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Effect of dietary boron supplementation on serum biochemical and carcass characteristics in broiler chicken

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Abstract
An experiment was conducted to ascertain the effect of graded level of dietary boron supplementation on serum biochemical and carcass characteristics in broiler chicken. A total of 240 day old broiler chicks (Vencob) of mixed sex (mean body weight 47.50± 0.26g) were used in completely randomize design with five treatments with four replication comprised of 12 birds (6 males and 6 Females) per replica. The five dietary treatments T_1, T_2, T_3, T_4 and T_5 were fed the basal diet with supplemental boron at 0, 25, 50, 75 and 100 mg/kg diet respectively. At the end of experiment blood samples were collected for serum biochemical and two birds per replication were selected randomly and sacrificed as per the standard protocol to study carcass characteristics. A significantly decreased (P<0.05) serum glucose and total cholesterol were observed in boron supplemented groups irrespective of doses as compared to control. The carcass characteristics were comparable (P>0.05) among the dietary treatment groups. It is concluded that supplementation of boron decreased the serum glucose and total cholesterol without affecting the carcass characteristics in broiler chicken.

Keywords: Boron, serum biochemical, carcass characteristics, broiler

Introduction
Boron was found in nature with its compound, especially as boric acids and borates [1]. In recent years, there are many investigations about using boron compounds in animal nutrition. Although high levels of boron has fatal effect, in deficiency of boron, it is seen that inadequate growth and abnormal bone development [2]. Boron has been known to play roles in the metabolism of other substances such as calcium, magnesium, triacylglycerols, glucose, amino acids, reactive oxygen species, hormones such as 17β-estradiol, calcitonin, 25-hydroxycholecalciferol [3, 4]. The distinctive chemical properties of boron allow it to form complexes with organic molecules containing hydroxyl groups, and therefore to interact with various metabolites and enzymes to influence cellular activity, hormone reception and transmembrane signaling [5, 6]. Boron may act as a metabolic regulator in several enzymatic systems [7]. However, biochemical functions of B are not fully understood in animals and man [8]. Although there were some studies investigating the effects of B on performance [9, 10, 11, 12, 13, 14], limited studies investigating the effects of different B compounds on blood parameters [14, 15, 16, 17] were performed in broilers. Similarly the contradictory results were reported with respect to the effect of boron on carcass characteristics in broiler birds [17, 18, 19]. Therefore the present study was undertaken to access the effect of graded level of boron supplementation on serum biochemical and carcass characteristics in broiler chicken.

Materials and Methods
Birds and Housing
A total of 240 day old broiler chicks (Vencob) of mixed sex (mean body weight 47.50± 0.26 g) were used for this experiment. At arrival to the house the chicks were weighed and randomly allotted to floor pens, each representing a replication. Birds were vaccinated against infectious bursal disease virus (GUMBORO I^+, Haster Biosciences limited Mehsana, Gujarat-India) and Newcastle disease virus (LaSota Strain, Venkateshwara Hatcheies Pvt. Ltd, Pune-India) via drinking water at 10 and 14 days of age, respectively. Each replica was supplied with a floor space of 1.5 m^2 (1.5×1.0 m) along with the provision of hanging feeder and waterer. Birds were reared in pens provided with litter material (rice husk and saw dust) to a depth of 5-6 cm. The house was well ventilated with adjustable windows and every effort was made to reproduce the commercial condition as much as possible. The room temperature was maintained at 33±1 ºC up to 7 days and gradually decreased to 26±1 ºC by 21 days. Thereafter the birds were kept at room temperature up to 6 weeks of age.

Experimental Design and Diets

The experiment was performed in completely randomize design with five treatments. Each treatment comprised of four replication with 12 birds (6 males & 6 Females) per replica. The basal diet was a corn-rice-soya based diet formulated to meet or exceed the nutrient requirement of broiler as per the ICAR (ICAR, 2013) recommendation. The birds were offered starter (1 to 21 days) and finisher (22 to 42 days) diet in mash form. The chicks received feed within 12 hours of hatching. The ingredient and nutrient composition of basal diet are given in Table. 1.

The five dietary treatment were comprised of T_1 (basal diet without any supplemental boron), T_2 (Basal diet+ 25 mg/kg boron), T_3 (Basal diet+ 50 mg/kg boron), T_4 (Basal diet+ 75 mg/kg boron) and T_5 (Basal diet+ 100 mg/kg boron). Boric acid (LOBA CHEMIE PVT. LTD, Mumbai-India) with 17.48% boron contain was used as a source of boron. The treatments T1, T2, T3, T4 and T5 were supplemented with 0, 0.143, 0.286, 0.429 and 0.572 gram of boric acid per kg basal diet, respectively. Respective amount of boric acid for each treatment was mixed with the basal diet as a premix prior to feeding the birds.

Table 1: Ingredients and chemical composition of basal diet fed to broiler chicken (g/kg as fed)

Ingredients	Starter	Finisher	Calculated	*Starter*	*Finisher*
Maize	525.000	545.000	ME (Kcal/kg)	3100.0	3200.0
Soya DOC 45%	364.000	311.000	Crude Protein (%)	22.0	20.0
Rice Polish	36.100	56.700	Lysine (%)	1.20	1.05
Oil-Veg	35.250	46.300	Methionine (%)	0.50	0.45
Corn starch	3.150	4.700	Threonine (%)	0.8	0.8
Salt	2.000	2.000	Calcium (%)	1.0	1.0
Sodium bi-carbonate	1.200	1.200	Available Phosphorous (%)	0.45	0.45
Dicalcium Phosphate	9.000	9.000	Fat (%)	6.5	8.0
LSP-Powder	13.500	13.500	Crude fibre (%)	4.9	4.8
Enzyme	0.300	0.300	***Analysed nutrient***	***Starter***	***Finisher***
DL-Methionine	2.300	2.000	Dry Matter	90.2	90.3
L-Lysine HCL	1.400	1.500	Organic Matter	93.75	94.23
L-Threonine	0.300	0.300	Crude Protein	21.860	19.94
Vitamin Premix [a]	2.000	2.000	Ether Extract	5.870	7.89
Trace Mineral Mixture[b]	1.000	1.000	Crude Fibre	4.510	4.37
Toxin Binder	1.000	1.000	Total Ash	6.25	5.77
Choline Chloride, 60%	0.500	0.500	NFE	61.51	62.03
Acidifier [c]	1.000	1.000	***Mineral Composition***	***Starter***	***Finisher***
Liver Tonic Hepatocare	1.000	1.000	Ca (%)	0.981	0.972
			Available P (%)	0.46	0.42
			Mg (%)	0.17	0.18
			Mn (mg/kg)	50.56	52.87
			Cu (mg/kg)	12.10	11.26
			Zn (mg/kg)	82.59	80.43
			Fe (mg/kg)	205.1	201.6
			Boron (mg/kg)	2.10	1.96

[a] Provides per kg of diet: trans-retinol 12000 IU; cholecalciferol 1500 IU; α-tocopherol acetate 75mg; Vitamin K3 5mg; Vitamin B1 3mg; Vitamin B2 6mg; Vitamin B6 5mg; Vitamin B12 0.03 mg; nicotinamide 40mg; pantothenic acid 10mg; folic acid 0.75mg; D-biotin 0.075 mg; choline 375 mg,

[b]Contained (per kg) Manganese 40g; Iron 40 g; Zinc 60 g; Copper 5 g; Cobalt, 0.2 g (all as sulfate salt); Iodine 0.5 g (as potassium iodide); Selenium 0.15 g (as sodium selenite)

[c]Acidifier Contains (per kg) ortho-phosphoric acid (400 g), formic acid (150 g), propionic acid, and calcium propionate (30 g) mixed with a carrier.

Blood Sample Collection

Blood was collected randomly from 40 birds (2 birds/replica) at the end of 6th weeks of age. Two ml of blood was collected early in the morning through puncturing the wing vein using clean, sterilized needle and placed in an eppendorf without any anticoagulant. They were allowed to clot at room temperature and then centrifuged at 2000 rpm for 15 minutes to harvest the serum.

Carcass Characteristics

At the end of experiment two birds per replication were selected randomly and sacrificed as per the standard protocol to study carcass characteristics. The birds were kept without any feed and offered only drinking water 12 hours prior to slaughter. The live body weights of the birds were taken just before slaughter. The birds were sacrificed by severing through neck and throat and bled completely. After complete bleeding the birds were decapitated at occipital-atlantal joint. The de-feathered carcasses were eviscerated manually following standard commercial procedures and weighed for carcass (including liver, gizzard and heart) yield. The dressing percentage was calculated as carcass yield × 100/live weight of the bird. Thereafter the liver, pancreas, kidney, spleen, heart and intestine were excised and weighed separately using an electronic balance for their absolute and relative yield.

Chemical Analysis of Feed and Serum

Serum samples were assayed for the concentrations of glucose, urea, creatinine, total cholesterol, total protein, albumin, tri-iodothyronine, thyroxin and the activities of Alanine transaminase, alkaline phosphatase and alanine aminotransferase using diagnostic kits (Span Diagnostics Ltd. Surat, India). The feed samples were taken in pre-weighed quartz crucible and oven dried at 80 ◦C for 24 h, decarbonized followed by ashing at 550-600 ◦C in a muffle furnace. The total ash was digested with 5 N hydrochloric acid on a hot plate for 15 min, cooled and filtered through Whattman filter paper (No. 41) into a polypropylene volumetric flask of desired volume. Mineral content in feed samples were

determined using the Microwave Plasma Atomic Emission Spectrometer (MP-AES) (MP-AES, Agilent 4200/4210; Perkin Stevens Creek Blvd, Santa Clara, California, USA) with analytical conditions as suggested by the manufacturer.

Statistical Analysis

Data generated in the study were analyzed using the Statistical Package for Social Sciences (SPSS, version 20.0 Chicago, USA) by one-way ANOVA and comparison of means was tested using Duncan's multiple range tests. The effects were considered to be significant at $P<0.05$.

Results and Discussion

The data pertaining to the serum biochemical are given in Table 2. A significantly decreased ($P<0.05$) blood glucose and total cholesterol were observed in boron supplemented groups irrespective of doses as compared to control. The serum urea, creatinine, total protein, albumin, tri-iodothyronine, thyroxin and the activities of Alanine transaminase, alkaline phosphatase and alanine aminotransferase were comparable ($P>0.05$) among the dietary treatment groups. Boron plays an important role in regulating enzymatic activity in pathways involved in energy substrate metabolism, glycolytic enzyme activity and release of insulin [20] believed to regulate the blood glucose level. In this study the decreased serum glucose and total cholesterol concentrations in the boron supplemented group is in agreement with previous studies reported a decrease in insulin, serum glucose and total cholesterol level in rat supplemented with boron at 10 to 100 mg/kg diet [21,22]. A decrease in glucose, insulin and total cholesterol was also reported in dog [23] supplemented with boron. On the other hand, supplementation with 1.4 mg/kg boron as orthoboric acid decreased the elevated plasma glucose levels in the chicks fed a diet with inadequate vitamin D [24]. The contradictory findings may be due to the dose of boron supplementation and vitamin D induced hyperglycemia [21]. Administration of boron as sodium borate was reported to decrease the concentration of glucose and insulin in blood [25]. In this study, the decrease in serum glucose concentration might be due to the binding of boric acid to hydroxyl groups of α-glucose [14]. Similarly the lipid lowering effect of boron in this study is in agreement with several other research findings reported the decreased serum cholesterol level in quail [26] and laying hen [27] fed a diet with supplemental boron at 10-240mg/kg and 5-400 mg/kg diet, respectively. The result of the present study about the serum parameters (protein, albumin, globulin, creatinine, uric acid, ALT, AST and ALP) are in agreement with findings of several researchers [25, 28, 29]. The data pertaining to the carcass characteristics of broiler chicken fed a diet with graded level of boron supplementation is given in Table 3. The average pre-slaughter weight (live BW g) of selected birds showed similarities ($P>0.05$) across the dietary treatment groups ranges between 1850.50±43.98 (T5) and 2012.50±65.74 (T2). The carcass characteristics and organ yields were comparable ($P>0.05$) across the different dietary treatment groups. The carcass yield (%) ranged between 69.29±2.29 to 73.28±1.58 and the dressing percentage between 53.03±1.01 to 58.96±3.37 across the treatments. There was no significant difference ($P>0.05$) in the absolute and relative yield of organs like liver, pancreas, kidney, spleen, heart and small intestine across the dietary treatment groups. The results of the present study with respect to the effect of boron supplementation on carcass characteristics in broiler chicken are in agreement with several other research findings [19,20,30] suggested supplemental boron did not influence the carcass characteristics in birds.

Conclusion

It is concluded that supplementation of boron decreased the serum glucose and total cholesterol without affecting the carcass characteristics in broiler chicken

Table 2. Effects of graded level of boron supplementation on serum biochemical in broiler chicken

Parameters	T_1	T_2	T_3	T_4	T_5	SEM	P value
Blood glucose mg/dl	232.29^{a}±3.48	219.35^{b}±2.79	221.04^{b}±2.79	221.60^{b}±4.55	217.53^{b}±2.04	3.242	0.045
BUN mg/dl	0.50±0.03	0.50±0.04	0.50±0.04	0.52±0.05	0.51±0.04	0.039	0.997
Creatinine mg/dl	0.42±0.01	0.43±0.02	0.43±0.02	0.44±0.01	0.41±0.02	0.016	0.752
Total protein g/dl	3.77±0.04	3.78±0.04	3.74±0.03	3.75±0.04	3.75±0.04	0.039	0.933
Albumin g/dl	1.77±0.02	1.78±0.02	1.76±0.01	1.76±0.02	1.76±0.02	0.018	0.931
Globulin g/dl	2.00±0.02	2.00±0.02	1.98±0.02	1.99±0.02	1.98±0.02	0.020	0.926
Cholesterol mg/dl	142.57^{a}±4.28	127.09^{b}±2.78	132.78^{b}±2.79	128.02^{b}±3.12	129.27^{b}±2.40	3.089	0.018
(ALT) U/L	8.29±0.11	8.37±0.09	8.28±0.04	8.33±0.13	8.31±0.07	0.092	0.962
(AST) U/L	67.90±2.05	66.89±5.36	67.42±2.84	66.67±2.35	66.26±5.74	3.986	0.999
(ALP) U/L	659.57±20.88	664.92±12.11	667.41±12.66	662.66±12.91	664.14±12.42	14.587	0.997
T3 ng/ml	1.18±0.01	1.19±0.02	1.20±0.03	1.17±0.01	1.17±0.01	0.019	0.725
T4 µg/ml	1.96±0.03	1.98±0.04	1.98±0.04	1.97±0.04	1.96±0.04	0.038	0.996

ALT: Alanine transaminase
AST: Aspartate transaminase
ALP: Alkaline phosphatase
T_3: Tri-iodothyronine
T_4: thyroxin
^{abcd} Mean with different superscript in a row differ significantly; SEM, standard error of mean

Table 3: Effects of graded level of boron supplementation on carcass characteristics in broiler chicken

	Treatments*						
Attributes	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**	**P value**
Live BW g	1912.50±68.84	2012.50±65.74	1950.00±45.64	1881.00±47.48	1850.50±43.98	55.382	0.317
Carcass weight (CW) g	1357.42±43.48	1389.49±16.61	1389.63±29.49	1380.60±63.81	1339.67±59.28	46.094	0.918

Carcass yield %	71.01±0.41	69.26±2.29	71.29±0.82	73.28±1.58	72.30±1.61	1.493	0.427
Dressed weight g	1068.56±18.89	1187.59±80.07	1162.50±23.93	1062.50±71.80	980.30±16.60	50.546	0.068
Dressing %	56.04±1.73	58.96±3.37	59.76±2.30	56.54±3.75	53.03±1.01	2.637	0.430
Liver g	62.31±6.50	59.47±0.90	56.93±4.97	59.19±2.60	65.19±6.48	4.832	0.781
Liver % of CW	4.58±0.41	4.28±0.16	4.08±0.29	4.30±0.15	4.91±0.59	0.354	0.525
Pancreas g	3.45±0.47	3.18±0.13	4.43±0.17	3.71±0.32	3.39±0.12	0.277	0.051
Pancreas % of CW	0.25±0.04	0.23±0.01	0.32±0.01	0.27±0.03	0.25±0.02	0.025	0.181
Kidney g	9.93±0.89	11.04±1.20	9.67±1.24	10.47±1.25	12.31±1.37	1.201	0.565
Kidney % of CW	0.73±0.07	0.80±0.09	0.69±0.08	0.76±0.10	0.93±0.15	0.097	0.494
Spleen g	1.98±0.14	1.51±0.07	1.73±0.15	2.04±0.50	1.59±0.04	0.247	0.490
Spleen % of CW	0.15±0.02	0.11±0.005	0.12±0.01	0.15±0.04	0.12±0.003	0.021	0.593
Heart g	10.63±0.27	10.40±0.57	8.83±0.94	10.41±0.80	12.00±0.71	0.700	0.079
Heart % of CW	0.79±0.03	0.75±0.04	0.64±0.06	0.77±0.09	0.90±0.06	0.063	0.130
Small intestine g	59.68±4.79	54.64±5.01	52.66±2.72	48.99±3.57	66.18±2.89	3.916	0.058
Small intestine % of CW	4.43±0.47	3.94±0.38	3.79±0.21	3.54±0.17	4.99±0.40	0.346	0.067

cw: carcass weight: organ % = organ weight*100/ carcass weight; SEM, standard error of mean

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