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Effect of Cottonseed Meal Fermented with Yeast on the Lipid-related Gene Expression in Broiler Chickens

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■Keywords

Fermented cottonseed meal, fermented feed, lipid metabolism, gene expression, broilers.

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ABSTRACT

Fermented cottonseed meal (FCSM) is widely used in poultry diets in China. This study was conducted to investigate the effect of FCSM on lipid-related gene expression in broilers. Initially, 180 broiler chickens (21-days-old, equal number of males and females) were randomly divided into three groups, with six pens per group and 10 birds per pen. The chickens in the control group were fed a diet containing unfermented cottonseed meal, and those in the treatment groups were fed with diets including either CSM fermented by *Candida tropicalis* (Ct group) or CSM fermented by *Candida tropicalis* plus *Saccharomyces cerevisae* (Ct-Sc group) until 64 days old. The results revealed that, compared with the control group ($p<0.05$), the mRNA expression of peroxisome proliferator-activated receptor alpha (PPAR-α) and lipoprotein lipase (LPL) were upregulated in the livers of Ct-Sc males. The expression of PPAR-α was also upregulated in the livers of Ct females. The expression levels of acetyl CoA carboxylase (ACC) and LPL in the liver of males and the expression of PPAR-α in the liver of females were significantly different between the Ct and Ct-Sc groups ($p<0.05$). However, gene expressions of fatty acid synthase (FAS) and liver fatty acid-binding protein (L-FABP) in the liver were not altered when the broilers were fed FCSM-supplemented diets ($p>0.05$). Likewise, the expressions of peroxisome proliferator-activated receptor gamma (PPAR-γ) and LPL in the abdominal fat were not altered by the FCSM-supplemented diets ($p>0.05$). The results in this study indicate that CSM fermented by *Candida tropicalis* and *Saccharomyces cerevisiae* effectively regulated the genes involved in fatty acid β-oxidation and triglyceride hydrolysis in male broiler chickens. Furthermore, the effects of the FCSM-supplemented diets were significantly different between bird sexes and between yeast strains used in the fermentation process.

INTRODUCTION

In modern broiler production, excessive fat deposition, especially of abdominal fat, is a major concern in the poultry industry (Zaboli *et al.*, 2013). Fat is a main form of energy deposition in the body; therefore, excessive fat deposition demands high energy supply and results in energy loss. This, in turn, causes an increase in overall feed cost as well as in reduced feed efficiency. Furthermore, the more body fat that a chicken has, the lower the possible lean meat yield of that chicken, which is highly problematic because lean meat has the high nutritional value and it is preferred by consumers (de Genova Gaya *et al.*, 2005; Duarte *et al.*, 2013). Accordingly, because low lean meat yield is undesirable for consumers, it is also causes processing losses. Therefore, it is evident that excessive fat in broilers is detrimental for the broiler industry.

Naturally, based on the aforementioned information, reducing excessive fat deposition is a priority for broiler producers and consumers. Nutritionists have tried to mitigate this problem using nutritional strategies, such as the dietary supplementation of plant extracts and probiotics, and some researchers have demonstrated that this is an efficient means of preventing excessive fat deposition. Huang *et al*. (2013) reported that the dietary supplementation of green tea polyphenols (extracted from tea plants) reduced abdominal and subcutaneous fat deposition in broiler chickens by regulating the expression of key genes related to lipid metabolism. Furthermore, Aluwong *et al*. (2013) showed that the supplementation of a yeast probiotic in broiler feeds significantly decreased abdominal fat weight by 16.67% in the group fed 1.5% and by 28.626% in the group fed 2.0% compared with the controls. Therefore, these results indicate the potential of specific diets and dietary supplements to prevent excessive fat deposition in broiler carcasses.

One such supplement widely used in poultry diets is fermented cottonseed meal (FCSM). Numerous studies have been conducted on the effect the supplementation of broiler diets with FCSM on growth performance, apparent nutrient digestibility, immune function, digestive enzyme activity, and intestinal tract bacteria and morphology (Nie *et al.*, 2011; Tang *et al.*, 2012; Sun *et al.*, 2013a,b). In our previous studies, we found that FCSM increased the levels of glycerol phospholipids, as determined by a metabolomics method based on liquid chromatography-mass spectrometry, as well as reduced blood triglyceride and total cholesterol levels in broiler chickens (Nie *et al.*, 2011, 2013). In addition, various studies have also shown that fermented products have a regulatory effect on lipid metabolism. Park *et al*. (2012) indicated that kimchi (Korean traditional fermented vegetable) fermented with *Weissella koreensis* had an anti-obesity effect on high-fat diet-induced obese mice. Moreover, Cha *et al*. (2013) showed that fermented soybean-based red pepper paste could decrease visceral fat and serum triglyceride concentrations in overweight adult humans. However, despite the demonstrated benefits of an FCSM-supplemented diet, there is little information on the effect of FCSM on lipid metabolism, and its mode of action is poorly understood, especially at the molecular level.

In broilers, fatty acid synthesis occurs mainly in the liver, and the adipose tissue is the primary site of fat storage as triglycerides, especially the abdominal fat tissue (Huang *et al*., 2008; Fouad & El-Senousey, 2014). Therefore, we investigated the effect of the dietary supplementation of FCSM on lipid-related gene expression of acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL), peroxisome proliferator-activated receptor alpha (PPAR-α), and liver fatty acid-binding protein (L-FABP) in liver tissue, as well as LPL and peroxisome proliferator-activated receptor gamma (PPAR-γ) in the abdominal fat tissue of broiler chickens.

MATERIALS AND METHODS

Substrate preparation and fermentation

The FCSM substrate was prepared according the method previously reported by Zhang *et al*. (2007). Cottonseed meal (CSM), corn flour, and wheat bran were used as fermentation substrate and were obtained from the Shihezi district (Xinjiang, China). The *Candida tropicalis* (*C. tropicalis*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) yeasts used for fermentation were provided by the Feed Science Institute, Zhejiang University. The substrate to be fermented was mixed at a CSM: corn flour: wheat bran ratio of 7:2:1 and moistened at a substrate: water ratio of 1:0.8. The substrate was then autoclaved at 112.6˚C for 20 min.

The treated substrate (1 kg) was inoculated with liquid yeast inocula (80 mL, 10^8 cells/mL) consisting of either *C. tropicalis* or two combined yeast strains (at volume ratio of *C. tropicalis* to *S. cerevisiae* of 3:7). The inoculated substrate was evenly blended and then placed in a plastic container (50cm long × 30cm wide ×12cm high), in which it was incubated at 30˚C for 48h in an incubator. At the end of the fermentation period, the fermented products were dried with a draught drying cabinet at 40˚C for 48h. As a control, the unfermented CSM underwent the same process, except an equal volume of sterile culture medium was added. The chemical compositions of unfermented CSM and FCSM by *C. tropicalis* treatment (Ct CSM) or by *C. tropicalis* and *S. cerevisiae* treatment (Ct-Sc CSM) were analyzed according to the AOAC (1999), and the free gossypol contents were determined by the standard method of the AOCS (2009).

Broilers, housing, and sample collection

A total of 300 one-day-old Chinese yellow-feathered chickens were purchased from Tecon Animal Science Bio-technology Co. LTD (Urumqi, China), housed in a brooder house for 14 days and fed a commercial diet. From days 14 to 21, the birds were fed a control diet for habituation to powdered feeds because of the experimental diets were fed as powder. On day 21, the birds were fasted for 12 h and then were weighed.

Out of the original 300 broilers, 180 birds (21-d-old) were selected according to similar weight and sex and overall lack of physical deformities. The selected birds were then randomly distributed in three treatments (groups) with six replicates (pens) of 10 birds each (equal number of males and females). Each of the three groups received a different diet: (1) a control diet with unfermented CSM (Cont group), (2) a diet with FCSM fermented with *C. tropicalis* (Ct group), or (3) a diet with FCSM fermented with *C. tropicalis* and *S. cerevisiae* (Ct-Sc group). The ingredients and nutritional composition of the diets are presented in Table 1 (Nie *et al.* 2015). The diets were free from any antibiotics. Birds were housed in common rearing conditions, under continuous light and had free access to feed and water during the rearing period. This experiment lasted 42 days and was divided into two phases (21-42 days and 43-64 days). The animal care and use protocol was approved by the Animal Welfare Committee of Shihezi University (Shihezi, China).

On day 64, 12 birds per group (six of each sex), which weight was close to the average weight of the group, were randomly selected and weighed. They were then killed by cervical dislocation (no anesthesia) in order to collect the liver and manually separate the abdominal fat from the gizzard and cloaca with surgical scissors. Both abdominal fat and liver were weighed and, and their weight relative to body weight was calculated. About 5 g of abdominal fat and liver tissue each were collected and immediately frozen in liquid nitrogen, and then stored at -80˚C for further analysis of mRNA expression.

Real-time quantitative PCR analysis

The total tissue RNA of the liver and abdominal fat was isolated using TRIzol reagent (Invitrogen, CA, USA) according to the manufacture's protocol, and the RNA concentrations were measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop, DE, USA). All isolated RNA samples were adjusted to the same concentration and then reverse transcribed to cDNA using a PrimeScriptTM RT reagent Kit (Takara, Shiga, Japan).

Real-time quantitative PCR was carried out in a 7500 Real Time PCR System (Applied Biosystems, CA, USA) using a SYBR-Green PCR Kit (Roche, Quebec, Canada), according to optimized PCR protocols. The PCR program was performed for 2 min at 95˚C, followed by 32 cycles (95˚C for 30s, 55˚C for 30s, 72˚C for 30s), and then extended at 72˚C for 10 min. The gene primers of ACC, FAS, LPL, PPAR-α, PPAR-γ, and β-actin were designed as described by Wu (2012), and the L-FABP was designed according to the chicken sequences in the GenBank (Table 2). These

Table 1 – Ingredients and nutrient composition of experimental diets (based on air-dry matter)

	Starter (days 21-42)			Finisher (days 43-64)		
Ingredient (%)	Cont	Ct	Ct-Sc	Cont	Ct	Ct-Sc
Yellow corn	64.35	64.70	64.65	67.95	68.30	68.25
Soybean meal	17.15	16.80	16.85	13.25	12.90	12.95
Rapeseed meal	2.00	2.00	2.00	2.00	2.00	2.00
Cottonseed meal (CSM)	6.00	6.00	6.00	6.00	6.00	6.00
Unfermented CSM	6.00	-	-	6.00	-	-
Fermented CSM	-	6.00	6.00	-	6.00	6.00
Cottonseed oil	1.00	1.00	1.00	1.50	1.50	1.50
Dicalcium phosphate	1.20	1.20	1.20	1.10	1.10	1.10
Limestone	1.30	1.30	1.30	1.20	1.20	1.20
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Nutrient content[2] (%)						
ME (MJ/kg)	11.94	11.96	11.95	12.23	12.25	12.24
Crude protein	18.70	18.73	18.75	17.11	17.15	17.16
Calcium	0.87	0.85	0.84	0.81	0.79	0.82
Available phosphorus	0.36	0.34	0.35	0.33	0.35	0.34
Met + Cys	0.68	0.68	0.68	0.63	0.63	0.63
Lys	1.04	1.03	1.03	0.89	0.89	0.89

ME, metabolizable energy; Cont, control group fed a unfermented cottonseed meal (CSM); Ct, group fed cottonseed meal fermented by *C. tropicalis*; Ct-Sc, group fed cottonseed meal fermented by *C. tropicalis* plus *S. cerevisiae*.

[1]Premix content (g or mg/kg diet): L-lysine·HCl, 2 g (1.5 g in finisher); DL-methionine, 1.1g (1 g in finisher); NaCl, 3 g; Choline chloride (50%), 1 g; Cu, 6 mg; Fe, 100 mg; Mn, 150 mg; Zn, 100 mg; Se, 0.3 mg; I, 0.4 mg; V_A, 25 mg; V_{D3}, 8 mg; V_E, 36 mg; V_K, 3 mg; V_{B1}, 2 mg; V_{B2}, 7.5 mg; V_{B6}, 4.5 mg; V_{B12}, 0.02 mg; V_{B5}, 12 mg; niacin, 50 mg; folic acid, 1.2 mg; biotin, 0.15 mg.

[2] ME, phosphorus, and amino acid contents were calculated; crude protein and calcium are analyzed values.

Table 2 – Gene specific primer sequence of the lipid-related metabolism for real-time PCR

Gene	Genbank number	Primer sequences (5′-3′)	Size (bp)
FAS	NM_205155.2	Forward: TCAGGGTGTTCTGGAATGCAA Reverse: AATCCTGGTGGGCAATCGTAG	142
LPL	NM_205282.1	Forward: AGTCAGAGTGAAGT CAGGCGAAAC Reverse: CTGCTCCAGGCACT TCACAAATA	115
ACC	NM_205505.1	Forward: CTGATGGTCTTTGCC AACTGGA Reverse: CACGATGTAGGCAC CAAACTTGA	87
PPAR-α	NM_001001464.1	Forward: TGCACTGGAACTGG ATGATAGTGA Reverse: TCCTACATTTACAAG ACCAGGACGA	88
PPAR-γ	NM_001001460.1	Forward: TGTGAAGTTCAACG CACTGGAATTA Reverse: GGAGCTCCAAAGCT TGCAACA	146
L-FABP	AF_380999	Forward: ATGAGCTTCACTGGAAAGTACGAG Reverse: TCTTGATGTCCTTA CCCTTCTGG	148
β-actin	NM_205518.1	Forward: ATTGTCCACCGCAA ATGCTTC Reverse: AAATAAAGCCATGCC AATCTCGTC	113

FAS, fatty acid synthase; LPL, lipoprotein lipase; ACC, acetyl-coenzyme A carboxylase; PPAR-γ, peroxisome proliferator-activated receptor alpha; PPAR-γ, peroxisome proliferator--activated receptor gamma; L-FABP, liver fatty acid-binding protein. All primers were designed as described by Wu (2012), except for the L-FABP primer.

primers were synthesized by BGI (Beijing, China). All samples were analyzed in triplicate, and the results are expressed as $2^{-\Delta\Delta Ct}$, according to the method of Livak and Schmittgen (2001).

Statistical analysis

Data analysis was performed using SPSS 16.0 (SPSS Inc., IL, USA). General Linear Models were used to assess the effects of FCSM and sex and their interactions on body weight (BW) and liver and abdominal fat weight relative to BW. Tissue mRNA expression data within the same sex were submitted to one-way analysis of variance. Differences among means were tested using Duncan's multiple range tests. The significance level of 0.05 was applied.

RESULTS

Chemical composition of unfermented CSM and of FCSM

The chemical composition of unfermented CSM and CSM fermented by *C. tropicalis* or by *C. tropicalis* and *S. cerevisiae* are shown in Table 3. Fermented CSM presented higher crude protein and crude ash contents than unfermented CSM. The free gossypol concentrations were reduced by 64.59% in Ct CSM (from 126.71 mg/kg to 44.87 mg/kg) and by 73.96% (from 126.71 mg/kg to 32.99 mg/kg) in Ct-Sc CSM compared with unfermented CSM.

Effect of FCSM on liver and abdominal fat relative weights

The effects of dietary supplementation with FCSM on liver and abdominal fat as a percentage of BW

Table 3 – Composition of unfermented CSM and two kinds of FCSM (based on air-dry matter)

Composition	Substrate	Ct CSM	Ct-Sc CSM
Day mater (%)	95.28	94.63	94.71
Crude protein (%)	34.15	37.63	37.49
Ether extract (%)	0.71	0.72	0.74
Crude ash (%)	5.32	5.76	5.85
Free gossypol (mg/kg)	126.71	44.87	32.99

CSM, cottonseed meal; Ct CSM, CSM fermented by *C. tropicalis*; Ct-Sc CSM, CSM fermented by *C. tropicalis* and *S. cerevisiae*. Values are the means of three replicates per treatment.

are presented in Table 4. The results reveal that liver and abdominal fat absolute and relative weights were not significantly ($p>0.05$) affected by the addition of FCSM to the broiler diets. However, fat relative weight decreased by 5.50% in the Ct group and 8.12% in the Ct-Sc group when compared with the Cont group ($p>0.05$). No significant interaction was detected between BW and abdominal fat relative weight ($p>0.05$). However, sex significantly affected both BW and abdominal fat relative weight, and the interaction group × sex was significant ($p<0.05$) for BW. Therefore, we analyzed the lipid-related mRNA expression within each sex in the liver and abdominal fat tissues.

Effect of FCSM on lipid-related mRNA expression in liver and abdominal fat tissues

The effects of the FCSM-supplemented diet on hepatic mRNA expression are presented in Figure 1. In the male broilers, the gene expression of hepatic PPAR-α and LPL was clearly upregulated in the Ct-Sc group compared with the Cont group ($p<0.05$), but the expressions of those genes were not altered when Ct-CSM was added to the diet. In the female broilers, the mRNA expression of PPAR-α in the liver tissue was

Table 4 – Effect of fermented cottonseed meal on the body weight, and liver and abdominal fat absolute and relative weights (six birds per sex per group).

	Group				Sex		Significance		
	Cont	Ct	Ct-Sc	SEM	Male	Female	Group	Sex	Group × Sex
BW (g)	2099.50	2089.50	2083.50	46.76	2275.89	1905.78	ns	*	*
LW (g)	39.95	37.25	38.38	1.62	41.12	35.93	ns	ns	ns
FW (g)	79.67	75.37	72.43	1.86	78.51	71.13	ns	ns	ns
LW (% of BW)	1.90	1.80	1.85	0.07	1.81	1.89	ns	ns	ns
FW (% of BW)	3.82	3.61	3.51	0.10	3.45	3.84	ns	*	ns

BW, body weight; LW, liver weight; FW, abdominal fat weight; Cont, control group fed a unfermented cottonseed meal (CSM); Ct, group fed cottonseed meal fermented by *C. tropicalis*; Ct-Sc, group fed cottonseed meal fermented by *C. tropicalis* plus *S. cerevisiae*; SEM, pooled standard error of mean. * $p<0.05$; ns, $p>0.05$.

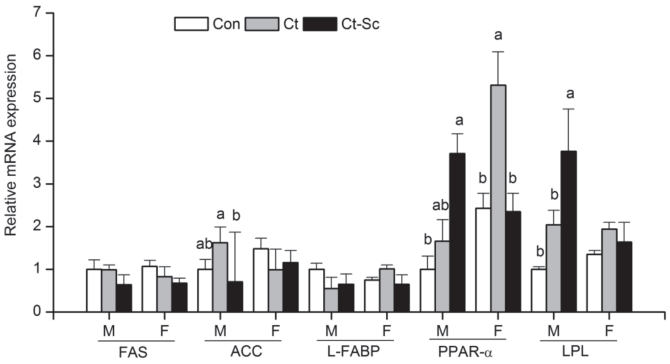

Figure 1. Effect of fermented cottonseed meal on lipid-related mRNA expression of FAS, ACC, L-FABP, PPAR-α, and LPL in the liver tissue.

M, males; F, females; FAS, fatty acid synthase; ACC, acetyl-coenzyme A carboxylase; L-FABP, liver fatty acid-binding protein; PPAR-α, peroxisome proliferator-activated receptor alpha; LPL, lipoprotein lipase. [a,b] Mean values for the same sex and gene with different letters are significantly different ($p<0.05$).

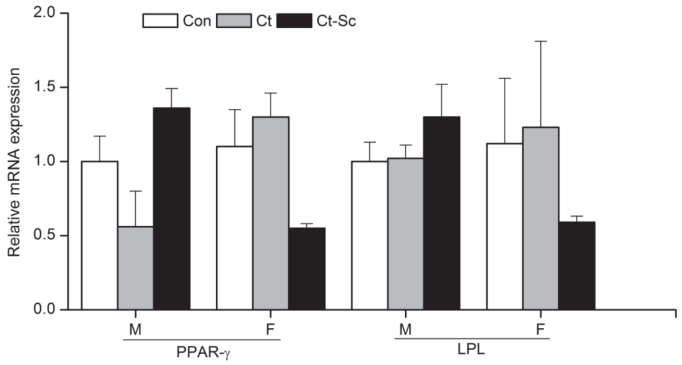

Figure 2. Effect of fermented cottonseed meal on lipid-related mRNA expression of FAS, PPAR-γ, and LPL in abdominal fat tissue

M, males; F, females; PPAR-γ, peroxisome proliferator-activated receptor gamma; LPL, lipoprotein lipase.

[a,b] Mean values for the same sex and gene with different letters are significantly different ($p<0.05$).

higher in the Ct group than in either the Cont group or Ct-Sc group ($p<0.05$). In addition, the hepatic gene expression of ACC in the male birds and PPAR-α in the female birds was significantly different between the Ct and Ct-Sc groups ($p<0.05$). No significant changes in FAS and L-FABP gene expressions were observed in the livers of either males or females among the three groups ($p>0.05$). Furthermore, the transcription levels of PPAR-γ and LPL were not altered in the abdominal fat tissue of either male or female broilers in the groups fed FCSM compared with the Cont group ($p>0.05$) (Figure 2).

DISCUSSION

In this study, *C. tropicalis* and *S. cerevisiae* were both used for CSM fermentation, which is a common practice. Moreover, our previous studies indicated that 6% supplementation of CSM fermented by these two strains did not negatively impact broilers (Zhang *et al.*, 2007; Nie *et al.*, 2011, 2012, 2013). Additionally, the results of the present study indicate that the CSM fermented by *C. tropicalis* or *C. tropicalis* and *S. cerevisiae* presented lower free gossypol content and a higher crude protein than unfermented CSM, which is consistent with the findings of Zhang *et al.* (2007). Similar results were reported by Tang *et al.* (2012), who showed that CSM fermented by *Bacillus subtilis* had lower free gossypol content and higher crude protein level relative to unfermented CSM. The free gossypol contents in all three experimental diets in the present study were lower than 30 mg/kg (data not shown), well below the level (100 mg/kg) allowed for poultry according to the EU standard and the report of Gadelha *et al.* (2014). In this trial, we primarily measured the effects of FCSM compared with unfermented CSM, and therefore, a gossypol control group was not included. Furthermore, yeast level was higher than $2'10^6$ cfu/g in the FCSM diets (data not shown).

FCSM influences lipid metabolism regulation, but it does so in a limited manner. More specifically, Henry *et al*. (2001) reported that CSM significantly increased abdominal fat content (as a percentage of body weight) in 7- to 21-d-old broilers. However, in a previous study, we showed that FCSM supplementation significantly reduced the abdominal fat content of 21- to 42-d-old broilers (Nie *et al.*, 2015), whereas in the current study, no significant differences were detected when the birds were reared up to 64 days. Therefore, this

indicates that the effect of FCSM on the regulation of lipid metabolism is limited. The different effects of CSM and FCSM on the abdominal fat content are associated with many metabolic products, such as essential amino acids, small-size peptides, and vitamins present in FCSM (Nie *et al.*, 2012; Tang *et al.*, 2012). In addition, the probiotics present in FCSM may also influence lipid metabolism regulation (Kalavathy *et al.*, 2003; Aluwong *et al.*, 2013).

In the present study, abdominal fat content was significantly different between broiler sexes. Therefore, gene expression in the tissues of male and female broilers were separately analyzed in order to elucidate the molecular mode of action of FCSM on lipid metabolism. Lipid metabolism maintains a state of equilibrium by regulating a variety of genes related to anabolism and catabolism, such as ACC, FAS, PPAR-α, LPL, L-FABP, and PPAR-γ (Zhao *et al.*, 2007; Zhang *et al.*, 2011). The expressions of the aforementioned genes were analyzed in this study, and the specific gene expression results are discussed below.

One such gene expression analysis was conducted on ACC expression. ACC is a rate-limiting enzyme of fatty acid synthesis and catalyzes acetyl-CoA to generate malonyl-CoA (Numa *et al.*, 1970; Richards *et al.*, 2003). FAS is also a critical enzyme in the control of lipogenesis from malonyl-CoA to palmitate (Joseph *et al.*, 2002). These two enzymes, ACC and FAS, are highly correlated to lipogenesis (Huang *et al*., 2008). The present results showed that for male broilers, the mRNA expression of ACC in the Ct group differed from that of the Ct-Sc group, which indicates that the fermentation of CSM with a single yeast strain or with combined yeast strains differently influences fatty acid synthesis. This regulation is occurs in the reactions from acetyl-CoA to malonyl-CoA, but not from malonyl-CoA to palmitate in fatty-acid synthesis.

The expressions levels of PPAR-α and LPL were also analyzed. PPAR-α, as a member of nuclear receptors, is highly expressed in the liver and plays an important role in regulating glucose and lipid metabolism or enhancing the related-gene expression in fatty acid oxidation (Lee *et al.*, 1995; Zhang *et al.*, 2011; Huang *et al.*, 2013). LPL, on the other hand, catalyzes triglycerides from circulating chylomicron and very low-density lipoprotein to generate fatty acids and glycerol for tissue utilization or storage (Zhao *et al.,* 2013). Again, this time due to the different changes in the PPAR-α and LPL expression levels in the liver tissue, there was a distinction between CSM fermented by *C. tropicalis* and CSM fermented by *C. tropicalis* plus *S. cerevisiae*. Furthermore, the upregulated expression of PPAR-α detected in this experiment indicates that fatty acid β-oxidation was higher in the males fed CSM fermented by *C. tropicalis* plus *S. cerevisiae* and in the females fed CSM fermented by *C. tropicalis*. The LPL expression level in the male birds was highest in the Ct-Sc group, which suggests that the combined fermentation of CSM enhances triglyceride hydrolysis, generating fatty acids and glycerol for energy supply. This consistent with the notion that fatty acid β-oxidation increases by upregulating the transcription level of PPAR-α. The obvious upregulation of hepatic PPAR-α and LPL was associated with better gain-to-feed ratio and nutrient digestibility when FCSM was added to the diet (Nie *et al.*, 2015), and the difference between the male and female broilers results in different growth rates.

L-FABP expression was also analyzed in this study. L-FABP participates in the transport of fatty acids and it is related to long-chain fatty acid (C16:0 and C18:3) contents, as well to total intramuscular fat in the pectoral muscle of ducks (He *et al.,* 2012). In broiler chickens, Wang *et al.* (2006) also demonstrated that the L-FABP gene is associated with abdominal fat weight and abdominal fat percentage. In our study, FCSM-supplementation did not significantly affect the transcription level of L-FABP in the liver tissue of broilers. This result indicates that FCSM did not influence the transport fatty acids to sites of β-oxidation and/or lipid synthesis via L-FABP expression.

PPAR-γ expression was also analyzed, and like L-FABP, it was not significantly influenced by FCSM-supplementation either. PPAR-γ is highly expressed particularly in the adipose tissue, and it promotes adipocyte differentiation (Mandrup *et al.,* 1997). Therefore, the change in PPAR-γ expression was examined in abdominal fat tissue considering the dietary supplementation with FCSM. The transcription level of PPAR-γ did not change after adding FCSM to the diet in this study. Therefore, FCSM supplementation did not alter adipocyte differentiation through the expression of the PPAR-γ gene.

All of the aforementioned results caused by FCSM supplementation are attributed to metabolites (Højer-Pedersen *et al.*, 2008) and probiotics (Aluwong *et al.,* 2013) present in the fermented substrate. Microbial fermentation produces many metabolites, including exoenzymes, vitamins, organic acids, small peptides, and an unknown active substance (Højer-Pedersen *et al.*, 2008; Nie *et al.*, 2012; Johnson, 2013), which may regulate the lipid metabolism of animals. Accordingly, the different mRNA expression levels of ACC, PPAR-γ, and LPL in the liver tissue were caused by the different

metabolites present in the CSM fermented using a single yeast or a yeast combination (Nie *et al.*, 2012). Moreover, Zhao *et al.* (2013) reported that probiotics (*Clostridium butyricum* and *Enterococcus faecium*) enhance the mRNA abundance of lipogenic genes in male broilers, including ACC, FAS, and malic enzyme in the liver tissue. However, in the present study, the expressions of FAS and L-FABP in the liver tissue were not altered and, therefore, do not agree with the results of Zhao *et al.* (2013). These differences may be attributed to differences in yeast strain, broiler breed, and feeding system.

CONCLUSIONS

The results of this study indicate that the dietary supplementation of CSM fermented with *C. tropicalis* plus *S. cerevisiae* produces a more obvious effect on the hepatic gene expressions of ACC and LPL in male than in female broilers. Furthermore, in male broilers, CSM fermented either with *C. tropicalis* or with *C. tropicalis* plus *S. cerevisiae* increased fatty acid β-oxidation and triglyceride hydrolysis by upregulating the expression levels of the PPAR-α and LPL genes. In female broilers, CSM fermented with *C. tropicalis* increased fatty-acid β-oxidation by upregulating the expression of the PPAR-α gene. Finally, FCSM supplementation did not affect gene transcription in the abdominal fat tissue of either male or female broiler chickens.

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