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Effect of creatine monohydrate on some blood and biochemical blood parameters of broiler chickens

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Abstract

A 42 days study was conducted on 240 day-old Ross broiler chicks allocated randomly to four dietary treatments to compare the effect of different levels of creatine monohydrate (CrH) on blood (RBC count, PCV, Hb concentration, MCV, MCH and MCHC) and biochemical traits (creatinine and uric acid). Two types of diets were used, starter diet was used from one to 21 day and then grower diet was used till the end of the experiment. Beside control group, CMH was incorporated into the diet of broilers at the rate of 4 g/kg (T1), 8 g/kg (T2) and 12 g/kg (T3). The results showed that blood traits (RBC count, PCV, Hb concentration, MCV, MCH and MCHC) of birds of all supplemented groups (T1, T2 and T3) were not different ($P<0.05$) compared to control group. No significant difference was observed among all experimental groups concerning H/L ratio. Significant differences ($P<0.05$) was seen among all supplemented groups concerning biochemical blood traits (creatinine and uric acid). In conclusion, supplementation broiler ration with CrH did not affect the status of the bird at the used levels of CrM.

Keywords: Creatine, broiler, RBC count, PCV, Hb concentration, MCV, MCH, MCHC

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Introduction

Human studies show 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). So creatine (Cr) is heavily involved in energy metabolism through the Cr and phospho creatine (PCr) system. This system functions as a backup to the adenosine diphosphate/adenosine triphosphate (ATP)-cycle to store and mobilize energy when required on short notice. In general, about 1.7% of the Cr and PCr pool is irreversibly converted to Cr each day and excreted in the urine (Wyss and Kaddurah-Daouk, 2000).

The animal's demand for Cr can be supplied either directly from animal protein (e.g., fish or animal by-product meal) in the diet or by endogenous synthesis. Farm animals fed diets containing reduced amounts of animal protein, or no animal protein at all, might be deficient in Cr. Hence, in view of the decreasing amounts of protein from animal-origin included in animal feeds, particularly in the Middle East countries, supplementation with Cr might restore the Cr load in tissues. Young fast-growing chicks because of their high need to supply Cr to growing muscles (Brosnan et al., 2009) and because the regeneration of ATP from the Cr and PCr system appears to be of paramount importance in the cardiac energy management of fast growing broilers (Nain et al., 2008).

In the last 20 years, Cr has become a very popular dietary supplement (Bird, 2003; Maughan et al., 2004) but despite its widespread use there is little evidence concerning possible side effects (Bizzarini and De Angelis, 2004; Poortmans et al., 2005). 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) but the possible side effects of Cr supplementation, such as renal

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dysfunction, and hepatotoxicity are still inconclusive (Bizzarini and De Angelis, 2004). Because urea, which is one of the metabolic products of Cr metabolism, is involved in the conversion of toxic compounds such as methylamine and formaldehyde, Cr supplementation can also be expected to influence this conversion (Poortmans et al., 2005). 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).

In general, renal diseases are characterized by the occurrence of morphological lesions at any degree of magnification and also by any biochemical abnormality (Gregory et al., 2003). Anemia is a frequent complication of chronic kidney disease (CKD) and is primarily due to failure of erythropoietin production to respond to decreased hemoglobin (Hb) concentration (Nissenson et al., 1991, Erslev et al., 1997). Therefore, plasma levels of urea and creatinine are classical markers of renal function because they represent a simple marker of glomerular filtration (Ghosh and Sil, 2007).

The aim of this study was to investigate the effects of Cr supplementation on some biochemical blood traits in broiler.

Materials and Methods

Layout of experiment

Day-old, straight run (unsexed) broiler chicks (Ross 308) (n =240) were purchased from a commercial hatchery and randomly assigned to floor cages, where the new hatched chicks were raised at poultry experimental fields of Bakrajo, Faculty of Agricultural Sciences, University of Sulaimany. Sawdust was used with the thickness of 5cm as litter. Temperature and humidity of the rooms was measured by electronic thermometers. Initially, room temperature was maintained at approximately 32°C and heat lamps were placed within each pen. Heat lamps were gradually raised and then turned off by the fourth week of the study. However, a ventilation fan was used to remove excessive moisture from within the building. Broilers were provided with a continuous light source (24 h/d) throughout the study. The experiment period lasted for six weeks, from the first day to the 21st d. All chicks were raised together and then chicks were separated into the treatments till the end of the experiment. Cages were randomly assigned to four treatment groups (4 replicates for each group and 15 chicks per replicate).

Feeding programs

Birds were provided access to both feed and water via plastic hanging feeders and a bell type drinker during the whole period of experiment. Two types of diets were used over the period of experiment according to NRC (1994). Starter diet was used from one to 21 day of age and then grower diet was used till the end of the experiment. The chickens were weighed individually before start of the experiment (21 d). Three graded levels of CMH (Micronized creapure, Degussa Food Ingredients, D-85354 Freising, Germany) was added to the diet at the rate of 4 g/kg (T1), 8 g/kg (T2) and 12 g/kg (T3) (Tables 1 & 2). Pens were visually inspected on a

Table 1: Ingredient of the starter diet used in the experiment

Ingredients (%)	Control	T1	T2	T3
Yellow Corn	58	58	58	58
Soya bean meal (48%)	27	27	27	27
Protein*	9	9	9	9
Wheat	4	3.6	3.2	3.2
Sunflower oil	1.5	1.5	1.5	1.5
DCP**	0.3	0.3	0.3	0.3
Salt	0.2	0.2	0.2	0.2
Total	100	100	100	100
	Analysis composition***			
Protein	22.00	22.00	22.00	22.00
ME (Kcal/Kg)	3045	3040	3040	3040
Calcium	0.74	0.74	0.74	0.74
Phosphorus	0.41	0.41	0.41	0.41
Lys.	1.3	1.3	1.3	1.3
Meth.	0.62	0.62	0.62	0.62
Meth. to Cyst. ratio	0.96	0.96	0.96	0.96

*Protein concentrate used in the diets was produced in Holland (WAFI) which contains: 40% crude protein, 2100 Kcal ME/Kg, 5% crude fat, 2% crude fiber, 6.5% calcium, 2.50% phosphorus, %3.85 lysine, 3.70% methionine, and 4% cystine; **DCP: Dicalcium phosphate; ***The Analysis composition of the diets was determined according to NRC (1994).

Table 2: Ingredient of the grower diet used in the experiment

Ingredients (%)	Control	T1	T2	T3
Yellow Corn	61	61	61	61
Soya bean meal 48%	24	24	24	24
Protein*	7	7	7	7
Wheat	5	4.6	4.2	3.8
Sunflower oil	2.5	2.5	2.5	2.5
DCP**	0.3	0.3	0.3	0.3
Salt	0.2	0.2	0.2	0.2
Creatine	0	0.4	0.8	1.2
Total	100	100	100	100
	Analysis composition***			
Protein	20.15	20.13	20.17	20.19
ME Kcal/Kg	3150	3143	3140	3137
Calcium	0.65	0.64	0.67	0.61
Phosphorus	0.33	0.35	0.32	0.31
Lys.	1.14	1.14	1.16	1.15
Me.	0.51	0.54	0.57	0.58
Meth. to Cyst. ratio	0.82	0.85	0.83	0.87

*Protein concentrate used in the diets was produced in Holland (WAFI) which contains: 40% crude protein, 2100 Kcal ME/Kg, 5% crude fat, 2% crude fiber, 6.5% calcium, 2.50% phosphorus, 3.85% lysine, 3.70% methionine, and 4% cystine; **DCP: Dicalcium phosphate; ***The Analysis composition of the diets was determined according to NRC (1994).

daily basis for morbidity and mortality, each of which was documented as it occurred. Blood samples from 10 chicks per group were obtained (at week four five and six of the experiment) by cervical dislocation. Blood samples in this study were divided into two parts; part one was used for blood traits which included total erythrocyte count (RBC), Packet Cell Volume (PCV), Hemoglobin concentration (Hb), Mean Corpuscular Value (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Heterophil to Lymphocyte ratio (H/L). The second part of the blood samples was used for biochemical serum traits. Serum was separated by centrifugation (1500 rpm for 10 minutes). The serums parameters include in this study were uric acid and creatinine. These traits were determined according to Al-Daraji et al. (2008).

Data were analyzed using XLStat, version 7.5, 2004. The significant difference between means of traits were determined using Duncan's multiple range test (Duncan, 1955) under the probability $P<0.05$.

Results

Results in Table 3 clarified the effect of CrM on some blood traits which included RBC count, PCV and Hb concentration in broiler fed different levels of CrM. Supplementary CrM did not affect ($P>0.05$) none of these traits in 4th, 5th and 6th week of the age of the broiler and also the total means of these traits.

Moreover, results of Table 3 also indicated that there was a no significant effect in all supplemented groups compared to control group in relation to all mentioned traits. No significant effects of Cr loading supplementation (T1, T2 and T3) on MCV, MCH and MCHC were observed during all experimental weeks (Table 4). Cr supplementations (T1, T2 and T3) during experimental periods led to increased levels creatinine and uric acid (Table 5). Cr caused a significant linear increase in creatinine concentration with increasing the level of CrM. H/L ratio did not change significantly between the control and treated groups.

Discussion

To our knowledge, this study is the first experimental study to examine the effect of CrM on the association between renal function and blood traits in broiler. It is obvious from the data of Table 3 and 4 that CrM did not alter RBC count, PCV, Hb, MCV, MCH and MCHC. We cannot rule out that alteration of blood traits may be only a reflection of the kidney disease. However, during kidney dysfunction, erythropoietin production is impaired, and all blood traits will be affected. Therefore, the only non hemodynamic mechanism of compensation is an increase in oxygen extraction, which has a limited effect (London et al., 2001).

As part of possible toxic mechanisms of Cr supplementation, we could consider the Cr accumulation into the tissue, which has low metabolic capacity to convert Cr into creatinine and is enzymatically capable of accomplishing the methyilation processes, contributing to the formation of cytotoxic substances such as formaldehyde and methylamine (Yu and Deng, 2000; Clayton et al., 2004). Additionally, long-term Cr supplementation stimulates down-regulation of Cr receptors (CT-1) in skeletal muscles, blocking any additional storage of this nutrient (Guerrero-Ontiveros and Wallimann, 1998). Greenhaff (1997) demonstrated that muscular Cr capitation is independently saturated after the loading phase, with or without exercise. Therefore, long-term Cr supplementation results in increased Cr concentrations in other organs that present very low basal Cr storage such as kidneys and liver (Ipsiroglu et al., 2001),which favour its conversion to cytotoxic compounds. In the present study, it is possible that supplemental levels of CMH were not high enough to be lethal or toxicant for the birds.

In the present study, it was shown that adding CrM to broiler diet did not affect ($P<0.05$) H/L ratio (Table 5). This is because CrM in the present study did not cause any stresses on the birds. Since an index to stress is the ratio of heterophils to lymphocytes (H/L) in blood (Maxwell, 1993). In birds, epinephrine levels can increase

Table 3: Effect of creatine monohydrate supplementation on RBC count, PCV and Hb concentration on broiler chickens at different weeks of age

Treatments	RBC ($\times 10^6$/µl)				PCV (%)				Hb (g/dl)			
	Weeks			Total	Weeks			Total	Weeks			Total
	4	5	6	average	4	5	6	average	4	5	6	Average
C	2.51 ±0.04^a	2.87 ±0.08^a	3.16 ±0.09^a	2.81 ±0.071^a	25.75 ±0.66^a	28.90 ±0.66^a	31.03 ±0.99^a	28.56 ±1.02^a	8.87 ±0.23^a	9.01 ±0.56	11.99 ±0.10^a	9.95 ±0.33^a
T1	2.60 ±0.04^a	2.94 ±0.03^a	3.14 ±0.05^a	2.89 ±0.04^a	26.37 ±0.47^a	29.1 ±0.83^a	31.07 ±0.93^a	28.84 ±0.80^a	9.02 ±0.14^a	9.1 ±0.55	12.02 ±0.33^a	10.04 ±0.4^a
T2	2.56 ±0.06	2.90 ±0.02^a	3.18 ±0.02^a	2.86 ±0.03^a	25.83 ±0.78^a	28.7 ±0.66^a	31.87 ±0.1^a	28.8 ±0.91^a	8.95 ±0.20^a	8.99 ±0.08	12.28 ±0.19^a	10.07 ±0.25^a
T3	2.62^a ±0.08^a	2.95 ±0.02^a	3.25 ±0.06^a	2.94 ±0.05^a	26.4 ±0.87^a	29.2 ±0.44^a	31.92 ±0.78^a	29.17 ±0.66^a	9.17 ±0.18^a	9.2 ±0.20	12.50 ±0.15^a	10.29 ±0.33^a

C: control group; T1, T2 and T3: adding CrM to the diet of broiler chicken at levels of 0.4, 0.8 and 1.2 %, respectively; ^{abc}Values within columns followed by different letters differ significantly ($P<0.05$).

Table 4: Effect of dietary supplementation of creatine monohydrate on MCV, MCH and MCHC of broiler chickens at different weeks of age

Treatments	MCV (femtoliter)				MCH (picogram)				MCHC (g/dl)			
	Weeks			Total average	Weeks			Total average	Weeks			Total Average
	4	5	6		4	5	6		4	5	6	
C	102.58 ±7.92 [a]	100.69 ±10.76[a]	101.40 ±12.21[a]	101.63 ±12.26[a]	35.33 ±4.05[a]	31.39 ±5.5[a]	a39.18 ±4.76[a]	35.40 ±5.33[a]	34.44 ±4.56[a]	31.17 ±3.98[a]	38.64 ±3.25[a]	b34.83 ±3.76[b]
T1	100.64 ±5.33 [a]	98.31 ±11.13[a]	100.55 ±10.04[a]	99.79 ±10.53[a]	34.42 ±3.32[a]	30.74 ±6.08[a]	A38.89 ±6.03[a]	34.74 ±7.04[a]	34.02 ±2.19[a]	31.27 ±3.45[a]	38.68 ±3.23[a]	34.81 ±3.99[a]
T2	100.89 ±8.52 [a]	98.96 ±9.23[a]	102.14 ±10.55[a]	100.69 ±9.67[a]	34.96 ±1.33[a]	31.05 ±5.59[a]	39.35 ±2.98[a]	35.20 ±6.65[a]	34.64 ±2.98[a]	31.32 ±2.89[a]	38.53 ±3.55[a]	34.96 ±5.09[a]
T3	100.94 ±5.33 [a]	98.98 ±8.56[a]	98.14 ±9.99[a]	99.21 ±9.87[a]	34.97 ±2.21[a]	31.18 ±4.48[a]	38.48 ±3.91[a]	35.12 ±6.34[a]	34.73 ±2.3[a]	31.50 ±2.60[a]	39.16 ±3.77[a]	35.27 ±4.10[a]

C: control group; T1, T2 and T3: adding CrM to the diet of broiler chicken at levels of 0.4, 0.8 and 1.2 %, respectively; [abc]Values within columns followed by different letters differ significantly (P<0.05)

Table 5: Effect of dietary supplementation of creatine monohydrate on Hetrophil to Lymphocyte ratio and plasma uric acid and creatinine concentration of broiler chickens at different weeks of age

Treatments	H/L Ratio			Uric acid (mg/dl)			Creatinine (mg/dl)		
	Weeks		Total average	Weeks		Total average	Weeks		Total average
	4	6		4	6		4	6	
C	0.330±0.09[a]	0.356 ±0.03[a]	0.343±0.04[a]	5.22±0.45[b]	4.98±0.24[b]	5.1±0.29[b]	1.01±0.04[b]	1.40±0.07[c]	1.20±0.03[c]
T1	0.348±0.08 [a]	0.366 ±0.06[a]	0.357±0.03[a]	6.53±0.43[a]	5.80±0.21[a]	6.16±0.36[a]	1.32±0.07[a]	1.60±0.09[b]	1.46±0.04[b]
T2	0.359±0.07[a]	0.364±0.03[a]	0.361±0.01[a]	6.89±0.35[a]	5.85±0.18[a]	6.37±0.33[a]	1.43±0.03[a]	1.65±0.09[a]	1.54±0.05[a]
T3	0.335±0.08[a]	0.356±0.08[a]	0.345±0.03[a]	7.08±0.54[a]	5.92±0.24[a]	6.50±0.34[a]	1.58±0.05[a]	1.66±0.1[a]	1.62±0.07[a]

C: control group; T1, T2 and T3: adding CrM to the diet of broiler chicken at levels of 0.4, 0.8 and 1.2%, respectively; [abc]Values within columns followed by different letters differ significantly (P<0.05)

within seconds of exposure to stress and glucocorticoids rise within minutes (Le Maho et al., 1992). In the field, plasma levels of glucocorticoids can increase by an order of magnitude within 20-30 min of capture, so unless handling time is very short, the effect of these investigator-imposed stressors may swamp other inputs. Elevation of corticosterone, the major glucocorticoid in birds, leads to a series of events that can enhance short-term survival, including redirected behaviour and mobilization of energy reserves (Wingfield et al., 1998). The half-life of these hormones is short (minutes to hours), so their levels drop and their effects disappear if the stressor is removed. This is functionally important because chronic stress and chronically elevated glucocorticoids can result in stress-related disease (Sapolskyr, 1992). The high rate of H/L ratio refers to the high level of stress hormone (Al-Daraji et al., 2008).

In the present study, CrM supplementation significantly increased concentration of uric acid and creatinine in the plasma. However, the alteration of these traits belongs to the side effects of Cr and the clinical trials studies concluded that there is no conclusive evidence to support the notion that both short and long-term Cr may adversely affect kidney and liver function in healthy individuals (Pline and Smith, 2005).

Conclusion

The results of the current study added to the growing body of knowledge regarding acute and chronic effects of Cr supplementation on blood traits and renal function using broiler model. Therefore, we concluded that supplementation at the levels of 4 g/kg, 8 g/kg and 12 g/kg have not negative effects on blood traits. However, further studies are necessary to clarify the metabolism of long-term Cr supplementation, as well as the possible side effects in other organs such as liver.

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