The effect of conjugated linoleic acids on the growth performance, carcase composition and meat quality of fattening rabbits

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ABSTRACT
This experiment was conducted to study the effects of dietary conjugated linoleic acids (CLAs) on the growth performance, fat metabolism, carcase composition and muscle quality of fattening rabbits. A total of 160 Minxinan black rabbits aged 75 days with body weights of 1658.9 ± 188.8 g were randomly divided into 4 groups. They were fed a basic diet supplemented with 0 (control), 0.5%, 1.0%, or 1.5% CLA for 50 days. Dietary CLA reduced the feed intake and serum triglyceride and cholesterol levels. In addition, the crude protein content in rabbit meat was increased, along with the cholesterol and the ether extract levels in the 1.5% depression group. Moreover, the fatty acid composition in rabbit meat was changed, and the content of polyunsaturated fatty acids/monounsaturated fatty acids (PUFAs/MUFAs) increased with the increasing levels of CLA addition. These findings indicate that CLA greatly improved the nutritional value of rabbit meat. From an economic point of view, CLA would normally be included at a concentration of 0.5% in the formulation of feeds for fattening rabbits.

HIGHLIGHTS
- Minxinan black rabbits were fed conjugated linoleic acid (CLA)-supplemented diets for 50 d.
- CLA decreased the feed intake of experimental rabbits.
- CLA has significant effects on fat deposition and serum triglyceride and cholesterol levels.
- CLA greatly improved the nutritional value of rabbit meat.
- The recommended CLA level is 0.5% for meat rabbits.

Introduction
With the continuous improvement of people's living standards, the number of patients with cardiovascular diseases such as obesity, hypertension and heart disease has increased. Increasing consumer knowledge of the link between diet and health has raised the awareness and demand for functional food ingredients. Rabbit meat has the characteristics of high digestibility, high protein, high lysine, low fat, low energy and low cholesterol. It is an ideal nutritional meat for patients with hypertension and cardiovascular disease (Dalle Zotte and Szendro 2011). Conjugated linoleic acid (CLA) is a mixture of several geometric and positionally conjugated isomers of linoleic acid and has received considerable attention for its potential to regulate fat metabolism, reduce cholesterol, inhibit atherosclerosis, improve immunity, improve bone density, prevent diabetes and promote growth in animals (Shen et al. 2013; Kim et al. 2016; Shen and McIntosh 2016; Chen et al. 2019). In addition, supplementing CLA in poultry diets has been suggested as a way to obtain CLA-enriched meat and egg products (Aydin and Cook 2009; Kumari et al. 2014). Therefore, we conclude that using CLA in rabbit feeding could enrich rabbit meat. This experiment was conducted to study the effects of dietary CLA on the growth performance, carcase yield and muscle quality of meat rabbits to provide a theoretical reference for the production of high-grade functional rabbit meat.

Materials and methods

Ethical approval
The experimental procedures were approved by the Shandong Academy of Agricultural Sciences Animal
Food or drinking water during the experiment.

Food and water. No antibiotics were added to the rabbits (De Blas and Mateos 2010), and the fatty acid composition of the basal diets (air-dry basis, %).

| Raw material composition | Content | Nutrient levels\textsuperscript{a} |
|--------------------------|---------|----------------------------------|
| Corn                      | 6.0     | Digestible energy (MJ/kg)        |
|                          |         | 10.32                            |
| Soybean meal              | 7.0     | Dry matter, DM                   |
|                          |         | 88.02                            |
| Wheat middling            | 5.0     | Crude protein                    |
|                          |         | 17.22                            |
| Barley                    | 6.0     | Ether extract                     |
|                          |         | 2.74                             |
| Wheat bran                | 15.0    | Crude fibre                      |
|                          |         | 17.04                            |
| Corn germ meal            | 15.0    | Neutral detergent fibre           |
|                          |         | 38.40                            |
| Peanut seedling           | 20.0    | Acid detergent fibre              |
|                          |         | 23.71                            |
| Sunflower meal            | 8.0     | Acid detergent lignin             |
|                          |         | 5.23                             |
| Rice hull powder          | 5.0     | Crude ash                        |
|                          |         | 8.12                             |
| Peanut shell powder       | 3.0     | Calcium                          |
|                          |         | 1.05                             |
| Soybean straw powder      | 5.0     | Total phosphorus                  |
|                          |         | 0.52                             |
| Premix \textsuperscript{b} | 5.0   | Lysine                           |
|                          |         | 0.04                             |
| Total                     | 100.0   | Methionine                        |
|                          |         | 0.47                             |

\textsuperscript{a}The premix provided the following per kg diet, vitamin A: 10,000 IU; vitamin D3: 2,000 IU; vitamin E: 50 mg; vitamin K3: 2.5 mg; vitamin B1: 5 mg; vitamin B2: 10 mg; nicotinic acid: 20 mg, pantothenic acid: 50 mg; folic acid: 2.5 mg; vitamin B12: 1 mg; choline chloride: 400 mg; Fe: 100 mg; Mn: 50 mg; Cu: 40 mg; Mn: 30 mg; I: 0.5 mg; Se: 0.05 mg; CaHPO4: 15,000 mg; NaCl: 5,000 mg; Lysine: 1,500 mg; Methionine: 1,500 mg; the rest is miscellaneous meal carrier complement.

\textsuperscript{b}Calculated values.

Care and Use Committee (SAAS-2020-05) and were conducted in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China).

**Animals, diet and feed chemical composition**

A total of 160 Minxinan black rabbits (half male and half female) aged 75 days with body weights of $1658.9 \pm 188.8$ g were randomly divided into 4 groups, with 10 replicates in each group and 4 rabbits in each replicate. They were fed a basic diet supplemented with 0 (control), 0.5%, 1.0%, 1.5% CLA, CLA containing fatty acids. The fat content of each carcase were collected to determine their physical properties, nutritional components and fatty acid composition.

**Determination of indicators and methods**

**Growth performance analysis**

At the beginning and end of the trial, the weight of each repeated rabbit was measured and the average daily gain was calculated. The average daily feed intake was calculated by dividing the total feed intake by the total test days and the number of test rabbits. The feed/gain ratio was calculated as feed intake/weight gain.

**Slaughter performance analysis**

Twelve hours prior to slaughter, the rabbits were stunned by electric shock and then slaughtered by bloodletting. After bleeding, the pelts, paws and full gastrointestinal tract were removed, and the commercial carcase was weighed (preserving the head, trachea, oesophagus, thoracic organs, liver, kidney and perirenal fat). The semiclean carcase weight was the commercial carcase weight after removing the head at the first cervical

| Fatty acids contents \textsuperscript{a} | Dietary CLA supplemental level (%) |
|------------------------------------------|----------------------------------|
| C14:0                                    | 0 (Control) 0.5 1.0 1.5           |
| C15:0                                    | 0.30 0.32 0.31 0.33              |
| C15:1                                    | 0.10 0.09 0.07 0.11              |
| C15:2                                    | 0.10 0.09 0.07 0.11              |
| C16:0                                    | 22.75 23.23 23.42 22.95          |
| C16:1                                    | 0.23 0.21 0.24 0.25              |
| C17:0                                    | 0.14 0.12 0.11 0.13              |
| C18:0                                    | 3.00 3.02 3.05 3.08              |
| C18:1 n-9                                 | 25.22 27.46 29.35 30.08          |
| C18:2 n-10                               | 30.10 33.15 35.95 37.54          |
| C18:3 n-3                                | 2.54 2.88 3.11 3.68              |
| CLA \textsuperscript{b}                  | ND 5.75 11.45 16.38              |
| C20:1                                    | 0.45 0.39 0.43 0.40              |
| C20:2                                    | 0.02 0.03 0.02 0.04              |
| C20:4                                    | 0.15 0.12 0.14 0.13              |
| C22:1 n-9                                | 0.58 0.52 0.55 0.59              |
| C22:2                                    | 0.12 0.10 0.15 0.14              |
| C24:0                                    | 0.05 0.07 0.06 0.04              |

\textsuperscript{a}n = 3 for groups, and data expressed as means.

\textsuperscript{b}CLA: conjugated linoleic acids, including cis-9, trans-11 and trans-10, cis-12 C18:2.

ND: not detectable.

1500 g for 10 min. The isolated serum samples were separated and stored at $-20^\circ$C for the determination of serum biochemical indices. At the same time, the longissimus thoracis et lumborum (LTL; between the 1st and 7th lumbar vertebra) muscles from both sides of each carcase were collected to determine their fatty acid composition.

**Sample collection and preparation**

At the end of the trial, 1 experimental rabbit was selected for each repetition, and 10 experimental rabbits in each treatment were selected for sample collection. A 10 mL blood sample was collected from the heart and then centrifuged at a centrifugal force of.
vertebra, removing the trachea and oesophagus, and retaining the liver, kidney and perirenal fat. The full clean carcase weight is the semiclean carcase weight after removing the heart, liver, kidney and perirenal fat. The heart, liver and kidney were also weighed. The commercial slaughter ratio, semiclean slaughter ratio, full clean slaughter ratio, heart index, liver index and kidney index were calculated by dividing their weights by the live weight before slaughter.

**Fat deposition and serum biochemical indices**

During slaughter, shoulder fat, perigastric fat and perirenal fat should be carefully stripped, weighed and calculated as their ratio to the live weight before slaughter and are relative weights, expressed as g/kg.

A sequential multiple analyser (Hitachi 7020, Japan) was used to analyse serum glucose, cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol following the manual of commercial protocols (Wako, Japan). The glucose content in serum was measured by the glucose oxidase endpoint method, and triglycerides were measured by the glycerol phosphate oxidase (GPO)-hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol (PAP) endpoint methods. In addition, using the cholesterol-PAP endpoint method to measure total cholesterol, the primary and secondary wavelengths were 505/670, and the measurement time was 300 s. For HDL cholesterol, the direct method-selective inhibition endpoint method was selected, while LDL cholesterol was measured by the direct method-surfactant removal endpoint method. The primary and secondary wavelengths were 570/700, and the measurement time was 300 s.

**Meat physical characteristics analyses**

The muscle colour (L*, a*, b*) was measured by an NR20XE colour difference metre (3NH Technology Co., Ltd.) in CIE-Lab output mode, set to the L*, a*, b* colour space and illuminant D65, with an observer angle of 2°, an aperture size of 5.0 mm and a closed cone. The chromameter was calibrated using a standardised white tile prior to measurement, and three measurements were taken across the face of the LTL for each sample. From the chromaticity coordinates, hue (H°) and chroma (C°) were calculated (McLellan et al. 1995).

At the end of the chilling period (chilling for 24 h at 4°C), the pHu values (pH measured at 24 h post-mortem) were measured by a pH metre (HI92240, Hanna Instruments, Padova, Italy) with a penetration electrode (Double-Pore cod. n° 32384003, Hamilton) at the fifth rib of the LTL muscle. Before measurement, the pH metre was calibrated with pH = 6.86 standard phosphate-buffered saline (PBS), and the probe was inserted into the muscle for a reading at 3 mm. The pH metre was calibrated with pH 4.01 and 6.86 buffers at 22°C. The whole LTL was removed from both sides of each carcase, cut into 2 cm thick pieces within 45–60 minutes after slaughter, and a 5 cm long strip with a width of 3 cm was trimmed and weighed (W1). One end of the meat sample (W1) was hooked with a thin wire to hang the meat sample vertically downwards in a plastic cup, and the loin samples were not close to the four walls and sealed with fresh-keeping film at 4°C under gravity. After 24 h, the samples were weighed (W2). At 24 h, the drip loss ratio (%) = 100*(W1–W2)/W1. To determine the cooking loss ratio and shear force, the whole LTL muscle samples were individually weighed (25–30 g) and recorded as the initial weight (M1). The weighted samples were individually vacuum-sealed in PVC bags and cooked for 10 min in a preheated water bath set at 80°C to ensure a core temperature of 75°C, detected by a thermometer equipped with a Type J (iron constantan) thermocouple (EUROTRON, Micrologger 2, Sesto San Giovanni, Italy). The cooked samples were then removed from the water bath, equilibrated at room temperature, removed from the bag, blotted dry using paper towels without any squeezing and reweighed (M2). The cooked meat percentage was calculated according to Honikel (1998) using the following equation: Cooking loss ratio (%) = 100%*(M1–M2)/M1. Shear force (N) was measured in triplicate using a Warner-Blatzler meat shear apparatus (C-LM, USA). Each sample was cores (Ø = 1.25 cm, thickness = 2 cm) obtained from loin samples (25–30 g), cut perpendicularly to the fibre direction and previously cooked in a water bath (80°C, 10 min) until reaching the core temperature of 75°C (measured with the thermocouples), and there was one cooking batch for the test.

**Meat chemical characteristics analysis**

The chemical composition of the meat was analysed using the LTL muscles following the procedures of AOAC International (2005). Prior to analyses, the samples were vacuum-packed, frozen at −20°C and stored for approximately two months until the start of analysis. Twenty-four hours before analysis, samples were thawed inside their vacuum bags at 4°C. The dry matter was determined by oven drying at 105°C (procedure 934.01), and the ether extract was determined by extraction with petroleum ether in a Soxtec 1043 apparatus (FOSS Tecator AB, Hogensa, Sweden; procedure 920.39). The protein content was determined using a Kjeltec Auto 1030 Analyser (procedure...
Lipid extraction and fatty acid analysis
Analyses were carried out on basal diets and LTL muscles. Total lipids were extracted according to Bligh and Dyer (1959) and directly transmethylated (Ichihara et al. 1996). Five grams of each sample was weighted, and 100 mg of pyrogallic acid and several zeolites were added, followed by 2 mL of 95% ethanol and 10 mL hydrochloric acid solution, and the sample was mixed well. The flask was placed into a water bath at 70°C ~ 80°C for hydrolysis for 40 min. The flask was shaken every 10 min to mix the particles adhered to the flask wall into the solution. After hydrolysis, the flask was removed and cooled to room temperature. For the hydrolysed sample, 10 mL of 95% ethanol was added and mixed well. The hydrolysate in the flask was transferred to the separating funnel, and the flask and plug were washed with 50 mL of ether petroleum ether mixture, after which the washing solution was poured into the separating funnel and covered. The mixture was shaken for 5 min and allowed to stand for 10 min. The ether layer extract was collected into a 250 mL flask. The above steps were repeated to extract the hydrolysate 3 times. Finally, wash the separating funnel with the mixture of ether and petroleum ether, collect it into a constant weight flask, steam the flask in a water bath and concentrate to dryness by rotary evaporator, and the residue is fat extract. In the fat extract, continue to add 2 mL of 2% sodium hydroxide methanol solution, water bath in 80°C water bath for 30 min, then add 3 mL of 14% boron trifluoride methanol solution, and water bath in 80°C water bath for 30 min. After the water bath incubation was completed and the temperature had returned to room temperature, 1 mL of n-hexane was added to the centrifuge tube, shaken and extracted for 2 min, allowed to stand for 1 h and allowed to stratify. The supernatant (100 μL) was diluted to 1 mL with n-hexane. A 0.45 μM filter membrane was tested on the machine after filtration. Fatty acid methyl esters (FAMEs) were analysed on a gas chromatograph (GC 7890 A; Agilent Technologies, Santa Clara, CA) according to Pirini et al. (2007).

For fatty acid determination, an SP-2560 (100 m × 0.25 mm × 0.20 μm; Supelco, Bellefonte, PA) chromatographic column was used. For the temperature rise procedure, the sample was maintained at 100°C for 13 min; 100°C~180°C, heating rate 10°C/min, and keeping for 6 min; 180°C~200°C, heating rate 1°C/min, keeping for 20 min; 200°C~240°C, heating rate 4°C/min, and keeping for 10.5 min. The injection port temperature was 250°C, the carrier gas flow rate was 0.5 mL/min. Split injection was performed; Detector was flame ionization detector (FID) and temperature: 250°C. For CLA determination, chromatographic column as fatty acid determination; The temperature rise procedure was as follows: 100°C was maintained for 13 min, the temperature was increased to 180°C at a rate of 10°C/min and maintained for 6 min. Then, the temperature was increased to 192°C at a rate of 1°C/min and maintained for 9 min. The temperature was then increased to 230°C at a rate of 3°C/min and maintained for 10 min. The injection port temperature was 240°C. Nitrogen was the carrier gas, and the flow rate was 1.3 mL/min. Split injection was performed, and the FID temperature was also 280°C. Data were processed using a Varian Star Chromatography Workstation, and each peak was identified with pure FAME standard mixtures (Sigma–Aldrich, USA). The content of fatty acids in diets was expressed as % of total fat acid, and the content of fatty acids in meat was expressed as mg/100 g tissue.

Statistical analysis
All the data were analysed by analysis of variance (ANOVA) and Duncan's test. A general linear model (GLM) procedure of SAS 9.1.3 statistical software (SAS Institute Inc., Cary, NC, USA) was used to assess the effects of the dietary CLA treatment on the growth performance, carcass yield and muscle quality and fatty acid profile of the meat. The orthogonal polynomial contrast test was performed to determine the linear and quadratic effects of the inclusion level of CLA in diets. The data are expressed as the mean and root mean square error (RMSE), n = 10 per group, and p < .05 was considered to be significant.

Results
Growth performance
The growth performance of the experimental rabbits is presented in Table 3. The dietary CLA supplementation level had a linear effect on the average daily feed intake and a quadratic effect on the feed/gain of meat rabbits (p < .05). With the increase in the addition level, the feed intake decreased, and the feed/gain ratio first decreased and then increased, with the lowest intake level in the 0.5% group. However, the final body weight and average daily gain were similar between groups (p > .05).
Slaughter performance

Dietary CLA supplementation had linear effects on the kidney weight and kidney ratio of experimental rabbits ($p < .05$), and the kidney weight and kidney index of the experimental group (0.5%, 1.0% and 1.5% group) with CLA were significantly higher than those of the control group. Furthermore, CLA supplementation of maternal diets had no linear or quadratic influence on the preslaughter live weight, commercial carcase weight, semiclean carcase weight, full clean carcass weight, heart weight, liver weight ($p > .05$), commercial slaughter ratio, semiclean carcase ratio, full clean carcase ratio, heart index, or liver index (Table 4, $p > .05$).

Fat metabolism

As shown in Table 5, dietary CLA supplementation level had linear or quadratic effects on the relative weight of shoulder fat, perigastric fat or perilrenal fat of meat rabbits ($p < .05$). With the increase in addition level, the relative weight of abdominal fat (the relative weights of perigastric fat and perilrenal fat) decreased significantly, and the relative weight of subcutaneous fat (the relative weight of shoulder fat) increased first and then decreased and reached the maximum in the 0.5% group. Dietary CLA supplementation had a quadratic effect on the contents of triglycerides and had a linear effect on cholesterol in serum ($p < .05$), and serum triglycerides and cholesterol in the experimental group (0.5%, 1.0% and 1.5% group) with CLA were significantly lower than those in the control group. However, dietary CLA supplementation had no linear or quadratic effects on serum glucose, HDL and LDL in meat rabbits ($p > .05$).

Physical characteristics of the LTL meat

The physical characteristics of the LTL meat of the meat rabbits are presented in Table 6. Dietary CLA supplementation level had linear effects on the pHu value and muscle colour ($a^*$, $b^*$ and $C^*$, $p < .05$), and the pHu value and $a^*$, $b^*$ and $C^*$ values of the 1.5% group were significantly lower than those of the control group. Nevertheless, dietary CLA supplementation level had no linear or quadratic effect on muscle colour ($L^*$, $H^*$), shear force, drip loss ratio or cooking loss ratio among the experimental rabbits ($p > .05$).

Chemical characteristics of the LTL meat

As shown in Table 7, dietary CLA supplementation had linear effects on the contents of crude protein, ether extract and cholesterol ($p < .05$). The crude protein levels of the experimental groups (0.5%, 1.0% and 1.5% group) were significantly higher than that of the control group, the cholesterol was significantly lower than that of the control group, and the ether extract of the high-dose addition group (1.5% group) was significantly lower than that of the control group.

Fatty acid composition of the LTL meat

The effects of the experimental diets on the fatty acid compositions of the LTL are reported in Table 8. Feeding CLA could linearly decrease the proportions of C16:1 (palmitoleic acid), C18:1 n-9 (oleic acid), C18:2 n-6 (linoleic acid), C20:2 ($\alpha$-linolenic acid), C20:3 n-6 (cis-8,11,14-eicosatrienic acid) and C22:1 n-9 (erucic acid, $p < .05$) and increase the proportions of C17:0 (heptadecanoic acid), C18:0 (stearic acid), C18:2 n-6 (linoleic acid), C20:2 (cis-11,14-eicosadienoic acid), C21:0 (heneicosanoic acid) and C24:0 (tetradecanoic acid, $p < .05$), but the proportions of C12:0 (lauric acid), C14:0 (myristic acid), C16:0 (palmitic acid) and C20:3 n-3 (alpha-linolenic acid) increased first and then decreased ($p < .05$). In addition, dietary CLA supplementation level had linear and quadratic effects on the percentage of CLA (including cis-9, trans-11 and trans-10, cis-12 C18:2, $p < .05$) and increased with increasing levels of CLA addition. The inclusion of CLA in diets could linearly and quadratically affect the percentage of total saturated fatty acids ($\sum$ SFA), total unsaturated fatty acids ($\sum$ UFA) and UFA/SFA ($p < .05$). Furthermore, the total monounsaturated fatty acids

| Table 3. Effects of dietary dietary conjugated linoleic acid (CLA)-supplemented level on growth performance of fattening rabbits. |
|---------------------------------------------------------------|
| **Items** | 0 (Control) | 0.5 | 1.0 | 1.5 | RMSE | ANOVA | Linear | Quadratic |
|-----------|-------------|------|------|------|------|--------|---------|-----------|
| Initial body weight (g) | 1652.8 | 1652.5 | 1666.9 | 1663.2 | 195.682 | .998 | .941 | .257 |
| Final body weight (g) | 2466.1 | 2409.4 | 2443.9 | 2393.9 | 345.556 | .927 | .619 | .857 |
| Average daily gain (g/d) | 16.27 | 16.86 | 15.54 | 14.61 | 5.092 | .782 | .540 | .600 |
| Average daily feed intake (g/d) | 164.89<sup>a</sup> | 154.18<sup>ab</sup> | 155.92<sup>ab</sup> | 139.25<sup>b</sup> | 28.283 | .025 | .042 | .123 |
| Feed/Gain | 10.46<sup>a</sup> | 9.86<sup>b</sup> | 10.72<sup>a</sup> | 10.99<sup>a</sup> | 2.014 | .033 | .986 | .044 |

<sup>a,b</sup>Different letters in the same row indicate significant differences ($p < .05$) between groups, $n = 10$ for each group. RMSE: Root mean square error.
Table 4. Effects of dietary conjugated linoleic acid (CLA)-supplemented level on slaughter performance of fattening rabbits.

| Items                        | Dietary CLA supplemental level (%) | RMSE | ANOVA | Linear | Quadratic |
|------------------------------|------------------------------------|------|-------|--------|----------|
| Pre-slaughter body weight (g)| 0 (Control) 0.5 1.0 1.5            |      |       |        |          |
|                             | 2402.5 2554.1 2495.9 2449.7       | 353.709 |       |        |          |
| Commercial carcass weight (g)| 1499.2 1583.9 1550.2 1518.7      | 215.220 |       |        |          |
| Semi clean carcass weight (g)| 1352.0 1436.2 1386.5 1371.4      | 202.943 |       |        |          |
| Full clean carcass weight (g)| 1168.5 1226.0 1203.3 1194.7      | 161.070 |       |        |          |
| Heart weight (g)             | 6.43 6.69 6.56 6.85               | 1.028 |       |        |          |
| Liver weight (g)             | 84.40 96.09 108.61 98.48          | 30.182 |       |        |          |
| Kidney weight (g)            | 13.38 17.11 19.40 16.95           | 3.417 |       |        |          |
| Semi clean slaughter ratio (%)| 62.51 62.05 62.11 62.01          | 1.730 |       |        |          |
| Full clean slaughter ratio (%)| 48.74 48.16 48.15 48.89         | 1.941 |       |        |          |
| Heart ratio (g/kg)           | 6.43 6.69 6.56 6.85               | 1.028 |       |        |          |
| Liver ratio (g/kg)           | 84.40 96.09 108.61 98.48          | 30.182 |       |        |          |
| Kidney ratio (g/kg)          | 13.38 17.11 19.40 16.95           | 3.417 |       |        |          |

a–cdifferent letters in the same row indicate significant differences \( p < .05 \) between groups, \( n = 10 \) for each group.

RMSE: Root mean square error.

Table 5. Effects of dietary conjugated linoleic acid (CLA)-supplemented level on fat metabolism of fattening rabbits.

| Items                        | Dietary CLA supplemental level (%) | RMSE | ANOVA | Linear | Quadratic |
|------------------------------|------------------------------------|------|-------|--------|----------|
| Fat deposition (g/kg)        | 0 (Control) 0.5 1.0 1.5            |      |       |        |          |
| Relative weight of shoulder fat | 5.97c 9.64a 8.95ab 6.75bc         | 2.612 | .009  | .942   | .031     |
| Relative weight of perigastric fat | 4.22a 3.88ab 3.59b 2.55b           | 1.462 | .041  | .002   | .005     |
| Relative weight of perirenal fat | 24.44a 23.07a 17.09ab 13.27b        | 7.888 | <.001 | .001   | .001     |
| Glucose (%)                  | 0.48 1.79 1.06 1.52               | 1.089 | .056  | .117   | .271     |
| Triglyceride (mmol/L)        | 5.80a 3.65b 3.45b 1.43c           | 2.011 | .024  | .111   | .014     |
| Cholesterol (mg/kg)          | 2.66a 1.60b 1.27 b 1.37 b         | 1.054 | .024  | .024   | .881     |
| HDL (mg/dL)                  | 0.54 0.44 0.37 0.45              | 0.215 | .392  | .224   | .070     |
| LDL (mg/dL)                  | 0.81 0.62 0.49 0.42              | 0.563 | .317  | .529   | .716     |

a–cdifferent letters in the same row indicate significant differences \( p < .05 \) between groups, \( n = 10 \) for each group.

RMSE: Root mean square error; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Table 6. Effects of dietary conjugated linoleic acid (CLA)-supplemented level on physical characteristics of the longissimus thoracis et lumborum (LTL) meat.

| Items                        | Dietary CLA supplemental level (%) | RMSE | ANOVA | Linear | Quadratic |
|------------------------------|------------------------------------|------|-------|--------|----------|
| pHu value                    | 0 (Control) 0.5 1.0 1.5            |      |       |        |          |
| Shear force (N)              | 25.48 25.87 25.77 25.77            | 2.070 |       |        |          |
| Drip loss ratio (%)          | 2.61 2.35 3.53 3.11               | 1.116 | .104  | .209   | .443     |
| Cooking loss ratio (%)       | 34.51 34.48 33.97 33.56            | 1.908 | .639  | .192   | .368     |
| Lightness (L*)               | 51.33 50.85 51.96 50.41            | 5.084 | .914  | .694   | .922     |
| Redness (a*)                 | 13.79a 13.22a 12.13ab 10.71b        | 2.538 | .041  | .021   | .065     |
| Yellowness (b*)              | 9.03a 8.75ab 8.90a 7.46b           | 1.427 | .049  | .040   | .024     |
| Chroma (C*)                  | 16.49a 15.88a 15.11ab 13.06b         | 2.792 | .041  | .027   | .085     |
| Hue (H*)                     | 33.31 33.77 36.70 34.76            | 3.158 | .097  | .150   | .316     |

a–bDifferent letters in the same row indicate significant differences \( p < .05 \) between groups, \( n = 10 \) for each group.

RMSE: Root mean square error.

Table 7. Effects of dietary conjugated linoleic acid (CLA)-supplemented level on chemical characteristics of the longissimus thoracis et lumborum (LTL) meat.

| Items                        | Dietary CLA supplemental level (%) | RMSE | ANOVA | Linear | Quadratic |
|------------------------------|------------------------------------|------|-------|--------|----------|
| Dry matter (%)               | 0 (Control) 0.5 1.0 1.5            |      |       |        |          |
| Crude protein (%)            | 24.39b 25.12a 25.47a 25.03a        | 0.582 | .225  | .059   | .113     |
| Ether extract (%)            | 1.56a 1.33ab 1.32ab 1.21b           | 0.333 | .002  | .041   | .024     |
| Crude ash (%)                | 1.23a 1.30 1.23 1.31               | 0.125 | .039  | .015   | .162     |
| Cholesterol (mg/kg)          | 550.87a 493.14b 483.61b 489.19b     | 45.380 | .317  | .143   | .341     |

a–bDifferent letters in the same row indicate significant differences \( p < .05 \) between groups, \( n = 10 \) for each group.

RMSE: Root mean square error.
Table 8. Effects of dietary dietary conjugated linoleic acid (CLA)-supplemented level on fatty acid composition of the longissimus thoracis et lumborum (LTL) meat (% of total fatty acids).

| Fatty acids contents | 0 (Control) | 0.5 | 1.0 | 1.5 | RMSE | ANOVA   | p-value |
|----------------------|-------------|-----|-----|-----|------|---------|---------|
| C12:0                | 0.03a       | 0.06a | 0.05a | 0.04ab | 0.011 | .017 | .465 | .010 |
| C14:0                | 1.63a       | 2.23a | 1.94ab | 1.71b  | 0.329 | .001 | .828 | <.001 |
| C14:1                | 0.16        | 0.17  | 0.12  | 0.10  | 0.067 | .127 | .065 | .063 |
| C15:0                | 0.46        | 0.50  | 0.47  | 0.48  | 0.049 | .178 | .289 | .505 |
| C16:0                | 27.38c      | 30.19a | 28.71b | 27.41c | 1.362 | <.001 | .475 | <.001 |
| C16:1                | 3.53a       | 2.84b | 2.20bc | 1.76c  | 0.758 | <.001 | <.001 | <.001 |
| C17:0                | 0.65        | 0.77b | 0.82ab | 0.86c  | 0.078 | <.001 | <.001 | <.001 |
| C18:0                | 10.67a      | 11.48b | 12.93a | 13.61a | 1.154 | <.001 | <.001 | <.001 |
| C18:1 n-9            | 23.66a      | 21.38b | 20.47bc | 19.84c | 1.484 | <.001 | <.001 | <.001 |
| C18:2 n-6            | 20.33a      | 21.25bc | 22.26ab | 23.63a | 1.921 | .004 | <.001 | <.001 |
| C18:3 n-3            | 1.02        | 1.18  | 1.11  | 1.08  | 0.236 | .552 | .659 | .698 |
| C18:3 n-6            | 0.07a       | 0.05b  | 0.04b  | 0.04ab | 0.009 | <.001 | <.001 | <.001 |
| CLA                  | 0.03c       | 0.05c  | 1.07  | 1.72c  | 0.303 | <.001 | <.001 | <.001 |
| C20:0                | 0.20        | 0.17  | 0.20  | 0.23  | 0.048 | .087 | .643 | .310 |
| C20:1                | 0.28ab      | 0.29a  | 0.25ab | 0.23b  | 0.058 | .024 | .005 | .012 |
| C20:2                | 0.32b       | 0.39a  | 0.4a  | 0.4a  | 0.062 | .002 | .006 | .004 |
| C20:3 n-3            | 0.04ab      | 0.05ab | 0.07a  | 0.05ab | 0.019 | .013 | .648 | .022 |
| C20:3 n-6            | 0.52a       | 0.38b  | 0.39b  | 0.40b  | 0.103 | .010 | .014 | .024 |
| C20:4 n-6            | 7.94        | 5.78  | 6.69  | 7.20  | 1.751 | .062 | .521 | .127 |
| C20:5 n-3            | 0.09        | 0.07  | 0.08  | 0.09  | 0.023 | .391 | .697 | .850 |
| C21:0                | 0.03b       | 0.03b  | 0.03b  | 0.04a  | 0.007 | <.001 | <.001 | <.001 |
| C22:0                | 0.20        | 0.17  | 0.20  | 0.23  | 0.048 | .087 | .144 | .046 |
| C22:1 n-9            | 0.34ab      | 0.20b  | 0.12c  | 0.12c  | 0.599 | .025 | <.001 | <.001 |
| C22:2                | 0.02        | 0.02  | 0.02  | 0.03  | 0.025 | .599 | .687 | .913 |
| C22:6 n-3            | 0.14        | 0.10  | 0.14  | 0.15  | 0.041 | .076 | .518 | .362 |
| C23:0                | 0.12        | 0.11  | 0.11  | 0.13  | 0.027 | .190 | .274 | .700 |
| C24:0                | 0.18b       | 0.17b  | 0.20ab | 0.24a  | 0.050 | .027 | .017 | .018 |
| C24:1                | 0.16        | 0.13  | 0.14  | 0.15  | 0.038 | .411 | .609 | .212 |

Summary

| ∑SFA | 41.36a | 45.71a | 45.46a | 44.76a | 1.310 | <.001 | <.001 | <.001 |
|∑UFA | 58.64a | 54.29b | 54.54a | 55.24b | 1.310 | <.001 | <.001 | <.001 |
|UFA/SFA | 1.418a | 1.194b | 1.2022b | 1.2362b | 0.066 | <.001 | <.001 | <.001 |
|∑MUFA | 28.14c | 25.07c | 23.30c | 22.16c | 1.949 | <.001 | <.001 | <.001 |
|∑PUFA | 30.50a | 29.28b | 31.24ab | 33.06a | 2.285 | .007 | .004 | .001 |
|PUFA/MUFA | 1.10c | 1.19bc | 1.35ab | 1.50a | 0.182 | <.001 | <.001 | <.001 |
|∑n-3 PUFA | 1.30 | 1.41 | 1.41 | 1.36 | 0.196 | .538 | .470 | .599 |
|∑n-6 PUFA | 28.86a | 27.46b | 29.38ab | 31.27a | 2.269 | .007 | .007 | .001 |
|n-6 PUFA/n-3 PUFA | 22.44 | 20.15 | 21.25 | 23.39 | 3.635 | <.001 | <.001 | <.001 |

**a**CLAs: conjugated linoleic acids, including cis-9, trans-11 and trans-10, cis-12 C18:2.

**Different letters in the same row indicate significant differences (p < .05) between groups, n = 10 for each group.**

**RMSE:** Root mean square error; **SFA:** saturated fatty acids; **UFA:** unsaturated fatty acids; **MUFA:** monounsaturated fatty acids; **PUFA:** polyunsaturated fatty acids.

Fatty acids contents (% of total fatty acids).

Discussion

CLA is an indispensable fatty acid with a high energy value. As one of the important factors that influences animal growth performance, dietary energy density has a close relationship with animal production performance (Panda et al. 2006; Wu et al. 2017; Wang et al. 2020). Previous studies showed that a CLA diet decreased the feed intake of fattening rabbits when the addition level of CLA exceeded 1.5%, and the lower feed intake might be due to the higher energy of CLA, which was consistent with research showing that an overdose addition level of CLA in the diet decreased the egg production of laying hens (Shang et al. 2005; Kim et al. 2007).

Animal growth is predominantly due to increases in protein synthesis, and the effects of diet composition on slaughter traits have been thoroughly investigated. The effect of dietary CLA inclusion depends not only on the extent and dose of CLA supplementation but also on the animal’s age (Corino et al. 2002, 2004). The growth performance and carcass characteristics of commercial meat rabbits at standard slaughter weight (2.5 kg, 76 days) were not affected by diets supplemented with 2.5 or 5 CLA g/kg. Our experiment demonstrated that CLA addition to the rabbit diet did not affect slaughter performance or carcass yield. This finding is in agreement with the research of other studies.
with other studies that found no significant differences in carcase net weight or eviscerated ratio from rabbits that were slaughtered at 2,500 g in weight (Peiretti and Meineri 2008). The diets did not significantly influence the carcase yield of the rabbits (Liu et al. 2017).

Many investigations have shown that CLA may have beneficial effects on diabetes, obesity, the inflammatory response, atherosclerosis, and glucolipid metabolism (Reynolds et al. 2008; Kennedy et al. 2010). Xu et al. (2003) reported that feeding diets supplemented with CLA to growing mice reduces body fat mass. CLA (especially the trans-10, cis-12 isomer) is reported to reduce body lipid deposition and regulate hepatic lipometabolism, as well as the inflammatory response and antioxidative capability in animals, referring to cis-9 and trans-11 isomers (Shen and McIntosh 2016). In addition, CLA supplementation reduced perirenal fat weight at a heavy slaughter weight and lowered the concentrations of serum triglycerides and cholesterol; our results are similar to Corino et al. (2002). Moreover, the results show that the blood glucose concentration in the experimental groups tended to increase, indicating improved insulin sensitivity in animals (Holtenius and Holtenius 2007; Hammon et al. 2009).

The main sensory properties of meat are its colour, juiciness, tenderness and flavour. Muscle pH and water-holding capacity exert a high influence on the technological and eating qualities of meat. In rabbits, the important factors affecting muscle pHu are identified as muscle type, age, slaughter method, carcase treatment post-mortem and animal diet (Dalle Zotte 2002). The main colour variability factors are the type of muscle, muscle pHu and myoglobin content, age and diet of the rabbits (Dalle Zotte et al. 1996) and even the activity undertaken by the animal (Gondret et al. 2009). Dietary CLA supplementation level had an effect on the pHu value, and muscle colour was found in our study, which may be related to CLA in terms of antioxidative activity and has been demonstrated in different animal models (Hanschke et al. 2016; Qi et al. 2018).

CLA has been reported to have a wide range of beneficial effects, including anticarcinogenic (Kelley et al. 2007), antiatherogenic (McLeod et al. 2004) and antiobesity (Whigham et al. 2007) activities. Previous studies have shown that CLA could enrich tissues by increasing their nutritional value and improving the human diet with a fatty acid (Sirri et al. 2003). In addition to the beneficial effects of CLA on human health, CLA can favourably modify the rabbit’s body composition (Corino et al. 2002, 2004) due to its potential to increase lean tissue deposition. In this study, we found that CLA could increase the crude protein content and decrease the ether extract and the cholesterol content in rabbit meat.

Compared to other meats, rabbit meat has high nutritional value as a result of its lipid component, characterised by comparatively low fat and cholesterol levels, higher unsaturated fatty acids (UFA) and the best ratio of n-6/n-3 polyunsaturated fatty acids (PUFA) (Dalle Zotte and Szendro 2011; Montero-Vicente et al. 2018; Petrescu and Petrescu-Mag 2018). However, Chamruspollert and Sell (1999) also reported a decrease in MUFA in egg yolks when the hen diet contained 5% CLA and claimed that changes in MUFA could be ascribed to CLA, which inhibits the delta-9 desaturase enzyme system that is responsible for saturated fatty acid desaturation, converting them into MUFA. In this way, saturated fatty acid accumulation was increased, and MUFA formation was reduced. Our results in LTL meat of fattening rabbits agreed with the findings of the above authors. Dietary CLA inclusion is an effective tool for increasing, in a dose-dependent manner, the amount of CLA in the intramuscular lipids of rabbits, with cis-9, trans-11 being the predominant isomer (Fiego et al. 2005; Petacchi et al. 2005). Regarding the chemical composition of rabbit meat, significant decreases in meat lipid content were evident only when rabbits were fed a high supplementation level of CLA (5 g/kg) and at heavy slaughter weight (Corino et al. 2004). It is possible to increase the CLA content in rabbit loin from 1.3 to 10.4 mg/100 g meat with a 5 g/kg supplementation of CLA in the diet (Corino et al. 2007). Supplementing CLA in poultry diets has also been suggested as a way to obtain CLA-enriched meat and egg products (Aydin and Cook 2009; Kumari et al. 2014). Our results are consistent with previous reports; there was a strong positive correlation between the deposition of CLA in rabbit meat and the amount of dietary supplement.

Conclusions

These findings indicate that CLA could affect fat deposition and serum triglyceride and cholesterol levels and greatly improve the nutritional value of rabbit meat. From an economic point of view, CLA would normally be included at 0.5% in the formulation of feed for fattening rabbits.

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Disclosure statement
The authors declare that they have no competing interests.

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Data availability statement
The data that support the findings of this study are available from the corresponding author (Email: shuxiagao_2004@163.com), upon reasonable request.

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