



Lower ω -6/ ω -3 Polyunsaturated Fatty Acid Ratios Decrease Fat Deposition by Inhibiting Fat Synthesis in Gosling

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ABSTRACT: The objective of the current study was to investigate the effects of dietary ω -6/ ω -3 polyunsaturated fatty acid (PUFA) ratios on lipid metabolism in goslings. One hundred and sixty 21-day-old Yangzhou geese of similar weight were randomly divided into 4 groups. They were fed different PUFA-supplemented diets (the 4 diets had ω -6/ ω -3 PUFA ratios of 12:1, 9:1, 6:1, or 3:1). The geese were slaughtered and samples of liver and muscle were collected at day 70. The activities and the gene expression of enzymes involved in lipid metabolism were measured. The results show that the activities of acetyl coenzyme A carboxylase (ACC), malic enzyme (ME), and fatty acid synthase (FAS) were lower ($p < 0.05$), but the activities of hepatic lipase (HL) and lipoprotein lipase (LPL) were higher ($p < 0.05$), in the liver and the muscle from the 3:1 and 6:1 groups compared with those in the 9:1 and 12:1 groups. Expression of the genes for FAS ($p < 0.01$), ME ($p < 0.01$) and ACC ($p < 0.05$) were higher in the muscle of groups fed diets with higher ω -6/ ω -3 PUFA ratios. Additionally, *in situ* hybridization tests showed that the expression intensities of the high density lipoprotein (*HDL-R*) gene in the 12:1 and 9:1 groups were significantly lower ($p < 0.01$) than that of the 3:1 group in the muscle of goslings. In conclusion, diets containing lower ω -6/ ω -3 PUFA ratios (3:1 or 6:1) could decrease fat deposition by inhibiting fat synthesis in goslings. (**Key Words:** ω -6/ ω -3 Polyunsaturated Fatty Acid, Lipid Metabolism, Goose)

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) not only are necessary nutrients for living organisms but also have important impacts on enzyme activity and gene expression (Sampath and Ntambi, 2005; Jump, 2008). PUFA-supplemented diets could regulate the activities of enzymes involved in lipid metabolism and inhibit the expression of genes related to fat synthesis, thus decreasing fat deposition (Clarke and Jump, 1994). Our previous study suggested that different ω -6/ ω -3 PUFA ratios had different effects on fat deposition in Yangzhou goslings (Wang et al., 2010). However, the underlying mechanism is not clearly understood.

Previous studies in humans have showed that an increase in the ω -6/ ω -3 PUFA ratio increase the risk for obesity. (Simopoulos, 2016). The higher ration of ω -6 PUFA could increase the membrane permeability, thus increase the cellular triglyceride (Ukropec et al., 2003). We hypothesized that the decreased fat deposition in PUFA-supplemented goslings might also be associated with changes in gene expression and enzyme activity related to fat metabolism. The activities of enzymes related to fat metabolism and the expression of genes critical for the synthesis and metabolism of lipids in gosling were examined in the present study. An *in situ* hybridization test was also conducted to measure the expression intensities of the high density lipoprotein receptor (*HDL-R*) and low density lipoprotein receptor (*LDL-R*) genes. The goal of our study was to provide a theoretical basis to help clarify the mechanisms controlling fat deposition in PUFA-supplemented gosling.

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MATERIALS AND METHODS

Animals and experimental design

This experiment was conducted at the Experimental Farm of Yangzhou University. All animal handling protocols were approved by the Yangzhou University Animal Care and Use Committee. One hundred and sixty 21-day-old healthy Yangzhou goslings (80 male and 80 female) of similar weight (0.407 ± 0.023 kg) were randomly divided into 4 groups. Each group contained 4 replicates of 10 birds each (5 males and 5 females). Birds in different groups were each fed a different diet (shown in Table 1) from day

21 to day 70. The four groups of goslings were raised in the same house with 16 small house (the ambient temperature is 25°C , humidity is 40%, stocking density is 10 birds per 3 m^2) and could have feed and water freely.

Birds were acclimated to their experimental diet over 7 days. The experimental diet made up 1/3 of the basal diet when the birds were 22 to 24 days old, 1/2 when they were 25 to 26 days old, and 2/3 when they were 27 to 28 days old. They were fed only the experimental diet after this point. Two birds (one male and one female) from each replicate of each group were randomly selected and slaughtered at the end of the experiment. All birds were fed in the same enclosure of one shelter. Additionally, no vaccinations were given throughout the experimental period.

Table 1. Composition and nutrient levels of the experimental diets (based on air-dried samples) (%)

Items	ω -6/ ω -3 PUFA ratio			
	12:1	9:1	6:1	3:1
Ingredients¹				
Corn	66.20	66.20	66.20	66.20
Soybean meal	17.30	17.30	17.30	17.30
Alfalfa powder	10.70	10.70	10.70	10.70
Peanut oil	1.30	1.28	1.16	1.06
Sunflower seed oil	0.16	0.15	0.16	0.08
Linseed oil	0.10	0.13	0.19	0.32
Palmitic acid	0.37	0.37	0.38	0.40
Oleic acid	0.07	0.07	0.11	0.14
CaH ₂ PO ₄	1.20	1.20	1.20	1.20
Limestone	0.60	0.60	0.60	0.60
L-Lys·HCl	0.35	0.35	0.35	0.35
Met	0.15	0.15	0.15	0.15
NaCl	0.50	0.50	0.50	0.50
Premix 1	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Nutrient levels (based on DM)²				
CP	14.34	14.34	14.34	14.34
Metabolic energy (MJ/kg)	11.70	11.70	11.70	11.70
Crude fiber	5.27	5.27	5.27	5.27
Ca	0.78	0.78	0.78	0.78
AP	0.40	0.40	0.40	0.40
SFA	0.67	0.67	0.66	0.66
MUFA	0.67	0.67	0.68	0.68
PUFA	0.66	0.67	0.65	0.66
n-6 PUFA	0.60	0.60	0.56	0.50
n-3 PUFA	0.05	0.07	0.09	0.16
ω -6/ ω -3 PUFA ratio	12.05:1	9.13:1	5.93:1	3.10:1

PUFA, polyunsaturated fatty acid; DM, dry matter; CP, crude protein; AP, available phosphorus; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, lactic acid.

¹ One kg of premix contains the following: vitamin A, 10,000 IU; vitamin D₃, 3,000 IU; vitamin E, 30 mg; vitamin K₃, 2 mg; vitamin B₁, 5 mg; vitamin B₂, 7 mg; vitamin B₆, 5 mg; vitamin B₁₂, 20 μg ; nicotinic acid, 38 mg; pantothenic acid, 9 mg; folic acid, 1 mg; biotin, 35 μg ; choline chloride, 6 g; Cu, 5 mg; I, 0.9 mg; Fe, 100 mg; Zn, 110 mg; Mn, 100 mg; Se, 0.15 mg; Co, 0.5 mg.

² LA, LNA and ω -6/ ω -3 PUFA ratio were all measured values, other values were calculated.

Composition of experimental diets

A maize-peanut meal diet was designed based on previous studies on this breed (Shi et al., 2007). The basal diet used maize, soyabean meal and Lucerne hay as raw material. We produced the experimental diets by mixing different amounts of 2% peanut oil, sunflower seed oil, linseed oil, palmitic acid and oleic acid into the basal diet to adjust the saturated fatty acid:monounsaturated fatty acid:polyunsaturated fatty acid (PUFA) ratio to 1:1:1 and the ratios of ω -6/ ω -3 PUFA to either 12:1, 9:1, or 6:1, 3:1. The composition and nutrient levels of the experimental diets are shown in Table 1.

Sample collection

Thirty-two geese were slaughtered on day 70. The liver and crureus were isolated and stored in liquid nitrogen before being analyzed for gene expression and enzyme activities.

Enzyme activity tests

Two grams of tissue were mixed with specific amounts of phosphate buffer saline (pH = 7.4) and fully homogenized at 2°C to 8°C . The homogenates were centrifuged at 2,000 to 3,000 rpm for 20 min, and the supernatant was collected for enzyme activity tests. Enzyme-linked immuno sorbent assay (ELISA) kits for acetyl-CoA carboxylase (ACC, No. H232), malic enzyme (ME, No. H233), and fatty acid synthase (FAS, No. H231) were purchased from Jiancheng Company (Nanjing, China). The experiment was conducted according to the kit's instructions. The correlation coefficients (R) of a linear regression between measured enzyme standards and expected concentrations were greater than 0.99. Coomassie brilliant blue assay kits (No. A045-2) were purchased from Jiancheng Company (Nanjing, China). The supernatant of each sample was diluted with normal saline to make a 1% solution, and protein content was measured according to the kit's instructions. Enzyme activity was expressed IU/mg of

tissue protein.

Test of hepatic lipase and lipoprotein lipase activity

Assay kits for hepatic lipase (HL assay kit, No. A067) and lipoprotein lipase (LPL, No. A068) were purchased from Jiancheng Company (Nanjing, China). The experiment was conducted according to the kit instructions. One activity unit was defined as the enzyme activity required to produce 1 mmol of free fatty acids per hour.

Extraction and reverse transcription of total RNA

Total RNA was extracted according to the instructions for the Trizol total RNA Extraction Reagent kit (TaKaRa Biotechnology Dalian Co., Ltd., Dalian, China). A spectrophotometer (NanoDrop 1000 spectrophotometer) was used to detect the purity and concentration of RNA. The 260/280 nm absorbance ratio was between 1.8 to 2.0 for all samples. Agarose gel electrophoresis was then used to determine RNA integrity. A 25 μ L reaction system was used to reverse transcribe RNA to cDNA.

mRNA expression analysis by real-time polymerase chain reaction

We used samples that were mixed at the same concentrations as the experimental samples to optimize the polymerase chain reaction (PCR) reaction conditions using different target genes, cycle numbers and proportions of the different internal control and target genes. The PCR reaction was carried out with an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR-Green real-time PCR Master Mix (TaKaRa Biotechnology Co. Ltd, China) according to the manufacturer's specifications. The housekeeping gene β -actin was used as the internal control (Table 2). The reaction system volume was 25 μ L and contained 2.5 μ L RT product and 12.5 μ L SYBR Green real-time PCR Master Mix. The final concentration of primers was 0.1 μ M. The parameters of the PCR reaction were as follows: Pre-denatured at 95°C for 5 min, 40 to 45 cycles of being denatured at 95°C for 30 s, annealed at 58°C to 61°C for 30 s, and extended at 72°C for 20 s. The results were expressed

as the $2^{-\Delta\Delta C(t)}$ values, where $\Delta\Delta C(t) = [C(t)_{ij} - C(t)\beta\text{-actin}_j] - [C(t)_{i1} - C(t)\beta\text{-actin}_1]$. $C(T)_{ij}$ and $C(T)\beta\text{-actin}_j$ refer to the objective gene (i) of the sample (j) and the housekeeping gene, respectively, and $C(T)_{i1}$ and $C(T)\beta\text{-actin}_1$ referred to the target gene and the housekeeping gene, respectively, of the control group. Each sample was run in triplicate, and ultrapure water was used as a negative control.

In situ hybridization test of mRNA for proteins involved in fat metabolism

In situ hybridization kits for HDL-R and LDL-R mRNA were purchased from Jiancheng Company (Nanjing, China) and used according to the kit instructions. Cells with brown granules or patches in the cytoplasm or nuclear membrane were considered positive for HDL-R or LDL-R expression. Samples were examined with an OLYMPUS microscope at 400 \times . From each sample, 5 complete and non-overlapping views were selected. The average number of positive cells out of 20 cells from each view were counted. The mean value of each slide was calculated and used as the value for the expression rate of the sample.

Statistical analysis

All statistical analyses were performed using SPSS 17.0. The significance of differences among treatments were evaluated by Duncan's multiple range test (Kapoor et al., 2004; Um et al., 2013). Data were analyzed using one-way analysis of variance. Statistical significance was set at $p < 0.05$. Data are presented as the mean \pm standard error of the mean.

RESULTS

Effects of ω -6/ ω -3 PUFA ratios on the activities of enzymes involved in fat metabolism

Table 3 shows that the activities of ACC in the liver and the crureus were highest ($p < 0.05$) in the 12:1 group. The 9:1 group had lower values than the 12:1 group ($p < 0.05$) but was still higher than the other groups. The lowest ($p < 0.05$) activities were in the 6:1 and 3:1 groups. Table 3 shows that the ME activity in the livers of the 12:1 and 9:1 groups

Table 2. Sequences of polymerase chain reaction primers

Gene name	ID in GeneBank	The sequence of primers (5' - 3')	Production (bp)
ACC	J03541	F: TCTCGCTTTATTATTGGTT R: CATTGTTGGCTATCAGGAC	312
ME	AF408407	F: GCTGCAATTGGTGGTGCTT R: ACTCTGCTTTGCTGGTAGGATTG	106
FAS	J04485	F: TGAAGGACCTTATCGCATTGC R: GCATGGGAAGCATTGTGTTGT	195
β -actin	L08165	F: TCGGTGACATCAAGGAGAAG R: TGCCAGGGTACATTGTGGTA	300

ACC, acetyl-CoA carboxylase; ME, malic enzyme; FAS, fatty acid synthase.

Table 3. The effects of ω -6/ ω -3 PUFA ratios on the activities of enzymes involved in fat metabolism in goslings (U/mg)

Item	Tissue	12:1	9:1	6:1	3:1	SEM
ACC	Liver	4.95 ^a	4.01 ^b	3.20 ^c	3.10 ^c	0.18
	Leg muscle	1.83 ^a	1.40 ^b	0.84 ^c	0.80 ^c	0.09
ME	Liver	4.30 ^a	3.86 ^{ab}	3.20 ^c	3.10 ^c	0.20
	Leg muscle	3.40 ^a	3.26 ^a	2.64 ^c	2.14 ^c	0.17
FAS	Liver	8.84 ^a	8.10 ^a	5.08 ^c	4.79 ^c	0.98
	Leg muscle	2.88 ^a	2.91 ^a	1.45 ^c	1.40 ^c	0.23
HL	Liver	56.77 ^c	64.17 ^{bc}	71.95 ^{ab}	75.68 ^a	3.79
	Leg muscle	2.35 ^c	3.01 ^{ab}	3.50 ^a	3.09 ^a	0.31
LPL	Liver	2.40 ^c	2.80 ^{bc}	3.07 ^{ab}	3.49 ^a	0.21
	Leg muscle	4.75 ^c	5.04 ^c	5.42 ^{bc}	6.21 ^a	0.35

PUFA, polyunsaturated fatty acid; SEM, standard error of the mean; ACC, acetyl-CoA carboxylase; ME, malic enzyme; FAS, fatty acid synthase; HL, hepatic lipase; LPL, lipoprotein lipase.

Values in the same row with the same superscript were not significantly different ($p > 0.05$); values in the same row with different superscripts were significantly different ($p < 0.05$); values with different superscripts were significantly different ($p < 0.01$).

were higher ($p < 0.05$) than in those of the 6:1 and 3:1 groups. The activity of FAS in the liver was higher than that in the crureus. The activities of FAS in the liver and crureus were higher ($p < 0.05$) in the 12:1 and 9:1 groups than they were in the 6:1 and 3:1 groups. However, activities of HL in the liver were highest in the 3:1 group and lowest in the 12:1 and 9:1 groups. The activity of HL in the crureus was only 1/20th of that in liver. The activity of HL in the liver and the crureus were higher in the 6:1 and 3:1 groups than in the 12:1 group ($p < 0.05$). In addition, the activity of LPL in the liver was higher ($p < 0.05$) in the 3:1 and 6:1 groups than ($p < 0.05$) in the 12:1 and 9:1 groups. The activity of LPL in the crureus was higher in the 3:1 group than ($p < 0.05$) in the 12:1, 9:1, and 6:1 groups.

Effects of ω -6/ ω -3 PUFA ratios on genes related to lipid metabolism

Table 4 shows that the gene expression of FAS, ACC, and ME, enzymes related to fat synthesis, were down-regulated with a decrease in the dietary ω -6/ ω -3 PUFA ratio. The gene expression of ACC in the 12:1 group was higher than in the other three groups ($p < 0.05$), whereas the gene

expression of ACC was significantly lower in the 3:1 group than in the other three groups ($p < 0.05$). Table 4 also shows that the expression of ME in the 12:1 and 9:1 groups was significantly higher than that in the 6:1 and 3:1 groups ($p < 0.01$). The expression of FAS in the 12:1 group was also significantly higher ($p < 0.01$) than that in the 6:1 and 3:1 groups.

The results of the *in situ* hybridization analysis of HDL-R mRNA (Figure 1 and Table 4) showed that the positive rates of HDL-R expression in the 12:1 and 9:1 groups were significantly lower ($p < 0.01$) than that of the 3:1 group. The positive expression rates in the 12:1 and 9:1 groups were different from that of the 6:1 group, but the difference was not significant ($p > 0.05$). The *in situ* hybridization images show that HDL-R labeling in the 12:1 and 9:1 groups was less intense than in the 6:1 and 3:1 groups. Figure 2 and Table 4 show that the positive rates of LDL-R expression between the different groups had a tendency to decrease with decreasing ω -6/ ω -3 PUFA ratios, but these differences were not statistically significant ($p > 0.05$).

DISCUSSION

Effects of ω -6/ ω -3 PUFA ratios on enzymes involved in fatty acid synthesis

The enzyme ACC is present in many organisms. It catalyzes a reaction that restricts the rate of the first stage of fatty acid synthesis. The rate of fatty acid synthesis in animals can change based on different nutritional and hormonal conditions, and ACC plays a major role in these changes (Kim and Tae, 1994; Chow et al., 2014). The current study showed that the activity of ACC in the liver was higher than that in muscle and that the activity of ACC in the liver of the 12:1 and 9:1 groups were both significantly higher than those in the other groups. The results showed that diets with a low ω -6/ ω -3 PUFA ratio may inhibit the activity of ACC in the liver and muscle.

The decarboxylation of malic acid catalyzed by ME is part of the citric acid-pyruvic acid cycle that enables acetyl-CoA to enter the cytosol from the mitochondria. The nicotinamide adenine dinucleotide phosphate (NADPH) that is produced is mainly used for fatty acid synthesis,

Table 4. The effects of ω -6/ ω -3 PUFA ratio on the expression genes critical to fat metabolism in goslings

Item	Tissue	12:1	9:1	6:1	3:1	SEM
ACC	Leg muscle	5.09 ^a	3.44 ^b	2.84 ^{bc}	2.39 ^c	0.33
ME	Leg muscle	5.90 ^a	4.86 ^{ab}	3.45 ^c	3.15 ^c	0.17
FAS	Leg muscle	7.62 ^a	6.92 ^{ab}	4.97 ^{cd}	3.98 ^d	0.89
HDL-R positive rates	Leg muscle	42.71 ^b	47.61 ^b	55.22 ^{ab}	58.79 ^a	1.35
LDL-R positive rates	Leg muscle	55.36	54.23	51.04	51.30	4.23

PUFA, polyunsaturated fatty acid; SEM, the standard error of the mean; ACC, acetyl-CoA carboxylase; ME, malic enzyme; FAS, fatty acid synthase; HDL-R, high density lipoprotein receptor; LDL-R, low density lipoprotein receptor.

Values in the same row with the same superscript were not significantly different ($p > 0.05$); values in the same row with different superscripts were significantly different ($p < 0.05$); values with different superscripts were significantly different ($p < 0.01$).

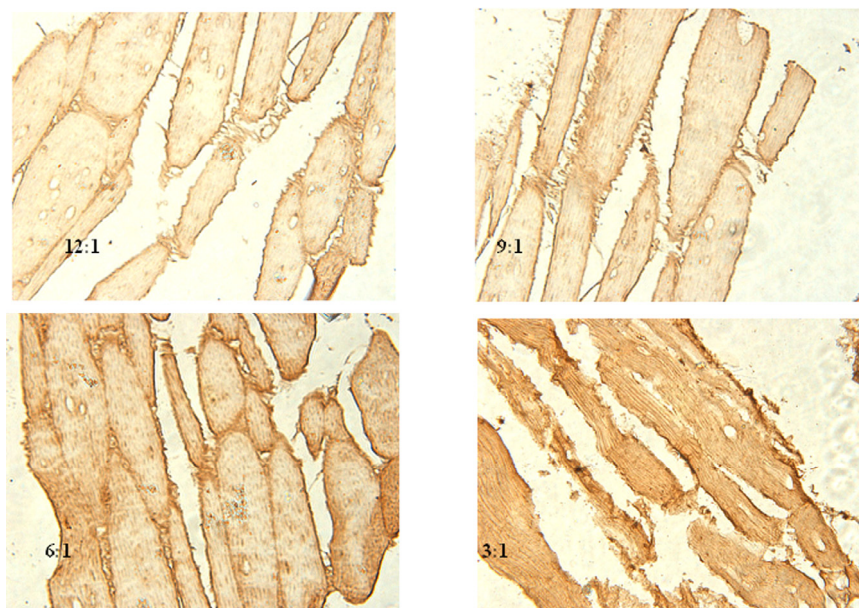


Figure 1. The gene expression of HDL-R in the leg muscle measured by *in situ* hybridization. Five to ten slides for each tissue were prepared, and images were taken using a binocular microscope (Olympus BX5; Olympus, Japan) coupled to a digital camera (Nikon H550L, Japan). The four images shown in Figure 1 were magnified 400 times. The *in situ* hybridization images show that the 12:1 and 9:1 groups had lower levels of labeling than the 6:1 and 3:1 groups. The positive rates of HDL-R expression in the 12:1 and 9:1 groups were significantly lower ($p < 0.01$) than that of the 3:1 group (shown in Table 2).

especially in birds, whose source of NADPH for fatty acid synthesis relies primarily on ME activity (Mourou et al., 2000). The present study showed that the difference between the activities of ME in liver and in muscle was not significant, and the effects of different diets on liver and muscle ME activity were similar. Rates in the 12:1 and 9:1

groups were significantly higher than those in the 6:1 and 3:1 groups. Our study also showed that diets with a low ω -6/ ω -3 PUFA ratio could inhibit the activity of ME.

The FAS catalyzes fatty acid synthesis. Mammalian FAS is an enzyme formed from multiple units with a molecular weight of 272 kDa (Guichard et al., 1992) and is

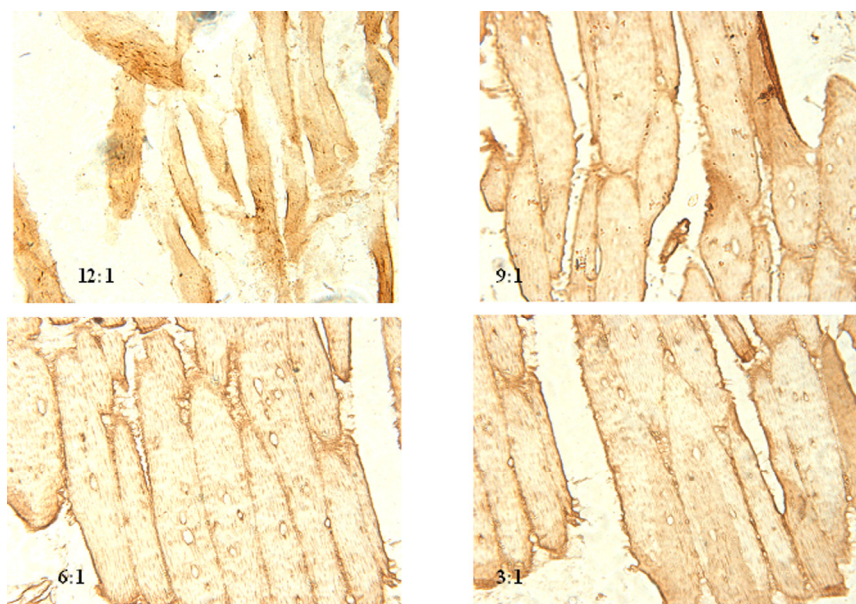


Figure 2. The gene expression of LDL-R in the leg muscle measured by *in situ* hybridization. Five to ten slides for each tissue were prepared, and images were taken using binocular microscope (Olympus BX5; Olympus, Japan) coupled to a digital camera (Nikon H550L, Japan). The four images shown in Figure 2 were all magnified 400. Figure 2 shows that the positive rates of LDL-R expression between the different groups had a tendency to decrease with decreasing ω -6/ ω -3 polyunsaturated fatty acid ratios (data shown in Table 2).

involved in the process of fatty acid synthesis. Previous studies have shown that PUFAs can inhibit the activities of the enzymes involved fatty acid synthesis (Foretz et al., 1999). The results of the present study suggest that a diet containing low ω -6/ ω -3 PUFA ratios (3:1 or 6:1) could inhibit this activity even more.

Effects of ω -6/ ω -3 PUFA ratios on enzymes involved in lipid metabolism

The HL is mainly involved in the metabolism of small lipoprotein particles such as very low density lipoprotein (VLDL) and chylomicron emulsion (CM) and the triglyceride (TG) component of VLDL residual grains. It modulates the reaction responsible for transferring cholesterol from the peripheral tissue to the liver where VLDL is converted to low density lipoprotein (LDL). The unesterified cholesterol accumulated in high density lipoprotein (HDL) is taken up by the liver with the help of HL. This could prevent the excess accumulation of cholesterol in the peripheral tissues of the liver (Connolly et al., 1999; Perret et al., 2002). The results of the present study showed that the activity of HL in the liver was more than 20 times that in the crureus. Feeding goslings with diets of different ω -6/ ω -3 PUFA ratios had significant effects. The 3:1 group had the highest HL activity suggesting that this diet promoted the absorption of VLDL and CM and of the TG component of VLDL residual grains, which may be beneficial to the health of the animal.

The LPL is synthesized in parenchymal cells in fat, myocardium, skeletal muscle and breast tissue. It also has functional similarities with HL. Its main physiological function is to catalyze the central TG of CM and VLDL and break the structure into fatty acids and monoglycerides for use in aerobic metabolism or for fat storage. In the present study, the activity of LPL in the crureus was higher than that in the liver. The ratios of ω -6/ ω -3 PUFA also had significant effects on LPL. The activity of LPL was highest in the group whose dietary ω -6/ ω -3 PUFA ratio was the lowest (3:1), and our results showed that this ratio improved the absorption of TG in the body. However, the results of the present study were different from those of a previous study (Zhang et al., 2009), where dietary n-6:n-3 PUFA ratios were 1.28, 5.03, 9.98, 68.62, which showed that the different n-6:n-3 PUFA ratios had no effect on the expression of LPL in the mouse liver. This might be due to difference in the species and the different n-6:n-3 PUFA ratios.

Studies have shown that PUFAs can have effects on the expression of genes (Van Deursen et al., 2009) and the activities of enzymes (Clarke, 2001) involved in fatty acid metabolism by affecting sterol regulatory element binding proteins (SREBPs) and peroxisome proliferator activated

receptors (PPARs). The present research also showed that the activities of enzymes involved in fatty acid synthesis and metabolism in groups with different ω -6/ ω -3 PUFA ratios in their diet were different, but the regulating mechanisms were still unknown. Therefore, we tested the expression of the genes involved in these processes.

Effects of ω -6/ ω -3 PUFA ratios on the expression of genes related to fat metabolism

The expression and activities of the genes involved in fatty acids metabolism, such as FAS, ACC, and ME, are closely related to the metabolism of fatty acids (Fang and Hillgartner, 1998), and their transcription has a common control element, which is directly affected by SREBP-1 (Richards et al., 2003). The expression levels of the 3 genes measured in the present study showed that the effects of different ω -6/ ω -3 PUFA ratios on these genes were similar, and our results agreed with those found in the chicken (Huang et al., 2007). In this study, the expression levels of enzymes involved in lipid metabolism, such as FAS, ME, and ACC, were higher in the groups fed diets with higher ω -6/ ω -3 PUFA ratios. This indicates that low ω -6/ ω -3 PUFA dietary ratios could inhibit lipid metabolism.

The HDL can transport the free cholesterol accumulated in peripheral tissues and the lipoproteins in circulation to cells in the liver and can accelerate the removal of cholesterol from cells, thus playing an important role in minimizing atherosclerosis (Khera et al., 2011; Mora et al., 2011). The quantity of HDL-R and its expression reflected the turnover rate and metabolism of HDL. In the present study, the expression of HDL-R was higher in the groups fed diets with lower ω -6/ ω -3 PUFA ratios than in those fed diets with higher ω -6/ ω -3 PUFA ratios. This increased the activity of HDL-R and accelerated the removal of HDL-cholesterol in the circulation.

The LDL is one of the main carriers of cholesterol in the blood. It primarily transports cholesterol to peripheral tissues. The quantity of LDL-R in cells correlates to the speed of LDL removal from the circulation. The sterol regulatory element is in the region of the LDL-R gene promoter 5 (Mora et al., 2011) and the cholesterol content of cells and affect this regulator and controls the synthesis of LDL receptors (Yang et al., 1995). The results of the present study showed a higher expression of LDL-R in the groups fed diets with higher ω -6/ ω -3 PUFA ratios. This might be because the lower ω -6/ ω -3 PUFA ratios inhibited the expression of LDL-R, and the opposite effect may have occurred in the high ratio groups. Thus, the LDL-R activity in groups fed diets with high ω -6/ ω -3 PUFA ratios increased. This may promote the expression of LDL-R, thereby clearing LDL-C and accelerating lipid metabolism. However, the differences between the groups were not

significant. The reasons for the observed LDL-R results were not clear in the current work; further studies are needed to elucidate the specific mechanisms.

CONCLUSION

The activities of the enzymes involved in fatty acid metabolism, such as ACC, ME, and FAS, were lower, but the activities of enzymes in the turnover of fat, such as HL and LPL, were higher, in groups fed diets with lower ω -6/ ω -3 PUFA ratios (3:1 and 6:1). The expression levels of genes involved in fatty acid metabolism, such as FAS, ME, and ACC, were higher in groups fed diets with higher ω -6/ ω -3 PUFA ratios. The expression of HDL-R in groups fed diets with lower ω -6/ ω -3 PUFA ratios was higher than those fed diets with higher ω -6/ ω -3 PUFA ratios, whereas the opposite pattern was observed in the expression of LDL-R. In conclusion, diets containing lower a ω -6/ ω -3 PUFA ratios (3:1 or 6:1) could decrease fat deposition in goslings by inhibiting fat synthesis

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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