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Supplementation of Whey Protein Concentrates and Creatine Monohydrate to Broiler Diet: Effects on Performance, Molecular Regulation of Muscle Building, Carcass Characteristics and Oxidative Status

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Abstract: The purpose of this study was to assess the effects of whey protein concentrates (WPC) and /or creatine monohydrate (CMH) addition to broiler diets on growth performance, gene expression regulating muscle building, carcass characteristics and oxidative status. One-day-old Ross chicks (N= 300) were assigned to six dietary treatments. The 1st treatment received control diet, the 2nd treatment received the control diet supplemented with 0.05% WPC, the 3rd treatment received the control diet supplemented with 0.05% CMH, the 4th treatment received the control diet supplemented with both WPC and CMH (0.05%, each), the 5th treatment received a reduced diet (RD) lower in metabolizable energy and crude protein (50 kcal/kg and 0.5%, respectively) and the 6th treatment received the RD supplemented with WPC and CMH (0.05%, each) for 42 days feeding trail. Each treatment was replicated in five pens of 10 birds each.The results showed that birds fed on either control or RD supplemented with WPC and/ or CMH exhibited a significant ($p<0.05$) increased cumulative body weight (BW), body weight gain (BWG), improved feed conversion ratio (FCR), elevated carcass yield and breast meat percentage compared with the control treatment. Where, the body abdominal fat was significantly ($p > 0.05$) decreased in groups fed on WPC and/or CMH.The expression of myognin and insulin like growth factor-1 (IGF-1) in muscle were up-regulated after addition of WPC and /or CMH. Supplementation of WPC and/or CMH did not significantly alter the serum creatine, urea and uric acids levels of broiler. The reduction of malondialdehyde (MDA) and increased values of reduced glutathione (GSH) in liver corresponding to antioxidant capacity were observed after 42 days feeding of WPC. In conclusion, addition of WPC and/or CMH to a conventional diets or RD for growing chickens exerts a growth-promoting action and induces a higher carcass yield and breast muscle percentage based on both chemical and molecular analysis and prompt desirable changes in bird's health.

Key words: Whey protein concentrates · Creatine monohydrate · Broiler performance · Gene expression · Antioxidant capacity

INTRODUCTION

In poultry farming, feedstuffs represent the main proportion of costs, energy and protein furnishing ingredients comprise the most of those costs. Using of low level of such feed ingredients may limit the energy and amino acid concentrations in the diet. This limitation can be reinforced with supplementation of diet with new rich energy and highly available amino acids sources. Creatine, a compound based on amino acids (arginine, glycine and methionine), produced in the liver, kidneys and pancreas may participate in this market because it is an essential precursor in the production of muscle energy, additionally favoring muscle growth [1]. Creatine helps to maintain the energy balance in cells and tissues by accepting high energy phosphate groups from adenosine triphosphate (ATP) to create phosphocreatine (PCr) and then releasing the high energy phosphate group to form ATP when energy demand is high [2]. As creatine continues to be broken down in the body's metabolic processes, many animals such as growing broilers are not able to produce enough creatine in intensive farming

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conditions [3]. Supplementation of creatine can spare arginine and improve growth performance of poultry [4]. Dietary inclusion of CMH (1200 mg/kg) for 14 days before slaughter significantly decreased drip loss, lactate content and glycolytic potential in the pectoralis major of broilers transported 3 h during the summer which maintain the meat quality [5]. The other enriched amino acids source is the whey proteins containing β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin and lactoperoxidase are highly bioavailable and quickly absorbed into the body. Their concentrations vary depending on breed, feeding system and season [6]. With special concern to whey protein concentrates containing 70% – 85% protein, up to 5% lactose and has higher concentration of branched-chain amino acids, leucine, isoleucine and valine which are highly concentrated in muscle tissue and are used to fuel working muscles and stimulate protein synthesis [7]. Besides, whey protein concentrates is an excellent source of lysine with considerable contents of methionine plus cystine. The last two play a key role in cellular antioxidant status, serving as precursors for glutathione [8]. In addition to their excellent nutritional value, whey protein possesses health related properties as immune enhancing effects, gastrointestinal hormone secretion, antimicrobial and antiviral actions and such other metabolic functions as muscle anabolism [9]. Feeding whey protein concentrate with 8 or 32 g/kg can be regarded as a method for elevating production efficiency and meat yield in broiler chickens [10]. Inclusion of 0.02% dried whey powder also exerts an improvement in body weight gain, feed conversion ratio to broiler chicks [11]. Hence, WPC and CMH certainly are an excellent source of amino acids. Consequently, they can be used for poultry diets in comparatively small amounts as a basic protein-supplying feedstuff, better overall health status and improved productivity of chickens. Therefore, the objective of this study was to evaluate the effect of dietary WPC and/or CMH on performance, muscular protein metabolic gene expression, carcass characteristics and oxidative status of broiler chickens.

MATERIALS AND METHODS

Bird Husbandry, Diets and Experimental Design: Three hundred One-day-old ROSS boiler chicks were purchased from commercial hatchery and incubated together in the first three days then weighted, wing banded and randomly assigned to floor pens within the experimental station of Faculty of Veterinary Medicine, Zagazig University. Basal control diet (Table 1) was formulated for growing broilers according to NRC [12] as well a reduced diet (RD) with decreasing level of metabolizable energy (50 kcal/kg diet) and crude protein (0.5%). A completely randomized design was used to allocate birds into six dietary treatments for 42 days feeding trail. The six treatments included, the 1st treatment received basal control diet, the 2nd treatment received the control diet supplemented with 0.05% WPC (500 gm/ton), the 3rd treatment received the control diet supplemented with 0.05% CMH (500 gm/ton), the 4th treatment received the control diet supplemented with 0.05% of each WPC and CMH (500 gm/ton of each), the 5th treatment received the reduced diet (RD) lower in metabolizable energy and crude protein (50 kcal/kg and 0.5%, respectively) and the 6th treatment received the RD supplemented with 0.05% of each WPC and CMH (500 gm/ton of each). Each treatment had five replicate pens of 10 chicks in each. Diets were fed in mash form and the bird had free excess to feed and water. CMH was purchased from APN, USA manufacture and WPC was available from Abbott Nutrition.

Growth Measurements and Carcass Evaluation: The birds were weighed by pen at the end of each feeding period (starter, grower and finisher) after 8 h. feed deprivation. Mean of FBW, BWG, FI and FCR were used to evaluate growth performance for the entire 42-day period. Mortality was considered in data analysis. At the end of the experiment, five birds were chosen from each dietary group (one from each replicate) fasted for 10 h and used for slaughter analysis. Abdominal fat pad was removed from the body cavity and weighed. Slaughter yield was recorded, breast and thigh muscle were also removed from the body, weighed and calculated on the basis of life body weight.

Meat Nutritional Composition Measurement: Parts from breast muscle samples were used for analysis of moisture, crude protein and intra muscular fat according to AOAC [13].

Measurement of Metabolic Parameters and Oxidative Status of Broiler: At the end of feeding trial, blood samples were taken from five birds (one bird from each replicate) and allowed to clot at 4°C and centrifuged at 3000×g for 10 min then the harvested serum samples were stored at -20°C until assayed for detection of creatine, urea and uric acid using commercial kits (Sigma Aldrich). As well, five liver samples collected and used for detection of antioxidant enzymes as reduced GSH and MDA concentrations with a colorimetric assay kits (Sigma Aldrich).

Table 1: Ingredient composition and calculated nutrient levels of the basal diets

	Starter		Grower		Finisher	
Ingredients (%)	Control	RD	Control	RD	Control	RD
Corn, Grain	58.00	60.00	61.90	63.90	64.80	66.80
Soybean meal	34.50	34.50	29.00	29.00	26.00	26.00
Corn gluten meal	1.40	0.22	2.60	1.50	2.20	1.00
Calcium CO3	1.30	1.30	1.25	1.25	1.20	1.20
Dical. Phos.	1.90	1.90	1.65	1.65	1.50	1.50
Phytase	0.00	0.00	0.00	0.00	0.00	0.00
Soybean oil	1.65	0.80	2.40	1.50	3.20	2.40
Common salt	0.20	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.35	0.35	0.32	0.32	0.32	0.32
DL-Methionine	0.25	0.25	0.23	0.23	0.18	0.18
L-Lysine HCl	0.20	0.20	0.20	0.20	0.15	0.15
Threonine	0.05	0.05	0.05	0.05	0.05	0.05
Premix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Calculated composition (%)						
M.E Kcal/Kg diet	2.998	2.948	3.105	3.052	3.181	3.133
Protein	21.54	21.03	20.00	19.52	18.54	18.00
E.E.	4.234	3.431	5.107	4.256	5.977	5.223
Calcium	1.00	1.00	0.92	0.92	0.86	0.86
Avail. Phos.	0.48	0.48	0.43	0.43	0.40	0.40

Premix[1] per kg of diet: vitamin A, 1 500 IU; vitamin D3, 200 IU; vitamin E, 10 mg; vitamin K3, 0.5 mg; thiamine, 1.8 mg; riboflavin, 3.6 mg; D pantothenic acid, 10 mg; folic acid, 0.55 mg; pyridoxine, 3.5 mg; niacin, 35 mg; cobalamin, 0.01 mg; biotin, 0.15 mg; Fe, 80 mg; Cu, 8 mg; Mn, 60 mg; Zn, 40 mg; I, 0.35 mg; Se, 0.15 mg. Nutrient level of the diets was based on NRC [34].

Table 2: Oligonucleotide PCR primers

Gene	Forward primer	Reverse primer	Product size
Myogenin	5'AGCAGCCTCAACCAGCAGGA-3'	5'- TCTGCCTGGTCATCGCTCAG -3'	179
Myostatin	5'- AGTAGCGATGGCTCTTTGGA -3'	5'CTGGGAATGTGACAGCAAGA-'3	427
IGF-1	5'- CACCTAAATCTGCACGC-3'	5'CTTGTGGATGGCATGATCT-3'	140
β-actine	5'- AATGAGAGGTTCAGGTGCCC -3'	5'- ATCACAGGGGTGTGGGTGTT -3'	409

Analysis of Gene Expression by Real-Time PCR: At the end of the trail five samples from pectorals muscle were collected rapidly, kept in liquid nitrogen and stored at -80 for molecular investigation. Molecular investigation of Myogenin, Myostatin, IGF-1 and β-actine gene expression were determined using a semi-quantitative Real-Time PCR according to Meadus [14]. Firstly, total RNA was isolated from muscles samples using the E.Z.N.A.™spin column RNA extraction kit (Omega Bio-Tech, Cat NO R6834-01, Canada) following the manufacturer's instructions. The extracted RNA was quantified by measuring the absorbance at 260 nm and its purity was checked by the ratio between the absorbance values at 260 and 280 nm which was 1.83, demonstrating the pure RNA. This was confirmed by electrophoresis on 1.5 % agarose gel containing ethidium bromide. Then 0.5 µg total RNA was denatured at 72 °C for 10 min, then incubated at 42 °C for 60 min in a 10 µl RT reaction mixture. The mixture contained 0.5 mM of each dNTP, 50 ng random hexamers and 100 U Superscript II reverse transcriptase which finally was heated to 95 °C for 5 min and chilled on ice. Then 2 µl cDNA was used for performing PCR in the presence of 0.2 mM of each dNTP, Taq polymerase buffer, 7.5 pM of each primer and 1.5 U of Taq polymerase. The conditions of PCR were initial denaturation at 95 °C for 5 min followed by 25 cycles of 94 °C, 30sec; 58 °C, 40 sec; 72 °C, 40 sec for myogenin, 94 °C, 30sec; 62 °C, 40 sec; 72 °C, 40 sec for myostatin94 °C, 30sec; 60 °C, 40 sec; 72 °C, 40 sec for IGF-1, 94 °C, 30sec; 56 °C, 40 sec; 72 °C, 40 sec for β-actine. PCR was performed with a 2720 thermocycler (Applied Biosystems, USA). PCR products were analyzed on a 1.5 % agarose gel in 90 mM Trisborate, 2 mM EDTA buffer (TBE), pH 8 and visualized by staining with ethidium bromide and UV transillumination and analyzed by gel documentation system (Bio Doc Analyze, Biometra, Germany). The values for the specific targets were normalized using those of ß-actin to express arbitrary units (AU) of relative abundance of the specific messages (i.e., relative expression).

Primer sequences of chicken Myogenin, Myostatin and β-actine were obtained from the published sequences Gabriela [15] and for IGF-1 from Del Vesco [16], Table 2.

Statistical Analysis: The experimental data were expressed as the mean ± SE and statistical significance was considered at ($P < 0.05$) as tested by one-way analysis of variance (ANOVA), using SPSS 18.0 and Excel 2013 in Microsoft.. Least square means showing significant differences were compared by Fisher's Protected LSD test.

RESULTS

Growth Performance: The cumulative results for final body weight (FBW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) for 42 days feeding trial are presented in Table 3. The broiler receiving diet supplemented with both WPC and CMH have significant ($P < 0.05$) increased final BW and BWG followed by groups fed on whey then group fed on creatine and finally group fed on RD supplemented with WPC and CMH in comparison with the control group and group fed on RD. Broilers belonging to diet supplemented by both WPC and CMH and WPC consumed overall lowered feed than birds from the control group and group fed on RD, but this did not negatively affect their BWG at the end of the experiment and gave better values of FCR. Consequently broiler groups fed on WPC and/or CMH were utilized their feed more efficiently than control and RD group. Moreover, addition of WPC and CMH to RD recorded improved growth performance results as did groups fed on control diet with WPC and CMH when compared with control group.

Post Slaughter Carcass Evaluation: Supplementation of WPC and / or CMH resulted in a significant ($P < 0.05$) higher percentage of carcass dressing, breast meat and decreased abdominal fat even in RD supplemented by them in comparison with control diet and RD (Table 4). Moreover, there was no significant difference between diet supplemented with WPC and RD supplemented with both WPC and CMH in carcass dressing percentage. As well no significant difference observed between diet supplemented with CMH and RD supplemented with both WPC and CMH in breast meat yield. In relation to thigh muscle yield, there is no significance difference between the different groups.

Meat Composition: Effects of dietary supplementation of WPC and/or CMH on broiler meat composition including moisture, crude protein and fat are shown in Table 5. There was no significant difference in the moisture content between different groups. However, crude protein content significantly ($p < 0.05$) increased in group supplemented with WPC and CMH then group supplemented with WPC and RD group supplemented with WPC and CMH followed by group supplemented with CMH when compared with control and RD group. In relation to fat content, addition of WPC and/or CMH significantly decreased the fat content even in RD supplemented by them with great effect in groups supplemented with both whey and creatine followed by group supplemented with WPC.

Table 3: Effect of dietary WPC and /or CMH on all over growth performance of broiler chicks (means ±SE)

Parameters	Control	WPC	CMH	CMH + WPC	RD	RD (CMH + WPC)
FBW(g per bird)	2466^{e}± 0.32	2575^{b}± 0.37	2542^{c}±0.25	2588^{a}± 0.44	2437^{f}±0.58	2512^{d}± 0.45
BWG (g per bird)	2383^{e}±0.32	2492^{b}± 0.37	2460^{c}± 0.24	2505^{a}± 0.49	2354^{f}± 0.58	2429^{d}± 0.45
FI (g per bird)	4227^{a}± 6.85	4071^{b}±12.79	4180^{a}±24.93	4071^{b}± 50.86	4241^{a}±32.16	4158^{ab}± 16.63
FCR	1.73^{b}±0.00	1.57^{d}±0.00	1.64^{c}±0.00	1.54^{e}±0.00	1.75^{a}±0.00	1.64^{c}±0.00

Within-row different superscript letters denote significant difference (P<0.05).

Table 4: Effect of dietary WPC and /or CMH on carcass characteristics of broiler chicks (means ±SE)

Parameters (%)	Control	WPC	CMH	CMH + WPC	RD	RD (CMH + WPC)
Dressing yield	71.6^{d} ±0.05	72.6^{b} ±0.06	72.3^{c} ±0.07	73.4^{a} ±0.05	71.0^{e}±0.20	72.8^{b}±0.05
Breast meat yield	29.7^{d} ±0.08	30.8^{b} ±0.04	30.7^{c} ±0.05	31.7^{a}±0.04	30.2^{e} ±0.05	30.8^{b}±0.04
Thigh meat yield	17.10±0.05	17.00± 0.10	17.00±0.11	17.30 ±0.11	16.90±0.15	17.20±0.11
Abdominal fat	1.83^{a} ±0.01	1.72^{b} ±0.01	1.73^{b}±0.01	1.67^{c} ±0.01	1.82^{a} ±0.01	1.66^{c}±0.01

Within-row different superscript letters denote significant difference (P<0.05).

Table 5: Effect of dietary WPC and /or CMH on meat nutritional composition (breast muscles, fresh basis) of broiler chicks (means ±SE)

Parameters (%)	Control	WPC	CMH	CMH + WPC	RD	RD (CMH + WPC)
Moisture	66.5±0.02	66.7±0.05	66.7±0.07	66.6±0.06	66.5±0.05	66.7±0.04
CP	18.8^{d}±0.04	20.4^{b}±0.07	19.7^{c}±0.09	21.4^{a}±0.04	18.5^{e}±0.07	20.3^{b}±0.04
EE	12.9^{a}±0.04	11.1^{c}±0.08	11.8^{b}±0.06	10.2^{d}±0.02	12.9^{a}±0.04	10.1^{c}±0.02

Within-row different superscript letters denote significant difference (P<0.05).

Table 6: Effect of dietary WPC and /or CMH on some metabolic parameters and oxidative status of broiler chicks (means ±SE)

Parameters	Control	WPC	CMH	CMH + WPC	RD	RD (CMH + WPC)
Creatine, mg/dl	3.22^{ab}±0.02	3.20^{b}±0.03	3.32^{a}±0.04	3.28^{ab}±0.04	3.20^{a}±0.03	3.28^{ab}±0.04
Urea, mg/dl	17.38^{ab} ±0.05	17.37^{ab} ±0.03	17.46^{a}±0.03	17.40^{a}±0.05	17.30^{b}±0.03	17.43^{ab}±0.02
uric acid, mg/dl	3.65^{ab}±0.04	3.67^{ab}±0.03	3.766^{a} ±0.03	3.746^{a} ±0.04	3.59^{b} ±0.04	3.726^{a}±0.03
GSH, mg/g tissue	0.23^{c}±0.00	0.34^{a}±0.00	0.23^{c}±0.00	0.34^{a}±0.00	0.22^{d}±0.00	0.33^{b}±0.00
MDA, mmol/g tissue	0.97^{a}±0.01	0.82^{c}±0.01	0.97^{a}±0.01	0.82^{c}±0.01	0.95^{b}±0.01	0.83^{c}±0.01

Within-row different superscript letters denote significant difference (P<0.05).

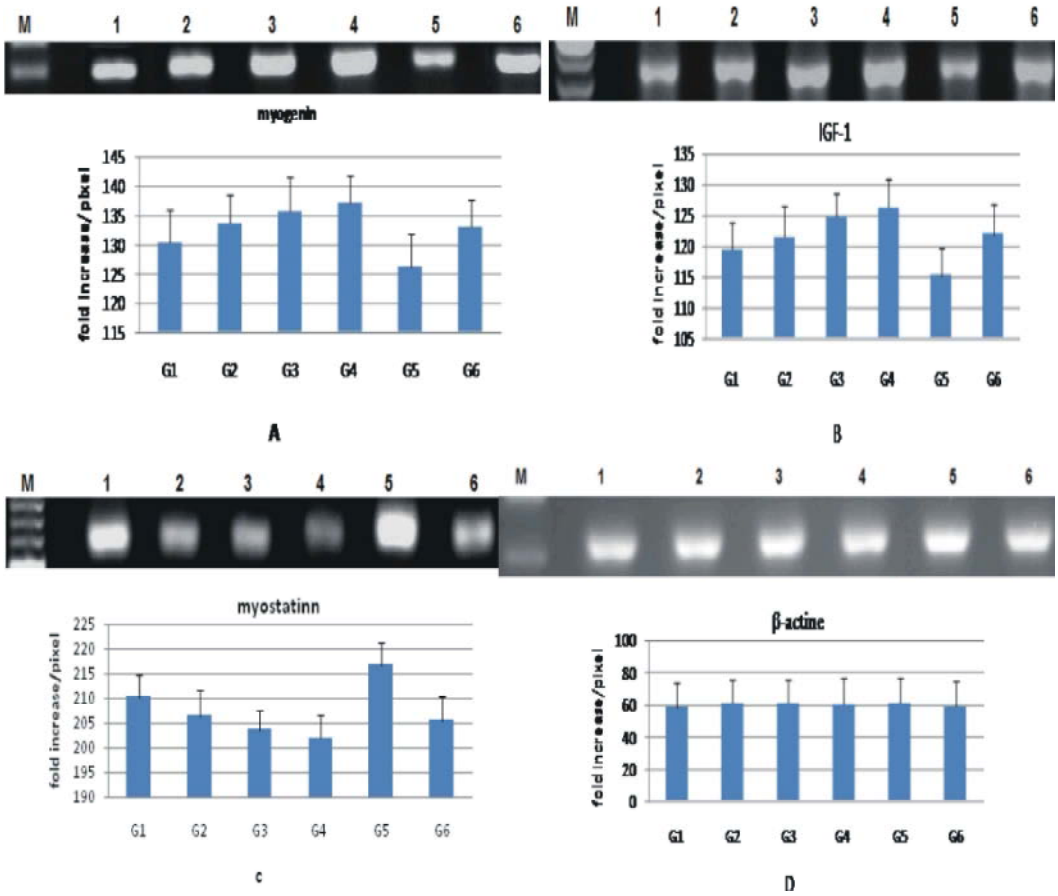

Fig. 1: The expression level of mRNAs of (a) Myogenin, (b) IGF-1, (c) Myostatin and (d) β-actine genes in the pectoralis muscle tissue of sp. M, marker; 1, control group; 2, CMH supplemented group; 3, WPC supplemented group; 4, CMH and WPC supplemented group;5, RD group; 6, CMH and WPC supplemented RD group.

Measurement of Some Metabolic Parameters and Oxidative Status of Broiler: Addition of CMH and/or WPC to broiler diet had no effect on blood creatine, urea and uric acid values when compared with control and RD group. However, supplementing of whey to the broiler diet significantly ($p<0.05$) increased reduced GSH levels and decreased the level of MDA in fresh liver tissue analyzed at the end of the feeding trial when compared with control (Table 6). Nevertheless, adding of creatine alone to broiler diet had no effect on reduced GSH and MDA levels.

Muscular Protein Metabolic Gene Expression: As shown in Fig. 1, both WPC and CMH supplementation up-regulated IGF-I gene expression and increased the expression of myogenin mRNA in muscle. Inversely, their addition was down-regulated mRNA expression for the muscle myostatin gene. Moreover, it was clear that addition of WPC alone had the best effect on muscular protein metabolic gene than creatine alone. Indeed, supplementation of WPC and CMH to RD improved the expression of the previous genes when compared with control and RD.

DISCUSSION

In prospect of properties and metabolic functions of both WPC and CMH, it was proposed that the dietary inclusion of both WPC and CMH would result in increased the breast muscle mass of broilers with maximum effect when WPC and CMH were combined together over those fed on the control diet and RD. Indeed, in the current experiment, treatment with both WPC and/or CMH increased the BWG of broiler even when supplemented to RD, despite lower feed intakes, compared with the birds in the control group. Consequently, broiler offered creatine and WPC in consecutive feeding phases had better FCR than control, with maximum productivity in group fed on both WPC and CMH. These effects also agreed by Kheiri *et al.* [11] who found that dietary inclusion of 0.02% dried whey powder to basal diet revealed an improvement in the BWG, FCR and decreased abdominal body fat of female broiler chicks. This may be explained by better nutrient digestibility and/or the capacity of the intestines to absorb nutrients. Moreover, increasing action of intestinotrophic hormone glucagon-like peptide 2 (GLP-2) which lead to better development of the intestinal mucosa and increase the relative intestinal weight, villus height, expression of nutrient transporter mRNA (including Na+/ glucose co-transporter-1 and peptide transporter-1 mRNA) in the small intestine and consequently, increase growth performance due to whey proteins [17,18]. Moreover, supplementation of dried whey had improved BWG of chickens when offered by level between 3–6 g. kg^{-1} [19, 20]. In addition, isolated whey peptides reported to increase rate of muscle growth [21, 22]. On other hand, inclusion 5% bone meal + creatine (600g/ton) improved BWG for 42 days broilers when compared with the bone meal diet with no creatine [1]. This may be clarified by an ability of creatine to make cellular hydration and increase fat free muscle as a result of enhancing protein synthesis. These effects somewhat resemble those reported by Halle *et al.* [23], who showed that creatine supplementation (1, 2, 5 and 10g/kg) to a corn-soybean based diet improved BWG compared with a control group in 35-day-old broilers. While Stahl *et al*. [24] found small but significant improvements in feed conversion after creatine loading. Additionally, creatine monohydrate increases bone mineral density [25]. Thus, creatine supplementation could be expected to increase weight gain and percentage lean.

In the present study the percentage of carcass dressing and breast meat were improved even in RD supplemented with WPC and or CMH. It was more prominent that the body abdominal fat was reduced by their WPC supplementation. Similar results agreed with Chen *et al*. [26] who found that inclusion of 5% Cr-Pyr and 3% creatine to broiler diet increased muscle protein synthesis and lowered abdominal fat as a portion of total BW, than in the control group. Mihic *et al*. [27] reported that CMH ingestion has significantly increased fat-free mass in humans. It is cleared that CMH supplementation increases body mass by increasing protein synthesis. In this way, creatine supplementation is equal to energy intake for increasing the storage of phosphocreatine, this result implies that when energy intake increases, protein accretion also increases linearly until it reaches an upper limit [28]. Whey proteins help muscle building through its contents from essential amino acids, on other hand decreasing intramuscular fat by whey proteins may be explained by balancing of blood sugar levels with energy source helping the body to burn fat. Majewska *et al*. [29] showed that use of whey increased liver weight percentage and decreased heart, abdominal fat and gizzard weight percentage in broiler chickens.

Another remarkable observation in this study was the up-regulated expression of IGF-I gene and myogenin mRNA in muscle and down regulated myostatin gene expression after inclusion of both CMH and WPC. IGF-I is known to stimulate proliferation, differentiation [30, 31] and protein synthesis in muscle cells [32]. It is well known that insulin-like growth factor-1 (IGF-1) signaling plays critical positive roles in skeletal muscle growth. Also, stimulation of muscle growth may be mediated by increased plasma IGF-I, which originates primarily from the liver or is produced locally in the muscle tissues [33, 34]. Skeletal muscle also produces IGF-I that acts as a paracrine and autocrine growth factor. Overexpression of IGF-I by injection of a plasmid or a viral construct containing IGF-I cDNA into a mouse muscle has been shown to increase muscle mass (15%) and to prevent sarcopenia in old mice [35]. Since the differentiation of myoblasts in culture is stimulated by creatine [36], we hypothesized that IGF-I might be involved in the effect of creatine. On contrast, myostatin pathways suppress muscle growth and/or induce muscle atrophy appears to act as negative regulator of muscle development [37, 38]. Myogenic is one of the most important genes concerned with muscle fiber formation and its expression level is related to muscle growth [39, 40].Thus, in this study, the up-regulation of IGF-I gene expression in breast muscle in WCP and /or CMH groups may be reflective of increased breast muscle weight. The previous studies indicated that

creatine may increase differentiation and protein synthesis in ovine myogenic satellite cells [36]. In addition, several human studies have shown that creatine increases muscle protein [41]. Also it was found that isolated whey peptides increased release of insulin-like growth factor, improved overall endocrine hormone response [42]. Moreover, Burke *et al.* [43] showed that the anabolic effects of creatine supplementation in humans might also be mediated by up-regulation of muscle IGF-I expression.

On other hand our study indicated that WPC had a significant role against oxidative stress but the creatine exerts no effect on it. As reduced GSH is the most important antioxidant in the cells. GSH serves several critical functions including antioxidant protection, detoxification of xenobiotics and/or their metabolites, regulation of cell cycle progression and apoptosis, storage of cysteine, conservation of redox potential, modulation of immune function and fibrogenesis [44]. From previous studies it has been reported that whey proteins exert several therapeutic effects on humans in a number of clinical trials as they are potential antioxidants, protect cells from ethanol damage, this protection includes their capacity to stimulate GSH synthesis a crucial intracellular antioxidant as whey proteins are a cysteine rich protein source which is the rate limiting step in GSH synthesis [45, 46]. It can be deduced that the better antioxidant status in the living body of broilers fed 32 g/kg WPC for 42 days and thus lower propagation of the radical mediated peroxidation reaction, was the most probable reason for the tendency towards reduced MDA formation in long-term frozen stored breast meat in this study [10]. This is similar to the report of Zommara *et al*. [47] demonstrating decreased thiobarbituric acid reactive substances (TBARS) concentrations in the liver of rats fed a diet with why powder products. Whey protein increase the level of GSH in rats liver [48] this depends on the fact that whey protein and alpha lactalbumin have a high content of cysteine and methionine [49] which are important antioxidants and are necessary for the glutathione synthesis that directly participates in the fight against inflammatory diseases [46]. Moreover, isolated whey peptides provide the following benefits increased intracellular glutathione and anti-aging antioxidants [50], improved immune function [51, 52] and improved gastrointestinal health [52, 53]. On other hand, Wang *et al*. [54] showed that supplementation of CMH to Arbor Acres broilers by 1200 mg/kg does not provide any significant protection via directly scavenging free radicals or increased antioxidant capacity of transported broilers.

CONCLUSION

The perceptions gained from using WPC and CMH are to verify the role of such feed supplement in muscle building when used in broiler chicks, indeed their supplementation increased the broiler productivity without any side effect. Moreover, their uses have a sparing effect on both protein and energy in broiler diet. In addition WPC exerts other health benefits which need further studying tools.

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