www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Research article

Effects of supplementing broiler diets with CreAMINO® on broiler performance, carcass traits and the expression of muscle growth related genes

AE Metwally[1], Doaa Ibrahim[1] and Safaa I Khater[2]

[1]Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, Egypt
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, Egypt

Article history
Received: 23 Nov, 2015
Revised: 13 Dec, 2015
Accepted: 16 Dec, 2015

Abstract

The present study was conducted to investigate the effects of CreAMINO® (96% Guanidinoacetic acid) addition into the broiler on growth performance, carcass trait, chemical composition of meat and molecular investigation of the expression of Myogenin, Myostatin and IGF-1 genes. Three hundred fifty one-day-old Ross 308 chicks were allocated to seven dietary treatments with 5 replicates containing 10 birds in each. Each feeding phase (starter, grower or finisher) was extended for two weeks. The treatments were control diet (without CreAMINO®), the 2nd, 3rd , 4th and 5th treatments were the same as control in addition to CreAMINO® was added on top with 0.6 g/kg to the control diet in starter only, grower only, finisher only and whole rearing period for (2nd, 3rd , 4th and 5th treatment, respectively). A control reduced metabolizable energy and crude protein diet (50 kcal/kg and 0.5%, respectively) was formulated to be the 6th treatment and CreAMINO® was added to the control reduced diet on top with 0.6 g/kg to be the 7th treatment. The results indicated that supplementation of broiler diets with CreAMINO® significantly improved bird performance. The best performance was seen when CreAMINO® was fed during the whole rearing period compared to control, rearing stages and reduced feed contents. Addition of CreAMINO® to the control. Adding CreAMINO® into reduced feed contents, fully compensated bird performance. CreAMINO® supplementation significantly improved carcass dressing, breast meat yield, reduced abdominal fat, and increased the protein content of the breast muscle. Moreover, feeding CreAMINO® had no negative effect on kidney function tests. CreAMINO® showed a powerful anabolic effect on skeletal muscle, indicated by the up regulation of Myogenin and IGF-1 genes and down regulation of Myostatin gene expression in the pectoral muscle. In conclusion, CreAMINO® improved broiler growth performance, carcass traits and had sparing effect on energy and protein in broiler diet.

Keywords: CreAMINO®, broiler performance, Guanidinoacetic acid, gene expression

To cite this article: Metwally AE, D Ibrahim and SI Khater, 2015. Effects of supplementing broiler diets with CreAMINO® on broiler performance, carcass traits and the expression of muscle growth related genes. Res. Opin. Anim. Vet. Sci., 5(11): 435-442.

***Corresponding author:** AE Metwally, Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44519, Sharkia, Egypt;
E. mail: drabdalla75@yahoo.com

Introduction

Creatine and its phosphorylated form, phosphor-creatine, is a naturally occurring component in the animal's body tissue and plays a major role in energy metabolism (Wyss and Kaddurah Daouk, 2000). It is assumed that about 50% of the daily creatine requirement is synthesized by de-novo synthesis while the remainder must be supplied by the feed (Lemme et al., 2007a). The need for creatine in diets for animals is well recognized. With the absence of animal proteins in pure vegetable diets, the risk for a creatine deficiency increased (Stahl et al., 2003). Creatine acts as a buffer for recycling of high energy phosphate groups for conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP); and as a mechanism to transport high energy phosphate group from mitochondrial sites of production to cytoplasmic sites of utilization (Brosnan et al., 2009). Therefore, the creatine/phosphocreatine function as a backup to convert ATP to ADP in order to store and mobilize energy when required on short notice, particularly in muscle cells (Lemme et al., 2007b). Highest levels in the body are found in skeletal muscle such as breast meat in broilers. In broilers, the primary requirement of creatine is to facilitate energy supply for growth of muscle. Therefore, a deficiency could be viewed as a limiting factor in broiler growth performance (Stahl et al., 2003).

As a precursor of creatine, guanidinoacetic acid (GAA), synthesized from arginine and glycine in the kidney, is also produced naturally in the body. In the liver, GAA is then converted to creatine through methylation by S-adenosyl-methione (Van Pilsum et al., 1972; Wyss and Kaddurah-Daouk, 2000) and finally transported to the target tissues (mainly muscle cells). Recently, positive effects on growth performance were noticed when broilers were administered GAA (Abudabos et al., 2014). The transformation of GAA into creatine was evidenced by an increase in creatine levels (in serum and muscle) (Lemme et al., 2007a&b; Ringel et al., 2008a; Michiels et al., 2012; Carvalho et al., 2013; Abudabos et al., 2014). Moreover, GAA could be beneficial in broiler diets because it may be able to spare arginine, which is considered to be the fifth limiting amino acid in typical corn-soybean diets for broilers (Dozier et al., 2008; Baker, 2009; Waguespack et al., 2009). Ringel et al. (2008b) proposed that GAA may have growth and feed efficiency promoting properties when added to corn-soybean meal diets in broilers.

From the previous studies on human it is observed that creatine can lead to an improvement in performance, (Hultman et al., 1996) stated that creatine supplementation increases total creatine in muscle and in absence of creatine supplementation, creatine level in muscle gradually declines to baseline level (this requires about 30 days). Creatine is metabolized into creatinine and excreted through kidney, therefore creatine and creatinine level must be carefully monitored. However, short term feeding to broiler (only at one feeding stage; starter, grower or finisher) or long term feeding (whole feeding period) should be investigated. Short term high intensity exercise, or repeated busts of power, can benefit from creatine supplementation, therefore, the sparing effect of creatine on energy and protein requirement must be studied. The effect of creatine on the muscle growth related genes must be studied also. Insulin-like growth factor 1 (IGF1) have extensive anabolic effects. The activation of it promotes protein synthesis and muscle development (Glass, 2003). Furthermore, myogenin is one of the most important genes concerned with the muscle fiber formation, its expression level is related to muscle growth (Koishi et al., 1995). Myostatin, is a potent inhibitor of muscle growth and is expressed in embryonic and adult skeletal muscle (McPherron et al., 1997).

CreAMINO® is a novel product and it contains 96% GAA. In this trial, we investigated the effect of supplementation of CreAMINO® (96% GAA) at different feeding stages (starter, grower or finisher) or the whole feeding period, under condition of reduced energy and reduced energy and protein on broiler performance, kidney function tests, carcass traits and molecular investigation of Myogenin, Myostatin and IGF-1 gene expression. Secondly the sparing effect of supplementation of CreAMINO® (96% GAA) on energy and protein requirement was also investigated.

Materials and Methods

The experimental design and diets

Three hundred fifty one-day-old ROSS chicks were purchased and incubated together in the first three days until body weight was 82.5g, then the chicks were allocated to seven treatments with 5 replicates containing 10 birds in each, to study the effect of adding CreAMINO® (Evonik Industries AG, Hanau, Germany) on broiler growth performance. The birds were reared from day 1 to 42 days of age. Each feeding phase (starter, grower or finisher) was extended for two weeks. The diet composition for each feeding phase (Table 1) was maintained according to the Ross manual Guide and CreAMINO® at the rate of 0.06% (0.6 g/kg diet) in the diet. Physically feed was in the form of mash in the whole period (starter, grower and finisher). Treatments were as follows:

Treatment 1: Control basal diet without CreAMINO®
Treatment 2: Control + 0.06% CreAMINO® in starter feed only (CreS)

Table 1: Composition of the basal diet

Ingredient	Starter	Grower	Finisher	Starter	Grower	Finisher
	Control			Reduced control		
Corn	58.0	61.9	64.8	60.0	63.9	66.8
Soybean Meal 48%	34.5	29.0	26.0	34.5	29.0	26.0
Corn gluten meal 60%	1.40	2.60	2.20	0.22	1.50	1.00
Calcium CO_3	1.30	1.25	1.20	1.30	1.25	1.20
Dical. Phos.	1.90	1.65	1.50	1.90	1.65	1.50
Phytase enzyme	0.005	0.005	0.005	0.005	0.005	0.005
Soybean oiL	1.65	2.40	3.20	0.80	1.50	2.40
Common salt	0.20	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.35	0.32	0.32	0.35	0.32	0.32
DL-Methionine	0.25	0.23	0.18	0.25	0.23	0.18
L-Lysine HCl	0.20	0.20	0.15	0.20	0.20	0.15
Threonine	0.05	0.05	0.05	0.05	0.05	0.05
Vit. and Min. Premix[1]	0.20	0.20	0.20	0.20	0.20	0.20
ME Kcal/Kg diet	2998	3105	3181	2948	3052	3133
Crude protein %	21.5	20.0	18.5	21.0	19.5	18.0
E.E. %	4.23	5.11	5.98	3.43	4.26	5.22
Calcium %	1.0	0.92	0.86	1.0	0.92	0.86
Avail. Phos. %	0.48	0.43	0.40	0.48	0.43	0.40

[1]Supplied per kilogram of diet: 11,000 IU vitamin A, 2,200 IU vitamin D3, 30 IU vitamin E (dl-α-tocopheryl acetate), 2.0 mg menadione, 1.5 mg thiamine, 6.0 mg riboflavin, 60 mg niacin, 4 mg pyridoxine, 0.02 mg vitamin B12, 10.0 mg pantothenic acid, 6.0 mg folic acid, 0.15 mg biotin, 0.625 mg ethoxyquin, 500 mg $CaCO_3$, 80 mg Fe, 80 mg Zn, 80 mg Mn, 10 mg Cu, 0.8 mg I, 0.3 mg Se.

Treatment 3: Control + 0.06% CreAMINO® in grower feed only (CreG)
Treatment 4: Control + 0.06% CreAMINO® in finisher feed only (CreF)
Treatment 5: Control + 0.06% CreAMINO® in all feeding phases (CreW)
Treatment 6: Control with reduced metabolizable energy and crude protein (50 kcal/kg and 0.5% respectively) (R-ve)
Treatment 7: Control with reduced metabolizable energy (50 kcal/kg) and crude protein (0.5%) + 0.06% CreAMINO® (RCre)

Housing and management

The experiment was carried out at experimental station of Faculty of Veterinary Medicine, Zagazig University, Egypt. The number of replicates and treatments were visible at each pen. The animals were housed in pens with ten birds each. Pens were equipped with feeders and drinkers. Feed and water were supplied *ad libitum*. During the first three days, the birds were provided with a temperature of 33ºC. The temperature was then gradually reduced by 1ºC every two days until the chicks were 20 days old, at which the temperature was maintained at 25ºC for the rest of the trial.

Measurements

All pens were checked for sick and dead birds on a daily basis. Identification number, pens, age, body weight of each dead bird was recorded on a pen record sheet. General health status and mortality were recorded. Body weights and feed intake were recorded every week on a pen basis subsequently, feed conversion ratio (FCR) was calculated.

After starter, grower and finisher periods, five birds from each group (one from each replicate) was used for blood sampling for kidney function tests using enzymatic kits.

After the starter, grower and finisher period, samples from pectoral muscle were collected rapidly, kept in liquid nitrogen and stored at -80ºC until molecular investigation of Myogenin, Myostatin and IGF-1 gene expression were determined using a semi-quantitative RT-PCR according to Meadus (2003). Primer sequences of chicken Myogenin, Myostatin and β-actine (Table 2) were obtained from the published sequences of Gabriel et al. (2003) and for IGF-1 from Del Vesco et al. (2013).

On the last day of the experiment, one bird of each replicate having body weights closest to the pen mean were selected, slaughtered and carcass traits, slaughter yield, breast meat, leg meat and abdominal fat were determined. Moreover, a sample from the pectoral muscle from each replicate was taken for meat chemical analysis (moisture content, crude protein, crude fat). Meat chemical analysis was determined according to the method described by the AOAC (2002).

Statistic analysis

Experimental data (body weights, weight gain, feed intake, FCR, blood sample, chemical analysis and carcass traits) were analyzed as a completely randomized block design by General Linear Model of SAS (SAS Institute Inc., Vers. 9.2):

$Yij = \mu + treatmenti + eij$

Where, Yij = observation value of the dependent variable, μ = overall mean, treatment *i* = effect of GAA treatments (1, 2, 3, ...7), eij = residual error.

Differences between treatments were assessed for statistical significance by Duncan's Test (treatment vs. eij) at P<0.05. Replicate was considered the experimental unit except for chemical, blood and carcass analysis data, where individual birds were used as experimental units. Mortality was considered in data analysis.

Results and Discussion

Performance parameters of the different treatments at starter, grower and finisher period as well as a cumulative for the whole rearing period are presented in Table 3. From the data illustrated in Table 3, it was found that addition of CreAMINO® significantly (P<0.05) improved broiler growth performance (body weight, body weight gain and feed conversation) at different growth stages. These results are in agreement with Lemme et al. (2007a), Ringel et al. (2008a&b), Abudabos et al. (2014) who found that addition of CreAMINO® significantly (P<0.05) improved FCR. It is also observed that CreAMINO® could significantly reduce and spare metabolizable energy (about 50 Kcal/Kg diet) and protein (about 0.5% crude protein). This is in line with the finding of Abudabos et al. (2014) who concluded that CreAMINO® supplementation in reduced-energy diets showed improvement in FCR, while the best performance was detected for the diet.

Reducing specification of feed by 50 Kcal metabolizable energy per kg diet and 0.5% CP absolutely had a significant (P<0.05) impact on FCR. Reducing feed specification had no significant reduction in

Table 2: Primer sequences of chicken Myogenin, Myostatin and β-actine

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'- CACCTAAATCTGCACGCT -3'	5'- CTTGTGGATGGCATGATCT -3'	140
β-actine	5'- AATGAGAGGTTCAGGTGCCC -3'	5'- ATCACAGGGGTGTGGGTGTT -3'	409

Table 3: Effect of adding CreAMINO® on broiler performance (body weight, body gain, feed intake and feed conversion ratio) at the different stages (starter, grower, finisher and cumulative)

	Control	CreS	CreG	CreF	CreW	R-ve	RCre
Starter							
BW (g)	582^{ab}±8.8	592^{a}±4.6	582^{ab}±12.4	582^{ab}±5.9	590^{a}±6.4	575^{b}±3.0	581^{ab}±5.2
BG (g)	499^{ab}±8.3	509^{a}±4.8	499^{ab}±12.2	499^{ab}±5.6	508^{a}±6.5	492^{b}±2.9	499^{ab}±5.3
FI (g)	650±9.5	648±7.8	650±12.8	648±5.6	645±5.3	652±5.8	640±6.5
FCR (g/g)	1.30^{b}±0.01	1.27^{c}±0.01	1.30^{b}±0.01	1.30^{b}±0.01	1.27^{c}±0.01	1.33^{a}±0.01	1.28^{c}±0.01
Grower							
BW (g)	1429^{c}±21.1	1476^{a}±11.9	1465^{abc}±33.8	1428^{c}±33.1	1473^{ab}±15.6	1430^{bc}±11.6	1467^{abc}±12.2
BG (g)	847^{b}±13.9	884^{a}±8.5	883^{a}±22.2	847^{b}±27.6	882^{a}±9.5	855^{ab}±10.1	886^{a}±10.1
FI (g)	1462^{ab}±19.8	1452^{ab}±15.2	1460^{ab}±36.1	1456^{ab}±37.2	1425^{b}±18.3	1481^{a}±19.4	1438^{ab}±9.7
FCR (g/g)	1.73^{a}±0.01	1.64^{bc}±0.01	1.65^{b}±0.02	1.72^{a}±0.01	1.61^{d}±0.01	1.73^{a}±0.01	1.62^{cd}±0.01
Finisher							
BW (g)	2647^{bc}±22.5	2714^{a}±40.2	2702^{ab}±37.0	2681^{abc}±40.4	2720^{a}±33.0	2633^{c}±23.9	2693^{abc}±16.4
BG (g)	1218^{bc}±9.9	1238^{ab}±30.5	1237^{abc}±9.7	1253^{a}±13.4	1247^{ab}±19.3	1203^{c}±16.8	1226^{abc}±8.4
FI (g)	2285^{ab}±18.0	2240^{ab}±53.4	2269^{ab}±23.0	2296^{a}±22.2	2222^{a}±42.1	2302^{a}±28.6	2222^{b}±21.7
FCR	1.88^{b}±0.14	1.81^{d}±0.06	1.83^{c}±0.07	1.83^{c}±0.11	1.78^{e}±0.08	1.91^{a}±0.04	1.81^{d}±.08
Cumulative							
BG (g)	2564^{bc}±22.2	2631^{a}±40	2619^{bc}±36.9	2598^{abc}±40.1	2637^{a}±33.4	2550^{c}±24.1	2610^{abc}±16.2
FI (g)	4297^{ab}±38.7	4240^{abc}±67.0	4279^{abc}±58.7	4301^{a}±41.9	4192^{c}±60.6	4335^{a}±43.7	4200^{bc}±23.0
FCR (g/g)	1.71^{e}±0.01	1.65^{b}±0.00	1.67^{c}±0.01	1.69^{d}±0.01	1.63^{a}±0.00	1.74^{f}±0.00	1.65^{b}±0.01

Means along the same row bearing different small letters are significantly different (p < 0.05).

Table 4: Carcass dressing, carcass parts as percentage and abdominal fat

	Control	CreS	CreG	CreF	CreW	R-ve	RCre
Carcass dressing (%)	70.5^{cd}±0.90	71.2^{bc}±0.44	71.7^{abc}±1.15	72.4^{ab}±0.61	73.1^{a}±0.49	69.7^{d}±0.77	72.8^{a}±0.22
Breast (%)	28.5^{bc}±0.83	29.9^{a}±0.61	29.7^{ab}±0.77	30.2^{a}±0.32	30.7^{a}±0.37	28.4^{c}±0.93	30.4^{a}±0.41
Thigh (%)	17.3±0.26	17.2±0.19	17.2±0.43	17.1±0.27	17.1±0.22	17.4±0.30	17.2±0.22
Drumstick (%)	12.2±0.24	12.4±0.11	12.3±0.13	12.3±0.29	12.2±0.30	12.4±0.25	12.3±0.24
Abdominal fat (%)	1.97^{a}±0.05	1.87^{cd}±0.04	1.89^{bc}±0.04	1.88^{c}±0.03	1.81^{d}±0.2	1.96^{ab}±0.02	1.84^{cd}±0.03

Means in the same row bearing different small letters are significantly different (P<0.05)

Table 5: Chemical analysis of broiler meat (breast meat)

	Control	CreS	CreG	CreF	CreW	R-ve	RCre
Moisture (%)	66.8±0.79	67.0±0.68	67.1±.28	66.8±0.35	67.0±0.42	66.8±0.17	67.0±0.15
Crude protein (%)	18.6^{b}±0.26	18.9^{bc}±0.46	19.3^{ab}±.27	19.4^{ab}±0.29	19.6^{a}±0.15	18.4^{c}±0.16	19.4^{ab}±0.13
Crude fat (%)	12.6^{a}±0.18	12.1^{bc}±0.19	12.0^{cd}±0.16	11.9^{cd}±0.17	11.6^{e}±0.08	12.4^{ab}±0.11	11.7^{de}±0.16

Means in the same row bearing different small letters are significantly different (P<0.05)

Table 6: Creatinine, urea and uric acids in blood serum (mg/dl) of broiler at the different stagess (starter, grower and finisher)

	Control	CreS	CreG	CreF	CreW	R-ve	RCre
Starter							
Creatinine	0.46±0.02	0.49±0.05	0.47±0.03	0.46±0.04	0.49±0.06	0.46±0.03	0.48±0.06
Urea	15.7±1.10	16.3±1.66	15.6±0.61	15.2±1.00	17.1±2.03	15.9±2.30	16.6±2.47
Uric acid	2.70±0.16	2.85±0.14	2.66±0.12	2.74±0.13	2.92±0.16	2.79±0.16	2.87±0.21
Grower							
Creatinine	0.55±0.03	0.57±0.06	0.57±0.05	0.54±0.05	0.58±0.04	0.53±0.05	0.55±0.06
Urea	16.4±1.90	17.4±2.27	16.8±1.23	18.0±1.66	16.9±2.31	15.8±2.06	16.5±0.77
Uric acid	3.10±0.19	3.05±0.15	3.14±0.16	3.07±0.33	3.17±0.19	3.04±0.35	3.12±0.31
Finisher							
Creatinine	0.56±0.03	0.59±0.04	0.55±0.06	0.56±0.06	0.58±0.09	0.54±0.05	0.57±0.08
Urea	19.1±1.40	18.6±0.91	17.9±1.18	18.7±1.72	18.5±2.81	16.6±1.90	18.3±2.13
Uric acid	3.74±0.21	3.91±0.41	3.84±0.19	3.92±0.43	3.87±0.22	3.74±0.31	3.84±0.24

growth, although the trend was clearly downwards across all phases. Similarly, adding CreAMINO® to the lower density diets did not result in significant changes; but there was a clear trend for growth performance to be better when CreAMINO® was included. These results are in agreement with results of Michiels et al. (2012) who reported that GAA supplementation (0.12%) increased the FCR when compared with a negative control diet. Dilger et al. (2013) reported that there was an improvement in FCR when feeding 0.12% GAA in Argnine deficient diets, which was comparable to 0.15% Creatine and 0.25% Argnine supplementation. The most consistent trend was seen when CreAMINO® was added in all phases. This is in line with Mousavi et al. (2013) who reported that the addition of 0.06% GAA improved FCR in the finisher period and the whole feeding period over 40 days in 4 and 3%, respectively, when compared with the Arginine adequate diet without added GAA and concluded that the GAA effect was most apparent in the finisher period. Mousavi et al. (2013) also reported that FCR was improved by 0.06% GAA supplementation in a nutritionally-complete diet.

Data presented in Table 4 indicated that addition of CreAMINO® into the broiler diet could significantly improve carcass dressing, breast meat as well as reduce abdominal fat when the product was added at all the growing stages. Surprisingly, there was no difference in breast meat yield and abdominal fat when compared with the control and reduced energy. However, breast meat yield was significantly higher when birds were fed CreAMINO® in the starter and finisher phases, and throughout the feeding cycle. This improvement was also seen when CreAMINO® was added to the reduced diets. For abdominal fat, the impact was similar, whereby CreAMINO® addition, significantly (P<0.05) reduced fat. The lowest level was seen in the birds fed CreAMINO® throughout the feeding cycle. Ringel et al. (2008a) and Michiels et al. (2012) reported more pronounced effect of the GAA on breast meat yield, which tended to increase with increasing dietary GAA level (Ringel et al., 2008a). In contrast, no effect of the GAA on carcass parameters was found in the study by Mousavi et al. (2013). It could be explained as GAA is a natural precursor of creatine, which is involved in cell energy metabolism, particularly in tissues with high and varying energy demand such as skeletal muscle (Michiels et al., 2012).

Using CreAMINO® in broiler diet leads to significant increase in crude protein and significant decrease in crude fat (Table 5), which resulted in the increased muscle mass in broiler. Moisture content in breast meat was unaffected by CreAMINO® addition. However, protein content was significantly (P<0.05) higher in birds fed CreAMINO® throughout the feeding cycle. Lowest protein content was seen in birds fed the reduced specification diets; and supplementation with CreAMINO® again more than compensated, bring protein up to the same level as CreAMINO® fed throughout. Breast fat content was significantly (P<0.05) reduced in all CreAMINO® supplemented diets. These results are compatible with the results of Michiels et al. (2012).

Data presented in Table 6 indicate that addition of CreAMINO® did not affect kidney function tests (Creatinine, Uera and Uric acid). This analysis was carried out to find the stress effect on kidneys. Hultman et al. (1996) postulated that as more creatine is formed in the productive tissues, more will be lost as creatinine and therefore exert greater stress on the kidney. These

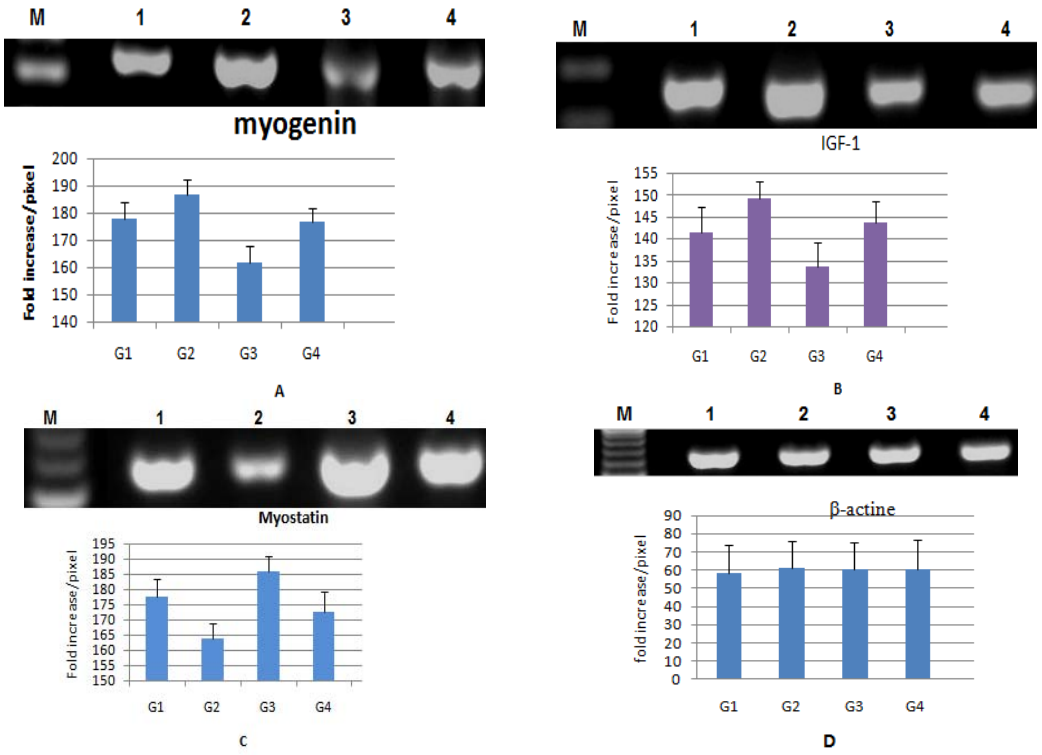

Fig. 1: The expression level of mRNAs of (a) Myogenin, (b) IGF-1, (c) Myostatin and (d) β-actine genes in the pectoralis muscle tissue of sp. M, marker *in starter period*. G1 control group, G2 CreAMINO® group, G3 reduced control and G4 reduced control supplemented with CreAMINO®

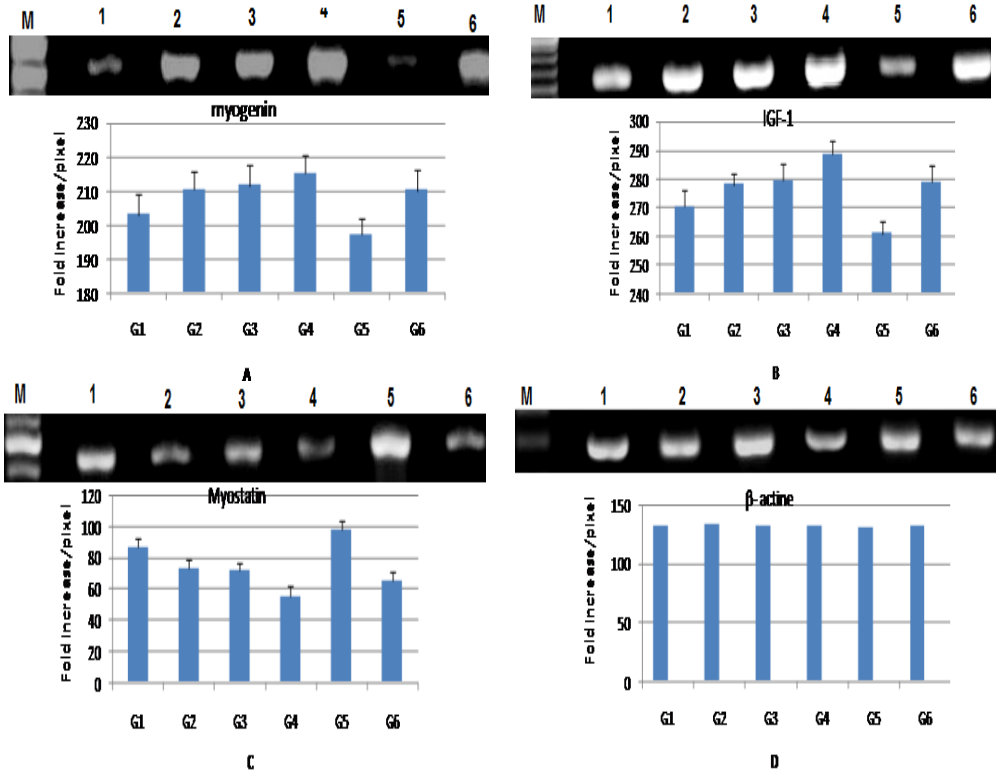

Fig. 2: The expression level of mRNAs of (a) Myogenin, (b) IGF-1, (c) Myostatin and (d) β-actine genes in the pectoralis muscle tissue of sp. M, marker in grower period. G1 control group, G2 CreAMINO® group at starter only, G3 CreAMINO® at grower only, G4 CreAMINO® at the whole period, G5 reduced control and G6 reduced control supplemented with CreAMINO®

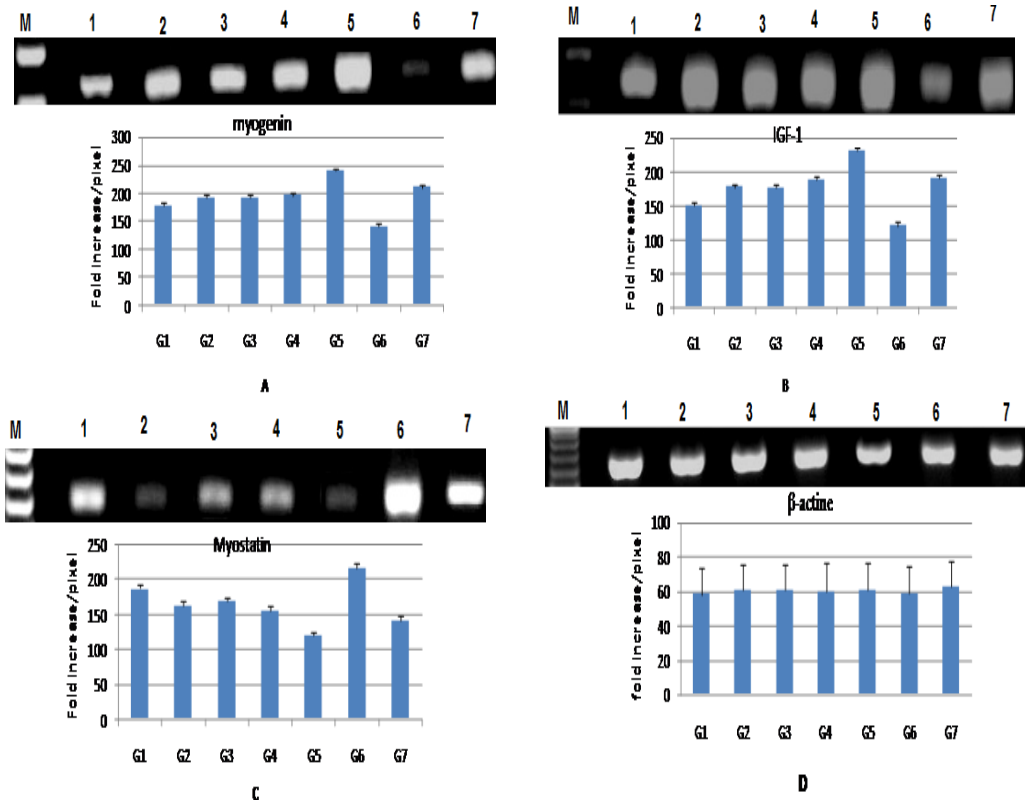

Fig. 3: The expression level of mRNAs of (a) Myogenin, (b) IGF-1, (c) Myostatin and (d) β-actine genes in the pectoralis muscle tissue of sp. M, marker *in finisher period.* G1 control group, G2 CreAMINO® group at starter only, G3 CreAMINO® at grower only, G4 CreAMINO® at finisher only, G5 CreAMINO® at the whole period, G6 reduced control and G7 reduced control supplemented with CreAMINO®

data clearly show no significant impact on serum creatinine, urea or uric acid in any treatment or any phase of growth.

From the results of molecular investigation of Myogenin, Myostatin and IGF-1 gene expression in pectoral muscle which are illustrated in Fig. 1, 2 and 3. It can be observed that CreAMINO® has a powerful anabolic effect on skeletal muscle, which is indicated by the up regulation of Myogenin and IGF-1 genes. The activation of IGF-1 genes promotes protein synthesis and muscle development (Glass, 2003). Furthermore, myogenin is one of the most important genes concerned with muscle fiber formation, its expression level is related to muscle growth (Koishi et al., 1995). CreAMINO® leads to down regulation of Myostatin gene expression in pectoral muscle. Myostatin is a potent inhibitor of muscle growth and is expressed in embryonic and adult skeletal muscle (McPherron et al., 1997). Therefore, CreAMINO® may decease protein degradation in broiler chickens and increase protein syntheses.

Conclusions

Supplementation of CreAMINO® significantly improved bird growth performance when added at different growth stages or during the entire period. The best results were observed when CreAMINO® was added during the entire period. Adding CreAMINO® into the bidet of birds having reduced specified feed (50 Kcal/kg diet metabolizable energy and 0.5% protein) compensated the reduced diet contents with no compromise on the performance. In addition, CreAMINO® improved breast meat yield and reduced abdominal fat, and increased protein content of breast muscle. There was no impact on serum metabolites of creatine and protein synthesis. Furthermore, CreAMINO® has a powerful anabolic effect on skeletal muscle.

Acknowledgement

The authors gratefully acknowledge and appreciate Evonik Industries AG, Hanau, Germany for the financial support of this study.

References

Abudabos AM, Saleh F, Lemme A and Hana AH Zakaria (2014) The relationship between guanidine acetic acid and metabolisable energy level of diets on performance of broiler chickens. Ital J Anim Sci, 13: 3269.

AOAC International (2002) AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals. Association of Official Analytical Chemists, Arlington, VA.

Baker DH (2009) Advances in protein-amino acid nutrition of poultry. Amino Acids 37: 29-41.

Brosnan JT, Wijekoon EP, Warford-Woolgar L, Trottier NL, Brosnan ME, Brunton JA, Bertolo RFP (2009) Creatine synthesis is a major metabolic process in neonatal piglets and has important implications for amino acid metabolism and methyl balance. J Nutr 139: 1292-1297.

Carvalho CMC, Fernandes, EA, Carvalho, AP, de Maciel, MP, Caires, RM, and Fagundes, NS (2013) Effect of creatine addition in feeds containing animal meals on the performance and carcass yield of broilers. Rev Bras Cienc Avic 15: 269- 275.

Del Vesco AP, Gasparino, E, Oliveira Neto, AR, Guimarães, SEF, Marcato, SMM, Voltolini, DM (2013) Dietary methionine effects on IGF-I and GHR mRNA expression in broilers. J Gen Mol Res 12: 6414-6423.

Dilger RN, Bryant-Angeoni K, Payne RL, Lemme A, Parsons CM (2013) Dietary guanidino acetic acid is an efficacious replacement for arginine for young chicks. Poult Sci 92: 171-177.

Dozier WA III, Kidd, MT, and Corzo, A (2008) Dietary amino acid responses of broiler chickens. J Appl Poultry Res 17: 157-167.

Gabriel JE, Alvaresa LE, Gobeta, MC, de Pazb, CCP, Packera, IU, Macaric, M and Coutinho, LL (2003) Expression of MyoD, myogenin, myostatin and Hsp70 transcripts in chicken embryos submitted to mild cold or heat. J Thermal Biol 28: 261–269.

Glass DJ (2003) Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. Nat Cell Biol 5: 87–90.

Hultman, E, Soderlund, K, Timmons, J A, Cederbald, G, and Greenhaff, PL (1996) Muscle creatine loading in men. J Appl Physio 81: 232 -237.

Koishi K, Zhang, M, McLennan IS, Harris AJ (1995) MyoD protein accumulates in satellite cells and is neurally regulated in regenerating myotubes and skeletal muscle fibers. Dev Dynamics 202: 244-254.

Lemme A, Ringel, J, Rostagno, HS, Redshaw, MS, (2007a) Supplemental guanidino acetic acid improved feed conversion, weight gain, and breast meat yield in male and female broilers. In Proc. XVI Eur Symp on Poultry Nutrition, Strasbourg, France. pp 1-4.

Lemme A, Ringel J, Sterk A, Young JF, (2007b) Supplemental guanidino acetic acid affects energy metabolism of broilers. pp 339-342 in Proc. XVI Eur Symp On Poultry Nutrition, Strasbourg, France.

McPherron AC, Lee SJ (1997) Double muscling in cattle due to mutations in the myostatin gene. PNAS 94: 12457–12461.

Meadus WJ (2003) A semi- Quantitative RT-PCR method to measure the in vivo effect of dietary conjugated linoleic acid on protein muscle PPAR gene expression. Biol Proced 5: 20-28.

Mousavi S, Afsar A, Lotfollahian H (2013) Effects of guanidinoacetic acid supplementation to broiler diets with varying energy contents. J Appl Poult Res 22: 47-54.

Michiels J, Maertens L, Buyse J (2012) Supplementation of guanidinoacetic acid to broiler diets: effects on performance, carcass characteristics, meat quality, and energy metabolism. Poult Sci 91: 402-412.

SAS Institute Inc 2008 SAS/STAT®9.2 User's Guide Cary, NC.

Stahl CA, MW Greenwood and EP Berg (2003) Growth parameters and carcass quality of broilers fed a corn-soybean diet supplemented with creatine monohydrate. Int J Poult Sci, 2: 404–408.

Ringel J, Lemme A, Araujo LF (2008a) The effect of supplemental guanidine acetic acid in Brazilian type broiler diets at summer conditions. Poultry Sci. 87(Suppl.1): 154.

Ringel J, Lemme AM, Redshaw S, Damme K, (2008b) The effects of supplemental guanidino acetic acid as a precursor of creatine in vegetable broiler diets on performance and carcass parameters. Poult Sci 87(Suppl 1): 72 (Abstr).

Van Pilsum JF, Stephens GC, Taylor D (1972) Distribution of creatine, guanidinoacetate and enzymes for their biosynthesis in the animal kingdom. Implications for phylogeny. Biochem J 126: 325-345.

Waguespack AM, Powell S, Bidner TD, Payne RL, Southern LL (2009) Effect of incremental levels of L-lysine and determination of the limiting amino acids in low crude protein corn-soybean meal diets for broilers. Poult Sci 88: 1216-1226.

Wyss M, Kaddurah-Daouk, R (20000 Creatine and creatinine metabolism. Physiol Rev 80: 1107-1213.