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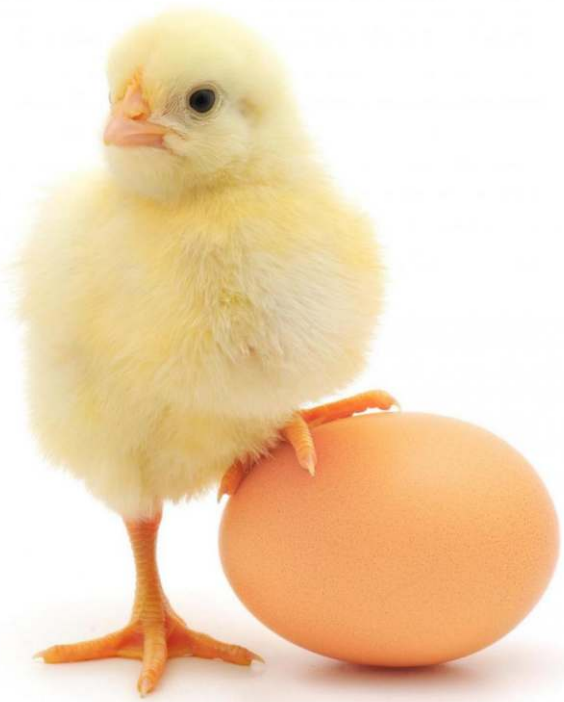

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Research Article

Effect of Dietary β-hydroxy-β-methylbutyrate Calcium on Carcass Cuts, Serum Biochemistry, Bone Characteristics and Digestive Tract pH of Broiler Chickens

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Abstract

Objective: This study aimed to investigate the effect of dietary β-hydroxy-β-methylbutyrate calcium (HMB-Ca) on the main carcass cuts, serum biochemistry, bone characteristics and digestive tract pH of broiler chickens. **Materials and Methods:** A total of 216 day-old chicks of strain Ross-308 were distributed into 4 groups dietary fed HMB-Ca at different concentrations (0.0, 0.1, 0.15 and 0.2%). The HMB-Ca was administered from 1-35 days of age. Duncan's multiple range test was used to compare means. **Results:** The results showed that 0.2% HMB-Ca increased ($p<0.05$) breast and thighs percentage, serum alkaline phosphatase (ALP), high-density lipoprotein (HDL) and femur and metatarsus phosphorus (P) but decreased serum cholesterol. Dietary HMB-Ca decreased ($p<0.01$) triglycerides (TG), very-low-density lipoprotein (VLDL) ($p<0.05$) and the pH of the crop and gizzard contents. The administration of 0.1 and 0.2% HMB-Ca increased ($p<0.05$) serum calcium (Ca). Dietary HMB-Ca had no effect on the length or weight of the femur and metatarsus bones. **Conclusion:** It is concluded that adding HMB-Ca, especially at the level of 0.2%, to the diet of broiler chickens may increase the development of breast and thigh muscles and decrease the levels of harmful serum lipids, in addition to its effect on the metatarsus bone mineral content and the pH of the contents of the digestive tract.

Key words: Beta-hydroxy-β-methylbutyrate calcium, carcass cuts, serum lipid profile, bone and serum mineral content, digestive tract pH

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The consumption of chicken meat and other products has increased recently because the consumer seeks healthy benefits of the proteins, fats, vitamins and minerals contained within[1]. This increase has motivated poultry breeders and researchers to selectively breed for rapid growth such that modern broiler chickens reach their desired weight a few days earlier than previous broiler chickens. Fancher[2] noted that during the past decade, the 42-day-old broiler has gained 0.55 kg more than in the past. This rapid increase in growth rate has probably resulted in the skeletal system being insufficient to support the heavy body weight[3] and has thus increased the need for dietary supplementation to support muscle growth. One of the most important of such compounds is β-hydroxy-β-methylbutyrate (HMB).

The HMB is a Lucien metabolite. A small amount of HMB is manufactured in the body[4]. Additionally, natural resources contain HMB, such as alfalfa, avocado, asparagus and other plants. The HMB has been studied by many researchers over the past two decades[5]. Many studies have demonstrated its benefits when administering HMB to domestic animals (including birds) and have reported increased body weight and muscle deposition, it is also an immunostimulator, reduces mortality rate and strengthens muscles in elderly animals and can be used to treat some medical conditions, such as muscle loss occurring as a result of glucocorticoid secretion[6]. Feeding broiler chickens a diet containing HMB results in increased growth rate and muscle mass, especially in the breast cut, this effect could be due to increased protein synthesis and decreased protein degradation[7]. Broilers fed 0.1% HMB on day 17.5 of incubation had 2.4% more breast muscle and 6.4% more leg muscle[8]. The effective period and mechanical influences of HMB on the body and muscle growth of broiler chickens remain unknown[9], but some evidence suggested the serum growth hormone and insulin-like growth factor (IGF) increase as a result of being fed HMB[3,9].

A modern broiler chicken suffers bone issues due to its high body weight. Some studies have evaluated the effect of supplementing HMB-Ca on bone characteristics. Tatara *et al.*[3] found that long-term administration of 0.05 g HMB-Ca kg^{-1} b.wt., increased the bone volumetric mineral density, which in turn had a positive effect on bone strength. Studies on the effect of HMB-Ca on the blood biochemistry and bone characteristics of broiler chickens are very limited; thus, this study was conducted to evaluate the effect of HMB-Ca on the serum lipid profile, serum alkaline phosphatase (ALP), calcium (Ca), phosphorus (P), femur and metatarsus weight, length and Ca and P contents.

MATERIALS AND METHODS

This study was carried out at the Poultry Farm of the Department of Animal Production, College of Agriculture, University of Baghdad, Iraq from 20/9/2015 to 25/10/2015. A total of 216 day-old, unsexed chicks of strain Ross-308 were randomly allocated to 12 floor pens in a clean and fumigated domestic fowl house. The pins were considered treatment replicates (3 replicates per treatment). Wood shavings were used as litter. Artificial lighting regimes were maintained 24 h day^{-1} during the study period. Water and mash feed were provided *ad libitum*. The house temperature was maintained at 34°C for the first 3 days and then decreased by 2°C each week until reaching 24°C. The chickens were vaccinated on days 1, 11, 20, 29 and 32 with ND-1B vaccines, ND-1B vaccines, Gumboro vaccines, ND Las Sota vaccines and ND-1B vaccines, respectively.

The HMB-Ca was added to the basal diet as powder (formulated and manufactured by SCITECH NUTRITION, Miami, USA) from 1-35 days of age. The experimental treatments were as follows: T1 was considered the control group without any addition and T2, T3 and T4 included the addition of 0.1, 0.15 and 0.2% HMB-Ca, respectively. The chickens were fed *ad libitum* two types of basal diet: The starter was administered from 1-21 days of age and contained 23% crude protein and 3027 kcal kg^{-1} Metabolizable Energy (ME) and the grower was administered from 22-35 days of age and contained 20% crude protein and 3195.3 kcal kg^{-1} ME (Table 1).

Blood samples were taken from the wing vein of 6 birds per replicate at 21 and 35 days of age. The samples were

Table 1: Ingredients and chemical calculations of the basal diet

Ingredient	Starter (%)	Grower (%)
Yellow corn	30	40
Wheat	28.25	24
Soybean meal (48%)	31.75	24.8
Protein concentrate	5	5
Vegetable oil	2.9	4.4
Limestone	0.9	0.6
Dicalcium phosphate	0.7	0.9
Salt	0.3	0.1
Premix	0.2	0.2
Total	100	100
Chemical calculated analysis*		
Crude protein	23	20
ME (kcal kg^{-1})	3027	3195.3
Lysine	1.2	1.1
Methionine	0.49	0.46
Cysteine	0.36	0.32
Methionine+cysteine	0.85	0.78
Calcium	0.85	0.79
Available phosphorus	0.45	0.49
C/P ratio	131.61	159.77

*According to NRC[13]

immediately centrifuged at 4000 rpm min^{-1} for 10 min and the serum was separated and refrigerated at -20°C until further analysis. Biochemical tests were performed using biochemical analyzer kits (Human, Germany) and a spectrophotometer, the total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), Ca, P and ALP contents were measured.

The LDL and VLDL were calculated as follows in Eq. 1 and 2[10]:

$$\text{VLDL} = \text{Triglycerides}/5 \quad (1)$$

$$\text{LDL} = \text{Total cholesterol-(HDL+VLDL)} \quad (2)$$

At the end of experiment, 6 birds per group were slaughtered, defeathered and eviscerated. The relative weight of the breasts, thighs, back, wings and neck was measured. The femur and metatarsus bones were separated to measure their weights, lengths and Ca and P contents.

The data were analyzed using Statistical Analysis System (SAS)[11] with a Completely Randomized Design (CRD). Duncan's multiple range test[12] was used to test the significant difference among treatments. Statistical significance was set at $p<0.05$ and $p<0.01$.

RESULTS

Carcass cuts: The relative weight of the carcass cuts indicated the administration of 0.2% HMB-Ca (T4) significantly ($p<0.05$) increased the weight of the breast and thigh cuts compared with the control, which had the lowest values; the administration of 0.1 and 0.15% HMB-Ca also increased the weight of the thigh and breast cuts compared with control, but the differences were not significant (Table 2).

The remaining carcass cuts, including the back, wings and neck, showed no significant differences in their relative weight between all experimental treatments.

Serum lipid profile: The serum lipid profile included cholesterol, TG, HDL, LDL and VLDL are present in Table 3. At 21 days of age, it was clear that none of the treated groups differed significantly from the control, but the cholesterol level in T2 was significantly ($p<0.05$) lower than that of T3 group. At 35 days of age, the cholesterol levels of T4 followed by T2 decreased significantly ($p<0.05$) compared with the control and T3.

Table 2: Effect of dietary HMB-Ca on the relative weight of broiler carcass cuts

Treatment groups	Relative weight (%)				
	Breasts	Thighs	Back	Wings	Neck
T1	34.23 ± 0.96^{b}	27.36 ± 0.98^{b}	18.24 ± 0.55	11.55 ± 0.74	5.34 ± 0.50
T2	35.81 ± 0.43^{ab}	29.04 ± 0.44^{ab}	18.90 ± 0.31	11.12 ± 0.16	5.55 ± 0.10
T3	35.43 ± 0.82^{ab}	29.49 ± 0.42^{ab}	18.49 ± 0.33	10.60 ± 0.23	4.77 ± 0.26
T4	36.95 ± 0.47^{a}	29.52 ± 0.61^{a}	19.02 ± 0.50	10.88 ± 0.25	5.64 ± 0.21
p-value	0.05	0.05	NS	NS	NS

[a,b]Means in the same row with different letters are significantly different at $p<0.05$, NS: Non-significant, T1: Control group, T2, T3 and T4: 0.1, 0.15 and 0.2% HMB-Ca, respectively

Table 3: Effect of dietary HMB-Ca on the serum lipid profile of broiler chickens

Parameters	Treatment groups				p-value
	T1	T2	T3	T4	
21 days of age (mg dL^{-1})					
Cholesterol	100.95 ± 4.47^{ab}	89.70 ± 6.40^{b}	110.63 ± 1.50^{a}	98.20 ± 5.40^{ab}	0.05
Triglycerides	158.33 ± 2.02^{a}	99.75 ± 0.83^{b}	111.00 ± 0.51^{b}	105.25 ± 8.51^{b}	0.01
HDL	42.56 ± 3.37^{ab}	37.25 ± 0.95^{b}	40.63 ± 2.68^{b}	49.06 ± 3.78^{a}	0.05
LDL	28.08 ± 6.94^{b}	32.50 ± 7.03^{ab}	47.80 ± 2.34^{a}	26.71 ± 7.14^{b}	0.05
VLDL	31.66 ± 0.40^{a}	19.95 ± 0.16^{b}	22.20 ± 0.10^{b}	21.05 ± 1.70^{b}	0.01
35 days of age (mg dL^{-1})					
Cholesterol	89.65 ± 6.43^{a}	66.65 ± 3.83^{b}	79.70 ± 2.45^{a}	60.93 ± 0.65^{b}	0.05
Triglycerides	69.00 ± 9.69^{a}	39.60 ± 1.67^{b}	50.25 ± 0.37^{b}	38.00 ± 0.75^{b}	0.01
HDL	36.30 ± 0.50	24.66 ± 0.54	28.76 ± 9.78	21.10 ± 0.66	NS
LDL	39.55 ± 8.31^{a}	34.06 ± 4.19^{ab}	40.88 ± 9.70^{a}	32.23 ± 1.22^{b}	0.05
VLDL	13.80 ± 1.93^{a}	7.92 ± 0.33^{b}	10.05 ± 0.07^{b}	7.60 ± 0.15^{b}	0.01

[a,b]Means in the same row with different letters are significantly different at $p<0.05$ and $p<0.01$, NS: Non-significant, T1: Control group, T2, T3 and T4: 0.1, 0.15 and 0.2% HMB-Ca, respectively

Table 4: Effect of dietary HMB-Ca on serum Ca, P and ALP

	Treatment groups				
Parameters	T1	T2	T3	T4	p-value
21 days of age					
Ca (mg dL^{-1})	8.76±0.13	8.56±0.38	8.13±0.12	7.83±0.92	NS
P (mg dL^{-1})	7.76±0.26	7.35±0.14	7.50±0.77	7.00±0.50	NS
ALP (IU L^{-1})	180.46±17.16^{bc}	152.39±2.88^{c}	202.05±4.34^{b}	325.55±14.23^{a}	0.05
35 days of age					
Ca (mg dL^{-1})	7.75±0.08^{b}	9.13±0.08^{a}	8.06±0.17^{b}	8.80±0.30^{a}	0.05
P (mg dL^{-1})	5.06±0.08	5.00±0.45	4.83±0.28	5.26±0.08	NS
ALP (IU L^{-1})	397.87±1.37^{a}	276.26±7.86^{b}	361.82±3.94^{ab}	424.22±16.32^{a}	0.05

^{a,b}Means in the same row with different letters are significantly different at p<0.05, NS: Non-significant, T1: Control group, T2, T3 and T4: 0.1, 0.15 and 0.2% HMB-Ca, respectively

Table 5: Effect of dietary HMB-Ca on some measurements of the metatarsus and femur bones

	Treatment groups				
Parameters	T1	T2	T3	T4	p-value
Metatarsus bone					
Length (cm)	7.13±0.23	7.00±0.15	6.83±0.14	7.23±0.21	NS
Weight (g)	10.33±1.11	8.91±0.35	8.80±0.31	9.09±0.17	NS
Ca (%)	2.67±0.07^{a}	2.28±0.0^{b}	2.73±0.02^{a}	2.76±0.02^{a}	0.01
P (%)	2.26±0.03^{b}	1.55±0.19^{c}	2.26±0.01^{b}	2.82±0.02^{a}	0.01
Femur bone					
Length (cm)	6.40±0.05	6.60±0.05	6.43±0.06	6.70±0.15	NS
Weight (g)	7.72±0.95	7.46±0.53	7.37±0.87	8.41±0.50	NS
Ca (%)	2.44±0.37	2.74±0.02	2.74±0.02	2.82±0.04	NS
P (%)	1.60±0.13^{b}	1.81±0.15^{ab}	2.03±0.10^{a}	2.08±0.02^{a}	0.05

^{a,b}Means in the same row with different letters are significantly different at p<0.05, NS: Non-significant, T1: Control group, T2, T3 and T4: 0.1, 0.15 and 0.2% HMB-Ca, respectively

All groups treated with HMB-Ca had significantly (p<0.01) lower serum TG levels compared to the control at 21 and 35 days of age. Treatment T4 had significantly (p<0.05) higher HDL levels at 21 days of age compared with T2 and T3, while the control group did not differ significantly from all treated groups. Treatment T4 had significantly (p<0.05) lower LDL levels compared with T3 at 21 days of age and compared with T3 and the control group at 35 days of age. All groups treated with HMB-Ca had decreased (p<0.01) VLDL levels compared with the control.

Serum Ca, P and ALP: Dietary treatment with HMB-Ca did not significantly affect the serum level of P at 21 and 35 days of age, while that of Ca was significantly different at 35 days of age: Ca levels in T2 and T4 were higher (p<0.05) than in the control and T3 (Table 4). Serum ALP increased significantly (p<0.05) in T4 compared to the control and other treatments at 21 days of age (Table 4).

Bone measurements: Femur bone weight, length and Ca level were not significantly affected by the administration of HMB-Ca, while the P level increased (p<0.05) in T4 and T3 compared to the control group, which in turn did not differ from T2.

The weight and length of the metatarsus bone did not differ significantly after the administration of HMB-Ca, while its Ca and P levels were significantly affected (p<0.01): T4 followed by T3 had higher Ca levels compared to T2, whose levels in turn were lower (p<0.01) than the control. Treatment T4 had significantly (p<0.01) higher P levels compared with the control and other treatments (Table 5).

pH for certain parts of the gut: The results of the statistical analysis of pH values of the content of certain parts of the digestive tract are shown in Table 6. Treatment groups T3 and T4 had significantly (p<0.05) lower pH values in crop, gizzard, duodenum and cecum compare with control group, whereas in large intestine, T2 had significantly (p<0.05) lower pH value compared with control and other groups.

DISCUSSION

Present study showed that adding 0.2% HMB-Ca to the diet of broiler chickens resulted in a significant increase in the weight of breast and thigh cuts. The results of the present

Table 6: Effect of dietary HMB-Ca on the pH of certain parts of the gut content

Gastrointestinal tract (mol L^{-1})	Treatment groups T1	T2	T3	T4
Crop	6.12±0.30[a]	5.40±0.23[b]	5.71±0.26[b]	5.85±0.34[b]
Gizzard	3.61±0.17[a]	3.30±0.35[b]	3.21±0.56[b]	3.33±0.27[b]
Duodenum	6.07±0.14[a]	6.05±0.08[a]	5.53±0.16[b]	5.61±0.06[b]
Cecum	7.99±0.83[a]	7.19±0.54[ab]	6.90±0.08[b]	6.91±0.52[b]
Large intestine	7.20±0.25[b]	6.74±0.00[c]	7.66±0.05[a]	7.32±0.04[ab]
p-value	0.05	0.05	0.05	0.05

[a,b]Means in the same row with different letters are significantly different at $p<0.05$, T1: Control group, T2, T3 and T4: 0.1, 0.15 and 0.2% HMB-Ca, respectively

study means that the high level of HMB-Ca used in this study was more beneficial than the lower levels used (0.1 and 0.15%). This increase could be due to the impact of HMB-Ca on muscle growth by increasing protein synthesis and decreasing protein degradation[7]. In addition, the gain in muscle weight may be due to the increased activity and efficiency of satellite cells[14], which may increase the weight of breast muscle; breast muscle is considered a more consumer-friendly cut because of its low fat content. The thigh percentage measured in this study agreed with that reported by Youbiao *et al.*[8] but disagreed with that reported by Qiao *et al.*[5], who found that HMB-Ca did not affect thigh cut percentage and attributed this result to the differences in breast and thigh muscle fibers, which in turn may impact the rate of protein transformation.

To explain the results of serum lipid profile, HMB-Ca plays a role in increasing the activity of the thyroid gland and in its secretion, thyroxin, which reduces the level of cholesterol in serum. In addition, the presence of calcium may increase bile acid secretion, leading to the increased use of endogenous cholesterol from the liver for cell regeneration[13]. Some studies have reported that the effect of HMB-Ca in reducing serum cholesterol may be due to the factors affecting the level of cholesterol also affecting TG[15]. Thus, the reduction of TG in serum in this study may be correlated with the reduction of total cholesterol. The reduction in VLDL in this study may be attributed to the significant reduction in TG due to the close relationship between these values.

Increasing the level of ALP in T4 suggest its high activity, which may be related to the low cholesterol level in serum[16] and is considered a reflection of increased protein synthesis in the liver[17].

The positive effect of HMB on bone strength when administered over a long time may be related to the increased bone tissue response, which depends on age and treatment duration[18]. Rodwell[19] reported that, in turkey, increased plasma concentrations of amino acids, especially proline and glutamate, as a result of adding HMB-Ca to the diet might be related to the increased density of bone minerals; proline will turn into hydroxyproline, which is an essential complement to collagen and protect it from protein degradation, while glutamate works as substrate of proline synthesis. Both proline and hydroxyl proline are shared in two-thirds of collagen installation; thus, glutamate and proline and hydroxyl proline are considered key amino acids responsible for the optimum quality of skeletal system.

Modulating the pH in certain parts of the gut content could be explained by a previous study of Wiaz *et al.*[20] found that HMB works to reduce the high pH of gut content. Thus, it is possible that HMG affects the stability of the composition of the intestinal flora (Gram-positive bacteria), which would be beneficial because HMB can serve as a substitute for antibiotics because it prevents the development of fungus and therefore it prevents the development of mycotoxins. HMB is an organic acid, organic acids and their salts work to inhibit the development of fungi and thus to restrict the possible evolution of toxic fungi[20].

CONCLUSION

It can be concluded from the results of this study that HMB-Ca appears to be safe for inclusion in the diet of growing broiler chickens under normal environmental conditions and that adding it, especially at the level of 0.2%, to the diet from 1-35 days of age stimulated the development of the main carcass muscles, decreases the level of harmful lipids in serum and increases the P level in the femur and metatarsus bones, in addition to its modification of the pH of certain parts of digestive tract content, which may prevent fungus development. Additional studies need to investigate the effect of HMB-Ca under heat stress conditions in hot climatic regions.

SIGNIFICANCE STATEMENTS

This study found that adding HMB-Ca to the diet of broiler chickens from 1-35 days of age is beneficial for developing breast and thigh muscles, which are the most consumer-friendly cuts in chicken carcasses, in addition to its positives effect of decreasing the levels of harmful lipids in serum and preventing the development of fungi in the gut. This study will help researchers to determine the critical effects of HMB as an organic acid on the blood and bone characteristics of broiler chickens that many researchers have not been able to explore. Thus, new studies may be carried out to determine the effect of HMB-Ca on egg yolk lipids or its effect under heat stress conditions.

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