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The effects of different levels of L-Carnitine and methionine on performance and blood metabolites in female broiler

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Abstract

The present study was conducted to determine the effects of different levels of L-Carnitine (Lohman 20% Carnitine) and methionine on female broiler chickens in a completely randomized experimental design with 12 treatments and 3 replicates in 36 pen with three levels of L-Carnitine (0, 75 and 150 mg.kg) and four levels of methionine (85, 100, 115 and 130 NRC%). At 42 d, 2 chickens from each replicate were randomly taken for measurements of body weight, serum triglyceride (TG), cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The highest body weight ($P<0.05$) was found at the level of 115% methionine + 150 mg/kg L-Carnitine with beneficial effect on serum Glu, TG, AST and ALP. In conclusion, we opined that the best combination was 115% methionine and 150 mg/kg L-Carnitine for optimum production in broiler.

Keywords: L-Carnitine; methionine; aspartate aminotransferase; glucose; triglyceride

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Introduction

L-Carnitine ($C_7H_{15}NO_3$) is present in both plasma and tissue as free Carnitine, or bound to fatty acids as acyl Carnitine derivatives (Bieber, 1988). It is a water soluble zwitterionic compound (161.2 MR). Its fundamental role in some aspect such as health and disease remain to be fully understood (Mast et al., 2000). Only the L-form of Carnitine is biologically active and occurs in nature and it has pharmacological and nutritional properties (Mardones et al., 1999). The first convincing evidence for Carnitine biosynthesis in animals was obtained from chick embryos, which contained significant amounts of Carnitine, however, it was not found in eggs (Bremer, 1983). Endogenous biosynthesis (in the kidney, liver and brain) occurs in small amounts, but appears sufficient to cover normal requirements. However, this is not the case in neonates (Rebouche, 1992; Keralapurath et al., 2010), where birds are under conditions of stress, higher performance and diets rich in fat (Rebouche, 1992; Rabie and Szilagyi, 1998). Two essential amino acids (lysine and methionine), three vitamins (ascorbate, niacin in the form of nicotin amide adenine dinucleotide and vitamin B6), and reduced iron (Fe^{2+}) are required as cofactors for the enzymes involved in the metabolic pathway of L-Carnitine synthesis (Borum, 1983; Rebouche, 1992). Reports of nutritional L-Carnitine deficiency are rare (Harpaz, 2005), and accumulation of toxic acyl-coenzyme (CoA) metabolites in mitochondria due to L-Carnitine deficiency impairs the citrate cycle, gluconeogenesis and fatty acid oxidation (Harpaz, 2005). L-Carnitine regulates cell membrane, enhances immunity and has metabolic role (Arslan and Citi, 2003). L-Carnitine synthesis occurs in liver, kidney and brain by combination of essential amino acids methionine and lysine. L-Carnitine transports long-chain fatty acid (LCFAs) to inner mitochondrial membrane and controls beta-oxidation of LCFAs (Bhargava and Sunde, 1971). L-Carnitine and its esters prevent toxic accumulation of FAs and acyl-coA among acetyl-coA production, therefore, FAs, especially in the

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liver, shift to triacylglycerol synthesis (AOAC, 1995). L-Carnitine affects energy production (Brouns and Vusse, 1998), increases fat metabolism and improves cell energy balance (Mast et al., 2000). Furthermore, L-Carnitine (esters) removes free radicals (Mast et al., 2000). L-Carnitine supplementation of diet results in more effective energy production through consumption of fat (Bell et al., 1992). Theoretically, an exogenous Carnitine supply can decrease the need for biosynthesis of L-Carnitine from methionine and lysine in poultry nutrition (La Count et al., 1995). An increased supply of Carnitine has been shown to spare branched-chain amino acids from oxidation in tissues (Owen et al., 1996).

Methionine is the first limiting essential amino acid in maize and soybean-based diets (Fancher and Jensen, 1989) for poultry. Chickens are unable to synthesize methionine in amounts necessary to sustain life and growth. Usually, large amounts of vegetable protein supplements are used in feeds, with low levels of animal and fish as protein source (North and Bell, 1990). Hence, it is necessary to add methionine to meet the bird's requirement, whether synthetic or herbal source.

Methionine and threonine are regarded as to be the first and third limiting amino acids in broilers fed practical corn-soybean meal diets (Ojano and Waldroup, 2002). Optimal levels of these amino acids are needed to support optimum growth and health of broilers. Nutrient requirement standards have been reported by the National Research Council (NRC, 1994) and through more than 25 years due to genetic selection, management practice and feed formulation changes birds have more rapid growth and better performance than previous years (Havenstein et al., 1994; Williams et al., 2000). On the other hand, NRC recommendation are usually based on the needs of healthy birds under ideal condition, but birds in commercial systems are normally exposed to different kinds of stresses, diseases and also the combination of environmental condition. Dietary levels of certain individual amino acids have been shown to affect immune response (Jeevanandam et al., 1990). Methionine is an amino acid that has proven immune regulatory action (Shini et al., 2005). There is some evidence that essential amino acid level in the feed higher than of NRC specifications needed to achieve optimal immune system and growth performances (Quentin et al., 2005). Increased AST, ALT and ALP activities are index of liver damage, therefore, their normal levels in serum is an indicator of healthy liver (Arslan and Citi, 2003). The aim of present research was to evaluate the effects of different levels of L-Carnitine plus methionine on body weight and some blood metabolites and liver enzymes on female broiler chickens for 42 days (starter and grower period) of their life.

Materials and Methods

A total 720 one day old Ross 308 female broiler were randomly distributed in a completely randomized experimental design with 12 treatments and 3 replicates in 36 pen with 3 L-Carnitine levels (0, 75 and 150 mg/kg) and 4 methionine levels (85, 100, 115 and 130 NRC %). Feeding and drinking were provided *ad libitum*. Experimental diets were prepared based on National Research Council (NRC) recommendations (Table 1). All diets were equivalent in energy and protein contents. At 42 days, 2 chickens from each replicate selected randomly for measurements of body weight, serum triglyceride (TG), cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL), glucose (GLU), Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). On day 42, two birds from each replicate were chosen at random and blood samples (4.0 ml) were collected from the brachial vein. Blood samples were collected from wing veins in test tubes and after stable clotting, centrifuged for separating serum samples. Serum was separated by centrifugation at 3000 g for 10 min and stored at -20°C until analysis. Serum Alkaline Phosphatase (ALP), Alanine Aminotransferase (SGPT or ALT), Aspartate Aminotransferase (SGOT or AST) were measured by specific commercial kits (Roche Diagnostica, Basel, Switzerland) using an auto analyzer (HITACHI 902 automatic auto-analyzer). All data was analyzed by SPSS (2011) software. Mean values were compared by Duncan multiple range test for significance. P value less than 0.05 was considered as significant.

Results

The results of body weight, GLU, TG, CHOL, LDL, HDL, AST, ALT and ALP are shown in Table 2. Significantly high body weight and GLU was found in 115% NRC + 150 mg/kg L-Carnitine and 130% NRC + 0 mg/kg L-Carnitine respectively. Highest TG and CHOL was found in birds fed 85% NRC + 0 mg//kg L-Carnitine and 130% NRC + 0 mg/kg L-Carnitine respectively. Highest LDL, HDL and AST were observed in group of birds treated with 130% NRC + 150 mg/kg L-Carnitine. Similarly, highest ALT and ALP was observed in birds fed 130% NRC + 0 mg/kg L-Carnitine.

Discussion

Previous reports found that there was no influence of L-Carnitine supplementation alone on live weight, feed consumption or feed conversion ratio in broilers (Kidd et al., 2009; Keralapurath et al., 2010). Based on our results, supplementation of broilers by 115% NRC methionine plus 150 mg/kg diet L-Carnitine increased body weight. The improvements in body weight gain of

Table1: Composition and proximate analysis of the starter and grower diets

Ingredient (%)	1	2	3	4	5	6	7	8	9	10	11	12
Corn	56.73	56.73	56.73	56.73	56.73	56.73	56.73	56.73	56.73	56.73	56.73	56.73
Soybean meal	30.38	30.34	30.20	30.30	30.26	30.22	30.22	30.18	30.15	30.15	30.11	30.07
Soybean oil	3	3	3	3	3	3	3	3	3	3	3	3
Gluten meal	6.31	6.31	6.31	6.31	6.31	6.31	6.31	6.31	6.31	6.31	6.31	6.31
Oyster shells	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88
Dical. Phosphorous	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Common Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
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
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
DL-Methionine	0.00	0.00	0.00	0.08	0.08	0.08	0.154	0.154	0.154	0.230	0.230	0.230
L- Carnitine*	0.00	0.038	0.075	0.00	0.038	0.075	0.00	0.038	0.075	0.00	0.038	0.075
Nutrient analysis												
Metabolizable Energy (Kcal.kg)	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125
Protein (%)	22.44	22.44	22.44	22.44	22.44	22.44	22.44	22.44	22.44	22.44	22.44	22.44
Ether Extract (%)	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62
Linoleic Aid(%)	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19
Calcium (%)	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Total phosphorus (%)	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Lysine (%)	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Methionine (%)	0.426	0.426	0.426	0.504	0.504	0.504	0.576	0.576	0.576	0.650	0.650	0.650

*supplement compositions: vit.A, 6600 IU, vit.D, 800IU, thiamin 10 mg, riboflavin 10 mg, pyridoxine 10 mg, cyanocobalamin 0.1 mg, vit.E 10 mg, biotin 1 mg, vit.K 1mg, niacin 100 mg, folic acid 1 mg, pentotenic acid 20 mg, choline chloride 450 mg, manganase 100 mg, ferrous 65 mg, zinc 65 mg, iodine 0.8 mg, cobalt 0.4 mg, selenium 0.25 mg, copper 15 mg;*L-carnitin: 20% carnitine, calcium carbonate

broilers observed may be due to added dietary L-Carnitine may be attributable to an improved utilization of diet, achieved through more efficient fat oxidation by L-Carnitine (Cyr et al., 1991). It is known that insulin-like growth factors (IGFs) stimulates growth rate in a number of animal species (Beccavin et al., 2001). Kita et al. (2002) found that when dietary L-Carnitine concentration increased from 0 to 1000 mg/kg in an adequate protein diet (200 g/kg), plasma IGF-I concentrations increased to stimulate body weight gain (Kita et al., 2005). The glucose lowering effect of L-Carnitine as seen in present study may be due to stimulation of IGF-I effect. There are reports indicating that L-Carnitine is a blood lipid lowering drug (hypolipidemic) which could decrease serum CHOL, TG, FFAs, phospholipids (Bell et al., 1992). These reports are partially in agreement with our results. Plasma fat lowering effects of L-Carnitine may be related to several processes including enhancing cholesterol turnover due to increased conversion of cholesterol to cholic acids and removing it via bile secretion or redistribution of modified cholesterol in the body, although the mechanism is unknown (Bell et al., 1992). L-Carnitine supplementation via drinking water at 100 and 200 mg/l in gooses and ducks had no significant effects on improvement of growth performance (body weight increase and food intake), and carcass traits (abdominal fat), serum cholesterol, fat, TG and glucose. Nevertheless, under L-Carnitine levels, abdominal fat composition changed in both animals (Arslan and Citi, 2004). These reports are partially in agreement with our results. Fasuyl and Alertor (2005) reported that better performance can still be obtained with adequate supplementation of essential amino acids especially methionine which has been identified to be in marginal quantities in most poultry diets. An improvement in broiler performance when methionine was added to a corn-soybean diet has been reported (Hesabi et al., 2006).

As a result, the serum fat lowering role of L-Carnitine in female broiler chickens has been emphasized in present study. Also, decrease of serum glucose level indicates the regulation role of L-Carnitine in broiler chickens. Decreased AST and ALP activity could be due to free radical scavenge effects of L-Carnitine which results in preventing liver damage. Mode of antioxidant effect of L-Carnitine can be summarized as following: L-Carnitine prevents oxidative stress and regulates nitric oxide, the cellular respiration (Brown, 1999) and the activity of enzymes involved in defence against oxidative damage (Kremser et al., 1995). L-Carnitine acts as an antioxidant in the protection of glutathione peroxidase, catalase and superoxide dismutase enzymes from further peroxidative damage (Kalaiselvi and Panneerselvam, 1998). L-Carnitine may have functions associated with scavenging of free radicals in cellular sites (Kalaiselvi and Panneerselvam, 1998; Citil et al., 2005). This result

Table 2: Effects L-Carnitine and methionine on body weight, some blood metabolites and liver enzymes of female broiler chickens at 42 day of age

	Body Weight (g)	GLU (mg/dl)	TG (g/dl)	CHOL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AST (u/l)	ALT (u/l)	ALP (u/l)
85% NRC + 0 mg//kg	1810.0^{f}	286.67^{abc}	235.67^{a}	97.33^{ab}	7.67^{ef}	45.67^{ef}	217.33^{de}	1.60^{cde}	6.66^{bcd}
85% NRC + 75 mg//kg	2221.7^{de}	303.67^{ab}	171.67^{b}	108.00^{bcd}	6.67^{r}	45.33^{f}	212.33^{def}	1.61^{cd}	8.02^{abc}
85% NRC + 150 mg/kg	2326.7^{cde}	271.67^{bcd}	163.00^{bc}	102.33^{cde}	10.00^{cde}	60.00^{de}	206.00^{ef}	1.87^{b}	4.84^{e}
100% NRC + 0 mg/kg	2425.0^{bc}	253.67^{bc}	150.67^{bcd}	117.00^{ab}	11.00^{cd}	61.67^{de}	311.00^{a}	1.59^{ab}	6.75^{bc}
100% NRC + 75 mg/kg	1953.3^{ef}	260.33^{de}	138.00^{de}	104.00^{cd}	12.67^{bc}	52.33^{cde}	254.00^{bc}	1.87^{b}	5.62^{def}
100% NRC + 150 mg/kg	2180.0^{def}	277.67^{bcd}	110.00^{ef}	90.33^{e}	11.33^{cd}	53.33^{cd}	205.00^{f}	1.60^{b}	6.15^{de}
115% NRC + 0 mg/kg	2566.7^{ab}	276.00^{cd}	113.00^{ef}	109.67^{bc}	9.67^{de}	55.33^{bcd}	281.67^{bc}	1.87^{b}	6.49^{cde}
115% NRC + 75 mg/kg	2131.7^{e}	284.33^{bc}	208.67^{ab}	120.33^{b}	8.68^{e}	61.33^{de}	223.67^{cde}	1.60^{cde}	6.57^{cd}
115% NRC + 150 mg/kg	2646.7^{a}	250.67^{f}	107.33^{f}	104.00^{cd}	14.67^{b}	57.67^{bc}	198.00^{g}	1.58^{e}	3.03^{f}
130% NRC + 0 mg/kg	2330.0^{cd}	303.68^{a}	165.00^{b}	124.67^{a}	15.33^{ab}	61.67^{ab}	253.33^{cd}	2.33^{a}	12.64^{a}
130% NRC + 75 mg/kg	2341.7^{bcd}	263.33^{cde}	142.33^{cde}	109.33^{bc}	6.67^{de}	55.33^{bc}	257.33^{bcd}	1.63^{c}	4.28^{ab}
130% NRC + 150 mg/kg	2243.3^{bc}	284.67^{bc}	145.00^{cd}	113.33^{abc}	20.67^{a}	73.00^{a}	291.00^{a}	2.00^{b}	11.99^{ab}
P value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
SEM	17.39	1.83	0.91	1.09	0.71	1.26	1.99	0.13	79.23

$^{a–f}$ Means followed by different superscript are significantly different (P<0.05)

emphasizes the protective role of L-Carnitine in chickens (and probably in other animals and human) especially in liver.

Glucose under 130% NRC methionine and 0 mg/kg diet L-Carnitine increased significantly. It indicates that methionine alone has no regulating effect on serum glucose. Serum TG at 115% NRC methionine plus 150 mg/kg diet L-Carnitine significantly decreased which indicates improving energy metabolism and prevention of liver damage. Since L-Carnitine is a relatively expensive feed supplement, the economic aspect and cost effectiveness must be considered if it is to be routinely applied in certain poultry applications. L-Carnitine is available in some countries as an oral preparation of a commercialized form. From a scientific standpoint, L-Carnitine can be used in broiler diets, at inclusion levels up to 50-200 mg/kg, to support its positive effects (Rabie and Szilagyi, 1998; Golzar Adabi et al., 2006).

Conclusion

Based on results of the present study, supplementation at the level of 115% NRC methionine (0.65%) plus 150 mg/kg L-Carnitine improved body weight and blood metabolites.

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