Effects of Dietary Chenodeoxycholic Acid Supplementation on Lipid Metabolism, Production Performance of Breeder Hens, and Muscle Development in Offspring (Postprint)

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Abstract

This study investigated the effects of dietary supplementation of chenodeoxycholic acid (CDCA) on lipid metabolism, production performance, and offspring muscle development in breeding chickens. A total of 80 14-week-old Dwarf Yellow breeding chickens were selected and randomly divided into 5 groups, with 16 replicates per group and 1 bird per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 10, 50, 100, and 500 mg/kg of CDCA, respectively, for a trial period of 4 weeks. During the last week of the trial, hatching eggs were collected for incubation, and the hatched offspring were divided into 5 groups according to the grouping of the breeding chickens, with a rearing period of 12 weeks. The results showed that: 1) Dietary CDCA supplementation had no significant effects on body weight, egg production rate, or egg quality in breeding chickens ($P>0.05$); compared with the control group, dietary supplementation with 100 mg/kg CDCA significantly increased serum triglyceride (TG) content ($P<0.05$) and significantly decreased serum very low-density lipoprotein (VLDL) content ($P<0.05$) in breeding chickens; compared with the control group, dietary supplementation with 100 mg/kg CDCA significantly decreased total bile acid (TBA) content in eggs on trial days 14 and 28 ($P<0.05$), and significantly increased egg cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) content ($P<0.05$). 2) Compared with the control group, dietary supplementation with 50 mg/kg CDCA significantly increased gastrocnemius muscle fiber density and liver index in male offspring ($P<0.05$); compared with the control group, dietary supplementation with 100 mg/kg CDCA significantly decreased average daily gain in female offspring, total protein deposition in gastrocnemius muscle of male offspring, and lactate dehydrogenase (LDH) activity in gastrocnemius

muscle of female offspring (P<0.05); compared with the control group, dietary supplementation with 500 mg/kg CDCA significantly decreased succinate dehydrogenase (SDH) activity in gastrocnemius muscle of female offspring and LDH activity in gastrocnemius muscle of male offspring (P<0.05). In conclusion, dietary CDCA supplementation had no significant effects on production performance of breeding chickens, but affected lipid metabolism and egg deposition, particularly TBA deposition, thereby exerting inhibitory effects on muscle development in offspring. Therefore, attention should be paid to the dosage and duration of CDCA application in breeding chicken diets.

Full Text

Effects of Dietary Chenodeoxycholic Acid on Lipid Metabolism and Performance of Breeding Hens and Muscle Development of Their Offspring

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Abstract

This experiment was conducted to investigate the effects of dietary chenodeoxycholic acid (CDCA) on lipid metabolism and performance of breeding hens and muscle development of their offspring. Eighty 14-week-old dwarf yellow breeding hens were randomly divided into 5 groups with 16 replicates per group and 1 bird per replicate. Breeding hens in the control group were fed a basal diet, while those in the experimental groups were fed the basal diet supplemented with 10, 50, 100, or 500 mg/kg CDCA for 4 weeks. Fertilized eggs were collected and hatched in the last week, and the offspring after hatching were divided into 5 groups according to the maternal grouping, with a feeding trial lasting for 12 weeks. The results showed as follows: (1) Dietary CDCA had no significant effects on body weight, laying rate, or egg quality of breeding hens (P>0.05). Compared with the control group, supplementation with 100 mg/kg CDCA significantly increased serum triglyceride (TG) content (P<0.05) and significantly decreased serum very low-density lipoprotein (VLDL) content (P<0.05) in breeding hens. Additionally, 100 mg/kg CDCA significantly decreased total bile acid (TBA) content in eggs on days 14 and 28 (P<0.05), while significantly increasing egg cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) contents (P<0.05). (2) Compared with the control group, supplementation with 50 mg/kg CDCA significantly increased gastrocnemius muscle fiber density in offspring and liver percentage in male offspring (P<0.05). Supplementation with 100 mg/kg CDCA significantly decreased average daily gain in female offspring, total protein deposition in gastrocnemius muscle of male

offspring, and lactate dehydrogenase (LDH) activity in gastrocnemius muscle of female offspring ($P<0.05$). Supplementation with 500 mg/kg CDCA significantly decreased succinate dehydrogenase (SDH) activity in gastrocnemius muscle of female offspring and LDH activity in gastrocnemius muscle of male offspring ($P<0.05$). In conclusion, dietary CDCA had no significant effect on performance of breeding hens but significantly affected lipid metabolism and deposition in eggs, particularly TBA deposition, which subsequently suppressed muscle development in offspring. Therefore, the dosage and duration of CDCA supplementation in breeding hen diets require careful consideration.

Keywords: chenodeoxycholic acid; breeding hens; performance; muscle development of offspring

Introduction

Embryonic skeletal muscle development determines postnatal muscle growth potential in animals. As oviparous animals, poultry rely entirely on nutrients within the egg for early growth and development, making their postnatal muscle development largely dependent on nutrient composition in the egg [1]. Previous studies have shown that nutritional levels in breeding hen diets can affect the concentration of nutrients deposited into eggs, subsequently influencing the expression of growth-regulating factors in offspring blood and tissues and ultimately affecting nutrient utilization efficiency in progeny [2]. Myofiber morphology (including density and diameter) in offspring is influenced by both maternal nutrition during embryonic development and postnatal environmental factors. Xu et al. [3] reported that low-energy maternal diets significantly increased myofiber density in breast and leg muscles of broiler offspring at 1, 28, and 56 days of age. Yan et al. [4] found that low-protein maternal diets significantly reduced myofiber diameter while increasing myofiber density in breast and leg muscles of offspring, with these effects being long-lasting and showing no compensatory response.

Bile acids are commonly used emulsifiers in animal production. Numerous studies have demonstrated that bile acids can promote lipid digestion and absorption, improve lipid metabolism, and enhance fat digestibility in poultry. Wu [5] supplemented broiler diets with different levels of bile acid composite emulsifiers and found that the emulsifier effectively improved broiler performance, with the most significant improvement observed at an addition level of 800 g/t bile acids. Liu et al. [6] investigated the effects of bile acids on performance and apparent metabolic rate of crude fat in "817" broiler chickens, reporting that bile acids significantly improved fat apparent metabolic rate during the early growth stage (1-20 days), with the experimental groups showing a 2.49% to 4.68% increase compared with the control group. However, the effects of dietary bile acid supplementation in breeding hens on nutrient deposition in eggs and offspring growth and development have not been reported.

Chenodeoxycholic acid (CDCA) is the primary component of bile acids. This study investigated the effects of dietary supplementation with different levels of CDCA on lipid metabolism, performance of dwarf yellow breeding hens, and muscle development of their offspring, providing a scientific basis for the rational use of CDCA in poultry production.

Materials and Methods

1.1 Experimental Materials and Diets CDCA (purity >99%) was purchased from Chengdu Kuanjing Biological Co., Ltd. The basal diet was a corn-soybean meal type diet provided by the experimental farm of South China Agricultural University, with composition and nutrient levels shown in Table 1 .

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

Items	1 to 4 weeks	5 to 12 weeks	14 to 18 weeks
Ingredients			
Dehulled soybean meal			
Corn			
Wheat bran			
Fish meal			
Limestone			
CaHPO4			
NaCl			
Lys			
Met			
Mineral premix[1]			
Multi-vitamin premix[2]			
Vitamin E			
Choline			
Fish liver oil			
Yeast powder			
Anti-molds			
Total			
Nutrient levels[3]			
ME (MJ/kg)			
CP			
Ca			
TP			
Lys			
Met			

[1] Mineral premix provided the following per kg of diets: Cu (as copper sulfate) 8 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 100 mg, Zn (as

zinc sulfate) 60 mg, I (as potassium iodide) 0.50 mg, Se (as sodium selenite) 0.40 mg, Co (as cobalt sulfate) 0.40 mg.

[2] Multi-vitamin premix provided the following per kg of diets: VA 4,000 IU, VB1 1 mg, VB2 3 mg, VB5 40 mg, VB6 2 mg, VB12 0.01 mg, VD3 1,000 IU, VE 10 IU, VK3 2 mg, biotin 0.05 mg, folic acid 0.5 mg, D-pantothenic acid 6 mg, nicotinic acid 20 mg, antioxidant 100 mg.

[3] ME was a calculated value, and others were measured values.

1.2 Experimental Design and Management Eighty 14-week-old healthy dwarf yellow breeding hens at the onset of lay were randomly divided into 5 groups with 16 replicates per group and 1 bird per replicate. The control group was fed the basal diet, while the experimental groups (Groups I, II, III, and IV) were fed the basal diet supplemented with 10, 50, 100, and 500 mg/kg CDCA, respectively. Hens were housed individually in cages with restricted feeding [60 g/(d · bird)] and free access to water under conventional management. The experiment consisted of a 1-week pre-trial period and a 4-week trial period (15-18 weeks of age). Artificial insemination was performed on all hens during the fourth week, and eggs were collected daily for 1 week before incubation according to the maternal grouping. The incubation period lasted 3 weeks. After hatching, offspring were divided into 5 groups corresponding to the maternal groups and raised for 12 weeks. At 4 weeks of age, offspring were separated by sex within each group, with male offspring having 6 replicates of 6 birds each and female offspring having 6 replicates of 4 birds each. Birds had free access to feed and water throughout the 12-week rearing period.

1.3 Sample Collection Eggs were collected daily at 09:00 and stored at 4°C. At the end of the fourth week of the breeding trial, 10 hens per group were randomly selected after fasting, and 10 mL of blood was collected from the wing vein. Serum was prepared by centrifugation at 3,500 r/min for 10 min for determination of lipid metabolism indices. At 12 weeks of age, offspring were weighed, and 2 birds per replicate with body weight close to the replicate average were selected for slaughter. The liver, breast muscle, and leg muscle were separated and weighed. The gastrocnemius muscle from the leg muscle was snap-frozen in liquid nitrogen and stored in an ultra-low temperature freezer for further analysis.

1.4 Measurement Indices and Methods

1.4.1 Performance The number of eggs laid by each hen was recorded accurately to calculate laying rate. Initial and final body weights of breeding hens were recorded. For offspring, feed intake and body weight gain were measured weekly from 4 weeks of age to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G). Carcass percentages were calculated using the following formulas:

Feed-to-gain ratio = ADFI/ADG
Breast muscle percentage (%) = 100 × (single-side breast muscle weight × 2)/live weight
Thigh muscle percentage (%) = 100 × (single-side thigh muscle weight × 2)/live weight
Abdominal fat percentage (%) = 100 × abdominal fat weight/live weight

1.4.2 Egg Quality and Deposition of Total Bile Acid (TBA), Total Cholesterol (TC), Triglyceride (TG), and Low-Density Lipoprotein Cholesterol (LDL-C) in Eggs Egg quality was determined using eggs collected on day 27 of the trial. Egg weight, shell strength, albumen height, yolk color, relative yolk weight, and Haugh unit were measured using an eggshell force gauge and a multifunctional egg quality analyzer (ORKA, Israel). Haugh unit was calculated as:

Haugh unit = 100 × log(H - 1.7W^0.37 + 7.60)

where H is albumen height (mm) and W is egg weight (g).

For determination of TBA, TC, TG, and LDL-C contents in eggs, 6 eggs per group were collected on days 7, 14, 23, and 28 of the trial. Yolk was separated from each egg and homogenized for TBA measurement on days 7, 14, 23, and 28, and for TC, TG, and LDL-C measurement on day 28. All indices were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

Sample preparation for TBA in eggs: Exactly 0.15 g of yolk was taken in a 2 mL centrifuge tube and diluted with anhydrous ethanol at a ratio of 1:9. After vortexing for 30 s, the sample was centrifuged at 4,000 r/min for 10 min. Then 600 L of supernatant was transferred to a 1.5 mL centrifuge tube, mixed with 600 L of anhydrous ethanol, and centrifuged at 4,000 r/min for 1 min. The resulting supernatant was collected as the test sample and stored at -20°C.

Sample preparation for TG, TC, and LDL-C in eggs: The procedure was the same as for TBA preparation, except that physiological saline was used as the diluent instead of anhydrous ethanol.

1.4.3 Serum Lipid Metabolism Indices of Breeding Hens Serum TC, TG, LDL-C, high-density lipoprotein cholesterol (HDL-C), and TBA contents were measured using assay kits from Nanjing Jiancheng Bioengineering Institute. Serum VLDL content was measured using an assay kit from Shanghai Jining Industrial Co., Ltd.

1.4.4 Metabolic Enzyme Activities in Offspring Gastrocnemius Muscle Gastrocnemius muscle samples were removed from the ultra-low temperature freezer, and exactly 50 mg of each sample was placed in a homogenization tube with homogenization beads and strong lysis buffer (Beijing Kangwei Century Co.). Samples were homogenized 4 times using a homogenizer (FastPrep-24, Carefreezy, USA) and then centrifuged at 12,000 r/min for 10 min at 4°C.

The supernatant was collected for determination of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.4.5 Myofiber Density in Offspring Gastrocnemius Muscle Gastrocnemius muscle sections were prepared using a cryostat (Leica, Germany). The cryostat temperature was set to -23°C. Samples were transferred from the ultra-low temperature freezer to the cryostat and allowed to equilibrate. Each sample was then trimmed into a 0.5 mm × 0.5 mm × 0.5 mm cube with a scalpel, fixed with embedding medium, and sectioned at a thickness of 10 m. At least 4 sections were prepared per sample. Sections were stained with routine hematoxylin-eosin (HE) staining and photographed under a 40× objective lens. Five random fields were selected per section, the number of fibers in each field was counted, and the average was calculated to determine the number of fibers per mm^2. Myofiber density was expressed as fibers/mm^2.

1.4.6 Total Protein Deposition in Offspring Gastrocnemius Muscle Gastrocnemius muscle samples were removed from the ultra-low temperature freezer, and exactly 50 mg of tissue was placed in a homogenization tube with homogenization beads and lysis buffer according to the kit instructions. Samples were homogenized 4 times using a homogenizer. The homogenate was divided into two equal portions: one portion was centrifuged at 12,000 r/min for 10 min at 4°C, and the supernatant was used for protein concentration determination using an assay kit from Nanjing Jiancheng Bioengineering Institute; the other portion was used for DNA extraction using a DNA extraction kit (Magen, USA). Total protein deposition in gastrocnemius muscle was expressed as the ratio of protein mass to DNA mass.

1.5 Statistical Analysis All data were analyzed using one-way ANOVA procedure in SPSS 17.0 software. Multiple comparisons were performed using LSD method. Results were expressed as "mean ± standard error." Differences were considered significant at $P<0.05$.

Results

2.1.1 Effects of Dietary CDCA on Body Weight and Laying Rate of Breeding Hens As shown in Table 2 , dietary supplementation with different levels of CDCA had no significant effects on body weight or laying rate of breeding hens ($P>0.05$).

Table 2 Effects of dietary CDCA on body weight and laying rate of breeding hens

Items	Control group	Group I	Group II	Group III	Group IV
Initial weight (kg)	0.85±0.02	0.85±0.02	0.85±0.02	0.85±0.02	0.85±0.02
Terminal weight (kg)	0.84±0.02	0.79±0.01	0.81±0.02	0.82±0.02	0.83±0.02
Laying rate (%)	56.17±2.11	55.33±5.06	55.03±1.26	45.86±4.41	50.17±4.19

In the same row, values with the same letter superscripts or no letter mean no significant difference (P>0.05), while values with different lowercase letter superscripts mean significant difference (P<0.05). The same applies below.

2.1.2 Effects of Dietary CDCA on Egg Quality of Breeding Hens As shown in Table 3 , dietary supplementation with different levels of CDCA had no significant effects on egg quality of breeding hens (P>0.05).

Table 3 Effects of dietary CDCA on egg quality of breeding hens

Items	Control group	Group I	Group II	Group III	Group IV
Eggshell hardness (kgf)	3.42±0.30	3.06±0.16	3.54±0.20	3.35±0.61	2.98±0.38
Albumen height (mm)	4.03±0.58	3.74±0.47	3.76±0.45	3.24±0.33	2.94±0.15
Yolk color	7.16±0.60	6.74±0.35	7.58±0.17	7.04±0.73	8.12±0.34
Haugh unit	74.24±5.22	70.96±3.07	70.56±4.01	68.00±2.76	63.92±1.57
Yolk weight percent (%)	33.62±0.53	32.29±1.17	33.62±0.42	31.46±1.32	33.72±0.75

2.2 Effects of Dietary CDCA on Serum Lipid Metabolism Indices of Breeding Hens As shown in Table 4 , dietary supplementation with 100 mg/kg CDCA significantly increased serum TG content (P<0.05), while supplementation with 10 and 100 mg/kg CDCA significantly decreased serum VLDL

content (P<0.05). Dietary supplementation with different levels of CDCA had no significant effects on serum TBA, TC, HDL-C, or LDL-C contents (P>0.05).

Table 4 Effects of dietary CDCA on serum parameters of lipid metabolism of breeding hens

Items	Control group	Group I	Group II	Group III	Group IV
TBA (mol/L)	28.78±2.28	23.59±3.5	28.81±2.02	22.56±1.26	29.82±3.11
TC (mmol/L)	1.95±0.13	2.18±0.22	1.92±0.07	2.25±0.84	2.23±0.58
TG (mmol/L)	7.79±1.27	6.97±0.91	6.47±0.28	14.28±4.49	7.19±1.51
HDL-C (mmol/L)	0.47±0.03	0.39±0.08	0.47±0.06	0.53±0.07	0.54±0.09
LDL-C (mmol/L)	1.03±0.08	1.10±0.10	0.99±0.08	0.92±0.15	1.25±0.19
VLDL (mol/L)	257.89±27.53	184.82±7.79	207.36±4.58	184.46±9.75	269.04±24.55

2.3 Effects of Dietary CDCA on TBA, TC, TG, and LDL-C Contents in Eggs of Breeding Hens As shown in Table 5 , compared with the control group, supplementation with 50 and 100 mg/kg CDCA significantly decreased TBA content in eggs on day 14 (P<0.05); supplementation with 10 and 50 mg/kg CDCA significantly decreased TBA content on day 23 (P<0.05); and supplementation with 100 mg/kg CDCA significantly decreased TBA content on day 28 (P<0.05).

Table 5 Effects of dietary CDCA on TBA content in eggs of breeding hens (mol/L)

Time (d)	Control group	Group I	Group II	Group III	Group IV
Day 7	941.87±39.92	813.68±54.96	812.63±18.83	826.92±26.53	1053.42±87.55
Day 14	930.18±35.74	841.58±21.77	771.84±52.37	767.37±59.50	902.11±34.97
Day 23	905.61±70.70	752.82±43.72	747.22±36.95	900.49±42.89	916.58±55.41
Day 28	1058.95±46.79	927.67±43.39	931.43±113.66	866.32±64.77	955.91±49.98

As shown in Table 6 , compared with the control group, supplementation with 100 mg/kg CDCA significantly increased TC and LDL-C contents in eggs (P<0.05), while having no significant effect on TG content (P>0.05).

Table 6 Effects of dietary CDCA on contents of TC, TG, and LDL-C in eggs of breeding hens (mmol/L)

Items	Control group	Group I	Group II	Group III	Group IV
TC	28.42±0.72	28.63±1.46	30.70±2.01	35.42±1.70	28.21±1.61
LDL-C	13.48±1.16	14.47±1.70	10.36±0.73	17.52±0.89	13.02±1.10
TG	69.98±2.19	70.48±3.60	71.10±2.29	69.60±1.93	69.44±0.60

2.4 Effects of Dietary CDCA on Performance of Offspring As shown in Table 7 , compared with the control group, supplementation with 100 and 500 mg/kg CDCA significantly decreased average daily gain in female offspring (P<0.05). Dietary supplementation with different levels of CDCA had no significant effects on 12-week body weight or feed-to-gain ratio in either male or female offspring (P>0.05).

Table 7 Effects of dietary CDCA on performance of offspring

Items	Control group	Group I	Group II	Group III	Group IV
Weight at 12 weeks (g)					
Male	848.20±20.88	830.00±23.19	827.87±34.37	832.17±26.73	779.29±9.13
Female	830.00±23.19	773.17±10.85	704.67±28.60	765.56±22.47	652.83±30.34
ADG (g)					
Male	18.01±0.33	17.85±0.43	17.48±0.69	18.71±0.88	16.75±0.42
Female	15.73±0.55	14.14±0.66	15.13±0.76	13.49±0.88	13.60±0.47
F/G (4-12 weeks)					
Male	3.24±0.08	3.35±0.09	3.21±0.06	3.49±0.18	3.99±0.19
Female	5.42±1.01	3.43±0.19	3.40±0.10	3.73±0.33	4.09±0.24

As shown in Table 8 , compared with the control group, supplementation with 50 mg/kg CDCA significantly increased liver percentage in male offspring (P<0.05). Dietary supplementation with different levels of CDCA had no significant effects on breast muscle percentage, thigh muscle percentage in male offspring, or liver percentage, breast muscle percentage, and thigh muscle percentage in female offspring (P>0.05).

Table 8 Effects of dietary CDCA on dressing percentage of offspring (%)

Items	Control group	Group I	Group II	Group III	Group IV
Liver percentage					
Male	2.32±0.09	2.61±0.09	2.49±0.10	2.60±0.07	2.45±0.09
Female	2.29±0.04	2.56±0.08	2.46±0.09	2.37±0.06	2.57±0.09
Breast muscle percentage					
Male	8.25±0.18	8.75±0.23	9.32±0.24	8.96±0.25	8.37±0.11
Female	8.16±0.23	8.42±0.38	8.74±0.36	9.32±0.15	8.62±0.25
Thigh muscle percentage					
Male	2.28±0.08	2.47±0.05	2.34±0.04	2.28±0.06	2.16±0.04
Female	2.42±0.09	2.40±0.08	2.20±0.05	2.32±0.08	2.15±0.06

2.5.1 Effects of Dietary CDCA on Total Protein Deposition in Offspring Gastrocnemius Muscle As shown in Table 9 , compared with the control group, supplementation with 10 and 100 mg/kg CDCA significantly decreased total protein deposition in gastrocnemius muscle of male offspring ($P<0.05$), while having no significant effect on female offspring ($P>0.05$).

Table 9 Effects of dietary CDCA on total protein deposition of gastrocnemius of offspring (g prot/ g DNA)

Items	Control group	Group I	Group II	Group III	Group IV
Male	200.41±13.15	146.61±14.21	170.26±8.88	161.77±12.76	167.64±10.34
Female	202.36±13.49	243.57±10.34	250.34±10.34	253.81±10.34	242.73±10.34

2.5.2 Effects of Dietary CDCA on Myofiber Density in Offspring Gastrocnemius Muscle As shown in Table 10 , compared with the control group, supplementation with 50 mg/kg CDCA significantly increased myofiber density in gastrocnemius muscle of offspring ($P<0.05$).

Table 10 Effects of dietary CDCA on fiber density of gastrocnemius of offspring (fibers/mm^2)

Items	Control group	Group I	Group II	Group III	Group IV
Male	233.51±13.58	229.35±12.62	269.52±3.18	217.87±2.76	236.37±8.38
Female	264.13±28.35	293.88±23.69	335.68±12.91	269.58±15.79	299.06±12.81

Figure 1 [Figure 1: see original paper] The section of gastrocnemius of offspring

2.5.3 Effects of Dietary CDCA on Metabolic Enzyme Activities in Offspring Gastrocnemius Muscle As shown in Table 11 , dietary CDCA supplementation had no significant effect on SDH activity in gastrocnemius muscle of male offspring (P>0.05), but supplementation with 500 mg/kg CDCA significantly decreased SDH activity in gastrocnemius muscle of female offspring (P<0.05). Supplementation with 500 mg/kg CDCA significantly decreased LDH activity in gastrocnemius muscle of male offspring (P<0.05), while supplementation with 50 and 100 mg/kg CDCA significantly decreased LDH activity in gastrocnemius muscle of female offspring (P<0.05).

Table 11 Effects of dietary CDCA on activities of lactate dehydrogenase and succinate dehydrogenase of gastrocnemius of offspring (U/mg prot)

Items	Control group	Group I	Group II	Group III	Group IV
LDH					
Male	1480.99±156.82	1310.39±53.13	1394.73±126.21	1232.67±65.27	1009.97±38.66
Female	1301.16±36.26	987.22±49.68	840.43±54.09	851.15±10.63	972.40±49.01
SDH					
Male	38.22±2.91	36.53±3.29	43.12±0.51	45.56±3.81	41.77±1.63
Female	72.81±6.71	67.43±6.21	59.16±4.40	53.17±3.43	71.45±2.08

Discussion

3.1 Effects of Dietary CDCA on Maternal Lipid Metabolism and Deposition of TBA, TC, TG, and LDL-C in Eggs Bile acids are synthesized from cholesterol in hepatocytes, making their content closely related to lipid metabolism in animals. In poultry, lipid metabolism directly affects lipid deposition in eggs. Qiu [7] demonstrated that hens with high plasma TG content had strong fat synthesis ability and high egg TC content, suggesting that plasma TG content could serve as an indirect selection indicator for egg TC content. Niu et al. [8] reported that feeding broiler breeder hens a high-energy diet during mid-lay significantly increased yolk TC content in embryos compared with the control group. In the present study, supplementation with 100 mg/kg CDCA significantly increased serum TG content and decreased serum VLDL content in breeding hens, while also significantly increasing egg TC and LDL-C contents. These findings are consistent with the reported relationship between serum TG and egg TC contents.

VLDL is the primary carrier for transporting TG and TC from the liver to peripheral tissues. Research has shown that laying hens have two types of VLDL: normal VLDL and very low-density lipoprotein Y (VLDLy), with 95% of yolk TC originating from VLDLy [9]. Under the action of estrogen, VLDL is converted to VLDLy, which can be specifically transferred from the liver to oocytes and deposited in yolk [10]. In this study, the significant increase in egg TC content was accompanied by a significant decrease in serum VLDL content, suggesting that VLDL may have been extensively converted to VLDLy, thereby promoting TC deposition in eggs.

Bile acids promote lipid digestion and absorption and increase hepatic bile secretion, but their deposition in poultry eggs and influencing factors have not been reported. This study found substantial bile acid deposition in breeding hen eggs, and supplementation with 100 mg/kg CDCA significantly decreased TBA content in eggs on day 14. Studies in mammals have shown that feeding C57BL/6 mice a high-fat diet supplemented with 0.25% cholic acid for 4 weeks increased serum TBA content compared with the high-fat group [11]. Zhang et al. [12] found that dietary deoxycholic acid supplementation inhibited hepatic cholesterol-7-hydroxylase 1 (CYP7A1) expression and bile acid synthesis in C57BL/6 mice. These results indicate that dietary bile acid supplementation can inhibit bile acid synthesis in mice. Therefore, it is speculated that dietary CDCA supplementation also inhibited bile acid synthesis in breeding hens, thereby affecting bile acid deposition in eggs.

3.2 Effects of Dietary CDCA on Offspring Performance and Muscle Development Normal embryonic development in poultry depends on nutrients in eggs, which originate from breeding hen diets and metabolism and are determined by the efficiency of nutrient deposition in eggs. CDCA is the primary component of bile acids. Our previous studies showed that in ovo injection of high-dose CDCA significantly increased gastrocnemius myofiber diameter and promoted myofiber growth and development in 42-day-old broilers, indicating that bile acid content in eggs regulates offspring growth [13].

The present study found that supplementation with 100 mg/kg CDCA significantly decreased TBA content in eggs on day 14. Correspondingly, supplementation with 50 mg/kg CDCA significantly increased gastrocnemius myofiber density in offspring; supplementation with 100 and 500 mg/kg CDCA significantly decreased average daily gain in female offspring; and supplementation with 10 and 100 mg/kg CDCA significantly decreased total protein deposition in gastrocnemius muscle of male offspring. These results further confirm that appropriate bile acid content in eggs promotes offspring muscle development, while reduced bile acid content impairs muscle development.

Muscle growth depends on the catabolism of energy substances within muscle cells. LDH activity reflects the intensity of anaerobic glycolysis of carbohydrates and indicates the capacity for glycogen anaerobic glycolysis in muscle fibers. SDH is an important metabolic enzyme in the mitochondrial tricarboxylic acid

cycle, and its activity indicates ATP generation capacity and reflects glycogen oxidation capacity in muscle fibers [14]. This study found that supplementation with 50 and 100 mg/kg CDCA significantly decreased LDH activity in gastrocnemius muscle of female offspring, while supplementation with 500 mg/kg CDCA significantly decreased SDH activity in gastrocnemius muscle of female offspring and LDH activity in gastrocnemius muscle of male offspring. These findings suggest that dietary CDCA supplementation may limit offspring muscle growth by inhibiting glycogen catabolism in muscle.

In summary, dietary supplementation with different doses of CDCA in breeding hens inhibited carbohydrate metabolism in offspring skeletal muscle to varying degrees, thereby affecting skeletal muscle growth.

In conclusion, dietary supplementation with different levels of CDCA had no significant effect on performance of breeding hens but significantly affected lipid metabolism and deposition in eggs, particularly bile acid deposition, which subsequently suppressed muscle development in offspring. Therefore, the dosage and duration of bile acid supplementation in breeding hen diets require careful consideration.

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