Histological and biochemical evaluation of supplementing broiler diet with β-hydroxy-methyl butyrate calcium (β-HMB-Ca)

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Summary

Two hundred and sixteen day-old Ross-308 broiler chicks were allocated into 4 groups to study the impacts of different concentrations (0.0, 0.1, 0.15 and 0.2%) of β-hydroxy-methyl butyrate calcium (β-HMB-Ca), on values of tri-iodothyronin (T3) and tetra-iodothyronin (T4) hormones, liver enzymes [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], uric acid, peroxide, malondialdihyde (MDA), fatty acids and some histological parameters of small intestine (thickness of mucosa, height of villi, thickness of villi, depth of epithelial crypts and epithelial height). The biochemical results did not show any significant effect on T3 and T4 hormones and ALT while there was significant ($P<0.01$) decrease of AST in groups 2 and 3 and significant ($P<0.05$) decrease in uric acid in groups 2, 3 and 4 in comparison to control. In the liver, peroxide value (PV) and free fatty acids (FFA) were significantly ($P<0.05$ and $P<0.01$ respectively) decreased in groups 2 and 3 compared to control. The histological changes indicate significant values ($P<0.05$) in all parameters of duodenum in group 2 and 3, while those parameters of jejunum showed significant values ($P<0.05$) in most parameters of groups 2 and 4. In conclusion, the addition of β-HMB-Ca to the broiler diet from age 1 to 35 days has improved the levels of liver function enzymes and uric acid in the serum and lowered the parameters of oxidation in the liver with improved the maturity, performance and secretory activities of the small intestine in broiler chickens.

Ker words: Biochemical, Broiler, β-hydroxy-methyl butyrate calcium, Histological

Introduction

β-hydroxy-methyl butyric (β-HMB) acid is sold as a dietary supplement which is provided in the form of free acid (β-hydroxy-methyl butyric (β-HMB-FA) acid), and as a monohydrate calcium salt of the conjugate base, calcium β-hydroxy-methyl butyrate (β-HMB-Ca, Caβ HMB) monohydrate (Fuller *et al*., 2015). It is naturally produced by thebody cells through the metabolism of L-leucine, a branched-chain amino acid (Kohlmeier, 2015) and is used as dietary supplement due to its role in reducing the loss of body mass (Wu *et al*., 2015). It exerts its function throughout by stimulating the production and inhibiting the protein breakdown in muscles (Wilkinson *et al*., 2013). In the field of poultry production the first application of HMB on growing broiler chickens has revealed faster growth rate, increase in muscle mass and decrease in mortality (Nissen *et al*., 1994), also β-HMB has important adjustment in increasing the rates of fattening and improving carcasses (Van-Koevering *et al*., 1994), β-HMB has suppressed the protein degradation in isolate chick muscles (Ostaszewski *et al*., 2000), some blood parameters involved pro-inflammatory cytokines, total feed intake, total body weight gain, total body weight and feed conversion ratio during certain time intervals have been investigated by (Mahfouz *et al*., 2016). On other hand many studies have been investigated the role of β-HMB in animals and human on different medical cases (Nissen and Abumrad, 1997), has been examined the acute effects of HMB-Ca in young pigs (Wan *et al*., 2016) in early postnatal period piglets (Nissen *et al*., 2000) in humans cases of cardiovascular (Helen *et al*., 2005), and (Lundholm *et al*., 1976) on cancer (Wilkinson *et al*., 2013) on skeletal muscle protein metabolism, also (Szcześniak *et al*., 2015) and (Brioche *et al*., 2016) in case of muscle wasting and aging. There were many organs of body systems including cardiovascular, central nervous, musculoskeletal and immune systems which involved in the studying the effect of β-HMB compounds. However, impacts of β-HMB-Ca, has not been yet investigated histologically on the digestive mucosa. The aims of this study were to evaluate the histological changes in the small intestine as well as some biochemical effects of β-HMB-Ca that occur in broiler chicken blood.

Materials and Methods

This study was carried out at Poultry Farm, Department of Animal Production, College of Agriculture, University of Baghdad. Two hundreds and sixteen broiler chicks (Ross-308) one day old, weighing (45 g) were housing in 12 floor pens with wood shaving litter. The chicks house temperatures were reducing from (34°C) at the first 3 days and decreased by 2°C each

week until reaching 24°C. The lighting cycle was 24 h/day. The protective program was as follows; on day 1, 11 and 32 of age the chicks were vaccinated against Newcastle disease (ND) (B1), on day 20 against infectious bursal disease (IBD) and on day 29 against ND (Las Sota). Since day old the chicks have randomly allocated into four groups: group 1 (control), groups 2, 3 and 4. Three replicates per each group (18 chicks/ replicate). The β-HMB-Ca has been added to the chicks' diet at the rates 0.1, 0.15 and 0.2% for groups 2, 3 and 4, respectively. The chicks were fed *ad libitum* with two types of basal diet, the first one was starter (1-21 days of age) consist of 23% Crude protein (CD) and 3027 kcal/kg diet metabolizable energy (ME) while the second diet was grower (22-35 days of age) consist of 20% CP and 3195.3 kcal/kg diet ME (Table 1). The chicks of groups 2, 3 and 4 have been fed on ration with β-HMB-Ca (as powder) for period extended from one until 35-day-old. The source of β-HMB-Ca was from SCITEC NUTRION, Miami, USA. Blood samples have been collected from the brachial vein at 5th weeks for estimation the levels of tri-iodothyronin (T3) and tetra-iodothyronin (T4) hormones (Britton *et al*., 1975), liver function enzymes [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] (Retiman and Frankel, 1957), serum uric acid (Teitz, 1987), peroxide value (PV), free fatty acids (FFA%) (Egan *et al*., 1981) and malondialdihyde (MDA) (Witta *et al*., 1970). At the ends of experiment, the birds have been euthanized by slaughtering and internal viscera including the organs of digestive tract have been examined and collected, then the tissue samples of small intestine (duodenum and jejunum) have fixed in 10% neutral buffered formalin for 10 days, then prepared for paraffin embedding technique and sectioned at 6 µm with rotary microtome, the tissue sections have stained with hematoxylin and eosin stain (Bancroft and Marilyn, 2008). Thirty intestinal villi were measured per each segment of duodenum and jejunum to measure; thickness of tunica mucosa, villus height, villus thickness, depth of epithelial crypts, epithelial height, villus height: crypt depth ratio. The tissue sections were examined by light microscopy and microphotography has been done by using Future Win Joe microscopic camera, the images have been analyzed and scored by using Fiji image analyzer system (Schindelin *et al*., 2015).

Statistical analysis

Data were analyzed by using SPSS (version 24.0). All numerical results have been expressed as the mean±standard error (SE). For comparisons, the statistical significance has assessed by ANOVA. The significance level has set at $P<0.05$ and $P<0.01$.

Results

Serum biochemical tests

At the end of the experiment the statistical analysis of blood chemistry revealed non-significant differences in values of serum T3, T4 and their ratio (T3/T4) among control and experimental groups (Table 2). The analysis

Table 1: Composition and chemical calculated analysis of basal diet

Ingredient	Starter %	Grower %	Ingredient	Starter %	Grower %
Yellow corn	30	40	Crude protein	23	20
Wheat	28.25	24	ME (kcal/kg)	3027	3195.3
Soybean	31.75	24.8	Lycine	1.2	1.1
Meal (48)	5	5	Methionine	0.49	0.49
Protein	2.9	4.4	Cysteine	0.36	0.32
concentrate	0.9	0.6	Methionine	0.85	0.78
Vegetable oil	0.7	0.9	+cysteine	0.85	0.79
Limestone	0.3	0.1	Calcium	0.45	0.49
Dicalcium	0.2	0.2	Available	131.61	159.77
phosphate	100	100	Phosphorus		
Salt			C/P ratio		
Premix					

Chemical calculated analysis according to NRC (1994)

Table 2: Effect of adding β-HMB-Ca to broiler diet on serum biochemical and liver tests. Data represents (mean±SE)

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	P-value
T3	1.57 ± 0.04	1.45 ± 0.08	1.57 ± 0.08	1.45 ± 0.02	NS
T4	2.63 ± 0.04	2.50 ± 0.05	2.16 ± 0.05	2.55 ± 0.02	NS
T3/T4 ration	0.59 ± 0.00	0.58 ± 0.04	0.61 ± 0.00	0.62 ± 0.04	NS
AST	184.33 ± 0.2^{a}	141.0 ± 0.58^{b}	144.0 ± 8.08^{b}	176.0 ± 4.61^{a}	0.01
ALT	5.0 ± 0.58	4.0 ± 0.48	5.0 ± 0.33	5.0 ± 0.50	NS
Uric acid	8.51 ± 1.10^{a}	5.13 ± 0.87^{b}	4.11 ± 0.92^{b}	5.66 ± 0.10^{b}	0.05
PV (meq/kg tissue)	19.93 ± 0.17^{a}	17.00 ± 0.58^{b}	17.20 ± 0.58^{b}	19.13 ± 1.09^{ab}	0.05
MDA (mmol/L)	0.757 ± 0.03	0.770 ± 0.30	0.760 ± 0.03	0.770 ± 0.01	NS
Free Fatty Acid %	0.846 ± 0.03^{a}	0.736 ± 0.03^{b}	0.680 ± 0.00^{b}	0.846 ± 0.03^{a}	0.01

Means in the same row with different letters are significantly at different at $P<0.05$ and $P<0.01$. NS: Non-significant, group 1: Control, groups 2, 3 and 4: 0.1, 0.15 and 0.2% β-HMB-Ca, respectively

Table 3: Effect of adding β-HMB-Ca to broiler diet on Histo-morphomertical measurements of duodenum. Data represents (mean±SE)

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	P-value
Thickness of tunica mucosa (µm)	2110.8 ± 12.3^{b}	2330.10 ± 9.5^{a}	2400.0 ± 11.1^{a}	2105.4 ± 8.5^{b}	0.05
Villus height (µm)	1720.0 ± 7.2^{b}	2150.2 ± 9.0^{a}	2250.2 ± 14.2^{a}	1820.0 ± 2.9^{ab}	0.05
Villus thickness (µm)	122.2 ± 5.2^{b}	214.0 ± 9.2^{a}	240.5 ± 6.3^{a}	227.4 ± 1.2^{a}	0.05
Depth of epithelial crypts (µm)	45.3 ± 1.1^{b}	73.2 ± 2.0^{a}	70.4 ± 2.2^{a}	54.0 ± 1.8^{ab}	0.05
Epithelial height (µm)	169.5 ± 4.0^{b}	255.1 ± 7.1^{a}	186.0 ± 3.1^{ab}	181.3 ± 2.4^{ab}	0.05
Villus height: crypt depth ratio	37.96^{b}	29.37^{a}	31.96^{a}	33.70^{b}	0.05

Means in the same row with different letters are significantly at different at P<0.05. NS: Non-significant, group 1: Control, groups 2, 3 and 4: 0.1, 0.15 and 0.2% β-HMB-Ca, respectively

Table 4: Effect of adding β-HMB-Ca to broiler diet on Histo-morphomertical measurements of jejunum. Data represents (mean±SE)

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	P-value
Thickness of tunica mucosa (µm)	1125.9 ± 6.6^{b}	1650.8 ± 9.5^{a}	1305.9 ± 10.2^{ab}	1435.8 ± 9.7^{a}	0.05
Villus height (µm)	750.9 ± 4.9^{b}	1225.9 ± 9.1^{a}	849.2 ± 8.2^{b}	1475.7 ± 2.9^{a}	0.05
Villus thickness (µm)	170.1 ± 2.1^{b}	260.5 ± 5.1^{a}	190.9 ± 7.2^{b}	206.3 ± 6.0^{a}	0.05
Depth of epithelial crypts (µm)	120.1 ± 3.2^{b}	195.8 ± 1.9^{a}	160.1 ± 2.6^{ab}	137.4 ± 2.3^{b}	0.05
Epithelial height (µm)	47.9 ± 2.5	51.7 ± 2.0	49.2 ± 1.7	52.2 ± 1.9	NS
Villus height: crypt depth ratio	6.25^{b}	6.26^{b}	5.30^{b}	10.74^{a}	0.05

Means in the same row with different letters are significantly at different at P<0.05. NS: Non-significant, group 1: Control, groups 2, 3 and 4: 0.1, 0.15 and 0.2% β-HMB-Ca, respectively

of liver function test indicating significant decreases (P<0.01) in values of AST in groups 2 and 3 in comparison to control and group 4 (Table 2), on other hand the values of ALT in groups dietary fed with HMB-Ca were still as that in control (Table 2). The present results have been displayed significantly decreased at (P<0.05) in uric acid in all groups (2, 3 and 4). Our result has been revealed significantly decreased at (P<0.05) in PV in groups 2 and 3 in comparison to control group (Table 2), also the FFA was significantly (P<0.01) decreased in groups 2 and 3. Our results revealed that, the values of MDA were non-significant in comparing between group's dietary fed with HMB-Ca and control.

Histological results

Duodenum

The histometrical measurements of this part of small intestine are present in (Table 3), there were significant (P<0.05) increase in the values of thickness of mucosa, height of the villi and depth of epithelial crypts in groups 2 and 3 in compared to control, on the other hand the value of the thickness of villi showed significant (P<0.05) increase in all groups (2, 3 and 4) in compared to control, while the group 2 showed significantly (P<0.05) increased epithelial height in comparison to control and there were no significant differences between groups 2, 3 and 4, (Figs. 1, 2, 3 and 4), while the villus height: crypt depth ratio was significantly decrease in groups 2 and 3. In jejunum, the statistical analysis are present in (Table 4), the results revealed significant (P<0.05) increase in the values of thickness of the mucosa, height and thickness of villi in groups 2 and 4. The value of depth of epithelial crypts was significantly (P<0.05) increased in group 2 only while the villus height; crypt depth ratio was significantly increased in groups 4 only, the values of epithelial height showed non-significant differences between experimental and control groups. The histological sections revealed marked increases in the depth of epithelial crypts, the lamina propria showed an increase in layers of these crypts (Figs. 5 and 6), the epithelium of this part of intestine showing increases in population of goblet cells (Fig. 7).

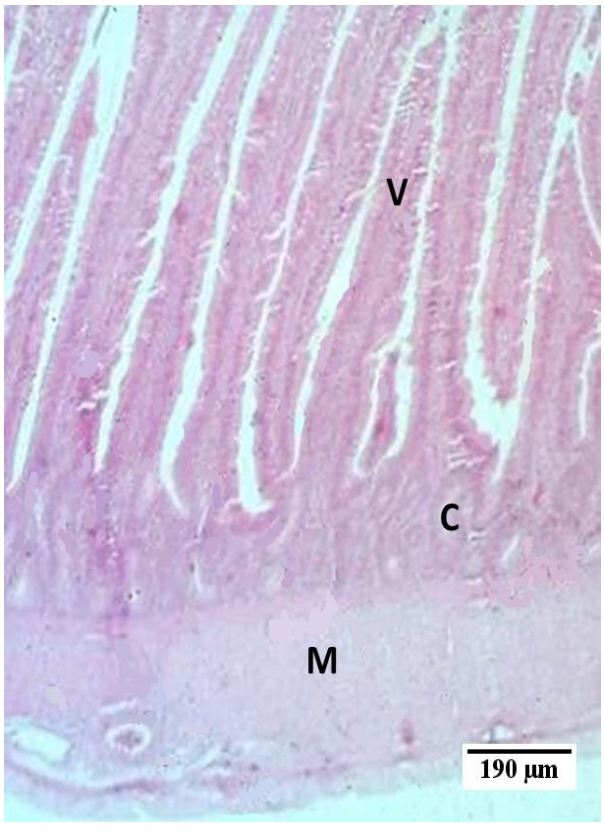

Fig. 1: Section of duodenum (control) shows: villus (V), epithelial crypts (C), and tunica muscularis (M), (H&E)

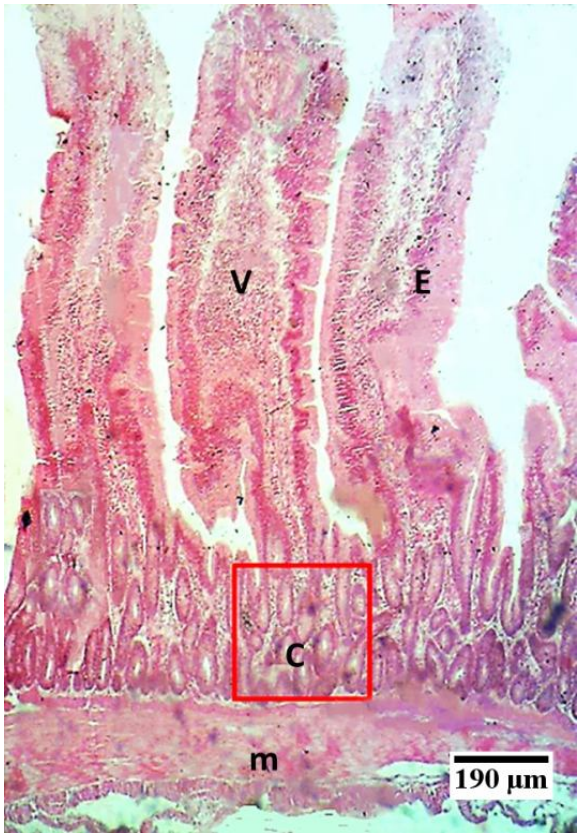

Fig. 2: Section of duodenum (group 2) shows: villus (V), epithelial crypts (C), epithelium (E), and tunica muscularis (m), (H&E)

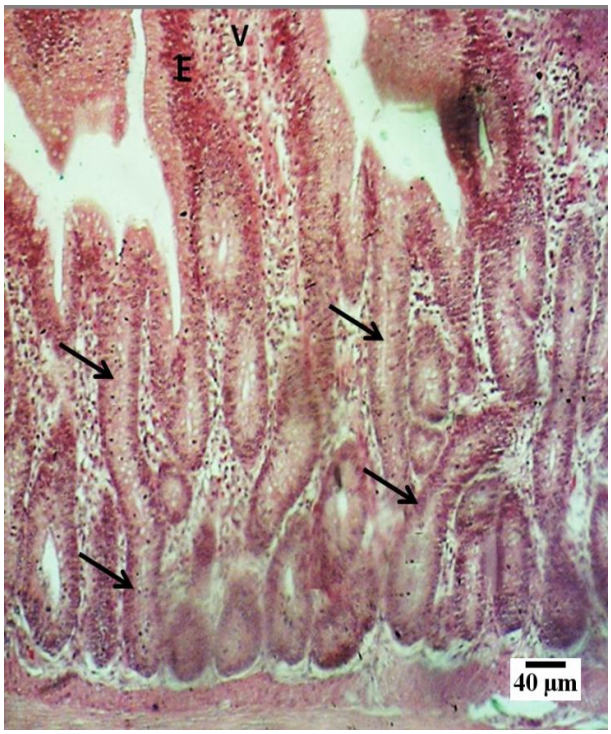

Fig. 3: Magnified section of red box area in Fig. 2 shows: villus (V), epithelium (E), and epithelial crypts (arrows), (H&E)

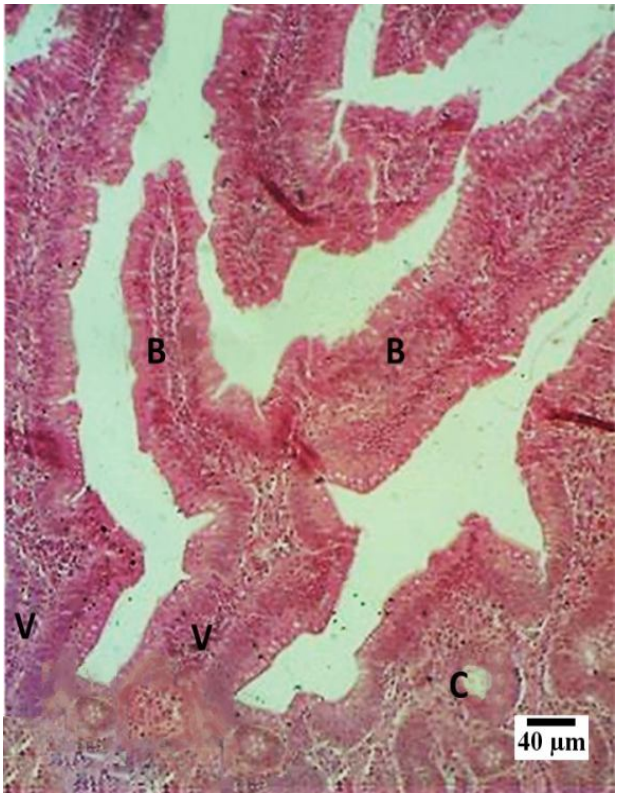

Fig. 4: Section of duodenum (group 3) shows: villus (V), villus branching parts (B), and epithelial crypts (C), (H&E)

Fig. 5: Section of jejunum (control) shows: villus (V), epithelial crypts (C), and tunica muscularis (m), (H&E)

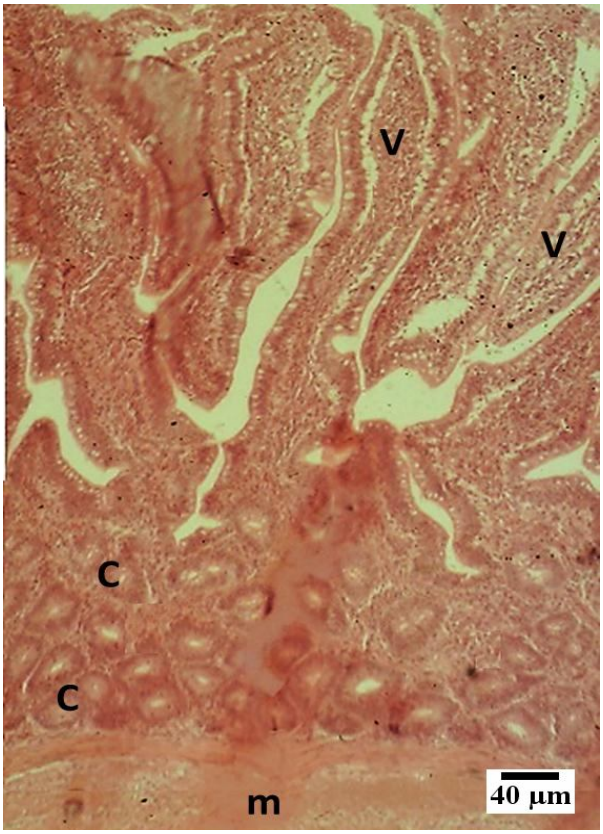

Fig. 6: Section of jejunum (group 2) shows: villus (V), epithelial crypts (C), and tunica muscularis (m), (H&E)

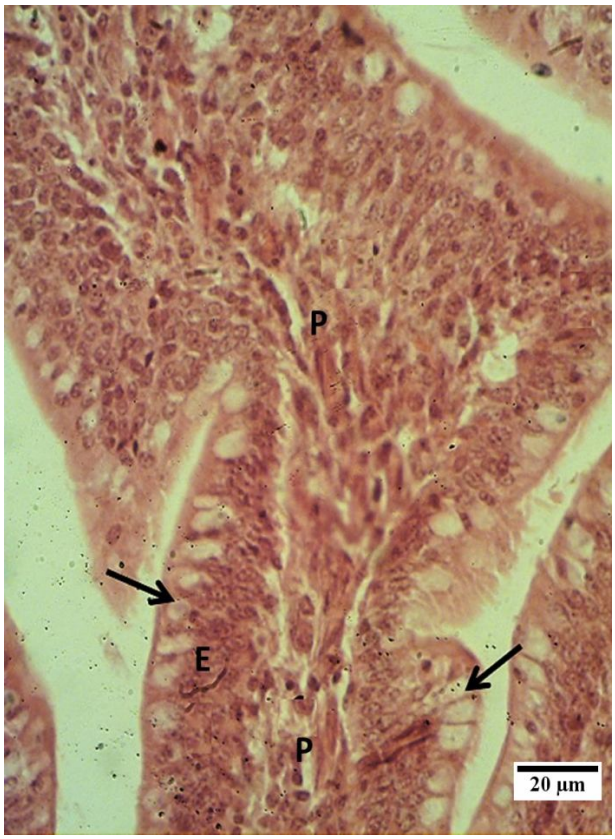

Fig. 7: Section of jejunum (group 2) shows: lamina propria within villus (P), epithelium (E), and goblet cells (arrows), (H&E)

Discussion

The statistical analysis of blood chemistry revealed non-significant differences in values of serum T3, T4 and their ratio (T3/T4), this result disagreed with result of (Moraes *et al*., 2014) who recorded marked decline in plasma levels of T3 hormone, this result suggests that the decreases was attributed for using the creatine as a compensatory in synthesis the activity (type 2) deiodinase which responsible for tissue conversion of T4 hormone into T3 hormone within tissue, records of (Buyse *et al*., 2009) has stated that, long term feeding of HMB did not affect serum T3 levels in chickens, which was contrary to our results, meanwhile the increase of these hormones was recorded by (Qiao *et al*., 2013; Mullur *et al*., 2014; Mahfouz *et al*., 2016) reported that the "dietary HMB-Ca had improved the growth throughout stimulating muscle development may be partly related to thyroid hormone changes in broiler chicks". The differences between the results of our study and those of other authors could be related to the differences in the bird's strains that used or to environmental factors or to the period of adding HMB-Ca to broiler diet. Results of liver function test indicating significant decreases in values of AST in groups 2, 3 and 4, such results were similar to that recorded by (Mahfouz *et al*., 2016), while current results disagree with the result of (Khudair and Al-Hussary, 2010) who referred to a relationship between the vaccination agonist viral disease (ND and IBD) and with elevation of AST. On other hand the values of ALT in groups, dietary fed with HMB-Ca were still as that in control (Table 2), this result was disagree (Mahfouz *et al*., 2016), ALT plays an important role in metabolism of glucose and amino acids as well as it increased when hepatocytes injury caused by toxicity, infection, alcoholism and liver steatosis (Wang *et al*., 2012), also (Gallagher *et al*., 2000) found no adverse effects from HMB supplementation on liver function test, lipid profile and renal function, also the significant decrease of ALT was recorded at 4% HMB-FA supplemented by (Fuller *et al*., 2014). The present results has been displayed significant decreased in uric acid in groups, this result agreed with result of (Qiao *et al*., 2013), uric acid is the end byproduct of nitrogen metabolism (Blachier *et al*., 2007) and reducing values of uric acid referring that, the HMB-Ca has been positively affected its metabolism, this suggested that, the HMB-Ca could interfere with production of xanthine throughout impairing releasing of phosphate molecules from adenosine and guanosine nucleotides (Pacher *et al*., 2006; Rodwell, 2009; Ferguson and Walters, 2011; Mandal and Mount, 2015), this result disagree with (Nissen *et al*., 2000) who revealed no effect of HMB seen for uric acid and blood urea nitrogen. Our results revealed that, the values of MDA in tall experimental groups were non-significant in compared to control, the MDA is one of several low-molecular-weight end products resulted from degradation of certain primary and secondary lipid peroxidation products and polyunsaturated fatty acids (Janero, 1990; Davey *et al*.,

2005), this result suggests that, the HMB-Ca is related with significant decreased of PV, such result disagrees with result reported significantly increased in plasma level of MDA when the amino acid L-leucine was used as supplements of HMB (EL-Kafoury *et al*., 2011), also our result parallel with (Siu *et al*., 2008) who reported that the muscle stress lead to greater increase content of MDA in young and aged rats, in association with increased of nitro tyrosine and catalase activities, those due to increased oxidative stress markers of MDA, H_2O_2 contents, also (Kondo *et al*., 2010) referred for greater increased in MDA in limb mice caused a modest increase in lipid peroxidation after stress exposure which related to oxidative damage of lipids. The present results revealed increase in thickness of intestinal mucosa which related with the increase in height of the villi, this result suggests that, the effect of the MBM-Ca was very important to increase the secretory activities of epithelial cells and epithelial crypts, in addition to increase the population of these crypts, also there were increase of cellular population associated with hypercellulartiy and vascularization of connective tissue within lamina propria of villi which were clearly obvious those lead to increase both the thickness and height of villi consequently many of villi appeared branched in compare with control (Figs. 2, 3, 4, 6 and 7) most parameters of duodenum in groups 2 (0.1%) and 3 (0.15%) have significantly affected histogenesis of these parts of small intestine, while these parameters showed less affection by HMB-Ca in jejunum except that of group 2 (0.1%), this suggested that the diet supplemented with 0.15% HMB-Ca has less effect on jejunum mucosa in compared to duodenum, the results of duodenum and jejunum suggest that, daily ingestion of HMB was important to increase the activities of gut and might be that the HMB-Ca have various positive ways that effected many of physiological roles included intracellular phosphocreatine functions which supported the energy buffer to prevent ATP depletion (Robertson *et al*., 2003), HMB's dose a mechanisms of action in the gut tissue may be associated with increase the cells wall integrity by up regulating cholesterol synthesis that permit maintenance of the plasma membrane during providing maximal cells growth and performance (Routhier and Stacy, 2007), so the HMB could metabolized into cholesterol and plays as well as raw material which necessary for the maintenance of intestinal epithelium (Mauch *et al*., 2001), the HMB enhances muscle protein synthesis (Eley *et al*., 2007) and regulates the growth hormone (GH) and/or insulin-like growth factor-1 (IGF-1) (Gerlinger-Romero *et al*., 2011), in addition increased expression of IGF-1 (Kornasio *et al*., 2009; Portal *et al*., 2011), study of (Holecek *et al*., 2009) proven the anabolic effect of HMB-Ca is related with proteolysis inhibition in proteasome that associated with protein synthesis in visceral tissues included digestive canal, on other hand (Tako *et al*., 2004; Foya *et al*., 2006) reported that the inoculation of eggs with HMB-Ca during late periods of chick embryo (E17 days) had enhanced the early development of gut.

The present study concluded the impacts of HMB-Ca which give good performance by recorded significant parameters in dietary supplementation of 0.1% and 0.15% (HMB-Ca), that affects primarily serum AST and uric acid and liver FFA and PV. The dietary supplementation of 0.1% and 0.15% HMB-Ca affects intestinal tissues and performance. The present study suggests finding more of immnunohistochemical investigations of HMB on the digestive enzymes and enteroendocrine cell population within intestinal mucosa.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Bancroft, JD and Marilyn, G (2008). *Theory and practice of histological techniques*. 1st Edn., London, Elsevier Limited. PP: 168-173.

Blachier, F; Mariotti, F; Huneau, JF and Tome, D (2007). Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. Amino Acids. 33: 547-562.

Brioche, T; Pagano, AF; Py, G and Chopard, A (2016). Muscle wasting and aging: experimental models, fatty infiltrations, and prevention. Mol. Aspects Med., 50: 56-87.

Britton, KE; Quinn, EV; Brown, BL and Eknis, PP (1957). A strategy for thyroid function tests. Br. Med. J., 3: 350-360.

Buyse, J; Swennen, Q; Vandemaele, F; Klasing, KC; Niewold, TA; Baumgartner, M and Goddeeris, BM (2009). Dietary β-hydroxy-β-methyl butyrate supplementation influences performance differently after immunization in broiler chickens. J. Anim. Physiol. Anim. Nutr., 93: 512-524.

Davey, MW; Stals, E; Panis, B; Keulemans, J and Swennen, RL (2005). High-throughput determination of malondialdihyde in plant tissues. Ana. Biochem., 347: 201-207.

Egan, H; Kirk, RS and Sawyer, R (1981). *Pearsons chemical analysis of foods*. 8th Edn., Edinburgh, UK, Churchill Livingstone. PP: 325-329.

Eley, HL; Russell, ST; Baxter, JH; Mukerji, P and Tisdale, MJ (2007). Signaling pathways initiated by beta-hydroxy-beta-methyl butyrate to attenuate the depression of protein synthesis in skeletal muscle in response to cachectic stimuli. Am. J. Physiol. Endocrinol. Metab., 293: 923-931.

EL-Kafoury, MA; Kalleny, NK and Hamam, GG (2011). Effect of amino acid L-leucine on the musculo-skeletal changes during cast-immobilization I adult male Albino rats. Physiological and histological study. Life Sci., 8: 876-892.

Ferguson, LD and Walters, MR (2011). Xanthine oxidase inhibition for the treatment of stroke disease: a novel therapeutic approach. Exp. Rev. Cardiovasc. Ther., 9: 399-401.

Foya, OT and Ferket, PR (2006). Effect of *in vivo* feeding egg with protein, beta-hydroxy-beta-methyl butyrate and carbohydrate on glycogen status and neonatal growth of turkeys. Poul. Sci., 85: 1185-1192.

Fuller, JC; Arp, LH; Diehl, LM; Landin, KL; Baier, SM and Rathmacher, JA (2014). Subchronic toxicity study of b-hydroxy-b-methyl butyric free acid in Sprague Dawley rats. Food Chem. Toxicol., 67: 145-153.

Fuller, JC; Sharp, RL; Angus, HF; Khoo, PY and Rathmacher, JA (2015). Comparison of availability and plasma clearance rates of β-hydroxy-β-methyl butyrate delivery in the free acid and calcium salt forms. Br. J. Nutri., 114: 1403-1409.

Gallagher, PM; Carrithers, JA; Goodard, MP; Schulze, KE and Trappe, SW (2000). β-Hydroxy-β-methyl butyrate ingestion, Part II: Effects on hematology, hepatic and renal function. Med. Sci. Sports Exerc., 32: 2116-2119.

Gerlinger-Romero, F; Guimarães-Ferreira, L; Giannocco, G and Nunes, MT (2011). Chronic supplementation of beta-hydroxy-beta methyl butyrate (HMB) increases the activity of the GH/IGF-I axis and induces hyperinsulinemia in rats. Growth Horm. IGF Res., 21: 57-62.

Helen, J; Mukerji, P and Michael, J (2005). Tisdale attenuation of proteasome-induced proteolysis in skeletal muscle by beta-hydroxy-beta-methyl butyrate in cancer-induced muscle. Loss. Cancer Res., 65: 277-283.

Holecek, M; Muthny, L; Kovarik, M and Sispera, L (2009). Effect of beta-hydroxy-beta-methyl butyrate (HMB) on protein metabolism in whole body and in selected tissues. Food and Chem. Toxicol., 47: 255-259.

Janero, DR (1990). Malondialdihyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med., 9: 515-540.

Khudair, IM and Al-Hussary, NA (2010). Effect of vaccination on some biochemical parameters in broiler chickens. Iraqi J. Vet. Sci., 24: 59-64.

Kohlmeier, M (2015). *Leucine nutrient metabolism: structures, functions, and genes*. 2nd Edn., USA, Academic Press. PP: 385-388.

Kondo, H; Yumoto, K; Alwood, JS; Mojarrab, R; Wang, A; Almeida, EA; Searby, ND; Limoli, CL and Globus, RK (2010). Oxidative stress and gamma radiation-induced cancellous bone loss with musculoskeletal disuse. J. Appl. Physiol., 108: 152-161.

Kornasio, R; Riederer, I; Butler-Browne, G; Mouly, V; Uni, Z and Halevy, O (2009). β-hydroxy-β-methyl butyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways. Biochim. Biophys. Acta BBA-Mol. Cell Res., 1793: 755-763.

Mahfouz, MK; Zyan, KAM and Abdel-Hamid, HH (2016). Biochemical effect of β-hydroxy-β-methyl butyrate calcium and creatine supplementations on some blood parameters, pro-inflammatory cytokines and growth performance of broiler chicks. Benha Vet. Med. J., 30: 39-50.

Mandal, AK and Mount, DB (2015). The molecular physiology of uric acid homeostasis. Annu. Rev. Physiol., 77: 323-345.

Mauch, DH; Nagler, K; Schumacher, S; Goritz, C; Muller, EC; Otto, A and Pfrieger, FW (2001). CNS synaptogenesis promoted by glia-derived cholesterol. Science. 294: 1354-1357.

Moraes, R; Bavel, DV; Moraes, BS and Tibiriçá, E (2014). Effects of dietary creatine supplementation on systemic microvascular density and reactivity in healthy young adults. Nutrition J., 13: 115-124.

Mullur, R; Liu, YY and Brent, GA (2014). Thyroid hormone regulation of metabolism. Physiol. Rev., 94: 355-382.

Nissen, S and Abumrad, NN (1997). Role of the leucine metabolite β-hydroxy β-methyl butyrate (HMB). J. Nutrt. Biochem., 8: 300-311.

Nissen, S; Fuller, JC; Sell, J; Ferket, PR and Rives, DV (1994). The effect of β-hydroxy-β-methylbutyrate on growth, mortality, and carcass qualities of broiler chickens. Poult. Sci., 73: 137-155.

Nissen, S; Sharp, L and Panton, M (2000). *B*-hydroxy-beta-methyl butyrate (HMB) supplementation humans is safe and may decrease cardiovascular risk factors. Am. Soc. Nutri. Sci., 30: 1937-1945.

NRC (1994). *National research council nutrent requirement for poultry*. 9th Edn., USA, National Academy Press. PP: 19-26.

Ostaszewski, P; Kostiuk, S; Balasińska, B; Jank, M; Papet, I and Glomot, F (2000). The leucine metabolite 3-hydroxy-3-methylbutyrate (HMB) modifies protein turnover in muscles of laboratory rats and domestic chickens *in vitro*. J. Anim. Physiol. Anim. Nutr., 84: 1-8.

Pacher, P; Nivorozhkin, A and Szabo, C (2006). Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol. Rev., 58: 87-114.

Portal, S; Zadik, Z; Rabinowitz, J; Pilz-Burstein, R; Adler-Portal, D and Meckel, Y (2011). The effect of HMB supplementation on body composition, fitness, hormonal and inflammatory mediators in elite adolescent volleyball players: a prospective randomized, double-blind, placebo-controlled study. Eur. J. Appl. Physiol., 111: 2261-2269.

Qiao, X; Zhang, HJ; Wu, SG; Yue, HY; Zuo, JJ; Feng, DY and Qi, GH (2013). Effect of β-hydroxy-β-methyl butyrate calcium on growth, blood parameters, and carcass qualities of broiler chickens. Poul. Sci., 92: 753-759.

Retiman, S and Frankel, S (1957). A colorimeteric method for determination of serum glutamic oxaloacetic pyruvic transaminase. Am. J. Clin. Path., 28: 56-63.

Robertson, SP; Twigg, SR; Sutherland-Smith, AJ; Biancalana, V; Gorlin, RJ; Horn, D; Kenwrick, SJ; Kim, CA; Morava, E; Newbury-Ecob, R; Orstavik, KH; Quarrell, OW; Schwartz, CE; Shears, DJ; Suri, M; Kendrick-Jones, J and Wilkie, AO (2003). Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. Nat. Genet., 33: 487-491.

Rodwell, VW (2009). Metabolism of purine and pyrimidine nucleotides. *Harper's illustrated biochemistry*.-28 e. New York, McGraw Hill. PP: 30-292.

Routhier, DD and Stacy, JJ (2007). HMB use and its relationship to exercise-induced muscle damage and performance during exercise. Int. Sport Med. J., 2: 68-77.

Schindelin, J; Rueden, CT and Hiner, MC (2015). The Image J ecosystem: an open platform for biomedical image analysis, Molecular Reproduction and Development, PMID 26153368 (on Google Scholar).

Siu, PM; Pistilli, EE and Always, SE (2008). Age-dependent increase in oxidative stress in gastrocnemius muscle with unloading. J. Appl. Physiol., 105: 1695-1705.

Szcześniak, KA; Ostaszewski, P; Fuller, JC; Ciecierska, A and Sadkowski, T (2015). Dietary supplementation of β-hydroxy-β-methylbutyrate in animals. J. Anim. Physiol. Anim. Nutr., 99: 405-417.

Tako, E; Ferkt, PR and Uni, Z (2004). Effect of *in vivo* feeding on carbohydrate and beta-hydroxy-beta-methyl butyrate on the development of chick intestine. Poult. Sci., 83: 2023-2028.

Teitz, NW (1987). *Fundamental of clinical chemistry*. 2nd Edn., Philadelphia, PA, Saunders Co., PP: 47-53.

Van-Koevering, MT; Dolezal, HG; Gill, DR; Owens, FN; Strasia, CA; Buchanan, DS; Lake, R and Nissen, S (1994). Effects of beta-hydroxy-beta-methyl butyrate on performance and carcass quality of feedlot steers. J. Anim. Sci., 72: 1927-1935.

Wan, H; Zhu, J; Su, G; Liu, Y; HUa, L; HU, L; Wu, C; Zhang, R; Zhou, P; Shen, Y; Lin, Y; Xu, S; Fang, Z; Che, L; Feng, B and Wu, D (2016). Dietary supplementation with β-hydroxy-β-methyl butyrate calcium during the early postnatal period accelerates skeletal muscle fiber growth and maturity in intra-uterine growth-retarded and normal-birth-weight piglets. Br. J. Nutri., 115: 1360-1369.

Wang, CS; Chang, TT; Yao, WJ; Wang, ST and Chou, P (2012). Impact of increasing alanine aminotransferase levels within normal range on incident diabetes. J. Formosan Med. Asso., 111: 201-208.

Wilkinson, DJ; Hossain, T; Hill, DS; Phillips, BE; Crossland, H; Williams, J; Loughna, P; Churchward-Venne, TA; Breen, L; Phillips, SM; Etheridge, T; Rathmacher, JA; Smith, K; Szewczyk, NJ and Atherton, PJ (2013). Effects of leucine and its metabolite β-hydroxy-β-methyl butyrate on human skeletal muscle protein metabolism. J. Physiol., 591: 2911-2923.

Witta, VC; Krause, GF and Bailey, ME (1970). A new extraction method for determination thiobarbituric acid values of pork and beef during storage. J. Food Sci., 35: 582-585.

Wu, H; Xia, Y; Jiang, J; Du, H; Guo, X; Liu, X; Li, C; Huang, G and Niu, K (2015). Effect of beta-hydroxy-beta-methyl butyrate supplementation on muscle loss in older adults: a systematic review and meta-analysis. Arch. Gerontol. Geriatrics. 61: 168-175.