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Effect of adding Creatine and Methionine on Japanese Quails Performance

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Abstract

The objective of this study was to evaluate the effect of creatine supplementation and additional plus 10% of the methionine requirement. All diets were isoenergetic and isonitrogenous and balanced with NRC recommendation. 336 ten day-old Japanese quail chicks unsexed were distributed according to a completely randomized experimental design into four treatments. Experimental treatments were :1) basal diet (control) , 2) basal diet + 6000 mg of creatine /kg feed , 3) basal diet + 1000 mg of additional methionine /kg feed and 4) basal diet + 6000 mg of creatine / kg feed and 1000 mg of additional methionine /kg feed. Each dietary treatment was designed to three replicate groups of 28 Japanese quail chicks and the experiment lasted 42 days. Birds were raised on litter and provided ad libitum access to both feed and water . chicks and feed were recorded to determine efficiency conversion ratio of feed , protein , energy , methionine and lysine . Inspected on a daily basis for mortality and mortality . During 42 days of the experiment , the body weight gain (BWG) , efficiency conversion ratio (ECR) for feed , protein , energy , methionine and lysine of chicks fed diets (treatments) 2,3,and 4 were significance ($P \leq 0.05$) to that of birds fed control diet (T1). On other hand there were no significant differences in the performance Traits among the 2,3 and 4 treatments.

Introduction

Creatine or methyl guanidine – acetic acid, is a naturally occurring nitrogen compound found primarily in skeletal muscle , and its phosphorylated from, phosphocreatine , plays a pivotal role in energy metabolism by donating its phosphate groups to adenosine diphosphate to regenerate adenosine triphosphate *[1,2 and 3]* .Oral consumption of creatine mono anhydrate by humans can elevate both muscle creatine and creatine phosphate contents by 32% and 20% respectively *[4]*. The effects of creatine mono hydrate on domestic animals (poly gastric and mono gastric) have also been evaluated. It is also help to improve the quality of chicken meat *[5,6 and 7]*. Creatine is normally produced in the liver, kidneys and pancreas from glycine , arginine and methionine *[8]*. For animals, phosphocreatine metabolism is an important energy buffering pool that is rapid , direct and efficient so that Adenosine Triphosphate remains stable at the cellular level. However , 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 modern intensive farming conditions *[9]*. Two

possible mechanisms suggest that creatine may enhance muscle performance and protein synthesis *[8]*. Due to an increase in the amount of energy stored as phosphocreatine , creatine – loaded muscle has the capacity to maintain normal physical function and to delay the onset of muscle fatigue*[9]*. Increased concentration of intramuscular phosphocreatine attack water into the muscle cell and increase the cell volume *[11]*. Creatine and its phosphorylated from , creatine phosphate , play an important role in cellular energy storage , buffering and trans port *[12]*. Nutritional sources for creatine are protein rich tissues of animal origin . Performance of farm animals raised for meat production is de find as weight gain , muscle growth and protein retention . However ,as creatine is predominantly found in muscle tissue *[1, 2 and 4]*. The 1 kg of broiler an average daily weight gain of about 70 g can be expected *[13 and 14]*. Since about 50% of the gain is muscle *[15]* . Containing on average 4.5 g creatine per kg *[1, 4 and 16]*, 157.5mg creatine arid required (net) per day for maintenance, for one kg broiler . Methionine is classified as the first limiting amino acid for the growth of broiler chickens given vegetable protein sources, maninely soybean meal. Methionine is essential amino acid for protein synthesis , a methyl group donor and is also precursor of cysteine and cysteine is required for the synthesis of reduced glutathione *[17]*. Glutathione (GSH) is synthesized mainly the liver *[18]* and has a crucial role in the detoxification of free radicals *[19 and 20]*. More than half of the methionine ingested is used for the synthesis of GSH in the liver *[21]* and GSH content in the cells is influenced by dietary Sulphur amino acids *[22]*. Methionine is very import anted for protein and polyamines (spermine and spermidine) synthesis and for working as methyl group donor for methylation reactions of DNA and other molecules *[23]*. On the other hand methionine is a precursors of L- creatine which play important roles in lipid and energy metabolism . Improvement in weight gain , feed conversion ratio , carcass characteristics or decrease in serum triglyceride in bird fed supplemented L-carnitine reported by researchers such as Lettner et al (1992) *[24]* and Xu et al (2011) *[25]*. The objective of this study was to determine the effects of creatine and additional methionine supplementation for growing Japanese quail upon its performance.

Material and Method

The experiment was carried out at the poultry Farm-Department of Animal Production- Agriculture College-Kirkuk University , Kirkuk-Iraq . 336 ten day-old unsexed Japanese quail distributed in a completely randomized design experiment with four treatments (1: basal diet , 2: basal diet + 6.0 gm creatine /kg diet , 3: basal diet + 1.0 gm methionine /kg diet and 4: basal diet + 6.0 gm of creatine + 1.0 gm of methionine /kg diet) of three replicates (floor pens) of 28 birds each . Feed and water were offered ad libitum. Diets were formulated to supply the quail chicks nutritional requirements according to the recommendations of N.R.C (1994) *[26]* . Table 1 show the ingredients and experimental diets . Birds , diet offer , and diet residues were weighted on days 10 , 17 , 24 , 31 , 38 , 45 and 52 to determine feed intake (FI) , Live body weight (LBW) , body weight gain (BWG) , feed protein energy , methionine and lysine intake (FI , PI , EI , MI , and LI) respectively and conversion ratio (FCR , PCR , ECR , MCR and LCR) respectively . The number of dead birds were daily recorded to calculate mortality percentage (%) . The GLM procedure of SAS software *[27]* was used for data analysis of variance as completely randomized design . The significant differences among the means were calculated by Duncan's multiple range *[28]*.

Table -1: Formulation and Calculated Composition of the experimental diets.

	% of diet			
Feed ingredient	***T1(basal diet)***	***T2***	***T3***	***T4***
Wheat	*58.72*	*58.02*	*58.52*	*57.92*
Soybean meal	*28.33*	*28.33*	*28.33*	*28.33*
Meat and bone meal	*10.00*	*10.00*	*10.00*	*10.00*
Vegetable oil	*2.50*	*2.50*	*2.50*	*2.50*
Nacl	*0.2*	*0.2*	*0.20*	*0.2*
Vit and Min premix	*0.10*	*0.10*	*0.10*	*0.10*
Enzymes	*0.10*	*0.10*	*0.10*	*0.10*
DL- Methionine	*0.10*	*0.10*	*0.20*	*0.20*
DL-Lysine	*0.05*	*0.05*	*0.05*	*0.05*
Creatine	*0.00*	*0.60*	*0.00*	*0.60*
Total	*100*	*100*	*100*	*100*
Calculated nutrients Values				
ME(kcal/kg)	*2900*	*2900*	*2900*	*2900*
CP(%)	*24.00*	*24.00*	*24.00*	*24.00*
Calcium (%)	*0.80*	*0.80*	*0.80*	*0.80*
Available P (%)	*0.30*	*0.30*	*0.30*	*0.30*
Methionine(%)	*0.50*	*0.50*	*0.61*	*0.61*
Lysine (%)	*1.30*	*1.30*	*1.30*	*1.30*
Creatine (%)	-	*0.60*	-	*0.60*

Results and Discussion

Growing Japanese quail performance results on day 52 are shown in the Tables 2 , 3 and 4. There were significant ($p \leq 0.05$) influence of adding 6.0 gm creatine /kg basal diet (T2) , additional 1.0 gm of DL-methionine /kg basal diet (T3) and 6.0 gm of creatine + addition 1.0 gm of DL-methionine /kg basal diet (T4) that compared with birds fed basal diet(T1) . The average of body weight at 52 days old and body weight gain were heavier as percentage (8.97 , 10.97 and 8.10 %) and (11.39 , 14.42 and 11.19) respectively for T2 , T3 and T4 by the birds fed control diet (T1) as shown in Table 2 . Daily intake of feed , Protein energy , Methionine and lysine the experiment period are shown in Table 3 . There was significant difference ($p \leq 0.05$) among treatments in consume of nutrients . This shows that supplementation 6.0 gm creatine / kg (T2) , 1.0 gm methionine / kg (T3) and 6.0 gm creatine / kg + 1.0 gm methionine / kg (T3) led to decrease feed and energy intake significantly($p \leq 0.05$) for the birds of the T2 , T3 and T4 as percentage (9.11 , 6.86 and 11.71 %) by the birds of the control group (T1), While the protein intake for the T4 birds (11.71%) were significantly lower than the birds of the (T1). There were no significant differences among the treatment for methionine and lysine intake. The values in the table (4) were referred to the supplementation 6gm of creatine to the basal diet (T2) , or 1gm methionine to the basal diet (T3) and 6gm creatine with 1gm methionine (T4) led to improve significantly($p \leq 0.05$) ability of conversion ratio of feed ,protein and energy as percentage which were (18.40 , 18.60 and 20.40%) ,(18.33 , 18.33 and 20.83%) and (15.68 , 15.89 and 17.96 %) for T2,T3and T4 by the T1 respectively. There were no significant differences among the treatments for methionine conversion ratio (table4). The ability of the birds for lysine conversion ratio for T2,T3 and T4 were better than the birds of the control group as percentages (18.46, 18.64 and 20 %) for the T2 ,T3 and T4 comparing that with (T1) . Methionine is an essential amino acid with at least four main roles that may be involved direct or indirect in the improvement of the quail performance . First, methionine participates in protein synthesis . Seconds , is a glutathione precursor . Third , methionine is required for synthesis of polyamines. Fourth, methionine is most important methyl group donor for methylation reactions of DNA and other molecules *[29,30, 31, 32 and 33]*. On other hand methionine and lysine acts as precursor

of L-Carnitine which can play important roles in lipid and energy metabolism in poultry *[34, 35, 36, 37, 32 and 33]*. On other hand the results conflicting the ability of creatine to stimulate myosin synthesis and muscle cannot synthesis creatine and depend on the liver for it *[38]*. Supplementation creatine (1,2,5 and 10 g / kg diet) improved weight gain compared with control group in 35 – d- old broilers *[29]*. Birds received either 0.00, 250.0 and 1000 mg Creatine Monohydrates for 21 days did not effect upon the daily weight gain , daily feed intake and feed efficiency*[8]*. Two possible mechanisms suggest that may creatine enhance muscle performance and protein synthesis *[10]*. On the other hand the feed efficiency of broiler chicks fed diet contained 0.63 % creatine was superior ($p \leq 0.05$) to that of birds fed diet contained 0.0% creatine; and creatine supplementation has been shown to increase total body weight during 5-6 weeks of the experiment *[40]*.

Table -2: Effect of supplementation of creatine and additional methionine upon growth traits of growing Japanese quail.

Treatments	***Final body weight at 52 days (g)***	***Body weight gain(g)***	***Mortality (%)***
T1 (control)	*199.91±1.52 b*	*150.09±2.68 b*	*2.38*
T2	*217.84± 1.72 a*	*167.18± 2.15 a*	*3.57*
T3	*221.85± 2.56 a*	*171.73± 1.89 a*	*2.38*
T4	*216.11±1.79 a*	*166.89± 1.80 a*	*2.38*

* Means with different letters in the same column are significantly different($p \leq 0.05$) .

Table -3: Effect of supplementation of creatine and additional methionine upon the feed , protein ,energy , methionine and lysine daily intake of growing Japanese quails.

Treatments	***Intake (g) of***				
	Feed	***Protein***	***Energy***	***Methionine***	***Lysine***
T1 (control)	*a 750.45±5.77*	*a 180.10±5.77*	*a 2176.31±5.73*	*a 3.75±0.56*	*a 9.75±1.15*
T2	*bc 682.09±5.75*	*ab 163.70±7.50*	*c 1978.61±9.81*	*a 3.41±0.55*	*a 8.86±0.50*
T3	*b 698.94±10.30*	*ab 167.74±4.04*	*b2024.47±13.85*	*a 4.26±1.15*	*a 9.08±0.52*
T4	*c 662.55±10.39*	*b 159.01±5.19*	*d1921.39±12.12*	*a 4.04±1.15*	*a 8.61±0.57*

* Means with different letters in the same column are significantly different($p \leq 0.05$) .

Table- 4: Effect of supplementation of creatine and additional methionine upon Conversion ratio of feed, protein , energy , methionine and lysine of the growing Japanese quails.

Treatments	***Conversion Ratio of***				
	Feed	***Protein***	***Energy***	***Methionine***	***Lysine***
T1 (control)	*a 5.00± 0.50*	*a 1.20± 0.02*	*a 14.50± 0.51*	*a 0.024±0.001*	*a 0.065± 0.001*
T2	*b 4.08± 0.50*	*b 0.98± 0.01*	*b 11.84± 0.51*	*a 0.019±0.001*	*b 0.053±0.001*
T3	*b 4.07± 1.15*	*b 0.98± 0.02*	*b 11.79± 0.57*	*a 0.025±0.001*	*b 0.053±0.001*
T4	*b 3.97± 1.15*	*b 0.95± 0.02*	*b 11.51± 0.53*	*a 0.024±0.001*	*b 0.052±0.001*

* Means with different letters in the same column are significantly different($p \leq 0.05$) .

Some blood physiological traits were shown in the table (5) . Supple mentation of creatine and additional methionine led to significant variation($p \leq 0.05$) between the treatments (1,2 and 3) when compared with the fourth treatment for (RBC) while the differences were significant($p \leq 0.05$) the treatments (1,2 and 4) for the (WBC) trait . On other hand the differences were no significant among the experimental treatments for the PCV (%) and Hb traits. Supplementation three levels of guanidine acetic acid(0.00 , 0.06 and 0.12%) to the broiler diets (basal diets) led to no significant differences among the dietary treatments for some blood parameters as PCV % and WBC numbers *[41]*.

Table-5: Effect of Supplementation of creatine and additional methionine upon some quail blood parameters.

Treatments	Pcv (%)	Hb (g/ 100 ml)	RBC (m^3 / 10^6)	WBC (m^3/ 10^3)
T1 (control)	a 50.00 ± 1.52	a 16.66 ± 0.50	a 5.78 ± 0.06	a 6.77± 0.27
T2	a 49.33 ± 2.08	a 16.44 ± 0.69	a 5.71 ± 0.07	b 5.92± 0.07
T3	a 46.00 ± 1.00	a 15.33 ± 0.33	a 5.70 ± 0.10	bc 5.34± 0.11
T4	a 45.00 ± 2.02	a 15.00 ± 0.67	b 5.25± 0.10	c 4.76 ± 0.38

* Means with different letters in the same column are significantly different($p \leq 0.05$) .

The were no significant effect of adding creatine and additional methionine upon some biochemical quail blood values (Total protein , Globulin and Glucose) as shown in the Table (6) , while there was significant differences($p \leq 0.05$) between the control treatment (T1) and the treatments (2,3 and 4) for the blood Albumen and cholesterol .There were no significant differences among three dietary groups (supplementation 0.00 ,0.06 and 0.12%) guanidine acetic acid in the concentration means of the broiler blood total protein , albumin and globulin *[41]*.

Table -6 : Effect of Supplementation of creatine and additional methionine upon some quail blood biochemical traits.

Treatments	Total protein (g/100ml)	Globulin (g/100ml)	Albumen (g/100ml)	Cholesterol (gm/100ml)	Glucose
T1 (control)	a 5.33± 0.41	a 3.62 ± 0.51	a 1.71± 0.14	a 278.61± 6.09	a 261.15±17.80
T2	a 4.91± 0.48	a 3.54 ± 0.46	b 1.36± 0.04	b 242.42± 15.86	a 257.92± 2.89
T3	a 4.51± 0.43	a 3.27± 0.47	b 1.34 ± 0.04	b 242.36± 6.06	a 252.57±18.13
T4	a 4.19 ± 0.34	a 2.84± 0.34	b 1.23± 0.00	b 230 ± 2.27	a 240.98±21.31

* Means with different letters in the same column are significantly different($p \leq 0.05$) .

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