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Betaine hydrochloride in broiler chicken: Effect on breast muscle cholestrol and tibial bone ash content

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Abstract
A trial was conducted to study the effect of dietary supplementation of betaine hydrochloride (betaine HCl) on growth and nutrient utilization in broiler chicken. The study was carried out for a period of six weeks in Vencobb 400 broiler chicks. One hundred – and – ninety two, day-old commercial broiler chicks were allotted to four groups, with four replications of 12 chicks each, randomly. The four groups were allotted to four dietary regimes and each replicate was randomly assigned to one of the four dietary treatments in this study. The experimental feed was formulated according to BIS (1992) [4] specifications and to the control ration (T_1), feed grade betaine HCl at 250, 500, 750 ppm was added respectively to form different rations T_2, T_3 and T_4 for different treatment groups. The birds in each group were maintained on their respective ration throughout the experimental period of six weeks. The proximate composition of breast muscle did not show any significant difference between groups except in the case of crude protein (P<0.01). The tibial bone analysis results indicated that the defatted bone weight, tibial bone ash, bone calcium and phosphorus levels were not influenced with different levels of betaine HCl supplementation.

Keywords: Broiler birds, betaine hydrochloride, muscle cholesterol

Introduction
Betaine is the most important carriers of preformed, transferablemethyl groups in diets for poultry. Methyl groups are required for synrhesis of numerous substances such as creatine, phosphatidyl choline, carnitine, adrenaline, methyl purines as well as methylated amino acids. Dietary betaine may be directly used as methyl group donor. Betaine can be used as methyl donor, through choline has to be converted in mitochondria into betaine. Due to its chemical structure, betaine shows the characteristics of a dipolar Zwitter ion resulting in osmoprotective properties. Accumulates in cell organelles and cells exposed to osmotic and ionic stress, thereby replacing inorganic ions and protecting enzymes as well as cell membranes from inactivation.

Konca *et al.,* (2008) [7] reported that betaine supplementation at the rate of 0.1 and 0.2 per cent in broiler diets did not affect the tibial bone weight, length, width, tibia breaking strength and tibial ash percentage. However the tibia width decreased chicks fed betaine 1 g / kg of feed as compared with control group fed without betaine. There is no available literature regarding the effect of betaine on bone properties.

Saunderson and Mackinlay, 1990 [9] suggested that supplementation of betaine decreased fat in poultry carcass in their experiment and Alirezaei *et al.,* (2012) [2] concluded that the betaine supplementation at the rate of 1 g / kg to the diet deficient in methionine improved meat quality and decreased lipid peroxidation in the breast muscle, compared to those fed control diet. Hence the objective of study is to investigate the breast muscle cholesterol and tibial bone ash content in broilers during the supplementation of betaine hydrochloride (betaine HCl) as feed additive.

Materials and Methods
The betaine HCl supplemented broilers were evaluated based on their Muscle cholesterol and tibial bone ash content. Straight run commercial broiler chicks (Vencobb-400) were utilized in the biological trial, from day old to six weeks of age. One hundred – and – ninety two, day-old Vencobb – 400 strain commercial broiler chicks were used as the experimental birds. All the birds were identified with wing bands placed in the wing web of the right wing on each bird on

day old, and were weighed individually. The birds were allotted to four dietary treatment groups, with four replications of 12 chicks each randomly in a Completely Randomized Design. An experiment was designed and conducted at the University Poultry and Duck Farm, College of Veterinary and animal sciences, Mannuthy, Kerala.

The experimental feed (in mash form) was formulated using corn and soyabean meal as per BIS (1992) [4] specifications. To the control ration (T_1), feed grade betaine HCl was added at 250, 500, 750 ppm to formulate rations T_2, T_3 and T_4 respectively, taking special care for proper mixing of betaine HCl. No growth promoting antibiotics was added to any rations.

Broiler starter ration were fed up to four weeks of age and then switched over to broiler finisher ration for the last two weeks. The birds were provided with feed and water *ad libitum*. Feed consumption by the birds in each replicate, was calculated weekly.

At the end of the experiment (42nd day) four birds (two males and two females) per treatment group were randomly selected, weighed and starved for 12 hours and slaughtered. The muscle samples collected from birds fed four treatment rations were chopped and minced with mortar and pestle. The total lipid was extracted from the muscle samples as per the method of Folch *et al.* (1957) [5] and concentration of cholesterol was estimated by Cholesterol Oxidase Peroxidase method suggested by Richmond (1973) [8] using LyphoCHEK- Cholesterol kit.

The tibia bones collected from birds fed four treatment rations were cleaned from adhering flesh and placed in boiling water for 30 minutes to remove the remaining muscles and connective tissues, dried at 100 ºC for 24 hours, defatted with acetone dried and ashed at 600 ºC/ 4 hours. Total ash, calcium and phosphorus contents were estimated as per AOAC (1990) [1]. The data collected on various parameters were statistically analyzed by Completely Randomized Design (CRD) as per the methods of Snedecor and Cochran (1994) [10] and the means of different experimental groups were also tested by using Duncan's Multible Range Test (DMRT) in SPSS Ver. 20.0.

Results and Discussion

The breast muscle cholesterol (mg/ dl) of experimental birds belonging to the groups T_1, T_2, T_3 and T_4 were 92.54, 80.50, 70.46 and 69.64 respectively and data represented in the Table 1. Average moisture percentage of breast muscle from birds belonging to four dietary treatments T_1, T_2, T_3 and T_4 were 71.91, 72.15, 71.86 and 72.21 and crude protein percentage were 85.07, 85.62, 85.51 and 86.68 respectively and data are presented in Table 2. The data on dry matter and ether extract contents of liver of birds fed four treatment rations T_1, T_2, T_3 and T_4 are presented in Table 3. The result of tibial bone weight, percentage of tibial bone ash and tibial calcium and phosphorus contents of birds fed four experimental rations T_1, T_2, T_3 and T_4 are presented in Table 4.

The group fed with rations T3 and T4 recorded significantly (P<0.01) lower breast muscle cholesterol as compared to other two treatments. The group fed T_1 ration recorded significantly higher breast muscle cholesterol (92.54 mg/dl) followed by those fed T_2, T_3 and T_4.

The result is in accordance with the findings of Saunderson and Mackinlay, (1990) [9] observed that betaine supplementation increased the lean meat and decreased the fat in poultry carcass. Alirezaei *et al.*, (2012) [2] also reported that betaine supplementation in methionine deficient diet improved meat quality and decreased lipid peroxidation in the breast muscle.

In the present study composition of breast muscle, such as moisture, dry matter, crude protein, ether extract, total ash and acid insoluble ash per cent of broiler chicken under different treatment groups From the result no significant difference was observed among different groups in any of the parameters except crude protein, on statistical analysis and it could be inferred that there was no major influence on the meat composition except crude protein with betaine HCl supplementation in broiler diet.

These findings complied with Attia *et al.*, (2005) [3] who reported that the dietary supplementation of betaine in slow growing chicken feed improved percentage of meat crude protein and also Zhan *et al*., (2006) [12] who reported that methionine and betaine supplementation in the methionine deficient diet had increased (P<0.05) the protein content of breast muscle by 2 per cent. Sun *et al*., (2008) [11] also reported that the betaine supplementation with 25 per cent methionine deficient diet resulted in higher (p<0.05%) muscle crude protein. Contrary to this, Hassan *et al.*, (2005) [6] and Konca *et al.*, (2008) [7] could not observe any effect on muscle crude protein by supplementation of betaine in broilers diet.

The findings of the present study that betaine HCl supplementation did not affect the breast muscle moisture, dry matter, ether extract, total ash and acid insoluble ash percentages are in accordance to Saunderson and Mackinlay (1990) [9], Attia *et al.*, (2005) [3] and Konca *et al.*, (2008) [7] in broilers and Hassan *et al.*, (2005) [6] in slow growing chicken who reported that betaine supplementation had no significant effect on breast muscle moisture, ether extract and total ash. Contrary to this, Zhan *et al*., (2006) [12] and Sun *et al*., (2008) [11] reported that supplementation of methionine and betaine in the methionine deficient broiler diet significantly increased fat contents.

Statistical analysis revealed no significant difference among the treatment groups for dry matter and ether extract contents of liver consequent to betaine supplementation. Statistical analysis revealed no significant difference among the treatment groups for weight of dried tibia, tibial ash, tibial calcium and tibial phosphorus percentage. The observation of the present study is in agreement with Konca *et al.*, (2008) [7] who found no significant difference in tibial bone weight and length with betaine supplementation in broiler diet.

Conclusion

It can be concluded that in the presented study indicated that the breast muscle cholesterol level and crude protein level were lower and higher respectively in birds supplemented with betaine HCl at 750 ppm. However, breast muscle proximate composition of moisture, ether extract, total ash, acid insoluble ash, liver dry matter and liver ether extract, dried tibia, tibial ash, tibial calcium and tibial phosphorus levels were not influenced with different levels of betaine HCl supplementation.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

The animal studies for the experiment have been approved by the ethics committee- COVAS, Mannuthy, Kerala and therefore have been performed in accordance with the ethics standards as applicable under institutional guidelines."

Table 1: Breast muscle cholesterol contents of birds maintained on four experimental rations, (mg / dl)

Lipid profile	Treatments[1]					
	T1	T2	T3	T4	F value	P value
Breast muscle cholesterol	92.54 [a] ±2.73	80.50 [b] ±3.14	70.46 [c] ±4.41	69.64 [c] ±0.55	12.381**	0.001

[1]Mean of 4 observations with SE

ns – non significant (P>0.05), ** significant at 0.01 level ($P\leq$ 0.01)

*Means bearing different superscripts within same column differ significantly ($P\leq$0.05)

Table 2: Breast muscle proximate composition of birds maintained on four experimental rations, %

Parameters*	Treatments[1]				F value	P value
	T1	T2	T3	T4		
Moisture	71.91 ±0.26	72.15 ±0.23	71.86 ±0.19	72.21 ±0.22	0.578 [ns]	0.640
Dry matter	28.09 ±0.26	27.85 ±0.23	28.14 ±0.19	27.79 ±0.22	0.578 [ns]	0.640
Organic dry matter	94.63 ±0.08	94.55 ±0.27	94.42 ±0.09	94.23 ±0.16	1.099 [ns]	0.387
Crude protein	85.07[a] ±0.26	85.62[b] ±0.13	85.51[ab] ±0.07	86.68[c] ±0.09	19.874**	0.001
Ether extract	2.26 ±0.08	2.50 ±0.12	2.60 ±0.02	2.76 ±0.10	1.449 [ns]	0.277
Total ash	5.37 ±0.08	5.45 ±0.27	5.58 ±0.09	5.77 ±0.16	1.099 [ns]	0.387
Acid insoluble ash	0.11 ±0.01	0.12 ±0.01	0.13 ±0.01	0.13 ±0.01	0.997 [ns]	0.428

*On dry matter basis except Moisture%.

[1]Each value is a mean of 4 observations.

ns – non significant (P>0.05)

**Means bearing different superscripts within same column differ significantly ($P\leq$0.01)

Table 3: Weight of dried tibia (g), tibial ash, tibial calcium and tibial phosphorus of birds maintained on four experimental rations, %

Parameters	Treatments[1]				F value	P Value
	T1	T2	T3	T4		
Weight of dried tibia	8.25 ±0.41	8.45 ±0.21	8.60 ±0.96	7.85 ±0.27	0.346[ns]	0.793
Tibial bone ash	43.72 ±0.38	43.07 ±0.52	43.04 ±0.48	43.62 ±0.24	0.724 [ns]	0.557
Tibial calcium	23.20 ±0.19	22.77 ±0.41	23.42 ±0.12	22.75 ±0.36	1.261 [ns]	0.332
Tibial phosphorus	10.01 ±0.14	10.33 ±0.26	9.86 ±0.14	9.89 ±0.40	0.676 [ns]	0.583

[1]Each value is a mean of 4 observations with SE

ns-non significant (P>0.05)

Table 4: Weight of dried tibia (g), tibial ash, tibial calcium and tibial phosphorus of birds maintained on four experimental rations, %

Parameters	Treatments[1]				F value	P Value
	T1	T2	T3	T4		
Weight of dried tibia	8.25 ±0.41	8.45 ±0.21	8.60 ±0.96	7.85 ±0.27	0.346[ns]	0.793
Tibial bone ash	43.72 ±0.38	43.07 ±0.52	43.04 ±0.48	43.62 ±0.24	0.724 [ns]	0.557
Tibial calcium	23.20 ±0.19	22.77 ±0.41	23.42 ±0.12	22.75 ±0.36	1.261 [ns]	0.332
Tibial phosphorus	10.01 ±0.14	10.33 ±0.26	9.86 ±0.14	9.89 ±0.40	0.676 [ns]	0.583

[1]Each value is a mean of 4 observations with SE

ns-non significant (P>0.05)

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