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Research Article

Effects of feed restriction on the slaughter performance, antioxidant activity, and meat quality of rabbits

Qi Lu[1], Jixiao Qin[1], Shuanglong Xie[1], Rui Chen[1], Yiqing Xu[1], Xu Wang[1], Di Zhou[2] ✉, Xingzhou Tian[1] ✉

[1] *Key Laboratory of Animal Genetics, Breeding and Reproduction in the Plateau Mountainous Region, Ministry of Education, College of Animal Science, Guizhou University, Guiyang 550025, China*

[2] *Guizhou Testing Center for Livestock and Poultry Germplasm, Guiyang 550018, China*

✉Address correspondence to Di Zhou, dizhougz@163.com; Xingzhou Tian, tianxingzhou@yeah.net

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Abstract: The objective of this study was to investigate the effects of feed restriction on the slaughter performance of rabbit, and antioxidant activity, chemical composition, physical characteristics, amino acid, fatty acid, and volatile compound contents of rabbit meat. A total of 48 male rabbits with similar body weights were randomly allocated into two equal treatment groups: the control group, which received *ad libitum* access to the feed (AL), and the treatment group, which received 80% of the feed consumed by the control (FR). The results indicated that there were no significant differences in the slaughter performance parameters between the two groups ($P > 0.05$). Compared with the AL treatment, the FR treatment significantly increased muscle superoxide dismutase activity, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, and crude protein content ($P < 0.05$), whereas it significantly decreased the content of malondialdehyde, water loss rate, and shear force ($P < 0.05$). Compared with those in the AL treatment group, the rabbits in the FR treatment group presented significantly greater levels of threonine, glutamate, alanine, tyrosine, histidine, proline, and total amino acids ($P < 0.05$). Moreover, the AL treatment resulted in significantly greater concentrations of $C_{16:0}$, $C_{16:1}$, $C_{18:1\ n\text{-}9c}$, and monounsaturated fatty acids ($P < 0.05$), whereas it resulted in significantly lower levels of $C_{18:2\ n\text{-}6c}$, $C_{20:4\ n\text{-}6}$, and polyunsaturated fatty acids than the FR treatment did ($P < 0.05$). Furthermore, the FR treatment resulted in significantly greater levels of benzaldehyde, octanal, decanal, hexadecane, carbamodithioic acid diethyl-methyl ester, 2-heptanone, *N*-ethyl-ethanamine, dimethyl sulfone, and *N*,*N*-diethyl-formamide than did the AL treatment ($P < 0.05$). Overall, mild feed restriction can increase meat quality by increasing muscle antioxidant activity and improving the physical characteristics, amino acid, fatty acid, and volatile compound contents of rabbits under the conditions of this experiment.

Keywords: rabbit; feed restriction; slaughter performance; antioxidant activity; meat quality

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1 Introduction

Feedstuff is the main cost in the production of rabbits, so decreasing feed intake while maintaining growth performance can increase the economic benefits for farmers[1]. Currently, growing rabbits are usually fed *ad libitum* to maximize production performance[2]. However, when rabbits are fed *ad libitum*, mortality rates and the incidences of various diseases (such as metabolic disorders and skeletal diseases) increase, which might have negative effects on the animal's health and decrease their performance and the economic benefits[3]. Moreover, *ad libitum* feeding system might enhanced the impact of digestive diseases, and increased the mortality and morbidity rates of rabbits in the post-weaning period[4–5]. Accordingly, limiting feed intake may be an effective method to improve body's health and reduce mortality rates in rabbits[6].

Feeding restriction is a management technique in which animals are artificially restricted in terms of their feed intake or the intake of a certain nutrient[7]. In general, feeding restrictions include controlling feed intake, nutrient level, and feeding time. Notably, feeding restrictions can regulate the deposition of animal nutrients and improve growth and development, meat quality, and carcass quality[8]. Feed restriction in rabbits is frequently used to prevent gastroenteric pathologies, which can be used in different ways in terms of feeding time or feeding level[9]. For example, Birolo et al.[10] reported that feed restriction improved digestive health and epizootic rabbit enteropathy in rabbits. Similarly, Birolo et al.[2] suggested reducing feed intake without changing daily weight gain could reduce the morbidity and mortality rates of rabbits in the fattening stage. In addition, Chodová et al.[11] reported that feed restriction had no effect on performance, carcass traits or meat quality, whereas it improved some morphological muscle fibre characteristics in weaned rabbits. Therefore, feed restriction could be a useful tool to improve biological and economic performance, consequently reducing production costs in rabbits[12].

Previous study showed that limiting feed intake is the most commonly used feed restriction method in meat rabbits[5]. For example, Alabiso et al.[13] reported that restricted rabbits had no side effects on muscle physical characteristics, which could be compensated for partly by a lower feed conversion ratio. In addition, Birolo et al.[14] showed that feed restriction did not impact on the growth, slaughter performance, and meat quality of crossbred rabbits. To the best of our knowledge, the effects of feed restriction was mainly focused on the physical characteristic parameters in growing rabbits[13,15]. However, feed restriction on other meat quality parameters, such as amino acids (AAs), fatty acids (FAs), and volatile compounds of rabbit meat in the fattening stage have rarely been reported. Therefore, the aim of this study was to investigate the effects of mild feed restriction on the slaughter

performance, antioxidant activity, chemical composition, physical characteristics, AAs, FAs, and volatile compounds of rabbits in the fattening stage. This study provides a scientific basis for the selection of feed restrictions to regulate the meat quality of growing rabbits.

2 Materials and methods

2.1 Animals, experimental design, and diet

Previous study indicated that excessive feed restriction can lead to reduced growth performance in rabbits[16]. Moreover, Gidenne et al.[17] reported that mild feed restriction, resulting in an approximately 20% reduction in feed intake, has the ability to reduce mortality and improve the digestive health of rabbits. A total of 48 male 8-week-old rabbits with similar body weights ((1 872.11 ± 180.85) g) were randomly assigned to two equal treatment groups. The control group received *ad libitum* access to the feed (AL), and the treatment group received 80% of the feed consumed by the control (FR). The restriction program was applied by giving a daily meal starting at 8 weeks. Before the start of the experimental period, a pretest was conducted to calculate the feed intake of 24 randomly fed rabbits. This provided the feed consumption for both groups during the restricted feeding period[16]. Moreover, daily feed intake was calculated, and the amount of rabbit feed was adjusted weekly. The feeding trial lasted for 7 weeks and included a 1-week pretrial period and a 6-week formal period. The feed of the rabbits in the AL group was gradually reduced to 80% over 4 d, and the rabbits were allowed to acclimate to 80% for 3 d before beginning the experimental period. Each treatment consisted of 12 replicates with 2 rabbits each (2 rabbits per cage). The rabbits were placed in pairs in cages (160 cm × 70 cm × 195 cm). During the entire experimental period, all rabbits had free access to water. The experimental diet was manufactured and pelleted with a 4 mm millstone by a granule presser at one time (Jixiang Animal Husbandry Machinery Co., Ltd., Xinxiang, China) using one batch of raw materials. A pelleted fattening diet was fed in equal amounts twice daily at 8:30 and 17:30. The diet was formulated to cover the nutritional requirements of growing rabbits according to the Chinese standard (NY/T 4049–2021 *Nutrient requirements of meat rabbit*). The ingredients and chemical composition of the basal diet are shown in Table 1.

Table 1 The ingredients and chemical composition of basal diet.

Ingredients	Content (%)	Chemical composition	Content
Corn	23.66	DM (%)	89.43
Soybean meal	6.65	CP (% of DM)	16.35
Rapeseed meal	3.22	GE (mJ/kg)	15.22
Cottonseed meal	3.15	Neutral detergent fiber (% of DM)	34.85
Wheat bran	15.84	Acid detergent fiber (% of DM)	21.36
Alfalfa	42.48	Ash (% of DM)	9.72
Calcium hydrogen phosphate	0.40		
Salt	0.35		
Stone powder	0.25		
Compound premix	4.00		

Note: Values represent the means of three replicates (n = 3). Compound premix contained per kg were as followed: vitamin D_3 10 KIU; vitamin A 150 KIU; vitamin E 300 IU; vitamin B_3 300 mg; vitamin K 20 mg; folic acid 4 mg; biotin 1 mg; calcium pantothenate 200 mg; I 3.5 mg; Fe 1 500 mg; Mn 500 mg; Cu 300 mg; Zn 1 500 mg.

2.2 Determination of chemical composition

The chemical compositions of the diet and muscle for moisture (method 934.01), and crude protein (CP; method 988.05) contents were determined according to the AOAC standards[18]. Dry matter (DM) content was calculated 100 minus moisture content.

The neutral detergent fibre and acid detergent fibre contents of the diet were detected following the methods of van Soest et al.[19]. The ether extract (EE; AOAC 920.39) of muscle was analysed via the Soxhlet method (with diethyl ether as the solvent) using an automatic fat detector (Soxtce 8000, Foss, Denmark). The gross energy (GE) of the diet and muscle was analysed by an adiabatic oxygen bomb calorimeter (WGR-WR3, Changsha BENTE Instrument Co., Ltd., Changsha, China).

2.3 Determination of slaughter performance

Ethical approval for this study was obtained from the Guizhou University Subcommittee of Experimental Animal Ethics (protocol number EAE-GZU-2024-E028). The slaughter experiment was performed at the Institute of Animal Nutrition and Feed Science (Guizhou University, Guiyang, China). Briefly, at the end of the experimental period, all rabbits were fasted for 12 h and weighed before they were slaughtered to determine the preslaughter weight (PSW). After that, all rabbits were injected air by the ear margin intravenous to cause thrombotic death. Next, all rabbits were slaughtered, and the skin was separated and weighed to determine the skin weight (SW). After that, the lungs, reproductive organs, intestines, stomach, trachea, spleen, oesophagus, and pancreas were removed and weighed to determine the half-bore weight (HBW). After that, the heart, liver, and kidneys were removed weighed to determine the full-bore weight (FBW). Additionally, the half-bore rate (HBR) and full-bore rate (FBR) were calculated via the formulas (1) and (2):

$$\text{HBR}\ (\%) = \frac{\text{HBW(g)}}{\text{PSW(g)}} \times 100 \quad (1)$$

$$\text{FBR}\ (\%) = \frac{\text{FBW(g)}}{\text{PSW(g)}} \times 100 \quad (2)$$

Moreover, the heart, liver, spleen, lungs, and kidneys were separated and weighed. The relative weights of the heart, liver, spleen, lungs, and kidneys were calculated according to the methods of Attia et al.[20], as shown in formula (3):

$$\text{Relative weight of organ}\ (\%) = \frac{\text{Fresh organ weight (g)}}{\text{PSW(g)}} \times 100 \quad (3)$$

2.4 Determination of antioxidant activity

The *longissimus thoracis et lumborum* (LTL) muscle antioxidant activity was detected according to the method of Tian et al.[21]. The LTL was separated and cut into pieces, and 0.1 g of muscle was weighed and added to 0.9 g of precooled phosphate-buffered solution. Next, all of the mixtures were ultrasonicated via a Bransonic® ultrasonic cleaner (Branson Ultrasonics Co., Danbury, CT, USA). After centrifugation at 3 000 × g for 15 min at 4 °C, the muscle supernatant was obtained for the measurement of total antioxidant capacity (TAC; Cat. No. A015-1-2), superoxide dismutase (SOD; Cat. No. A001-1-2) activity, glutathione peroxidase (GPX; Cat. No. A005-1-2) activity, catalase (CAT; Cat. No. A007-1-1) activity, DPPH free radical scavenging activity

(Cat. No. A153-1-1), and malondialdehyde (MDA; Cat. No. A003-1-2) content. All kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and all antioxidant activity parameters were measured according to the manufacturer's instructions.

2.5 Determination of physical characteristics

The physical characteristics were analysed according to the methods of Yu et al.[22] and Wang et al.[23], with minor modifications. Briefly, the right LTL muscle was separated and kept at 4 °C for 24 h after slaughter to determine its physical characteristics. The muscle pH value was detected using a portable pH meter (Matthäus, Eckelsheim, Germany) with an automatic temperature compensation function. The muscle colour, including lightness (L^*), redness (a^*), and yellowness (b^*), was detected by using a portable colorimeter with a measured area diameter of 8 mm, standard illumininant D65, an 8° observer angle, and zero and white calibration. Three measurements per sample distributed on the muscle cross-section surface were taken after 30 min of air exposure to allow blooming. The a^* and b^* values were subsequently used in the calculation of chroma (C^*) and hue angle (H) via the formulas (4) and (5):

$$C^* = (a^{*2} + b^{*2})^{1/2} \tag{4}$$

$$H(°) = \arctan\frac{b^*}{a^*} \tag{5}$$

Drip loss was detected after the samples were attached to plastic netting and suspended in an inflated plastic bag for 24 h at 4 °C, and drip loss was the difference in weight before and after suspension and was expressed as a percentage of the initial weight. Approximately 4 g of LTL muscle was weighed, placed between 17 layers of filter paper, and then pressed with a 30 kg weight for 5 min by using a meat press (Tenovo Meat-1, Tenovo International Co., Ltd., Beijing, China). The water loss rate was calculated from the sample weight difference before and after the application of pressure. A cylindrical sample was collected from the central part of each muscle using 1.27-cm-diameter cylindrical cores. Each sample was sheared three times, and the peak force needed to shear the sample across fibres was recorded using a tenderness metre (Xielikeji Co., Ltd., Harbin, China) with a load cell of 15 kg and running at a crosshead speed of 200 mm/min.

2.6 Determination of AAs profile

The left LTL muscles were kept at −80 °C and then lyophilized via a vacuum freeze dryer (LYOQUEST-85 Plus, Telstar Industry, Spain). After that, all of the muscles were ground with a high-speed grinder to prepare the muscle powder for further analysis of AAs, FAs, and volatile compounds. The AAs profiles were detected as described by Luo et al.[24]. Briefly, 0.1 g of muscle powder was weighed, transferred into a hydrolysis tube, mixed with 10 mL of 6 mol/L HCl solution, and three drops of phenol were added. The mixture was subjected to vacuum after being filled with nitrogen; this step was repeated 3 times, and the mixture was then sealed. After that, the mixture was kept in an electric heating blast drying oven for hydrolysis at 110 °C for 22 h and cooled to room temperature. Next, the mixture was filtered with medium-speed filter paper and diluted in a 50 mL volumetric flask. Then, 1 mL of the mixture was collected and dried under reduced pressure in a drying box, added to 1 mL of a 0.2 mol/L of buffer solution of sodium citrate (pH 2.22), and filtered through a 0.45 μm filter membrane. The individual AAs profile was analysed via an AAs automatic analyser (Biochrom 30, Biochrom Ltd., Cambridge, UK). In this study, aspartate (Asp), threonine (Thr), serine (Ser), glutamate (Glu), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), lysine (Lys), arginine (Arg), and proline (Pro) were detected. Moreover, the sum of these AAs contents was set at the total AAs (TAAs) content. All of the determinations were carried out in triplicate.

2.7 Determination of FAs profiles

The FAs profile was analysed in accordance with the internal standard method outlined by Guo et al.[25], with minor modifications. Briefly, 2 g of muscle powder was weighed and added to 0.4 mL of 5.00 mg/mL methanol glycerol triundecanoate as an internal standard. The total lipids were extracted using diethyl ether-light petroleum (1:1, *V*/*V*) solution, and then, 1.6 mL of 2 g/100 mL NaOH in methanol was added to prepare fatty acid methyl esters (FAME). Next, 4 mL of heptane and 10 mL of saturated sodium chloride solution were added to the mixture, which was subsequently centrifuged at 4 000 × *g* for 10 min at 4 °C. After that, 1 mL of the supernatant was collected, 0.8 g of Na_2SO_4 was added, mixed evenly, and centrifuged at 4 000 × *g* for 10 min at 4 °C. The supernatant was harvested and filtered through a 0.45 μm nylon syringe, and individual FAs profiles were measured with gas chromatography (GC; Agilent 6890, Agilent Technologies, Santa Clara, CA, USA) with a polydicyanopropyl siloxane capillary column (100 m × 0.25 mm, 0.20 μm) and a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). The injector temperature was 270 °C, and the ionization detector temperature was 280 °C. The column oven temperature was held at 45 °C for 4 min, increased to 180 °C at 10 °C/min, held at 180 °C for 6 min, increased from 180 to 200 °C at 1 °C/min, held at 200 °C for 6 min, increased to 230 °C at 4 °C/min, and held at 230 °C for 10.5 min. The carrier gas was nitrogen with a 100:1 split ratio. The peaks of individual FAMEs were identified and quantified by comparison of the retention times and peak areas with known external standards.

2.8 Determination of volatile compounds

The volatile compounds present in the rabbit meat were detected using the headspace solid-phase microextraction method described by Yang et al.[26] and Yuan et al.[27], with minor modifications. Briefly, 2 g of rabbit meat was weighed and put into a headspace injection bottle (20 mL, Supelco). The 2 cm-50/30 μm DVB/CAR/PDMS StableFlex was inserted into the sample vial and equilibrated at 80 °C for 60 min. Then, the volatile compounds were detected by using a GC-mass spectrometer (GC-MS; HP6890/5975C, Agilent Technologies, Santa Clara, CA, USA) under chromatographic conditions. The chromatographic column used was an Agilent HP-5MS elastic quartz capillary column (60 m × 0.25 mm, 0.25 μm) with an initial temperature of 45 °C for 2 min, which was increased to 185 °C at a rate of 3.5 °C/min and then increased to 310 °C at a rate of 10 °C/min. The running time was 54.5 min; the transfer line temperature was set at 250 °C, the precolumn pressure

was 16.39 psi, and the solvent delay time was 3 min. Helium (99.999%) with a constant flow rate of 1 mL/min was used as the carrier gas. MS was performed via electron impact ionization at 230 °C at 70 eV, the interface temperature was 280 °C, the quadrupole temperature was 150 °C, the emission current was 34.6 μA, the multiplier voltage was 1 858 V, and the mass range was 29–500 amu. The individual volatile compounds were identified in accordance with mass spectra and linear retention indices from NIST20 and Wiley275, and the relative mass fractions of each chemical component were determined using peak area normalization method.

2.9 Statistical analysis

The sample size analysed by the SAS 9.1.3 software, and 12 replicates resulted in a 0.05 significance level and 0.80 power[28]. The cage was considered an experimental unit, and the average of the data was calculated for two rabbits in each cage for further statistical analysis; thus, each treatment had 12 replicates (n = 12). All data were calculated of two rabbits in each cage, and the means of these data were used for further analysis. The variance homogeneity was analysed by Levene's test method, and normal distribution was analysed by Kolmogorov-Smirnov method, and all data were variance homogeneity and normally distributed. Therefore, all the parameters were analysed using SAS 9.1.3 software (SAS Institute Inc., Cary, NC, USA) and student's t-test[28]. The level of significance was set to $P < 0.05$.

3 Results and discussion

3.1 Slaughter performance

There were no significant differences in the carcass yield parameters (including PSW, SW, HBW, HBR, FBW, and FBR) or visceral organ (including heart, liver, spleen, lungs, and kidneys) weight parameters between the two groups ($P > 0.05$) (Table 2).

Table 2 Effect of feed restriction on slaughter performance in rabbit.

Slaughter performance	AL	FR	SEM	*P* value
Carcass yield				
PSW (g)	2 685	2 757	93.514	0.593
SW (g)	303	298	7.239	0.592
HBW (g)	1 840	1 851	49.842	0.883
HBR (%)	68.50	67.69	1.314	0.665
FBW (g)	1 364	1 347	38.263	0.744
FBR (%)	50.78	49.25	1.048	0.314
Visceral organ				
Heart weight (g)	7.35	7.47	0.275	0.763
Heart relative weight (%)	0.273	0.275	0.011	0.907
Liver weight (g)	60.36	59.28	3.107	0.809
Liver relative weight (%)	2.24	2.20	0.123	0.817
Spleen weight (g)	1.41	1.45	0.140	0.836
Spleen relative weight (%)	0.053	0.054	0.006	0.894
Lung weight (g)	22.33	20.63	1.707	0.487
Lung relative weight (%)	0.832	0.749	0.059	0.326
Kidney weight (g)	14.83	15.25	0.488	0.552
Kidney relative weight (%)	0.552	0.563	0.022	0.722

Note: AL, rabbits were fed *ad libitum* level; FR, rabbits were fed 80% *ad libitum* level; SEM, standard error of mean.

3.2 Antioxidant activity

There were no significant differences in the muscle TAC, GPX or CAT activities between the two treatment groups ($P > 0.05$) (Figure 1). The SOD activity and DPPH free radical scavenging activity of the FR treatment were greater than those of the AL treatment ($P < 0.05$). In contrast, the MDA content was significantly lower in the FR treatment than in the AL treatment ($P < 0.05$).

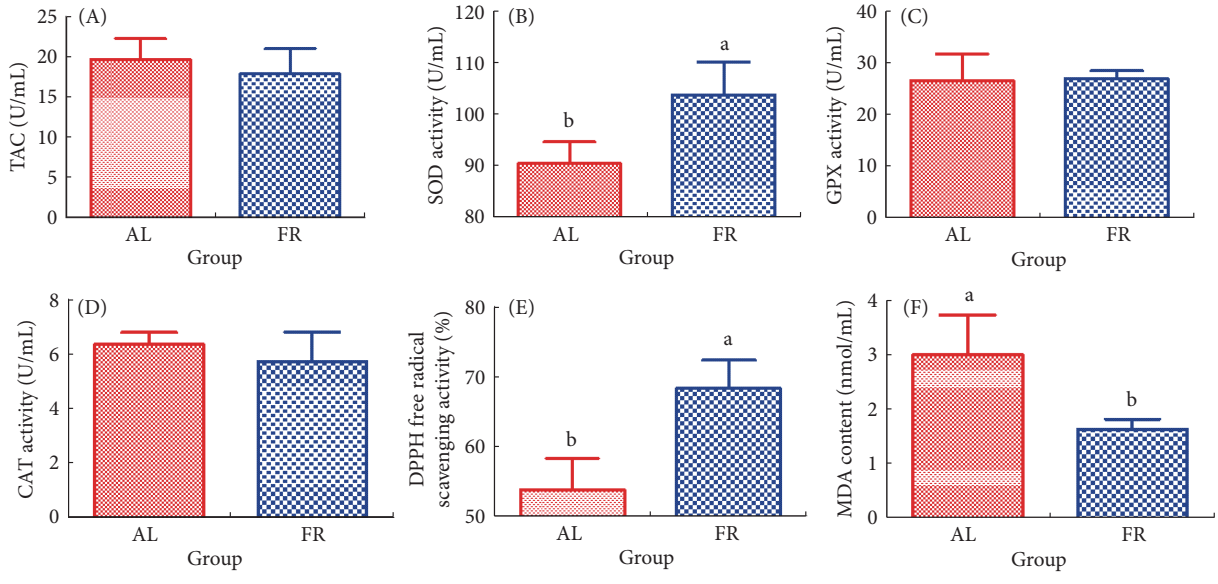

Figure 1 Effect of feed restriction on muscle antioxidant activity parameters in rabbit. (A) TAC; (B) SOD activity; (C) GPX activity; (D) CAT activity; (E) DPPH free radical scavenging activity; (F) MDA content. Data reported as mean ± SD (n = 12). Different lowercase letters mean significant difference ($P < 0.05$). AL, rabbits were fed *ad libitum* level; FR, rabbits were fed 80% *ad libitum* level.

3.3 Chemical composition and physical characteristics

No significant differences were detected in the moisture content, DM content, GE, or EE content between the two groups ($P > 0.05$) (Figure 2). However, compared with the AL treatment, FR treatment led to a significant increase in the level of CP ($P < 0.05$).

In terms of physical characteristics, there were no significant differences in pH, L^*, a^*, b^*, C^*, H, or drip loss between the two groups ($P > 0.05$) (Figure 3). Interestingly, the FR treatment resulted in significantly lower water loss rates and shear forces than the AL treatment ($P < 0.05$).

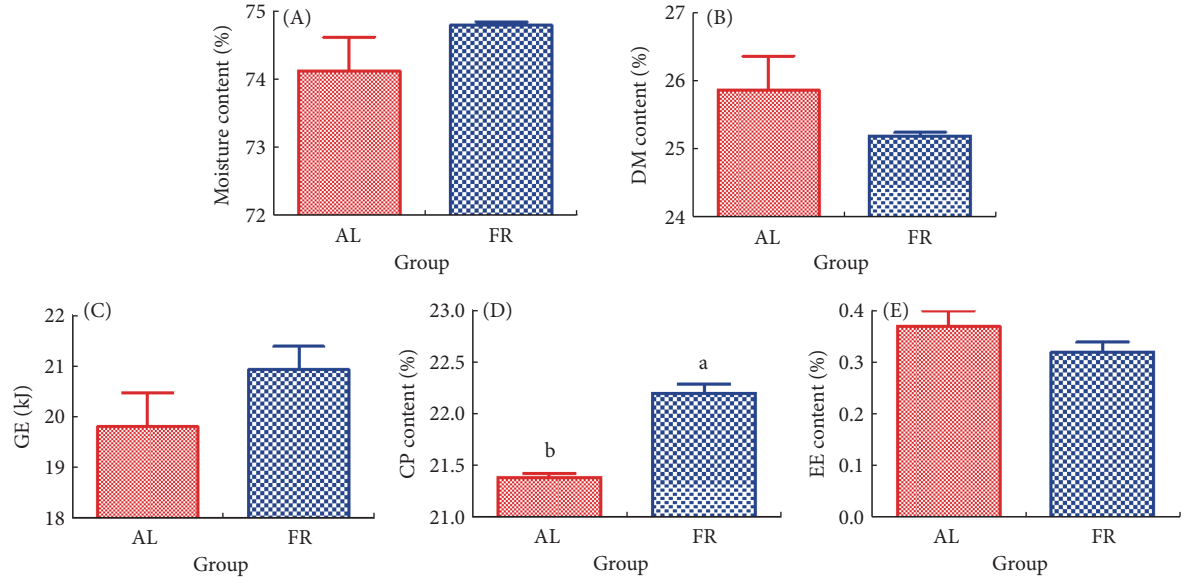

Figure 2 Effect of feed restriction on muscle chemical composition in rabbit. (A) Moisture content; (B) DM content; (C) gross energy (GE); (D) crude protein (CP) content; (E) ether extract (EE) content. Data reported as mean ± SD (n = 12). Different lowercase letters mean significant difference ($P < 0.05$). AL, rabbits were fed *ad libitum* level; FR, rabbits were fed 80% *ad libitum* level.

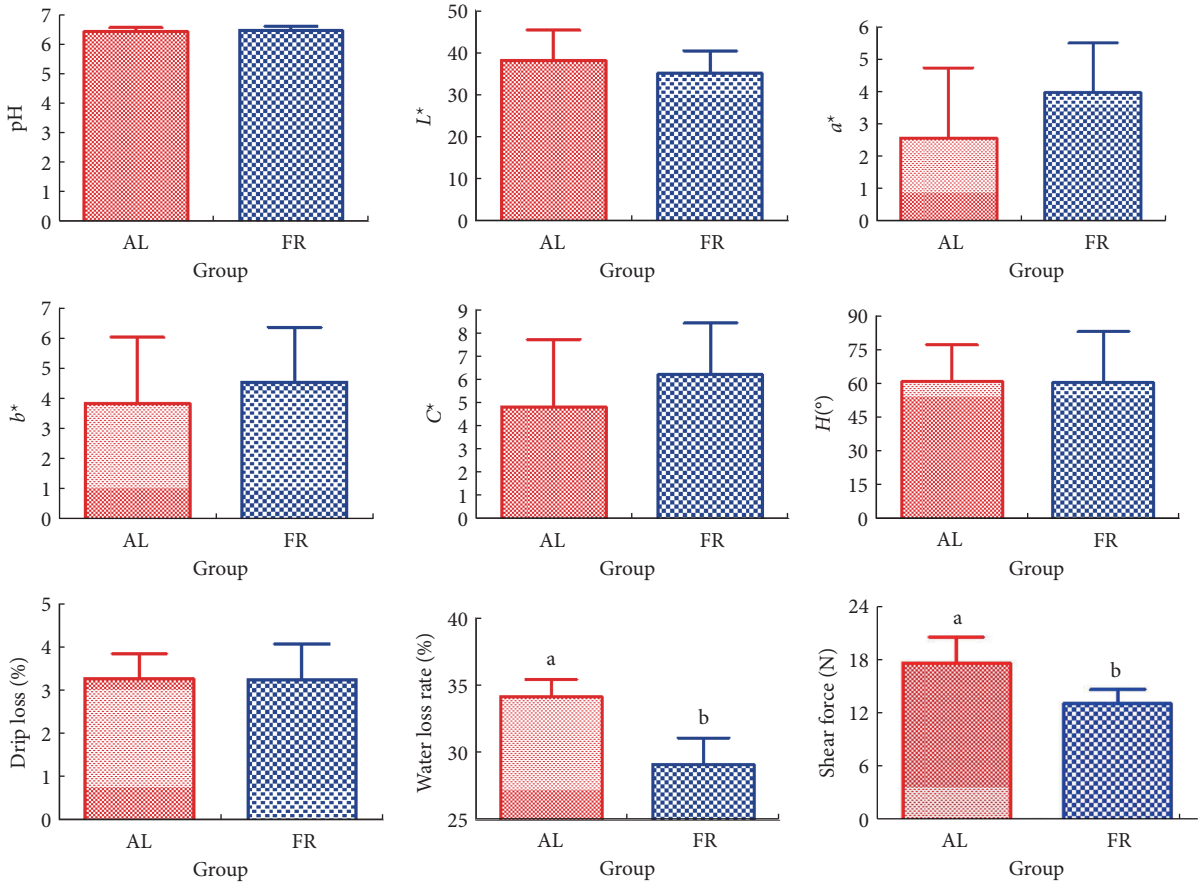

Figure 3 Effect of feed restriction on muscle physical characteristics in rabbit. Data reported as mean ± SD (n = 12). Different lowercase letters mean significant difference ($P < 0.05$). AL, rabbits were fed *ad libitum* level; FR, rabbits were fed 80% *ad libitum* level.

3.4 AAs profile

There were no significant differences in the Asp, Ser, Gly, Val, Met, Ile, Leu, Phe, Lys, and Arg contents between the AL and FR treatments ($P > 0.05$) (Table 3). However, the FR treatment resulted in significantly greater levels of Thr, Glu, Ala, Tyr, His, Pro, and TAAs than did the AL treatment ($P < 0.05$).

Table 3 Effect of feed restriction on muscle AAs profile in rabbit (%).

AAs	AL	FR	SEM	*P* value
Asp	7.18	7.34	0.070	0.178
Thr	3.52[b]	3.75[a]	0.051	0.032
Ser	3.74	3.78	0.035	0.433
Glu	12.48[b]	13.77[a]	0.136	0.003
Gly	3.71	3.82	0.066	0.299
Ala	4.66[b]	4.83[a]	0.039	0.038
Val	4.12	3.90	0.063	0.064
Met	1.76	1.79	0.037	0.511
Ile	3.70	3.73	0.056	0.718
Leu	6.84	6.70	0.115	0.446
Tyr	2.31[b]	2.86[a]	0.054	0.002
Phe	3.56	3.71	0.048	0.093
His	2.21[b]	2.40[a]	0.033	0.015
Lys	7.42	7.44	0.069	0.801
Arg	5.37	5.29	0.041	0.247
Pro	2.75[b]	3.11[a]	0.061	0.014
TAAs	75.49[b]	78.07[a]	0.254	0.002

Note: In the same row, values with different lowercase letter superscripts mean significant difference ($P < 0.05$). AL, rabbits were fed *ad libitum* level; FR, rabbits were fed 80% *ad libitum* level; SEM, standard error of mean.

3.5 FAs profile

No significant differences were detected in the contents of $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, $C_{18:0}$, $C_{18:3\ n\text{-}6}$, $C_{18:3\ n\text{-}3}$, $C_{20:3\ n\text{-}6}$, $C_{20:5\ n\text{-}3}$, $C_{22:5\ n\text{-}3}$, $C_{22:6\ n\text{-}3}$, or SFA between the two treatments ($P > 0.05$) (Table 4). Compared with the FR treatment, the AL treatment had significantly greater ($P < 0.05$) contents of $C_{16:0}$, $C_{16:1}$, $C_{18:1\ n\text{-}9c}$, and monounsaturated FA (MUFA), whereas significantly lower ($P < 0.05$) contents of $C_{18:2\ n\text{-}6c}$, $C_{20:4\ n\text{-}6}$, and polyunsaturated FA (PUFA) were detected. Moreover, $C_{20:2}$ was not detected in the AL treatment group.

Table 4 Effect of feed restriction on muscle FAs profile in rabbit (% total fatty acid methyl esters).

FAs	AL	FR	SEM	*P* value
$C_{12:0}$	0.19	0.15	0.015	0.135
$C_{14:0}$	3.39	3.58	0.102	0.254
$C_{15:0}$	0.78	0.76	0.015	0.282
$C_{16:0}$	29.72[a]	29.00[b]	0.149	0.027
$C_{16:1}$	2.86[a]	2.31[b]	0.065	0.004
$C_{17:0}$	0.30	0.32	0.032	0.721
$C_{18:0}$	5.65	6.08	0.413	0.499
$C_{18:1\ n\text{-}9c}$	21.21[a]	19.43[b]	0.172	0.002
$C_{18:2\ n\text{-}6c}$	27.18[b]	29.08[a]	0.258	0.007
$C_{18:3\ n\text{-}6}$	0.20	0.17	0.013	0.241
$C_{18:3\ n\text{-}3}$	3.80	4.01	0.099	0.217
$C_{20:2}$	nd	0.11		
$C_{20:3\ n\text{-}6}$	0.34	0.34	0.007	0.589
$C_{20:4\ n\text{-}6}$	3.94[b]	4.21[a]	0.040	0.009
$C_{20:5\ n\text{-}3}$	0.12	0.14	0.014	0.534
$C_{22:5\ n\text{-}3}$	0.23	0.24	0.015	0.681
$C_{22:6\ n\text{-}3}$	0.080	0.077	0.004	0.607
SFA	40.03	39.89	0.432	0.827
MUFA	24.07[a]	21.73[b]	0.164	0.001
PUFA	35.90[b]	38.38[a]	0.352	0.008

Note: In the same row, values with different lowercase letter superscripts mean significant difference ($P < 0.05$). AL, rabbits were fed *ad libitum* level; FR, rabbits were fed 80% *ad libitum* level. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. nd, not detected; SEM, standard error of mean.

3.6 Volatile compounds

A total of 24 volatile compounds were detected in this study, including 3 alcohols, 8 aldehydes, 4 alkanes, 1 carboxylic acid, 1 ester, 1 ketone, and 6 other compounds (Table 5). There were no significant differences in the relative contents of ethanol, 1-octen-3-ol, 2-ethyl-1-hexanol, 3-methyl-butanal, 2-methyl-butanal, hexanal, heptanal, nonanal, tridecane, tetradecane, pentadecane, acetic acid, 3-ethyl-2,5-dimethyl-pyrazine, benzenepropanenitrile, or *N*,*N*-dibutyl-formamide between the two treatments ($P > 0.05$). Interestingly, the FR treatment resulted in significantly greater relative contents of benzaldehyde, octanal,

Table 5 Effect of feed restriction on muscle volatile compounds in rabbit.

Volatile compounds	Molecular formula	Relative molecular mass	Relative content (%)		SEM	*P* value
			AL	FR		
Alcohols (3)						
Ethanol	C_2H_6O	46.04	2.81	2.89	0.361	0.880
1-Octen-3-ol	$C_8H_{16}O$	128.12	4.49	3.27	0.945	0.414
2-Ethyl-1-hexanol	$C_8H_{18}O$	130.14	5.44	6.15	1.023	0.650
Aldehydes (8)						
3-Methyl-butanal	$C_5H_{10}O$	86.07	0.37	0.49	0.108	0.458
2-Methyl-butanal	$C_5H_{10}O$	86.07	0.42	0.51	0.063	0.351
Hexanal	$C_6H_{12}O$	100.09	2.59	1.71	0.265	0.080
Heptanal	$C_7H_{14}O$	114.1	0.58	0.50	0.134	0.713
Benzaldehyde	C_7H_6O	106.04	0.60[b]	0.93[a]	0.079	0.041
Octanal	$C_8H_{16}O$	128.12	0.60[b]	1.36[a]	0.172	0.034
Nonanal	$C_9H_{18}O$	142.14	6.56	5.76	0.460	0.286
Decanal	$C_{10}H_{20}O$	156.15	0.22[b]	0.67[a]	0.061	0.006

Table 5 (*Continued*)

Volatile compounds	Molecular formula	Relative molecular mass	Relative content (%)		SEM	*P* value
			AL	FR		
Alkanes (4)						
Tridecane	$C_{13}H_{28}$	184.22	0.35	0.31	0.056	0.612
Tetradecane	$C_{14}H_{30}$	198.24	13.14	12.22	1.398	0.664
Pentadecane	$C_{15}H_{32}$	212.25	1.57	1.72	0.136	0.504
Hexadecane	$C_{16}H_{34}$	226.27	3.32^{b}	4.28^{a}	0.184	0.022
Carboxylic acids (1)						
Acetic acid	$C_2H_4O_2$	60.02	4.43	5.60	0.656	0.276
Esters (1)						
Carbamodithioic acid diethyl-methyl ester	$C_6H_{13}NS_2$	163.05	7.81^{b}	10.19^{a}	0.595	0.047
Ketones (1)						
2-Heptanone	$C_7H_{14}O$	114.10	0.22^{b}	0.55^{a}	0.043	0.005
Others (6)						
N-Ethyl-ethanamine	$C_4H_{11}N$	73.09	6.71^{b}	10.27^{a}	0.850	0.041
Dimethyl sulfone	$C_2H_6O_2S$	94.01	3.96^{b}	7.47^{a}	0.787	0.035
N,*N*-Diethyl-formamide	$C_5H_{11}NO$	101.08	0.70^{b}	1.14^{a}	0.078	0.017
3-Ethyl-2,5-dimethyl-pyrazine	$C_8H_{12}N_2$	136.10	0.90	0.86	0.040	0.456
Benzenepropanenitrile	C_9H_9N	131.07	1.86	2.23	0.275	0.403
N,*N*-Dibutyl-formamide	$C_9H_{19}NO$	157.15	1.17	1.55	0.146	0.142

Note: In the same row, values with different lowercase letter superscripts mean significant difference ($P < 0.05$). AL, rabbits were fed *ad libitum* level; FR, rabbits were fed 80% *ad libitum* level; SEM, standard error of mean.

decanal, hexadecane, carbamodithioic acid diethyl-methyl ester, 2-heptanone, *N*-ethyl-ethanamine, dimethyl sulfone, and *N*,*N*-diethyl-formamide than did the AL treatment ($P < 0.05$).

4 Discussion

Feed restriction might be a good strategy for rabbit management because it could decrease the feeding cost and reduce health risks[29]. Chodová et al.[30] reported that although greater feed restriction could reduce growth and slaughter weight, mild restriction did not have a negative effect on carcass composition in rabbits. Moreover, Larzul et al.[31] suggested that feed restriction did not impact on carcass yield in rabbits. In the present study, feed restriction had no effect on carcass yield or visceral organs, indicating that mild feed restriction (80% AL) had no adverse effect on slaughter performance parameters in rabbits. This elimination of any differences may be attributed to the compensatory growth of rabbits in the feed restriction group[32]. Moreover, the effects of feed restriction on carcass traits are usually coupled with the final body weight, possibly because feed restriction did not affect body weight (data not shown) compared with that of the AL group[33]. Consistent with our findings, Birolo et al.[34] reported that feed restriction did not affect the slaughter results or carcass traits of growing rabbits. Similarly, Meo et al.[9] reported that mild feed restriction (approximately 10%) did not induce differences in slaughter weight throughout the entire productive cycle of rabbits.

Numerous studies have shown that feed restriction can effectively enhance the body's immune function, antioxidant capacity, and disease resistance, thereby improving health in animals[35–37]. For example, Chen et al.[38] reported that 30% energy restriction could improve blood SOD activity, thus enhancing the antioxidant potential of broilers. Similarly, we found that feed restriction improved muscle antioxidant activity by increasing the activities of SOD and DPPH free radical scavenging and reducing the concentrations of MDA in rabbits. This may be due to feed restriction enhancing DNA repair ability, altering gene expression, and reducing the metabolic rate of animals, thereby increasing their antioxidant potential[39]. Another explanation might be that feed restriction has beneficial effects on the humoral immune response, which could promote a longer-lasting immunoglobulin Y anti-bovine serum albumin response and enhance the immune function of the animal body[40]. These findings are in agreement with those of Zhuang et al.[16], who reported that 70%–85% feed restriction could decrease the jejunum MDA content during the entire period, potentially increasing the antioxidant capacity of rabbits. However, Savary-Auzeloux et al.[41] reported that feed restriction reduced the TAC and that the TAC increased after *ad libitum* refeeding in lambs. The difference could be due to the different animals, feed restriction periods, feed restriction methods, and feeding conditions.

The chemical composition is an important characteristic of meat quality. Specifically, the chemical composition of meat is influenced by various factors, among which feed restriction is one of the most important factors[33]. Birolo et al.[10] reported that restricted rabbits presented compensatory growth and increased empty body gains in CP, EE, and GE at 69 d of age throughout a trial. Gidenne et al.[42] demonstrated that an intake restriction tended to increase nutrient digestibility (for example, CP) in rabbits. Hence, we found that feed restriction increased muscle CP content, possibly because feed restriction increased CP apparent digestibility and nitrogen retention and thus decreased nitrogen excretion in rabbits[43]. However, we did not detect CP digestibility in this study, and this parameter needs to be investigated in the future. In agreement with the results of the present study, Xiccato[44] reported that feed restriction resulted in a higher muscle CP content than did *ad libitum* feed in rabbits. Similarly, Crespo et al.[43] reported that feed restriction could increase the muscle CP content in growing rabbits at the slaughtering age.

Tenderness is an important sensory indicator of meat quality and it affects the nutritional and edible qualities of muscle[45]. Interestingly, increasing the activity of muscle antioxidants, such as SOD, GPX, and CAT, could protect the integrity of protein and enzyme structure and function in muscle and thus improve muscle tenderness[46]. Hence, we found that feed restriction reduced the water loss rate and shear force, suggesting that feed restriction might increase muscle tenderness in rabbits. This was possibly because feed restriction increased the activity of antioxidant enzymes and decreased the levels of oxidative products in muscle (Figure 1), subsequently increased the muscle tenderness. However, the underlying mechanisms deserve further investigation.

The protein in rabbit meat has high nutritional value because it contains various essential AAs. In addition, protein metabolism is regulated mainly by the concentration of AAs in animals[47]. AAs and their metabolites in tissues can be used for body function or participate in signal transduction processes[48]. Interestingly, a previous study reported that restricted feeding could stimulate protein metabolism in muscle tissue, causing the protein in the muscles of the body to be mobilized and broken down into AAs[49]. In the present study, we found that feed restriction treatment increased various muscle AAs, possibly because restricting feeding reduced the decarboxylation rate and transamination metabolism, thereby inhibiting protein degradation and further increasing the muscle AAs content[50]. However, the reason needs to be studied further. Another possible explanation is that feed restriction increased the concentration of CP (Figure 2) and thus improved the AAs content in rabbits.

PUFAs play important roles in immune function, intestinal development, energy metabolism, and gene expression regulation in animals[51–52]. Specifically, feeding restriction increased the proportion of PUFAs in their overall structure[53]. Wang et al.[54] reported that high-protein and high-energy feed inhibited FAs synthesis in animals. Liu et al.[55] reported that dietary supplemented with a low-energy diet presented lower lipase activity in the duodenum and ileum of animals. On the basis of the results of previous studies, we assumed that reducing feed intake appropriately might inhibit lipase activity and thus promote FAs synthesis in animals. In the present study, feed restriction increased the muscle PUFAs content of rabbits, possibly because feeding restriction might reduce the content of white adipose tissue and thus promote the production of the adipogenic cytokine lipid ketone[56]. Moreover, the high antioxidant activity might prevent lipid oxidation, and thus improved muscle PUFAs in animals[57]. Thus, another mechanism may be due to feeding restriction resulted in enhanced muscle some antioxidant activity parameters, thereby improving PUFAs content in rabbits. Consistent with our results, Metzger et al.[58] reported that feed restriction improved the muscle PUFAs content in rabbits.

Volatile compounds can impart specific flavours, aromas, or mouthfeel to meat and thus affect consumer purchasing decisions[59–60]. In particular, an increase in PUFAs content may have a negative effect on the oxidative stability of meat, and most volatile compounds have been identified as a result of lipid degradation[61]. van Ba et al.[62] reported that a high level of PUFAs in muscle might result in increased levels of volatile compounds. This occurred because PUFAs oxidation is a free radical chain reaction that results in the formation of volatile compounds, such as aldehydes, ketones, and alcohols, which then promote lipid oxidation and affect the flavour of meat[63]. Hence, PUFAs lipid oxidation is one of the main pathways for the formation of volatile compounds in meat, and the content and types of PUFAs directly affect the formation of meat flavour[64]. In the present study, we found that feed restriction increased the contents of benzaldehyde, octanal, decanal, hexadecane, 2-heptanone, etc., suggesting that it might improve muscle volatile compounds in rabbits. This could be because the feed restriction treatment improved the PUFAs content of the rabbit meat (Table 4), and the PUFAs were oxidized into volatile compounds, such as alcohols, hydrocarbons, and aldehydes, thus increasing the volatile compound content[65].

5 Conclusion

The results of the present study revealed that feed restriction was an effective strategy for improving the meat quality of rabbits at the late stage under the conditions of this experiment, because: 1) it enhanced muscle antioxidant activity and reduced oxidation products, 2) it increased muscle CP content and decreased the water loss rate and shear force, and 3) it improved the contents of some AAs, FAs and volatile compounds. However, we needed to consider the differences between LTL and hind leg meats in further studies. Moreover, we also did not consider the impact of sex differences on meat quality of rabbits, which was a potential limitation in this study. Further studies are needed to determine the mechanism by which feed restriction affects meat quality from the perspective of microbiomics and metabolomics.

Conflict of interest

The authors report that there are no conflicts of interest to declare in this manuscript.

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