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Carcass Traits and Immune Response of Broiler Chickens Fed Dietary L-Carnitine, Coenzyme Q_{10} and Ractopamine

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ABSTRACT

This study was conducted to evaluate the effects of coenzyme Q_{10}, L-carnitine and ractopamine supplementation, alone and in combinations, on carcass traits and immune response of broiler chickens. Five hundred and twelve one-day-old Ross 308 male broiler chickens were randomly allocated into eight treatments with four replicates each. A 2×2×2 factorial arrangement was applied, with two levels of coenzyme Q_{10} (0 and 40 mg/kg), two levels of L-carnitine (0 and 200 mg/kg) and two levels of ractopamine (0 and 10 mg/kg). The birds were reared until day 42 of age under standard conditions. Blood samples were collected at the end of grower and finisher periods from the wing vein. Four birds per group were sacrificed at day 42 of age. Except for carcass yield, other carcass traits were not significantly affected ($p>0.05$) by different levels of coenzyme Q_{10}, L-carnitine, or ractopamine. Immune response parameters were significantly ($p<0.05$) different between the treatments. The lowest antibody titers against Newcastle disease virus and relative spleen weight were observed in control group. The results of this study suggest that addition of coenzyme Q_{10} and L-carnitine to broiler diets has benefit effect on immune response of broiler chickens.

INTRODUCTION

Physiological additives aid the normal development of physiological functions (Hassan *et al.,* 2011). As early as 1963, beta-adrenergic agonists have been used in broiler diets (Cunning, 1963). The dietary administration of the beta-adrenergic agonist ractopamine, a potent growth promoter, has been shown to increase protein accretion and decrease fat deposition in turkeys (Wellenreiter & Tonkinson, 1990). Various beta-adrenergic agonists have been shown to be capable of improving weight gain when added to the feed of domestic species. The feed additive ractopamine is a beta-adrenergic agonist licensed for use in cattle and swine diets by the US Food and Drug Administration in 2003. The positive effects of beta-adrenergic agonists on the performance of meat-producing animals, including poultry, are well documented in several experiments (Yousefi *et al.,* 2011).

Coenzyme Q (2,3-dimethoxy, 5-methyl, 6-polyisoprene parabenzoquinone) is present in all membranes of cell. Ubiquinone or coenzyme Q_{10} is a vitamin – like substance which is found in small amounts in a wide variety of foods and is synthesized in all tissues. Ubiquinone is the coenzyme of at least three mitochondrial enzymes (complexes 1, 2 and 3). Mitochondrial enzymes of the oxidative phosphorylation pathway are essential for the production of the high oxidative energy phosphate containing compound, adenosine-tri-phosphate (ATP), upon which all cellular function depend (Ahmad *et al.,* 2010). Coenzyme Q_{10} acts as a non-specific stimulant of the immune host defense system. Coenzyme

Q_{10} has been shown to protect experimental animals against tumor growth and to enhance the immunity to viruses. In a study with eight chronically-ill human patients, the administration of coenzyme Q_{10} at 60 mg/day was associated with significant increases in serum levels of immunoglobulin G after 27-98 day of treatment (Folkers, 1982). The results of a study showed that the dietary combination of coenzyme Q_{10} and L-carnitine reduce packed cell volume and ascites mortality in broiler (Asadi *et al.,* 2013).

The chemical structure of L-carnitine (ß-hydroxy γ-trimethyl amino butyrate) was elucidated in 1927. Carnitine was synthesized *in vivo* from lysine and methionine in the kidney and testes (rat), skeletal muscle (sheep), brain (man), and liver in all mammals. During carnitine synthesis, lysine provides the carbon chain and the nitrogen atom, while methionine provides the methyl groups (Arsalan, 2006). The major role of L-carnitine appears to be the transport of long-chain fatty acids into the mitochondria for ß-oxidation. In addition to its role in the oxidation of fatty acids, L-carnitine was shown to exhibit immunomodulatory effects. Mast *et al.* (2000) showed that dietary L-carnitine supplementation increased the production of antigen-specific immunoglobulin G in broiler chickens. This amine is present at high concentrations in the lymphocytes, where it is involved in apoptosis inhibition and in the proliferative response to mitogens (Desimone *et al.,* 1994).

The aim of this experiment was to investigate the effects of the dietary supplementation of coenzyme Q_{10}, L-carnitine and ractopamine, individually and in combination, on the carcass traits and immune parameters of broiler chickens.

MATERIALS AND METHODS

In this study, 521 one-day-old male Ross 308 broilers were obtained from a commercial hatchery. Birds were randomly distributed into eight treatment groups with four replicates of 16 chicks each. The experiment was conducted according to a completely randomized design in a 2×2×2 factorial arrangement, consisting of two levels of ractopamine (0, 10 mg/kg), two levels of coenzyme Q_{10} (0, 40 mg/kg), and two levels of L-carnitine (0, 200 mg/kg). Treatments included T_1: basal diet (control), T_2: basal diet+ ractopamine, T_3: basal diet +coenzyme Q_{10}, T_4: basal diet + L-carnitine, T_5: basal diet + ractopamine + coenzyme Q_{10}, T_6: basal diet + ractopamine + L-carnitine, T_7: basal diet +coenzyme Q_{10}+L-carnitine, and T_8: basal diet + ractopamine +coenzyme Q_{10}+L-carnitine.

The diets were based on corn and soybean meal, formulated according to the recommendations of the Ross 308 manual, and offered as mash. The feedstuffs and nutrient composition of the starter (days 1-10), grower (days 11-24), and finisher (days 25-42) are presented in Table 1.

Table 1 – Composition and nutrient levels of the starter, grower and finisher diets

Ingredients (%)	Starter (1-10)	Grower (11-24)	Finisher (25-42)
Corn grain	52.28	51.80	59.00
Soybean meal (44% CP)	36.37	34.90	28.20
Soybean oil	3.90	5.07	4.60
Fish meal	3.07	5.00	5.00
Di-calcium phosphate	2.09	1.19	1.10
Oyster shell	0.77	0.89	0.91
Vitamin/mineral premix*	0.60	0.50	0.50
Dl- methionine	0.33	0.24	0.22
L-Lysine	0.21	0.11	0.13
Salt	0.20	0.17	0.20
$NaHCO_3$	0.11	0.12	0.10
L- Threonine	0.07	0.01	0.04
Calculated nutrient contents (%)			
ME (kcal/kg)	3025	3150	3198
Crude protein	22.4	22.4	20.0
Calcium	1.00	0.90	0.87
Available phosphorus	0.50	0.45	0.43
Methionine	0.68	0.57	0.52
Methionine+cystine	1.03	0.95	0.87
Lysine	1.41	1.34	1.20
Threonine	0.94	0.88	0.82

*Supplied per kg of diet:
Vitamin A 12000IU, Vitamin E 10mg, Vitamin D 2200IU, niacin 35mg, D-pantothenic acid 12mg, riboflavin 3.63mg, pyridoxine 3.5mg, thiamine 2.4mg, folic acid 1.4mg, biotin 0.15mg, Vitamin B 0.03mg, Manganese 60mg, Zinc 40mg, Iron 1280mg, Copper 8mg, Iodine 0.3mg, Selenium 0.2mg

Birds were housed in an environmentally-controlled room. House temperature was initially maintained at 32 ˚C and then gradually reduced to 23-24 ˚C for the remaining of the experimental period. Air relative humidity was maintained at 65%. A continuous lighting program was applied for the first three days, after which a 23-h light: 1-h dark cycle was provided. Feed and fresh water were available *ad libitum*.

Feed was withdrawn three hours before slaughter. At the end of experiment (6 weeks), one bird per pen, which body weight was nearest to the average in the pen, was sacrificed by severing the jugular vein Carcass and edible parts, including heart and liver, yields were calculated relative to body weight at sacrifice.

Birds were individually vaccinated using a commercial Newcastle disease virus (NDV) vaccine on days 8 (B_1) and 18 (LaSota). Blood samples of one bird per replicate were collected from the wing vein at days 24 and 42 of

age. Sera were separated and stored at -20 ˚C before analysis. Antibody titers against Newcastle disease were measured by hemagglutination-inhibition test (Allan and Gough, 1974). Antibody titer values were $\log_2(x)$ transformed before statistical analysis.

Blood samples were collected from the wing vein in sterile test tubes containing anticoagulant (sodium citrate) at the end of experimental period to determine white blood cell count (WBC) and the absolute numbers of each leukocyte type.

Statistical analysis was performed using SPSS 20 for Windows. Firstly, the normality of data distribution was verified using the Kolmogorov-Smirnov test. Data were subjected to analysis of variance using the GLM (general linear model) procedure, means were compared by Duncan's Multiple Range test. Statistical significance was accepted when $p<0.05$.

RESULTS AND DISCUSSION

The effect of coenzyme $Q_{10,}$ ractopamine, and L-carnitine on carcass traits are presented in Table 2. As shown in Table 2, the dietary supplementation of these compounds had no effect on breast yield, thigh yield, or heart and liver percentages. This finding was in agreement with report of Farhangfar *et al.* (2010), who did not detect any effect of coenzyme Q_{10} at level of 20mg/kg of feed on carcass, thighs, or breast yields. Honda *et al.* (2010) also showed that liver weight was not influenced by coenzyme Q_{10} supplementation. In agreement with our findings, Akbariazad *et al.* (2010) observed that the use of L-carnitine in broiler chicks had no significant effects on carcass traits. In contrast, Kidd *et al.* (2009) verified a significant increase in the leg yield of broilers fed L-carnitine. These differences probably are related to the level of L-carnitine supplementation, broiler sex, and composition of experimental diet.

Among the eight treatments, the control broilers presented the lowest carcass yield, which was significantly relative to treatment 5 (ractopamine + coenzyme Q_{10}), treatment 6 (ractopamine+ L-carnitine), and treatment 8 (ractopamine + coenzyme Q_{10}+ L-carnitine). The higher carcass observed in the present study is consistent with the findings of Moslemipur *et al.* (2012), with the addition of terbutaline, a beta-adrenergic antagonist to broiler diets. Differences in broiler sex, type and level of beta-adrenergic agonist, and diet compositions may explain the differences between the results obtained in our experiment and in other studies.

Newcastle antibody titers determined at days 24 and 42 of age, white blood cell (WBC) counts, spleen relative weight, and heterophil to lymphocyte ratio are summarized in Table 3. The results showed that WBC counts were not affected by ractopamine, coenzyme Q_{10} or L-carnitine supplementation. These results are in agreement with an earlier report by Akbariazad *et al.* (2010), who showed that the dietary inclusion of L-carnitine at levels of 125 and 250 ppm had no significant effect on the WBC count of broilers. On the other hand, Karadeniz *et al.* (2008) found an

Table 2 – Effects of supplemental dietary ractopamine, coenzymeQ_{10} and L-carnitine on carcass traits of broiler chickens. (mean ± standard deviation)

Parameter			Carcass (%)	Thigh (%)	Breast (%)	Heart (%)	Liver (%)
Ractopamine (mg/kg)							
0			73.4±0.9[b]	29.4±0.9	35.1±1.2	0.57±0.09	2±0.25
10			74.4±1.1[a]	29.9±0.9	34.3±1.2	0.6±0.06	2±0.18
Coenzyme Q_{10}(mg/kg)							
0			73.6±1.2	29.6±0.8	34.7±1.3	0.57±0.08	1.95±0.17
40			74.2±1.07	29.7±1	34.7±1.2	0.61±0.07	2.1±0.23
L-caraitine (mg/kg)							
0			73.8±1.19	29.4±0.9	34.4±1.16	0.58±0.07	2.1±0.23
200			74.1±1.2	29.9±0.9	35±1.25	0.6±0.09	2±0.2
RXQ10XL-C							
0	0	0	72.8±0.5[b]	29.5±0.4	35.03±0.4	0.56±0.08	1.97±0.18
10	0	0	74.1±1.3[ab]	29.8±0.3	33.4±1.1	0.55±0.03	1.99±0.6
0	40	0	73.6±1.4[ab]	29.9±0.9	34.6±1.6	0.6±0.09	2.12±0.33
0	0	200	73.4±0.75[ab]	29.1±1	35.3±0.9	0.54±0.12	1.9±0.29
10	40	0	74.7±0.61[a]	29.8±1.5	34.2±0.25	0.6±0.07	2.19±0.26
10	0	200	74.2±1.8[a]	30.2±1	34.8±1.7	0.63±0.05	1.94±0.1
0	40	200	73.7±1.06[ab]	29.3±0.5	35.1±1.8	0.58±0.08	2.11±0.2
10	40	200	74.7±0.8[a]	29.6±0.9	35±0.96	0.63±0.08	2±0.16

Values with different superscripts in the same column for each section are significantly different ($p<0.05$).

Table 3 – Effects of supplemental dietary ractopamine, coenzymeQ_{10} and L-carnitine on immune response of broiler chickens. (mean ± standard deviation)

Parameter			WBC[1] (x/µl)	Heterophil/ Lymphocyte	Newcastle titer (day 24) Log 2	Newcastle titer (day 42) log 2	Spleen relative weight (%)
Ractopamine (mg/kg)							
0			26350±4026	0.44±0.06	2.61±0.41	2.3±0.46	0.121±0.029
10			26356±3048	0.46±0.08	2.57±0.2	2.2±0.35	0.13±0.018
Coenzyme Q_{10}(mg/kg)							
0			27244±3258	0.44±0.05	2.47±0.32^{b}	2.13±0.4	0.119±0.024
40			25462±3632	0.46±0.08	2.71±0.27^{a}	2.37±0.38	0.133±0.022
L-caraitine (mg/kg)							
0			26256±2474	0.47±0.07	2.5±0.37	2.12±0.42	0.119±0.024
200			26450±4400	0.43±0.06	2.67±0.23	2.38±0.36	0.133±0.022
RXQ10XL-C							
0	0	0	27125±3492	0.42±0.02^{b}	2.13±0.37^{b}	1.87±0.36^{b}	0.09±0.014^{b}
10	0	0	25775±1300	0.42±0.07^{b}	2.45±0.15^{ab}	1.89±0.21^{b}	0.13±0.018^{a}
0	40	0	26275±1676	0.49±0.05^{ab}	2.87±0.37^{a}	2.58±0.5^{a}	0.125±0.026^{a}
0	0	200	26550±5026	0.44±0.07^{b}	2.73±0.29^{a}	2.4±0.4^{ab}	0.125±0.023^{a}
10	40	0	25850±3518	0.55±0.06^{a}	2.57±0.19^{a}	2.16±0.18^{ab}	0.132±0.017^{a}
10	0	200	29525±1857	0.48±0.02^{ab}	2.57±0.19^{a}	2.36±0.33^{ab}	0.132±0.02^{a}
0	40	200	25450±6231	0.41±0.08^{b}	2.74±0.2^{a}	2.36±0.33^{ab}	0.147±0.02^{a}
10	40	200	24275±2995	0.4±0.05^{b}	2.67±0.3^{a}	2.4±0.4^{ab}	0.127±0.02^{a}

Values with different superscripts in the same column for each section are significantly different ($p<0.05$). [1]WBC, White blood cell.

increase in WBC counts when adding L-carnitine to broiler diets. These differences are probably due to the levels of L-carnitine supplementation, broiler sex, and composition of the experimental diets. Results of Table3 indicate that the spleen relative weight of control broilers was significantly lower than that of the other groups ($p<0.05$). The spleen is a secondary lymphoid organ that filters bloodborne antigens, and it is a major site of immune response. These results agree with those of Hassan *et al.* (2011), who obtained higher spleen relative weight when adding 100 mg L-carnitine/kg diet compared with the control group.

Antibody titers against Newcastle disease at day 24 of age were significantly higher when coenzyme Q_{10} was added to the diet ($p<0.05$). Tanner (1992) observed that coenzyme Q_{10} protected animals against tumor growth and enhanced their immunity against viruses. Many experiments indicated that coenzyme Q is an important component for the optimal function of immune system (Lookwood *et al.,* 1995). Among the eight treatment groups, the control group presented the lowest antibody titers against Newcastle. These results indicate that use of L-carnitine had positive effect on the immune response. Similar results were obtained by Akabariazad *et al.* (2010), who reported that antibody titers against Newcastle disease measured in 350-day-old broilers significantly increased by dietary L-carnitine supplementation. Mast *et al.* (2000) also found that dietary L-carnitine supplementation (100 mg/kg) appeared to be beneficial in enhancing specific humoral response in broiler chickens. Geng *et al.* (2007) also demonstrated that serum IgG titers increased when L-carnitine was individually supplemented, and that lysozyme activity increased when L-carnitine and Coenzyme Q_{10} were added together to broiler diets. In contrast, Kheirkah *et al.* (2009) did not find any differences in anti-NDV or anti-SRBC titers in broiler chickens fed with diets supplemented with L-carnitine at 100 mg/kg and 200 mg/kg. This difference probably is related to broiler genetic strain, day of blood collection, and composition of experimental diets.

The results of the present study suggest that dietary supplementation of coenzyme Q_{10} individually or combined with L-carnitine has positive effects on the humoral immune response of broilers. In addition, dietary ractopamine supplementation significantly improved carcass yield.

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