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Changes in the triglyceride metabolism in the thigh muscle and liver of broilers injected intravenously with angiopoietin-like protein 4

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Abstract: Angiopoietin-like protein 4 (ANGPTL4) is a potential circulating mediator connecting nutritional factors and fat metabolism, however, information is lacking on the exact role of ANGPTL4 on triglyceride metabolism in the thigh muscles and livers of broilers. The objective of this study was to determine the changes in the triglyceride metabolism in the thigh muscles and livers of broilers injected intravenously with ANGPTL4. In experiment 1, 36 male Arbor Acres broilers at 35 days of age were randomly allocated into six treatments with six replicates. The broilers were subjected to intravenous injection of polyhistidine-small ubiquitin-related modifier-ANGPTL4 (His-SUMO-ANGPTL4) once at a dose of 0, 20, 100, 500, 2 500, or 12 500 ng/kg body weight (BW), respectively. The results showed that the injection of His-SUMO-ANGPTL4 at a dose of 500, 2 500 and 12 500 ng/kg BW decreased ($P < 0.05$) the broilers' heart-fatty acid-binding protein (H-FABP) mRNA expression in the thigh muscle. All of the His-SUMO-ANGPTL4 broiler injected groups had a lower ($P < 0.05$) adipocyte-fatty acid-binding protein mRNA expression in the thigh muscle. In experiment 2, 18 male Arbor Acres broilers at 35 days of age were randomly allocated into three treatments with six replicates. The broilers were given an injection of normal saline (NS), His-SUMO, or His-SUMO-ANGPTL4 (500 ng/kg BW) once. The results showed that the broilers receiving an injection of ANGPTL4 at a dose of 500 ng/kg BW decreased ($P < 0.05$) the H-FABP mRNA expression in the thigh muscle. Besides, the broilers given an injection of ANGPTL4 at a dose of 500 ng/kg BW increased ($P < 0.05$) the fatty acid synthase mRNA expression and activity and decreased ($P < 0.05$) the microsomal triglyceride transfer protein mRNA expression in the liver. However, the concentrations of the high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, insulin, growth hormone and leptin in the serum were not affected by the ANGPTL4 injection. In conclusion, ANGPTL4 has the ability to change the triglyceride metabolism in the thigh muscles and livers of broilers.

Keywords: biochemical parameters; chicken; hormone; lipid metabolism-related enzyme; recombinant chicken angiopoietin-like protein 4

With the wide use of feed additives and the continuous improvement of the feed nutrition level, the fat deposition of broilers increases day by day. However, the mediator and its mechanism between the nutritional factors and the fat metabolism of broilers are not yet clear. In order to promote the fat deposition of broilers and improve the production performance of broilers, it is very important to reveal the mechanism of the nutritional factors regulating the fat metabolism in broilers,

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thus, looking for the medium connecting the nutritional factors and the fat metabolism in broilers is one of the important links.

Angiopoietin-like protein 4 (ANGPTL4), also known as the peroxisome proliferator-activated receptor γ angiopoietin-related protein or fasting-induced adipose factor, is a kind of secreted protein with high biological activity (Alex et al. 2013; Altun et al. 2018). Studies in mice and humans have found that ANGPTL4 is closely related to the fat metabolism, and factors such as microorganisms, fasting and high fat feeding can affect its secretion (Mandard et al. 2004; Grootaert et al. 2012). However, the ANGPTL4 sequence on chickens has a low homology with other species, and information is lacking on the exact role of ANGPTL4 on the fat metabolism in broilers. Previous studies have found that ANGPTL4 can regulate the breast muscle triglyceride metabolism in broilers (Zhao et al. 2021). In addition, some studies in recent years have shown that the regulation of intestinal microorganisms on the triglyceride metabolism in the thigh muscles and livers of broilers is accompanied by changes in the secretion of ANGPTL4 (Zhao et al. 2013; Zhao et al. 2017; Zhao et al. 2018). Therefore, ANGPTL4 may play a role in regulating the triglyceride metabolism in the thigh muscle (such as lipodieresis and lipid uptake) and liver (such as lipogenesis and lipid transfer) of broilers, and can be used as a medium to connect the nutritional factors and fat metabolism of broilers. In order to verify this hypothesis, this experiment studied the changes in the triglyceride metabolism in the thigh muscles and livers of broilers injected intravenously with angiopoietin-like protein 4.

MATERIAL AND METHODS

Ethical approval

The animal care and use protocol was approved by the Animal Care and Use Committee of the Linyi University (Linyi, Shandong, China).

Description of recombinant chicken ANGPTL4

Recombinant chicken ANGPTL4 was produced by the *Escherichia coli* expression system and the target gene encoding Ala19-Pro478 is expressed with a polyhistidine-small ubiquitin-related modifier (His-SUMO) tag at the N-terminus.

Birds and treatments

One-day-old male Arbor Acres broiler chicks were obtained from a commercial hatchery and housed in an environmentally controlled room. The feeding management, ingredients and nutrient composition of the experimental diets were the same as described by Zhao et al. (2021).

Experiment 1: at 35 days of age, 36 healthy starving broilers (fasted for 12 h with access to water) with a similar body weight (BW) (2.17 ± 0.03 kg) were randomly divided into six groups of six birds. Broilers in group 1 were intravenously injected (inferior pterygoid vein) with normal saline (NS, 0.75%) once, and the broilers in groups 2–6 were intravenously injected with 20, 100, 500, 2 500 and 12 500 ng/kg BW of the recombinant chicken ANGPTL4 (His-SUMO-ANGPTL4) once (denoted as the control, ANGPTL4 20, ANGPTL4 100, ANGPTL4 500, ANGPTL4 2 500, and ANGPTL4 12 500, respectively).

Experiment 2: at 35 days of age, 18 healthy starving broilers (fasted for 12 h with access to water) with a similar BW (2.18 ± 0.04 kg) were randomly divided into three groups of six birds. The three groups of broilers were intravenously injected (inferior pterygoid vein) with NS, His-SUMO and His-SUMO-ANGPTL4 once (recombinant chicken ANGPTL4 at a dose of 500 ng/kg BW), respectively.

Sample collection

Blood samples were taken from the inferior pterygoid vein of all the birds at 30 min after the intravenous injection using sterilised needles and non-heparinised tubes. The serum of the blood samples was collected as described by Zhao et al. (2013) and was then stored in 0.5-ml Eppendorf tubes at −20 °C. After the blood collection, all the broilers were immediately deeply anesthetised with pentobarbital sodium (30 mg/kg BW, *i.v.*) and slaughtered by exsanguination. Tissue samples were obtained from the thigh muscle and the liver of each broiler. Some of the samples were quickly put into an RNAfixer (RP1302, BioTeke Co. Ltd, Beijing, China), and after being kept at 4 °C overnight, they

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were transferred to the condition of –20 °C for long-term preservation for the total RNA extraction. The other samples were washed with ice-cold NS, quickly frozen in liquid nitrogen, and then stored at –40 °C to determine the enzyme activity.

Real-time quantitative PCR analysis of gene expression

The real-time quantitative polymerase chain reaction (PCR) analysis of the gene expression in the thigh muscle and liver was carried out according to the method described by Zhao et al. (2016). The primer sequences of the fatty acid transport protein 1 (FATP1), heart-fatty acid-binding protein (H-FABP), adipocyte fatty acid-binding protein (A-FABP), adipose triglyceride lipase (ATGL), carnitine palmitoyltransferase 1 (CPT1), carnitine palmitoyltransferase 2 (CPT2), lipoprotein lipase (LPL) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were the same as those described by Zhao et al. (2021). The primer sequences of the fatty acid synthase (FAS), malic enzyme (ME), acetyl-CoA carboxylase (ACC), apolipoprotein B (ApoB) and microsomal triglyceride transfer protein (MTTP) were the same as those described by Zhao et al. (2016).

Enzyme activity assays

The activities of the LPL (EC 3.1.1.34) and hormone-sensitive lipase (HSL, EC 3.1.1.79) in the thigh muscle and the activities of the FAS (EC 2.3.1.85) and ACC (EC 6.4.1.2) in the liver was assayed using the same procedure as described by Zhao et al. (2013).

Determination of the serum biochemical parameters and serum hormones levels

The contents of the high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) in the serum were determined by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

The serum insulin (INS), growth hormone (GH) and leptin (LEP) were measured by a radioimmunoassay (RIA) (Beijing North Institute of Biological Technology, Beijing, China).

Data calculations and statistical analysis

All data were analysed by an analysis of variance (ANOVA) in the Statistical Analysis System (SAS) v9.4 software (SAS Institute, Inc., Cary, NC, USA), and multiple comparisons were made by Duncan's multiple-range test. Orthogonal polynomial contrasts were used to determine the linear and quadratic responses of the broilers to the His-SUMO-ANGPTL4 levels. $P < 0.05$ representing the significant difference.

RESULTS

Different recombinant chicken ANGPTL4 levels on the lipid metabolism-related gene expression and enzyme activity in the thigh muscles of the broilers

As the levels of recombinant chicken ANGPTL4 increased from 0 to 12 500 ng/kg BW, the H-FABP and A-FABP mRNA expression in the thigh muscles of the broilers decreased quadratically ($P < 0.05$) (Table 1). The H-FABP mRNA expression in the thigh muscles of the broilers in ANGPTL4 500, ANGPTL4 2 500 and ANGPTL4 12 500 were lower ($P < 0.05$) than those of the broilers in the control, ANGPTL4 20 and ANGPTL4 100, and the thigh muscles of the broilers in ANGPTL4 500 had the lowest H-FABP mRNA expression. The broilers of all of the recombinant chicken ANGPTL4 injected groups had a lower ($P < 0.05$) A-FABP mRNA expression in the thigh muscles than those of the control group broilers. However, no difference in the A-FABP mRNA expression was observed among the recombinant chicken ANGPTL4 injected groups. Besides, the FATP1, ATGL, CPT1, CPT2 and LPL mRNA expression and the LPL and HSL activities in the thigh muscles of the broilers were not significantly affected by the injection of the recombinant chicken ANGPTL4 (Table 1, Table 2).

Angiopoietin-like protein 4 on the serum biochemical parameters and hormone levels of the broilers

As shown in Table 3 and Table 4, no differences in the concentrations of the HDL, LDL, VLDL,

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Table 1. Effect of the different recombinant chicken ANGPTL4 levels on the lipid metabolism-related gene expression in the thigh muscles of the broilers[1]

Item	Recombinant chicken ANGPTL4 (ng/kg)						SEM	*P*-value	Linear	Quadratic
	0	20	100	500	2 500	12 500				
Fatty acid transport protein 1	1.00	0.93	1.01	1.02	1.19	1.04	0.150	0.914 9	0.768 1	0.496 4
Heart-fatty acid-binding protein	1.00[a]	0.94[a]	1.06[a]	0.25[c]	0.51[bc]	0.59[b]	0.097	< 0.000 1	0.188 1	0.024 3
Adipocyte-fatty acid-binding protein	1.00[a]	0.48[b]	0.63[b]	0.57[b]	0.36[b]	0.58[b]	0.103	0.003 9	0.499 1	0.048 7
Adipose triglyceride lipase	1.00	1.09	0.99	0.89	0.86	0.78	0.106	0.412 7	0.071 0	0.122 8
Carnitine palmitoyltransferase 1	1.00	0.90	0.94	0.73	0.84	0.74	0.090	0.221 4	0.120 3	0.249 8
Carnitine palmitoyltransferase 2	1.00	0.81	0.92	0.90	0.79	0.83	0.131	0.890 7	0.598 4	0.680 9
Lipoprotein lipase	1.00	1.21	1.02	1.06	0.78	0.96	0.223	0.846 3	0.660 4	0.473 3

[1]Data are means for six chickens

[a–c]Means within a row with different letters differ significantly ($P < 0.05$)

Table 2. Effect of the different recombinant chicken ANGPTL4 levels on the lipid metabolism-related enzyme activities in the thigh muscles of the broilers[1]

Item	Recombinant chicken ANGPTL4 (ng/kg)						SEM	*P*-value	Linear	Quadratic
	0	20	100	500	2 500	12 500				
Lipoprotein lipase (IU/mg prot)	0.30	0.33	0.29	0.29	0.31	0.27	0.020	0.520 3	0.141 5	0.314 8
Hormone-sensitive lipase (IU/mg prot)	1.28	1.32	1.22	1.21	1.32	1.65	0.181	0.558 6	0.047 8	0.144 3

[1]Data are means for six chickens

Table 3. Effect of the recombinant chicken ANGPTL4 on the serum biochemical parameters of the broilers[1]

Item	NS	His-SUMO	His-SUMO-ANGPTL4	SEM	*P*-value
High-density lipoprotein (mmol/l)	2.50	2.32	2.30	0.118	0.392 6
Low-density lipoprotein (mmol/l)	0.74	0.64	0.62	0.075	0.449 8
Very low-density lipoprotein (μmol/l)	39.22	48.03	47.57	4.339	0.295 3

[1]Data are means for six chickens

Table 4. Effect of the recombinant chicken ANGPTL4 on the serum hormone levels of the broilers[1]

Item	NS	His-SUMO	His-SUMO-ANGPTL4	SEM	*P*-value
Insulin (μIU/ml)	4.63	5.61	5.87	0.461	0.158 7
Growth hormone (ng/ml)	3.40	3.06	3.40	0.185	0.345 0
Leptin (ng/ml)	1.25[a]	0.65[b]	0.61[b]	0.081	< 0.000 1

[1]Data are means for six chickens

[a,b]Means within a row with different letters differ significantly ($P < 0.05$)

INS and GH in the serum was observed among the NS, His-SUMO and His-SUMO-ANGPTL4 injected birds. The broilers had a lower ($P < 0.05$) LEP level in the serum than that of NS injection broilers after the His-SUMO or His-SUMO-ANGPTL4 injection, however, no difference in the LEP level in the serum was observed between the His-SUMO and His-SUMO-ANGPTL4 injected birds.

Angiopoietin-like protein 4 on the lipid metabolism-related gene expression and enzyme activity in the thigh muscles of broilers

As shown in Figure 1 and Figure 2, no differences in the mRNA expression of the FATP1, ATGL, CPT1, CPT2 and LPL and in the activities

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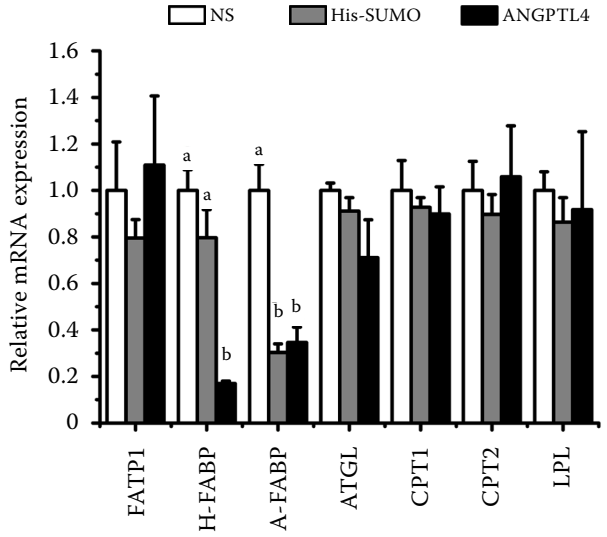

Figure 1. Effect of ANGPTL4 (500 ng/kg BW, *i.v.*) on the lipid metabolism-related gene expression in the thigh muscles of the broilers

[a,b]Means with different letters differ significantly ($P < 0.05$)

At 30 min after the intravenous injection, the fatty acid transport protein 1 (FATP1), heart-fatty acid-binding protein (H-FABP), adipocyte-fatty acid-binding protein (A-FABP), adipose triglyceride lipase (ATGL), carnitine palmitoyltransferase 1 (CPT1), carnitine palmitoyltransferase 2 (CPT2), and lipoprotein lipase (LPL) mRNA expression in the thigh muscles were measured for the normal saline (NS), His-SUMO, or His-SUMO-ANGPTL4 (ANGPTL4). The value of each treatment is the mean of six chickens, and the vertical bar represents the standard error

of the HSL and LPL in the thigh muscles was observed among the NS, His-SUMO and His-SUMO-ANGPTL4 injected birds. The broilers had a lower ($P < 0.05$) H-FABP mRNA expression in the thigh muscles than those of the NS or His-SUMO injection broilers after the His-SUMO-ANGPTL4 injection, however, no difference in the H-FABP mRNA expression in the thigh muscle was observed between the NS and His-SUMO injected birds. The broilers had a lower ($P < 0.05$) A-FABP mRNA expression in the thigh muscles than those of the NS injection broilers after the His-SUMO or His-SUMO-ANGPTL4 injection, however, no difference in the A-FABP mRNA expression in the thigh muscles was observed between the His-SUMO and His-SUMO-ANGPTL4 injected birds.

Angiopoietin-like protein 4 on the lipid metabolism-related gene expression and enzyme activity in the livers of broilers

As shown in Figure 3 and Figure 4, no difference in the mRNA expression of the ACC and ApoB and the activity of the ACC in the livers was observed among the NS, His-SUMO and His-SUMO-ANGPTL4 injected birds. The broilers had a higher ($P < 0.05$) FAS mRNA expression and activity in the livers than those of NS or His-SUMO injected broilers after the His-SUMO-ANGPTL4 injection, however, no difference in the FAS mRNA expres-

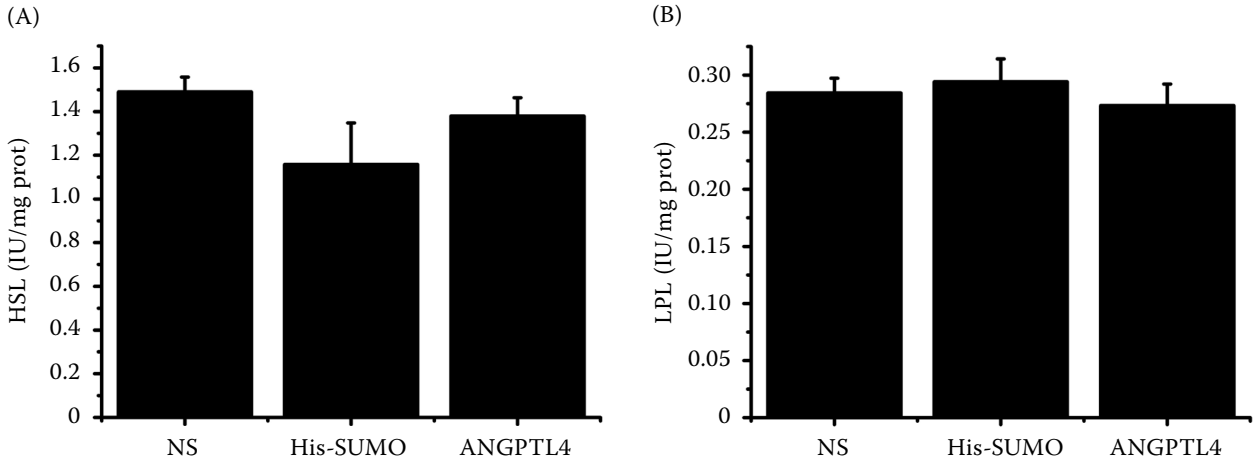

Figure 2. Effect of ANGPTL4 (500 ng/kg BW, *i.v.*) on the lipid metabolism-related enzyme activities in the thigh muscles of the broilers

At 30 min after the intravenous injection, the hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL) activities in the thigh muscles were measured for the normal saline (NS), His-SUMO, or His-SUMO-ANGPTL4 (ANGPTL4). The value of each treatment is the mean of six chickens, and the vertical bar represents the standard error

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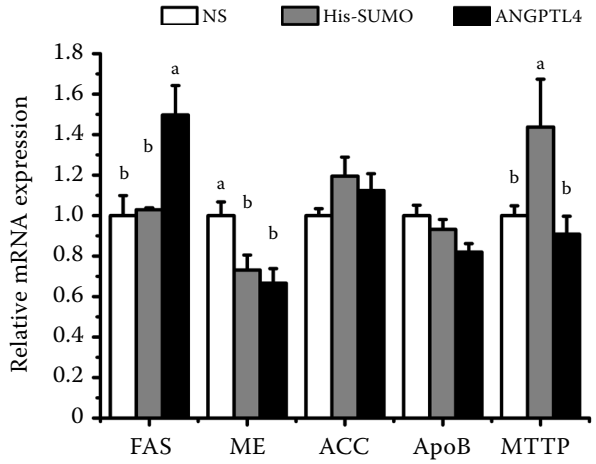

Figure 3. Effect of ANGPTL4 (500 ng/kg BW, *i.v.*) on the lipid metabolism-related gene expression in the livers of the broilers

[a,b]Means with different letters differ significantly ($P < 0.05$)

At 30 min after the intravenous injection, the fatty acid synthase (FAS), malic enzyme (ME), acetyl-CoA carboxylase (ACC), apolipoprotein B (ApoB), and microsomal triglyceride transfer protein (MTTP) mRNA expression in the livers were measured for the normal saline (NS), His-SUMO, or His-SUMO-ANGPTL4 (ANGPTL4). The value of each treatment is the mean of six chickens, and the vertical bar represents the standard error

sion and activity in the liver was observed between the NS and His-SUMO injected birds. The broilers had a lower ($P < 0.05$) ME mRNA expression in the livers than that of the NS injected broilers after the His-SUMO or His-SUMO-ANGPTL4 injection, however, no difference in the ME mRNA expression in the livers was observed between the His-SUMO and His-SUMO-ANGPTL4 injected birds. The His-SUMO-ANGPTL4 injected broilers had a lower ($P < 0.05$) MTTP mRNA expression in the livers than those of the His-SUMO injected birds, however, no difference in the MTTP mRNA expression in the livers was observed between the NS and His-SUMO-ANGPTL4 injected birds.

DISCUSSION

Effect of the angiopoietin-like protein 4 on the lipid metabolism-related gene expression and enzyme activity in the thigh muscles of the broilers

Lipid metabolism and its regulation in broilers are quite different from those in mammals. Fat in broilers mainly comes from the synthesis in the liver and the digestion and absorption in the intestine, while the synthesis of fat in the thigh muscle is very limited. Therefore, it is more important to study the process of lipodieresis and the lipid uptake in the thigh muscles of broilers. Hormone-sensitive lipase and ATGL are the key enzymes that catalyse the hydrolysis of TG stored in adipose tissue into free fatty acid and glycerol (Zimmermann et al. 2004; Chen et al. 2018). Fatty acid β-oxidation oc-

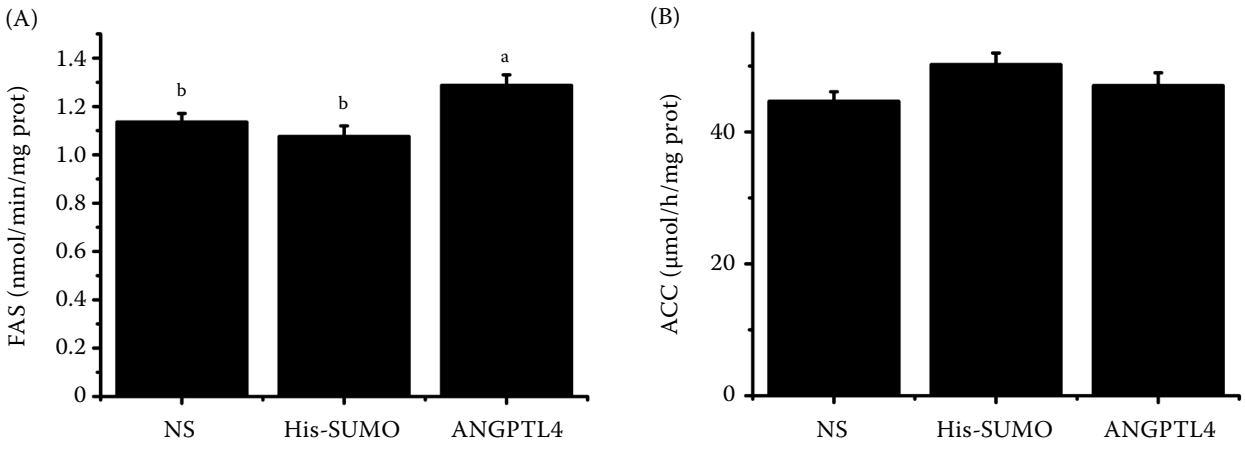

Figure 4. Effect of ANGPTL4 (500 ng/kg BW, *i.v.*) on the lipid metabolism-related enzyme activities in the livers of the broilers

[a,b]Means with different letters differ significantly ($P < 0.05$)

At 30 min after the intravenous injection, the fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) activities in the livers were measured for the normal saline (NS), His-SUMO, or His-SUMO-ANGPTL4 (ANGPTL4). The value of each treatment is the mean of six chickens, and the vertical bar represents the standard error

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curs mainly in the mitochondria, and CPT1 and CPT2 play important roles in the process of long-chain fatty acids (LCFAs) crossing the mitochondrial membranes (Ge et al. 2019). Therefore, HSL, ATGL, CPT1 and CPT2 are the four major enzymes involved in lipolysis. The similar mRNA expression of ATGL, CPT1 and CPT2 and activity of HSL in the thigh muscles among all the groups of broilers in this study indicated that the intravenous injection of ANGPTL4 had no effect on the lipodieresis in the thigh muscles of the broilers.

Fatty acid transport protein 1, H-FABP and A-FABP have been proven to facilitate the LCFA uptake and utilisation in the skeletal muscles (Tunim et al. 2021). Besides, some studies have reported that the expression of H-FABP and A-FABP were closely related to the deposition of the intramuscular fat in broilers (Li et al. 2008; Ye et al. 2009). Lipoprotein lipase catalyses the hydrolysis of TG carried by circulating chylomicrons and VLDL in the blood into glycerol and fatty acids, thus providing raw materials for the TG synthesis to the adipose tissue and promoting fat deposition (Cai et al. 2009; Ge et al. 2019). The results of this study showed that the H-FABP and A-FABP mRNA expression in the thigh muscles quadratically decreased by the His-SUMO-ANGPTL4 intravenous injection at levels up to 12 500 ng/kg BW. The results demonstrated that the broilers' intravenous injection of His-SUMO-ANGPTL4 had a lower H-FABP and A-FABP mRNA expression than the control, indicating that ANGPTL4 may play a role in reducing the uptake and utilisation of LCFA. To further confirm this idea, we used the His-SUMO tag as a negative control in order to rule out the tag effect on the thigh muscle lipid metabolism regulation. The lower thigh muscle H-FABP mRNA expression in the group injected with His-SUMO-ANGPTL4 in this study indicated ANGPTL4 indeed plays an important role in the thigh muscle H-FABP mRNA expression regulation. However, the ability of His-SUMO-ANGPTL4 and His-SUMO to stimulate the thigh muscle A-FABP mRNA expression indicated that the decrease in the A-FABP mRNA expression in the thigh muscle was caused by His-SUMO tag in His-SUMO-ANGPTL4, but not by ANGPTL4 itself.

Studies on the effects of ANGPTL4 on the lipid metabolism-related gene expression and enzyme activity in the skeletal muscles of animals are mostly focused on mice, and no relevant reports have been found on broilers. Mandard et al. (2006) observed that the ATGL mRNA expression in the ANGPTL4 transgenic mice increased by 50%. Greiner and Backhed (2011) reported that ANGPTL4 not only inhibited the activity of LPL, but also increased the lipolysis of the skeletal muscles. However, our study found that ANGPTL4 regulates the lipid metabolism in the thigh muscles of broilers by decreasing the LCFA uptake and utilisation in the thigh muscles of broilers. The discrepancy among these studies may be attributed to several factors such as the animal species and physiological stages and the increase in ANGPTL4.

Effect of the angiopoietin-like protein 4 on the serum biochemical parameters and hormone levels of the broilers

Most of the lipid transport in the blood is in the form of lipoproteins. High-density lipoproteins are mainly involved in the reverse transport of TC. Low-density lipoproteins are mainly involved in the transport of endogenous TC. Very low-density lipoproteins are mainly associated with the transport of endogenous TG and TC (Ohkawa et al. 2020). The similar HDL, LDL and VLDL in the serum among all the groups of broilers in this study indicated that the intravenous injection of ANGPTL4 had no effect on the serum lipid transport.

Insulin, GH and LEP are important hormones involved in the fat metabolism in broilers. Insulin can promote fat deposition in broilers, while GH and LEP can reduce fat deposition in broilers (Zhao et al. 2013). In this study, the serum LEP levels of the broilers injected with His-SUMO-ANGPTL4 and His-SUMO were significantly lower than those of the broilers injected with NS, but there was no significant difference in the serum LEP levels between the broilers injected with His-SUMO-ANGPTL4 and the broilers injected with His-SUMO. The results indicated that the decrease in the serum LEP level was caused by His-SUMO tag in recombinant chicken ANGPTL4, but not by ANGPTL4 itself.

Studies on the effects of ANGPTL4 on the blood biochemical parameters and hormone levels of animals are mostly focused on mice, and no relevant reports have been found on broilers. Ge et al. (2004) observed that adenovirus-mediated

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overexpression of ANGPTL4 caused an increase in the plasma VLDL levels in mice. Koster et al. (2005) reported that there were no significant differences in the plasma insulin and leptin in the fed or fasted states between ANGPTL4 transgenic mice and wild-type mice. Mandard et al. (2006) observed that the plasma VLDL and HDL levels in 6 h fasted ANGPTL4 transgenic mice were significantly higher than those of the wild-type mice. Lichtenstein et al. (2007) reported that the plasma levels of HDL, LDL and insulin in 24 h fasted ANGPTL4 transgenic mice were not significantly different from those in the wild-type mice. Singh et al. (2018) observed that the plasma HDL level was similar in the wild-type mice and brown adipose tissue conditional ANGPTL4 knockout mice. The discrepancy among these studies may be attributed to several factors such as the animal species and physiological stages, duration of fasting, and the increase in ANGPTL4.

Effect of the angiopoietin-like protein 4 on the lipid metabolism-related gene expression and enzyme activity in the livers of the broilers

For broilers, *de novo* lipogenesis mainly occurs in the liver, and most of the endogenous lipids are derived from the liver. It is well recognised that FAS, ME, and ACC are three main indicators that reflect the *de novo* lipogenesis (Zhao et al. 2016). The higher liver FAS mRNA expression and activity of the broilers injected with His-SUMO-ANGPTL4 in this study indicated that ANGPTL4 could improve the ability of broiler's liver to catalyse acetyl-CoA and malonyl-CoA to produce LCFA. However, the ability of His-SUMO-ANGPTL4 and His-SUMO to weaken the thigh muscle ME mRNA expression indicated that the decrease in the ME mRNA expression in the thigh muscle was caused by the His-SUMO tag in the recombinant chicken ANGPTL4, but not by ANGPTL4 itself.

The TG content in the liver is the net product of the hepatic lipogenesis and lipid transfer. A large number of studies have shown that ApoB and MTTP are two main indicators of lipid transfer (Han et al. 2011; Wang et al. 2013). The higher thigh muscle MTTP mRNA expression of the broilers injected with His-SUMO in this study indicated ANGPTL4 plays an important role in inhibiting the TG delivery to the nascent apoB molecules during the assembly of the lipoprotein particles.

Information on the effect of ANGPTL4 on the lipid metabolism in the liver of broilers is lacking. Xu et al. (2005) and Mandard et al. (2006) reported that the adenovirus-mediated expression of ANGPTL4 induced fatty liver and hepatomegaly in mice. They concluded that ANGPTL4 can regulate lipid metabolism in the liver of animal. The liver fat content is the net outcome of lipogenesis and the lipid transfer in the liver. The results of the fatty liver and hepatomegaly in ANGPTL4 transgenic mice indicated that ANGPTL4 has the effect of leading to higher hepatic lipogenesis than hepatic lipid transfer in animals. This is consistent with the ANGPTL4 effects on the lipid metabolism-related gene expression and enzyme activity in the livers of the broilers in this study, suggesting that ANGPTL4 may induce hepatic steatosis in broilers by increasing hepatic lipogenesis and decreasing the hepatic lipid transfer in broilers.

CONCLUSION

In conclusion, we demonstrated that ANGPTL4 could reduce the LCFA uptake and utilisation in the thigh muscles, increase the hepatic fat synthesis and decrease the transport of hepatic fat in broilers. This finding raises an interesting possibility that ANGPTL4 might play an important role in regulating the intramuscular fat in the thigh muscles and fat deposition in the livers of broilers.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Alex S, Lange K, Amolo T, Grinstead JS, Haakonsson AK, Szalowska E, Koppen A, Mudde K, Haenen D, Al-Lahham S, Roelofsen H, Houtman R, van der Burg B, Mandrup S, Bonvin AM, Kalkhoven E, Muller M, Hooiveld GJ, Kersten S. Short-chain fatty acids stimulate angiopoietin-like 4 synthesis in human colon adenocarcinoma

https://doi.org/10.17221/134/2021-CJAS

cells by activating peroxisome proliferator-activated receptor γ. Mol Cell Biol. 2013 Apr;33(7):1303-16.

Altun O, Dikker O, Arman Y, Ugurlukisi B, Kutlu O, Ozgun Cil E, Aydin Yoldemir S, Akarsu M, Ozcan M, Kalyon S, Ozsoy N, Tukek T. Serum Angiopoietin-like peptide 4 levels in patients with hepatic steatosis. Cytokine. 2018 Nov 1;111:496-9.

Cai Y, Song Z, Zhang X, Wang X, Jiao H, Lin H. Increased de novo lipogenesis in liver contributes to the augmented fat deposition in dexamethasone exposed broiler chickens (Gallus gallus domesticus). Comp Biochem Physiol C Toxicol Pharmacol. 2009 Aug 1;150(2):164-9.

Chen G, Gao Z, Chu W, Cao Z, Li C, Zhao H. Effects of chromium picolinate on fat deposition, activity and genetic expression of lipid metabolism-related enzymes in 21 day old Ross broilers. Asian-Australas J Anim Sci. 2018 Apr;31(4):569-75.

Ge H, Yang G, Yu X, Pourbahrami T, Li C. Oligomerization state-dependent hyperlipidemic effect of angiopoietin-like protein 4. J Lipid Res. 2004 Nov;45(11):2071-9.

Ge XK, Wang AA, Ying ZX, Zhang LG, Su WP, Cheng K, Feng CC, Zhou YM, Zhang LL, Wang T. Effects of diets with different energy and bile acids levels on growth performance and lipid metabolism in broilers. Poult Sci. 2019 Feb 1;98(2):887-95.

Greiner T, Backhed F. Effects of the gut microbiota on obesity and glucose homeostasis. Trends Endocrinol Metab. 2011 Apr 1;22(4):117-23.

Grootaert C, Van de Wiele T, Verstraete W, Bracke M, Vanhoecke B. Angiopoietin-like protein 4: Health effects, modulating agents and structure-function relationships. Expert Rev Proteomics. 2012 Apr 1;9(2):181-99.

Han CC, Wang JW, Pan ZX, Tang H, Xiang SX, Wang J, Li L, Xu F, Wei SH. Effect of liver X receptor activation on the very low density lipoprotein secretion and messenger ribonucleic acid level of related genes in goose primary hepatocytes. Poult Sci. 2011 Feb;90(2):402-9.

Koster A, Chao YB, Mosior M, Ford A, Gonzalez-DeWhitt PA, Hale JE, Li D, Qiu Y, Fraser CC, Yang DD, Heuer JG, Jaskunas SR, Eacho P. Transgenic angiopoietin-like (Angptl)4 overexpression and targeted disruption of Angptl4 and Angptl3: Regulation of triglyceride metabolism. Endocrinology. 2005 Nov 1;146(11):4943-50.

Li WJ, Li HB, Chen JL, Zhao GP, Zheng MQ, Wen J. Gene expression of heart- and adipocyte-fatty acid-binding protein and correlation with intramuscular fat in Chinese chickens. Anim Biotechnol. 2008 Jul 7;19(3):189-93.

Lichtenstein L, Berbee JF, van Dijk SJ, van Dijk KW, Bensadoun A, Kema IP, Voshol PJ, Muller M, Rensen PC, Kersten S. Angptl4 upregulates cholesterol synthesis in liver via inhibition of LPL- and HL-dependent hepatic cholesterol uptake. Arterioscler Thromb Vasc Biol. 2007 Nov 1;27(11):2420-7.

Mandard S, Zandbergen F, Tan NS, Escher P, Patsouris D, Koenig W, Kleemann R, Bakker A, Veenman F, Wahli W, Muller M, Kersten S. The direct peroxisome proliferator-activated receptor target fasting-induced adipose factor (FIAF/PGAR/ANGPTL4) is present in blood plasma as a truncated protein that is increased by fenofibrate treatment. J Biol Chem. 2004 Aug 14; 279(33):34411-20.

Mandard S, Zandbergen F, van Straten E, Wahli W, Kuipers F, Muller M, Kersten S. The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity. J Biol Chem. 2006 Jan 13;281(2):934-44.

Ohkawa R, Low H, Mukhamedova N, Fu Y, Lai SJ, Sasaoka M, Hara A, Yamazaki A, Kameda T, Horiuchi Y, Meikle PJ, Pernes G, Lancaster G, Ditiatkovski M, Nestel P, Vaisman B, Sviridov D, Murphy A, Remaley AT, Sviridov D, Tozuka M. Cholesterol transport between red blood cells and lipoproteins contributes to cholesterol metabolism in blood. J Lipid Res. 2020 Dec 1;61 (12):1577-88.

Singh AK, Aryal B, Chaube B, Rotllan N, Varela L, Horvath TL, Suarez Y, Fernandez-Hernando C. Brown adipose tissue derived ANGPTL4 controls glucose and lipid metabolism and regulates thermogenesis. Mol Metab. 2018 May 1;11:59-69.

Tunim S, Phasuk Y, Aggrey SE, Duangjinda M. Gene expression of fatty acid binding protein genes and its relationship with fat deposition of Thai native crossbreed chickens. Anim Biosci. 2021 Apr;34(4):751-8.

Wang XJ, Li Y, Song QQ, Guo YY, Jiao HC, Song ZG, Lin H. Corticosterone regulation of ovarian follicular development is dependent on the energy status of laying hens. J Lipid Res. 2013 Jul 1;54(7):1860-76.

Xu A, Lam MC, Chan KW, Wang Y, Zhang J, Hoo RL, Xu JY, Chen B, Chow WS, Tso AW, Lam KS. Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. Proc Natl Acad Sci. 2005 Apr 26; 102(17):6086-91.

Ye MH, Chen JL, Zhao GP, Zheng MQ, Wen J. Associations of A-FABP and H-FABP markers with the content of intramuscular fat in Beijing-You chicken. Anim Biotechnol. 2009 Dec 23;21(1):14-24.

Zhao X, Guo Y, Guo S, Tan J. Effects of Clostridium butyricum and Enterococcus faecium on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. Appl Microbiol Biotechnol. 2013 Jul; 97(14):6477-88.

https://doi.org/10.17221/134/2021-CJAS

Zhao X, Zhang S, Shen YR, Guo YM, Shi SR. Changes in liver triglyceride metabolism in broiler chickens with cold-induced ascites syndrome. Europ Poult Sci. 2016 Aug 17;8: 10 p.

Zhao X, Ding X, Yang Z, Shen Y, Zhang S, Shi S. [Effects of Clostridium butyricum on lipid metabolism in the thigh muscle of broiler chickens]. [J Anim Nutr]. 2017 Aug;29:2884-92. Chinese.

Zhao X, Ding X, Yang ZB, Shen YR, Zhang S, Shi SR. Effects of Clostridium butyricum on breast muscle lipid metabolism of broilers. Ital J Anim Sci. 2018 Oct 2; 17(4):1010-20.

Zhao X, Huang H, Ding X, Yang Z, Hou Y, Wang H. Angiopoietin-like protein 4 regulates breast muscle lipid metabolism in broilers. Poult Sci. 2021 Jul;100(7): 9 p.

Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science. 2004 Nov 19;306(5700):1383-6.

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