Biochemical effect of β-hydroxy-β-methylbutyrate calcium and creatine supplementations on some blood parameters, pro-inflammatory cytokines and growth performance of broiler chicks

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ABSTRACT

One hundred and sixty-five one-day old Cobb broiler chicks were allocated into 3 groups to investigate the effect of dietary 0.1% β-hydroxy- β-methylbutyrate calcium (HMB) and 0.1% creatine on some blood parameters, pro-inflammatory cytokines and growth performance parameters in terms of total feed intake (TFI), total body weight gain (TBWG), total body weight and feed conversion ratio (FCR) during certain time intervals (21st day and 42nd day) versus control group (0 treatment). Some biochemical changes were recorded at the 42nd day among creatine supplemented group in the form of significantly high results of both triglycerides and T3 in group 3 (creatine) than that of the control group on the same day at P value > 0.05. HMB supplemented group showed significantly lower results of ALT (mean) than that of control group at (*P* value > 0.05) on the 21st day which became insignificant at the 42nd day. Dietary 0.1% HMB-Ca recorded better growth performance parameters in terms of TFI (4.527), TBWG (2.515), TBW (2.555) and TFCR (1.8) if compared to creatine supplemented group of (4.664, 2.508, 2.549 and 1.86) respectively and the control group of TFI equaled to 4.7432, TBWG equaled to 2.156, TBW equaled to 2.1987 and TFCR equaled to 2.2 at the end of the experiment. HMB and creatine supplementations had no effect on other blood parameters, *viz.* AST, urea, creatinine, total protein, FFA, T4, T3/T4 ratio, TSH, uric acid, cholesterol, HDL and LDL, and pro-inflammatory cytokines namely IL-6, IL-2 and TNF-α.

Keywords: HMB-Ca, Creatine, Broiler chicks, TFI, FCR, TBWG, TBW.

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1. INTRODUCTION

β-Hydroxy-β-methylbutyrate (HMB) is a bioactive metabolite formed from the breakdown of the essential branched amino acid, leucine. Leucine and its metabolite, keto-isocaproate (KIC), appear to inhibit protein degradation. This anti-proteolytic effect is believed to be mediated by HMB (Portal et al., 2011), which has gained interest in the animal and human research field in the last two decades**.** In domestic animals, HMB has been shown to convey beneficial effects in various species. Nissen et al. (1994a) first discovered that feeding HMB to broiler chickens resulted in faster growth rate, reduction in mortality, and increase in muscle deposition. The same research group also obtained positive results with HMB such as increasing body mass in growing lambs (Nissen et al., 1994b), and improving carcass yield in feedlot steers (Van-Koevering et al., 1994). Subsequently, extensive studies have been conducted on HMB supplementation in protein metabolism. Inclusion of HMB was reported

to suppress the protein degradation in isolated rat and chick muscles (Ostaszewski et al., 2000). Currently, HMB is widely used as an ergogenic supplement mainly in bodybuilders and power athletes who seek to improve performance or enlarge muscle mass (Portal et al., 2010). On the other side, human studies have shown that creatine monohydrate (CrM) supplementation has a number of biochemical and physiological effects. Intracellular phosphocreatine (PCr) functions as an energy buffer to prevent ATP depletion in the skeletal muscle (Robertson et al., 2003). Thus, creatine (Cr) is heavily involved in energy metabolism through the Cr and phosphocreatine (PCr) system. This system functions as a backup to the adenosine diphosphate/adenosine triphosphate (ATP) cycle to store and mobilize energy when required. The animal's demand for Cr can be supplied either directly from animal protein (e.g., fish or animal byproduct meal) in the diet or by endogenous synthesis. In the last 20 years, Cr has become a very popular dietary supplement (Maughan et al., 2004). Recent findings also highlight the effect of Cr supplementation on the increase of skeletal muscle and brain total Cr and PCr concentrations with an even greater degree of increase seen in organs with low baseline Cr content such as kidney and liver (Ipsiroglu et al., 2001). In animals, the effects of Cr supplementation on renal and hepatic structure and function have not been well established. Whereas some studies did not report any alteration in renal and hepatic function after Cr supplementation (Taes et al., 2003; Tarnopolsky et al., 2003), others have observed that it can speed up renal and hepatic disease progression (Tarnopolsky et al., 2003; Ferreira et al., 2005). Data on the influence of HMB and creatine supplementations, particularly on blood parameters and pro-inflammatory cytokines, are still scarce and inconsistent in broiler chicks. The aim of the current research was to investigate the biochemical effect of HMB and creatine on some blood parameters, pro-inflammatory cytokines as well as the effect of each other on growth performance (total feed intake (TFI), total body weight gain (TBWG), total body weight (TBW) and feed conversion ratio) in broiler chicks at certain time intervals (21st day and 42nd day).

2. MATERIAL AND METHODS

2.1. Birds and experimental design:

One hundred and sixty-five one-day old Cobb broiler chicks were commercially obtained and allocated into 3 groups to 3 dietary treatment (supplementation) groups; 1- control group (0 treatment), 2- 0.1% beta-hydroxy-beta-methylbutyrate (HMB-Ca) (ISOLABS, USA) and 3- 0.1% creatine (SAN Corporation, CA, USA). Each treatment group had 55 chicks. All groups were housed into three separate ventilated rooms and maintained on a 24-h constant-light program and temperature. Birds were fed on well-balanced diet as described by NRC (1994). Starter diet was given till the 14th day of age after that chicks were fed on grower diet which was given till the 28th day of age when chicks were then fed on finisher diet till the end of the experiment (42nd day of age). Feed and water were provided *ad libitum* throughout the experiment.

2.2. Growth performance parameters:

Feed intake (FI): The FI was calculated by dividing the amount of feed consumed in gram (by a certain group) during the week by the number of chicks of this group during the same week, where the TFI was only recorded. Body weight gain: The gain in body weight per week was calculated by subtracting the body weight between the two successive weeks, where the total body weight gain (TBWG) was the only recorded at the end of the experiment. Body weight was monitored at certain time (weekly to the 6th week), where the total body weight (TBW) was the

only recorded at the end of the experiment. Feed conversion ratio (FCR) was calculated by dividing the average amount of feed consumed in grams (per a chick) during the week by the average weight gain in grams during the same week (Lambert et al., 1936). FCR = average feed intake (g) bird per week/ average body weight gain (g) bird per week, where the only TFCR was recorded.

2.3. Blood sampling:

Blood samples were collected from the jugular vein at the 21st and 42nd days of age where five chicks were randomly taken from each group. Blood samples were then allowed to clot by leaving it undisturbed at room temperature for 15-30 minutes. Serum samples were separated after centrifuging at 1800 rpm for 10 minutes and then divided into small aliquots and stored at –20°C until examined.

2.4. Blood parameters:

Liver function tests (ALT and AST) which were performed according to Young (2001). Total proteins and uric acid were performed according to Tietz (1995) and Young (2001). Kidney function tests (creatinine and urea) were performed according to Burtis et al. (1999). Lipid profiles (cholesterol, triglyceride, HDL and LDL) were performed according to Kaplan (1984), Young (1995) and Young (2001). Thyroid gland functions: Thyroid stimulating hormone (TSH) ELISA kit: A competitive enzyme immunoassay technique for quantitative determination of chicken TSH concentrations in serum and plasma. The microtiter plate provided in this kit has been pre-coated with an antibody specific to TSH. It is a commercial kit obtained from BioSource Company (San Diego, CA, USA). Chicken thyroxine (T4) ELISA kit: It is a double antibody sandwich ELISA Kit. Microtiter plates were pre-coated with chicken T4 monoclonal antibody and the detecting antibody was polyclonal antibody labeled with biotin. This kit was made for *in vitro* quantitative detection in chicken serum, plasma or cell culture supernatant and organizations in the natural and recombinant T4 concentration. The kit was obtained from BioSource Company, San Diego, CA, USA. Chicken Triiodothyronine (T3) ELISA kit: It is a competitive enzyme immunoassay utilizing a monoclonal anti-T3 antibody and a T3-HRP conjugate. This T3 ELISA kit is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Chicken T3. It was obtained from BioSource Company, San Diego, CA, USA. Chicken Free Fatty Acid (FFA) ELISA kit: It is a competitive enzyme immunoassay utilizing a monoclonal anti-FFA antibody and an FFA-HRP conjugate. This FFA ELISA kit is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Chicken FFA. It was obtained from BioSource Company, San Diego, CA, USA.

Pro-inflammatory cytokines: Chicken Interleukin2 (IL-2) ELISA kit: It is quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-2 has been pre-coated onto a microplate. This kit was used for quantitative determination of chicken interleukin 2 (IL-2) concentration in serum, plasma and tissue homogenates. It is a commercial kit obtained from BioSource Company (San Diego, CA, USA). Chicken Interleukin 6 (IL-6) ELISA kit: It is a sandwich-ELISA applied *in vitro* for quantitative determination of Chicken IL-6 concentrations in serum, plasma and other biological fluids. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to IL-6. It was obtained from BioSource Company, San Diego, CA, USA. Chicken TNF-α (Tumor Necrosis Factor Alpha) ELISA Kit: It is a sandwich-ELISA applied *in vitro* for quantitative determination of chicken TGF-β1 concentrations in serum, plasma and other biological fluids. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to TGF-β1. The kit was

obtained from BioSource Company, San Diego, CA, USA.

2.5. Statistical analysis:

All the following analyses were performed using IBM® SPSS® Statistics, Version 21, IBM Corporation, 2012, under the environment of Windows® 8.1, Microsoft Corporation. Two-way analysis of variance (ANOVA): Raw data were statistically tested for the normal distribution using SPSS different statistical parameters. Unfortunately, the assumption of normal distribution of data was violated (right Skewness) as indicated by the SPSS' Shapiro-Wilk and Kolmogorov-Smirnov tests at P< 0.05. Data were then transformed using log base 10 + 1 to follow normal distribution. Two-way ANOVA with post hoc test using the least significant difference (LSD) were used to study the statistical significant differences in the means of phases; phase 1 (21 day old) and phase 2 (42 day old chicks), blood parameters & pro-inflammatory cytokines categories and the interaction between each other as independent factor and their effect on results in chickens as dependent factor and to study the statistical significant differences in the means of growth performance parameters (TFI, TBWG, TBW and FCR) of control group as well as treatment groups at the end of the experiment (42nd day).

3. RESULTS:

Two-way ANOVA was performed on transformed raw data. It revealed statistically significant differences of blood parameters and pro-inflammatory cytokines during the 2 phases (21 d and 42 d) at P value less than 0.05, as shown in Table (1), Figure (1) and (2). To specify where the statistical significant differences are located, pairwise comparisons were done in the form of post hoc test (group of *T* tests) using LSD at *P*< 0.05 and confidence interval of 95% for blood parameters & pro-inflammatory cytokines categories within time intervals (21 d and 42 d) as in Table (2). This revealed low statistically significant differences in the means of ALT between the control group and group 2 (HMB) at the 21st day, where the results of ALT (mean) were significantly higher in the control group than in group 2 (HMB) at P = 0.031. Then again, the significant results obtained at the 21st day between the control group and group 2 of ALT parameter became insignificant at the 42nd day (*P* value > 0.05). Once again, there was high statistically significant differences in the means of triglycerides at the 42nd day between the control group and group 3 (creatine) at P value = 0.001, where the result (mean) of triglycerides was significantly higher in group 3 (creatine) than that of the control group. There were statistically significant differences in the means of T3 between the control group at the 42nd day and group 3 (creatine) at P value of 0.000, indicating significantly higher results (mean) of T3 in group 3 (creatine) than those of control group). Table (3) specifies where the statically significant differences of weight between treatment groups were located, where a post hoc test (pairwise comparison) using LSD at $p < 0.05$ and confidence interval of 95% was performed. Very high statistically significant variation was noticed between the control group as well as the treatment group and between treatment groups each other at *P* =0.000000 throughout the experiment, where the recorded overall mean differences of weight were significantly higher in treatment groups than in the control group and in group 2 (HMB) than that of group3 (creatine). Moreover, the overall weight mean difference was significantly better between the control group and group 2 (HMB) than that between the control group and group 3 (creatine). The increased total body weights were significantly higher at the 6th week in HMB supplemented group (2.555 Kg) than that of creatine supplemented group

Table (1). Comparison of blood parameters and pro inflammatory cytokines' average means during certain intervals of the experiment

Blood parameter & pro-inflammatory cytokines	21st day			42nd day		
	G1 (control)	G2 (HMB)	G3 (creatine)	G1 (control)	G2 (HMB)	G3 (creatine)
ALT	9.4±2.07[a]	6±2.73 [a,b]	8.2±3.34 [b,c]	6.4±1.14 [a,b]	5.2±2.48 [a]	6±3.00 [a]
AST	153.4±15.50[a]	144±8.71 [a,b]	153.2±11.30[a]	152.4±9 [a]	137.8±16 [b,c]	157.8±8.58[a]
Total protein	2.25±0.38[a,b]	2.17±0.56 [a]	2.32±0.18 [a]	2.90±0.33 [a]	3.65±0.23 [a]	2.75±0.23 [a]
Creatinine	0.34±0.046[a]	0.29±0.019[a]	0.28±0.054 [a]	0.38±0.037 [a]	0.32±0.053[a]	0.31±0.033[a]
Urea	8.2±0.83[a]	7.2±1.64 [a, b]	10±1.22 [a,b]	8.2±0.44 [a]	7±0.70 [a]	8.8±1.09 [a]
Uric acid	7.8±0.60[a]	5.4±0.13 [a]	7.5±0.19 [a]	8.24±0.35 [a]	6.74±0.40 [a]	6.44±0.25 [a]
Cholesterol	`114.2±13.4[a]	116.6±10.5[a]	118±15.1 [b]	133.2±8.52 [b]	137.6±14.9[a]	138.6±31 [b]
Triglyceride	62.2±5.80[b]	72.2±14.80[b,c]	61±18.73 [a]	62.4±17.81 [a]	52.8±5.71 [b]	102.6±12.5[b]
HDL	82.26±4.43[a]	85.76±4.08[a]	89.26±8.13 [a]	66.76±1.78 [a]	69.76±1.08[a]	58.26±2.2 [a]
LDL	19.5±16.24[a]	16.6±12.48[a]	16.5±10.75 [a,b]	53.96±10.41[b]	57.28±15.6[b]	59.82±30.6[a]
T3	17.10±5.32[a]	25.55±19.8[a,c]	18.42±5.81 [a,b]	18.44±10.1 [a]	24.49±10.9[a]	32.79±14.3[a]
T4	21±6.34[a,c]	27.10±7.10[a]	25.38±4.69 [a,b]	30.8±6.7 [a,c]	34.1±8.7 [a]	33.1±11.4 [a]
T3/T4 ratio	0.82±0.26[a]	0.93±0.72 [b]	0.70±0.13 [a]	0.56±0.19 [a]	0.71±0.25 [a,b]	1.12±0.74 [a]
TSH	1.64±0.31[a]	1.86±0.45 [a]	1.61±0.39 [a,b]	1.44±0.27 [a]	1.08±0.11 [a]	1.17±0.06 [a]
IL2	0.32±0.060[a]	0.24±0.063[a]	0.27±0.15 [a]	0.32±0.073 [a,b]	0.22±0.033[a]	0.28±0.049[a]
IL6	30.43±6.74[a,b]	36.42±4.45[a]	29.12±2.27 [b,c]	27.8±5.05 [a]	24.5±9.59 [a]	29.7±11.3 [a]
TNF-α	73.79±19.55[a]	66.88±6.81[b,c]	74.54±16.76[a]	85.6±21.6 [a]	57.7±20.3 [a]	41.5±12.9 [a]
FFA	3.88±0.15[a]	4.40±0.32 [b]	3.51±0.93 [a]	5.66±0.65 [b]	5±2.17 [a]	5.26±0.90 [b]

Data are revealed as (Mean ± S.E). S.E = Standard error.
Mean values with superscript letters are statistically significant difference at $P< 0.05$

Table (2). Post Hoc test (pairwise comparisons) using the least significant difference (LSD) to compare multiple mean differences of blood parameters and pro inflammatory cytokines within time intervals (21 d and 42 d).

Phases	(i) Blood parameters & pro inflammatory cytokines	(j) Blood parameters & pro inflammatory cytokines	Mean difference (i-j)[a]	(P value)[b]
(21 d)	G1 (ALT)	G2 (ALT)	0.1992±0.092[a]	0.031
(42 d)	G1 (Trig.)	G3 (Trig.)	-0.2225±0.064[a]	.001
	G1 (T3)	G3 (T3)	-0.2550±0.071[a]	.000

a: The mean difference± Standard error: significant at P value less than 0.05. b. Adjustment for multiple comparisons: Least Significant Difference (LSD).

Table (3): Post Hoc test (Pairwise Comparisons) using LSD to compare multiple mean differences of chick weights at certain time intervals

Treatment groups (i)	Treatment groups (j)	Overall mean (LG10) difference of weight (i-j)	(*P* value)[b]
G1	G2	-180.2724±0.302[a]	0.000000
G1	G3	-170.0057±0.302[a]	0.000000
G2	G3	10.2667±0.303[a]	0.000000

Data are revealed as overall mean difference of weight (Mean ± S.E). S.E = Standard error
Mean values with superscript letters are statistically significant at $P< 0.05$.

Table (4) Total feed conversion ratio, total body weight gain, total body weights and total feed intake for control group as well as treatment groups at the end of the experiment.

Performance parameters	G1 (control)	G2 (HMB)	G3(creatine)
TFI	4.743±0.037[a]	4.527±0.098[a]	4.664±0.090[b]
TBWG	2.156±0.037[a]	2.515±0.085[a]	2.508±0.025[a]
Total FCR	2.2±0.004[a]	1.8±0.0076[b]	1.86±0.008[b]
TBW	2.198±0.010[a]	2.555±0.015[a]	2.549±0.032[b]

(Mean ± S.E). TFCR = total feed conversion ratio. TBWG= total body weight gain. S.E = Standard error. TFI= total feed intake. TBW= total body weight. Mean values with superscript letters are statistically significant at $P< 0.05$.

(2.549 Kg), while the control group recorded a significant lower total body weight in comparison with other groups (2.198 Kg). Table (4) and figure (3) record a better (significant) total feed intake (TFI), total body weight gain (TBWG), total body weight (TBW) and total feed conversion ratio (TFCR) in group 2 (HMB) and group 3 (creatine) of (4.527, 2.515, 2.555 and 1.8) and (4.664, 2.507, 2.549 and 1.86) respectively specially if compared with the control group (group 1) of TFI equal to 4.743, TBWG equal to 2.156 and TBW equal to 2.198 and TFCR equal to 2.2 at the end of the experiment. In conclusion, group 2 (HMB) showed better performance followed by group3 (creatine). All treatment groups recorded significant TFI, TBWG, TBW and TFCR better than the control group.

4. DISCUSSION

Biochemical blood parameters are indicators for the physiological, pathological, and nutritional status of the animal. They have the potential of being used to elucidate the impact of nutritional factors and additives supplied in the diet (Ashour et al., 2014). Table (1) and Figures (1-2) reveal statistically significant differences (2-way ANOVA) of blood parameters, *viz.* AST, ALT, urea, creatinine, total protein, FFA, T3, T4, T3/T4 ratio, TSH, uric acid, cholesterol, triglycerides, HDL and LDL and pro inflammatory cytokines namely IL-6, IL-2 and TNF-α of chicks treated with HMB, creatine and the combination of creatine and HMB in comparison of the control group (0 treatment) during certain time intervals (21 d and 42 d) at $P < 0.05$. Table (2) revealed significantly higher results of ALT (mean) in the control group as compared to group 2 (HMB) with P value = 0.031 on the 21st day. This may be attributed to increased metabolic rate in the liver, indicating liver toxicity, especially when exposed to drugs, viral or bacterial agents (Takahashi et al., 1995). In the current study, vaccination against New Castle was carried out at the 20th day of the experiment with Lasota strain, where it could be the reason why ALT was elevated. This finding is in agreement with the results reported by Khudair and Al-Hussary (2010), who denoted that vaccinated chickens showed a significant elevation in the level of globulin and activities of aspartate aminotransferase (AST), ALT and lactate dehydrogenase (LDH) compared with their values in non-vaccinated chickens. That finding (elevated ALT) fortunately became insignificant at the 42nd day. There were no statistical significant differences recorded between the control group and the other treatment groups or between treatment groups with each other in ALT profile either at the 21st day or the 42nd day. Except for ALT significant changes was noticed between the control group and groups 2 (HMB), there were no significant changes recorded in blood parameters and pro-inflammatory cytokines at the 21st day between the control group and the other treatment groups. High statistically significant differences in the mean of triglycerides was recorded at the 42nd day between the control and group 3 (creatine) at P value = 0.001, where the result (mean) of triglycerides was significantly higher in group 3 (creatine) than that of the control group as shown in Table (2). This finding contradicts the studies which reported that creatine had a little effect on lipid metabolism specially serum triglycerides in broilers as recorded by Chen et al. (2011) as well as the fact that creatine supplementation at doses of 0.5g/kg/day produced no significant changes in laboratory measurements of triglycerides compared to the control group in rats (Baracho et al., 2015). However, Earnest et al. (1996) reported decreased triglycerides (26%) in hypercholesterolemic men and women (32 y to 70 y) supplemented with creatine 5g/day for 56 days. The mechanism of creatine hypolipidaemic effects remains enigmatic, however, creatine may promote

acute increases in peripheral and/or hepatic insulin sensitivity and/or post-receptor signaling in post prandial hyperglycemic non-diabetic subjects. This may faster decrease in *de novo* triglyceride production as stated by Steiner and Vranic, (1982). There was significant higher results (mean) of T3 in group 3 (creatine) than that of the control group. The mechanism through which creatine exerts its effects on thyroid hormones is yet to be investigated. These results are contrary to the results revealed by Moraes et al. (2014), who observed a reduction in plasma levels of T3 and an increase in T4 levels in healthy young adults, where the authors suggested that the main cause of lowering the plasma T3 level was the creatine supply which contributed to a compensatory reduction in T3 synthesis by decreasing the activity of the type 2 deiodinase responsible for tissue conversion of T4 to T3 in tissues such as the kidneys and skeletal muscles (Mullur et al., 2014), where, a week of creatine supplementation in diet significantly increased creatinine and creatine kinase (CK) plasma levels, and simultaneously reduced globulins and T3 plasms levels, mimicking a condition that characterizes impaired renal function. There were no significant changes recorded in blood parameter and pro inflammatory cytokines at the 42nd day between the control group and other treatment groups rather than those mentioned before.

HMB supplemented group showed better performance in terms of better TFI and TBWG, TBW and TFCR followed by group 3 (creatine) as shown in Table (3). All treatment groups recorded significant growth performance parameters better than the control group. The reasons behind better performance of group 2 supplementary treated with HMB will be discussed as follows. Tendency in weight gain was observed when HMB was consumed at 0.1% (Peterson et al., 1999). Nissen et al. (1994) explained that the increase in performance of broilers fed a diet supplemented with HMB was possibly due to meeting the HMB requirement for metabolic function in those chicks. Studies have shown that administration of HMB into the amnion of late-term avian embryo beneficially affected chick BW (Uni et al., 2005; Foye et al., 2006b) through enhancing early gut development (Foye et al., 2006a). Furthermore, HMB may enhance chick growth by acting as an immune-modulator. For instance, Peterson et al. (1999) reported that HMB supplementation tended to improve several immunological functions in broilers, and speculated that such immunological improvement may enhance growth performance. HMB has also been adopted as an alternative supplement to combat protein degradation. Regarding meat producing livestock, the effects of HMB on different meat-producing animals are not consistent. In the present study, the chicks were fed HMB from day 1. A significant increase in average body weight was observed in chicks fed 0.1% HMB-Ca diet at both the 21 and the 42 days of age compared with chicks fed the control diet. In chickens, between 1 to 6 weeks of age, protein degradation was slower than protein synthesis as evidenced by increases in muscle growth during this period (Lauterio et al., 1986). It was suggested that HMB might affect muscle growth because it increased protein synthesis and decreased protein degradation (Uni et al., 2005). However, HMB-Ca supplementation did not significantly affect the leg muscle yield. Ostaszewski et al. (2000) reported that HMB appeared to suppress protein breakdown more in white muscle fibers than in red muscle fibers in the rat. The reason for this phenomenon may be attributed to the fact that the rate of protein turnover in different skeletal muscles varies with the composition of fiber types (Baillie and Garlick, 1991). It has been postulated that HMB may regulate protein metabolism either through hormonal

receptor effects (Pause et al., 1994; Tsuruzoe et al., 1998 and Tatara, 2008), or it might be attributed to modulating the enzyme responsible for muscle tissue breakdown. Better growth performance recorded in the current research in group 2 supplemented with HMB matched the results of (Qiao et al., 2013).

Creatine monohydrate is an amino acid derivative that has become a popular sports supplement used to increase muscle performance (Wyss and Kaddurah-Daouk, 2000) in humans. The role of creatine in the better performance of group 3 (creatine) than that of the control group as recorded may be due to that the mechanisms suggest that creatine may enhance muscle performance and protein synthesis due to an increase in the amount of energy stored as phosphocreatine. Creatine loaded muscle has the capacity to maintain normal physiological function and to delay the onset of muscle fatigue (Casey et al., 1996). Increased concentrations of intramuscular phosphocreatine attract water into muscle cell and increase the cell volume (Hultman et al., 1996). Earlier research has suggested that creatine can help the body quickly provide ATP through the creatine-phosphocreatine energy shuttle system, improve the muscle ATP concentration and buffer muscle lactic acid accumulation (Bessman and Carpenter, 1985). Additionally, creatine monohydrate increases bone mineral density (Antollic et al., 2007). Thus, creatine supplementation could be expected to increase weight gain and percentage lean. Balsom et al. (1995) showed that human athletes consuming 20 g of CrM/day for 6 days had increased body mass by 1.1 kg in the study done by Moghadam et al. (2008) to clarify the effects of creatine monohydrate (CrM) supplementation on growth performance and to evaluate histopathological lesions in rats and broiler chickens. The authors concluded that feeding CrM, for 15 days had no effect on body weight gain in rats but had significant elevation in chickens like the current study and the difference between the two species is probably due to differences in the metabolism and function of creatine. This was obvious in a six-week study conducted by Stahle et al. (2003) to determine the feed efficiency and carcass quality of broilers supplemented creatine monohydrate where Stahle et al. (2003) stated that the feed efficiency of chicks fed diet (0.63% creatine weeks 4-6) was superior to that of birds fed diet without creatine (control), during week four of the experiment and authors concluded that feed efficiency was improved from the third week to the fourth one. The current study was contrary to the results stated by Xia et al. (2012), who indicated that CMH supplementation didn't affect the average daily weight gain, average daily feed intake or feed efficiency and breast muscle or thigh muscle percentage of broilers.

5. Conclusion:

Based on the results of the current research, the authors concluded that dietary supplementation of 0.1% HMB-Ca improved growth performance parameters in terms of TFI, TBWG, TBW and TFCR compared to creatine supplemented group and the control group. HMB-Ca offers significant lower total feed intake (low dietary costs), significant better total feed conversion ratio, better total body weight gain and subsequently better average total body weights. The better growth performance offered by HMB is followed by the performance of creatine (0.1%) compared to the control group. The performance of HMB as well as creatine in broiler chicks needs further work to investigate the histopathological changes induced by each one in the doses tried and even higher doses. Creatine induce some biochemical changes in blood parameters in the form of significantly high results of both triglyceride and T3 at the end of the experiment in creatine supplemented group. Significant high results

of ALT (mean) in the control group than in group 2 (HMB) on the 21st day during feeding on grower diet were observed where, that significant results became fortunately insignificant at the 42nd day. Rather than these changes, HMB and creatine supplementations had no effect on the other blood parameters, *viz.* AST, urea, creatinine, total protein, FFA, T4, T3/T4 ratio, TSH, uric acid, cholesterol, HDL and LDL, and pro inflammatory cytokines namely, IL6, IL2 and TNF-α.

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