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Effects of dietary lipids and *Clostridium butyricum* on serum lipids and lipid-related gene expression in broiler chickens

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The effect of dietary lipids and Clostridium butyricum *supplementation on circulating lipids and the transcription of selected genes involved in lipid metabolism were investigated in broiler chickens. One hundred and sixty-day-old broilers (Arbor Acres) were divided into four treatment groups in a 2 × 2 factorial arrangement and fed four diets with two lipid sources (soybean oil or fish oil) and two levels of* C. butyricum *(0 or 5 g/kg) for a period of 6 weeks. Serum concentrations of total cholesterol and low-density lipoprotein cholesterol were lower ($P < 0.05$) in broilers fed diets containing fish oil than in those fed diets containing soybean oil. Fish oil significantly reduced ($P < 0.05$) hepatic fatty acid synthase and lipoprotein lipase (LPL) activities in abdominal fat. In contrast, fish oil significantly increased ($P < 0.05$) breast muscle LPL activity and hepatic peroxisome proliferator-activated receptor-α and LPL gene expression. The addition of* C. butyricum *significantly increased ($P < 0.05$) LPL activity in abdominal fat and liver-type fatty acid-binding protein gene expression in jejunal mucosa. The results of this study indicated that the reduced abdominal fat in broilers fed fish oil as observed may be due to augmented hepatic fatty acid catabolism and lower hepatic fat synthesis. The increased intramuscular fat content in breast muscle of broilers fed* C. butyricum *as described may be the result of enhanced fatty acid uptake.*

Keywords: *Clostridium butyricum*, fish oil, lipid-related genes expression, broiler chicken

Implications

Dietary lipid composition is an important determinant of poultry meat quality. Previous studies focused mainly on the fatty acid profile of meat rather than the mechanisms through which dietary manipulations change lipid deposition. This study shows that the increased intramuscular fat content in breast muscle of broilers fed *Clostridium butyricum* as described in Yang *et al.* (2010) may be associated with enhanced fatty acid uptake.

Introduction

Consumers are becoming increasingly aware of the nutritional quality and health benefits of the foods they consume. The poultry industry has made significant efforts to decrease the total amount of abdominal fat deposited in broiler chickens through genetics, management and nutrition. Studies in poultry nutrition have shown that the fatty acid profile of poultry meat can be modulated by dietary outcome (Hulan *et al.*, 1989; Fébel *et al.*, 2008; Ponte *et al.*, 2008). Previous studies focused mainly on the fatty acid profile of meat (dietary outcome) rather than the mechanisms through which dietary manipulations change lipid deposition. *Clostridium butyricum* is a typical butyric acid-producing, spore-forming, gram-positive anaerobe, which is found in soil and in the intestines of healthy humans and animals. This *C. butyricum* is a probiotic bacteria used clinically to prevent disturbances of gastrointestinal microbiota, to treat diarrhea, to enhance humoral immune response and to promote digestion in humans (Ito *et al.*, 1997; Seki *et al.*, 2003; Isono *et al.*, 2007). Our previous experiments demonstrated that fish oil diets reduced abdominal fat in broiler chickens, whereas *C. butyricum* supplementation increased intramuscular fat content in breast muscle (Yang *et al.*, 2010). However, the mechanisms for these effects remain unclear.

Liver is the main site of *de novo* fatty acid synthesis in avian species (O'Hea and Leveille, 1968). Hepatic lipogenesis and the export of lipid are crucial steps related to lipid metabolism in broilers (Hillgartner *et al.*, 1995; Kersten, 2001). It has been reported that some lipogenic genes, such as fatty acid synthase (FAS), sterol regulatory element

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binding protein-1c (SREBP-1c) and peroxisome proliferator-activated receptors (PPARs), participated in the hepatic response to nutritional intervention (Daval *et al*., 2000). The development of adipose tissue depends on the availability of serum triglycerides (TG) that are hydrolyzed before their utilization by adipocytes. TG, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are principal substrates in lipid metabolism.

There have been numerous reports on how fish oil affects fatty acid metabolism in mammals (Ribeiro *et al*., 1991; Kim *et al*., 1999; Yu *et al*., 2010), but few have focused on birds. Moreover, the effects of dietary *C. butyricum* on the expression of genes linked to fatty acid metabolism have not been examined in chickens. This study was conducted to investigate the effects of fish oil and *C. butyricum* supplementation on the profile of circulating lipids and the transcription of selected genes involved in fat metabolism in adipose tissue, liver and breast muscle of broiler chickens. We monitored the relative mRNA levels of SREBP-1c, FAS, PPARs, lipoprotein lipase (LPL) and fatty acid binding protein (FABP) in selected tissues as well as the levels of TG, TC, LDL-C and high-density lipoprotein cholesterol (HDL-C) in serum.

Material and methods

Birds, livestock management and diets

A total of 160 day-old male chicks (Arbor Acres) with body weights (BWs) 42.4 ± 2.1 g were purchased from a commercial hatchery (Huadu Broiler Company, Beijing, China). Chicks were randomly divided into four treatment groups consisting of five replicates (cages) with eight birds per replicate. Each replicates were housed in wire mesh cages (90 cm × 70 cm × 45 cm) with individual feed-troughs and a common water-trough. All chickens had free access to water and feed. The temperature inside the poultry house was maintained at 35°C during the first 3 days, between 28°C and 30°C during the subsequent 2 weeks, and at 25°C during the last 3 weeks of the study. Throughout the experimental period, 24-hour constant light was provided. Experimental protocols were approved by the China Agricultural University Animal Care and Use Committee. The experimental period lasted 42 days. The four groups of birds were fed diets containing (a) soybean oil (Beijing Red Star Starch Co., Beijing, China) without *C. butyricum* supplementation (SO), (b) soybean oil with *C. butyricum* supplementation (SO + CB), (c) fish oil (menhaden fish oil, Fujian Gaolong Co., Fujian, China) without *C. butyricum* supplementation (FO) or (d) fish oil with *C. butyricum* supplementation (FO + CB). The amount of lipid added to the diet was 25 g/kg for 0 to 21 days and 300 g/kg for 22 to 42 days. The composition and analysis of fatty acid contents were as described by Yang *et al*. (2010). The raw powder of the non-toxic bacterial strain *C. butyricum* was provided by Qingdao East Sea Pharmaceutical Co. Ltd, China. The product (bacterial spores) contained viable bacteria at 1.6×10^{10} cfu/g. The dried *C. butyricum* powder was diluted with glucose to a concentration of 2×10^8 cfu/g before being mixed into broiler diets at 5 g/kg of diet. Diets SO + CB and FO + CB contained finally 1×10^9 cfu of viable *C. butyricum* per kg.

Animal sacrifice and tissue sampling

At day 42, one chicken from each pen replicate was randomly selected and sacrificed by exsanguination under deep sodium pentobarbitone anesthesia (30 mg/kg BW, i.p.). Blood samples (one bird per pen) were collected from the wing vein and centrifuged (3000 × **g** for 15 min at 4°C) to separate sera. Serum samples were frozen at −30°C for further analysis. The abdominal fat, breast muscle and liver samples were removed and immediately frozen in liquid nitrogen and stored in a freezer at −80°C for future mRNA determination or enzyme activity analysis. Jejunal samples were cut longitudinally to expose mucosa and washed three times in ice-cold phosphate-buffered saline to remove the mucus and digesta. The jejunal mucosa was scraped gently, quickly frozen in liquid nitrogen and stored at −80°C until mRNA determination.

Biochemical assays

The serum concentrations of TC, TG, HDL-C and LDL-C were measured by colorimetric enzymatic methods using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

LPL activity in abdominal fat and muscle tissue was determined by colorimetric enzymatic methods using a commercial diagnostic kit (Jiancheng Bioengineering Institute, Nanjing, China). One unit of enzyme activity was defined as 1 μmol of nonesterified fatty acid released from 1 mg of tissue protein per hour. FAS activity was determined as described by Moibi *et al*. (2000). Frozen liver tissue samples were pulverized in liquid nitrogen and homogenized (30 s at 4°C) in three volumes of phosphate bicarbonate buffer (70 mM $KHCO_3$, 85 mM K_2HPO_4, 1 mM DTT, pH 8). The homogenate was centrifuged at 10 000 × **g** for 10 min and the supernatant was centrifuged at 105 000 × **g** for 60 min at 4°C to obtain liver tissue cytosol. The protein content was assayed by the Bradford method (Bradford, 1976). The supernatant fraction was brought to saturation with ammonium sulfate solution (containing 3 mM EDTA and 1 mM β-mercaptoethanol) and stirred for 60 min on ice. The precipitate was collected by centrifugation at 10 500 × **g** for 60 min at 4°C. FAS activity was determined in duplicates by measuring the malonyl-CoA- and acetyl-CoA-dependent oxidation of NADPH using a UV-visible automated spectrophotometer equipped with a temperature controller set at 30°C. For each assay, reference (blank) and sample cuvettes were measured simultaneously and the decrease in absorbance at 340 nm was recorded over time. The change in concentration of NADPH during oxidation was calculated as described by Moibi *et al*. (2000). FAS activity was expressed as nanomoles NADPH oxidized per minute per milligram of protein.

Real-time quantitative PCR analysis of gene expression

Total RNA of the abdominal fat, breast muscle, liver samples or jejunal mucosa was extracted using TRIzol reagent (Invitrogen Company, Carlsbad, CA, USA) according to the manufacturer's instructions. The yield and quality of the RNA

Table 1 *Primers used for the quantification of the mRNA expression by real-time PCR*

Name	Sequence of forward (FP) and reverse (RP) primers	Genbank accession number	Product size (bp)
LPL	FP 5′-TTGGTGACCTGCTTATGCTA-3′ RP 5′-ATTGCTGCCTCTTCTCCTTT-3′	X14670	187
H-FABP	FP 5′-CGACAAGGCGACGGTGAA-3′ RP 5′-AAAGGGCGGAGGGAAGCAG-3′	AY648562	142
L-FABP	FP 5′-GAAGGGTAAGGACATCAA-3′ RP 5′-TCGGTCACGGATTTCAGC-3′	AF380999	219
PPAR-β	FP 5′-GCTTTGTGACCCGTGAGT-3′ RP 5′-GTGCTCGGAGGATGTTGT-3′	AF163810	213
PPAR-α	FP 5′-GATTTCCTGCAGTAAAGGG-3′ RP 5′-GATTTCCTGCAGTAAAGGG-3′	AF163809	222
PPAR-γ	FP 5′-GAGGCAGTGCAGGAGATTAC-3′ RP 5′-CTCTTCAGAAACTCCCGTGT-3′	AF163811	194
SREBP-1c	FP 5′-GCAGAAGAGCAAGTCCCTCAA-3′ RP 5′-TCGGCATCTCCATCACCTC-3′	AY029224	104
FAS	FP 5′-CCAACGATTACCCGTCTCAA-3′ RP 5′-CAGGCTCTGTATGCTGTCCAA-3′	J03860	170
β-actin	FP 5′-GATGAAGCCCAGAGCAAAG-3′ RP 5′-ACATACATGGCTGGGGTGTT-3′	NM_205518	236

LPL = lipoprotein lipase; H-FABP = heart-fatty acid binding protein; L-FABP = liver fatty acid binding protein; PPAR-β = peroxisome proliferator-activated receptors-β; PPAR-α = peroxisome proliferator-activated receptors-α; PPAR-γ = peroxisome proliferator-activated receptors-γ; SREBP-1c = sterol regulatory element binding protein-1c; FAS = fatty acid synthase.

were determined spectrophotometrically using A_{260} and A_{280} measurements and by electrophoresis on 1.5% agarose gels. Reverse transcription reactions (25 μl) consisted of 2 μg of total RNA, 100 U of Moloney-murine leukemia virus reverse transcriptase, 10 mmol of dNTP, 20 U of RNAsin ribonuclease inhibitor and 1 μg of random primers (all agents from MBI Fermentas, Canada) in distilled water and buffer supplied by the manufacturer. After incubation for 60 min at 42°C, the reverse transcriptase was inactivated at 70°C for 5 min.

The cDNAs were amplified by real-time PCR using the Applied Biosystem ABI-PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA). Each PCR reaction contained 2 μl of cDNA mixed with 1 μmol of each primer, 10 μl of SYBR Green PCR master Mix (ABI Company, Foster City, CA, USA) and 6 μl of sterile super-distilled water in a final volume of 20 μl. The thermocycle protocol consisted of 2 min at 55°C and 10 min at 94°C, followed by 40 cycles of 15 s denaturation at 95°C and 60 s annealing/extension at 60°C. The gene-specific primers for LPL, heart-type-FABP (H-FABP), liver-type-FABP (L-FABP), PPAR-β, PPAR-α, PPAR-γ, SREBP-1c, FAS and β-actin are listed in Table 1. The relative standard curve method was used to estimate mRNA abundance. Standard curves were derived from serial dilutions of untreated samples. β-actin was used as the internal reference gene and the mRNA expression of target genes was normalized to β-actin mRNA expression. All the samples were analyzed in triplicates and the mean values of these triplicate measurements were used for calculations of mRNA expression.

Statistical analysis

All analyses were performed with SPSS 16.0 software. The data were analyzed by two-way analysis of variance with lipids and *C. butyricum* as the fixed factors. Differences were considered significant at $P < 0.05$, although probability values up to $P < 0.10$ are shown in the text if the data suggest a trend.

Results

Serum parameters

The effects of dietary lipids and *C. butyricum* supplements on the serum concentrations of TC, TG, HDL-C and LDL-C are listed in Table 2. The type of dietary lipid had a clear effect on serum concentrations of TC and LDL-C in broiler chickens. Serum concentrations of TC and LDL-C were lower for broilers fed diets containing fish oil than those fed diets containing soybean oil ($P < 0.05$). The addition of *C. butyricum* tended to reduce serum concentrations of TG ($P = 0.056$) and TC ($P = 0.088$). There was no significant interaction effects on serum concentrations of TC, TG, HDL-C and LDL-C ($P > 0.05$) between dietary lipid and *C. butyricum*.

Hepatic FAS activity and LPL activity

The effects of dietary lipids and *C. butyricum* addition on hepatic FAS activity and LPL activity in abdominal fat and breast muscle of broiler chickens are presented in Table 3. The dietary lipid type had a clear effect on hepatic FAS activity and LPL activity in abdominal fat and breast muscle. Fish oil reduced hepatic FAS activity and LPL activity in abdominal fat ($P < 0.05$), but increased LPL activity in breast muscle ($P < 0.05$). The addition of *C. butyricum* significantly increased LPL activity only in abdominal fat ($P < 0.05$). There was no significant interaction effect on these parameters ($P > 0.05$).

Table 2 *Effects of dietary lipids and* Clostridium butyricum *on concentrations of TC, TG, HDL-C and LDL-C in serum of broiler chickens*[1]

	Treatment					Lipid		CB (g/kg of diet)		*P*-value		
Item (mM)	SO	SO + CB	FO	FO + CB	s.e.m.	Soybean oil	Fish oil	0	5	Lipid	CB	Lipid × CB
TG	0.60	0.57	0.57	0.54	0.009	0.58	0.55	0.59	0.55	NS	0.056	NS
TC	3.78	3.55	3.47	3.43	0.047	3.67	3.45	3.62	3.49	*	0.088	NS
HDL-C	2.45	2.54	2.49	2.54	0.033	2.47	2.54	2.50	2.52	NS	NS	NS
LDL-C	1.09	0.86	1.00	0.83	0.037	1.05	0.84	0.98	0.92	**	NS	NS

[1] $n = 5$ per treatment group.
TC = total cholesterol; TG = triglycerides; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SO = dietary soybean oil without *C. butyricum* supplementation; SO + CB = dietary soybean oil with *C. butyricum* supplementation (1×10^9 cfu/kg); FO = dietary fish oil without *C. butyricum* supplementation; FO + CB = dietary fish oil with *C. butyricum* supplementation (1×10^9 cfu/kg); CB = *C. butyricum*; lipid × CB = interaction between lipids and *C. butyricum* treatment.
*$P < 0.05$; **$P < 0.01$; NS at $P > 0.05$.

Table 3 *Effects of dietary lipids and* Clostridium butyricum *on liver FAS activity and LPL activity in abdominal fat and breast muscle of broiler chickens*[1]

	Treatment					Lipid		CB (g/kg of diet)		*P*-value		
Item	SO	SO + CB	FO	FO + CB	s.e.m.	Soybean oil	Fish oil	0	5	Lipid	CB	Lipid × CB
Hepatic FAS activity (nM NADPH/min/mg protein)	1.39	1.04	0.51	0.42	96.9	1.21	0.47	0.95	0.73	**	NS	NS
Breast muscle LPL (U/mg protein)	0.97	1.53	1.56	1.74	0.058	1.25	1.65	1.27	1.64	**	**	NS
Abdominal fat LPL (U/mg protein)	4.78	4.97	3.97	4.45	0.146	4.88	4.21	4.38	4.71	*	NS	NS

[1] $n = 5$ per treatment group.
FAS = fatty acid synthase; LPL = lipoprotein lipase; SO = dietary soybean oil without *C. butyricum* supplementation; SO + CB = dietary soybean oil with *C. butyricum* supplementation (1×10^9 cfu/kg); FO = dietary fish oil without *C. butyricum* supplementation; FO + CB = dietary fish oil with *C. butyricum* supplementation (1×10^9 cfu/kg); CB = *C. butyricum*; lipid × CB = interaction between lipids and *C. butyricum* treatment.
*$P < 0.05$; **$P < 0.01$; NS at $P > 0.05$.

Lipid metabolic gene expression

The effects of dietary lipids and *C. butyricum* addition on lipid metabolic gene expression in abdominal fat, breast muscle, liver and jejunal mucosa are shown in Table 4. The lipid type had a clear effect on hepatic PPAR-α and LPL gene expression in breast muscle tissue. Hepatic PPAR-α and LPL gene expression were higher in broilers fed diets containing fish oil than in those fed diets containing soybean oil ($P < 0.05$). Fish oil tended to reduce hepatic FAS gene expression ($P = 0.081$). The addition of *C. butyricum* significantly increased L-FABP gene expression only in jejunal mucosa ($P < 0.05$). There was a significant interaction effect on PPAR-β gene expression in breast muscle tissue. The mRNA levels of PPAR-β in breast muscle tissue were highest for broilers fed diets containing fish oil supplemented with 5 g of *C. butyricum* per kg diet ($P < 0.05$). Dietary lipids and *C. butyricum* did not significantly influence the expression of the other genes examined (Table 4).

Discussion

Concentrations of serum lipids and lipoproteins are indicative of the metabolic regulations in a steady state and, especially, of the basal adjustment of fatty acid circulation between the adipose tissue and the liver (Mossab *et al.*, 2002). *C. butyricum* tended to reduce serum concentrations of TG and TC in the present study. Limited research focusing on the effect of probiotics on lipid metabolism of chicks indicated that the serum level of cholesterol was significantly decreased by adding a mixture of Bacillus, Lactobacillus, Streptococcus, Clostridium, Saccharomyces and Candida into the diet (Endo *et al.*, 1999). Previous studies reported that a fish oil diet significantly decreased serum levels of cholesterol and TG (An *et al.*, 1997; Castillo *et al.*, 2000), while fish oil reduced serum TC levels, but not cholesterol, in our study. This discrepancy may be attributed to the difference in control diet. In our study, the control treatment was a soybean oil-based diet rather than the high-saturated-fat (palm oil) diet used in previous studies.

Fatty acid-binding protein plays a key role in transporting long-chain fatty acids (LCFA) in gut. There are two main FABPs in the small intestine, the intestinal type (I-FABP) and the liver-type (L-FABP). Previous reports indicated that I-FABP was not essential for fatty acid absorption (Vassileva *et al.*, 2000) and that L-FABP exhibited a higher affinity for unsaturated LCFA than I-FABP (Richieri *et al.*, 1994). In addition, L-FABP had the ability to increase fatty acid uptake (Prows *et al.*, 1995), so we examined the changes in L-FABP mRNA expression in jejunum under changing dietary conditions. Indeed, this study represents the first examination of the effect of *C. butyricum* on fatty acid metabolism-linked

Table 4 *Effects of dietary lipids and* Clostridium butyricum *on mRNA abundance of lipogenic genes in tissues of broiler chickens*[1]

	Treatment					Lipid		CB (g/kg of diet)		*P*-value		
Item ($\times 10^{-2}$)	SO	SO + CB	FO	FO + CB	s.e.m.	Soybean oil	Fish oil	0	5	Lipid	CB	Lipid × CB
Jejunal L-FABP	1.01	1.81	0.74	2.00	0.185	1.41	1.37	0.87	1.91	NS	*	NS
Abdominal fat												
LPL	0.153	0.122	0.104	0.089	0.0131	0.128	0.106	0.138	0.097	NS	NS	NS
PPAR-γ	0.165	0.096	0.089	0.078	0.0233	0.127	0.087	0.131	0.084	NS	NS	NS
Liver												
PPAR-α	1.15	0.61	1.58	1.45	0.133	0.88	1.52	1.37	1.03	*	NS	NS
SREBP-1c	1.22	0.91	1.16	0.79	0.145	1.07	0.97	1.19	0.85	NS	NS	NS
FAS	14.3	10.6	13.4	10.7	0.86	13.9	10.7	12.5	12.0	0.081	NS	NS
Breast muscle												
LPL	3.24	6.00	4.58	6.87	0.472	3.91	6.43	4.62	5.73	***	NS	NS
PPAR-β	1.10	0.41	0.06	2.05	0.291	0.75	1.06	0.58	1.23	NS	NS	*
H-FABP	1.06	1.81	1.55	3.32	0.404	1.44	2.43	1.30	2.57	NS	NS	NS

[1] $n = 5$ per treatment group.
SO = dietary soybean oil without *C. butyricum* supplementation; SO + CB = dietary soybean oil with *C. butyricum* supplementation (1×10^9 cfu/kg); FO = dietary fish oil without *C. butyricum* supplementation; FO + CB = dietary fish oil with *C. butyricum* supplementation (1×10^9 cfu/kg); CB = *C. butyricum*; Lipid × CB = interaction between lipids and *C. butyricum* treatment; L-FABP = liver-type fatty acid-binding protein; LPL = lipoprotein lipase; PPAR-γ = peroxisome proliferator-activated receptor-γ; PPAR-α = peroxisome proliferator-activated receptor-α; SREBP-1c = sterol regulatory element binding protein-1c; FAS = fatty acid synthase; H-FABP = heart-fatty acid binding protein.
*$P < 0.05$; **$P < 0.01$; NS at $P > 0.05$.

gene expression in broiler chickens. The results indicated that *C. butyricum* increased L-FABP mRNA expression in jejunum. H-FABP has been shown to facilitate LCFA uptake and utilization in skeletal muscles (Corcoran *et al.*, 2007). In the present study, the transcription levels of H-FABP were not significantly affected by *C. butyricum* and dietary lipid type, indicating no effect on fatty acid transport into muscle cells.

In chickens, *de novo* lipogenesis occurs primarily in the liver, and most of the body's endogenous lipids are of hepatic origin (O'Hea and Leveille, 1968). FAS is a key enzyme involved in the *de novo* synthesis of LCFA (Back *et al.*, 1986), so we examined FAS activity and FAS mRNA levels in liver. Although no significant difference in FAS expression was observed between dietary lipid groups, broilers fed fish oil diets showed a trend toward down-regulated FAS gene expression, further indicating that fish oil reduced hepatic synthesis of fatty acids. Previous study also demonstrated reduced lipogenesis with dietary fish oil (Wong *et al.*, 1984). Studies found that fish oil inhibited lipid synthesis by decreasing the expression of FAS genes in the liver of mice (Ren *et al.*, 1997; Kim *et al.*, 1998). The present results are consistent with these findings. *C. butyricum* did not influence FAS activity or FAS mRNA levels in liver, indicating no effect on liver fatty acid synthesis.

The SREBP-1c transcription factor acts as a key regulator of fatty acid synthesis by regulating multiple enzymes required for fatty acid biosynthesis (Brown and Goldstein, 1997). The mRNA level of SREBP-1c was not significantly influenced by lipid type or *C. butyricum* addition. This contradicts previous studies demonstrating that polyunsaturated fatty acids (PUFA) inhibited lipogenic gene transcription by suppressing SREBP-1c expression (Kim *et al.*, 1999; Xu *et al.*, 1999). Xu *et al.* (1999) also indicated that the SREBP-1 mRNA content of cells was reduced by 20:4n-6 fatty acids in a dose-dependent manner, whereas 18:1n-9 had no effect. However, the exact amount of 20:4n-6 fatty acids was not determined by Yang *et al.* (2010). The content of 20:4n-6 fatty acids was only about 1.6 g/kg of the total fatty acid in fish oil (He *et al.*, 2007). In our study, fish oil constituted 25 g/kg of the total diet, implying 20:4n-6 fatty acid levels of about 0.004 g/kg in the present diet. This small dose (about 0.004 g/kg) of 20:4n-6 fatty acids may be a cause for the lack of effect on SREBP-1c expression.

PPAR-α, PPAR-γ and PPAR-β belong to a family of nuclear hormone receptors that regulate the expression of genes involved in glucose and lipid metabolism. The PPAR-α isoform was highly expressed in liver, but not in chicken skeletal muscle (Meng *et al.*, 2005), whereas PPAR-γ was mainly localized to adipose tissue and immune cells and PPAR-β was ubiquitously expressed in all tissues. Activation of PPAR-α increases lipid oxidation, thus reducing tissue fatty acid contents (Corcoran *et al.*, 2007). It is known that fish oil can induce fatty acid oxidation by increasing the expression of PPAR-α (Nakatani *et al.*, 2002). In the present study, the mRNA level of PPAR-α was significantly increased by the fish oil diet, thus possibly enhancing fatty acid oxidation in liver. The PPAR-β isoform increased fatty acid oxidation in skeletal muscle (Krämer *et al.*, 2007). PUFAs are potential ligands for PPAR-β activation, and a recent study indicated that porcine PPAR-β mediated the lipolytic effects of dietary fish oil, resulting in reduced body fat deposition (Yu *et al.*, 2010). However, fish oil did not influence expression of PPAR-β in breast muscle or PPAR-γ expression in abdominal fat of broiler chickens. The mRNA levels of PPAR-α in liver, PPAR-γ in abdominal fat and PPAR-β in breast muscle were also not affected by *C. butyricum*, again indicating that *C. butyricum* had little if any effect on fatty acid oxidation.

LPL is responsible for the hydrolysis of plasma lipoproteins, a rate-limiting step in lipid transport into peripheral tissues (Goldberg, 1996). Overexpression of muscle LPL has been associated with increasing intramuscular triacylglycerol accumulation and fat deposition in mammals (Voshol *et al*., 2001). Although no significant difference in LPL expression in breast muscle was found in broilers fed the diet supplemented with *C. butyricum*, a trend toward upregulated LPL gene expression was found, and LPL activity in muscle tissue was significantly increased by *C. butyricum* addition. This response may be the causation for the increased intramuscular fat in breast muscle of chicken feed supplementary *C. butyricum* in a previous report (Yang *et al*., 2010). LPL activity and mRNA expression in breast muscle were significantly increased by fish oil in the present work, but fish oil did not significantly influence the intramuscular fat in breast muscle as our previous study demonstrated (Yang *et al*., 2010). This may have been the result of the reduced synthesis of fatty acids in liver under the fish oil diet. Adipose expression of genes involved in adipogenesis (i.e. LPL) was decreased in transgenic mice fed fish oil (Yu *et al*., 2010). Fish oil reduced the activity of LPL, but did not influence the expression of LPL in abdominal fat in the present study.

Lipid accumulation is the net result of the balance between dietary absorbed fat, endogenous fat synthesis and fat catabolism via β-oxidation (Sanz *et al*., 2000). According to the present results, the reduced abdominal fat in broiler chicks fed the fish oil diet as described by Yang *et al*. (2010) may be ascribed to augmented hepatic fat catabolism and lowered hepatic endogenous fat synthesis. In contrast, the increased intramuscular fat content in breast muscle evoked by *C. butyricum* supplementation as observed by Yang *et al*. (2010) may be associated with enhanced fatty acid uptake.

In conclusion, fish oil reduced endogenous fat synthesis in liver by down-regulating FAS expression and activity, as well as by inducing fatty acid oxidation through increased expression of PPAR-α. The net result was a reduction in the abdominal fat of broiler chickens as observed by Yang *et al*. (2010). *C. butyricum* enhanced fatty acid uptake by enhancing L-FABP expression in jejunum and by upregulating LPL expression and activity in breast muscle, resulting in increased intramuscular fat content as described by Yang *et al*. (2010).

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