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The Gradual Affection of Creatine Monohydrate Supplemented at Different Protein Level in Diets of Broilers on Performance, Blood Biochemical Parameters and some Specific Meat Characteristics

Research Article

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ABSTRACT

This study was carried out to evaluate how broiler chickens respond to corn-soybean meal-based diets enriched with creatine monohydrate (CMH). The effects of CMH at protein levels were observed on performance, nutrients retention, meat water holding capacity (WHC) and blood biochemical parameters in broiler chickens. Three hundred twenty-day-old chicks (Ross 308) were organized in a 2 × 4 factorial arrangement in a completely randomized design, having 4 replicates and 10 birds in each cage (repeat). Diets formulated for two levels of protein (at an amount recommended for Ross 308 and also an amount being 10% higher than the recommendation catalog Ross 308, 2019). Four levels of CMH were used, amounting to 0, 0.1, 0.3 and 0.5% of the diet. In all of the experiment periods the performance results showed that the highest body-weight gain and the lowest feed conversion ratio were observed in high levels of CMH (0.3 and 0.5%) ($P<0.05$). Feeding the birds with a diet containing protein (high level) led to the highest growth performance ($P<0.05$). Regarding N loss and nitrogen retention (g $bird^{-1}$ day^{-1}), the interaction between CMH and protein was significant. Apparent metabolizable energy (AME), N intake (g $bird^{-1}$ day^{-1}) and N retention (% intake) were affected by protein ($P<0.05$). The highest WHC was observed in birds that were fed on a diet containing higher amount of protein ($P<0.05$). Birds fed with 0.3% or 0.5% CMH showed an increase in blood creatinine ($P<0.05$), as compared to birds fed with 0 or 0.1% CMH. In conclusion, the growth performance of broilers improved by adding 0.3% CMH to the diet, and also birds fed to protein (high level) increased their growth performance.

KEY WORDS broiler, creatine monohydrate, nitrogen retention, performance, protein.

INTRODUCTION

Nutrition is a physiological satisfier that affect the growth performance of chickens. In fact, nutrition comprises nearly 70% of the production costs. The economic benefits of broilers largely depends on factors such as high-quality feed ingredients, knowledge about their amino acid composition and the formulation of diets that can optimally sustain poultry and their productive functions (Kidd and Tillman, 2016). Proteins are one of the most expensive ingredients in broiler diets and, thus, it seems that maximizing the efficiency and intake of protein and amino acids in diets can be essential for reducing the costs of feed and for maximizing lean meat production by a proper balance of amino acids (Han and Lee, 2000). Furthermore, energy can be considered as a major limiting factor in the growth of chickens, especially regarding muscle growth and development (Ahmadipour *et al.* 2018). In general, the poultry industry can grow diversely and meet the global demand for high-quality animal protein through improvements in nutritive efficiency. Known as a "semi-essential nutrient", creatine is a guanidine compound generated by the liver and kidney through body functions and it is used in meat-containing diets (Terjung *et al.* 2000). This multifunctional nitrogen compound performs several functions in the body. It serves as a cellular buffer (Newsholme and Beis, 1996), with key roles in energy metabolism (Wyss and Kaddurah-Daouk, 2000). It improves neuromuscular control (Iqbal *et al.* 2015), conserves endogenous precursors (Ostojic *et al.* 2014) and increases protein synthesis (Shankaran *et al.* 2016). Creatine is also known for its ability to increase water retention in human muscles (Juhn, 1999). It can cause a higher water content and a better WHC in pigs, postmortem (Young *et al.* 2005).

Researchers have reported that dietary creatine, as a supplement, tends to have a positive impact on the quantity and quality of broiler chicken meat in different conditions (Wang *et al.* 2015; Zhang *et al.* 2017; Fosoul *et al.* 2018; Yang *et al.* 2019). Broiler chicken are mostly fed with diets that are plant-based and contain little or no dietary creatine. In animals, approximately 1.7% of the total body is phosphorcreatine and creatine which are spontaneously broken down on a daily basis (Wyss and Kaddurah-Daouk, 2000). Creatine synthesis requires three amino acids: glycine, methionine and arginine, and three enzymes: L-arginine: glycineamidino transferase (AGAT), methionine adenosyl transferase (MAT) and guanidinoacetate methyltransferase (GAMT) (Brosnan *et al.* 2011).

Here, we hypothesized that 1) some feed-grade amino acid usage and requirements such as methionine and arginine could be spared and reduced by supplementing creatine monohydrate (CMH), 2) improving performance by increasing dietary protein and 3) a balance can be reached between nutrients (energy and protein) in response to dietary CMH (used at graded levels) and protein (at an amount that is 10% higher than the Ross 308 recommendation). The present study is an attempt to examine how graded levels of CMH and different levels of protein can, individually and in combination, affect growth performance, energy status, nutrient retention and meat quality in broiler chicks.

MATERIALS AND METHODS

Bioethical

All experiment procedures used were confirmed by the Animal Welfare Committee of the Department of Animal Science, University of Tehran. They were approved by the Animal Welfare Committee of the Department of Animal Science (Approval number: 91-01-18022).

Bird housing and treatments

Generally, three hundred twenty-day-old chicks (Ross 308, female /male) (40±2 g) were organized in a 2 × 4 factorial arrangement in a completely randomized design, having four replicates and 10 birds in each cage (repeat). In this experiment, two levels of protein (Ross 308 recommendation and 10% higher than the recommendation) and four levels of CMH (0, 0.1, 0.3 and 0.5 % of diet) were tested. All birds were placed in 32 cages (each measuring 1×1.1 m).

The basal diet was formulated to meet nutritive requirements of the broiler chickens as recommended by the management guide on Ross 308 broilers. For the first ten days of their life, the birds were fed with a starter diet. From day 11 to 24, they received a grower diet and, then, a finisher diet was used from day 25 to 42 (Table 1). Feed and water were provided *ad libitum*. Temperature and relative humidity were maintained within an optimum range (Catalog Ross 308). A photoperiod of 24 h light/d was maintained from day 1 to 3 and then a photoperiod of 23 h light/1 h darkness until the birds became 42 days old. The ventilation rate was 0.12 m/s during the whole period. The initial house temperature was 32 ˚C which gradually decreased and reached 20 ˚C by the time the birds had become 42 days old.

Growth performance

Feed intake (FI) and body weight were recorded during the starter (0–10 d), grower (11–24 d) and finisher (25–42 d) periods in each group. The feed conversion ratio (FCR) was also calculated by the ratio of feed intake to weight gain.

Nitrogen intake-retention and digestibility of apparent metabolizable energy

Apparent metabolizable energy and the rates of nitrogen intake and retention were evaluated from day 28 to 31 (Schneitz *et al.* 1998; Kaur *et al.* 2008). For this purpose, chromium oxide (3 g/kg of diet) was used as a marker. After being fed for 3 days, the chicks were adapted to diets containing chromium oxide. Excreta samples were collected daily from day 28 to 31. The excreta samples were dried at 45 ˚C for 72 h. The gross energy of excreta and feed samples were determined by using a bomb calorimeter (PARR 1261).

The amount of nitrogen was determined by Kjeldo Automatic (KjeldoAutoAnalyzer 1030). Chromic oxide concentrations in the feed and excreta were determined using a procedure described by Fenton and Fenton (1979). The apparent metabolizable energy (AME), nitrogen (N) intake and retention were calculated using the following equation (Schneitz *et al.* 1998; Kaur *et al.* 2008).

$$AME\ (kcal/kg) = GE_{diet} - [GE_{feces} \times Cr_2O_{3diet}/Cr_2O_{3\ feces}]$$

Where:
Cr_2O_{3diet}: concentration of chromium oxide in the diet (%).
Cr_2O_{3fecal}: concentration of chromium oxide in the feces digesta (%).
GE_{diet}: gross energy of the diet (kcal/kg).
GE_{feces}: gross energy content of the feces (kcal/kg).

N intake (g $bird^{-1}$ day^{-1})= nitrogen content of feed (%) × Feed intake per bird (g)

Excreta content (g $bird^{-1}$ day^{-1})= (chromium content of feed (%) / chromium content of feces (%)) × feed intake per bird (g)

N loss (g $bird^{-1}$ day^{-1})= excreta content(g) × nitrogen content of feces (%)

N retention (g $bird^{-1}$ day^{-1})= N intake – N loss

N retention (ercentage of nitrogen intake)= (N retention (g $bird^{-1}$ day^{-1}) / N intake (g $bird^{-1}$ day^{-1})) × 100

Energy intake (kcal)= feed intake per bird (g) × metabolizable energy content of the diet

N retention (g MJ^{-1} ME intake)= (N retention (g $bird^{-1}$ day^{-1}) / Energy intake(kcal)) × 100

Water holding capacity (WHC)

Water holding capacity (WHC) was determined by centrifugation, according to Bertram *et al*. (2001) on samples collected 4 h after slaughter. In each bird, one sample was obtained from the thigh muscle and another sample from the breast. Each sample weighed about 1 g and was centrifuged at 360 ×g for 15 min. The expressible juice was defined as water loss and was presented as a percentage of the initial weight of the original sample. Total moisture content was determined in duplicate, according to AOAC (2005). The WHC was calculated as the fraction of water retained by the meat (1 - (expressible juice/total moisture content)).

Serum biochemistry parameters

On day 42, two birds (female or male) per replicate were randomly selected and blood samples were collected from the brachial vein. The blood samples were centrifuged at 200 × g for 10 min to obtain the serum. Then, the procedure involved measuring the amounts of albumin, total protein and uric acid by the photometric method, whereas the amount of creatinine was determined by the IFCC/DGKC method (AOAC, 1990).

Statistical analysis

The data were tested for normality using the Shapiro-Wilk test. All data were analyzed in a 2 × 4 factorial arrangement in a completely randomized design using general linear models (GLM) (SAS, 2003). Significant differences among mean values were determined by Tukey's test ($P<0.05$).

RESULTS AND DISCUSSION

Table 2 reports the changes in growth performance in response to treatments (Table 2). Protein levels higher than the catalog recommendation improved weight gain in different periods (d 0–14, d 15-24 and d 25-42) ($P<0.05$). Likewise, the birds that were fed with high levels of protein had a lower food conversion ratio (FCR)during d 15-24 and d 25-42 ($P< 0.05$). During the three periods (d 0–14, d 15-24, and d 25-42), feed intake was not affected by protein levels. During d 0 to 42, however, feeding with protein at an amount that is 10% higher than that recommended in strain 308 Ross caused an increase in weight gain ($P<0.05$) and a decrease in FCR ($P<0.05$) as compared to birds fed with the protein recommendation in strain 308 Ross. During d 10-24 and d 25-42, the interaction effects of CMH × protein were not significant in affecting the amounts of feed intake, weight gain and feed conversion. Nevertheless, birds fed with 0.3% or 0.5% of CMH experienced an increase in weight gain ($P<0.05$) and a decrease in FCR ($P<0.05$) compared with birds fed without CMH or with 0.1% CMH.

As shown in Table 3, the results indicated that AME, N intake (g $bird^{-1}$ day^{-1}) and N retention (% of intake) increased by feeding higher protein levels ($P<0.05$). Birds fed with 0.3% or 0.5% CMH had an increase in N retention (% of intake) ($P<0.05$) compared with birds fed without CMH or with 0.1% CMH. Similarly, levels with 0.3% or 0.5% CMH had higher AME than levels 0 and 0.1% CMH ($P<0.05$) (Table 3). Although, the effects of interactions between CMH and protein were not significant on AME, N intake (g $bird^{-1}$ day^{-1}) and N retention (% of intake) ($P>0.05$), But the interactions between CMH and protein significantly affected N loss (g $bird^{-1}$ day^{-1}) and nitrogen retention (g $bird^{-1}$ day^{-1}, and g MJ^{-1} ME intake) ($P<0.05$; Table 3). The treatments caused a variety of changes in the WHC of breast and thigh muscles (Table 4). The results indicated that WHC in the breast and thigh muscles were significantly affected by graded levels of CMH ($P<0.05$).

Although, the interactions between graded levels of CMH and protein had no substantial effect on WHC in the breast and thigh muscles, nevertheless birds that were fed with higher protein levels had higher WHC than birds fed with the recommended amount of protein in strain 308 Ross ($P<0.05$).

Table 1 Ingredients and nutrient composition of experimental diets in the starter (0-10 d) and grower (11-24 d) periods

	Starter (0-10 d)								**Grower (11-24 d)**							
Protein (%)	21.46				23.60				20.30				22.37			
Creatine (%)	None	0.1	0.3	0.5	None	0.1	0.3	0.5	None	0.1	0.3	0.5	None	0.1	0.3	0.5
Ingredient																
Corn (%)	55.48	55.48	55.48	55.48	50.69	50.68	50.67	50.67	58.42	58.42	58.42	58.42	53.87	53.86	53.85	53.83
Soybean meal (%)	37.47	37.15	36.50	35.86	39.40	39.09	38.45	37.81	32.82	32.82	32.80	32.15	35.58	35.27	34.63	34
Corn gluten meal (%)	1.79	1.99	2.41	2.82	4.84	5.07	5.51	5.93	2.92	2.92	2.93	3.35	5.12	5.34	5.79	6.24
Soy oil (%)	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
Dical. Phos (%)	1.70	1.70	1.70	1.71	1.65	1.65	1.66	1.66	1.53	1.46	1.32	1.33	1.27	1.27	1.28	1.28
Limestone (%)	1.53	1.53	1.53	1.53	1.54	1.54	1.54	1.54	1.44	1.41	1.36	1.36	1.37	1.37	1.36	1.36
Creatine monohydrate (%)	0	0.10	0.30	0.50	0	0.10	0.30	0.50	0	0.10	0.30	0.50	0	0.10	0.30	0.50
Vitamin premix (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine (%)	0.23	0.23	0.23	0.23	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.06	0.06	0.06	0.06
L-Lysine HCl (%)	0.07	0.08	0.10	0.13	0	0	0.0009	0.02	0	0	0.0006	0.02	0	0	0	0
Common salt (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Calculated analysis																
AMEn, kcal/kg	2950	2950	2950	2950	2950	2950	2950	2950	3050	3050	3050	3050	3050	3050	3050	3050
CP (%)	21.46	21.46	21.46	21.46	23.60	23.60	23.60	23.60	20.30	20.30	20.30	20.30	22.37	22.37	22.37	22.37
Met (%)	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Lys (%)	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Ca (%)	1.024	1.024	1.024	1.024	1.024	1.024	1.024	1.024	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87

Continuation of Table 1 Ingredients and nutrient composition of experimental diets in the finisher period (25-42 d)

	Finisher (25-42 d)							
Protein (%)	18.40				20.24			
Creatine (%)	None	0.1	0.3	0.5	None	0.1	0.3	0.5
Ingredient								
Corn (%)	63.44	63.43	63.42	63.42	59.38	59.37	59.35	59.34
Soybean meal (%)	29.35	29.03	28.40	27.76	30.98	30.66	30.03	29.40
Corn gluten meal (%)	1.53	1.76	2.20	2.62	4.07	4.30	4.74	5.19
Soy oil (%)	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30
Dical. Phos (%)	1.23	1.23	1.24	1.24	1.19	1.19	1.20	1.20
Limestone (%)	1.29	1.29	1.29	1.29	1.30	1.30	1.30	1.30
Creatine monohydrate (%)	0	0.10	0.30	0.50	0	0.10	0.30	0.50
Vitamin premix (%)[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix (%)[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL–Methionine (%)	0.12	0.12	0.12	0.12	0.05	0.05	0.05	0.05
L–Lysine HCl (%)	0	0	0	0.02	0	0	0	0
Common salt (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100	100	100	100
Calculated analysis								
AMEn, kcal/kg	3100	3100	3100	3100	3100	3100	3100	3100
CP (%)	18.40	18.40	18.40	18.40	20.24	20.24	20.24	20.24
Met (%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Lys (%)	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Ca (%)	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82

[1] Supply per kg feed: vitamin A: 4400000 IU; vitamin D: 72000 IU; vitamin E: 14400 IU; vitamin K: 2000 mg; B_{12}: 640 mg; B_1: 612 mg; B_2: 3000 mg; B_5: 4896 mg; B_3: 12160 mg; B_6: 612 mg; Biotin: 2000 mg and Cholin: 260 g.
[2] Supply per kg feed: Mn: 64.5 g; Zn: 33.8 g; Fe: 100 g; Cu: 8 g; I: 640 g; Co: 190 mg and Se: 8 g.
AMEn: apparent metabolizable energy corrected for nitrogen.

Table 2 Effects of graded levels of CMH and protein on the growth performance of broiler chicks

Items	BW gain (g/bird per d)				Feed intake (g/bird per d)				Feed conversion (g feed/g gain)			
	0-10 d	11-24 d	25-42 d	0-42 d	0-10 d	11-24 d	25-42 d	0-42 d	0-10 d	11-24 d	25-42 d	0-42 d
Protein[1] + creatine												
Recom[1] + none	16.17	43.58	74.75	48.77	23.33	81.88	151.92	92.67	1.44	1.87	2.03	1.90
Recom + 0.1%	17.42	43.78	75.01	49.19	22.53	82.33	152.18	92.82	1.30	1.88	2.02	1.88
Recom + 0.3%	15.81	44.51	77.73	50.42	23.47	82.45	152.23	93.26	1.49	1.85	1.96	1.84
Recom + 0.5%	17.08	45.04	77.92	50.51	24.10	82.48	152.38	93.53	1.41	1.83	1.95	1.85
High + none	17.60	44.85	79.73	49.98	24.08	81.97	152.07	93.86	1.37	1.82	1.9	1.87
High + 0.1%	17.17	45.67	80.59	50.60	23.77	82.45	152.24	93.87	1.38	1.80	1.88	1.85
High + 0.3%	17.96	47.16	82.35	52.14	24.16	82.55	152.30	93.97	1.34	1.75	1.85	1.80
High + 0.5%	17.78	47.38	82.54	52.43	23.98	82.85	152.48	94.22	1.35	1.74	1.84	1.79
Pooled SEM	1.28	0.73	0.71	0.56	1.21	0.71	0.41	0.69	0.08	0.03	0.01	0.02
Main effect												
Protein												
Recom	16.62^{b}	44.23^{b}	76.35^{b}	49.72^{b}	23.36	82.29	152.18	93.07^{b}	1.41	1.86^{a}	1.99^{a}	1.87^{a}
High	17.63^{a}	46.27^{a}	81.30^{a}	51.29^{a}	24	82.46	152.27	93.98^{a}	1.36	1.78^{b}	1.87^{b}	1.83^{b}
Pooled SEM	0.64	0.36	0.35	0.28	0.60	0.35	0.20	0.34	0.04	0.01	0.009	0.01
Creatine												
None	16.88	44.22^{b}	77.24^{b}	49.37^{b}	23.71	81.93	151.99	93.27	1.40	1.85^{a}	1.96^{a}	1.88^{a}
0.1%	17.3	44.73^{b}	77.80^{b}	49.89^{b}	23.15	82.39	152.21	93.34	1.34	1.84^{a}	1.95^{a}	1.87^{a}
0.3%	16.88	45.83^{a}	80.04^{a}	51.28^{a}	23.81	82.50	152.26	93.61	1.42	1.80^{b}	1.90^{b}	1.83^{b}
0.5%	17.43	46.21^{a}	80.23^{a}	51.47^{a}	24.04	82.66	152.43	93.87	1.38	1.79^{b}	1.89^{b}	1.82^{b}
Pooled SEM	0.90	0.52	0.50	0.40	0.86	0.50	0.29	0.49	0.05	0.02	0.01	0.01
							P-value					
Protein	0.03	0.0001	0.0001	0.0001	0.15	0.50	0.51	0.0012	0.11	0.0001	0.0001	0.0002
Creatine	0.76	0.0001	0.0001	0.0001	0.52	0.21	0.24	0.30	0.29	0.0009	0.0001	0.0001
Protein × creatine	0.30	0.28	0.50	0.60	0.73	0.97	0.99	0.85	0.07	0.45	0.28	0.57

[1] Protein requirement: Ross 308 recommendation and 10% higher than the recommendation (High).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Graded levels of CMH and protein had a range of effects on blood parameters (Table 5). Nonetheless, the effects of interactions between graded levels of CMH and protein were not significant on any of the blood parameters. Birds fed with protein higher than the recommended requirements strain 308 Ross had higher uric acid levels the blood (P<0.05). Birds fed with 0.3% or 0.5% CMH showed an increase in creatinine (P<0.05) as compared to birds fed without CMH or with 0.1% CMH. However, graded levels of CMH had no substantial effect on the amounts of albumin, total protein and uric acid in the blood (P>0.05).

Nutritionists are progressively looking for new approaches that can promote the availability of nutrients for broiler chickens. The present study was conducted to investigate the effect of graded levels of CMH and different levels of protein on growth performance, energy status, nutrient retention and meat quality in broiler chicks. The interaction between CMH and protein was not significant in relation to growth performance (Table 2).

However, adding 0.3 or 0.5% CMH to the diet improved body weight gain and feed conversion ratio. These findings are consistent with previous results reported by Lemme *et al.* (2007), Nahashon *et al.* (2005), Niu *et al.* (2009) and Waldroup *et al.* (2005).

Creatine plays the role of a carrier in the form of phosphocreatine. It enhances energy delivery to specific tissues, such as the muscles and heart, which are characterized by high demands for energy (Wyss and Kaddurah-Daouk, 2000). In this regard, creatine loading has been widely studied because of its potential ergogenic effects on muscular performance.

For instance, creatine supplementation in the human diet can increase the total creatine content in muscles (Harris *et al.* 1992; Hultman *et al.* 1996) and enlarge muscle mass (Vandenberghe *et al.* 1997). Young *et al.* (2007) observed an increase of protein synthesis in primary myotubes when the myotubes were exposed to CMH, while protein degradation remained unaffected.

Table 3 Effects of graded levels of CMH and protein on AME, nitrogen intake and nitrogen retention of broilers chicks

Items	AME (kcal/kg)	N intake (g bird^{-1} day^{-1})	N loss (g bird^{-1} day^{-1})	Nitrogen retention N retention (g bird^{-1} day^{-1})	N retention (% of intake)	N retention (g MJ^{-1} ME intake)
Protein + creatine						
Recom[1] + none	2997.71	4.60	1.92^{a}	2.67^{e}	58.14	0.56^{e}
Recom + 0.1%	3035.04	4.61	1.88^{ab}	2.72^{de}	59.09	0.57^{de}
Recom + 0.3%	3153.67	4.61	1.77^{bc}	2.84^{cd}	61.62	0.60^{cd}
Recom + 0.5%	3155.29	4.62	1.67^{c}	2.95^{c}	63.84	0.62^{c}
High + none	3068.24	5.96	1.87^{ab}	4.08^{b}	68.57	0.86^{b}
High + 0.1%	3070.73	5.96	1.81^{ab}	4.15^{b}	69.55	0.87^{b}
High + 0.3%	3213.09	5.97	1.50^{d}	4.47^{a}	74.87	0.94^{a}
High + 0.5%	3246.39	5.98	1.43^{d}	4.53^{a}	75.91	0.96^{a}
Pooled SEM	20.43	0.01	0.08	0.08	1.63	0.01
Main effect						
Protein						
Recom	3085.42^{b}	4.61^{b}	1.81^{a}	2.80^{b}	60.67^{b}	0.59^{b}
High	3149.61^{a}	5.96^{a}	1.65^{b}	4.31^{a}	72.23^{a}	0.91^{a}
Pooled SEM	10.21	0.007	0.04	0.04	0.81	0.008
Creatine						
None	3032.97^{b}	5.28	1.90^{a}	3.38^{b}	63.36^{b}	0.71^{b}
0.1%	3052.88^{b}	5.29	1.85^{a}	3.43^{b}	63.32^{b}	0.72^{b}
0.3%	3183.38^{a}	5.29	1.63^{b}	3.65^{a}	68.24^{a}	0.77^{a}
0.5%	3200.84^{a}	5.30	1.55^{b}	3.74^{a}	69.88^{a}	0.79^{a}
Pooled SEM	14.44	0.01	0.05	0.05	1.15	0.01
				P-value		
Protein	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Creatine	0.0001	0.30	0.0001	0.0001	0.0001	0.0001
Protein × creatine	0.07	0.99	0.02	0.03	0.27	0.03

[1] Protein requirement: Ross 308 recommendation and 10% higher than the recommendation (High).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Moreover, Amer *et al.* (2018) showed that body weight gain and FCR improved in Balady chicks which were fed with CMH supplementation. However, Zhang *et al.* (2014) reported that dietary supplementations at different CMH levels tend to have no significant effect on body weight gain (BWG) and FCR. The inconsistency in these results could be ascribed to the differences in creatine bioavailability, supplementation dose and experimental duration. It is proven that both dietary protein and energy can boost the performance of broiler chickens. Liu *et al.* (2016) explained how protein is more important than carbohydrates and lipids in increasing the rate of body weight gain in broiler chickens, while asserting that a balance between protein and energy is essential for optimal feed efficiency. The experimental results of the current research revealed that feeding birds on a higher amount of protein is likely to make their feed intake higher, followed by an enhanced growth, than feeding them on a low protein diet.

Previously published papers shown that reducing the dietary protein content can lead to poor growth performance in broiler chickens (Waldroup *et al.* 2005; Dean *et al.* 2006; Namroud *et al.* 2008; Hernandez *et al.* 2012). These negative effects can be attributed to a less limiting AA content in reduced protein diets.

Nagaraj *et al.* (2007) observed that the protein content in a diet would have exerted significant effects on BWG and feed efficiency by the time broiler chicks become 42 days old.

In an experiment, Leu *et al.* (2017) reported that the effects of protein are more limited in diets with high lipid concentrations, which is consistent with our results. They reported that the genetic potential of broilers can be better actualized in high-protein diets because broiler chickens fed with 400 mg/kg protein diets exhibited an average FCR of 1.139 g/g, thereby showing a 23% improvement *vs.* 2014 Ross 308 performance objectives.

Table 4 Effects of graded levels of CMH and protein on blood serum parameters of broiler chicks

Items	Albumin (g/dL)	Total protein (g/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)
Protein + creatine				
Recom + non	1.80	2.97	0.57	4.20
Recom + 0.1%e	1.92	3.02	0.65	4.27
Recom + 0.3%	1.95	3.05	0.72	4.30
Recom + 0.5%	1.97	3.10	0.75	4.32
High + none	1.85	3.02	0.62	4.97
High + 0.1%	1.95	3.05	0.67	5
High + 0.3%	1.97	3.10	0.75	5.20
High + 0.5%	2.02	3.17	0.77	5.30
Pooled SEM	0.13	0.10	0.05	0.17
Main effect				
Protein				
Recom	1.91	3.03	0.67	4.27[b]
High	1.95	3.08	0.71	5.11[a]
Pooled SEM	0.06	0.05	0.02	0.08
Creatine				
None	1.82	3	0.60[c]	4.58
0.1%	1493	3.03	0.66[b]	4.63
0.3%	1.96	3.07	0.73[a]	4.75
0.5%	2	3.13	0.76[a]	4.81
Pooled SEM	0.09	0.07	0.03	0.12
		P-value		
Protein	0.45	0.19	0.1	0.0001
Creatine	0.09	0.08	0.0001	0.06
Protein × creatine	0.99	0.97	0.95	0.47

[1] Protein requirement: Ross 308 recommendation and 10% higher than the recommendation (High).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

So there is a possibility that in the present experiment, the birds fed with a protein level higher than the Ross 308 catalog were able to express their genetic potential.

Our findings revealed that interactions between graded levels of CMH and protein (10% higher than Ross 308 Recommendation *vs*. Ross 308 Recommendation) can significantly affect N loss (g bird^{-1} day^{-1}), nitrogen retention (expressed as N retention (g bird^{-1} day^{-1})). It appears that CMH in high-protein diets can improve the utilization of protein and energy e.g. diet for growing chicks. The provision of ‘extra’ functional amino acids with increased protein concentrations may explain the changes in performance observed in extreme dietary protein concentrations (Liu *et al.* 2017). Zhang *et al.* (2017) reported that dietary CMH is likely to elevate the energy status in pectoralis main muscles of transport-stressed broiler chickens, which is agrees with our results. Also, CMH represents one of the most important nitrogen-containing compounds in protein and energy metabolism (Navratil *et al.* 2009). Thus, it is not surprising that providing a proper dosage of CMH and giving slightly more protein can enhance the growth performance of broiler chickens.

In the present study, the interactions between graded levels of CMH and protein did not affect meat quality in any negative way. The capability of meat in accumulating water in and among its fibers is an intricate property that is determined by structural and biochemical changes which happen throughout the growth and transformation of muscles. Our results showed that birds fed with higher protein levels (i.e. 10% higher than the Ross 308 Recommendation) have higher values of WHC compared with low level of protein. According to previous research, protein denaturation is considered as a major determinant of WHC in meat (Offer and Knight, 1988; Pietrzak *et al.* 1997; Bowker and Zhuang, 2013).

Protein denaturation is characterized by a rapid, continuous decline in the pH of postmortem meat. In effect, this process can negatively influence WHC. It is a common observation that changes in pH and WHC are closely interconnected (Dransfield and Sosnicki, 1999; Fletcher, 1999; Wilkins *et al.* 2000). These changes are partly defined by the energy status of the muscles at slaughter that it is highly influenced by the duration of transportation and stress before and during slaughter (Fletcher, 1999).

Table 5 Effect of graded levels of CMH and protein on water holding capacity in breast and thigh meat

Items	Breast (%)	Thigh (%)
Protein + creatine		
Recom[1] + none	65.15	69.47
Recom + 0.1%	65.52	69.63
Recom + 0.3%	65.72	71.12
Recom + 0.5%	66.14	71.43
High + none	65.75	70.97
High + 0.1%	65.90	71.27
High + 0.3%	66.64	72.13
High + 0.5%	67.20	72.69
Pooled SEM	0.56	0.45
Main effect		
Protein		
Recom	65.63[b]	70.41[b]
High	66.37[a]	71.76[a]
Pooled SEM	0.28	0.22
Creatine		
None	65.45[c]	70.22[b]
0.1%	65.71[bc]	70.45[b]
0.3%	66.18[ab]	71.62[a]
0.5%	66.67[a]	72.06[a]
Pooled SEM	0.39	0.32
	P-value	
Protein	0.001	0.0001
Creatine	0.002	0.0001
Protein × creatine	0.63	0.54

[1] Protein requirement: Ross 308 recommendation and 10% higher than the recommendation (High).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Many compounds that are involved in metabolism, e.g. glycogen, lactate, creatine and ATP, are indirectly associated with the WHC value in meat (Offer and Knight, 1988; Northcutt *et al*. 1994). In a recent study, an increase was reported in the ability of meat to retain water because of adding CMH to the diet. Previous reports have indicated that dietary CMH can improve creatine and phosphocreatine contents in muscles which could, in turn, generate more ATP and enhance meat quality by reducing unwanted processes involved in transport-induced glycolysis and lactic acid accumulation in muscles (Wang *et al*. 2015; Zhang *et al*. 2017). There are variations among different studies which could be explained by differences in the amounts of CMH consumption, or source of animal and the duration of CMH in diets.

In our experiment, the results were more promising when protein was added to the diet at an amount that was 10% more than the recommendation strain for 308 Ross. This higher amount of protein increased uric acid levels in the broilers blood. Glycine is not only involved in protein synthesis as a non-essential amino acid but is also required for the synthesis of uric acid, creatine, DNA and RNA.

The increase in uric acid concentrations in the blood was statistically associated with the increase in dietary protein levels in this experiment. This is probably caused by increased levels of glycine and methionine (amino acids) in the diet.

Namroud *et al*. (2008) reported an increase in plasma uric acid in broilers that were fed on high-protein diets. This increase occurred in response to an increase in glycine content. Glycine is one of the suppliers of the nitrogen group in the uric acid construct, while methionine has the methyl group required for the synthesis of uric acid (Sturkie and Griminger, 1976).

Thus, the most of uric acid is produced related in high level of protein. In this study, birds that were fed on 0.3% or 0.5% CMH showed an increase in creatinine, as compared to birds that were fed without CMH or with 0.1% CMH. In line with our results, Lemme *et al*. (2007) stated that creatinine levels can increase significantly in broilers when using higher levels of guanidinostatic acid (i.e. the precursor of creatine monohydrate).

CONCLUSION

In conclusion, substantial improvements are observed in maximum growth performance, meat WHC and nutritive retention (i.e. protein and energy) when using dietary protein at an amount that was 10% more than the recommendation level for strain 308 Ross. On the other hand, birds fed with CMH (0.3 and 0.5%) developed higher growth performance, WCH and nutritive retention, as compared to other groups (0 and 0.1%). Interactions between CMH and protein have significant effects on nutritive retention (protein and energy), thereby improving growth performance.

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