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EVALUATION OF *CPT-1* GENE EXPRESSION WITH TREATMENT DIET OF L-CARNITINE ON BROILER CHICKS USING REAL-TIME PCR ASSAY

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Abstract

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Body fat of broiler chickens is one of the major concerns in poultry industry. Carnitine palmitoyltransferase I (CPT-1) also known as carnitine is a rate-limiting enzyme of fatty acid oxidation in animals. The objective of the present study was to evaluation of L-Carnitine diet on *CPT-1* gene expression of broiler chicks using Real-time PCR assay. 675 Ross male broiler chicks were randomly distributed into 45 pens and feeding with diets contain three levels of L-carnitine (0, 60 and 120 mg/kg^{-1}). At the end of day 45 four chickens were selected randomly and tissue samples (liver, pectoral and thigh) were obtained from each bird. Total RNA was isolated from the tissue samples and reverse transcribed to cDNA using kit. After cDNA synthesis, *CPT-1* gene expression in liver tissue, pectoral, and thigh muscle of broiler chicks were evaluated using Real-time PCR assay. The results of this study showed that liver had maximum expression of *CPT-1* gene (2.09) in diet with 60 mg/kg^{-1} L-carnitine. In liver, pectoral and thigh tissues the diet contain 0 mg/kg^{-1} of L-carnitine show not any changes in activity of *CPT-1* gene and 120 mg/kg^{-1} of L-carnitine had a low range of *CPT-1* gene expression compared with 60 mg/kg^{-1} (P values <0.05). These findings indicated that the diet contains 60 mg/kg^{-1} of L-carnitine is useful for decrease of adipose and increase of protein content of the **muscle** tissues in broiler chicks.

Key words: L-carnitine, *CPT-1* gene, Broiler chicks, Real-time PCR

Abbreviations: CPT - Carnitine palmitoyltransferase

Introduction

The broiler chicken industry is a main source of animal protein and body fat of broiler chicks are one of the major concerns in chicken industry (Mersmann, 2002). The supply of such aminos, like L-carnitine, must be supplemented exogenously. L-Carnitine (Carnitor) is an amino acid, which plays a vital role in the metabolism of fat. It functions as a transporter of fatty acids into the mitochondria, where they are oxidized and converted to energy (Ji et al., 1996; Harpaz, 2005). L-carnitine is available in diary products, red meat, avocado, and tempeh. L-carnitine is synthesized in the kidneys and liver, from two essential amino acids, lysine and methionine. This synthesis requires the presence of vitamins B6, niacin, and iron (Ozório et al., 2001; Xu et al., 2003).

After birth, a dramatic increase in fatty acid oxidation occurs in the heart, which has been attributed to an increase in L-carnitine levels and a switch from the liver (L) to muscle (M) isoform of carnitine palmitoyltransferase CPT-1 (Onay-Besikci et al., 2003). CPT-1 is located on the outer membrane of the mitochondria, catalyzes the transfer of the acyl group from acylcoenzyme-A complexes to carnitine, producing acylcarnitine. Acylcarnitine is then transported to the inner mitochondrial membrane by a second protein, carnitine acylcarnitine translocase. A second carnitine palmitoyltransferase (CPT-2) reverses the transacylation reaction on the inner mitochondrial membrane and regenerates acyl-CoA (Skiba-Cassy et al., 2007).

In mammalian and animal species, two different kinds of isoforms of CPT-1 have been described. They vary in tissues

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(such as liver, pancreatic, muscle, and kidney), and distribution and enzymatic properties. The liver isoform (L-CPT-1 or CPT-1A or α) is expressed ubiquitously but at higher levels in the liver, kidneys, pancreatic islets, intestine and brain. By contrast, the muscle isoform (M-CPT-1 or CPT-1B or β) is restricted to skeletal muscles, heart, adipose tissue and the testis (Brown et al., 1997).

In humans, two different genes residing on separate chromosomes (11q13 and 22q13.3, respectively) encode liver and muscle CPT-1 (Britton et al., 1997). The human *L-CPT-1* gene is constituted of 19 exons ranging from 62 to 195 bp in size with 18 introns varying from 0.5 to 8.9 kb in size (Gobin et al., 2002). The human *M-CPT-1* consists of two 5′ non-coding exons (exons 1A and 1B), 18 coding exons and 1 3′ non-coding exon spanning approximately 10 kb (Yamazaki et al., 1997). The size of the M-CPT-1 transcript (~ 3 kb) is smaller than that of L-CPT-1 (~ 4.7 kb). Human L-CPT-1 and M-CPT-1 genes encode 88.4 and 87.8 kDa proteins, respectively, exhibiting only 63% amino acid identity (Yamazaki et al., 1996).

In mammals such as broiler chicks, both L- and M-CPT-1 genes are subject to dietary and hormonal regulation in tissues highly concerned by fatty acid β-oxidation such as liver, muscle and heart. In adult liver, the level of fatty acid β-oxidation is mainly regulated at the level of *CPT-1* gene expression. Stimuli like fat feeding, fasting, diabetes or treatment with peroxisomal or mitochondrial proliferating agents increase both mRNA expression and activity of CPT-1 without affecting CPT-2 (Britton et al., 1997; Louet et al., 2001).

In broiler chicks, mitochondrial CPT-1 activity has been characterized mostly in the liver and rarely in muscle (Blomstrand et al., 1983; Ishii et al., 1985; Lien and Horng, 2001). The purpose of present study was to evaluation of *CPT-1* gene expression with treatment diet of L-Carnitine on broiler chicks using Real-time PCR assay.

Materials and Methods

Collection of samples and diet

In present study, 675 Ross male broiler chicks were randomly distributed into 45 pens, allocated to nine dietary treatment groups with 5 replicates for each treatment. The trail was conducted using 3 × 3 factorial arrangement for treatments (three replicates per treatment) in a completely randomized design with three levels of L-carnitine as the main effect. Diets were formulated to contain three levels of L-carnitine (0, 60 and 120 mg/kg^{-1}). The ingredients and composition of the experimental diets are shown in Table 1.

At the end of experiment (day 42), from each replicate four chickens were selected randomly and blood samples was taken from jugular vein. Serum was separated (centrifuged 10 min; 5000 g) and stored at -20°C before analysis.

RNA extraction and cDNA synthesis

Total RNA was isolated from the liver, pectoral, and thigh tissues using a Qiagen RNA extraction kit (Qiagen, Ltd., Crawley, UK). Extracted RNA was immediately used or stored at –70ºC until needed. The total isolated RNA was

Table 1
Ingredients and composition of the experimental diets

Diet	1	2	3	4	5	6	7	8	9
L-Carnitine, mg kg^{-1}	0	60	120	0	60	120	0	60	120
Ingredient, %									
Basal Portion[1]	99.959	99.959	99.959	99.959	99.959	99.959	99.959	99.959	99.959
L-carnitine premix[2]	0	0.015	0.03	0	0.015	0.03	0	0.015	0.03
Premix free L-canitine[3]	0.018	0.009	0	0.018	0.009	0	0.018	0.009	0
Sand	0.023	0.017	0.011	0.022	0.016	0.01	0.0225	0.0165	0.0105
Chemical analysis, %									
ME, kcal kg^{-1}	3100	3100	3100	3100	3100	3100	3100	3100	3100
CP, %	19.375	19.375	19.375	19.375	19.375	19.375	19.375	19.375	19.375
Ca, %	0.871	0.871	0.871	0.871	0.871	0.871	0.871	0.871	0.871
P, %	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339
Met + Cys, %	0.6975	0.6975	0.6975	0.6975	0.6975	0.6975	0.6975	0.6975	0.6975

[1] Basal portion contained (%); 64.67 corn, 25.74 soybean meal (CP: 44%), 4.21 fish meal (CP: 60.05), 2.737 soybean oil, 1.2 oyster shell, 0.51 dicalcium phosphate, 0.042 DL-methionine, 0.2 salt, 0.15 sodium bicarbonate, 0.25 mineral premix and 0.25 vitamin premix. [2]Content: 60% Lcarnitine L-tartrate (40% pure L-carnitine). [3]Content: lactose, starch and cellulose microcrystal.

measured at 260 nm optical density according to the method described by Sambrook and Russell (Sambrook and Russell, 2001). 5 µg of RNA was reverse transcribed to cDNA with a first strand cDNA synthesis kit (Fermentas, Germany) according to the manufacturer's protocols.

Real-time PCR assay

After cDNA synthesis, Real-time PCR was performed in 25 µL of Universal PCR Master Mix (Roche Applied Science, Indianapolis, IN, USA) using a RotorGene 6000 instrument (Corbett Research, Australia). A typical 25 µL reaction contained: 1:75000 dilution of SYBR Green I (Molecular Probes, USA), 10 mM Tris-HCl (pH 8.8), 150 mM KCl, 0.1% Triton X-100, 2-5 mM $MgCl_2$, 100 µM each dNTP, 0.5 µM concentration of each forward and reverse primer (BioNeer Corporation, South Korea), and 2.5 ng of template DNA. The reaction mixture was initially incubated for 10 min at 95°C. Reaction mixtures were incubated for an initial denaturation at 95°C for 10 min, followed by 45 thermal cycles. Each cycle consisted of 95°C for 20 s, 64°C for 20 s, and 72°C for 20 s.

Primers were designed according to the published sequence for *CPT-1* gene (accession number: DQ314726). The sequences of the sense and antisense primers were as follows: CPT-1-F: 5′-TGACGTCGATTTCTGCTGCTTC-3′ and CPT-1-R: 5′-CCACGTAGAGGCAGAAGAGGT-3′.

Statistical analysis

Analysis of data related to dietary treatment groups were performed using the SPSS version 17.0-computer software (SPSS, Chicago, IL). Also, comparison and influence of three levels of L-carnitine (0, 60 and 120 mg/kg^{-1}) diet on *CPT-1* gene expression in liver, pectoral and thigh tissues were examined by T test statistical analysis. P values <0.05 were considered significant.

Results

In present study three levels of L-carnitine (0, 60, and 120 mg/kg^{-1}) were used for feeding of broiler chicks and effects of these diets on *CPT-1* gene expression in liver tissue, pectoral, and thigh muscle evaluated by Real-time PCR technique. *CPT-1* gene activity in these tissues of broiler chicks after feeding by varies level of L-carnitine using Real-time PCR is shown in Figure 1.

The results of Real-time PCR showed that liver had maximum activity of *CPT-1* gene (2.09) in diet with 60 mg/kg^{-1} L-carnitine. In liver, pectoral and thigh tissues the diet contain 0 mg/kg^{-1} of L-carnitine show not any changes in expression of *CPT-1* gene and 120 mg/kg^{-1} of L-carnitine had a low level of *CPT-1* gene activity compared with 60 mg/kg^{-1} (P values <0.05).

Discussion

The physiological role of L-carnitine is to transport long-chain fatty acids across the mitochondrial membrane and to subsequently facilitate β-oxidation of long-chain fatty acids for energy production (Lien and Horng, 2001). In cells, excess of metabolic fuel is converted into fatty acids in cytosol and oxidized later in mitochondria to generate ATP and acetyl-CoA. In fatty acid synthesis, catalytic formation of malonyl-CoA (precursor for long-chain fatty acyl-CoA, LCFA-CoA) from acetyl-CoA by Acetyl-CoA carboxylase (ACC1) is the rate-limiting step. The translocation of LCFA-CoA from cytosol to mitochondria catalyzed by two carnitine palmitoyltransferases (CPT-1 & CPT-2) and regulated by ACC-2, is the rate-limiting step of mitochondrial fatty acid β –oxidation (Lettner et al., 1992).

In the present study, three levels of L-carnitine (0, 60 and 120 mg/kg^{-1}) were used for feeding of broiler chicks and effects of these diets on *CPT-1* gene expression in liver tissue, pectoral, and thigh muscle evaluated by Real-time PCR method. The findings of this study showed that maximum activity of CPT-1 gene in diet with 60 mg/kg^{-1} L-carnitine were in liver. In liver, pectoral and thigh tissues the diet contain 0 mg/kg^{-1} of L-carnitine show not any varies in expression of CPT-1 gene and 120 mg/kg^{-1} of L-carnitine had a low level of CPT-1 gene activity compared with 60 mg/kg^{-1}. According to the previous researches, L-carnitine can reduce body fat in pigs (Newton and Haydon, 1989; Kachura et al., 1995; Kaudo et al., 1995; Owen et al., 2001). Results indicated the effect of L-carnitine on chickens did not assign equal. Cart-

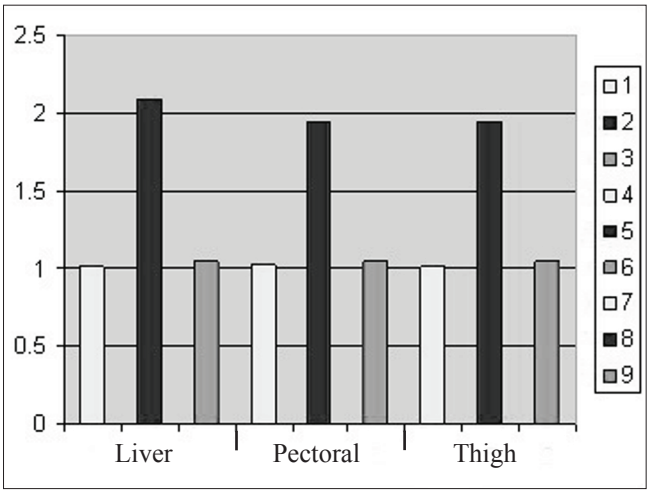

Fig. 1. *CPT-1* gene activity in liver tissue, pectoral, and thigh muscle of broiler chicks after feeding by three levels of L-carnitine (yellow: 0 mg/kg^{-1}, red: 60 mg/kg^{-1}, and blue: 120 mg/kg^{-1})

wright (1986) reported that L-carnitine did not have affected on abdominal fat in broiler chicks during 5 to 7 weeks of age (Cartwright, 1986). Moreover, Rabie et al. (1997), and Xu et al. (2003) found that L-carnitine increased breast muscle yield and leg meat yield and reduced abdominal fat content (Rabie et al., 1997; Xu et al., 2003).

The research of Perdomo et al. indicated that L-CPT-1 over-expression failed to decrease intracellular triacylglycerol, diacylglycerol, ceramide, or long-chain acyl-CoA (Perdomo et al., 2004). The study of Skiba-Cassy and coworkers showed that the regulation of fatty acid oxidation is probably not impaired in fat chicken. The absence of fasting stimulation of M-CPT-1 mRNA expression, which is at variance with the situation observed in mammals, suggests that during fasting, chicken muscles preferentially use ketone bodies as fuel, at least in the short term (Skiba-Cassy et al., 2007). The method of their study is same to the present research. The findings of de Barros Reis on increased L-CPT-1 activity and altered gene expression in pancreatic islets of malnourished adult rats showed that the activity of L-CPT-1 in pancreatic islets increased in the low protein (LP), although the L-CPT-1 mRNA levels were unaffected by malnutrition. The susceptibility of enzyme to inhibition by malonyl-CoA was unaltered and the content of malonyl-CoA was reduced in LP cells (de Barros Reis et al., 2008).

Conclusions

In conclusion, *CPT-1* gene was strongly expressed in liver **of** broiler chicks. The results showed that the diet with 60 mg/kg^{-1} of L-carnitine in liver and muscle tissues had maximum activity of *CPT-1* gene. These findings indicated that the diet contains 60 mg/kg^{-1} of L-carnitine is useful for increase of protein content of the muscle tissues and decrease of adipose tissue in these birds.

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