Effects of Clostridium butyricum on Lipid Metabolism in Broiler Chicken Leg Muscle (Postprint)

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Abstract

This experiment aimed to investigate the effects of Clostridium butyricum on fat metabolism in the leg muscle of broiler chickens. A total of 192 one-day-old Arbor Acres broiler cocks were selected and randomly divided into 2 groups with 6 replicates per group and 16 chickens per replicate. The control group was fed a basal diet, while the experimental group was fed the basal diet supplemented with 1×109 CFU/kg Clostridium butyricum. The experimental period lasted 42 days. The results showed that, compared with the control group: 1) dietary supplementation of Clostridium butyricum significantly increased the intramuscular fat content in the leg muscle of 21-day-old broilers ($P<0.05$), but had no significant effect on the intramuscular fat content in the leg muscle of 42-day-old broilers ($P>0.05$). 2) dietary supplementation of Clostridium butyricum significantly decreased the hormone-sensitive lipase activity in the leg muscle of 21-day-old broilers ($P<0.05$), significantly increased the lipoprotein lipase activity in the leg muscle of 21-day-old broilers ($P<0.05$), and showed a tendency to increase the lipoprotein lipase activity in the leg muscle of 42-day-old broilers (0.05

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Full Text

Effects of *Clostridium butyricum* on Thigh Muscle Lipid Metabolism of Broilers

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Abstract

This experiment investigated the effects of dietary *Clostridium butyricum* supplementation on thigh muscle lipid metabolism in broiler chickens. A total of 192 one-day-old male Arbor Acres (AA) broilers were randomly allocated into two groups with six replicates per group and 16 broilers per replicate. The control group received a basal diet, while the treatment group received the basal diet supplemented with 1×10 CFU/kg *Clostridium butyricum*. The experiment lasted 42 days. Compared with the control group, dietary *Clostridium butyricum* supplementation significantly increased thigh muscle intramuscular fat content at 21 days of age ($P<0.05$), but had no significant effect at 42 days of age ($P>0.05$). Supplementation significantly decreased hormone-sensitive lipase (HSL) activity at 21 days ($P<0.05$), significantly increased lipoprotein lipase (LPL) activity at 21 days ($P<0.05$), and tended to increase LPL activity at 42 days ($0.05<P<0.10$). Serum free triiodothyronine (FT3) content was significantly decreased at 21 days ($P<0.05$). Additionally, adipose triglyceride lipase (ATGL) mRNA expression in thigh muscle was significantly downregulated at 21 days ($P<0.05$). These results indicate that dietary *Clostridium butyricum* supplementation can positively influence intramuscular fat deposition by modulating lipid metabolism-related enzyme activities and gene expression in broiler thigh muscle, particularly during the early growth phase.

Keywords: *Clostridium butyricum*; broilers; thigh muscle; lipid metabolism

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1. Materials and Methods

1.1 Experimental Design and Animals A total of 192 one-day-old male Arbor Acres (AA) broilers were randomly divided into two dietary treatment groups with six replicates per group and 16 birds per replicate. The control group was fed a basal diet formulated according to NRC (1994) standards, while the treatment group received the basal diet supplemented with *Clostridium butyricum* at a concentration of 1×10 CFU/kg. The *C. butyricum* strain (CCTCC M 2011384) was provided at a purity of 5×10 CFU/g. The feeding trial lasted 42 days.

1.2 Diet Composition The basal diet composition and nutrient levels are presented in Table 1 . The vitamin premix provided per kilogram of diet: VA 12,500 IU, VD 2,500 IU, VK 2.65 mg, VB 2.00 mg, VB 6.00 mg, VB 0.025 mg, VE 30 IU, biotin 0.0325 mg, folic acid 1.25 mg, pantothenic acid 12.00 mg, and nicotinic acid 50.00 mg. The mineral premix provided per kilogram of diet: Cu 8.00 mg, Zn 75.00 mg, Fe 100.00 mg, Mn 100.00 mg, Se 0.15 mg, and I 0.35 mg.

1.3 Sample Collection At 21 and 42 days of age, birds were weighed after a 12-hour fast. Blood samples were collected and centrifuged to obtain serum.

Thigh muscle samples were rapidly excised, frozen in liquid nitrogen, and stored at -80°C for subsequent analysis of enzyme activities and gene expression.

1.4 Laboratory Analyses 1.4.1 Intramuscular Fat Content
Intramuscular fat content was determined using standard extraction methods.

1.4.2 Enzyme Activity Assays
Lipoprotein lipase (LPL) activity was measured using a commercial assay kit, with one unit defined as 1 mol of free fatty acid released per hour. Hormone-sensitive lipase (HSL) activity was determined according to established protocols [6].

1.4.3 Serum Hormone Analysis
Serum free triiodothyronine (FT3) and free thyroxine (FT4) concentrations were measured using radioimmunoassay kits.

1.4.4 Gene Expression Analysis
Total RNA was extracted from thigh muscle using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA). Real-time PCR was performed using TaKaRa reagents with gene-specific primers (Table 2). Target genes included heart-type fatty acid binding protein (H-FABP), adipocyte fatty acid binding protein (A-FABP), adipose triglyceride lipase (ATGL), carnitine palmitoyltransferase 1 (CPT1), CPT2, long-chain acyl-CoA dehydrogenase (LCAD), and LPL. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the reference gene. Relative expression was calculated using the $2^{(-\Delta\Delta Ct)}$ method.

1.5 Statistical Analysis Data were analyzed using SAS 9.2 software. Differences between groups were assessed by t-test. Results are expressed as means ± standard deviation. Statistical significance was declared at $P<0.05$, with trends noted at $0.05<P<0.10$.

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2. Results

2.1 Intramuscular Fat Content Dietary *C. butyricum* supplementation significantly increased thigh muscle intramuscular fat content at 21 days of age ($P<0.05$) but had no significant effect at 42 days ($P>0.05$) (Fig. 1 [Figure 1: see original paper]).

2.2 Lipid Metabolism Enzyme Activities Supplementation significantly decreased HSL activity at 21 days ($P<0.05$) while significantly increasing LPL activity at 21 days ($P<0.05$). At 42 days, LPL activity showed an increasing trend ($0.05<P<0.10$), though HSL activity was not significantly affected ($P>0.05$) (Fig. 2 [Figure 2: see original paper]).

2.3 Serum Hormone Contents Serum FT3 concentration was significantly reduced at 21 days in the *C. butyricum* group ($P<0.05$), while FT4 levels remained unchanged. No significant differences were observed for either hormone at 42 days ($P>0.05$) (Fig. 3 [Figure 3: see original paper]).

2.4 Gene Expression ATGL mRNA expression in thigh muscle was significantly downregulated at 21 days ($P<0.05$). However, no significant differences were detected for H-FABP, A-FABP, CPT1, CPT2, LCAD, or LPL mRNA expression at either time point ($P>0.05$) (Fig. 4 [Figure 4: see original paper]).

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3. Discussion

The present study demonstrates that dietary *C. butyricum* supplementation modulates lipid metabolism in broiler thigh muscle, particularly during the early growth phase (1-21 days). The significant increase in intramuscular fat content at 21 days coincided with enhanced LPL activity and reduced HSL activity, suggesting a shift toward fat storage. The downregulation of ATGL mRNA expression further supports reduced lipolysis. These effects may be mediated through alterations in thyroid hormone status, as evidenced by decreased FT3 levels.

Previous studies have reported that *C. butyricum* improves growth performance and alters cecal microbiota composition [2-4]. The current findings extend these observations by revealing direct effects on muscle lipid metabolism. The time-dependent nature of the response, with effects diminishing by 42 days, suggests that early intervention with probiotics may be critical for programming lipid deposition patterns.

The lack of significant changes in most gene expression levels, despite altered enzyme activities, indicates potential post-transcriptional regulation. The trend toward increased LPL activity at 42 days, without corresponding mRNA changes, supports this hypothesis. Further research is needed to elucidate the molecular mechanisms underlying these observations.

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4. Conclusion

Dietary supplementation with *Clostridium butyricum* at 1×10 CFU/kg positively affects intramuscular fat deposition in broiler thigh muscle by regulating key lipid metabolism enzymes and gene expression, particularly during the first 21 days post-hatch. These findings provide a basis for using probiotics to improve meat quality in poultry production.

References

[1] GREINER T, BÄCKHED F. Effects of the gut microbiota on obesity and glucose homeostasis[J]. *Trends in Endocrinology and Metabolism*, 2011, 22(4): 117–123.

[2] ZHANG B K, YANG X, GUO Y M, et al. Effects of dietary lipids and *Clostridium butyricum* on the performance and the digestive tract of broiler chickens[J]. *Archives of Animal Nutrition*, 2011, 65(4): 329–339.

[3] YANG C M, CAO G T, FERKET P R, et al. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens[J]. *Poultry Science*, 2012, 91(9): 2121–2129.

[4] ZHAO X, GUO Y M, GUO S S, et al. Effects of *Clostridium butyricum* and *Enterococcus faecium* on growth performance, lipid metabolism, and cecal microbiota of broiler chickens[J]. *Applied Microbiology and Biotechnology*, 2013, 97(14): 6477–6488.

[5] NRC. *Nutrient Requirements of Poultry*[S]. 9th rev. ed. Washington, D.C.: National Academy Press, 1994.

[6] [Chinese reference - title not fully decipherable][D]. [University publication], 2014: 36–37.

[7] WANG X J, SONG Z G, JIAO H C, et al. Dexamethasone facilitates lipid accumulation in chicken skeletal muscle[J]. *Stress*, 2012, 15(4): 443–456.

[8] CAI Y L, SONG Z G, ZHANG X H, et al. Increased de novo lipogenesis in liver contributes to the augmented fat deposition in dexamethasone-exposed broiler chickens (*Gallus gallus domesticus*)[J]. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 2009, 150(2): 164–169.

[9] HUANG J B, ZHANG Y, ZHOU Y B, et al. Effects of epigallocatechin gallate on lipid metabolism and its underlying molecular mechanism in broiler chickens[J]. *Journal of Animal Physiology and Animal Nutrition*, 2015, 99(4): 719–727.

[10] DRUYAN S, CAHANER A, ASHWELL C M. The expression patterns of hypoxia-inducing factor subunit -1, heme oxygenase, hypoxia upregulated protein 1, and cardiac troponin T during development of the chicken heart[J]. *Poultry Science*, 2007, 86(11): 2384–2389.

[11] [Chinese reference - title not fully decipherable][D]. [University publication], 2015: 38–46.

[12] LI W J, LI H B, CHEN J L, et al. Gene expression of heart- and adipocyte-fatty acid binding protein correlation with intramuscular fat in Chinese chickens[J]. *Animal Biotechnology*, 2008, 19(3): 190–194.

[13] YE M H, CHEN J L, ZHAO G P, et al. Associations of A-FABP and H-FABP markers with the content of intramuscular fat in Beijing-You chicken[J].

Animal Biotechnology, 2009, 21(1): 14-24.

[14] ALI Y B, CARRIÈRE F, VERGER R, et al. Continuous monitoring of cholesterol oleate hydrolysis by hormone-sensitive lipase and other cholesterol esterases[J]. *Journal of Lipid Research*, 2005, 46(5): 994-1000.

[15] ZIMMERMANN R, STRAUSS J G, HAEMMERLE G, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase[J]. *Science*, 2004, 306(5700): 1383-1386.

[16] LEE K, SHIN J, LATSHAW J D, et al. Cloning of adipose triglyceride lipase complementary deoxyribonucleic acid in poultry and expression of adipose triglyceride lipase during development of adipose in chickens[J]. *Poultry Science*, 2009, 88(3): 620-630.

[17] WANG X J, LIN H, SONG Z G, et al. Dexamethasone facilitates lipid accumulation and mild feed restriction improves fatty acids oxidation in skeletal muscle of broiler chicks (*Gallus gallus domesticus*)[J]. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 2010, 151(4): 447-454.

[18] WANG X J, SONG Z G, JIAO H C, et al. Skeletal muscle fatty acids shift from oxidation to storage with dexamethasone treatment in chickens[J]. *General and Comparative Endocrinology*, 2012, 179(3): 319-330.

[19] GOLDBERG I J. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis[J]. *Journal of Lipid Research*, 1996, 37(4): 693-707.

[20] VOSHOL P J, JONG M C, DAHLMANS V E H, et al. In muscle-specific lipoprotein lipase-overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body muscle-specific glucose uptake[J]. *Diabetes*, 2001, 50(11): 2585-2590.

[21] YOSHIDA K, SHIMIZUGAWA T, ONO M, et al. Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase[J]. *Journal of Lipid Research*, 2002, 43(11): 1770-1772.

[22] MANDARD S, ZANDBERGEN F, VAN STRATEN E, et al. The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity[J]. *The Journal of Biological Chemistry*, 2006, 281(2): 934-944.

[23] PUCCI E, CHIOVATO L, PINCHERA A. Thyroid and lipid metabolism[J]. *International Journal of Obesity*, 2000, 24(Suppl. 2): S109-S112.

[24] [Chinese reference - title not fully decipherable][D]. [University publication], 2008: 13.

Table 1
Composition and nutrient levels of basal diets (air-dry basis), %

Table 2
Primers for real-time PCR

Figure 1 [Figure 1: see original paper]
Effects of *Clostridium butyricum* on thigh muscle intramuscular fat content of broilers

Figure 2 [Figure 2: see original paper]
Effects of *Clostridium butyricum* on thigh muscle lipid metabolism enzyme activities of broilers

Figure 3 [Figure 3: see original paper]
Effects of *Clostridium butyricum* on serum hormone contents of broilers

Figure 4 [Figure 4: see original paper]
Effects of *Clostridium butyricum* on thigh muscle lipid metabolism related gene expression of broilers

Note: Figure translations are in progress. See original paper for figures.