveals a low number of muscle fibers per unit area compared with the other sections of the treated groups. Moreover, the treatment of betain+DL-methionin and treatment of betain+DL-methionin+ cholin showed remarkable hypertrophy and hyperplasia of the muscle fibers indicative of a positive response to the applied treatments.

B- Liver histology:

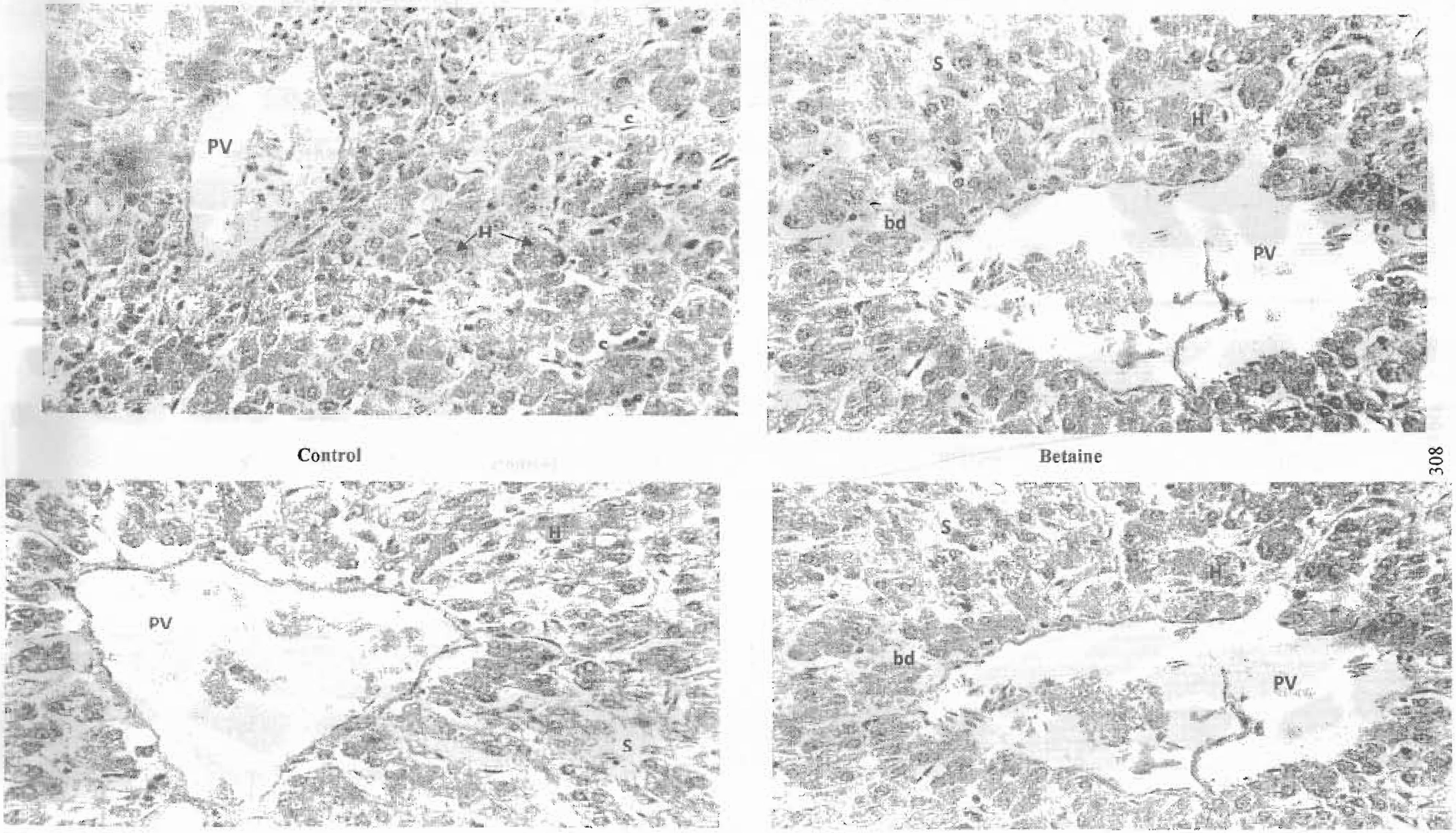
Liver sections showed normal hepatic parynchema of the control treatment group (Fig.2). The size of the central vein and the hepatocytes arrangement indicate an ideal structure of the liver, although same necrotic areas could be seen. This may be due to the higher metabolic activity. Furthermore, there is a moderate hypertrophy of the hepatocytes especially in the treatments of betaine and betaine +DL-methionine + choline, while in the treatment of betaine +DL-methionine +choline there were many binucleated cells accompanied with an obvious increase in the central vein. Also,this treatment showed some necrotic areas and more infilterable fluids in between the hepatocytes. In all sections, however, there were more or less dark-stained eosinophilic and (or) lymphocytic cells aggregations surrounding or near the central veins, especially in the treatment groups.

In general, the above mentioned histological observations support the performance data where the growth performance of the treatment groups was significantly improved without any undesirable effects on the liver histology and other related blood parameters.



Betaine + DL Methionine   Betaine + DL Methionine + Choline

Fig.(1): Histology of breast muscles of broilers treated with different methyl donor compounds. (H&E x 100).



Control   Betaine

Betaine + DL Methionine   Betaine + DL Methionine + Choline

Fig.(2): Histology of liver tissues of broilers treated with different methyl donor compounds. H:hepatic cells, P: portal vein, S: sinusoids; (H&E x 100).

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إتحاد البيتاين و المركبات المعطية لمجموعات المثايل يحسن نمو و ينظم أيض اللبيدات و يحمى كبد كتاكيت التسمين

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[1]قسم إنتاج دواجن –كلية الزراعة جامعة الزقازيق

[2]قسم الكيمياء الحيوية كلية الزراعة جامعة عين شمس

في هذة الدراسة تم إختبار تأثير البيتاين و دل مثيونين و الكولين على نمو و أيض الدهون ووظائف الكبد و أكسدة الدهون فى كتاكيت التسمين و كذلك خصائص الذبيحة. تم تقسيم عدد 240 طائر إلى 4 مجموعات مجموعة الكنترول غير المعاملة بالإضافة إلى 3 مجموعات معاملة بمحلول من 1جم بيتاين أو 0.5 حم بيتاين + 0.5 جم دل مثيونين او 0.33جم بيتاين + 0.33 جم دل مثيونين + 0.33 جم كولين / لتر . و قد اشارت النتائج إلى أن زيادة وزن الطيور المعاملة بالبيتاين معنويا بلمقارنة بالكنترول إستبدال بعض البيتاين بالمركبين دل مثيونين و الكولين لا يؤدي إلى أي إختلافات معنوية فى معدلات زيادة الوزن أوضحت النتائج أن البيتاين و المركبات المعطية لمجموعات المثايل تؤدي إلى إنخفاض فى نسبة الدهون فى بطن الذبيحة. وقد أشارت نتائج التحليلات البيوكيميائية لعينات البلازما أن المعاملات الثلاثة أدت الى إلى إنخفاض فى الدهون الكلية و الجلسريدات الثلاثية و الكلوستيرول و الكلوستيرول منخفض الكثافة. كما أوضحت النتائج إنخفاض مستوى أكسدة اللبيدات فى أنسجة الكبد حيث أدت المعاملات إلى إنخفاض فى تركيز المالونادإي ألدهيد سواء في أنسجة الكبد أو البلازما. بالإضافة إلى هذا أدت معاملات البيتاين الثلاثة إلى إنخفاض مستوي نشاط إنزيمات الكبد فى السيرم ممايعكس التأثير الواقى للمعاملة بالبيتاين و الماد المعطية لمجموعات المثايل الأخرى. نتائج الفحص الهستولوجي للكبد و عضلات الصدر تدعم نتائج النمو وخصائص الذبيحة. تخلص الدراسة لأن المعاملات الثلاثة تحسن نمو كتاكيت التسمين من خلال تنظيم تمثيل الدهون و حماية أنسجة الكبد.